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Structuring factors of the ant communities in the Segura River Basin

Factores Estructuradores de las Comunidades de Hormigas en la Cuenca del Segura

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“Biodiversity is our most valuable but least appreciated resource”

Edward O. Wilson

Photo credit for the previous page: Chema Catarineu

To my parents Leonardo and Frarén, to my wife Nuria and to my children Sofía, Gonzalo and Leo. I am very proud and fortunate to have had a shared life with all of them.

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Resumen

Previous page: *Camponotus sylvaticus* (photo credit: Manuel Martínez Manzano)

Factores estructuradores de las comunidades de hormigas en la Cuenca del Segura

Introducción

Las hormigas son el resultado de un gran éxito evolutivo. Son muy abundantes en los ecosistemas terrestres y llegan a constituir una fracción muy importante de la biomasa animal. Su importancia ecológica es incuestionable: actúan como ingenieras de ecosistemas, tienen efectos en la estructura y composición del suelo, afectan a los ciclos de materia y energía, son los principales depredadores de insectos y otros artrópodos, son los principales carroñeros de pequeños invertebrados, participan en la dispersión de semillas, y mantienen numerosas relaciones simbióticas con plantas, otros insectos, hongos o bacterias.

Durante las últimas décadas, las hormigas han tenido un creciente interés entre los ecólogos por su utilidad como bioindicadores en ecosistemas terrestres. Las características que hacen de las hormigas unos excelentes bioindicadores son su ubicuidad, su gran abundancia, su importancia en el funcionamiento de los ecosistemas, su sensibilidad ante los cambios ambientales y su facilidad de captura e identificación.

Objetivos y estructura de la Tesis

El principal objetivo de esta Tesis es el estudio de los factores que estructuran las comunidades de hormigas en la Cuenca del Segura.

Cuando iniciamos esta tesis, el conocimiento acerca de la mirmecofauna del sureste ibérico era bastante limitado, así que fue necesario hacer, en primer lugar, un estudio faunístico y zoogeográfico de las comunidades de hormigas (Capítulo I), basándonos en la bibliografía existente y, sobre todo, en los muestreos sistemáticos y no sistemáticos que llevamos a cabo durante los años 2010-2016. Durante estos muestreos, apareció una especie desconocida que resultó ser una especie nueva (*Temnothorax ansei* sp.n.) que se describe en el Capítulo IV.

Este estudio sirvió de base para el siguiente paso: estudiar los gradientes de diversidad de hormigas e identificar los principales factores responsables de los patrones de biodiversidad (Capítulo II).

Finalmente, para una mejor comprensión de los factores estructuradores, no sólo hay que tener en cuenta los gradientes ambientales regionales sino también los efectos locales de los diferentes cambios antropogénicos. Con este fin, se abordó el estudio de los efectos de una reforestación en la Sierra del Picarcho (Murcia) sobre las comunidades de hormigas.

Por tanto, los principales objetivos de esta Tesis fueron:

1) Realizar un estudio faunístico y zoogeográfico de las hormigas de la Cuenca del Segura.

2) Estudiar los gradientes de biodiversidad taxonómica, funcional y filogenética, así como sus interrelaciones en la Cuenca del Segura, e identificar los principales factores responsables de los patrones de biodiversidad.

3) Determinar cómo los cambios en la superficie del suelo y en la vegetación, provocados por una reforestación en la Sierra del Picarcho (Región de Murcia, Spain) afectan a las comunidades de hormigas.

La Tesis se estructura en cuatro capítulos:

I) Zoogeografía de las hormigas de la Cuenca del Segura.

II) El filtro ambiental en una región semiárida controla los gradientes de diversidad y la estructura filogenética de las comunidades de hormigas

III) Efectos de una reforestación con pinos, asociada a cambios en el suelo, en las comunidades de hormigas de una estepa semiárida.

IV) Una nueva especie de hormiga*, Temnothorax ansei* sp.n., de los ambientes áridos del sureste de España.

A continuación, se desarrolla un breve resumen de cada uno de los capítulos.

Capítulo 1. Zoogeografía de las hormigas de la Cuenca del Segura

En este capítulo, se presenta el primer listado de especies de hormigas de la Cuenca del Segura, completado a partir de tres fuentes:

1) Revisión de la bibliografía existente (23 publicaciones con citas sobre hormigas con referencia al área de estudio).

2) Muestreo sistemático con trampas de caída en 60 localidades de la Cuenca. En cada localidad se instaló un transecto con 10 pitfalls separadas 10m entre sí. Las 600 pitfalls estuvieron activas durante una semana (en julio de 2014 y de nuevo en julio de 2015)

3) Muestreo no sistemático mediante trampas de caída y muestreo manual en 103 localidades diferentes durante el periodo 2012–2016.

A partir de estos datos, hemos contabilizado un total de 110 especies de hormigas, pertenecientes a 30 géneros. Veintidós de las especies registradas son citadas por primera vez para la Cuenca del Segura.

Mediante una revisión bibliográfica para determinar su distribución geográfica actual, se han clasificado las especies presentes en la Cuenca del Segura en sus diferentes corotipos. La composición zoogeográfica está dominada por las especies de la Zona Mediterránea (75,2%), seguidos por los de la Zona de los Bosques mixtos y caducifolios (19,1%). Sólo aparecen seis especies cosmopolitas (5,71%). Actualmente hay una mayor proporción de especies de la zona de Bosques mixtos y caducifolios en altitudes medias y altas de la Cuenca del Segura, donde el clima es más frío y húmedo. Un 41% de las especies de hormigas de la Cuenca del Segura están también presentes en el Magreb.

Los elementos zoogeográficos más representados son los Ibéricos (20%), Holomediterráneos (17,1%) y Mediterráneos occidentales (13,3%). Los elementos eurocaucásicos y eurosiberianos occidentales tienden a estar más asociados a los bosques con mayores precipitaciones, mientras que los elementos surpaleárticos parecen estar más asociados a ecosistemas más parecidos a bosques y zonas esteparias con precipitaciones intermedias.

La existencia de todos estos diferentes orígenes zoogeográficos en la Cuenca del Segura está relacionada con su posición entre África y Europa; su compleja historia geotectónica, paleogeográfica y paleoclimática durante los últimos siete millones de años; su compleja geomorfología; y su gran diversidad de hábitats.

En base a estudios acerca de las comunidades de hormigas y de otros taxones, se proponen diferentes explicaciones acerca del origen de los diferentes corotipos de hormigas presentes en la Cuenca del Segura.

Capítulo 2. El filtro ambiental en una región semiárida controla los gradientes de diversidad y la estructura filogenética de las comunidades de hormigas

Nuestro principal objetivo en este capítulo fue estudiar los gradientes de diversidad taxonómica, funcional y filogenética de las comunidades de hormigas de la Cuenca del Segura, así como determinar cuáles son los principales procesos que estructuran esas comunidades.

Con este fin, seleccionamos 10 cuadrículas UTM de 10×10 km, representativas de la variedad climática, geológica y geomorfológica de la Cuenca del Segura. En cada una de estas cuadrículas, se instalaron 6 transectos (3 en zonas forestales y 3 en zonas de matorrales). Los transectos estaban formados por 10 trampas de caída, separadas 10 m entre sí, que permanecieron activas durante una semana en julio de 2014 y una semana en julio de 2015. Para las 54 especies de hormigas que aparecieron en los muestreos, preparamos una tabla de diez rasgos funcionales y un árbol filogenético, basándonos en la bibliografía existente. A partir de los datos obtenidos, calculamos diferentes índices de diversidad taxonómica, funcional y filogenética a dos escalas especiales (transecto y cuadrícula). También estudiamos los gradientes climáticos, geográficos y ecológicos.

En la Cuenca del Segura, existe un gradiente ambiental general que va desde las zonas altas, frías, más húmedas y con más vegetación hacia las zonas de menor altitud, más cálidas y secas, y con menos vegetación. Este gradiente es un importante factor estructurador de las comunidades de hormigas que afecta notablemente a los diferentes índices de diversidad. La precipitación parece ser el principal factor ambiental que controla la diversidad taxonómica, ya que las zonas más húmedas tienen una mayor producción primaria, abundancia y riqueza de especies. La diversidad taxonómica está muy correlacionada con la diversidad filogenética, pero la correlación con la diversidad funcional es menor.

La estructura filogenética es sobredispersa en las zonas de mayor altitud y humedad de la Cuenca, y agrupada en las zonas más bajas y áridas. Las dos subfamilias de hormigas dominantes en el área respondieron de manera diferente al gradiente ambiental general: Formicinae es mucho más sensible a este gradiente que Myrmicinae. Posiblemente estas diferencias de sensibilidad sean responsables de los importantes cambios de diversidad filogenética y de estructura de las comunidades a lo largo del gradiente.

Se comprobó la existencia de conservadurismo filogenético del nicho, lo que permite hipotetizar que las duras condiciones de aridez en las zonas bajas de la Cuenca suponen un filtro ambiental que agrupa la estructura filogenética de las comunidades de hormigas. En las zonas de mayor altitud y humedad, por el contrario, otros factores como las relaciones interespecíficas (principalmente competencia) pueden ser responsables de la estructura filogenética sobredispersa.

Las relaciones entre los diferentes índices de diversidad, los factores y el filtro ambientales provocado por las condiciones de aridez son más evidentes a escala de cuadrícula que a escala de transecto ya que, a escalas menores, las interacciones interespecíficas (principalmente la competencia) y los factores ambientales a pequeña escala tienen más relevancia como factores estructuradores de las comunidades.

Capítulo 3. Efectos de una reforestación con pinos en las comunidades de hormigas en una estepa semiárida.

Los cambios en el suelo y en la vegetación pueden afectar a las comunidades de hormigas, pero la relativa importancia de cada uno de estos factores en diferentes hábitats no es muy conocida. En particular, la información acerca de los efectos de las reforestaciones sobre las comunidades de artrópodos es particularmente escasa. En este trabajo se estudiaron los efectos de una reforestación en la Sierra del Picarcho (Región de Murcia) sobre sus comunidades de hormigas.

En mayo de 2012 fueron instalados tres transectos de 20 trampas de caída separadas 10m entre sí. En cada transecto, 10 de las trampas de caída se localizaban en la zona reforestada mientras que otras 10 estaban en el espartal sin alterar. Las trampas estuvieron activas durante una semana de cada una de las estaciones del año (mayo, agosto y noviembre de 2012 y febrero de 2013). A lo largo de estos transectos se caracterizó también la vegetación y la superficie del suelo.

Los cambios en la vegetación, causados por la reforestación produjeron un descenso en la cobertura de *Stipa tenacissima* y de cistáceas y un incremento de la cobertura de *Pinus halepensis*. La preparación mecánica del terreno hizo que aumentara la presencia de piedras superficiales y rocas semienterradas en la superficie del suelo del área reforestada, debido a la fractura del horizonte petrocálcico con el subsolado. Por el contrario, en el espartal sin alterar la superficie del suelo era más lisa y con menor cobertura de piedras y rocas.

La riqueza de especies de hormigas y la abundancia resultaron mayores en la reforestación que en el espartal sin alterar y un mayor número de especies mostraron respuestas positivas a la reforestación que negativas. Las causas subyacentes a estos cambios en la comunidad de hormigas de la reforestación están posiblemente relacionadas con los cambios en la vegetación y en la estructura del suelo. El incremento de la cobertura de pinos, posiblemente proporciona una mayor disponibilidad de alimentos a las hormigas, mientras que la mayor presencia de piedras superficiales permite disponer de refugios para las colonias de hormigas.

Capítulo 4. *Temnothorax ansei* sp.n. (Hymenoptera: Formicidae), una nueva especie de hormiga propia de los ambientes áridos del sureste de España.

En este capítulo se describe una nueva especie de hormiga del género *Temnothorax* basándonos en evidencias morfológicas y biológicas. La especie ha sido encontrada en dos localidades del sureste de España: Pulpí (Almería) y Lorca (Murcia).

Esta especie pertenece al grupo *laurae* en base a las siguientes características: ojos grandes, quetas largas y erectas en el mesosoma, peciolo y postpeciolo, cabeza rectangular y presencia de surco mesopropodeal. Las características específicas de la nueva especie son: color marrón castaño, pilosidad decumbente, cabeza y pronoto lisos y brillantes. Las reinas son pequeñas, similares en tamaño a las obreras. Los hábitos de esta nueva especie son nocturnos y crepusculares y habita en hábitats semiáridos con escasa cobertura vegetal dominada por *Stipa tenacissima* L. (Pulpí) o por caméfitos y pequeños matorrales (Lorca).

Teniendo en cuenta la morfometría de reinas y obreras, así como en su parcial poliginia, observada tanto en su hábitat como en el laboratorio, se sugiere que la formación de colonias de esta nueva especie ocurre probablemente mediante la estrategia de Fundación Dependiente.

En base a la morfología externa de las obreras, se incluyen unas claves para la identificación de las cinco especies ibéricas conocidas de *Temnothorax* del grupo *laurae*.

General Introduction

Previous page: *Polyergus rufescens* (photo credit: Pavel Krásenský, from www.AntWeb.org).

Background

Ecological importance of ants

Ants are a great evolutionary success, being extremely abundant and accounting for an important proportion of the total animal biomass in terrestrial ecosystems (Hölldobler & Wilson, 1990). Ants are also the most diverse group of all eusocial insects (Hölldobler & Wilson, 1990), with 13,512 species described (Bolton, 2019) and thousands of species yet to be described (Bolton et al., 2006). The ecological importance of ants is outstanding: they are ecologically dominant, act as ecosystem engineers, produce effects on soil structure, and affect the flow of energy and matter (Folgarait, 1998). "*They run much of the terrestrial world as the premier soil turners, channelers of energy, dominatrices of the insect fauna…*" (Hölldobler & Wilson, 1990).

The bioturbation caused by ants, increases the soil drainage, aeration, and water-holding capacity, and it can be globally compared to that caused by earthworms (Folgarait, 1998). Ants increase nutrients around its nest (mainly nitrogen and phosphorus), creating better conditions for microorganisms, microarthropods and some plant species (Wagner et al., 1997; Frouz & Jilková, 2008). Ants influence the carbon cycling, producing greenhouse gases as carbon dioxide or methane in their nests, and increasing soil respiration (Jurgensen et al., 2008). Ants also can influence chemistry of the litter and its decomposition, changing the food webs of fungivorous and bacterivorous invertebrates and microbial decomposers (Hunter et al., 2003).

Ants occupy multiple trophic levels. Some ant species are indirect herbivores (collecting honeydew) or direct herbivores (collecting seeds). Other ant species are specialiced predators, feeding on herbivore arthropods (being secondary consumers) or feeding on predator arthropods (being tertiary consumers). Most of them are omnivorous, combining different food sources as predation, scavenging or honeydew collection (Mooney & Tillberg, 2010). Ants are the main predators of other insects and small invertebrates (Hölldobler & Wilson, 1990), and they are preys, at the same time, for other predators as spiders, reptiles, amphibians, or birds (Retana et al., 1991). In many different habitats, ants are the main scavengers of small arthropod corpses (Retana et al., 1991).

Ants have developed complex symbiotic relationships with diverse organisms. Mutualistic relationships have been important for the evolutionary and ecological success of ants, including interactions with plants, insects, fungi and bacteria (Mooney & Tillberg, 2010). There are a lot of insects and other arthropods who are dependent on the ant societies, known as myrmecophiles (Hölldobler & Wilson, 1990). The myrmecophily is a very diverse phenomenon, including relationships of mutualism, parasitism or inquilinism.

One of the mutualistic relationships between ants and plants, myrmecochory, influence considerably the plant community structure. Some plant species provide seeds with a lipid-rich nutritional part (elaiosoma) consumed by ants who, in return, disperses these seeds (Giladi, 2006). This is an almost widespread phenomenom, present in more than 11,000 plant species (Lengyel et al., 2010). Ants also can act as pollinators in some cases (Rostás & Tautz, 2011).

In summary, ants provide important ecosystem services necessary for the maintenance and functioning of terrestrial ecosystems (Del Toro et al., 2012; Figure 1).

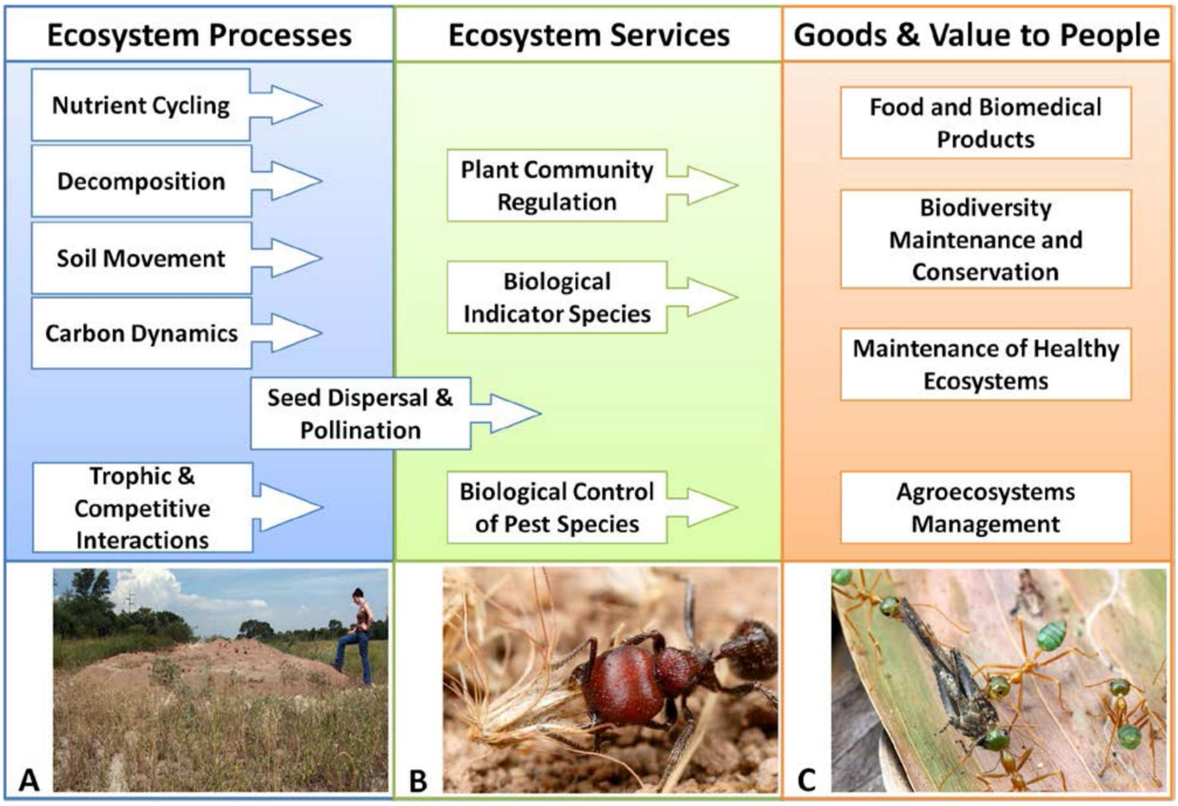

Figure 1: Concept map linking ecosystem processes and services to the products and goods valued by people. Developed from Mace et al. (2012) to include examples of ant-mediated ecosystem processes and services. (A) Leaf-cutter ant nest mound; (B) harvester ant transporting grass seed; (C) weaver ants attacking a grasshopper. Photo credits: Alexander Wild. (Figure from Del Toro et al., 2012).

Zoogeography of the ants of the Segura River Basin

The knowledge about the zoogeography of the ants of our study area, the Segura River Basin (Figure 2), was very limited at the beginning of this Thesis. Catarineu & Tinaut (2012) quoted 77 ant species in the first checklist for this area, but regarding only the Región de Murcia (60% of the area of the whole basin). There was also a few sub regional ant checklists of other nearby areas, but none of them deals in depth with ant zoogeography.

Figure 2: Map of Spain with the Segura River Basin outlined

There are also very few publications focusing on the zoogeography of European ants, but there is an increasing level of knowledge about ant species distribution. Czechowsky et al. (2002) and Czechowsky et al. (2012) classified the Polish ants into three main zoogeographical classes, corresponding with the three main vegetation zones in the Palaearctic realm: coniferous forest (taiga) zone, mixed and deciduous forest zone, and Mediterranean zone sensu lato. Within each zone, these authors categorized the ant species into different zoogeographic elements. Considering their current geographical distribution in the Palaearctic and Saharo-Arabian realms, and according with Czechowsky et al. (2002) and Czechowsky et al. (2012), the native ant species in the Segura River Basin can be classified into two different zones and 14 zoogeographical elements (Table 1; Figure 3).

Table 1. Zoogeographical composition of the native ant fauna of the Segura Basin (modified from Czechowsky et al., 2012).

Zoogeographical zones	**Zoogeographical elements**
Zone of the mixed and deciduous forest	
	Central and Southern European
	Central and Southern European/Maghrebian
	Euro-Caucasian
	Euro-Caucasian/Maghrebian
	Euro-West-Siberian
	Euro-West-Siberian/Maghrebian
	South Paleartic
Mediterranean zone	
	Holomediterranean
	Iberian
	Iberian/Maghrebian
	North-Mediterranean
	North-West Mediterranean
	Tethyan
	West-Mediterranean

The existence of ant species belonging to different chorotypes depends on the geologic, paleogeographic, climatic and evolutionary history of the region, and also on the current ecological and environmental factors. The Segura River Basin, has had clearly a complex paleogeographic and paleoclimatic history (Carrión et al., 2010) and currently has important contrasts in altitude, lithology, precipitation, temperature or human occupation, generating a great diversity of environments that allows a high biodiversity. The climatic oscillations during the Pleistocene Ice Ages are known to have had an important influence on the zoogeographic history of Europe, driving the repeated contraction/expansion of the zoogeographical ranges (Hewitt, 2011). After the last glacial maximum period (20–14 ky BP), some species expand their range northward from their Iberian refugia. The Baetic Ranges, where the Segura River Basin is located, were one of the Iberian Refugia (Gómez Lunt, 2007; Hewitt, 2011)

The ant species from the mixed and deciduous forest zone in the Segura River Basin possibly suffered contraction in their ranges during the ices ages, and survived in southern refuges as the Baetic ranges. After the last ice age, this species expand northward or tracked suitable habitats along elevation gradients in the mountain ranges.

Many of the Iberian endemic species possibly evolved during the Pleistocene Ice Ages from Tertiary species, surviving in the different Iberian refugia as the Baetic ranges. The mountainous geomorphology was particularly important in these processes of isolation, genetic divergence and allopatric speciation (Huseman et al., 2013).

The Maghreb was also an important speciation center and there are many Ibero-African ant species that colonized the Iberian Peninsula from North Africa and vice versa. Two paleogeographical events allowed the exchange of ants between Africa and the Iberian Peninsula. The first event was the separation of parts of the Baetic region from the Iberian mainland and its connection by the south to Africa during the Baetic crisis 16–14 Mya (Veith et al., 2004). The second event was the closure of the Mediterranean-Atlantic connections due to tectonic processes during the Messinian Salinity Crisis 5.96–5.33 Mya (Veith et al., 2004; Hewitt, 2011). The Iberian/Maghrebian and the West-Mediterranean elements might represent Tertiary species that colonized the Iberian Peninsula, and in some cases southern France, from North Africa during these events.

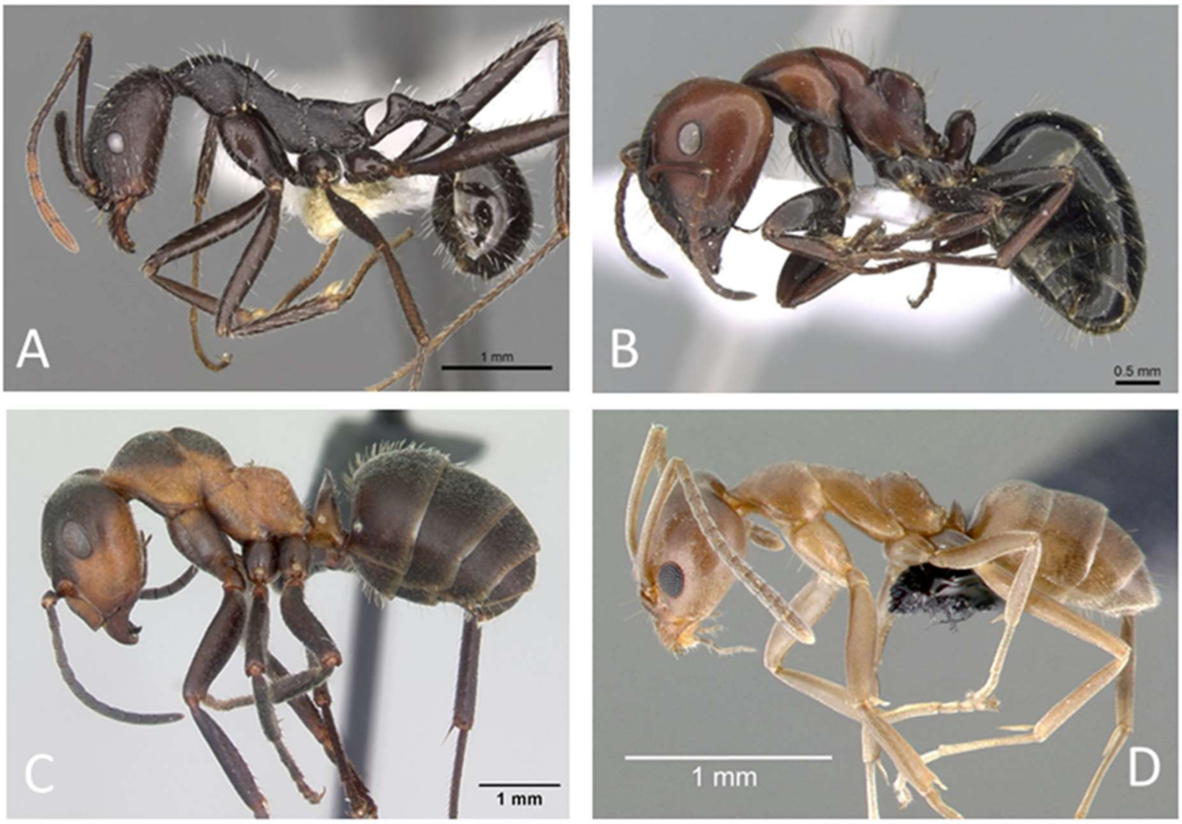

Figure 3. A) *Aphaenogaster iberica* Emery 1908, an Iberian endemism; B) *Camponotus ruber* Emery 1925, an Ibero/Maghrebian species; C) *Formica pratensis* Retzius, 1783, a South Palaeartic species; D) *Linepithema humile* Mayr, 1868, an invasive species. Photo credit: A) Shannon Hartman; B) Estella Ortega; C-D) April Nobile; all of them from www.AntWeb.org.

During the current geological epoch, the Anthropocene, the increase of air and maritime transport has led the introduction of invasive exotic species across the world. Nevertheless, only six cosmopolitan ant species are known in the Segura River Basin and none of them seems to have much ability for invasion in this region. It is likely that harsh conditions of the semi-arid areas of the Segura River Basin are not suitable for the invasion of the exotic species.

There is a greater proportion of species from the mixed and deciduous forest in the higher altitude areas, where the climate is wetter and cooler. Conversely, there are more species of Mediterranean ants in the lower altitude, hotter and dryer areas, possibly because they are more thermophilic species. This is possibly related to the phylogenetic diversity gradient discussed in Chapter II. We should take this point into account, because the evolutionary origin of species and their elevational distribution seem to be important in determining vulnerability to the ongoing climate change.

Ant community drivers

Understanding the drivers that originate and control spatial variation in the diversity and the composition of communities has been a central goal in ecology, biogeography and evolutionary biology since the nineteenth century (Lomolino, 2001). The predictions of increasing global mean temperatures and higher frequency of extreme weather events as a result of climate change (Pachauri et al., 2014) and its notable implications for biodiversity and ecosystem services (Cardinale et al., 2012) urge improving the knowledge about biodiversity controls, especially those related directly or indirectly with climate variables.

More than 30 hypotheses have been proposed to explain patterns of biodiversity (Sanders, 2002; Hawkins et al., 2003; Dunn et al., 2009; Szewczyk & Mc Cain, 2016). They can be grouped into four main categories related with environmental factors (mainly climate), space, evolutionary/biogeographic history and biotic processes (McCain & Grytnes, 2010).

Ants are an excellent candidate to study biodiversity gradients, because they are abundant, easy to sample (Figure 4), well known, have a very important functions in the ecosystems (Hölldobler & Wilson, 1990; Folgarait, 1998; Dunn et al., 2009) and have been the subject of gradient studies for more than 60 years (Dunn et al., 2009). Despite these efforts, we are still far from clearly understanding how the driving forces that control the diversity and structure of ant communities work.

Figure 4. Field techniques used in this Thesis for sampling ants. (A) Digging the hole for a pitfall trap. (B) Pitfall trap installed and geolocated. (C) Hand collecting with an aspirator. (D) Ants feeding on a bait. (Photo credits: Chema Catarineu).

Environmental factors, mainly climate, are one of the most important structuring forces for ant communities. Different habitat variables has been cited as ant community drivers: vegetation composition (Gotelli & Ellison, 2002), vegetation cover (Retana y Cerdá, 2000; Blatrix et al., 2016), vegetation density (Bernardou et al., 2014), habitat type (Kumschick et al., 2009; Del Toro, 2013), habitat complexity (Lassau & Hochuli, 2004; Sarty et al., 2006; Pacheco & Vasconcelos, 2012), net primary productivity (Kaspari et al., 2000), light availability (Gotelli & Ellison, 2002), aspect of slopes (Bernardou et al., 2014), proportion of bare ground (Munyai & Foord, 2012), or soil attributes (Boulton et al., 2005; Kaspari et al, 2008; Catarineu et al., 2018). Finally, anthropogenic disturbances have undoubtedly an increasing importance in the ant community structures (Boulton et al., 2005; Philpott et al., 2010; Liu et al., 2016; Arnan et al., 2018).

When only species with certain traits and successful evolutionary strategies can persist in a harsh environment, we are talking about Environmental or Habitat filtering. Environmental filtering is mainly produced by the climate variables, and those related to temperature are especially important for ant communities: mean temperature (Retana y

Cerdá, 2000; Kaspary et al., 2003; Longino & Colwell, 2011; Machac et al., 2011; Munyai & Foord, 2012; Bernardou et al 2014), diurnal temperature range (Paknia & Pfeiffer, 2014), maximum temperature of the warmest month (Del Toro, 2013) or temperature seasonality (Kaspari et al., 2000). Climatic variables related with water availability are also important, especially in arid and semiarid environments: precipitation (Sanders et al., 2003; Paknia & Pfeiffer, 2014), or aridity calculated as the difference between actual and potential evapotranspiration (Arnan et al., 2018).

In the Segura River Basin, there is a general environmental gradient, which is an important force structuring ant diversity, from the higher, colder and wetter areas to the lower, hotter and dryer areas. Precipitation seems to be the more important environmental factor controlling ant taxonomic diversity, allowing a higher primary production and a greater diversity in the wetter areas (Figure 5).

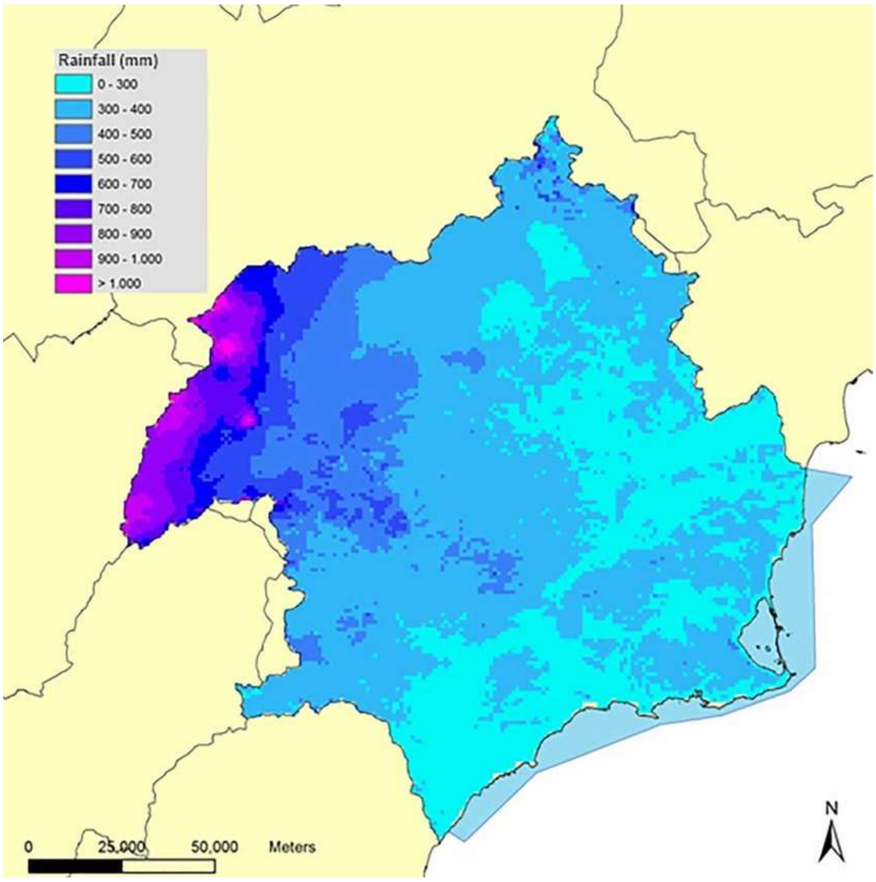

Figure 5. Spatial distribution of average yearly rainfall (mm/year) in the Segura River Basin (1980/81-2011/12). Source: SRBD, Ministry of Agriculture and Fisheries, Food and Environment (Spain).

Spatial factors are another important structuring force shaping ant communities. The dispersal limitations of ants have a strong effect on their community structure (Arnan et al., 2015). With a more limited dispersal, communities' similarity tend to be inversely associated with spatial distance (Paknia & Pfeiffer, 2014). The species-area relationship hypothesis (Rosenzweig, 1995) predicts that, on a mountain gradient, the mountain base should harbour mores species, because it cover more area, than the mountain tops. Some authors have proposed the available area as an important driver structuring ant communities (Sanders, 2002; Kaspary et al., 2003). Conversely, the spatial constraint hypothesis (Colwell et al., 2004) predict that, on a mountain gradient, the midpoints harbour more species, because there is more overlap of species ranges.

The complex interactions between ant species and the different spatial and environmental factors generate gradients of ant diversity at local or regional scales. Patterns of ant diversity along altitudinal gradients are the most studied in relation to ant communities. Szewczyk & McCain (2016), reviewed 67 datasets on ant elevation diversity, and concluded that ant elevational diversity is controlled by several factors, changing in number and identity by precipitation levels. The most common patterns were mid-elevation peaks in diversity followed by low-elevation plateaus and monotonic decreases with elevation (Szewczyk & McCain, 2016).

Evolutionary/biogeographic history (as demonstrated for the Segura Basin on Chapter 1) and biotic interactions are also important factors underlying community assembly, but to reveal the relative importance of each one, we need functional and phylogenetic approaches.

Ant taxonomic, functional and phylogenetic gradients

To disentangle the complex mechanism structuring the communities and the interactions between ecological and evolutive processes, community ecologist take into account not only the taxonomic diversity (TD) but also functional biodiversity (FD; Webb et al., 2002; Petchey & Gaston, 2006), phylogenetic biodiversity (PD; Faith 1992, Webb et al. 2002) and its interrelationships (Figure 6).

Analysing how functional and phylogenetic diversity indices relate with the environmental factors and how are the interrelationships between these different indices, provides important insights for the understanding of the mechanisms that control diversity patterns. Phylogenetic diversity reflects the accumulation of evolutionary history of a community (Webb et al., 2002; Swenson, 2011; Miller et al., 2017), while functional diversity reflects the morphological, physiological and ecological traits of a community, that generally concerns the range of things that organisms do in communities and ecosystems (Petchey-Gaston, 2006).

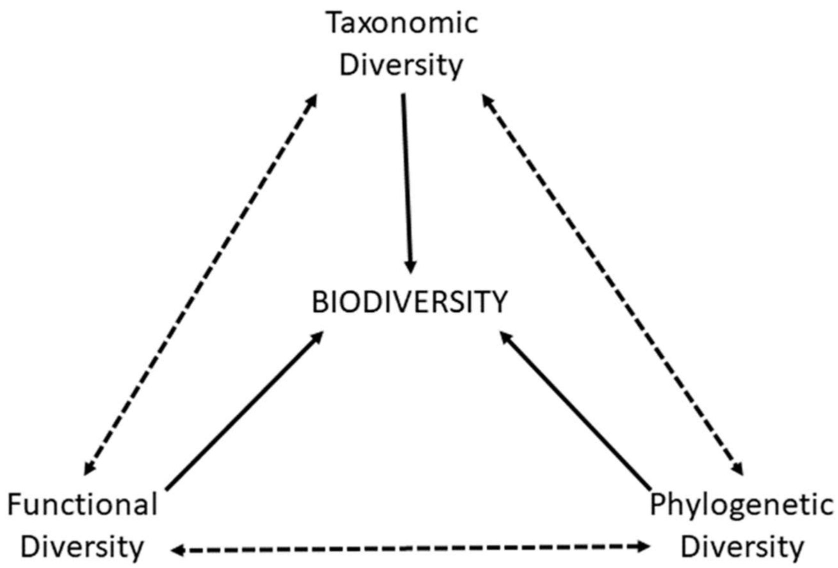

Figure 6. The three main components of biodiversity. Modified from Swenson (2011).

Examining the phylogenetic community structure and studying the evidence of niche conservatism is very useful to understand the processes driving community assembly (Webb et al., 2002; Cavender-Bares et al., 2009). When functional traits are evolutionarily conserved, a clustered phylogenetic distribution implies that the role of environmental filter is predominant shaping communities, and phylogenetic overdispersion (evenness) implies that interspecific competition are the main structuring factors (Webb et al. 2002; Kraft et al., 2007). When traits are convergent, environmental filter should produce phylogenetic overdispersion, and competition should produce phylogenetic random or clustered patterns (Kraft et al. 2007; see Table 2).

To properly understand the causes underlying biodiversity patterns, we have to take into account different scales of analyses (Ricklefs, 2004). At the regional scale, biodiversity is the result of the evolutionary processes (speciation, extinction and dispersal processes; Swensson 2011), modulated by the climatic and geological history (Cavender-Bares et al., 2009; Lessard et al., 2011). At higher resolution spatial scales, the different patterns of biodiversity becomes dominated by the environmental filters (Ricklefs, 2004; Cavender-Bares et al., 2009), while at the local scale, species interaction (competition, predation, mutualism…) are likely the main drivers (Cavender-Bares et al., 2009; see Figure 7).

Table 2. Patterns of phylogenetic structure predicted to be produced by various community assembly processes when traits of interest are phylogenetically conserved or convergent. Modified from Kraft et al. (2007).

Assembly process	**Phylogenetic structure**	
	Traits conserved	Traits convergent
Limiting similarity	Overdispersed	Random or clustered
Environmental filtering	Clustered	Overdispersed
Neutral assembly	Random	Random

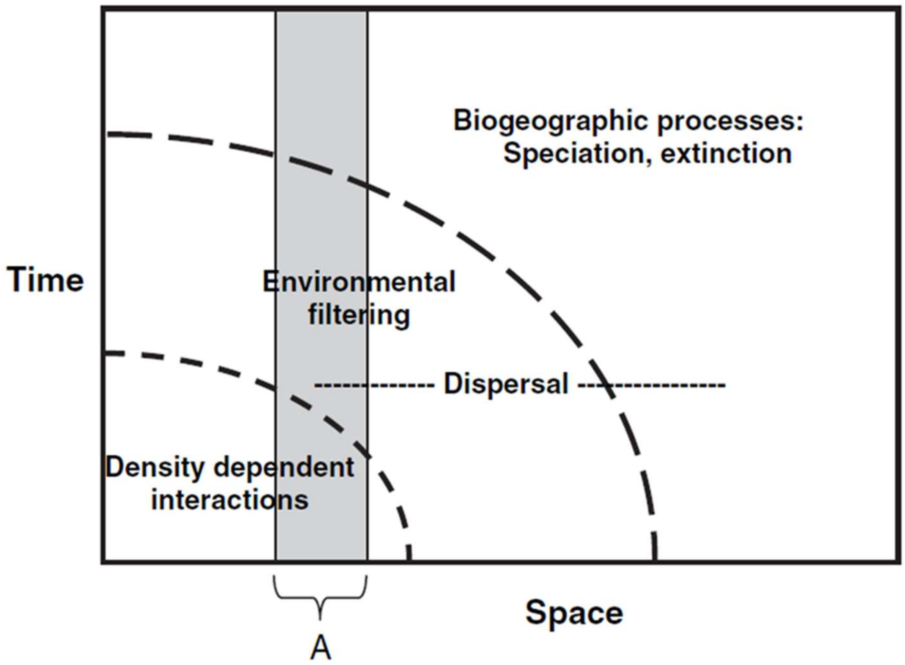

Figure 7. Processes driving community structure operate over varying temporal scales and depend fundamentally on the spatial scale of analysis. At the broader spatial scales, species distributions are determined largely by biogeographic processes involving speciation, extinction and dispersal. At decreasing spatial scales, environmental filtering is the main force. At the neighbourhood scales, density-dependent processes (e.g. competition) operate most intensively. At a given spatial scale (e.g. A), species distribution depends on multiple factors which may be difficult to tease apart. Source: Cavender-Bares et al. (2009).

The knowledge about ant phylogeny and evolution is developing rapidly. Moureau & Bell (2013), using a molecular dataset inferred the molecular phylogenetic relationships of 295 ant species, including members of all extant subfamilies. From this phylogeny, and from other 38 references, Arnan et al. (2016) built a complete phylogeny for 154 European ant species. We do not yet have reliable estimates of branch length for all the Iberian ant species, and the standard approach in this case is ultrametrize the phylogenetic tree (all the path-lengths from the root to the tips are equal) through the Grafen's rho transformation.

There is also an increasing knowledge about ant traits. Arnan et al. (2014) selected 10 traits that reflect different dimensions of the functional niche, including morphology, life history and behaviour and completed a species-trait database for 124 ant South-Western European ant species. Arnan et al. (2016) found evidence of niche conservatism in most of the functional traits of European ants, both at the continental and at the regional scale.

Phylogenetic and functional approaches are an undoubtedly useful tools to predict community responses to climate change, and it should be apply in future works on ant community ecology.

Thesis outline, Objectives and hypothesis

Our main initial objective was to study the structuring factors of the ant communities in the Segura River Basin. But, when we considered initiate this PhD Thesis, the knowledge about southeast Iberian ants was considerably low. There were very few researchs focusing on the myrmecofauna in the Segura River Basin. The first checklist that partially covers the area was about the ants of the Región de Murcia (Catarineu & Tinaut, 2012). We needed a faunistic study, a work that is becoming less and less attractive for researchers, but is the necessary basis on which we can build the ecological studies. Therefore, we began doing a faunistic and zoogeographic study of the ants of the Segura River Basin.

To complete the ant faunistic inventory (Chapter I), and for further zoogeographic and ecological studies we implement:

(i) a literature review (23 papers that cite South eastern Iberian ants)

(ii) systematic sampling of 60 locations spread over ten 10×10 km UTM squares representative of the climatic, geological and geomorphological variation of the region

(iii) non-systematic sampling by pitfall trapping and hand collecting over the whole region, adding up to 103 different localities during the period 2010–2016

During the systematic sampling, we detected an unknown ant species that we were unable to identify. We described it as a new ant species (*Temnothorax ansei* sp.n.; Chapter IV)

Once we had implemented the ant inventory, we conducted a zoogeographic study of the ant fauna of the Segura River Basin (Chapter I). In this study, we detected that a general environmental gradient was an important factor structuring ant communities in terms of the species pools of different biogeographic origins.

These studies were the basis for the next step: to examine the gradients of taxonomic, functional and phylogenetic diversity and the phylogenetic structure of the ant communities, at two different spatial scales, in the Segura River Basin (Chapter II). In this study, it becomes more evident that the general environmental gradient is very important determining the structure of the ant communities.

Finally, for a better understanding of the structuring factors underlying ant communities structure, and to predict their future evolution facing the global change, we must not only bear in mind the regional environmental gradients, but also the local effects of the different anthropogenic changes. For this purpose, we conducted a local case-study about the effects of a reforestation with *Pinus halepensis* of a semiarid *Stipa tenacissima* grassland in the southern pediment of Sierra del Picarcho (Murcia) on the local ant assemblage.

Summarizing, the main objetives for this Thesis were:

1) Faunistic and zoogeographic study of the ants of the Segura River Basin.

2) To study the ant gradients of Taxonomic, Functional and Phylogenetic diversity and its relationships in the Segura River Basin. To identify the main environmental drivers of ant diversity patterns.

3) To determine how changes in soil surface and vegetation caused by reforestation in Sierra del Picarcho (Región de Murcia, Spain) affect the ant assemblage.

To achieve this objetives, the present Thesis is composed of four chapters:

1) Zoogeography of the Ants (Hymenoptera: Formicidae) of the Segura River Basin.

2) Environmental filtering in a semiarid region underlies ant diversity gradients and phylogenetic structure.

3) Effect of pine reforestation associated with soil disturbance on ant assemblages (Hymenoptera: Formicidae) in a semiarid steppe.

4) A New Ant Species, *Temnothorax ansei* sp.n. (Hymenoptera: Formicidae) from the Arid Environments of South-eastern Spain.

The objectives and hypotheses of each chapter are detailed below.

Zoogeography of the ants of the Segura River Basin (Chapter I)

In this chapter, the main goal was to present the first checklist of the ant species of the Segura River Basin, and to analyse the zoogeographical composition.

The particular objectives were:

1) To compile a checklist of ant species in the Segura River Basin based on a review of the literature and material collected during sampling conducted from 2012 to 2016.

2) To classify the ant species of the Segura River Basin into zoogeographic chorotypes.

3) To analyse if zoogeographic chorotypes are related to climatic gradients in the Segura River Basin.

Environmental filtering in a semiarid region underlies ant diversity gradients and phylogenetic structure (Chapter II)

In this chapter, the main goal was to examine the gradients of taxonomic, functional and phylogenetic diversity and the phylogenetic structure of the ant communities, at two different spatial scales, in the Segura River Basin.

Four predictions were tested:

1) Environmental gradients significantly determines the taxonomic, functional and phylogenetic ant diversity in the Segura River Basin.

2) Precipitation is the main environmental factor explaining the taxonomic, functional and phylogenetic ant diversity patterns in the Segura River Basin

3) General environmental factors better explains variation of the diversity indices at the larger scale than at the lower scale.

4) Ant communities in the semi-arid areas would be phylogenetically clustered indicating environmental filtering (if traits are conserved).

Effects of a pine reforestation associated with soil disturbance on ant assemblages in a semiarid steppe (Chapter III)

In this chapter, the main goal was to study the effects of a 16-year old reforestation with *Pinus halepensis* of a semiarid *Stipa tenacissima* grassland on the local ant assemblage, looking specifically at the effects of changes in the soil and vegetation. The study was carried out in the Sierra del Picarcho (Region of Murcia, south eastern Spain)

The particular objectives were:

1) To determine how reforestation following Mechanical Site Preparation affects the structure of the surface of the soil and vegetation.

2) To determine how reforestation affects an epigeal ant assemblage and species, particularly their diversity and abundance.

3) To determine which changes at the surface of the soil and in the vegetation are likely to cause changes in the ant assemblage.

A new ant species, Temnothorax ansei sp.n. from the arid environments of South-eastern Spain (Chapter IV)

In this chapter, the main goal was to describe a new ant species in the genus *Temnothorax*, species group *laurae*, based on morphological evidences supported by other biological information.

The particular objectives were:

1) To describe the tree castes of this new species (worker, queens and males), and to comment the taxonomic position and diagnosis.

2) To study some aspects regarding the biology and ecology of this new species.

3) To propose a dichotomous key to the Iberian *laurae* group species.

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Chapter 1

Zoogeography of the Ants (Hymenoptera: Formicidae) of the Segura River Basin.

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Zoogeography of the Ants (Hymenoptera: Formicidae) of the Segura River Basin.

Abstract:

In this chapter, we present the first checklist of the ant species of the Segura River Basin based on a review of specific literature and biological material collected during field work conducted from 2012 to 2016. Our findings recorded 110 species that belong to 30 genera of ants and twenty two of these species have been recorded for the first time in this area. The zoogeographical composition is dominated by the species of the Mediterranean zone (75.2%), followed by the mixed and deciduous forest zone (19.1%). The most important zoogeographic elements are: Iberian (20%), Holomediterranean (17.1%) and West-Mediterranean (13.3%). There are only six cosmopolitan species (5.71%). There is a greater proportion of species from the mixed and deciduous forest zone in the high-mid altitudes in the Segura River Basin, where the climate is cooler, and more humid. The Euro-Caucasian and Euro-West Siberian elements have a tendency to be more associated to forest with a higher precipitation, whilst the South Palearctic elements seem to be more associated to ecosystems more similar to the forest-steppe zone with intermediate precipitation. The existence of these different zoogeographic origins in this area is probably linked with: the position between Africa and Europe; the complex geotectonic, paleogeographic, and paleoclimatic history during the last 7 My; the complex geomorphology; and the high climate and habitat diversity. Based on ant studies and other taxa, possible explanations of the zoogeographic origin of these ant chorotypes are proposed.

Introduction

The Mediterranean Basin, including the Iberian Peninsula, is one of the most important biodiversity hotspots in the world, with a high level of endemism (Médail & Quézel, 1997; Myers et al., 2000; Gómez & Lunt, 2007; Hewitt, 2011). Médail and Quézel (1997), based on floral endemism, identified ten Mediterranean Basin hotspots, and the Baetic-Rifan complex (including the Baetic ranges), one of them being.

Our study area, the Segura River Basin, is located in the central sector of southeastern Iberian Peninsula (Figure 1) and is representative of the environmental diversity of the area. It is framed in the western border by the Baetic ranges, a result of the convergence between the African and Iberian plates. The Baetic ranges intercept Atlantic cyclones in such a way that at some points of the mountainous area rainfall reaches 900-1,200 mm per year, whilst a rain shadow lies immediately to the east and dominates the region. The Segura River Basin (19,025 km^2) has large orographic variety, with mountains that reach up to 2,000 m.a.s.l., as well as contrasting coastal areas and extensive plains and valleys (Confederación Hidrográfica del Segura, 2017).

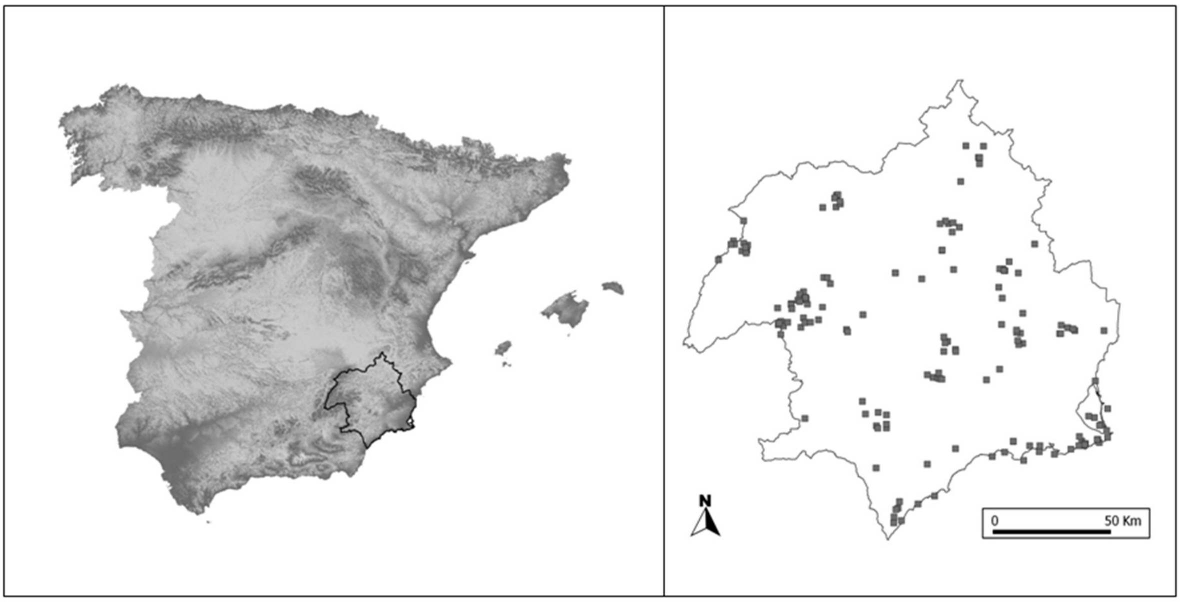

Figure 1. Map of Spain with the Segura River Basin outlined (on the left) and a map of the Segura River Basin with the 163 localities sampled indicated with grey squares (on the right).

Mountain lithology consists of limestone, dolomite and shale, and other metamorphic siliceous materials, whilst the plains and valleys between mountains are usually filled with marls, alluvial, and colluvial quaternary deposits and some volcanic outcrops (Mellado et al., 2002). The territory is very singular, both for the Iberian Peninsula and Europe as a whole, and is dominated by a Mediterranean semiarid climate, yet, also presents strong rainfall and temperature gradients (Vidal-Abarca et al., 1987). The vegetation, predominantly xerophytic, is a product of climate aridification during mid-to-late Holocene and human induced degradation (Carrión et al., 2010).

The contrasts in altitude, lithology, precipitation, temperature and also in relation to human occupation, generate a great diversity of environments that allows a high biodiversity. The complex paleogeographic and paleoclimatic history of the area (Carrión et al., 2010) has also contributed to the existence of a rich biodiversity.

The Iberian ant fauna is progressively more widely known, although this knowledge is still very fragmentary and limited to studies of specific areas. There are only a few sub regional checklists of the myrmecofauna in the southeastern Iberian Peninsula (Tinaut, 1981; Ortiz & Tinaut, 1988; Espadaler, 1997a; Espadaler, 1997b; Catarineu & Tinaut, 2012; Del Campo et al., 2014). There are also very few publications focusing on the zoogeography of European ants: northern Europe (Baroni-Urbani & Collingwood, 1977), Greek islands (Collingwood, 1993), Poland (Czechowski et al., 2002; Czechowski et al., 2012), Ukraine (Radchenko, 2011), and Montenegro (Karaman, 2011). Dynamic lists, such as Antmaps (Antmaps.org, 2016), also provide species distribution information. The southeastern Iberian Peninsula is included in only four publications mentioning ant zoogeography, in the Sierra Nevada (Tinaut, 1981), Cabo de Gata (Tinaut et al., 2009), SE-Spain (Piñero et al., 2011), and Región de Murcia (Catarineu & Tinaut, 2012).

The main objectives of this paper are:

1) to compile a checklist of ant species in the Segura River Basin based on a review of the literature and material collected during sampling conducted from 2012 to 2016.

2) to classify the ant species of the Segura River Basin into zoogeographic chorotypes.

3) to analyze if zoogeographic chorotypes are related to climatic gradients in the Segura River Basin.

Material and methods

Ant data sources

The checklist presented is based on three different sources: (i) a literature review (23 papers that cite Southeastern Iberian ants, see Table 1); (ii) systematic sampling of 60 locations spread over ten 10×10 km UTM squares representative of the climatic, geological and geomorphological variation of the region (in each location, a 100-m transect was set up, consisting of 10 pitfall traps separated by 10 metres and open for one week in July 2014 and July 2015); pitfall traps were polystyrene tubes (2 cm diameter, 10.5 cm long) filled with 5 ml of 50:50 propylene glycol and water solution with a few drops of liquid soap added to reduce surface tension; (iii) non-systematic sampling by pitfall trapping and hand collecting over the whole region, adding up to 103 different localities during the period 2010–2016 (see Figure 1 for the 163 locations, and Table S1 for the whole information about locations and dates).

All ant samples were sorted and identified to species level using a stereo microscope and Iberian ant taxonomic keys (Hormigas.org, 2016), taxonomic revisions of some ant genera (Seifert, 1988, 1992, 2002), species descriptions, and high-resolution pictures provided by Antweb (2017). It was not possible to identify species of *Leptanilla* Emery, *Proformica* Ruzsky, and *Solenopsis* Westwood due to a lack of modern revisions and complete taxonomic keys in these genera. In relation to *Tapinoma* cf *nigerrimum* and *Tetramorium* cf *caespitum*, recently Seifert et al. (2017) and Wagner et al. (2017) confirmed the existence of different cryptic species within these species complexes found in the Iberian Peninsula. Unfortunately, there is not a simple nor precise method for phenotypical species delimitation in these species complex as we have no access to the specific optical equipment required for this task. Identified ants were transferred to vials with ethanol 70º, or were mounted. All specimens are deposited in the premises of Asociación de Naturalistas del Sureste (Murcia, Spain) and some duplicates samples in the University of Córdoba (Área de Ecología, Córdoba, Spain).

Zoogeographic categories

A review of the present geographic distribution of the Segura River Basin ants was undertaken, based mainly on the following sources: Czechowski et al. (2002); Glaser (2006); Csösz et al. (2007); Karaman (2011); Czechowski et al. (2012); Seifert (2012a, 2012b); AntArea (2015); Antmaps (2016); Hormigas.org (2016); Janicki et al. (2016); Antweb (2017); and Lebas et al. (2017). Considering their current geographical distribution in the Palaearctic and Saharo-Arabian zoogeographic realms (sensu Holt et al., 2013), the native ant species were classified into two main zoogeographical classes corresponding to the two main Palaearctic vegetation zones, in accordance with Czechowski et al. (2002) and Czechowski et al. (2012): (I) mixed and deciduous forest zone; and (II) the Mediterranean zone sensu lato. Within each zone, species ranges were classified into the following scheme:

Elements represented in the Segura River Basin ants (modified from Czechowski et al., 2012):

I. Mixed and deciduous forest zone.

1) The Central and Southern European element (CSE)- species distributed mainly in Southern and Central Europe.

2) The Central and Southern European/Maghrebian element (CSE/M)- species distributed mainly in Southern and Central Europe and also in the Maghreb.

3) The Eurocaucasian element (EC)- species distributed mainly in the zone of deciduous forest, partly in mixed forest areas within Europe and the Caucasus; possibly reaching Asia Minor and the Near East.

4) The Eurocaucasian/Maghrebian element (EC/M)- Eurocaucasian species also present in the Maghreb.

5) Euro-West-Siberian element (EWS)- species widely distributed in Europe (usually also in the Caucasus) and West Siberia, reaching the east Altai Mts.

6) Euro-West-Siberian/Maghrebian element (EWS/M)- Euro-West-Siberian species also present in the Maghreb.

7) The South-Palaeartic element (SP)- generally Trans-Palaeartic forms, often distributed from the Atlantic to Pacific Oceans, whose ranges cover mainly the southern part of the forest zone and the forest-steppe zone; ecologically associated with dry forest or dry grasslands.

II. Mediterranean zone sensu lato.

8) The Tethyan Element (T)- species with a wide area that includes wholly or at least mostly the Mediterranean region, south of central and eastern Europe, the Caucasus, Asia Minor, the Middle East, Iran, Kazakhstan and Middle Asia.

9) The Holomediterranean element (HM)- species distributed in the European Mediterranean Region, south of central and eastern Europe, North Africa and, in Asia Minor (or in a significant part of this area). They can also occasionally reach Near and Middle East, Central Europe, and the southern part of East Europe.

10) The North-Mediterranean element (NM)- species distributed in the European Mediterranean Region but absent in North Africa.

11) The West-Mediterranean element (WM)- species distributed in the West-Mediterranean Region: Iberian Peninsula, France, the Apennine Peninsula and the Maghreb. In some cases, they are also present in Balearic Islands, Corsica, Sardinia or Sicily.

12) The North-West Mediterranean element (NWM)- species distributed in the Iberian Peninsula and southern France. They can also reach the northern Apennine Peninsula.

13) The Iberian elements (I)- species distributed only in the Iberian Peninsula. We include some Iberian sensu lato species, that can also reach the French Pyrenees or the Balearic Islands.

14) The Iberian/Maghrebian elements (I/M)- species distributed in the Iberian Peninsula and also in the Maghreb.

Relation of zoogeographic chorotypes to environmental variability

Data sources are heterogeneous and the sampling effort was heavily unbalanced across the region. Therefore, we adopted a strategy designed to deal with this problem. Firstly, we resampled data at 10×10 km resolution on the UTM grid in order to obtain a higher number of species by sampling unit and a more homogeneous definition of the locality concept. In total there are data from fifty-seven 10×10 km UTM squares. Then, we studied the relationship between the proportion of species belonging to the class of mixed and deciduous forests and the precipitation (computed as the mean of the localities sampled within a 10×10 km UTM square) by logistic regression, being the number of success the number of species belonging to the class of mixed and deciduous forests, and the number of trials the total number of species. The species richness of a particular chorotype is clearly influenced by sampling intensity, however one can expect that the proportion of species in a chorotype is independent of the sampling intensity although values on less sampled areas are not as reliable. Moreover, the Fisher scoring algorithm for logistic regression weighs observations proportional to the number of species, allocating low weights to sampling units with low sampling intensity and high weights to adequately sampled units. Therefore, estimates of parameters is much more influenced by best sampled localities, while marginal information of other areas is not absolutely neglected.

Mean annual precipitation and temperature data for the whole basin were obtained from Worldclim 2 (Fick & Hijmans, 2017), and altitude from ASTER satellite imagery. There was a very high correlation (>0.95) between the three variables as a result of the natural correlation of precipitation (positive) and temperature (negative) with altitude in the area, but it was probably exacerbated by the interpolation methodology in Worldclim 2 as altitude is incorporated in the interpolation algorithm. Because of redundancy between the three variables, we chose precipitation as the target climatic predictor variable.

Localities with ant data are a biased sample of the basin. Highest altitudes (>1200 m) are overrepresented in the sample (Figure S1.a) while low-mid altitude (200-1100 m) are in general underrepresented, but lowest altitudes (<200 m) are again overrepresented. Since the correlation between altitude and precipitation (positive) and temperature (negative) is high in the area, this means the coldest and rainiest areas are overrepresented in the sample, while intermediate temperature and precipitation are underrepresented except in the warmest areas (Figure S1b-c). The sampling bias is a consequence of much higher proportion of natural habitats on the mountain ranges than in the inter-range plains and valleys, mostly occupied by agricultural lands (López-Bermúdez et al., 1986) which were rarely sampled.

A second approach to better understanding the relationship between chorotypes and environmental variability was carried out using elements instead of zones as zoogeographic units. At this higher zoogeographic resolution, the number of species per chorotype becomes rather reduced, therefore we used only data obtained from the systematic sampling of ten 10×10 km UTM squares (item ii; Ant data sources). These squares were selected as representing the diversity of climates, lithologies and geomorphology of the basin, after clustering data with those variables using k-means method (data not shown). Sampling intensity was exactly the same across the 10 squares and therefore data are strictly comparable. In this analysis, we grouped CSE and CSE/M; EC and EC/M; and CSW and CSW/M (CSE, EC/M and EWS/M had only 1, 1 and 2 species respectively). Then, we calculated the richness per chorotype element (permitting the grouping indicated) and the richness matrix by element by 10×10 km UTM square was submitted to a non-metric multidimensional analysis (NMDS; Bray-Curtis distance; dimensions=2; stress=0.13). Coordinates in the NMDS space are represented on the geographic space of the basin and sampling unit coordinates on NMDS axis 1 and 2 were individually regressed on precipitation.

Results

The checklist of the Segura River Basin ants includes 110 ant species from 30 genera of five subfamilies (Dolichoderinae, Formicinae, Leptanillinae, Myrmicinae, and Ponerinae). Two subfamilies were dominant: Myrmicinae with 53 species and Formicinae with 46 species (Table 2). The most represented genera are *Temnothorax* Mayr (19 species), *Camponotus* Mayr (14 species), *Lasius* Fabricius (8 species), *Formica* Linnaeus, and *Plagiolepis* Mayr (6 of each). Twenty two species were recorded for the first time in this area (Table 1).

One species previously reported from the area is excluded: *Lasius niger* L. There are only two records of *L. niger* for the Segura River Basin (Collingwood & Yarrow, 1969; Martínez et al., 2002), and these records possibly correspond to *Lasius grandis* Forel, because the first record was before Seifert (1992) raised *L. grandis* to species, and the second was based on one queen only. Seifert (1992) argues that in most mesophilic areas of Spain *L. niger* is completely displaced by *L. grandis* and our findings appear to support him. One new species was discovered as a result of our field work, *Temnothorax ansei*, recently described by Catarineu et al. (2017).

There are three undescribed species in the checklist: *Camponotus* sp.1 (a parasite of *Camponotus pilicornis* Roger); *Plagiolepis sp.1* (a parasite of *Plagiolepis schmitzii* Forel); and *Temnothorax* sp.1. *Camponotus* sp.1 and *Plagiolepis* sp.1 are being described by X. Espadaler (X. Espadaler, personal communication); *Temnothorax* sp.1 is being described by A. Tinaut (A. Tinaut, personal communication).

Table 2: The composition of the ant fauna of the Segura River Basin, with species and genus numbers/percentages given for each subfamily.

Subfamily	**No. of genera (%)**	**No. of species (%)**
Dolichoderinae	3 (10.0)	6 (5.5)
Formicinae	10 (33.3)	46 (41.8)
Leptanillinae	2 (6.7)	2 (1.8)
Myrmicinae	13 (43.3)	53 (48.2)
Ponerinae	2 (6.7)	3 (2.7)
Total	**30**	**110**

Zoogeographical composition

Table 3 shows the zoogeographical composition of the ant fauna in the Segura Basin. For this classification, we consider only 105 species, discarding those not identified as species (*Leptanilla* sp., *Proformica* sp., *Solenopsis* sp., *Tapinoma* cf *nigerrimum* and *Tetramorium* cf *caespitum*). Three quarters of the species (79 spp., 75.2%) are associated with the Mediterranean zone, while nearly a fifth (20 spp., 19.05%) are associated with the mixed and deciduous forest zone. The other species are Cosmopolitan (6 spp., 5.7%), tramp species introduced in this region with larger distribution globally, which on the other hand indicates that 94.3% of the species collected can be considered as native from the Segura River Basin region. The four principal zoogeographical elements are clearly: Iberian (21 spp., 20 %), Holomediterranean (18 spp., 17.1%), West-Mediterranean (14 spp., 13.3%) and North-West Mediterranean (11 spp., 10.5%). Over two-fifths of the Segura Basin ants are also encountered in the Maghreb region (43 spp., 41%).

Table 3. Zoogeographical composition of the ant fauna of the Segura River Basin (south-west Spain). It includes only the 105 species in which it has been possible to reach the species level.

Zoogeographical zones	**Zoogeographical elements**	**Acronym**	**n**	**%**
Zone of the mixed and deciduous forest	Central and Southern European	CSE	1	0.95
	Central and Southern European/Maghrebian	CSE/M	3	2.86
	Euro-Caucasian	EC	5	4.76
	Euro-Caucasian/Maghrebian	EC/M	1	0.95
	Euro-West-Siberian	EWS	3	2.86
	Euro-West-Siberian/Maghrebian	EWS/M	2	1.90
	South Palearctic	SP	5	4.76
			20	**19.05**
Mediterranean zone	Holomediterranean	HM	18	17.14
	Iberian	I	21	20.00
	Iberian/Maghrebian	I/M	5	4.76
	North-Mediterranean	NM	6	5.71
	North-West Mediterranean	NWM	11	10.48
	Tethyan	T	4	3.81
	West-Mediterranean	WM	14	13.33
			79	**75.24**
Cosmopolitan	Cosmopolitan	C	**6**	**5.71**
Total number of species			**105**	**100.00**

Chorotypes and environmental variability

There is a clear relationship between the proportion of ant species in the mixed and deciduous zone and precipitation (Figure 2). It is rare to find species of this zone in areas with less than 350 mm of annual precipitation. With rainfall over 450 mm these species were only absent in 10×10 km UTM squares with very low intensity sampling (very low total observed ant richness consequently). With rainfall between 300-400 mm (the most frequent precipitation range in the region, Figure S1.c) there is a considerable spread of the proportion of species in the class. It may be related to the large environmental differences between south and north-face of the mountain ranges, the latter faces being considerably mesic as compared to areas on flat or south-faced slopes, and therefore having denser vegetation, less insolation and higher humidity.

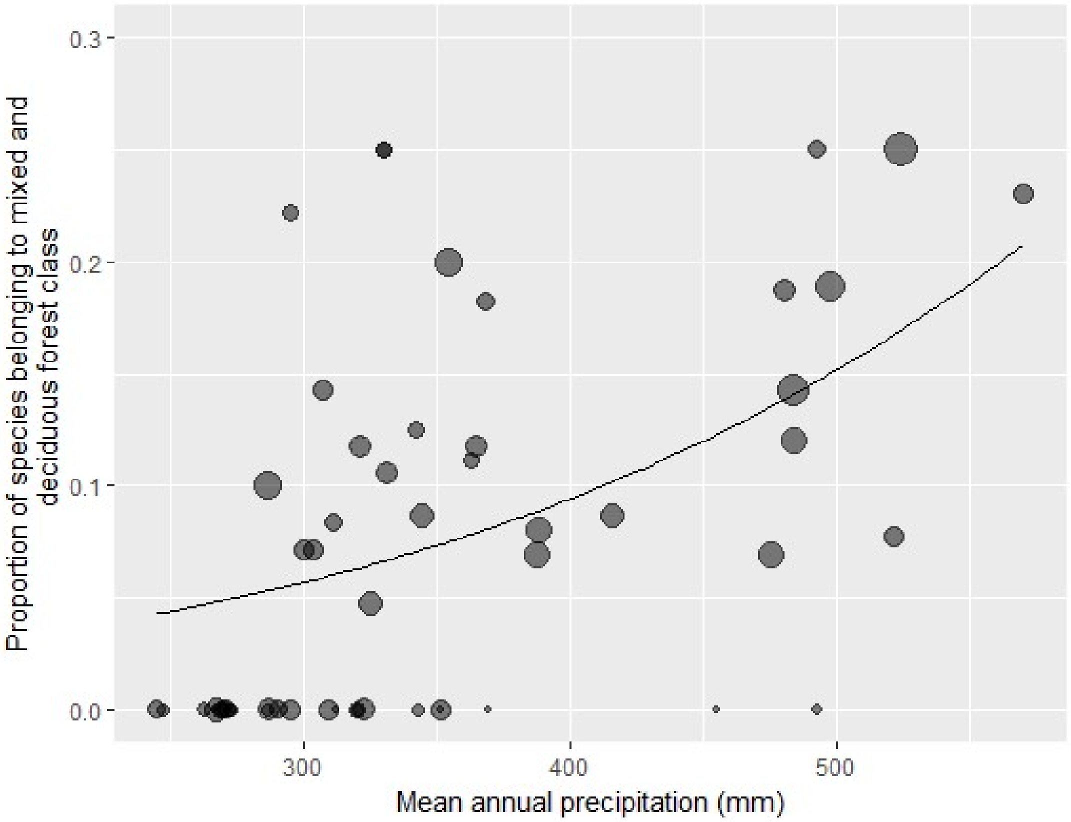

Figure 2. Relationship between proportion of species on the mixed and deciduous forest class and mean annual precipitation. Solid line, logistic regression model expectation. Dots, empirical data grouped on 10×10 km UTM squares. Size of dots is proportional to the total number of ant species recorded on the square. Two squares with one and two species, respectively, are not represented on the figure as they are out of range (proportion of mixed and deciduous forest class 0.5 and 1). Intercept: -4.4427 ± 0.5479, z= -8.108, P=<0.001; Precipitation: 0.0054 ± 0.0013, z= 4.271, P=<0.001.

Focusing on elements only for homogeneously sampled 10×10 km UTM squares, the pattern that appears clearly separates most of the mixed and deciduous forest zone elements from the Mediterranean elements (Figure 3). The first axis (NMDS1) separates Euro-Caucasian (EC + EC/M) and Euro-West Siberian (EWS + EWS/M) elements from the rest. The second axis (NMDS2) clearly separates South Paleartic (SP), EC and EWS elements from the rest, but EC and EWS and SP are on opposite sides of the axis (Figure 3). NMDS1 separates presumably forest associated elements EC and EWS from the rest, while NMDS2 separates clearly SP element more associated to steppe Eurosiberian ecosystems. This is clearer on the geographical representation of the NMDS coordinates of the 10×10 km UTM squares (Figure 4a and 4b). The Euro-Caucasian and Euro-West Siberian elements seem to be more associated with subhumid and humid forests on the NW- Segura River Basin mountains. The South Paleartic elements seem to be more associated to the N- Segura River Basin, an area of transition to the central Spain plateau, characterized by mid-height mountains surrounded by relatively high valleys and plains more similar to the forest-steppe zone with more open forests and grasslands (Figure 4a and 4b).

Both axes have a clear relation with precipitation (Figure 5). Thus NMDS1 coordinate is linearly negatively correlated with precipitation (P = 0.04; linear regression; R^2 adj = 0.61), while NMDS2 shows a peak at intermediate precipitations which is better fitted by a linear regression with linear and quadratic terms of precipitation as predictors (P = 0.02; R^2 adj = 0.79).

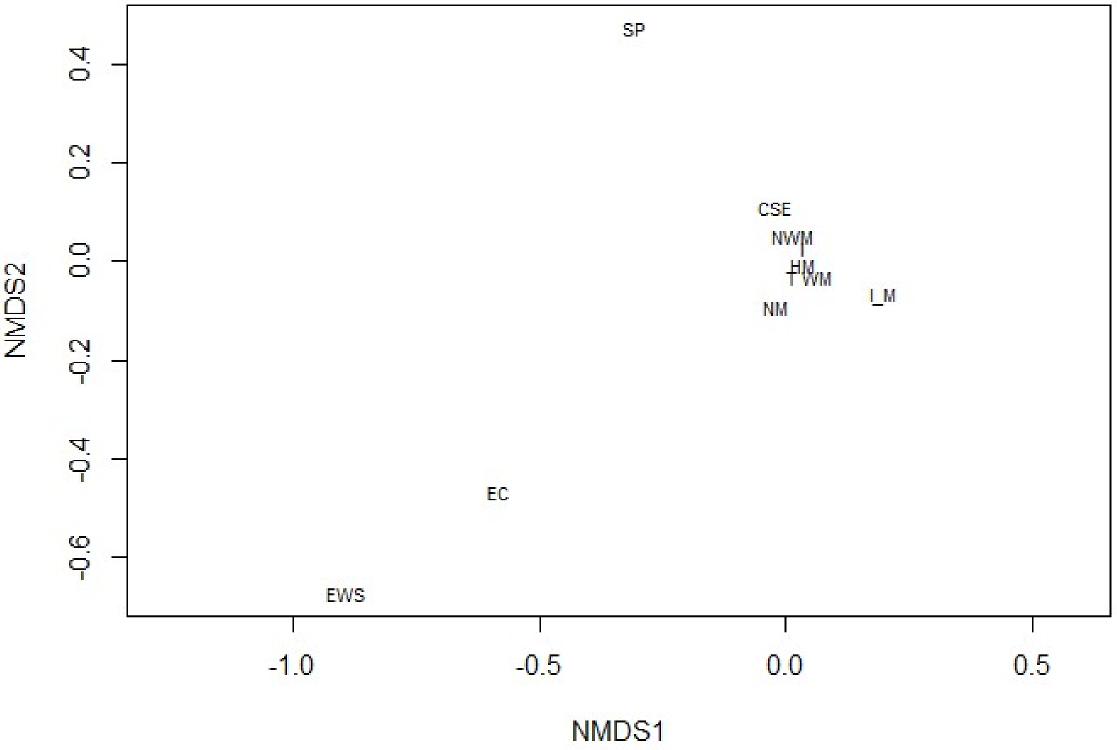

Figure 3. Non metric multidimensional scaling (NMDS) of zoogeographic elements on 10×10 km UTM squares representing the geological, geomorphological and climatic variation of the Segura River Basin. CSE= Central and Southern European, EC= Euro-Caucasian, EWS= Euro-West Siberian, HM= Holomediterranean, I= Iberian, I/M= Iberian/ Maghrebian, NM= North-Mediterranean, NWM= North-West Mediterranean, SP= South-Paleartic, T= Tethyan, WM= West-Mediterranean.

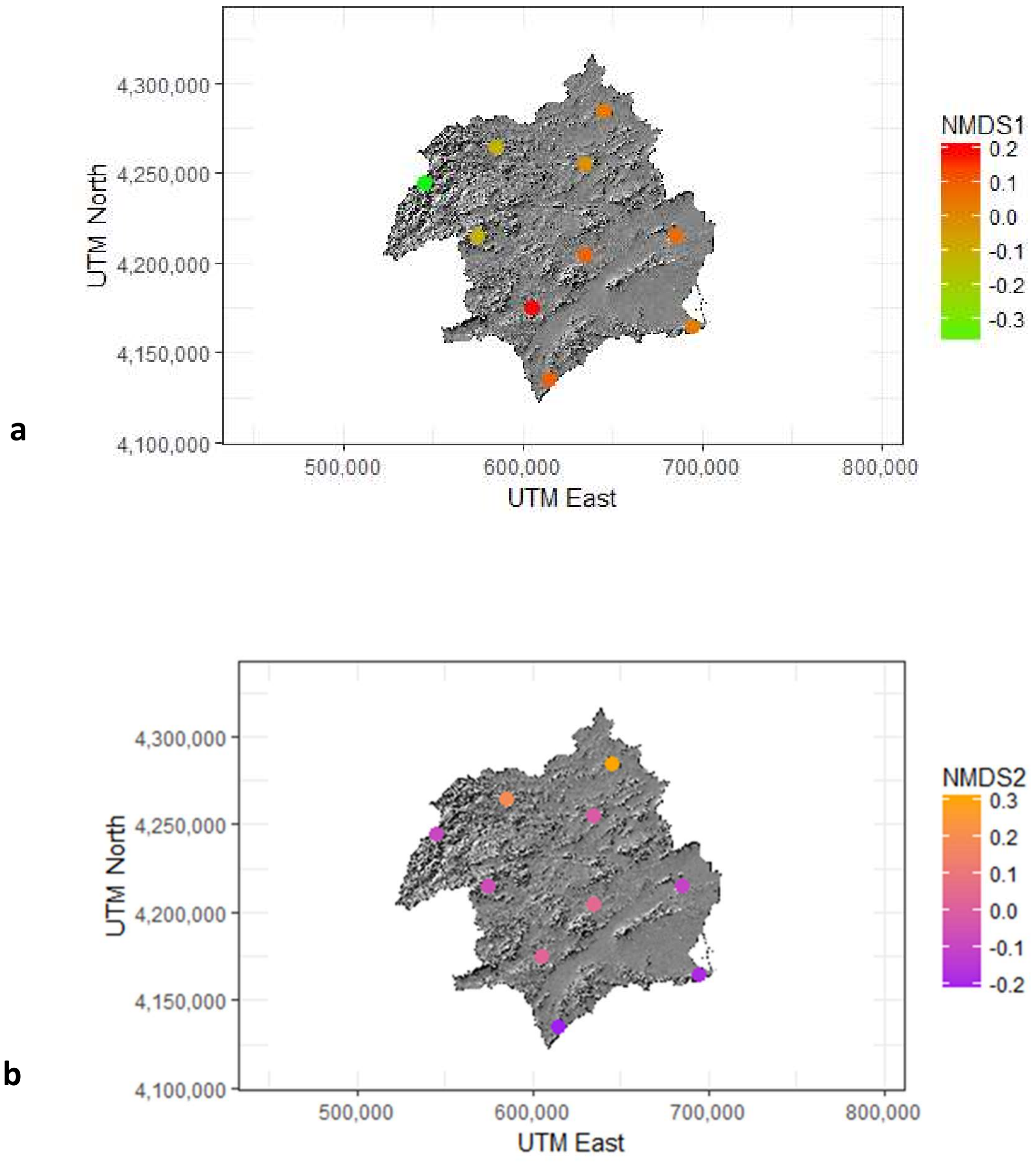

Figure 4. Geographical representation of coordinates of 10×10 km UTM squares of the systematic sampling (see Methods) on NMDS space of Figure 3. (a) Coordinates on axis NMDS1. (b) Coordinates on axis NMDS2.

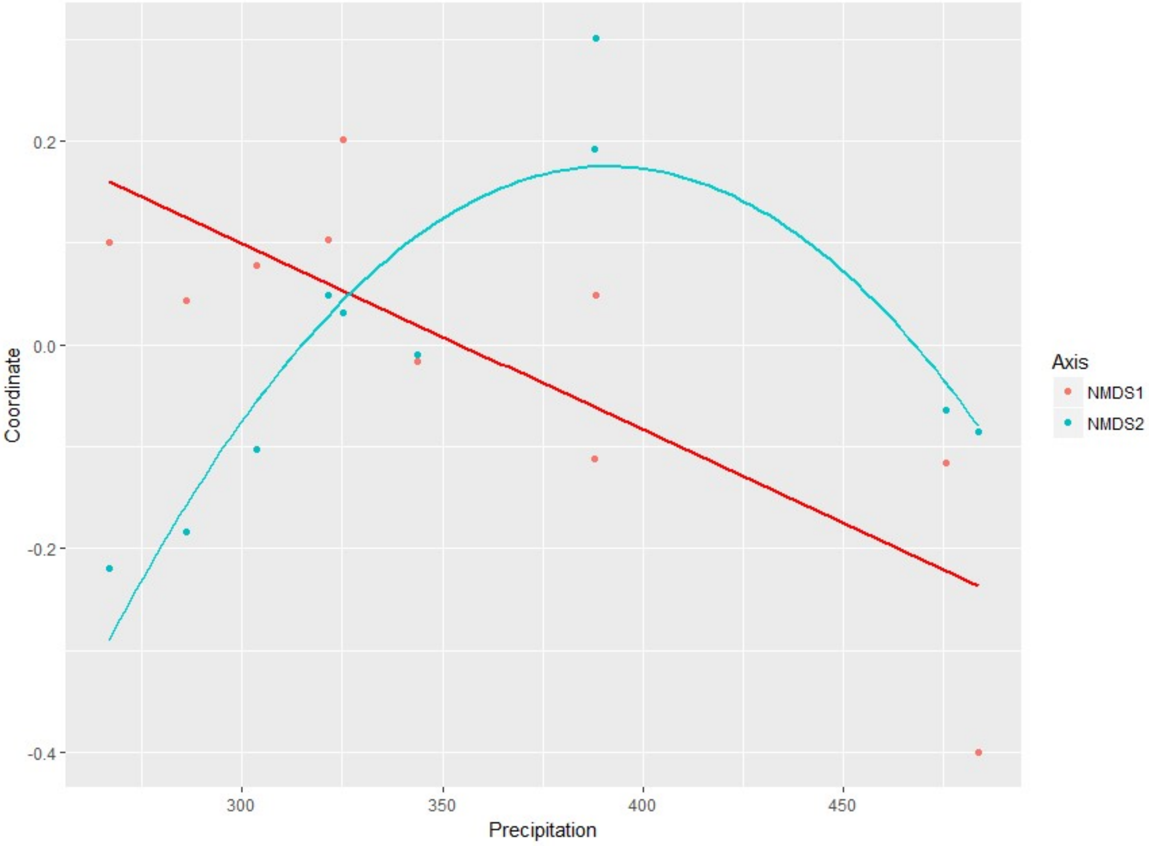

Figure 5. Coordinates of 10×10 km UTM squares of the systematic sampling (see Methods) on NMDS space of Figure 3 against precipitation. Dots, empirical data; lines fitted regression lines between NMDS coordinates in each axis and precipitation (see Methods).

Discussion

At this point 110 ant species belonging to 30 genera and five subfamilies were recorded in the Segura River Basin but the actual species number is undoubtedly higher. A greater sampling effort, the study of hypogaeic species such as *Leptanilla* spp., the delimitation in the *Tapinoma* cf *nigerrimum* and *Tetramorium* cf *caespitum* species complexes, and the revision of genera such as *Proformica* or *Solenopsis*, will increase the inventory of species. Ant diversity in the Segura River Basin seems similar to other Iberian Peninsula regions like the Comunitat Valenciana (108 sps, 23,255 km^2, Mediterranean coast; Del Campo et al., 2014) or Burgos (99 sps, 14,022 km^2, northern plateau in the transition between Mediterranean and Cantabrian ranges with oceanic climate; García & Cuesta, 2017).

The Segura River Basin ant fauna is formed by species with different evolutionary and zoogeographic origins (two zoogeographical zones and 14 zoogeographical elements; Table 3). The existence of these different zoogeographic origins in this area is probably linked with: the position between Africa and Europe; the complex geotectonic, paleogeographic, and paleoclimatic history during the last 7 My; the complex geomorphology; and the high climate and habitat diversity.

Ant species from the mixed and deciduous forest zone

The ant species from the mixed and deciduous forest zone in the Segura River Basin accounted for 19.1% of the species inventoried. These species possibly suffered contraction in their ranges during the ice ages and survived in several southern refuges, such as the Baetic ranges.

The climatic oscillations during the Pleistocene Ice Ages, and particularly over the last 2 My, are known to have had an important influence on the zoogeographic history of Europe driving the repeated contraction/expansion of the zoogeographical ranges (Hewitt, 2011). The Iberian Peninsula was one of the most important refuges in Europe during the Pleistocene Ice Ages (Hewitt, 2000, 2001, 2011; Gómez & Lunt, 2007). After the last glacial maximum period (20-14 ky BP) some species have expanded their range northward from their Iberian refugia, or tracked suitable habitats higher up along elevation gradients within mountain ranges (Gómez & Lunt, 2007; Hewitt, 2001). The Baetic ranges, where the Segura River Basin is located, were one of the Iberian refugia (Carrión et al., 2003; Gómez & Lunt, 2007, Hewitt, 2011).

Consistent with this, in the NMDS analysis, both NMDS1 and NMDS2 axis are mainly determined by zoogeographic elements of mixed and deciduous forest class (Figure 3). There is a greater proportion of species from the mixed and deciduous forest zone in the high-mid altitudes in the Segura River Basin, where the climate is cooler, the precipitation regime is subhumid or humid, and vegetation is dominated by *Pinus* L. and *Quercus* L.

forests (Figures 2, 4 and 5). This happens especially with Euro-West Siberian (EWS + EWS/M) and Eurocaucasian (EC + EC/M) elements. South Paleartic (SP) species have a similar pattern but peaks at intermediated precipitation/elevation and, especially at the transition zone between the Baetic ranges and valleys typical of the Segura Basin and the Central Spain plateau. CSE elements, on the contrary, appear clearly grouped with typically Mediterranean elements. Therefore, the general pattern of the class shows a clear gradient of elements where species are mainly associated to Eurosiberian forests (EC, EWS) than those on the transitional areas forest –steppe (SP) and then transitional to Mediterranean and CSE elements.

Some Euro-Caucasian and Euro-West-Siberian species were recorded only at high altitudes, with more humid climate: *Temnothorax unifasciatus* Latreille (EC/M, 1,161 m.a.s.l.); *Polyergus rufescens* Latreille (EWS, 858–1,282 m); *Strongylognathus testaceus* Schenck (EWS, 2,000 m); *Camponotus fallax* Nylander (EWS/M, 1,280–1,286 m). Other species classified under the same zoogeographical elements, were regularly collected at high altitudes but also at lower altitudes in humid habitats such as gallery forests or gardens: *Myrmica specioides* Bondroit (EC, 640–1,275m); *Temnothorax affinis* Mayr (EC, 640–1,100 m); *Formica rufibarbis* Fabricius (EWS, 40–1,359m); and *Camponotus vagus* Scopoli (EWS/M, 640-1,356 m). Some SP species were also collected only at high altitudes: *Formica pratensis* Retzius (1,208-1,280 m); *Formica sanguinea* Latreille (1,280 m); and *Lasius carniolicus* Mayr (1,563 m).

Global climate change is probably inducing altitudinal shifts of some ant species that will track suitable habitats along elevation, but the ant species currently limited to the mountain-tops have no further elevation range to track habitats with suitable climatic conditions. Talavera et al. (2014), studying a recently described and endemic ant from the Balearic Islands (*Lasius balearicus* Talavera et al., 2014) state that this species, elevationally constrained to island summits, is endangered by climate change and is potentially facing extinction. This could also be the case of species that inhabit only at high altitudes in the Segura River Basin which could be in danger of local extinction, as *F. pratensis* (Tinaut et al., 2011).

Ant species from the Mediterranean zone

The species from the Mediterranean zone accounted for three quarters of the species collected (79 spp., 75.2%), with the Iberian species, the most commonly represented zoogeographical element (21 spp., 20%). The Iberian Peninsula is known to present high endemism of plants (25–30%; Castroviejo et al., 1986), Iberian carabid (43.1 %; Serrano et al., 2003), and other taxa as reptiles, amphibians, mammals, fishes or butterflies (Abellán & Svenning, 2014). Many of these endemic species possibly evolved during the Pleistocene Ice Ages from Tertiary species, when conditions in the different Iberian refugia enabled long periods of allopatry, with speciation occurring as a result of adaptive selection and genetic drift processes (Huseman et al., 2013). Gómez and Lunt (2007) stated that many species and species complex show strong genetic subdivisions in the Iberian Peninsula which provides evidence of their isolation. A Pleistocene origin is also proposed for other endemic taxa as Dytiscidae water beetles: most Iberian endemics have an apparent recent origin, differing less than 2% in mDNA from their sister species, which implies an origin from Middle to Late Pleistocene (Ribera, 2003). A possible example of the Pleistocene speciation may be found in *Cataglyphis* Foerster ants: Villalta et al. (2017) investigated the phylogeography of the *Cataglyphis albicans* group and suggested the existence of at least three clades in the Iberian Peninsula and five in the Maghreb. The three Iberian clades are monophyletic and parapatric, and estimation of divergence times suggests a speciation process initiated after the Messinian Salinity Crisis and the last reopening of the Gibraltar Straits (≈5,33My). Other Iberian ant endemics such as *Aphaenogaster iberica* Emery and A. dulcineae Emery could also be derived from a North-African ancestor, bearing in mind that nowadays there are 40 *Aphaenogaster* Mayr native species and morphospecies in Morocco (Antmaps, 2016).

Medail and Quézel (1997) proposed the Baetic Rifan complex, which includes the Baetic ranges, as the most important areas of plant biodiversity inside the Mediterranean Basin Hotspot. Gómez-Campo et al. (1984) state that the Baetic ranges display the highest plant biodiversity in continental Europe with great Iberian endemic species richness, many of which are Baetic ranges endemic species. This high endemism is the result of the long-term survival, genetic divergence and speciation in these refugia (Gómez & Lunt, 2007; Hewitt, 2011). The mountainous geomorphology is particularly important in these processes of isolation, divergence and speciation during the past 3My (Hewitt, 2000). The number of endemic plants in the Baetic ranges is outstanding: 418 taxa (Pérez-García et al., 2012) and these endemics concentrate on highland disjunct areas (Mota et al., 2002). There are also a lot of species of terrestrial animals that are local endemics, as the amphibian *Alytes dickhilleni* Arntzen & García-París or the Spanish lizard *Algyroides marchi* Valverde (Gómez & Lunt, 2007). Piñero et al. (2011) studying the arid areas of south-east Iberian Peninsula found a 8.4% insect and a 9.6% spiders species endemic to this area. In the case of ants, Piñero et al. (2011) report 9.5% of endemic species but, in the Segura River Basin, we found only four endemic ants of the south-east Iberian Peninsula, accounting for 3.6% of the total number of species: *Camponotus haroi* Espadaler, *Goniomma collingwoodi* Espadaler, *Temnothorax ansei* and *Temnothorax cristinae* Espadaler.

Holomediterranean elements account for 17.1% (18 species). These species are possibly Tertiary species that found suitable habitats and survived the Pleistocene Ice Ages in the Iberian Peninsula and other refuges around the Mediterranean Basin. The paleoecological similarities of the Maghreb and Southern Europe have led to a circum-Mediterranean distribution of many taxa (Huseman et al., 2013).

There are 43 species of the Segura Basin ants also present in the Maghreb (CSE/M, EC/M, EWS/M, HM, I/M, T and WM) accounting for 41% of the recorded species. The presence of Ibero-North African species has been reported in other taxonomic groups, for example, the Baetic ranges have the highest values of Ibero-North African plants in the Iberian Peninsula (Gómez-Campo et al., 1984) and this occurrence is also found in spiders and in various insect orders (Piñero et al., 2011). The Maghreb was also an important differentiation and speciation center during the Pliocene and Pleistocene, where many taxa may have evolved or survived and later expanded to the Iberian Peninsula and Europe (Huseman et al., 2013)

Two paleogeographical events allowed the exchange of terrestrial organisms between Africa and the Iberian Peninsula. The first event was the separation of parts of the Baetic region from the Iberian mainland and its connection by the south to Africa during the Baetic crisis 16–14 Mya (Veith et al., 2004). The second event was the closure of the Mediterranean-Atlantic connections due to tectonic processes during the Messinian Salinity Crisis 5.96–5.33 Mya (Veith et al., 2004; Agustí et al., 2006; Hewitt, 2011; Gibert et al., 2013). The Strait of Gibraltar has been an effective barrier to genetic exchange since this event, although there is evidence that some terrestrial reptiles or amphibians crossed during the Pleistocene, possibly with lower sea levels during the glacial maxima (Veith et al., 2004; Hewitt, 2011; Huseman et al., 2013). The I/M and WM ants in the Segura Basin might represent Tertiary species that colonized the Iberian Peninsula, and in some cases southern France, from North Africa during the Messinian Salinity Crisis. There are no ant species in the Segura River Basin also present in the Afrotropical realm. Serrano et al. (2003), studying Iberian Carabidae (Coleoptera), state that the Iberian Peninsula is poor in Afrotropical elements, probably because of the strong isolation derived from the Sahara Desert.

The proportion of species from the Mediterranean zone is greater in the lowland areas, and decreases with the altitude in the Segura River Basin (Figure 2), possibly because they are more thermophilic species. In the NMDS analysis, all the Mediterranean elements are strongly aggregated together and with the Central and Southern European element, only Iberian/Maghrebian elements are lightly separating from the rest (Figure 3).

Cosmopolitan ant species

The cosmopolitan ant species are all introduced tramp species, and account only for 5.7% (6 species). None of them seem to have much ability for invasion in the Segura River Basin. The most widespread, *Linepithema humile* Mayr, as well as the scarcer *Pheidole indica* Mayr and *Cardiocondyla mauritanica* Forel are present only in degraded coastal habitats and in anthropogenic environments such as gardens. *Paratrechina longicornis* Latreille was collected at only two sites in urban habitats, while *Strumigenys membranifera* Emery was collected at a single site in a semiarid and altered area. It is likely the harsh conditions of the semi-arid areas of the Segura River Basin are not suitable for the invasion of the exotic species.

Future directions

Unfortunately, zoogeographical studies are so scarce that we are not able to compare the zoogeographical ant composition of the Segura Basin to other Mediterranean regions. Further comparative phylogeographical analyses are needed to elucidate the range changes and the evolutionary history of the myrmecofauna in the Iberian Peninsula during the Cenozoic. There is also further research required on the zoogeographic consequences of the changes we currently face living in the present epoch, the Anthropocene. The Segura River Basin has suffered during thousands of years the effects of deforestation, fire, and pastoralism. During the last decades, the area has suffered dramatic ecosystem changes caused by uncontrolled urban development, expansion of intensive agriculture, abandonment of extensive livestock farming, inadequate reforestations, soil degradation, habitat fragmentation, and a deficiency in the conservation management (Piñero et al., 2011). Climate change is already occurring and, as it progress, will also cause ecosystem changes with important effects on biodiversity. Some species will suffer a rapid loss of suitable habitat and face extinction (Thomas et al., 2004; Wilson et al., 2005). Other species are less vulnerable or may even be favoured by these changes. The evolutionary origin of species and their elevational distribution seem to be important in determining vulnerability to climate change. Management for biodiversity conservation requires monitoring ecosystem changes and their effects on the biocenosis. For this task, ants could be crucial bioindicators in the coming years.

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Table 1. Checklist of the ant species of the Segura River Basin. The list is arranged alphabetically by subfamily, genus and species. Species names in bold characters refer to species recorded for the first time in this area. Zoogeographical classes (ZC) and Zoogeographical elements (ZE) abbreviations: 1=mixed and deciduous forest zone, 2= Mediterranean zone, 3= Cosmopolitan, CSE= Central and Southern European, CSE/M= Central and Southern European/Maghrebian, C= Cosmopolitan, EC= Euro-Caucasian, EC/M=Euro-Caucasian/Maghrebian, EWS= Euro-West Siberian, EWS/M= Euro-West-Siberian/Maghrebian, HM= Holomediterranean, I= Iberian, I/M= Iberian/ Maghrebian, NM= North-Mediterranean, NWM= North-West Mediterranean, SP= South-Palearctic, T= Tethyan, U= Unknown, WM= West-Mediterranean, ?=zoogeographical element questionable. Elevation range units: masl. Bibliographic references are as follows: a= Emery 1924, b= Santschi 1932, c= Collingwood & Yarrow 1969, d= Martínez & Espadaler 1986, e= Cerdá 1988, f= Seifert 1988, g= López et al. 1990, h= López, 1991, i= Seifert, 1992, j= Haro et al. 1995, k= Dahbi et al. 1996, l= Espadaler 1997c, m= Martínez et al. 1997, n= Martínez et al. 2002, o= Morcillo et al. 2006, p= Espadaler et al. 2007, q= Tinaut et al. 2011, r= Catarineu & Tinaut 2012, s= Martínez et al. 2012, t= Arcos et al. 2013, u= Del Campo et al. 2014, v= Asociación Ibérica de Mirmecología 2016, x= Catarineu et al. 2017, y= present paper. For locations, see Table S1.

Subfamily	**Scientific valid name**	**Z.C.**	**Z. E.**	**Altitude range (masl)**	**References**
DOLICHODERINAE	*Bothriomyrmex meridionalis* (Roger, 1863)	2	WM	275-1223	r, y
	Linepithema humile (Mayr, 1868)	3	C	1-1148	c, n, o, r, t, y
	Tapinoma erraticum (Latreille, 1798)	1	SP	2	r
	Tapinoma madeirense Forel, 1895	2	NWM	248-1429	r, v, y
	Tapinoma cf nigerrimum (Nylander, 1856)	2	?	2-1507	b, c, r, v, y
	***Tapinoma nigerrimum* (Nylander, 1856)**	2	NWM	339-392	y
FORMICINAE	*Camponotus aethiops* (Latreille, 1798)	2	T	640-1282	c, r, v, y
	Camponotus cruentatus (Latreille, 1802)	2	WM	858-1442	r, y
	Camponotus fallax (Nylander, 1856)	1	EWS/M	1280-1282	r, v, y
	***Camponotus figaro* Coll. & Yarrow, 1969**	2	I	858-1359	y
	Camponotus foreli Emery, 1881	2	I/M	1-1280	b, m, r, v, x, y
	***Camponotus haroi* Espadaler, 1997**	2	I	484	y
	Camponotus lateralis (Olivier, 1792)	2	HM	51-1359	r, v, y
	Camponotus micans (Nylander, 1856)	2	WM	286-1113	b, r, u, y
	Camponotus piceus (Leach, 1825)	2	NM	248-1507	b, c, r, v, y
	Camponotus pilicornis (Roger, 1859)	2	NM	590-1442	c, p, r, y
	Camponotus ruber Emery, 1925	2	I/M	15-238	r, t
	Camponotus sp1 Espadaler in prep.	2	I	1148-1155	p, r

Subfamily	Scientific valid name	Z.C.	Z. E.	Altitude range (masl)	References
	Camponotus sylvaticus (Olivier, 1792)	2	WM	1-1406	b, c, d, n, r, t, v, x, y
	Camponotus vagus **(Scopoli, 1763)**	1	EWS/M	640-1356	y
	Cataglyphis iberica (Emery,1906)	2	I	1-1282	e, j, k, l, m, n, r, t, v, x, y
	Cataglyphis piliscapa (Forel, 1901)	2	NWM	?	c
	Cataglyphis rosenhaueri **Santschi, 1925**	2	I	905-1304	y
	Cataglyphis velox Santschi, 1929	2	I	640-1406	r, v, y
	Colobopsis truncata (Spinola, 1808)	2	HM	1280-1282	r, v, y
	Formica cunicularia Latreille, 1798	1	EC	42-1406	c, r, v, y
	Formica decipiens Bondroit, 1918	2	I	242-1282	r
	Formica frontalis **Santschi, 1919**	2	I	1356	y
	Formica pratensis Retzius, 1783	1	SP	1077-1282	q, r, v, y
	Formica rufibarbis Fabricius 1793	1	EWS	42-1359	r, v, y
	Formica sanguinea Latreille, 1798	1	SP	1280	v
	Iberoformica gerardi **(Bondroit, 1917)**	2	I	1356	y
	Iberoformica subrufa (Roger, 1859)	2	I	15-1359	b, r, v, x, y
	Lasius alienus (Foerster, 1850)	1	SP	?	c
	Lasius brunneus (Latreille, 1798)	1	EC	1282	r
	Lasius carniolicus **Mayr, 1861**	1	SP	1563	y
	Lasius cinereus Seifert, 1992	2	NWM	640-2000	i, r, v, y
	Lasius emarginatus (Olivier, 1792)	1	EC	495	r
	Lasius grandis Forel, 1909	2	WM	2-1307	c, n, r, t, v, y
	Lasius lasioides **(Emery, 1869)**	2	HM	248	y
	Lasius myops Forel, 1894	1	CSE/M	793-1661	r, y
	Paratrechina longicornis (Latreille, 1802)	3	C	73	r, y
	Plagiolepis grassei Le Masne, 1956	2	NWM	400	s
	Plagiolepis pygmaea (Latreille, 1798)	1	CSE/M	87-1429	c, n, r, t, v, y
	Plagiolepis schmitzii Forel, 1895	2	HM	2-1341	n, r, t, v, x, y

Subfamily	Scientific valid name	Z.C.	Z. E.	Altitude range (masl)	References
	***Plagiolepis sp1* Espadaler in prep.**	2	I/M	2-392	y
	***Plagiolepis taurica* Santschi, 1920**	1	SP	437-1046	y
	Plagiolepis xene Stärcke, 1936	1	CSE	107-1280	n, r, t, y
	Polyergus rufescens (Latreille, 1798)	1	EWS	858-1282	r, y
	***Proformica ferreri* (Bondroit, 1918)**	2	I	472	y
	Proformica nasuta (Nylander, 1856)	2	NWM	?	c
	Proformica sp	?	?	640-923	y
LEPTANILLINAE	*Leptanilla sp*	?	?	242-1258	x, y
	Leptanilla theryi Forel, 1903	2	I/M	?	r
MYRMICINAE	*Aphaenogaster dulciniae* Emery, 1924	2	NWM	329-1155	c, r, y
	Aphaenogaster gibbosa (Latreille, 1798)	2	HM	277-1492	r, v, y
	Aphaenogaster iberica Emery, 1908	2	I	1-2000	b, c, l, m, n, r, t, v, x, y
	Aphaenogaster senilis Mayr, 1853	2	WM	15	r
	Cardiocondyla batesii Forel, 1894	2	HM	2-714	c, r, x, y
	Cardiocondyla mauritanica Forel, 1890	3	C	2-10	r
	Crematogaster auberti Emery, 1869	2	WM	1-1359	a, c, r, t, v, x, y
	Crematogaster laestrygon Emery, 1869	2	HM	2-465	r
	Crematogaster scutellaris (Olivier, 1792)	2	HM	10-1359	b, r, t, v, y
	Crematogaster sordidula (Nylander, 1849)	2	HM	192-1359	a, c, r, t, y
	Goniomma blanci (André, 1881)	2	NWM	392-1280	r, v, y
	***Goniomma collingwoodi* Espadaler, 1997**	2	I	472	y
	Goniomma hispanicum (André, 1883)	2	WM	244-640	r, v, x, y
	Goniomma kugleri Espadaler, 1986	2	I	244-465	x, y
	Messor barbarus (Linnaeus, 1767)	2	WM	1-1359	b, l, m, n, r, v, x, y
	Messor bouvieri Bondroit, 1918	2	NWM	1-1313	b, c, r, v, x, y
	Messor capitatus (Latreille, 1798)	2	HM	248-1442	r, v, y
	Messor structor (Latreille, 1798)	1	T	1200	c, y

Subfamily	Scientific valid name	Z.C.	Z. E.	Altitude range (masl)	References
	Monomorium andrei Saunders, 1890	2	I/M	2-192	r, t, y
	Monomorium subopacum (Smith, 1858)	2	HM	1-275	r, y
	Myrmica aloba Forel, 1909	2	NWM	905-1307	c, f, r, y
	Myrmica specioides Bondroit, 1918	1	EC	248-1275	v, y
	Oxyopomyrmex saulcyi Emery, 1889	2	WM	198-881	x, y
	Pheidole indica Mayr, 1879	3	C	10-16	r
	Pheidole pallidula (Nylander, 1849)	2	T	1-1442	a, c, n, o, r, t, v, x, y
	Solenopsis latro Forel, 1894	2	HM	192-793	c, r, t
	Solenopsis sp.	4	U	6-1359	n, r, v, y
	Strongylognathus caeciliae Forel, 1897	2	I	238	r, x
	***Strongylognathus testaceus* (Schenck, 1852)**	1	EWS	2000	y
	Strumigenys membranifera Emery, 1869	3	C	154	n
	Temnothorax affinis (Mayr, 1855)	1	EC	640-1077	v, y
	Temnothorax angustulus (Nylander, 1856)	2	HM	640	v, y
	Temnothorax ansei Catarineu, Barberá & Reyes-López, 2017	2	I	244-591	x
	***Temnothorax blascoi* (Espadaler, 1997)**	2	I	854-1339	y
	Temnothorax cristinae (Espadaler, 1997)	2	I	107-787	l, r, t, x, y
	***Temnothorax curtulus* (Santschi, 1929)**	2	I/M	1307-1356	y
	Temnothorax formosus (Santschi, 1909)	2	WM	465-1359	r, v, x, y
	Temnothorax gredosi (Espadaler & Collingwood, 1982)	2	I	248	r
	***Temnothorax kraussei* (Emery, 1916)**	2	HM	1307	y
	***Temnothorax kutteri* (Cagniant, 1973)**	2	NWM	120	y
	Temnothorax luteus (Forel, 1874)	2	NM	79-1406	c, x, y
	Temnothorax niger (Forel, 1894)	2	NM	2-1077	r, x, y
	Temnothorax pardoi (Tinaut, 1987)	2	WM	248-1406	r, v, y
	Temnothorax racovitzai (Bondroit, 1918)	2	NM	79-1359	r, t, x, y

Subfamily	Scientific valid name	Z.C.	Z. E.	Altitude range (masl)	References
	Temnothorax recedens (Nylander, 1856)	2	HM	107-1291	r, t, v, y
	***Temnothorax* sp 1 Tinaut in prep.**	2	I	1231	y
	Temnothorax specularis (Emery, 1916)	2	NM	44-1359	v, x, y
	***Temnothorax unifasciatus* (Latreille, 1798)**	1	EC/M	1162	y
	Temnothorax universitatis (Espadaler, 1997)	2	I	465	x
	Tetramorium biskrense Forel, 1904	2	HM	88-1406	v, y
	Tetramorium cf caespitum (Linnaeus, 1758)	4	U	1-2000	g, h, l, m, r, v, y
	Tetramorium forte Forel, 1904	2	WM	392-1507	r, y
	Tetramorium semilaeve André, 1883	2	HM	2-1359	c, g, h, l, m, r, x, y
PONERINAE	*Hypoponera eduardi* (Forel, 1894)	3	C?	?	c
	Ponera coarctata (Latreille, 1802)	2	HM	500	c, r
	***Ponera testacea* Emery, 1895**	1	CSE/M	1155-1280	y

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Supplementary material

Table S1. Ant cites. Segura River Basin (SE-Spain; 2012-2016) is available on:

https://www.researchgate.net/publication/333023799_Table_S1xlsx

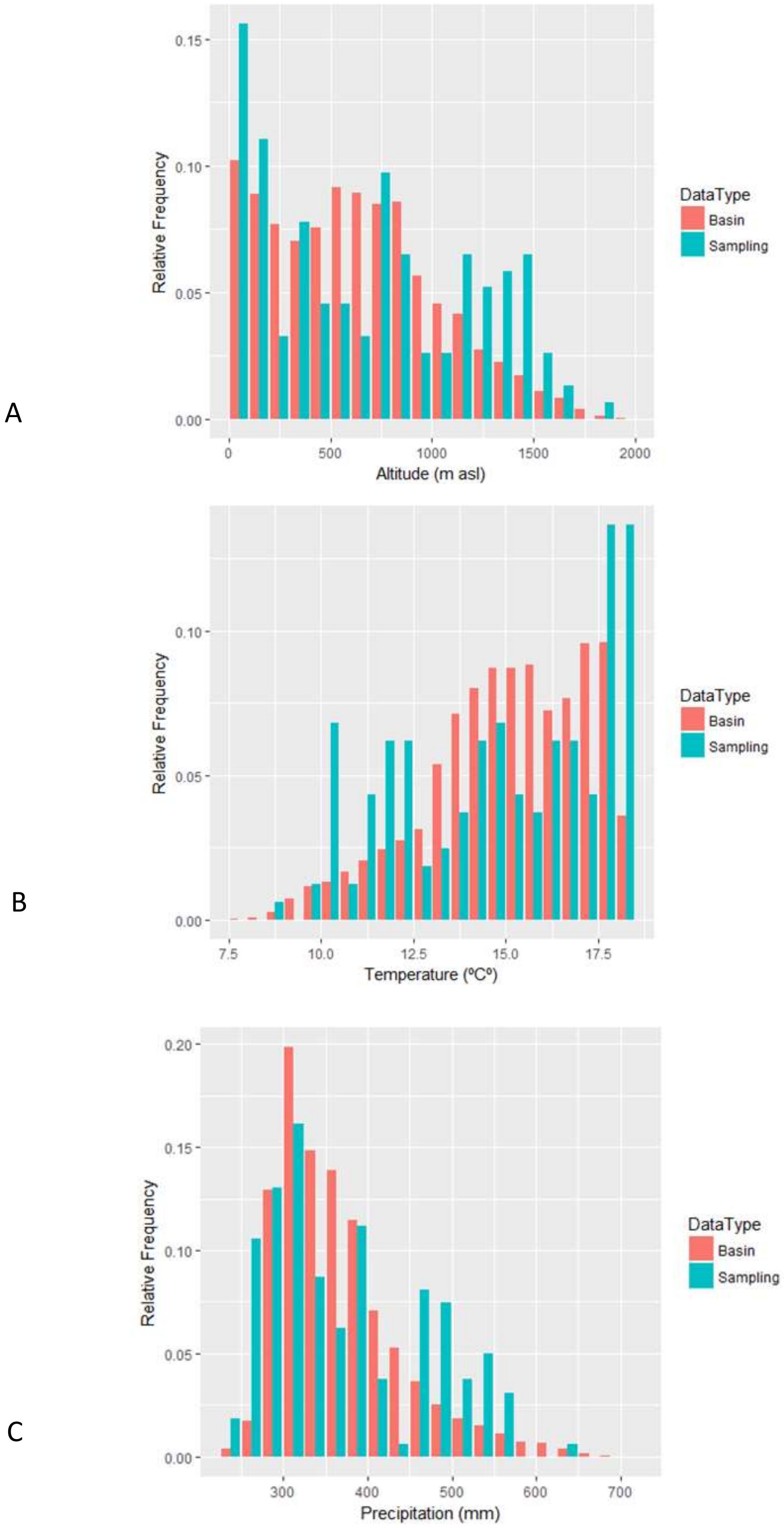

Figure S1. Relative Frequency of Altitude (A), Temperature (B) and Precipitation (C) in the Segura River Basin and in the sampling points.

Chapter 2

Environmental filtering in a semiarid region underlies ant diversity gradients and phylogenetic structure.

Photo credit for the previous page: Shannon Hartman, from www.AntWeb.org (*Polyergus rufescens*, *Camponotus cruentatus* and *Tapinoma nigerrimum*); Kiko Gómez, from www.hormigas.org (*Lasius myops* and *Formica pratensis* and *Colobopsis truncata*); Estrella Ortega, from www.AntWeb.org (*Plagiolepis schmitzii*, *Iberoformica subrufa* and *Aphaenogaster iberica*); Michael Branstetter, from AntWeb.org (*Strongylognathus testaceus*); Michele Esposito, from www.AntWeb.org (*Temnothorax ansei*); Will Ericson, from www.AntWeb.org (*Messor barbarus*); Elham Kashani, from www.AntWeb.org (*Pheidole pallidula*); Michael Branstetter, from www.AntWeb.org (*Goniomma blanci*), California Zach Lieberman, from www.AntWeb.org (*Temnothorax cristinae*).

Environmental filtering in a semiarid region underlies ant diversity gradients and phylogenetic structure.

Abstract

We studied ant communities with 60 pitfall trap transects (100-m long) on the Segura River Basin (SE Spain). Transects were distributed at 10 localities (defined as 10 x 10 km quadrats) representing the environmental variation of the basin. Thus, the study was carried out at two spatial scales, locality and transects. There is a general environmental gradient, which is an important force structuring ant diversity, from the higher, colder, wetter and better-vegetated areas to the lower, hotter, dryer and poorly vegetated areas. Precipitation seems to be the more important environmental factor controlling ant taxonomic diversity, allowing a higher primary production and diversity in the wetter areas. Taxonomic diversity is highly correlated with phylogenetic diversity, but the correlation with functional diversity is weaker. We found an overdispersed phylogenetic structure in the higher areas and a phylogenetically clustered structure at low elevations areas. Two dominant subfamilies on the area responded differently to the main environmental gradient, Formicinae being much more sensitive than Myrmicinae. These differences in sensitivity are behind important changes on phylogenetic diversity and structure of the communities along the gradient. As we detected niche conservatism, we hypothesized that the harsh conditions of aridity in the low areas constitute an environmental filter clustering the phylogenetic structure, while the interspecific competition could be the main force shaping the overdispersed phylogenetic structure of the higher altitude, cooler and wetter environments. The relationships between the diversity indices and the environmental factors, and the effects of the aridity environmental filter are more evident at the coarser spatial scale because, at the lower scale, species interactions (especially competition) and probably very local environmental factors have a major relevance as community drivers.

Introduction

Understanding the drivers that originate and control spatial variation in the diversity and the composition of communities has been a central goal in ecology, biogeography and evolutionary biology since the nineteenth century (Lomolino, 2001; Ricklefs, 2004; Swenson, 2011; Sundqvist et al., 2013; Szewczyk & McCain, 2016).

More than 30 hypotheses have been proposed to explain patterns of biodiversity (Sanders, 2002; Hawkins et al. 2003; Dunn et al., 2009b; Szewczyk & McCain, 2016). They can be grouped into four main categories related with climate, space, evolutionary/biogeographic history and biotic processes (McCain & Grytnes, 2010). In more recent times, a better understanding of diversity takes into account not only the taxonomic diversity (TD) but also functional biodiversity (FD; Webb et al., 2002; Petchey & Gaston, 2006) and phylogenetic biodiversity (PD; Faith, 1992; Webb et al., 2002). The predictions of increasing global mean temperatures and higher frequency of extreme weather events as a result of climate change (IPCC, 2014), and its notable implications for biodiversity and ecosystem services (Cardinale et al., 2012), urge improving the knowledge about biodiversity controls, especially those related directly or indirectly with climate variables.

To properly understand the causes underlying biodiversity patterns, we have to take into account different scales of analyses (Ricklefs, 2004). At the regional scale, biodiversity is the result of the evolutionary processes (speciation, extinction and dispersal processes; Swensson, 2011), modulated by the climatic and geological history (Wiens & Donoghue, 2004; Cavender-Bares et al., 2009; Lessard et al., 2011). At lower spatial scales, the different patterns of biodiversity becomes dominated by the environmental filters (Ricklefs, 2004; Cavender-Bares et al., 2009; Swensson, 2011), while at the local scale, species interaction (competition, predation, mutualism…) are likely the main drivers (Cavender-Bares et al., 2009). At these lower scales, PD and FD may become more relevant to the assembly of the communities.

Phylogenetic community structure is appropriate to understand the processes driving community assembly (Cavender-Bares et al., 2009). When functional traits are evolutionarily conserved, a clustered phylogenetic distribution implies that the role of environmental filter is predominant shaping communities, and phylogenetic overdispersion (evenness) implies that interspecific competition are the main structuring factors (Webb et al., 2002; Kraft et al., 2007). When traits are convergent, environmental filter should produce phylogenetic overdispersion, and competition should produce phylogenetic random or clustered patterns (Kraft et al., 2007).

Ants are an excellent candidate to study biodiversity gradients, because they are abundant, easy to sample, well known, have very important functions in the ecosystems (Hölldobler & Wilson, 1990; Folgarait, 1998; Dunn et al., 2009b) and have been the subject of gradient studies for more than 50 years (Dunn et al., 2009b). Despite these efforts, we are still far from clearly understanding how the driving forces that control the diversity and structure of ant communities work.

In relation to ants, environmental filtering is mainly produced by the climate variables, and those related to temperature are especially important: mean temperature (Retana & Cerdá, 2000; Kaspary et al., 2003; Longino & Colwell, 2011; Machac et al., 2011; Munyai & Foord, 2012; Bernardou et al., 2014), diurnal temperature range (Paknia & Pfeiffer, 2014), maximum temperature of the warmest month (Del Toro, 2013) or temperature seasonality (Kaspari et al., 2000). Climatic variables related with water availability are also important, especially in arid and semiarid environments: precipitation (Sanders et al., 2003; Paknia & Pfeiffer, 2014) or aridity calculated as the difference between actual and potential evapotranspiration (Arnan et al., 2018).

Different habitat variables has been cited as ant community drivers: vegetation composition (Gotelli & Ellison, 2002), vegetation cover (Retana & Cerdá, 2000; Blatrix et al., 2016), vegetation density (Bernardou et al., 2014), habitat type (Kumschick et al., 2009; Del Toro, 2013), habitat complexity (Lassau & Hochuli, 2004; Sarty et al., 2006; Pacheco & Vasconcelos, 2012), net primary productivity (Kaspari et al., 2000), light availability (Gotelli & Ellison, 2002), available area (Sanders, 2002; Kaspary et al., 2003), aspect of slopes (Bernardou et al., 2014), proportion of bare ground (Munyai & Foord, 2012), or soil attributes (Boulton et al., 2005; Kaspari et al., 2008; Catarineu et al., 2018b). Finally, anthropogenic disturbances have undoubtedly an increasing importance in the ant community structures (Boulton et al., 2005; Philpott et al., 2010; Liu et al., 2016; Arnan et al., 2018).

The complex interactions between ant species and the different spatial and environmental factors generate gradients of ant diversity. In this study, we examined the gradients of taxonomic, functional and phylogenetic diversity and the phylogenetic structure of the ant communities, at two different spatial scales (100-m transect; 10 x 10 km “locality”), in the Segura River Basin (southeastern Iberian Peninsula), where the complex paleogeographic and paleoclimatic history (Carrión et al. 2010) has contributed to the existence of a rich biodiversity. Moreover, Segura River encompasses some of the most arid areas of Europe (< 200 mm of mean annual precipitation) but yet it has strong climate gradients (rainfall range < 200 mm to >1000 mm) in a reduced space. Thus, it is an adequate area to study the role of environmental filters and limiting similarity (competition) on the assemblage of ant communities and how diversity components (TD, FD, PD) relate to these processes.

The aim of this chapter was to study the gradients of taxonomic, functional and phylogenetic ant diversity in the Segura River Basin and find out the main processes determining it. We tested four predictions:

1) Environmental gradients significantly determines the taxonomic, functional and phylogenetic ant diversity in the Segura River Basin.

2) Precipitation is the main environmental factor explaining the taxonomic, functional and phylogenetic ant diversity patterns in the Segura River Basin.

3) Environmental factors better explains variation of the diversity indices at the larger scale than at the lower scale.

4) Ant communities in the semi-arid areas would be phylogenetically clustered indicating environmental filtering (if traits are conserved).

Materials and methods

Study area

The Segura River Basin (19,025 km^2), is located in the southeastern of the Iberian Peninsula (Figure 1). This area has a large orographic variety, with mountains that reach up to 2,000 m.a.s.l., as well as contrasting coastal areas and extensive plains and valleys. The Mediterranean semiarid climate dominates the territory (mostly < 350 mm of rainfall per year), yet, also presents strong rainfall and temperature gradients (Vidal-Abarca et al., 1987). The Baetic ranges, in the western border, intercept Atlantic cyclones in such a way that at certain points of the mountainous area rainfall reaches 900–1,200 mm per year, whilst a rain shadow lies immediately to the east. The contrasts in precipitation, temperature, altitude, lithology, and in relation to human occupation, generate a great diversity of environments that allows a high biodiversity. The vegetation is predominantly xerophytic, due to climate aridification during mid-to-late Holocene and human induced degradation (Carrión et al., 2010).

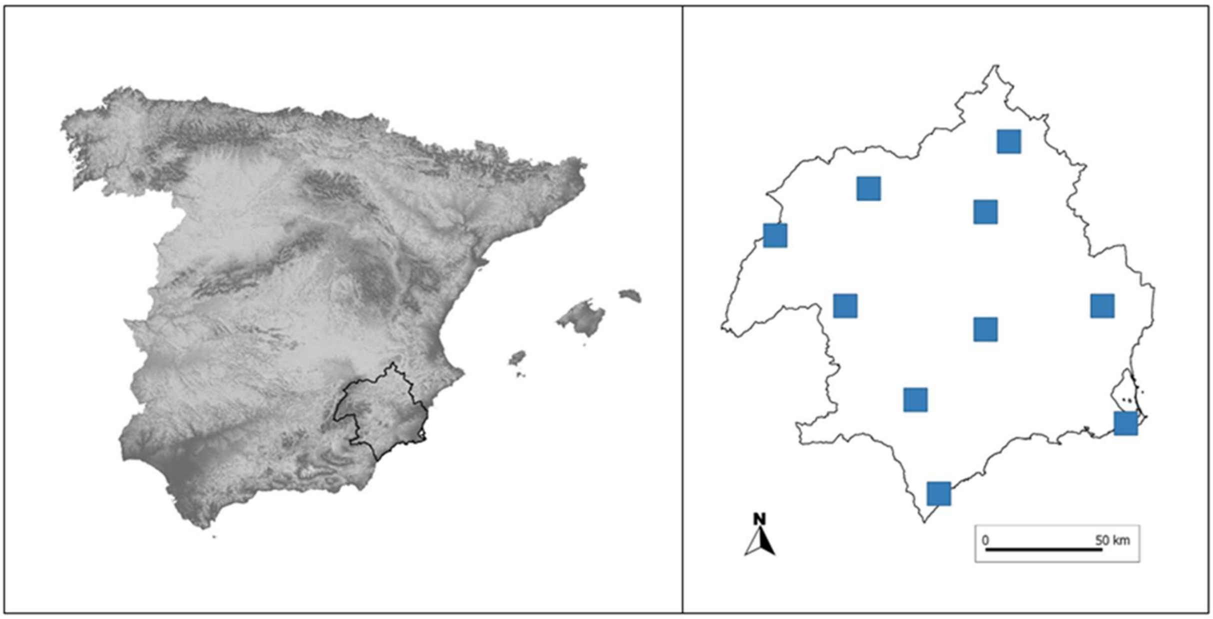

Figure 1. Map of Spain with the Segura River Basin outlined (on the left) and a map of the Segura River Basin with the ten 10x10 km UTM squares in blue (on the right).

Experimental design and sampling procedure

We selected ten 10×10 km UTM squares (henceforth 'localities') representative of the climatic, geological and geomorphological variation of the Segura River Basin (Figure 1). In each locality, we selected six sites to set up the transects, three in forests or woodlands and three in shrublands or grasslands (the dominant grassland is that of *Stipa tenacissima* being physiognomically more similar to shrublands than to mesic grasslands). Most of the region is on the semiarid domain and forest/woodland distribution is patchy and frequently linked to mesoscale geomorphic variation like the duality south/north faced slopes. In the most and least arid localities it was not possible to maintain the balanced sampling between grassland/shrubland and forest/woodland and therefore we placed five transects on shrubland and one on woodland on the most arid locality and one transect on shrubland and five on forests on the rainiest one. The 60 transects were 100-m long, consisting of 10 pitfall traps separated by 10 meters and were open for one week on July 2014 and July 2015. Pitfall traps were polystyrene tubes (2 cm diameter, 10.5 cm long) filled with 5 ml of 50:50 propylene glycol and water solution with a few drops of liquid soap added to reduce surface tension.

All ant samples were sorted and identified to species level using a stereo microscope and Iberian ant taxonomic keys (Gómez & Espadaler, 2007), taxonomic revisions of some ant genera (Seifert, 1988, 1992), and species descriptions and high-resolution pictures provided by AntWeb (Fisher, 2010). Reproductive ants were not considered for the analyses. For more details about the identification process and the specimen deposit, see Chapter I.

Experimental design let to test hypotheses at two spatial scales: the locality and the transect. The calculation of diversity indices was carried at both scales. First, we calculated indices aggregating pitfalls per transect and then aggregating transects per locality. Note also that sampling was carried out twice (2014 and 2015) and in the present analysis we calculated indices at each spatial scale per year and then one average value was calculated for both years per spatial scale and used for further analyses.

Taxonomic, functional and phylogenetic diversity indices

Indices were calculated based only on species composition not taking into account abundance. Species richness (SR) is the index for taxonomic diversity.

Following Arnan et al. (2014), we selected 10 ant functional traits, related to morphology and behaviour, to characterize the ant functional niche (Table 1), Arnan et al. (2014) and Arnan et al. (2016) provide two ant trait databases for European ant species, that include the traits of 37 out 54 ant species present in our sampling. For the other 17 captured species, we gathered information about ant functional traits from the scientific literature (Santschy, 1909; Emery, 1925; Cagniant, 1973; Espadaler, 1996; Espadaler, 1997; Arcos et al., 2013; Trager, 2013; Monnin et al., 2014; Seifert et al., 2014; Lebas et al. 2016; Catarineu et al., 2017; Barech et al., 2018), two myrmecologists (Xavier Espadaler and Alberto Tinaut) and from our own data. For some species with scarce available information, we completed the trait gaps with the known data of a similar species. For more information about the method to characterize the traits of the different species, see Table S1, and for the whole ant trait database of the ant species of the Segura River Basin, see Table S2.

We calculated two indices of functional diversity (FD) using the *FD* package (Laliberté et al., 2014) in R. Functional dispersion (FDis; Laliberté & Legendre, 2010) is the mean distance of individual species to the centroid of all species in the community in the space of the functional traits, and it is very highly correlated to Rao's Q quadratic entropy (Laliberté & Legendre, 2010). Functional divergence (FDiv; Villéger et al., 2008), in its form unweighted by abundance, represents how species spread along a functional trait axis. Although FDiv and FDis conceptually resemble, its correlation on simulation studies is moderate (Laliberté & Legendre, 2010). FDis is by design unaffected by SR, a desirable property in the context of studying covariation of taxonomic and functional diversity.

Table 1. Ant trait data. Modified from Arnan et al. (2014). We use the same traits, although Colony Size is ln-transformed and the definition of Worker Polymorphism is different [this deffinition was wrong in Arnan et al (2014); personal communication of the authors]. (a) Coded with numbers in the range 0–1 implying order of importance but not real proportion of food; 0 means absent or unimportant; 1 means dominant. (b) As defined by Arnan et al. (2014).

Acronym	Trait	Subtrait	Scale of mesasurement	Type of variable for Gower's dissimilarity calculation	Weight for Gower's dissimilarity
WS	Worker size	NA	Worker body size from the tip of mandibles to tip of the gaster (mm)	Continuous	1
WP	Worker polymorphism	NA	Range of worker size divided by the mean worker size	Continuous	1
CS	Colony size (ln-transformed)	NA	Mean number of workers per colony, ln transformed	Continuous	1
Ds	Diet	Seeds	Fuzzy estimation of the importance of seeds in diet [a]	Ordinal	1/3
Di	Diet	Insects	Fuzzy estimation of the importance of insects in the diet	Ordinal	1/3
Dlf	Diet	Liquid foods	Fuzzy estimation of the importance of insects in the diet	Ordinal	1/3
CFT	Colony foundation type	NA	Independent > Independent and dependent > dependent	Ordinal	1
nQ	Number of queens	NA	Polygyny > Polygyny and Monogyny > Monogyny	Ordinal	1
nN	Number of nests	NA	Polydomy > Polydomy and Monodomy > Monodomy	Ordinal	1
Diur	Strict diurnality	NA	Strictily diurnal (1); Non strictly diurnal (0)	Binary	1
Dom	Dominance	NA	Dominant species (1); subordinate species (0)	Binary	1
FSi	Foraging strategy	Individual(b)	Individual foraging strategy (1); other strategy (0)	Binary	1/3
FSg	Foraging strategy	Group(b)	Group foraging strategy (1); other strategy (0)	Binary	1/3
FSc	Foraging strategy	Collective(b)	Collective foraging strategy (1); other strategy (0)	Binary	1/3

Arnan et al. (2016) proposed an European ant phylogeny for 154 ant species using both molecular and morphological data, containing 39 out of 54 species present in our study. Based in this phylogeny, we made a phylogenetic tree for this 39 species with Mesquite ver 3.4 (Maddison & Madisson, 2018), and then we added the 15 remaining species based on additional references (Moureau et al., 2006; Muñoz-López et al., 2012; Espadaler et al., 2013; Ward et al., 2014; Ward et al., 2016; Catarineu et al., 2017; Prebus, 2017; Gómez et al., 2018; for more information see Figure S1). The tree was ultrametrized applying Grafen's Rho transformation to branch lengths (Carneiro et al., 2014), using the function ‘compute.brlen’ from R package *ape* (Paradis & Schliep, 2018).

We calculated three indices of phylogenetic diversity using the *picante* package (Kembel et al., 2010) in R. Faith phylogenetic diversity (PD; Faith, 1992) is the sum of branch lengths of the phylogenetic tree linking all the species in a local community. Mean pairwise distance (MPD; Webb et al., 2002; Swenson, 2014) is the mean phylogenetic pairwise distance between species in a local community. Mean nearest taxon distance (MNTD; Webb et al., 2002; Swenson, 2014) is the mean phylogenetic distance separating each species in the local community from its closest relative. It has been shown that PD has a positive relation to SR, MNTD a negative one, while MPD does not covary with SR (Miller et al., 2017). Therefore, in order to have comparable phylogenetic diversity indices across SR gradients, they were calculated the standardized versions of the three indices: phylogenetic diversity index, PDI (standardized version of PD), net relatedness index, NRI (for MPD) and nearest taxon index NTI (for MNTD). Standardization of indices was achieved by

$$StandardizedIndex(SI) = \frac{Index - \mu Index}{\sigma Index}$$

where µIndex and σIndex are, respectively, the mean and the standard deviation of index values obtained repeatedly shuffling tip labels of the phylogenetic tree and extracting a number of samples (999 in our case) with a SR identical to the reference community. NRI and NTI according to the convention are the -SI (sign changed). Thus, a positive value for PDI indicates that the community has a larger PD than expected while positive values for NRI and NTI indicate lesser MPD or MNTD (respectively) than expected.

Recently, Sandel (2018) demonstrated that assumption that standardization removes richness-dependence of phylogenetic diversity indices is incorrect: this means that studies that seek to compare the strength of environmental-biotic filtering over environmental gradients should not simply compare index values if there is substantial variation in richness along the gradient (Sandel, 2018). This dependence is removed by rarefying all samples to the smallest SR sample (Sandel, 2018). At transect scale this procedure is non-viable as poorest samples have a SR = 2. However this dependence is weak on low richness values and therefore we consider it of minor importance at transect scale. At the locality scale, we recalculated PDI, NRI and NTI rarefying localities (per year) to the lowest SR locality. It was carried out taking 1000 random samples of the community rarefied to the SR of the poorest locality (per year) and calculating their mean value. These rarefied versions of the standardized indices are named rPDI, rNRI and rNTI.

Climatic, ecological and geographic variables

Climatic variables were obtained from the nearest meteorological station of the State Agency of Meteorology of Spain (http://www.aemet.es; see Figure S2 and Table S3) to every of the localities; and normalized difference vegetation index (NDVI) from the MODIS NDVI time-series data through the University of Natural Resources and Life Sciences at Vienna (http://ivfl-info.boku.ac.at/). Climatic variables calculated from time series were: mean annual precipitation (Prec) and its coefficient of variation (CVPrec); mean annual temperature (MeanTemp), mean maximum daily temperature in the hottest month (MaxTempM) and mean minimum daily temperature in the coldest month (MinTempM). For calculating NDVI variables we downloaded data between 1/10/2013 to 30/09/2015 (the two hydrological years encompassing the sampling times) from pixels (250 x 250 m) where the transects were located. As MODIS captures one image each 16 days, there was a total of 52 images per transect. The mean NDVI (NDVI) per transect and its coefficient of variation (CVNDVI) were calculated for the cited period. As climate variables are taken from meteorological stations which altitude differ from transect ones, MeanTemp and MaxTempM were corrected by the universal gradient of temperature dropping with altitude of 0.55ºC per 100 m (e.g. Körner 2007). However, we left MinTempM unaltered as minimum temperature gradients are locally affected by thermal inversions in winter, especially on mountainous areas like Segura River Basin.

Data analysis

Climate variables in the region strongly covary, as it does vegetation with climate. This results from one general altitudinal (cooler and wetter in the mountains) and geographical gradient NW (higher, cooler and wetter) to E-SE-S gradient associated to the Foehn effect, as Baetic ranges (≈2000 m) intercept Atlantic cyclones moving W-E towards the Mediterranean coast. In order to describe these relationships, we submitted the set of climate, topographical and NDVI variables to a PCA (at each spatial scale). PCs of this analysis are named $PC1_{env}$ and $PC2_{env}$ henceforth.

Diversity indices were analysed individually in order to know what environmental variables better predict each index using linear models. Multicollinearity is high in the set of environmental variables as shown in the results section. As a result, model structure based on stepwise procedures to select best predictors out of the pool of environmental variables is unstable and may arrive to different 'best models' because marginal differences of the data. Those models could be overinterpreted on the basis of which variables entered or not in them. In order to obtain ecologically more meaningful models we proceeded in two steps. First step consisted on building regression models (locality) or mixed models (transects) for each index, using as predictors the scores of localities or transects (depending on the scale) on $PC1_{env}$ and $PC2_{env}$. On the transect case (mixed model), we include the locality as a random factor. The result of this first step is a base model for each diversity index keeping as predictors $PC1_{env}$, and/or $PC2_{env}$, if their regression coefficients were statistically significant ($p < 0.05$). These base models represent the relations of one diversity index to the general gradient(s) of the environmental factors, with no discrimination about which individual environmental variable may be more relevant. On the second step, we used the base model for each diversity index to explore the possible influence of individual variables once general environmental gradient(s) was (were) taken into account. To achieve this goal, the base model was introduced on a forward stepwise procedure as a fixed base, and variables to be introduced in the stepwise procedure were the pool of environmental variables plus the habitat factor (forest/woodland vs. grassland/shrubland) on the transect scale. At the locality scale, this variable makes no sense as by experimental design both type of habitats are present at any locality. With this two steps procedure, it is first assured the detection of the relation of diversity indices to the general environmental gradient(s) and then possible more intense relations with particular environmental variables once the general gradient was accounted for.

After first analyses, it become evident that habitat (forest/woodland vs. grassland/shrubland) had a relevant role on explaining patterns of phylogenetic diversity as well as the different behaviour of the two main ant subfamilies (Formicinae vs Myrmicinae) in respect of the environmental gradients. We modelled the SR of the two main subfamilies in a similar way to the global SR. Moreover, at the transect scale, habitat selection is explored by the Indicator Value Analysis (Dufrene & Legendre, 1997). Indicator value (indval) per species, year and habitat was calculated with the package *labdsv* (Roberts, 2016) of R. Differences between indval for forest/woodland and grassland and shrubland (Dindval_F_S) per species were averaged between years. A positive value indicates a preference of the species by wooded habitats. Number of species in each subfamily with preference per forest/woodland habitat was calculated. Finally, we estimated the association of diversity indices to species richness per subfamily by the Pearson correlation coefficient.

The functional characters may be related with the phylogeny. In order to detect it they were represented the different traits in relation to phylogenetic tree, and then the phylogenetic correlogram was calculated using *phylosignal* package (Keck et al., 2016) of R.

Results

A total of 14,499 ants (excluding reproductives) of 54 species, 20 genera and three subfamilies were collected in the two samplings periods (5,489 in 2014, and 9,010 in 2015; see Table S2). Myrmicinae (32 species and 10 genera) was the most diverse subfamily followed by Formicinae (19 species and 8 genera) and Dolichoderinae (3 species and 2 genera). The most diverse genera were *Temnothorax* (14 species) and *Camponotus* (7 species). The ant species pool recorded in the whole Segura River Basin reaches 110 (Catarineu et al., 2018), which doubles the species number recorded in this study.

Environmental and diversity gradients

Environment PCA at transect scale clearly shows the general climatic gradient (Figure 2 and Table 2). $PC1_{env}$ accounts for 59.2% of variance and clearly separates in the negative side, areas with high elevation, high precipitation, high NVDI, low CVNVDI, low MeanTemp and low MinTempM; and in the positive side areas with the opposite characteristics. Nevertheless, the factor loadings are higher for temperature and elevation than for NDVI or rainfall, indicating that this gradient represent better the altitude changes that other spatial (less important) variations of the rainfall pattern. In this sense $PC2_{env}$ (accounting 18.7% of variance) is mainly associated to MaxTempM (negative side, factor loading -0.86) but also for (less tightly) to higher precipitation (positive side, factor loading 0.43) and NDVI (0.45). On Figure 3 (a-b) they are represented on the geographical space the coordinates of the environmental variables on the principal components. From this figure, it is clear that $PC1_{env}$ is associated to the pattern of rain shadow and termicity NW to E-SW-SE (mountains to coast), while $PC2_{env}$ seems related to continentality well represented on the NE area dominated by mid-altitude plains relatively isolated from the sea influence either cooling effects of high mountains (NW and running NW to SE). Environment PCA at locality scale is very similar: $PC1_{env}$ accounts for 64.8 % of variance and $PC2_{env}$ accounts for 19.8 %, both adding up to 84.6%, and biplot is nearly identical to the transect one (data not shown).

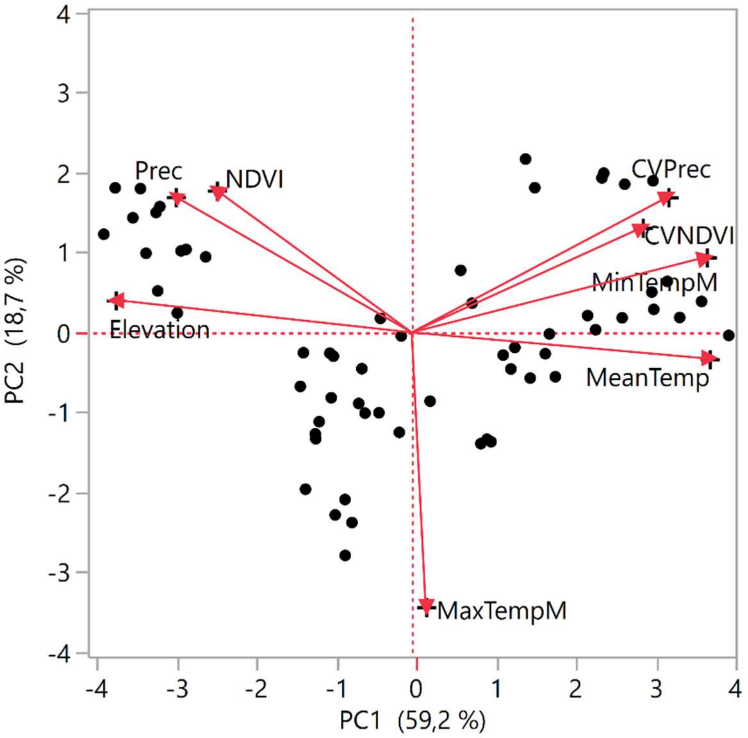

Figure 2. PCA Analysis of the main environmental variables at transect scale.

Table 2. Factor loadings on the PCA of climate and vegetation variables of the transects.

	PC1$_{env}$	**PC2$_{env}$**
Elevation	-0.93	0.10
Prec	-0.75	0.43
CVPrec	0.81	0.43
MinTempM	0.93	0.24
MeanTemp	0.94	-0.08
MaxTempM	0.04	-0.86
CVNDVI	0.73	0.33
NDVI	-0.62	0.45

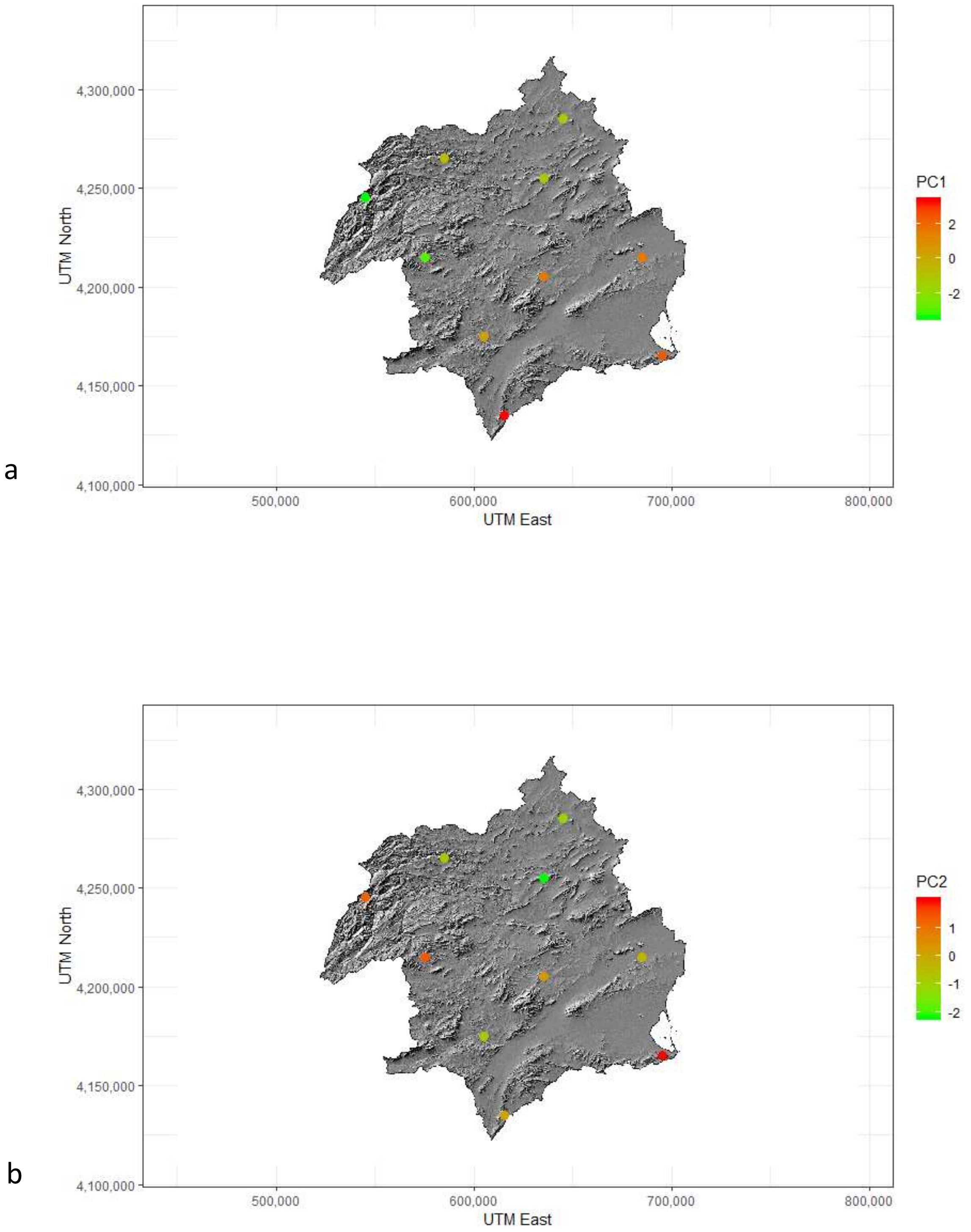

Figure 3. Environment principal components represented on the geographical space. (a) Mean values of $PC1_{env}$ per locality; (b) Mean values of $PC2_{env}$ per locality.

Ant abundance (log transformed, and measured at transect scale) is negatively correlated with $PC1_{env}$ ($P < 0.001$; R^2adj 0.33; note that in order to obtain a R^2 value, we fitted a standard regression, not a mixed model, taking into account the nesting of transects within localities; results of mixed model and standard regression were quite similar in terms of coefficients and P values and therefore the R^2 approximation is reasonable). Ant SR is also negatively correlated with $PC1_{env}$ ($P < 0.001$; R^2adj 0.43; also at the transect scale and also through a standard regression approximation like with ant abundance). There is considerably higher ant abundance and SR in the higher, colder and wetter areas (Figures 4 – 5).

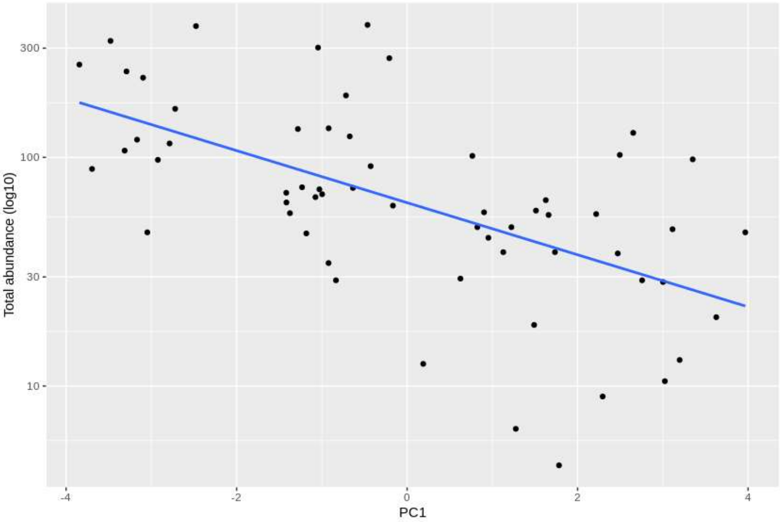

Figure 4. Correlation between $PC1_{env}$ and Total ant abundance (log transformed), at the transect scale, in the Segura River Basin

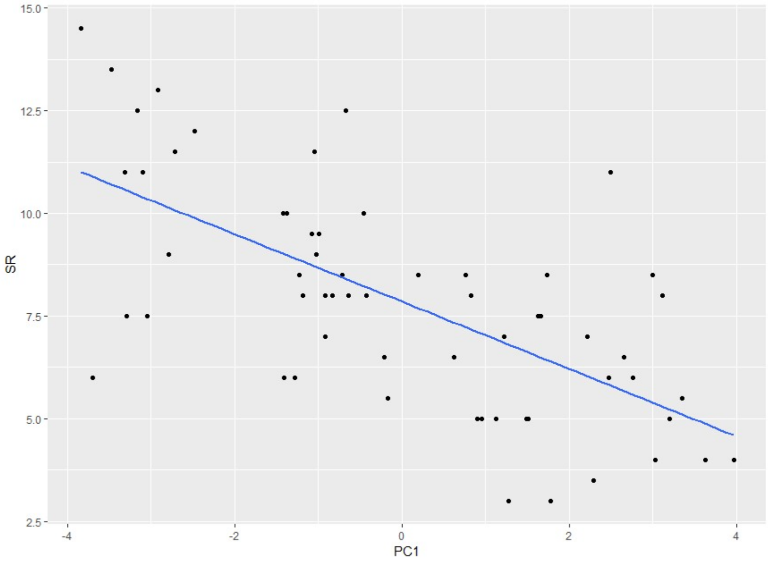

Figure 5. Correlation between $PC1_{env}$ and ant Species Richness, at the transect scale in the Segura River Basin.

The correlations between the different diversity indices are shown in Tables S4–S5. PD is positively correlated to SR at both scales, but especially at the locality scale (0.94). MPD is also positively correlated to SR, albeit moderately (0.66) and only at the locality scale. MNTD is negatively and also moderately correlated to SR (-0.61), but only at the transect scale. There is no correlation between SR and the functional diversity indices at the locality scale. At the transect scale, there is a lower correlation between SR and FDiv (0.47) and between SR and FDis (0.38). The correlation between the PD and FD indices at the locality scale is low, only MNTD to FDiv (0.5) or rPDI and FDis (0.53) are moderately correlated. At the transect scale, the correlations are a bit higher: FDiv is correlated to PD (0.52); and FDis is correlated to PD (0.55), to MPD (0.59) and to NRI (0.49).

Individual models for diversity indices

In Table 3, they are shown the models relating diversity indices to environmental gradients (first step of modelling; see Methods). In respect to $PC1_{env}$ (the dominant environmental gradient, ≈60% of variance), at both spatial scales SR and PD significantly increase as environment was higher altitude, wetter, cooler and with denser vegetation (note that they have negative coefficients; as positive values of $PC1_{env}$ implies lower altitude, dryer and hotter environments, a negative coefficient has a *negative* effect towards the positive side of $PC1_{env,}$ and a *positive* effect towards de negative side). MPD shows the same trend at both scales, but with a weaker evidence ($p = 0.09$) at the transect scale. NRI and rNRI significantly follow the opposite trend at the locality scale. MNTD in respect to $PC1_{env}$ decreases, at the transect scale, towards environments higher in altitude, wetter and cooler, while NTI follows the same trend, but only at the locality scale. In respect of $PC2_{env}$, no one of its regression coefficients on any diversity index was significant at the $P < 0.05$ level.

On the second step of modelling (Table 4), at the transect scale, models get structurally richer. The model of SR includes also precipitation (positive effect). PD and PDI are favoured by the presence of forest/woodland while NTI show the reverse trend and is also negatively associated to CVNDVI. MPD is negatively associated to CVPrec, while NRI is positively associated to this variable. FDiv is positively associated to NDVI.

At the locality scale, however, only two models change in the second step of modelling. SR incorporates CVPrec in addition to $PC1_{env}$ (Coefficient 42.317; $P = 0.020$) and rNRI, which was not associated to $PC1_{env}$ or $PC2_{env}$, is significantly influenced by CVPrec (coefficient 6.44; $P = 0.01$). In Figure 6, CVPrec is represented on the geographical space.

Table 3. Models relating diversity indices to environmental gradients (first step of modelling; see Methods)

	$PC1_{env}$				*$PC2_{env}$*			
	Transect scale		*Locality scale*		*Transect scale*		*Locality scale*	
	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value
SR	**-0.810**	**<0.001**	**-1.611**	**<0.001**	0.334	0.176	0.991	0.739
PD	**-0.181**	**0.003**	**-0.276**	**0.004**	0.040	0.693	0.035	0.240
PDI	0.022	0.712	-0.149	0.096	-0.057	0.580	0.096	0.519
rPDI	NA	NA	-0.062	0.319	NA	NA	0.005	0.961
MPD	-0.018	0.087	**-0.016**	**0.018**	-0.020	0.268	-0.008	0.420
NRI	0.067	0.190	**0.215**	**0.016**	0.143	0.108	0.104	0.425
rNRI	NA	NA	**0.174**	**0.018**	NA	NA	0.089	0.416
MNTD	**0.047**	**<0.001**	0.002	0.751	0.008	0.711	-0.005	0.578
NTI	-0.041	0.527	**0.225**	**0.008**	-0.090	0.429	-0.115	0.389
rNTI	NA	NA	0.070	0.198	NA	NA	-0.017	0.850
FDis	-0.001	0.724	0.001	0.517	-0.004	0.337	-0.003	0.352
FDiv	-0.004	0.157	-0.003	0.352	0.003	0.533	-0.004	0.400

Table 4. Models relating diversity indices to environmental factors at the transect scale (second step of modelling; see Methods)

	$PC1_{env}$		***Habitat***		***Prec***		***CVPrec***		***NDVI***		***CVNDVI***	
	Coef	P	Coef	P	Coef	P	Coef	P	Coef	P	Coef	P
SR	-0.513	0.012			0.006	0.043						
PD	-0.146	0.012	0.414	0.01								
PDI			0.491	0.01								
MPD							-0.820	0.021				
NRI							4.08	0.026				
MNT	0.047	<0.001										
NTI			-0.624	0.00							-4.048	0.04
FDis												
FDiv									0.122	0.018		

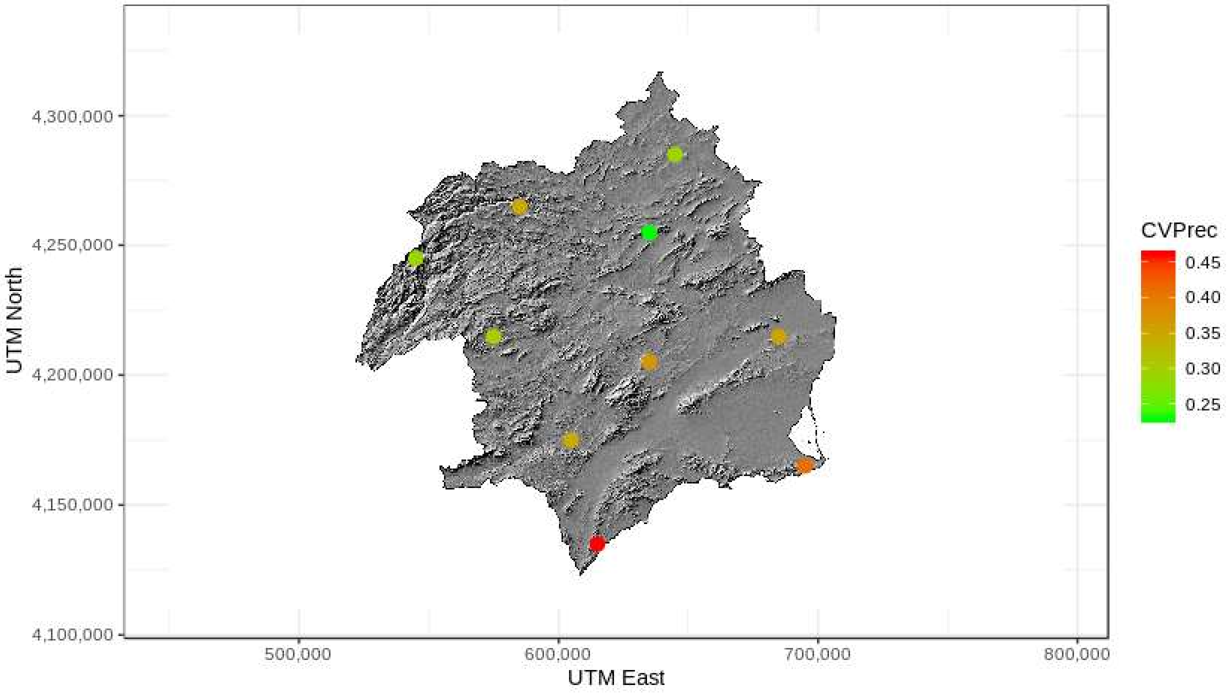

Figure 6. Coefficient of variation of mean annual precipitation (CVPrec) values represented on the geographical space.

Relationship of subfamilies to environmental variables and diversity indices to subfamilies

At the transect scale, Formicinae species richness shows a strong negative relationship with the main environmental gradient ($PC1_{env}$; $p = <0.001$). There were less species towards warm and arid extreme of the gradient. Myrmicinae shows a similar trend, but evidence was weaker ($p = 0.06$), and the intensity of this relationship (value of the coefficient of regression) was nearly four times higher for Formicinae (-0,615 vs. -0,166; see Figure 7). Furthermore, the predictive power of this relationship was much higher on Formicinae (adjusted $R^2 = 0.52$) than on Myrmicinae (0.061). For both subfamilies $PC2_{env}$ effect was far from the standard value of statistical significance ($p = 0.20$ and 0.15; Formicinae and Myrmicinae, respectively). When $PC1_{env}$ is maintained fixed in the model and the rest of environmental variables are stepwise selected, Formicinae SR increases with elevation (Table 5; note that introducing Elevation on the model strongly reduces the coefficient of regression for $PC1_{env}$, a logical consequence of the association of elevation to $PC1_{env}$). On the other hand, minimum temperature is incorporated in the model for Myrmicinae (positive effect, higher minimum temperature increases number of Myrmicinae species). Yet both models strongly differ on predictive power (adjusted $R^2 =$ 0.56 and 0.10; Formicinae and Myrmicinae, respectively).

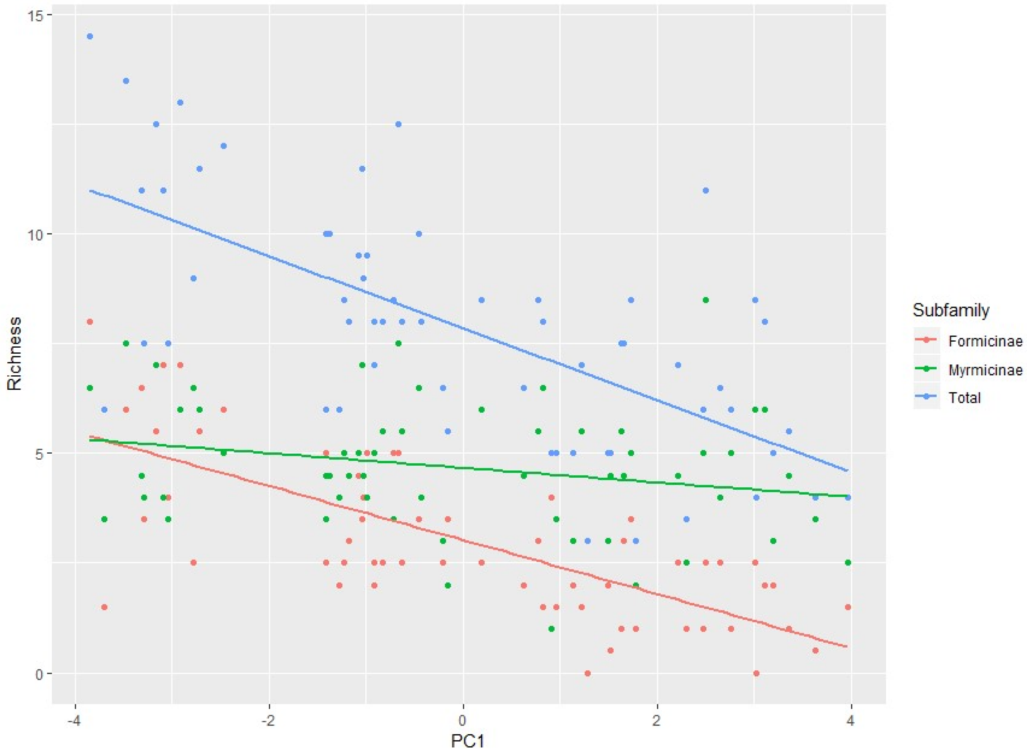

Figure 7. Correlation between $PC1_{env}$ and ant Species Richness, at the transect scale, in the Segura River Basin, including Formicinae and Myrmicinae species richness.

Table 5. Models relating SR indices (of the two main subfamilies) to environmental factors at the transect scale ($PC1_{env}$ is maintained fixed in the model, and the rest of environmental variables are stepwise selected)

	$PC1_{env}$		Elevation		MinTempM	
	Coefficient	P	Coefficient	P	Coefficient	P
Formicinae	-0.205	0.364	0.002	0.036		
Myrmicinae	-0.635	0.090			0.389	0.036

Mean differences indicator value (*indval*) for wooded and treeless habitats was 0.081 for Formicinae and 0.006 for Myrmicinae, t-test is not statistically significant but 95% confidence interval of the difference of differences is -0.007 to 0.216, giving some evidence of preference of Formicinae as a group for forest/woodland. Moreover, number of species with preference for forest, when compared with preferred for open habitats, was larger on Formicinae but equilibrated on Myrmicinae (Table 6). Formicinae SR is higher in the forests/woodlands than in the shrublands/grasslands, while Myrmicinae SR is very similar in both habitats (Figure 8).

Table 6. Number of species of the main subfamilies with preference for forest/woodland and grassland/shrubland habitats according to indicator value analysis.

	Forest/woodland	Grassland/shrubland
Formicinae	13	6
Myrmicinae	16	16

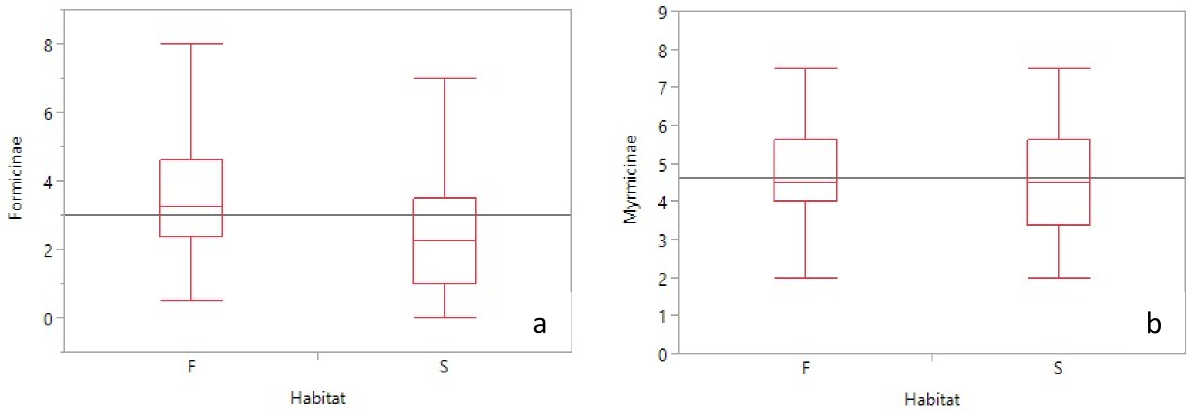

Figure 8. Boxplot of species richness of Formicinae (a) and Myrmicinae (b) by habitat at the transect scale. F = forest/woodland; S = shrubland/grassland. Horizontal lines represent mean values across both habitats.

At the locality scale, the picture is similar, both subfamilies are negatively related to $PC1_{env}$, but coefficient is triple to Formicinae (Table 7). R^2 is 0.84 for Formicinae versus 0.51 on Myrmicinae. Interestingly, evidences for $PC2_{env}$ relating to Myrmicinae are close to standard level of significance. When other variables are tested, only CVPrec is into the model for Formicinae (Table 8).

Correlation between the species richness per subfamily and diversity indices at both scales is shown in Table 9. There are marked differences between subfamilies in some phylogenetic indices, especially in MPD: Formicinae has a notably larger contribution to the variation of this index than Myrmicinae. Differences are more relevant at the transect scale.

Table 7. Model relating SR (of the two main ant subfamilies) to environmental gradients, at the locality scale.

	$PC1_{env}$		$PC2_{env}$	
Formicinae	-1,111	<0.001	0.357	0.257
Myrmicinae	-0.364	0.040	0.579	0.062

Table 8. Model relating SR (of the two main ant subfamilies) to environmental variables, at the locality scale.

	$PC1_{env}$		CVPrec	
Formicinae	-1.617	<0.001	22.231	0.022

Table 9. Correlation between the species richness per subfamily and diversity indices at both scale at both spatial scales.

	Transect		**Locality**	
	Formicinae	Myrmicinae	Formicinae	Myrmicinae
SR	0,85	0,75	0,96	0,85
PD	0,59	0,67	0,88	0,76
PDI	-0,21	0,03	0,60	0,49
MPD	0,19	0,02	0,72	0,28
MNTD	-0,59	-0,44	-0,23	-0,42
NRI	-0,07	0,06	-0,73	-0,26
NTI	0,16	-0,06	-0,83	-0,61
rPDI	NA	NA	0,34	0,29
rNRI	NA	NA	-0,72	-0,27
rNTI	NA	NA	-0,45	-0,34
FDiv	0,41	0,35	-0,24	-0,46
FDis	0,31	0,24	-0,14	-0,23

Functional traits, phylogenetic signals, and phylogenetic structure

Continuous functional traits are clearly associated to subfamilies and also at lower levels. In general, Formicinae have larger and more polymorphic workers (Figure 9). Barplots of diet traits along phylogeny (Figure S3), phylogenetic correlograms (Figure S4) and trait values along phylogeny (Figures S5–S11) are shown in the Supplementary material section.

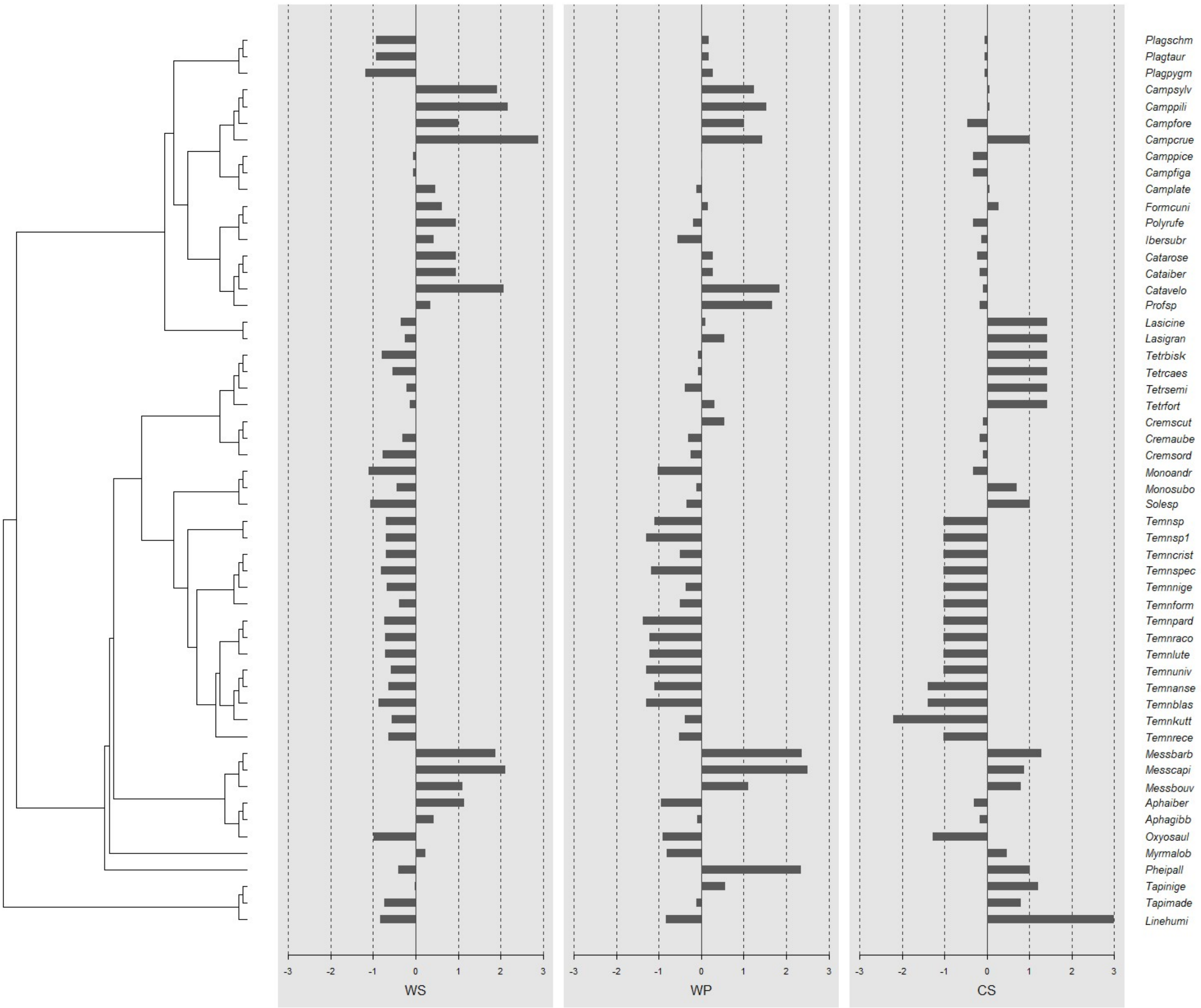

Figure 9. Barplot of the standardized traits along phylogeny. WS= worker size; WP= worker polymorphism, CS= Colony size.

The relationships between the $PC1_{env}$, and the PDI, NRI and NTI indices, at the locality scale, are shown in Figure 10. PDI decreases with $PC1_{env}$. NRI and NTI, conversely, increases with $PC1_{env}$, with negative values in the higher altitude, cooler and wetter areas, indicating overdispersed phylogenetic structure, and with positive values in the lower altitude, hotter and dryer areas, indicating clustered phylogenetic structure. The transition areas, from negative to positive values, occurs between 500–700 m altitude, 16ºC of mean temperature and 300–400 mm of annual precipitation for the NRI indice; and between 220–500 m altitude, 17ºC of mean temperature and 250–350 mm of annual precipitation for the NTI indice.

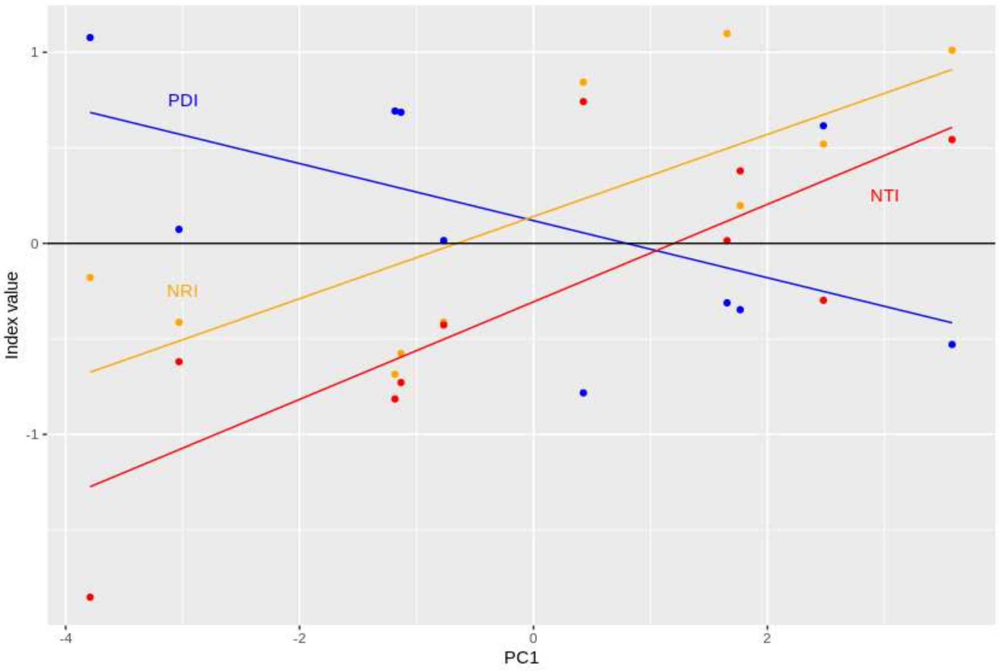

Figure 10. Relationships between the $PC1_{env}$, and the PDI, NRI and NTI indices, at the locality scale.

Discussion

In the Segura River Basin, we found a general environmental gradient (PC1env; Figures 2–3 and Table 2) from areas with high elevation, high precipitation, high NVDI, low CVNVDI, low MeanTemp and low MinTempM to areas with the opposite characteristics (from the NW mountains to the SW-S-SE coasts). As we expected, this general environmental gradient is an important force structuring ant diversity in our study area. There is also another weaker gradient of continentality ($PC2_{env}$; Figures 2–3 and Table 2) mainly associated with MaxTempM (from the NE mid-altitude plain to the SE coasts). Del Toro (2013), studying ant communities in Eastern North America, state that MaxTempM is a better predictor of ant species richness than altitude, but this is not our case.

The general environmental gradient ($PC1_{env}$) is significantly related to SR, PD and MNTD at the transect scale, but note that PD and MNTD are strongly dependent on SR (Miller et al., 2017). At the locality scale significant association of this gradient was to SR, PD, MPD, NRI, rNRI and NTI. In this case, except for PD, the indices are standardized by SR and responses should reflect real processes independent of SR. As we expected, there are a richer response of indices at the locality scale when compared to transect scale to the general environmental gradient. This is probably caused by local factors at the local scale, as detailed vegetation composition and physiognomy, soils, lithology, microclimatic setting, etc., that are not reflected in this coarse scale environmental analysis, but also to different intensity of the processes shaping ant communities at different scales, as we argue next when looking at the detailed patterns of taxonomic, functional and phylogenetic biodiversity.

Taxonomic diversity

The SR model at the first step of modelling shows that SR decreases with $PC1_{env}$ at both scales (Table 3). SR increases following the basic regional gradient towards higher elevation, higher precipitation, higher NVDI, lower CVNVDI, lower MeanTemp and lower MinTempM. This increase is most evident at the locality scale where the environmental factors play a greater role, than at the transect scale where the species interactions operate most intensively (Cavender-Bares et al., 2009).

SR has a monotonic increase along $PC1_{env}$ (Figure 5). Szewczyk and McCain (2016), state that the most common patterns of ant species richness in altitudinal gradients were mid-elevation peaks in diversity followed by low-elevation plateaus and monotonic decreases with elevation, so the pattern in our study area seem to be uncommon. Monotonic increases in ant species richness has been cited in arid environments (Sanders et al., 2003), related with higher precipitation and primary production in higher areas. But we need to bear in mind that our altitude range is 0–1,410 m, and we cannot exclude that there exists a mid-elevation peak above the upper range of our study area, taking into account that the summit of the mountains in the Segura River Basin reaches 2000 m. Flores et al. (2018), studying the elevational gradients of ant species richness in Guadarrama (Central Spain), found a mid-elevation peak at 1,100–1,400m.

Elevation does not affect intrinsically the taxonomic diversity, but the abiotic factors that covary with it does. In the Segura basin, temperature (within the studied range) does not seem to be the main driver underlying ant taxonomic diversity. Ants are termophilic species (Hölldobler & Wilson, 1990) and, in other studies, ant SR is positively correlated with temperature (Hawkins et al., 2003) while in the Segura River Basin, the relationship is the opposite.

Precipitation and NVDI seem to be the more important drivers controlling ant taxonomic diversity. At the second step of modelling, and at the locality scale, in areas with a higher precipitation than expected along the basic regional gradient, SR has a further increase (Table 4). Species richness increases with precipitation in a majority of taxa, but this does not seem to be the case in ants (Dunn et al., 2009a), except when rainfall is very low (Delsinne et al., 2010). Precipitation, in semi-arid areas, is well known to have strong influence on primary production (Paknia & Pfeifer, 2014), and therefore, the semi-arid climate in the Segura River Basin implies that precipitation should be a very important driver for ant diversity, allowing higher primary production in the wetter areas. The higher NDVI indicate greater net primary productivity (Kerr & Ostrovsky, 2003) in the higher altitude areas. When prymary productivity is higher, the availability of resources leads to an increase in biomass of organisms that depend on those resources (Clarke & Gaston, 2006). We found that ant abundance is negatively correlated to $PC1_{env}$ (Figure 4), i.e. there is more ant abundance in the higher, cooler and wetter areas. The more-individual hypothesis (Srivastava & Lawton, 1998) predicts that more productive sites can support higher abundance within a community and thus, more species richness, as is the case in our study area.

Evolutionary and climatic history may also be an underlying cause for the reduced diversity in the lower lands at the regional scale compared with the higher lands. The climatic oscillations during the Pleistocene Ice Ages had an important influence on the species zoogeography and, after the last glacial period some species tracked their optimal climate upwards, but at the lowest elevations did not exist species to replace those that moved upward (Gómez & Lunt, 2007; Szewczyc & McCain, 2016).

Functional diversity

At the transect scale (Table S4), there is a low positive correlation between FDiv and PD (0.52) and FDis and PD (0.55). There is also a low positive correlation between FDis and MPD (0.59), and negative between FDis and NRI (-0.49). At the locality scale (Table S5), we found no correlation between the FD indices and PD or TD and only a low correlation between FDiv and MNTD (0.5) and FDis and rPDI (0.53). Arnan et al. (2016) found a consistent relationship, albeit weak, between FD and PD in European ant communities while other authors suggest that there are mismatches between these diversity indices (Devictor et al., 2010; Safi et al., 2011)

Contrary to our expectations, FDis and FDiv are not correlated with the environmental gradients at the first step of modelling. These results are inconsistent with Arnan et al. (2015), who stated that European ants FD responded better to the environmental gradients than TD or PD did. Although these authors used Rao's coefficient to asses FD instead FDiv or FDis, it has been shown that FDis and Rao's coefficiente are very highly correlated (Laliberté & Legendre, 2010) and therefore difference in metrics does not seem to be the cause of disparity in results. Thus, the disparate spatial scales of both studies as well as the ranges of the environmental gradients and maybe the geological history may explain these differences.

At the second step of modelling, FDiv has a positive correlation with NDVI (Table 4). FDiv represents how species spread along a functional trait axis, so areas with higher NDVI show higher spread of species on the functional space. NDVI correlates with net primary productivity (Kerr & Ostrovsky, 2003) and also with canopy structure (Gamon et al., 1995) in sparse vegetation habitats (leaf area index, LAI < 2), which is the case for most of the samples. On the other hand NDVI was significantly higher on forest/woodland than on shrubland/grassland (t test, P < 0.001), but also differed between localities when controlling for habitat (two-way ANOVA; Habitat, P < 0.001; Locality P < 0.001; Habitat x Locality, P = 0.709), that is to say, structural complexity of both habitat increases along the main environmental gradient. In more structurally complex habitats, possibly there will also be more functional niches and so more FDiv.

Phylogenetic diversity and phylogenetic structure

We detected significant phylogenetic signals in the continuous functional traits (Figure S4), so we hypothesized that there is an important degree of niche conservatism in the Segura River Basin ants. This is consistent with Arnan et al. (2016), which found a high degree of niche conservatism evidence in European ant communities. The existence of niche conservatism, allows us to assume that interspecific competition could explain the phylogenetic overdispersion, and environmental filtering could explain the phylogenetic clustering.

As expected, PD closely tracks the behaviour of SR in respect to the general environmental gradient, increasing towards the higher, wetter and colder areas. The standardized indice of PD (PDI) and its rarefied version (rPDI) also follows the same trend at the locality scale (Table 3). Therefore, there is a gradient of phylogenetic diversity decreasing towards the lower and more arid areas. Arnan et al. (2018), studying the Brazilian Caatinga ant communities also found that PD decreased with aridity.

Interestingly however, PD increases proportionally more on forests, and its standardized version PDI is clearly related with forests at the transect scale (Table 4). The underlying cause of this higher PD in forest seem to be that Formicinae has a higher SR in the forests than in the shrublands, while Myrmicinae SR is very similar in both habitats (Figure 8). Thirteen out of 18 Formicinae species present higher indicator values for the forest, in contrast to 16 out of 32 Myrmicinae species (Table 6). Sanders et al. (2003) state that ant species in the subfamilies Dolichoderinae and Myrmicinae were more common at lower elevation than at higher elevation, while ant species in the subfamily Formicinae were more common at high elevation. In our study, the two main subfamilies Myrmicinae and Formicinae has a higher SR in high elevation, but Formicinae relation to main environmental gradient, as well as to elevation, is much more intense than for Myrmicinae, and also has higher values than expected in the forests, increasing the PD. Forest patches seems to act as refuges for Formicinae species as main environmental gradient gets warmer and dryer. It has to be noted that, on the region, forest patches on the semiarid domain are usually associated to favourable micro and mesoclimatic conditions at north-faced slopes, and even on wettest areas density of forest notoriously varies with these mesoclimatic conditions.

MPD and its standardized versions NRI and rNRI indicate phylogenetic clustering towards the warm and dry extreme of the general environmental gradient at the locality scale. At the transect scale, MPD decreases (and NRI increases) with CVPrec (Table 4), an indicator of ecosystem productivity variability (CVPrec to CVNDVI correlation is 0.81), and also a surrogate of the gradient from the coast to inner parts of the basin (Figure 6).

The behaviour of MNTD and its related standardized indices (NTI and rNTI) is different. At the transect scale, MNTD is positively related to general environmental gradient (larger distances to nearest neighbours on the warm and arid side of the gradient) suggesting more competitive exclusion in the arid sectors. However, because the strong dependence of MNTD on SR (Miller et al., 2017) and the lack of relation between NTI and the general environmental gradient at the transect scale, this conclusion may be wrong.

NTI, at the transect scale, has an interesting negative relation with forest and CVNDVI. This result suggests that the nearest neighbour phylogenetic structure is overdispersed, and there may be more competitive exclusion on forests and on the areas with higher oscillation in productivity at the transect scale. While this increase of limiting similarity at the transect scale when environment becomes harsher and more variable (higher CVNDVI) is easy to understand, we do not find a clear explanation for the similar and simultaneous effect of forests, although it has to be observed that at the transect scale NTI correlation to PDI is -0.92, and it was shown PDI at that scale is positively associated to forests. Thus, the sequence is that PD increases towards the humid and cool extreme of the gradient, but proportionally more on forests, this results in a clear (positive) association of PDI with forests and hence in a (negative) relation with NTI in that habitat. So, it could be that niche space in forests let higher PD and it indirectly produces dispersion of the nearest neighbour phylogenetic structure, not necessarily related to increase of limiting similarity. Nevertheless, this conjecture needs to be specifically studied.

NRI and NTI, at the locality scale, increases from the highest to the lowest areas (Table 3, Figure 10). Taking into account NRI and NTI indices, we detected a shifting pattern of phylogenetic structure along the basic environmental gradient ($PC1_{env}$), from phylogenetic overdispersion in the higher areas to phylogenetic clustering in the low areas. Ant species are less closely related than expected in the higher altitude areas, and more closely related in the lower altitude areas. The transition zone occurs between 220–700 m altitude, 16–17ºC of mean temperature, and 250-400 mm of annual precipitation. Above this transition zone, we detected phylogenetic overdispersion, and under it we detected phylogenetic clustering. Note that this overdispersion or clustering is referred to a regional standard, and it is strictly relative to the whole phylogenetic structure of the region. Nevertheless the important point is the matching between trend from ‘overdispersion’ to ‘clustering’ and the environmental gradient.

NRI and NTI phylogenetic structure measures provide us different information. NRI informs about general phylogenetic structure of the community, while NTI informs about the nearest neighbour phylogenetic structure of the community. At the locality scale, both are correlated positively with the general environment gradient and the interpretation is the same: overdispersed phylogenetic structure in the higher altitude, wetter and colder areas clustered phylogenetic structure in the lower altitude, dryer and warmer areas. Climatic factors impose a general environmental filter that vary relatively little among transects within a locality. Closer species are “packed” together by environmental filtering, and can share the whole area of the locality without direct competition between them, being segregated by subtler habitat variation or simple spatial segregation. But at the transect scale, NTI, more sensitive to limiting similarity (Kraf et al., 2007), shows that, there are more competition and overdispersed nearest neighbour phylogenetic structure in areas with higher CVNVDI. NRI, more sensitive to environmental filtering (Kraf et al., 2007), shows that there are more clustering in areas with higher CVPrec. It has to be noted that CVPrec and CVNDVI are highly correlated (0.81; Figure 2).

As expected, the harsh conditions of aridity at low elevations areas constitute an environmental filter in our study area that underlies the general phylogenetically clustered structure, nuanced by limiting similarity at the transect scale on localities where environmental filter is more rigorous. The ant species are more closely related than expected by chance, and possibly share phylogenetically conserved traits that allow them to survive in areas with low precipitation, high temperatures and low primary productivity. Delsine et al. (2010) state that aridity could not be stressful enough to constitute an phylogenetic environmental filter for ant communities, bearing in mind that aridity-tolerance traits are widespread in the ant phylogeny, but our results show the opposite in our study case, covering a broad range of precipitation (203–746 mm).

In the higher, cooler and wetter environments of the Segura River Basin , interspecific competition seems to be more important shaping the overdispersed phylogenetic structure, assuming that related taxa compete more intensely and are less likely to coexist (Webb et al., 2002; Cavender-Bares et al., 2009).

We did not detect phylogenetic clustering at the highest altitudes. Machac et al. (2011), studying elevational gradients in ant phylogenetic structure in USA and Austria, state that there was a critical mean temperature at ~10ºC, at which the main structuring force change from competitive interactions to environmental filtering caused by low temperatures. In our case, temperatures did not reach this critical limit: the coolest mean annual temperature in our study sites was 12.48ºC.

As expected, the relationships between the diversity indices and the environmental factors and the effects of the aridity environmental filter in the Segura River Basin are more evident at the locality scale because, at the transect scale, species interactions (especially competition) and probably very local environmental factors, have major relevance as community drivers (Cavender-Bares et al., 2009).

The three diversity components, Taxonomic, Phylogenetic and Functional, should be taken into consideration for a better insight about the drivers shaping the biological communities. Further research with ants and other taxa are needed if we want to predict how species and communities will respond to ongoing climate change.

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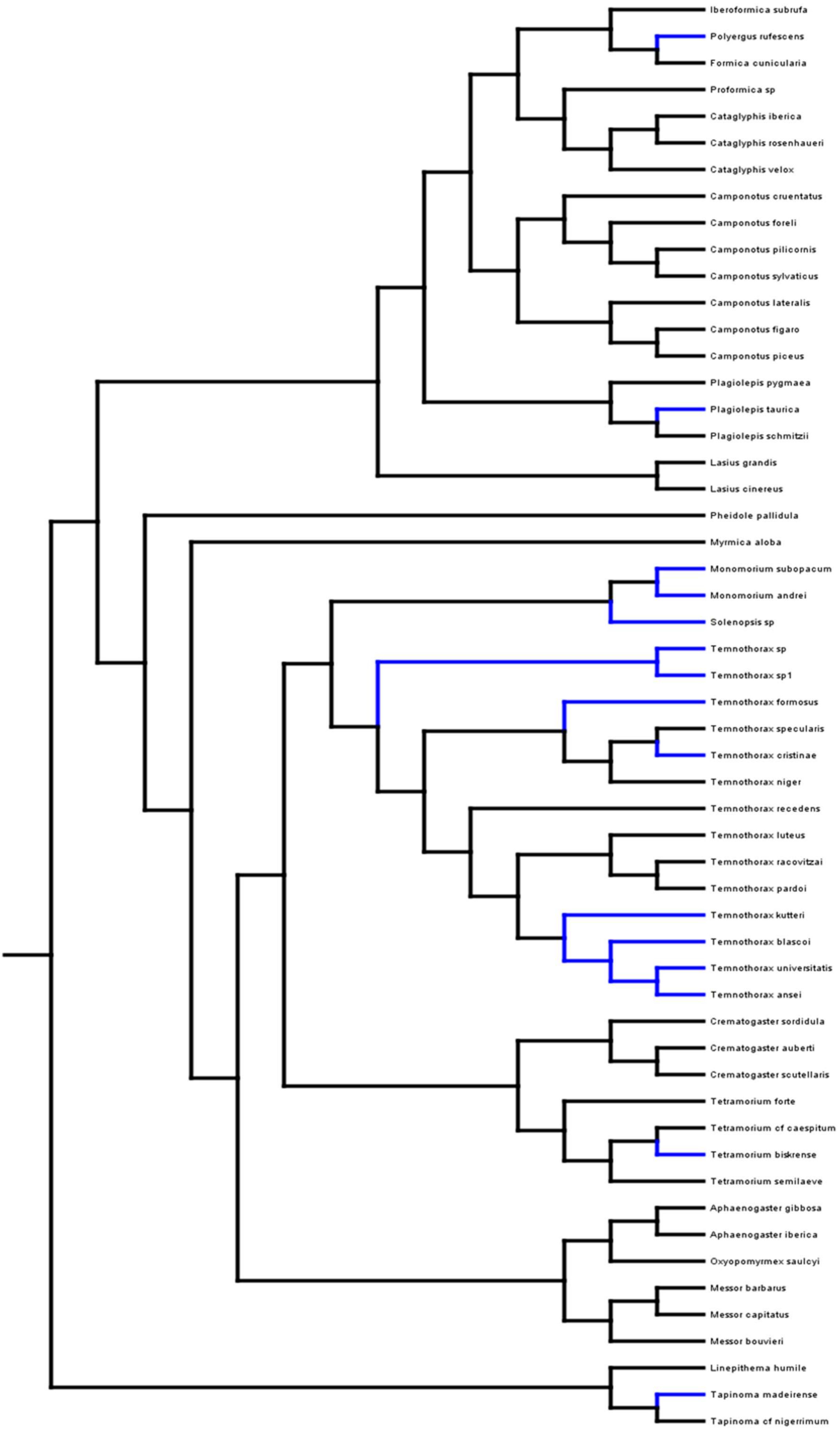
Webb, C.O., Ackerly, D.D., McPeek, M.A. & Donoghue, M.J. (2002). Phylogenies and community ecology. Annual review of ecology and systematics, 33(1): 475–505.

Wiens, J.J. & Donoghue, M.J. (2004). Historical biogeography, ecology and species richness. Trends in ecology & evolution, 19(12): 639–644.

Supplementary material

Figure S1 (in the next page). Phylogenetic tree of the ants of the Segura River Basin. It has been reconstructed with Mesquite 3.4 (Maddison & Madisson, 2018), based on the phylogeny proposed by Arnan et al. (2016) for european ants. From this phylogeny, we reconstructed a new tree, eliminating the species that were not present in the Segura River Basin and adding 15 species (in blue color) that were not present in the phylogeny of Arnan et al. (2016), but were present in the Segura River Basin. Six species of *Temnothorax* were incorporated based on Catarineu et al. (2017), Prebus (2017) and morphological data. The two species of *Temnothorax* that we could not identify to species level, were placed at the beginning of the *Temnothorax* branch. *Solenopsis* sp, *Polyergus rufescens* and the two species of *Monomorium* were incorporated to the tree according with different authors (Moreau et al., 2006, Ward et al., 2014; Ward et al., 2016). *Tetramorium biskrense* was incorporated in the same branch than *Tetramorium* cf *caespitum*. This is also the case for *Tapinoma madeirense*, incorporated in the same branch than *Tapinoma* cf *nigerrimum*. Finally, *Plagiolepis taurica* was incorporated in the same branch than *Plagiolepis schmitzii*, based on morphological criteria (Espadaler et al., 2013).


Iberoformica subrufa
Polyergus rufescens
Formica cunicularia
Proformica sp
Cataglyphis iberica
Cataglyphis rosenhaueri
Cataglyphis velox
Camponotus cruentatus
Camponotus foreli
Camponotus pilicornis
Camponotus sylvaticus
Camponotus lateralis
Camponotus figaro
Camponotus piceus
Plagiolepis pygmaea
Plagiolepis taurica
Plagiolepis schmitzii
Lasius grandis
Lasius cinereus
Pheidole pallidula
Myrmica aloba
Monomorium subopacum
Monomorium andrei
Solenopsis sp
Temnothorax sp
Temnothorax sp1
Temnothorax formosus
Temnothorax specularis
Temnothorax cristinae
Temnothorax niger
Temnothorax recedens
Temnothorax luteus
Temnothorax racovitzai
Temnothorax pardoi
Temnothorax kutteri
Temnothorax blascoi
Temnothorax universitatis
Temnothorax ansei
Crematogaster sordidula
Crematogaster auberti
Crematogaster scutellaris
Tetramorium forte
Tetramorium cf caespitum
Tetramorium biskrense
Tetramorium semilaeve
Aphaenogaster gibbosa
Aphaenogaster iberica
Oxyopomyrmex saulcyi
Messor barbarus
Messor capitatus
Messor bouvieri
Linepithema humile
Tapinoma madeirense
Tapinoma cf nigerrimum

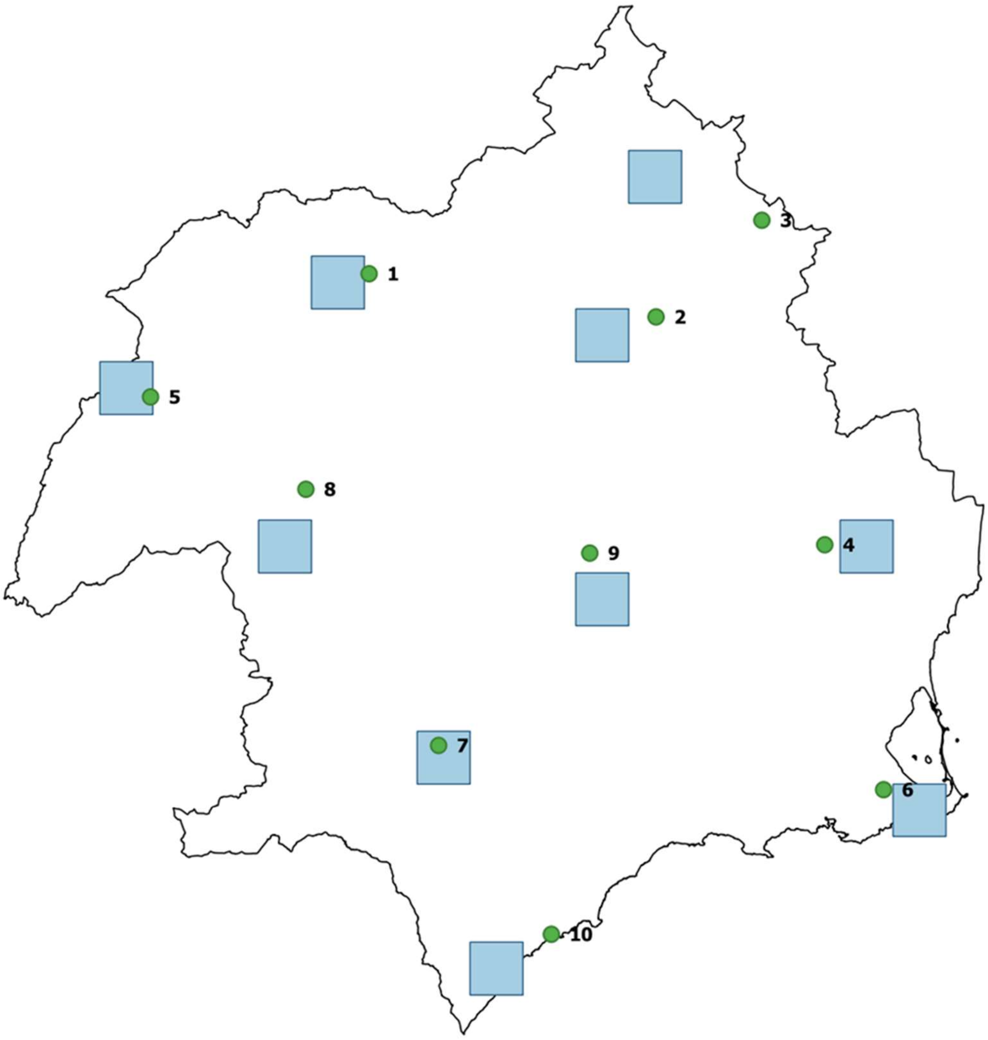


Figure S2. Map of the Segura River Basin with the ten meteorological stations whose data we have used in green dots. 1: 7092 Lietor; 2: 7138D Jumilla La Estacada; 3: 7275 Yecla CH Segura; 4: 7244 Desamparados; 5: 7059 Arguellite; 6: 7016 El Algar; 7: 7205 Embalse de Puentes; 8: 7113 Campo de S. Juan; 9: 7168 Emb. De la Cierva; 10: 7002 Águilas Diputación. The 10x10 km UTM squares are also indicated in blue squares.

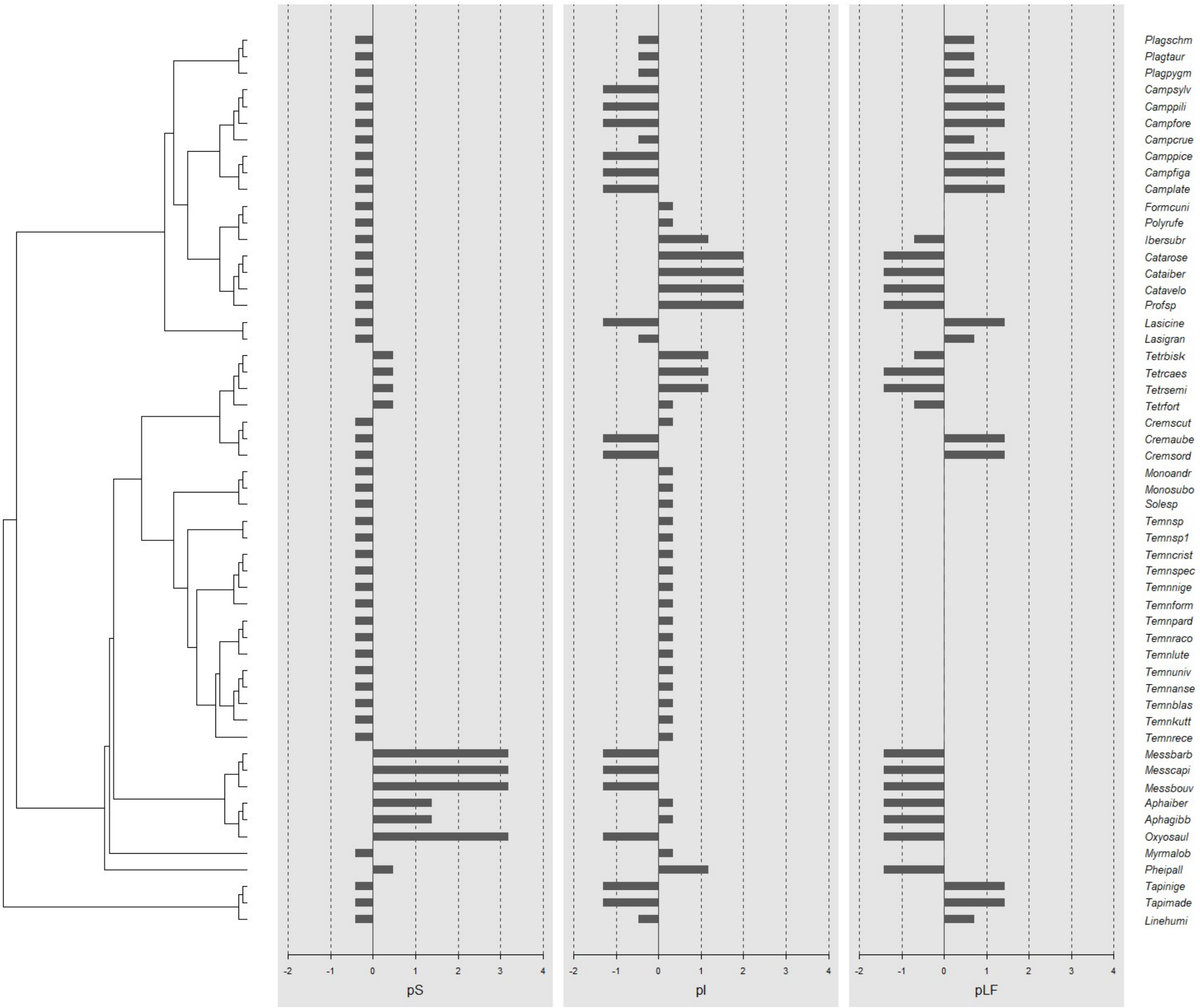


Figure S3. Barplot of the diet traits along phylogeny. pS= proportion of seeds in diet; pI= proportion of insects in diet; pLF= proportion of liquid foods in diet.

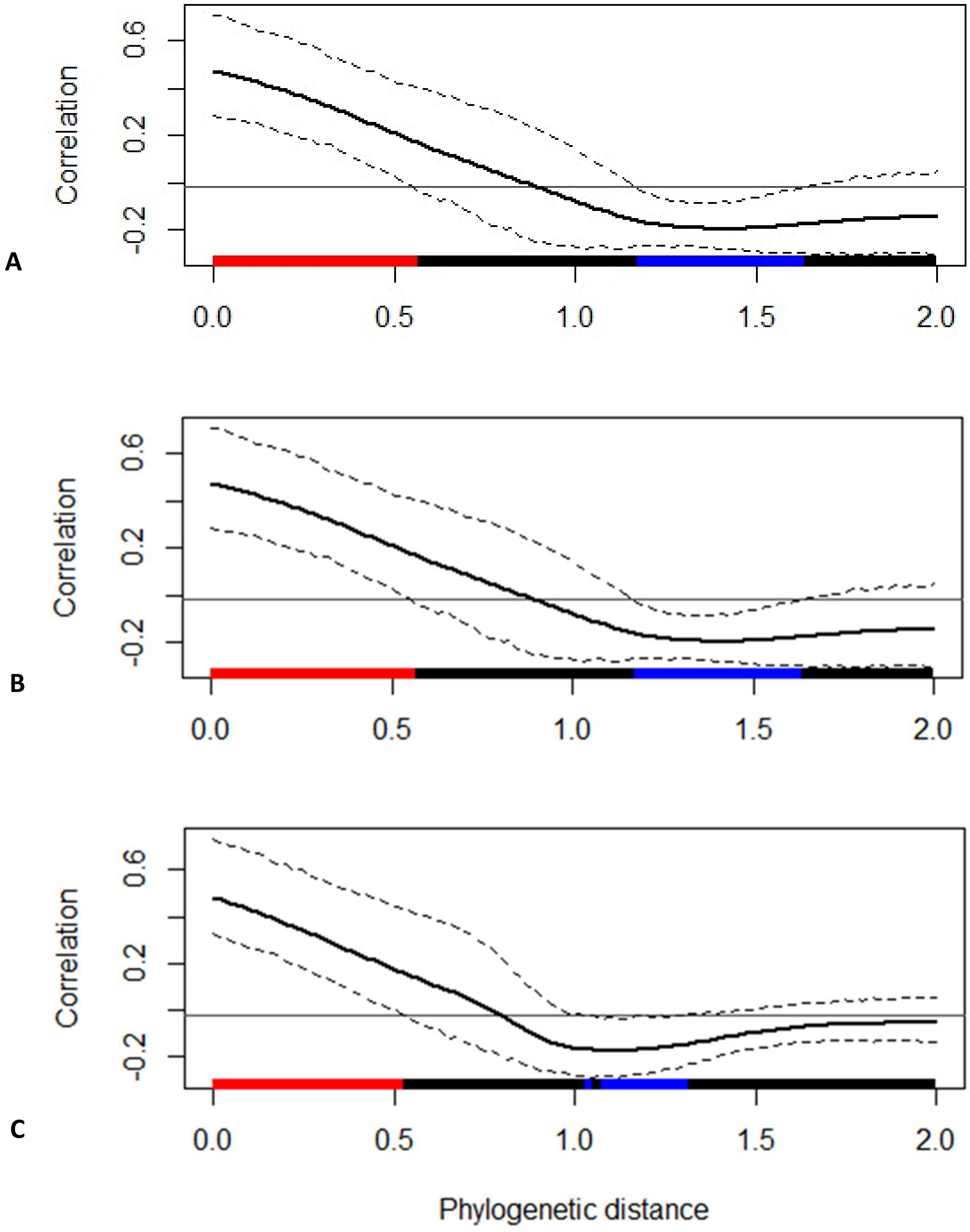


Figure S4. Phylogenetic correlograms. A) worker size, B) worker polymorphism; C) colony size

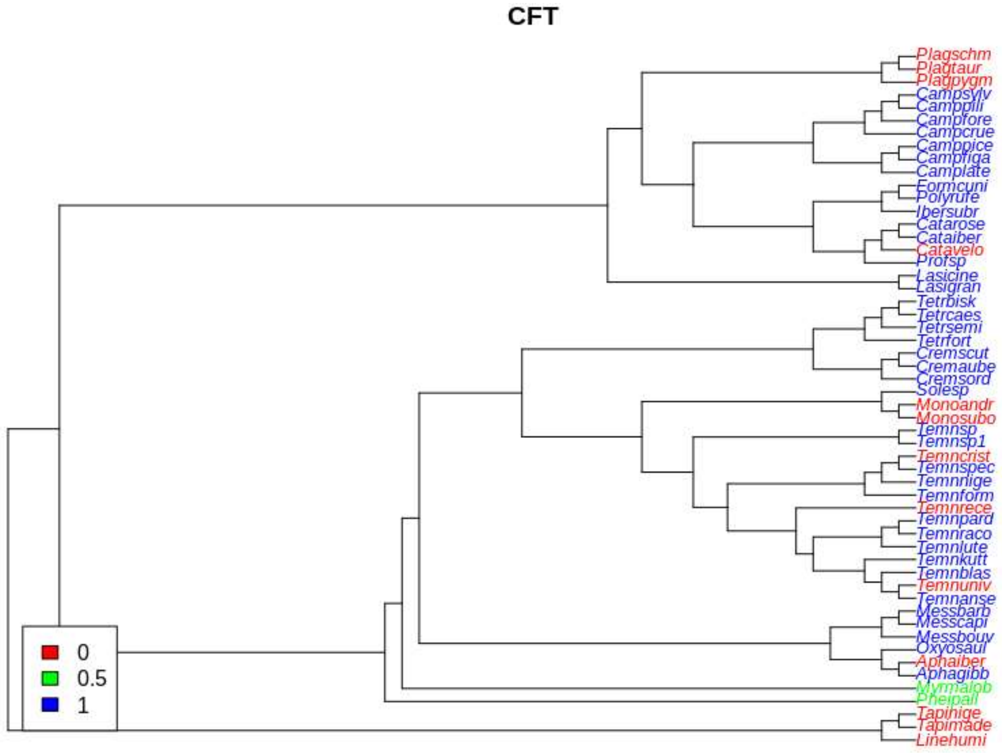


Figure S5. Colony Fundation Type values along phylogeny. 0=DCF; 0.5=Both DCF and ICF; 1=ICF

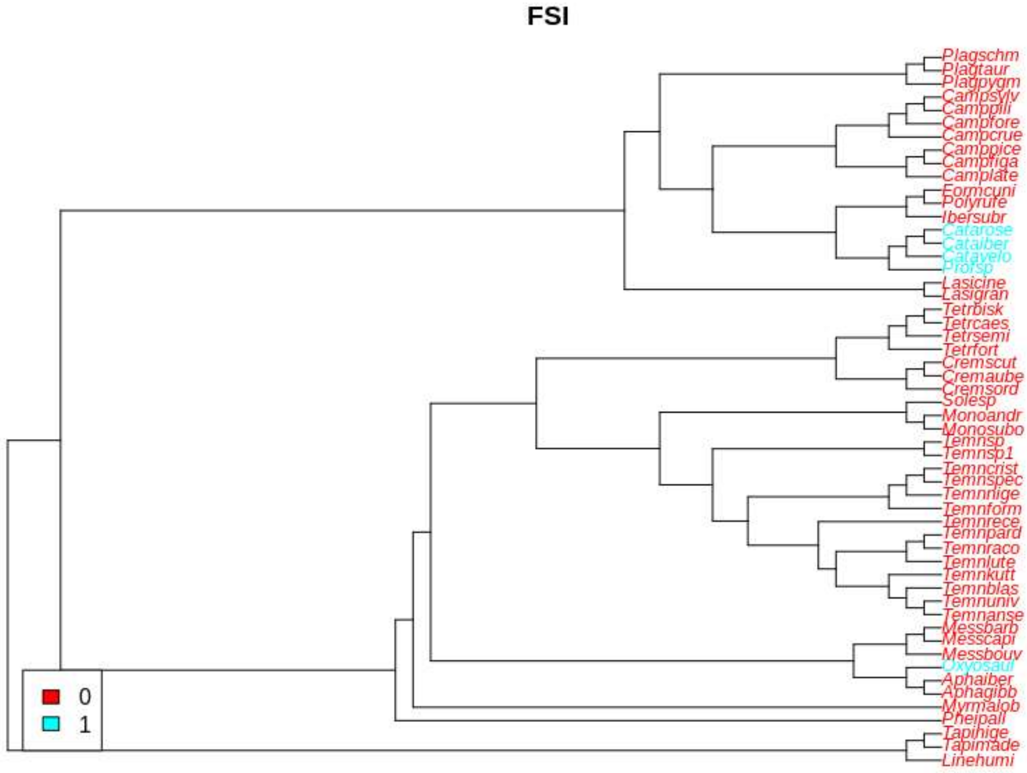


Figure S6. Individual foraging strategy values along phylogeny. 0=No; 1=Yes

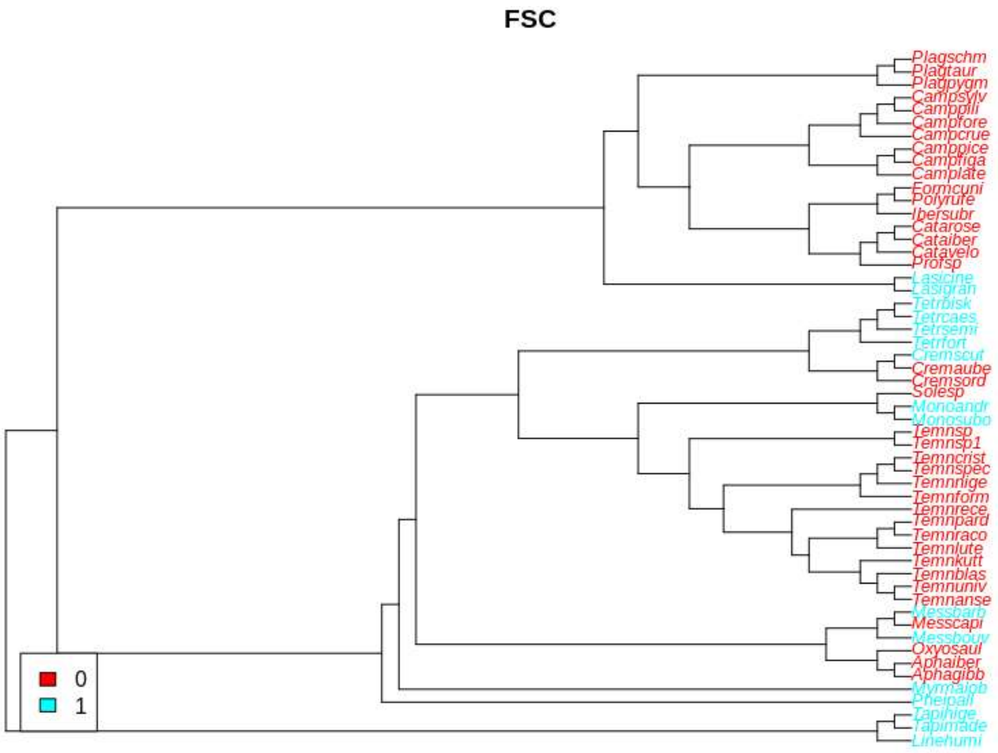


Figure S7. Collective foraging strategy values along phylogeny. 0=No; 1:Yes

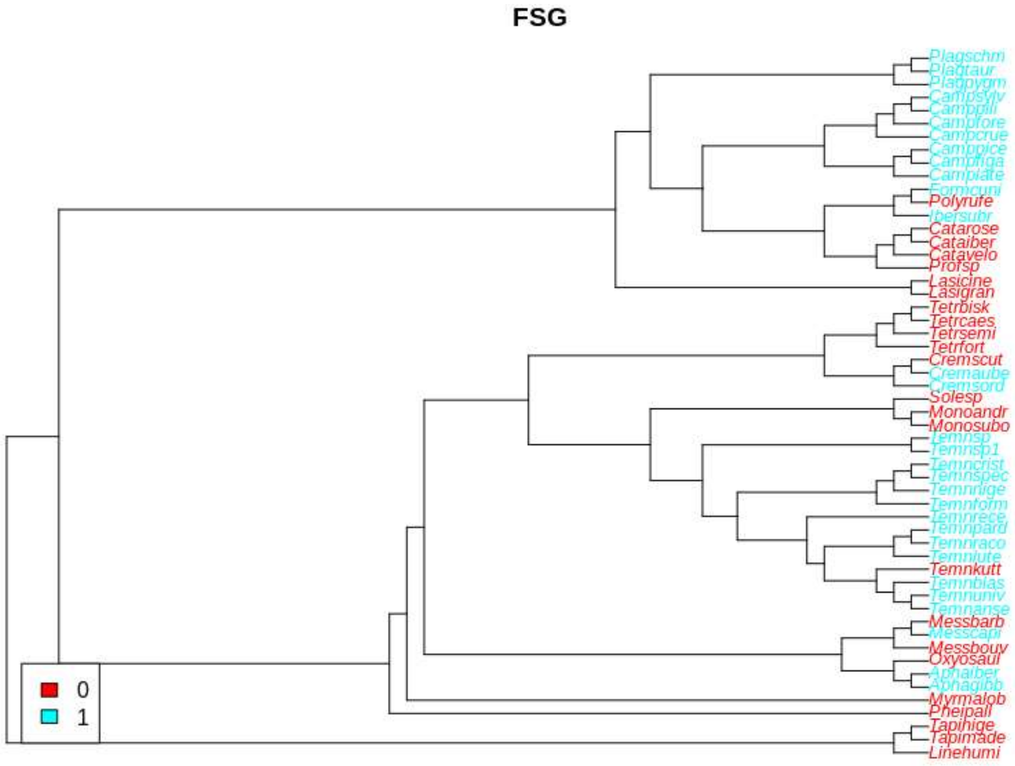


Figure S8. Group foraging strategy values along phylogeny. 0=No; 1=Yes

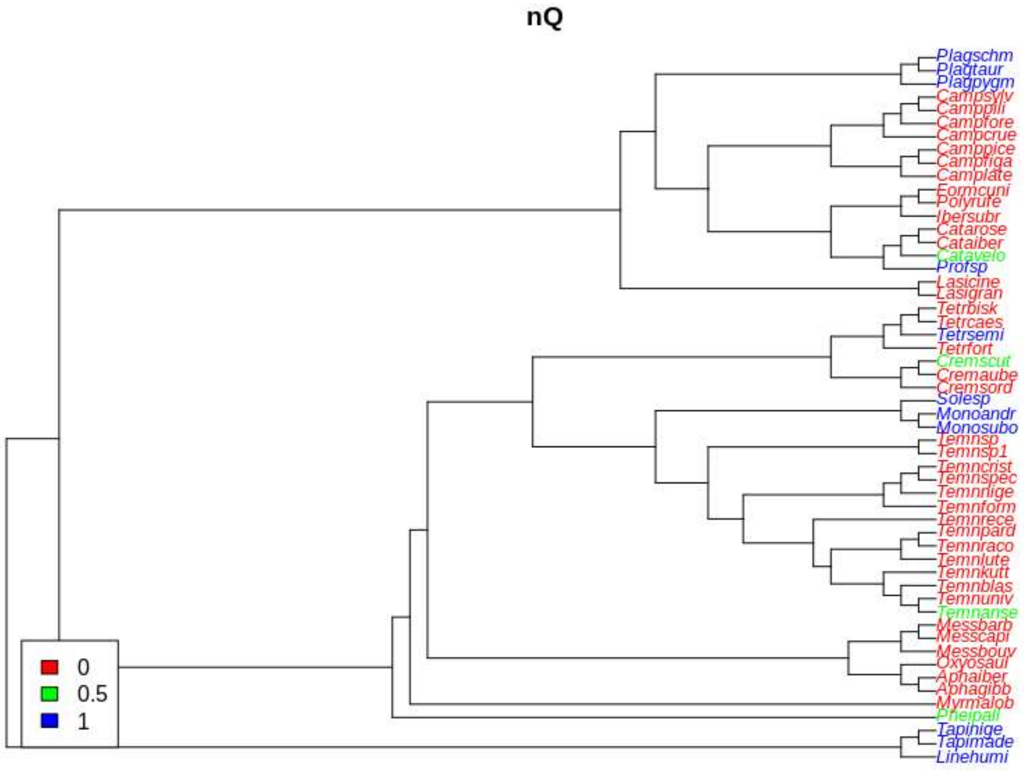


Figure S9. Number of queens values along phylogeny. 0=Monongyny; 0.5=Both monogyny and polygyny; 1=Polygyny

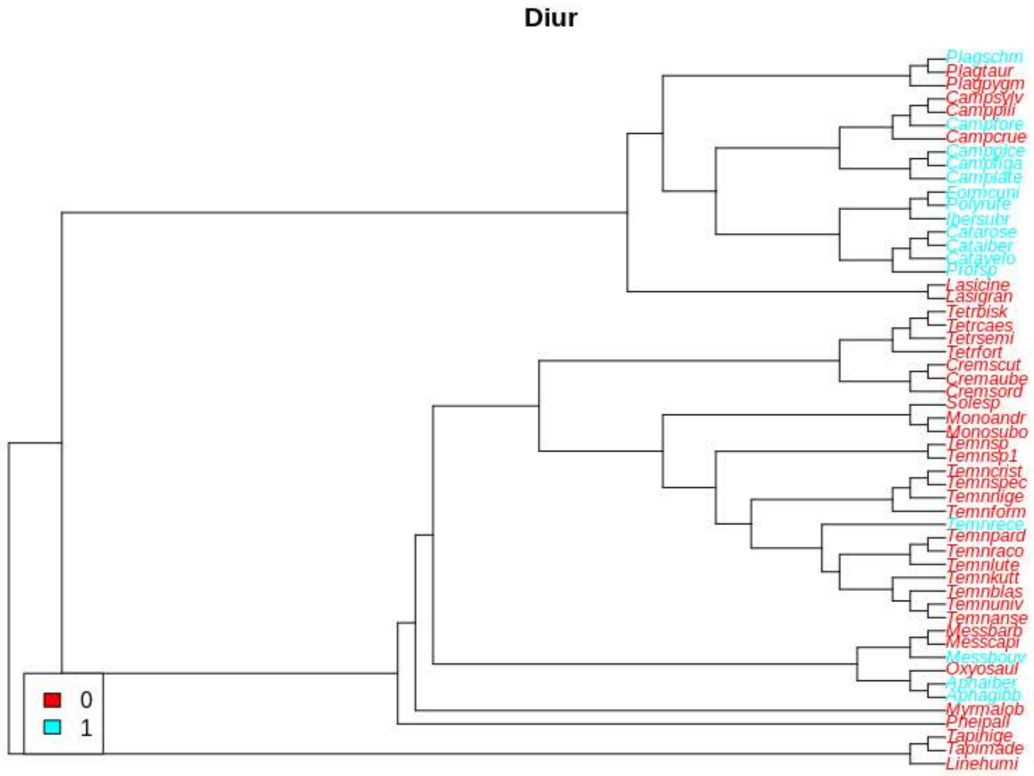


Figure S10. Diurnality values along phylogeny. 0=Nonstrictly diurnal; 1=Strictly diurnal.

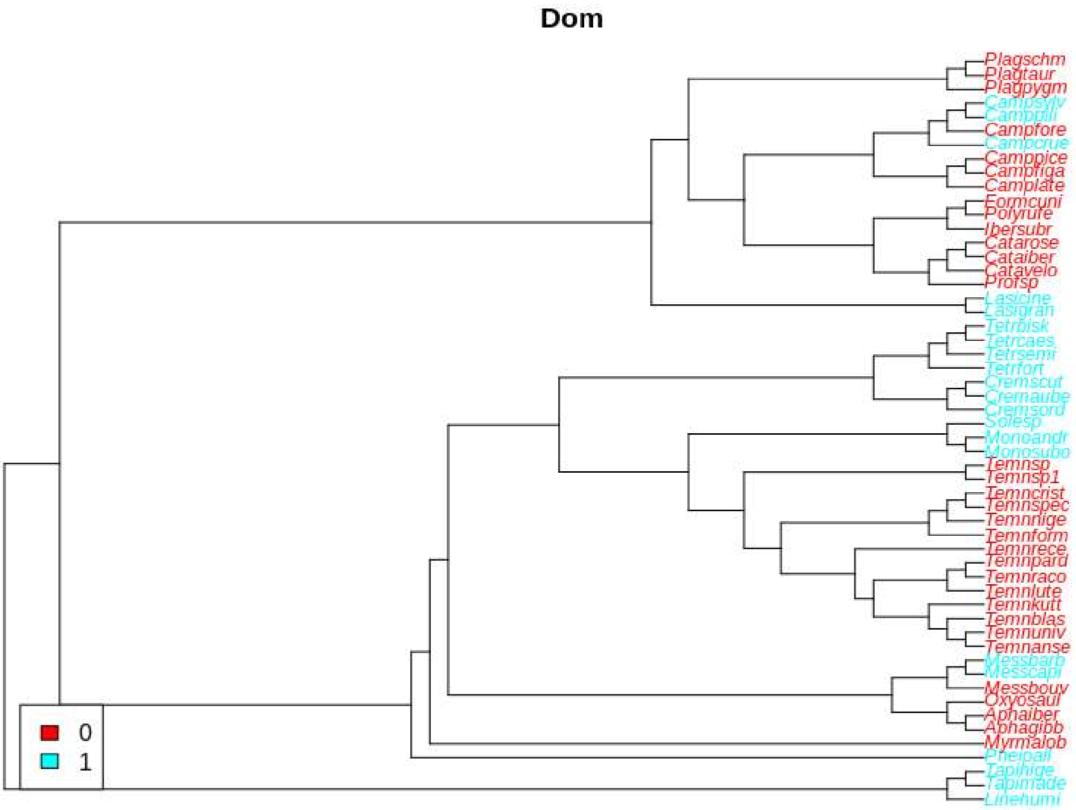


Figure S11. Behaviour of dominance values along phylogeny. 0=Subordinate; 1=Dominant

Table S1. Origin of the information to complete the species-trait database.In this table appears the list of the ant species cited in our study and the references from which the information has been obtained. When the information is scarce or incomplete, we cite the similar species that has been used to complete the information, as well as the references that have been used. In observations, we comment some aspects related to how we completed de data of the different species.

Species	**References**	**Similar species**	**References for similar species**	**Observations**
Aphaenogaster gibbosa	Arnán et al 2014			
Aphaenogaster iberica	Arnán et al 2014			
Camponotus cruentatus	Arnán et al 2014			
Camponotus figaro	not available	*Camponotus piceus*	Arnán et al 2014	
Camponotus foreli	Arnán et al 2014			
Camponotus lateralis	Arnán et al 2014			
Camponotus piceus	Arnán et al 2014			
Camponotus pilicornis	Arnán et al 2014			
Camponotus sylvaticus	Arnán et al 2014			
Cataglyphis iberica	Arnán et al 2014			
Cataglyphis rosenhaueri	Arnán et al 2014			
Cataglyphis velox	Arnán et al 2014			
Crematogaster auberti	Arnán et al 2014; Lebas et al 2016			
Crematogaster scutellaris	Arnán et al 2014			
Crematogaster sordidula	Arnán et al 2014			
Formica cunicularia	Arnán et al 2014			
Iberoformica subrufa	Arnán et al 2014			
Lasius cinereus	Arnán et al 2014			
Lasius grandis	Arnán et al 2014			
Linepithema humile	Arnán et al 2014			
Messor barbarus	Arnán et al 2014			
Messor bouvieri	Arnán et al 2014			

Species	**References**	**Similar species**	**References for similar species**	**Observations**
Messor capitatus	Arnán et al 2014			
Monomorium andrei	Lebas et al 2016; Barech et al 2018	*Monomorium salomonis*	Arnán et al 2014	
Monomorium subopacum	Lebas et al 2016	*Monomorium salomonis*	Arnán et al 2014	
Myrmica aloba	Arnán et al 2014			
Oxyopomyrmex saulcyi	Arnán et al 2014			
Pheidole pallidula	Arnán et al 2014			
Plagiolepis pygmaea	Arnán et al 2014; Lebas et al 2016			
Plagiolepis schmitzii	Arnán et al 2014; Lebas et al 2016			
Plagiolepis taurica	Lebas et al 2016	*Plagiolepis schmitzii*	Arnán et al 2014	
Polyergus rufescens	Trager 2013; Lebas et al 2016.	*Formica rufibarbis, Formica cunicularia*	Arnán et al 2014	Feeding data as their hosts species.We also used own data
Proformica sp	Lebas et al 2016; Tinaut 2018 pers. Comm.	*Proformica ferreri, Proformica nasuta*	Arnán et al 2014; Lebas et al 2016	
Solenopsis sp	Monin et al 2014; Espadaler 2018, pers. comm.	*Solenopsis fugax*	Lebas et al 2016	
Tapinoma madeirense	*Emery 1925*	*Tapinoma erraticum*	*Arnán et al 2014*	
Tapinoma cf *nigerrimum*	Arnán et al 2014; Lebas et al 2016			
Temnothorax ansei	Catarineu et al 2017			We also used own data
Temnothorax blascoi	Espadaler 1996.	other *Temnothorax* species	Arnán et al 2014	We also used own data
Temnothorax cristinae	Arcos et al 2013	*Temnothorax specularis*	Arnán et al 2014	
Temnothorax formosus	Santschi 1909; Lebas et al. 2016			
Temnothorax kutteri	Cagniant 1973; Lebas et al. 2016			We also used own data
Temnothorax universitatis	Espadaler 1997			We also used own data

Species	**References**	**Similar species**	**References for similar species**	**Observations**
Temnothorax luteus	Seifert et al 2014			
Temnothorax niger	Arnán et al 2016			
Temnothorax pardoi	Arnán et al 2016			
Temnothorax racovitzai	Arnán et al 2014			
Temnothorax recedens	Arnán et al 2014			
Temnothorax specularis	Arnán et al 2014			
Temnothorax sp	not available	other *Temnothorax* species	Arnán et al 2014	We completed the data with the most common trait values of the species of the genus.
Temnothorax sp1	not available	other *Temnothorax* species	Arnán et al 2014	We completed the data with the most common trait values of the species of the genus.
Tetramorium biskrense	Lebas et al. 2016	other *Tetramorium* species	Arnán et al 2014	We completed the data with the most common trait values of the species of the genus.
Tetramorium cf *caespitum*	Arnán et al 2014			
Tetramorium forte	Arnán et al 2016			
Tetramorium semilaeve	Arnán et al 2014			

Table S2. Ant trait matrix. WS=Worker size (worker body size from the tip of mandibles to tip of the gaster in mm); WP= worker polymorphism (range of worker size divided by the mean worker size) ; CS= colony size (mean number of workers per colony, ln transformed); pS=proportion of seeds in diet (0-1 proportion); pI=proportion of insects in diet (0-1 proportion); pLF= proportion of liquids foods in diet (0-1 proportion). CFT= colony foundation type (0: DCF; 0.5: Both DCF and ICF; 1: ICF); nQ=number of queens (0: Monongyny; 0.5: Both monogyny and polygyny; 1: Polygyny); nN=Number of nests (0: Monodomy; 0.5; Both monodomy and polydomy; 1: Polydomy); Diur= diurnality (0: Nonstrictly diurnal; 1: Strictly diurnal); Dom= behaviour of dominance (0: Subordinate; 1: Dominant); FSI= Individual foraging strategy (0: No; 1: Yes); FSC= Collective foraging strategy (0: No; 1: Yes); FSG= group foraging strategy (0: No; 1: Yes).

Species	**WS**	**WP**	**CS**	**pS**	**pI**	**pLF**	**CFT**	**nQ**	**nN**	**Diur**	**Dom**	**FSI**	**FSC**	**FSG**
Aphaenogaster gibbosa	4.90	0.41	6.48	0.50	0.5	0	1	0	0	1	0	0	0	1
Aphaenogaster iberica	6.40	0.19	6.25	0.50	0.5	0	0	0	0	1	0	0	0	1
Camponotus cruentatus	10.0	0.80	8.52	0.00	0.25	0.75	1	0	0	0	1	0	0	1
Camponotus figaro	3.90	0.44	6.21	0.00	0	1	1	0	0	1	0	0	0	1
Camponotus foreli	6.10	0.69	5.99	0.00	0	1	1	0	1	1	0	0	0	1
Camponotus lateralis	5.00	0.40	6.91	0.00	0	1	1	0	0	1	0	0	0	1
Camponotus piceus	3.90	0.44	6.21	0.00	0	1	1	0	0	1	0	0	0	1
Camponotus pilicornis	8.50	0.82	6.91	0.00	0	1	1	0	0	0	1	0	0	1
Camponotus sylvaticus	8.00	0.75	6.91	0.00	0	1	1	0	0	0	1	0	0	1
Cataglyphis iberica	6.00	0.50	6.48	0.00	1	0	1	0	1	1	0	1	0	0
Cataglyphis rosenhaueri	6.00	0.50	6.40	0.00	1	0	1	0	1	1	0	1	0	0
Cataglyphis velox	8.30	0.90	6.62	0.00	1	0	0	0.5	0.5	1	0	1	0	0
Crematogaster auberti	3.40	0.35	6.48	0.00	0	1	1	0	0	0	1	0	0	1
Crematogaster scutellaris	4.05	0.57	6.62	0.00	0.5	0.5	1	0.5	0.5	0	1	0	1	0
Crematogaster sordidula	2.45	0.37	6.62	0.00	0	1	1	0	0	0	1	0	0	1
Formica cunicularia	5.30	0.47	7.24	0.00	0.5	0.5	1	0	0.5	1	0	0	0	1
Iberoformica subrufa	4.90	0.29	6.55	0.00	0.75	0.25	1	0	0	1	0	0	0	1
Lasius cinereus	3.30	0.45	9.21	0.00	0	1	1	0	0	0	1	0	1	0
Lasius grandis	3.50	0.57	9.21	0.00	0.25	0.75	1	0	0	0	1	0	1	0
Linepithema humile	2.30	0.22	11.92	0.00	0.25	0.75	0	1	1	0	1	0	1	0
Messor barbarus	7.90	1.04	8.99	1.00	0	0	1	0	0	0	1	0	1	0

Species	**WS**	**WP**	**CS**	**pS**	**pI**	**pLF**	**CFT**	**nQ**	**nN**	**Diur**	**Dom**	**FSI**	**FSC**	**FSG**
Messor bouvieri	6.30	0.71	8.16	1.00	0	0	1	0	0	1	0	0	1	0
Messor capitatus	8.40	1.07	8.29	1.00	0	0	1	0	0	0	1	0	0	1
Monomorium andrei	1.75	0.17	6.21	0.00	0.5	0.5	0	1		0	1	0	1	0
Monomorium subopacum	3.10	0.40	8.01	0.00	0.5	0.5	0	1	1	0	1	0	1	0
Myrmica aloba	4.50	0.22	7.60	0.00	0.5	0.5	0.5	0	0	0	0	0	1	0
Oxyopomyrmex saulcyi	2.00	0.20	4.61	1.00	0	0	1	0	0	0	0	1	0	0
Pheidole pallidula	3.20	1.03	8.52	0.25	0.75	0	0.5	0.5	0	0	1	0	1	0
Plagiolepis pygmaea	1.60	0.50	6.68	0.00	0.25	0.75	0	1	0.5	0	0	0	0	1
Plagiolepis schmitzii	2.10	0.48	6.68	0.00	0.25	0.75	0	1	0.5	1	0	0	0	1
Plagiolepis taurica	2.10	0.48	6.68	0.00	0.25	0.75	0	1	0.5	0	0	0	0	1
Polyergus rufescens	6.00	0.38	6.21	0.00	0.5	0.5	1	0	0	1		0	0	0
Proformica sp	4.75	0.86	6.48	0.00	1	0		1	0	1	0	1	0	0
Solenopsis sp	1.85	0.34	8.52	0.00	0.5	0.5	1	1	0	0	1	0	1	0
Tapinoma madeirense	2.50	0.40	8.16	0.00	0	1	0	1	1	0	1	0	1	0
Tapinoma nigerrimum	4.00	0.58	8.85	0.00	0	1	0	1	1	0	1	0	1	0
Temnothorax ansei	2.70	0.15	4.38	0.00	0.5	0.5	1	0.5	0	0	0	0	0	1
Temnothorax blascoi	2.25	0.10	4.38	0.00	0.5	0.5	1	0	0	0	0	0	0	1
Temnothorax cristinae	2.60	0.30	5.01	0.00	0.5	0.5	0	0	0	0	0	0	0	1
Temnothorax formosus	3.25	0.30	5.01	0.00	0.5	0.5	1	0	0	0	0	0	0	1
Temnothorax kutteri	2.88	0.33	3.00	0.00	0.5	0.5	1	0	0	0		0	0	0
Temnothorax universitatis	2.85	0.10	5.01	0.00	0.5	0.5	0	0	0	0	0	0	0	1
Temnothorax luteus	2.55	0.12	5.01	0.00	0.5	0.5	1	0	0	0	0	0	0	1
Temnothorax niger	2.65	0.34	5.01	0.00	0.5	0.5	1	0	0	0	0	0	0	1
Temnothorax pardoi	2.50	0.08	5.01	0.00	0.5	0.5	1	0	0	0	0	0	0	1
Temnothorax racovitzai	2.55	0.12	5.01	0.00	0.5	0.5	1	0	0	0	0	0	0	1
Temnothorax recedens	2.70	0.30	5.01	0.00	0.5	0.5	0	0	0	1	0	0	0	1
Temnothorax specularis	2.35	0.13	5.01	0.00	0.5	0.5	1	0	0	0	0	0	0	1
Temnothorax sp	2.60	0.15	5.01	0.00	0.5	0.5	1	0	0	0	0	0	0	1
Temnothorax sp1	2.60	0.10	5.01	0.00	0.5	0.5	1	0	0	0	0	0	0	1
Tetramorium biskrense	2.40	0.41	9.21	0.25	0.75	0.25	1	0	0	0	1	0	1	0

Species	**WS**	**WP**	**CS**	**pS**	**pI**	**pLF**	**CFT**	**nQ**	**nN**	**Diur**	**Dom**	**FSI**	**FSC**	**FSG**
Tetramorium cf. caespitum	2.90	0.41	9.21	0.25	0.75	0	1	0	0	0	1	0	1	0
Tetramorium forte	3.75	0.51	9.21	0.25	0.5	0.25	1	0	0	0	1	0	1	0
Tetramorium semilaeve	3.60	0.33	9.21	0.25	0.75	0	1	1	0	0	1	0	1	0

Table S3. Climatic data. Prec = Average annual rainfall; SD Prec = Prec. Standard deviation; Mean Temp= Average annual temperature; MaxTempM =Average maximum daily temperature in the hottest month; MinTempM= Average minimum daily temperature in the coldest month.

Meteorological station	**Public institution**	**UTM X**	**UTM Y**	**DATUM**	**Elevation**	**Prec**	**SD Prec**	**Mean Temp**	**Max TempM**	**Min TempM**
7092 Lietor	AEMET	590.903	4.266.658	ETRS89	641	382.44	134.21	16.44	34.67	2.16
7138D Jumilla La Estacada	AEMET	645.174	4.258.484	ETRS89	445	296.57	68.43	16.23	35.27	1.38
7275 Yecla CH Segura	AEMET	665.189	4.276.789	ETRS89	605	301.65	88.76	15.06	33.73	0.66
7244 Desamparados	AEMET	677.082	4.215.329	ETRS89	26	287.67	102.78	18.31	33.49	4.64
7059 Arguellite	AEMET	549.575	4.243.331	ETRS89	980	746.12	216.55	14.4	33.34	0.79
7016 El Algar	AEMET	688.213	4.168.946	ETRS89	40	356.53	147.62	18.38	30.93	7.14
7205 Emb. de Puentes	AEMET	604.050	4.177.324	ETRS89	450	295.29	102.97	16.96	34.24	2.77
7113 Campo de S. Juan	AEMET	578.954	4.225.840	ETRS89	1150	498.53	151.98	12.48	30.75	-0.98
7168 Emb. De la Cierva	AEMET	632.644	4.213.744	ETRS89	395	334.65	123.09	17.72	32.95	5.72
7002 Águilas Diputación	AEMET	625.377	4.141.490	ETRS89	20	203.13	92.89	19.92	33.19	7.62

Table S4. Correlations between the diversity indices at the transect scale.

	PD	SR	MPD	MNTD	PDI	NRI	NTI	FDiv
SR	0.83							
MPD	0.53	0.19						
MNTD	-0.20	-0.61	0.37					
PDI	0.51	-0.05	0.70	0.65				
NRI	-0.52	-0.09	-0.89	-0.36	-0.81			
NTI	-0.48	0.02	-0.50	-0.71	-0.91	0.56		
FDiv	0.52	0.47	0.32	-0.12	0.22	-0.23	-0.23	
FDis	0.55	0.38	0.59	0.02	0.37	-0.49	-0.29	0.47

Table S5. Correlations between the diversity indices at the locality scale

	PD	SR	MPD	MNTD	PDI	NRI	NTI	rPDI	rNRI	rNTI	FDiv
SR	0.94										
MPD	0.75	0.66									
MNTD	0.00	-0.28	0.05								
PDI	0.87	0.66	0.66	0.45							
NRI	-0.75	-0.66	-0.99	-0.10	-0.67						
NTI	-0.94	-0.84	-0.67	-0.28	-0.92	0.69					
rPDI	0.70	0.42	0.67	0.52	0.90	-0.67	-0.70				
rNRI	-0.75	-0.65	-1.00	-0.06	-0.66	1.00	0.67	-0.67			
rNTI	-0.76	-0.50	-0.60	-0.61	-0.96	0.61	0.84	-0.95	0.60		
FDiv	-0.08	-0.29	0.14	0.50	0.18	-0.17	0.02	0.38	-0.15	-0.33	
FDis	0.08	-0.12	0.42	0.20	0.23	-0.40	0.03	0.53	-0.42	-0.33	0.81

Chapter 3

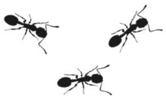
Effect of pine reforestation associated with soil disturbance on ant assemblages (Hymenoptera: Formicidae) in a semiarid steppe

Previous page: Sierra del Picarcho grasslands (photo credit: Chema Catarineu)

Effect of pine reforestation associated with soil disturbance on ant assemblages (Hymenoptera: Formicidae) in a semiarid steppe

Abstract

Soil and changes in vegetation may affect ant assemblages, but the relative importance of each in different habitats is not well characterized. In particular, information on the effects of ecological restoration on arthropods is scarce. It was decided, therefore, to study how reforestation may affect an ant assemblage. Ants were sampled in area that had been reforested and adjacent grassland using pitfall traps. Soil surface and vegetation were characterized. The disturbance of the vegetation caused by reforestation resulted in a decrease in the cover of *Stipa tenacissima* and Cistaceae and an increase in the cover of pine. The mechanical preparation of the site also resulted in changes in the soil surface, with an increase in the cover of stones and rocks. Ant species richness and abundance were greater at the reforested site than in the grassland and more species showed a positive than a negative response to reforestation. The underlying causes of this pattern are mainly related to changes in vegetation and structure of the soil surface and are associated with the increase in the cover of pine, which most probably provided additional food resources, and the greater cover of stones and rocks that provided more shelter for the ant assemblage.



Introduction

Grasslands dominated by esparto grass *Stipa tenacissima* L. with a sparse cover of vegetation, are one of the commonest ecosystems in semiarid regions in the Western Mediterranean basin (Barberá et al., 2006; Maestre et al., 2007; Cortina et al., 2009). *S. tenacissima* is a perennial tussock grass in which the live biomass is usually surrounded by thick layers of standing dead grass (Maestre et al., 2007). The physiognomy of *S. tenacissima* grasslands is more similar to shrub land than to mesic grassland. The effect of grazing on *S. tenacissima* is low because this grass has a low palatability, but these grasslands have been intensively managed for producing fibre (esparto) used for making baskets, shoes, mats, etc, from prehistoric times until the 1950–1960s. Although this practice has now been mostly abandoned, the abandonment has not resulted in any major changes in the habitat (Barberá et al., 2006; Maestre et al., 2007). Many authors consider esparto grass steppes to be degraded former oak or pine open forests or shrub land dominated by, e.g., *Pistacia lentiscus* L. (Costa, 1973; Valdés & Herranz, 1989; Maestre et al., 2007).

During the twentieth century, land managers in Spain promoted the planting of *Pinus halepensis* Mill., supposedly to boost succession. From the 1960s to 1990s most of the reforestation-afforestation in Spain was done using Mechanical Site Preparation (MSP), a broad category of site preparation typically involving the use of large heavy machinery with attached implements for preparing an area and its soil for planting trees (Löf et al., 2012). MSP causes a range of adverse effects, which are reported by different authors (Ortigosa, 1991; Chaparro & Esteve-Selma, 1995; Ternan et al., 1996). Löf et al. (2012) state that MSP can lead to different effects on soil characteristics, such as loss of carbon, changes in the decomposition of organic matter, disturbance of nutrient pools or increase in water loss, and also an increased risk of soil erosion. MSP may increase plant biodiversity, at least temporally (Haeussler et al., 1999; Löf et al., 2012), but there are few studies on the effects of MSP on arthropod communities (Bellocq et al., 2001; Bird et al., 2004).

As in other arid and semi-arid areas on the Iberian Peninsula, esparto grass steppes are inhabited by a great diversity of invertebrates. Two insect groups dominate: Coleoptera (mainly Tenebrionidae) and, more specifically, Formicidae (Doblas-Miranda et al., 2007; Piñero et al., 2011). Ants are dominant organisms in terrestrial ecosystems, have very important functions, make up a great part of the animal biomass and are ecosystem engineers (Hölldobler & Wilson, 1990; Folgarait, 1998; Read & Andersen, 2000; Frouz & Jilková, 2008; Del Toro et al., 2012).

Vegetation and soil may influence ant assemblages. Shrub encroachment of grasslands results in an increase in ant diversity and/or abundance, both in mesic and semiarid grasslands (Nash et al., 2000; Bestelmeyer, 2005; Azcárate & Peco, 2012; Wiezik et al., 2013). However, the reforestation of semiarid grasslands by pines may reduce the abundance and diversity of ants, although this effect is much lower when tree cover is sparse (Corley et al., 2006). Soil characteristics may be especially relevant to understanding ant assemblages in semiarid landscapes (Bestelmeyer & Wiens, 2001). Nevertheless, there are very few studies and little current literature that deals with the direct and indirect effects of soil properties on ant assemblages. In the current study, MSP simultaneously disturbed both the soil and vegetation, which provided the opportunity to study the effects of both factors on ant assemblages.

We studied the effects of a 16-year old reforestation with *P. halepensis* of a semiarid *S. tenacissima* grassland on the local ant assemblage, looking specifically at the effects of changes in the soil and vegetation. The objectives of this study were: i) to determine how reforestation following MSP affects the structure of the surface of the soil and vegetation; ii) to determine how reforestation affects an epigeal ant assemblage and species, particularly their diversity and abundance; iii) to determine which changes at the surface of the soil and in the vegetation are likely to cause changes in the ant assemblage.

Material and methods

Study Site

This study was carried out in the Sierra del Picarcho (Region of Murcia, south-eastern Spain, 394 m a.s.l, 38º20'N, 1º29'W; Figure 1). Soils are mostly Petric Calcisols (FAO-ISRIC-ISSS, 1998), characterized by an A-Ckm1-Ckm2 profile, with a thick petrocalcic horizon (hardpan) at a depth of 40–70 cm. The topography of the area is mostly flat, with slopes not exceeding 6% (Barberá et al. 2006). The climate is semiarid Mediterranean. At the nearby meteorological station of La Carrichosa (6.22 km S, 254 m a.s.l., data 1995–2012) the following annual mean values were recorded: temperature 17.2ºC, relative humidity 64%, rainfall 253 mm and evapotranspiration 1166 mm (SIAM-IMIDA, 2017).

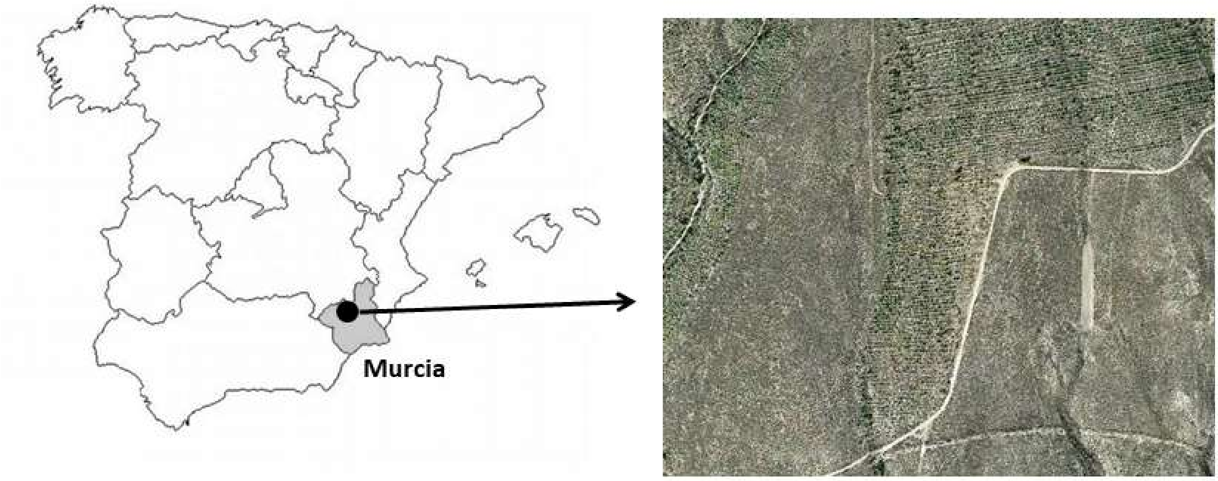


Figure 1. Map showing the location of the study area.

The vegetation in the study area is steppe grassland (Figure 2), dominated by the esparto grass (*Stipa tenacissima*). There was a partial reforestation with *Pinus halepensis* in the 1960s and 1970s, although the trees developed poorly, probably due to rooting difficulties associated with the petrocalcic horizon. In July 1994, the study area suffered a catastrophic wildfire. Our study site is located close (1–2 km) to the eastern limit of the fire. At the end of 1996, the area was patchily reforested again using *P. halepensis*. The planting of pines was preceded by mechanical preparation of the soil by subsoiling to 1–1.5 m along the plantation rows, causing a significant disturbance both to the soil surface and profile. One-year old tree seedlings were planted in rows and spaced 2.5–3 m apart. The 19-ha plot studied was an experimental plot, which was subjected to a large number of different mechanical subsoiling techniques, which differed mainly in the way organic matter was added to each line of trees (for details see Barberá et al., 2005). Pine growth was slow and mortality high. After 16 years, the trees were rarely more than 3 m high and their cover sparse (Figure 3). Mechanical preparation of the terrain combined with the presence of a shallow petrocalcic horizon resulted in large slabs of rock and stones being brought to the surface, creating a particular microtopography. Original soil surface was smooth with a varying density of small stones on the surface, while the subsoiled area is anfractuous at the microscale with a lot of large stones and rocks at the surface, which resulted from the breaking up of the hardpan during subsoiling.

Figure 2. Steppe grassland at the study site.

Figure 3. Reforested area at the study site.

Sampling

Three 200-m transects were established in May 2012 (Figure 4). Each transect consisted of 20 pitfall traps set 10 metres apart. Ten traps were placed in the reforested area, and 10 in the grassland. Because of the patchy structure of the reforested area, the distance of a pitfall from the edge of the grassland/reforested area may be less than the distance to the middle point of the transect (Figure 4).

The ants were sampled using pitfall traps. Polystyrene tubes (2 cm diameter, 10.5 cm long) were installed two weeks before the traps were set in order to avoid the effects of digging-in (Greenslade, 1973), with the lip of the tube flush with the soil surface. Tubes were filled with 5 ml of 50:50 propylene glycol and water solution with a few drops of liquid soap added to reduce surface tension (Bestelmeyer et al., 2000; Calixto et al., 2007). Pitfall traps were set for one week each season, in May, August, November 2012 and February 2013.

All ants were identified and counted using a stereo microscope. Identified ants were transferred to vials containing 70% ethanol, or were mounted. All specimens are deposited at the premises of Asociación de Naturalistas del Sureste (ANSE; Murcia, Spain).

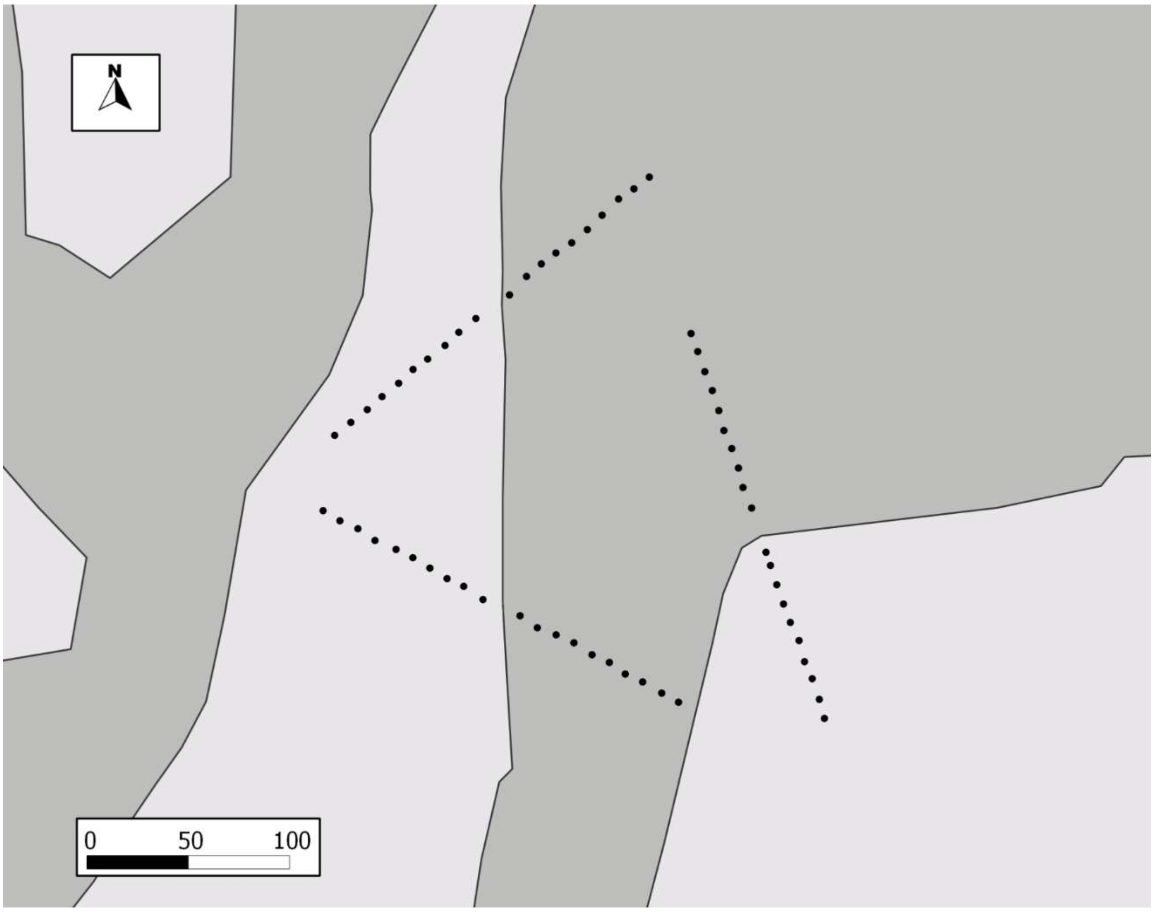


Figure 4. Transects. Light gray: grassland; dark gray: reforested area

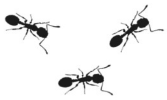
To characterize the vegetation and soil surface one transect was established alongside each transect with the pitfall traps in March 2013. The purpose of these transects was to record the general characteristics of the composition of the vegetation and structure of the soil surface. Each transect was 8-m long, centred on each pitfall point and aligned with the original pitfall transect. At 17 points, at 50-cm intervals, type of soil surface (cover of soil -no stones or rocks on soil surface-; superficial stones -hereafter stones-; or in-ground rocks -hereafter rocks-), species of plants and their height were recorded. Only perennial species were included in the analysis. Plants were categorized into functional groups (Table 1).

Table 1. Environmental variables and error function used for modelling response of the environmental and the ant assemblage variables to reforestation in the framework of Generalized Linear Mixed Models (see Material and methods). Tree and perennial grass functional groups were not tested as each one is represented by only one species (*Pinus halepensis* and *Stipa tenacissima*, respectively). Note that error functions for total vegetation and functional groups were tested using a Poisson error as species in the same functional group can be overlaid on the same sampling point.

ENVIRONMENTAL VARIABLE	Functional group	Error function
Soil surface		
Smooth soil surface cover		Binomial
Superficial stones cover		Binomial
Inground rock cover		Binomial
Vegetation		
Plant species richness		Poisson
Number of plant strata (NPS)		Poisson
NPS on points with vegetation cover		Poisson
Total vegetation cover		Poisson
Shrub cover		Poisson
Chamaephyte cover		Poisson
Pinus halepensis cover	Tree	Binomial
Stipa tenacissima cover	Perennialgrass	Binomial
Rosmarinus officinalis cover	Shrub	Binomial
Anthyllis terniflora cover	Shrub	Binomial
Cistus clusii cover	Shrub	Binomial
Fumana ericoides cover	Chamaephyte	Binomial
Fumana thymifolia cover	Chamaephyte	Binomial
Helianthemum violaceum cover	Chamaephyte	Binomial
Olea europea cover	Shrub	Binomial
Sideritis bourgeana cover	Chamaephyte	Binomial

Data analysis

In order to determine the effects of reforestation on the ant assemblage and infer the possible causes, we carried out two analyses. The first was for the whole year at the level of the assemblage. Numbers of each species caught in each pitfall on the four sampling sessions were summed and log-transformed. This matrix was submitted to non-metric multidimensional scaling (NMDS) followed by an Adonis test (McArdle and Anderson, 2001) to check if the between-group (grassland vs. reforestation) and within-group similarities differed significantly. Adonis is a permutation test that partitions the sum of squares for distance matrices in a way analogous to MANOVA. The number of permutations was set to 999. Relation between NMDS axes and environmental variables was tested using Spearman's rank correlation. These analyses were carried out using the



vegan package (Oksanen et al., 2018) in R (R Core Team, 2018). The second Analysis aimed to test the effects on the abundance of individual species as well as the abundance of the assemblage (all species summed) and species richness. It was hierarchically carried out in two steps: (i) testing if reforestation using MSP is associated with changes in soil and vegetation variables as well as in ant species richness and ant total abundance for each species; (ii) testing if ant species richness, total abundance and species specific variation were associated with the variation in the environmental variables that were significantly different in the two habitats, as tested in (i).

We hypothesized that these changes may be expressed in terms of three possible models (Figure S1): (i) a shift in the levels of the different variables of interest in each habitat (factor); (ii) a linear trend (positive or negative) in the variable from inner part of the reforested area to that of the grassland; (iii) a unimodal trend with a maximum or a minimum value of the variable of interest close to the ecotone. In relation to environmental variables, to test for the most complex case (unimodal response) a generalized linear mixed model was fitted to each variable with transect as a random factor and signed distance to the ecotone set to zero and distance to inner part of the grassland assigned a positive sign and that to the inner part of reforested area a negative sign and its squared value as fixed factors. If the effect of the quadratic signed distance was statistically significant (indicating a unimodal response) the model was retained, otherwise we tested the alternative (less complicated) linear trend model with the signed distance without the quadratic term. Again, if the signed distance parameter was significant the model was retained, otherwise we tested for the shift model, which includes the habitat as a qualitative factor. For each response variable, the generalized linear mixed model was fitted with an error function appropriate for the nature of data (binomial, Poisson, normal; details in Table 1). Models for ant species richness and total abundance were tested similarly but because ants were sampled seasonally the fixed part of the generalized linear mixed model includes habitat and season. Ant species richness was tested at two spatial scales: pitfall and habitat × transect (10 aggregated traps in a particular habitat along a particular transect). For the species' abundance models, as autumn and winter abundances per individual species were very low, a full model, including habitat and season, is usually over parametrized. Therefore, for each species, we tested a model that included the year-round aggregated abundance and four seasonal models. Abundance data were log-transformed prior to analyses.

Generalized linear mixed models were fitted using the GLIMMIX procedure in SAS 9.4. Details of the statistically significant models are presented in Table S1. In the main text only graphical expressions of the models are shown.

Next, we determined whether the abundance and richness of ant species was correlated with environmental variables that significantly varied between habitats, using Spearman's rank correlation with the Benjamini-Hochberg correction for multiple comparisons (Benjamini & Hochberg, 1995). This correction is less conservative than the

Bonferroni one and is based on controlling the false discovery rate (FDR). We set FDR in this study to 0.2, that is, we accept a maximum rate of false tests of 20%.

Results

Environmental changes induced by reforestation

Soil surface characteristics clearly vary between habitats (Figures 5–6; expected values of models are shown in Table S1). The cover of rocks was low and constant in the grassland and increased sharply towards the inner part of the reforested area. The cover of soil increased linearly from the centre of the reforested area to that of the grassland, while stone cover peaked close to the ecotone.

Changes in vegetation were not associated with differences in species richness or general physiognomy, but were with total vegetation, functional groups and, most importantly, species cover (Figure 7; predicted values of the models are presented in Table S1). Of course, pine cover was only recorded in the reforested area. The two subdominant shrubs showed opposite linear trends. In the inner part of the grassland the cover of *Cistus clusii* and *Rosmarinus officinalis* was similar, whereas that of *C. clusii* decreased towards the inner part of the reforested area while that of *R. officinalis* increased. As a result, the total shrub cover remains unchanged. *Stipa tenacissima* was clearly dominant in grassland but far less abundant in the reforested site, to the point that in the inner part its cover was similar to that of *R. officinalis*. Total chamaephyte cover had a similar trend, but only one species of chamaephyte differed significantly between habitats: *Fumana thymifolia*. The cover of this tiny species was very low except in the inner part of the grassland. Interestingly, total perennial plant cover was similar in the inner parts of the forest and grassland but showed a minimum in the ecotone close to the reforested area.

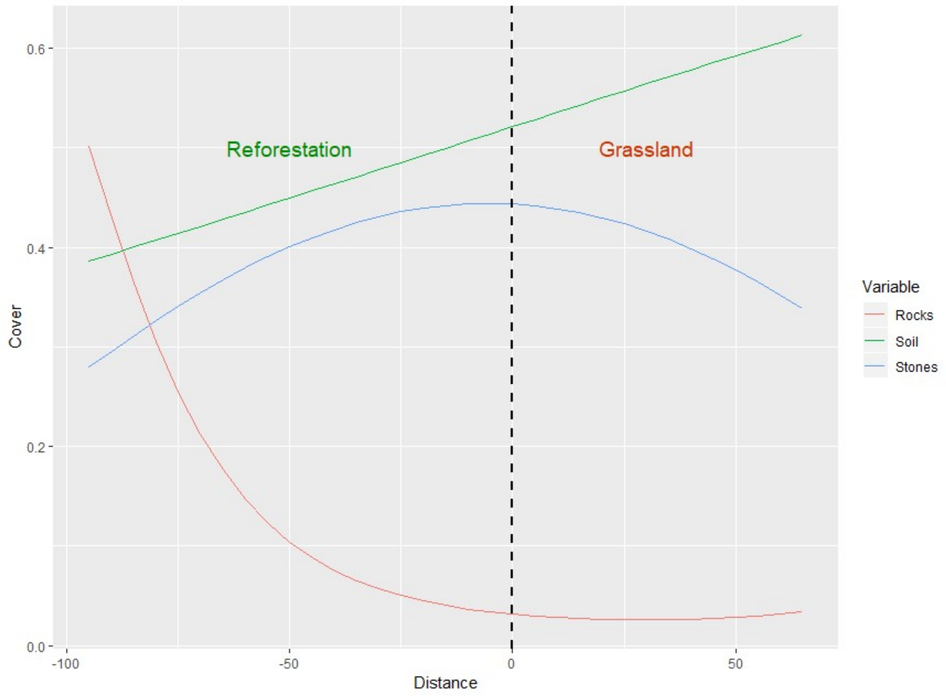


Figure 5. Soil surface cover. Values predicted by the models (Table S1).

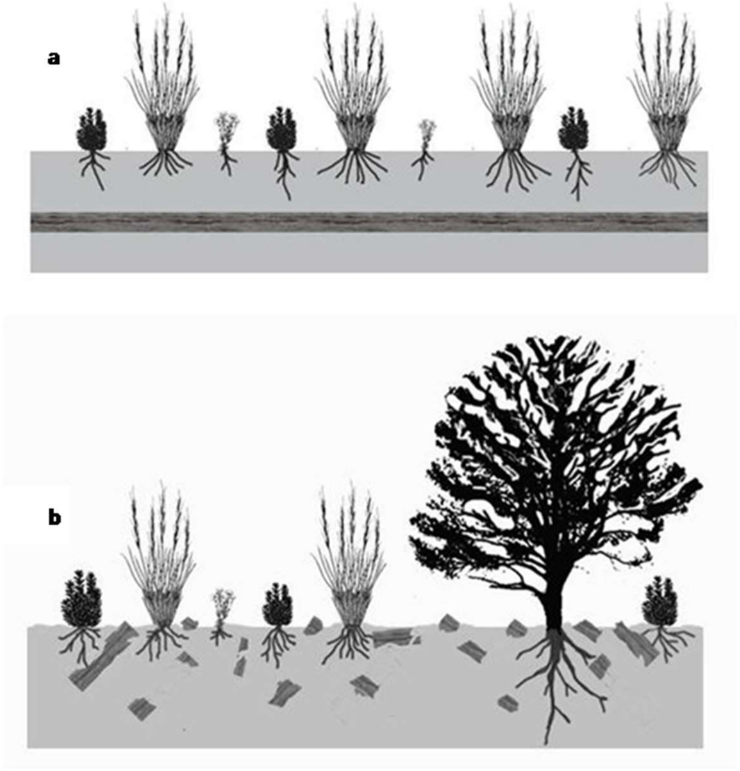


Figure 6. Soil profile in the grassland (a) and reforested area (b). Note that the petrocalcic horizon (Ckm) was lifted and fragmented by mechanical reforestation

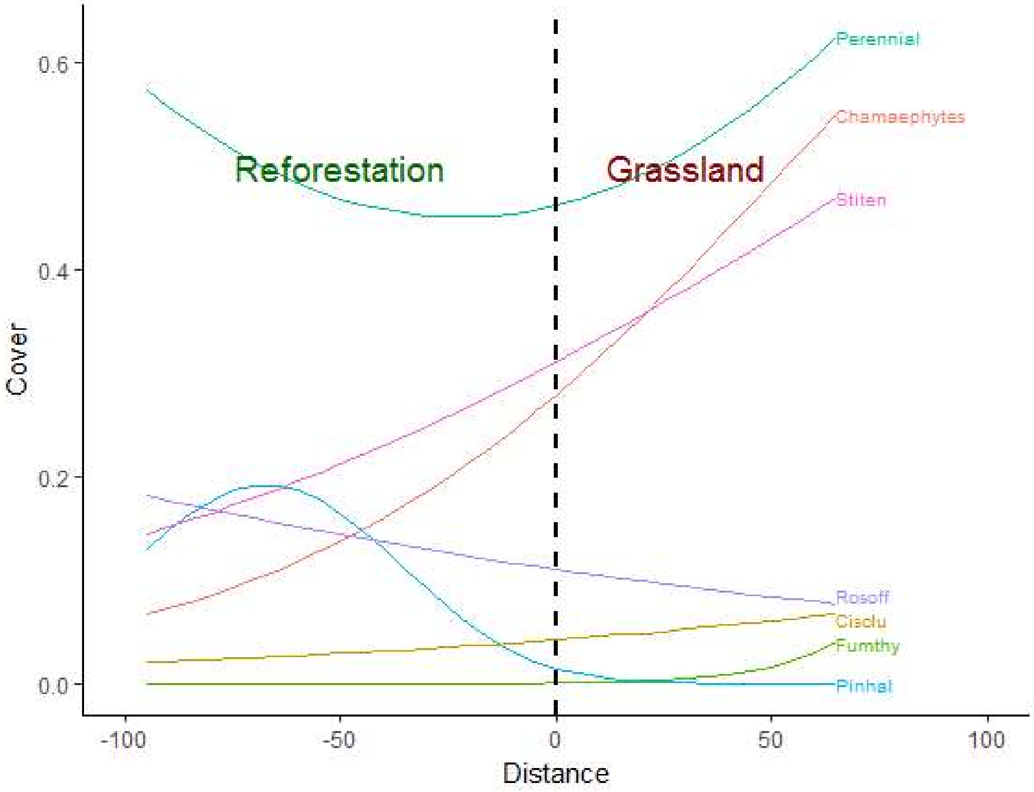


Figure 7. Components of the cover of vegetation. Values predicted by the models (Table S1). Cisclu, *Cistus clusii*; Fumthy, *Fumana thymifolia*; Pinhal, *Pinus halepensis*; Rosof, *Rosmarinus officinalis*; Stiten, *Stipa tenacissima.*

Effect of reforestation on ant assemblages

A total of 13 species of ants belonging to 11 genera were recorded in this study (Table 2). Ten species were found in both habitats and three only in the reforested habitat. As *Temnothorax racovitzai* was only caught twice it was not included in the analysis.

Species richness at the habitat × transect scale was significantly higher in the reforested area (Figure 8). The pattern did not differ between seasons. At the pitfall scale it is possible to detect a linear trend of decreasing richness from the inner part of the reforested area to that of the grassland (Figure 9; predicted values of models are presented in Table S1). Total ant abundance follows a very similar pattern (Figure S2; predicted values of models are presented in Table S1).

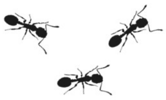


Table 2. Total number of each species of ant captured in the pitfall traps in grassland (G) and the reforested area (R).

Ant species	**Captures**	
	G	**R**
Aphaenogaster iberica Emery, 1908	1	15
Camponotus foreli Emery, 1881	4	18
Camponotus sylvaticus (Olivier, 1792)	0	12
Cataglyphis iberica (Emery, 1906)	75	111
Crematogaster auberti Emery, 1869	55	18
Goniomma blanci (André, 1881)	23	2
Messor bouvieri Bondroit, 1918	37	94
Pheidole pallidula (Nylander, 1849)	15	48
Plagiolepis schmitzii Forel, 1895	52	53
Tapinoma nigerrimum (Nylander, 1856)	178	234
Temnothorax racovitzai Bondroit, 1918	0	2
Temnothorax specularis Emery, 1916	5	5
Tetramorium semilaeve André, 1883	0	6
TOTAL	**445**	**618**

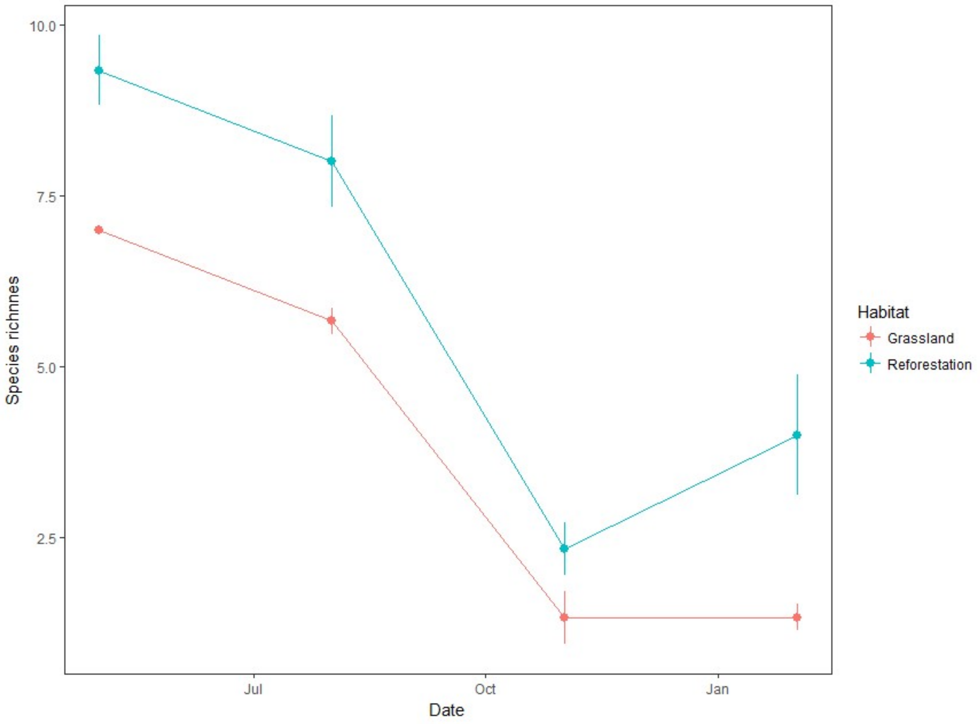


Figure 8. Ant species mean richness along the transect. Bars indicate standard error as calculated from model in Table S1.

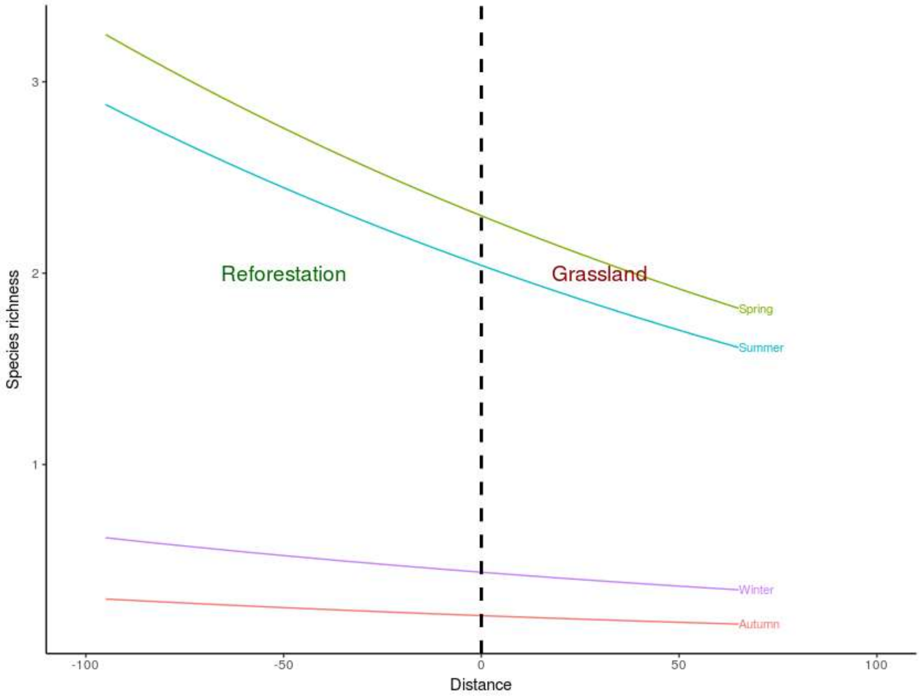


Figure 9. Ant species mean richness per pitfall trap. Values predicted by the models (Table S1).

Reforestation had significant effects on the year-round abundance of several species and in spring and summer, when ants are much more active, (Figures 10 and S3; predicted values of models are presented in Table S1). *Plagiolepis schmitzii* and *Gonioma blanci* were the only species that linearly increased in abundance from the inner part of the reforested area to that of the grassland. *Plagiolepis schmitzii* shows this trend year-round and in summer, whilst *G. blanci* shows this trend only in spring. More species decrease in abundance from the inner part of the reforested area to that of the grassland: *Aphaenogaster iberica* year-round and in both spring and summer, *Camponotus sylvaticus* year-round and in summer. For *Camponotus foreli* the trend also linearly decreased from the reforested area to the grassland year-round and in spring but in summer the response was unimodal with a maximum in the ecotone. This kind of unimodal response is also characteristic of *Tetramorium semilaeve* year-round and in summer.

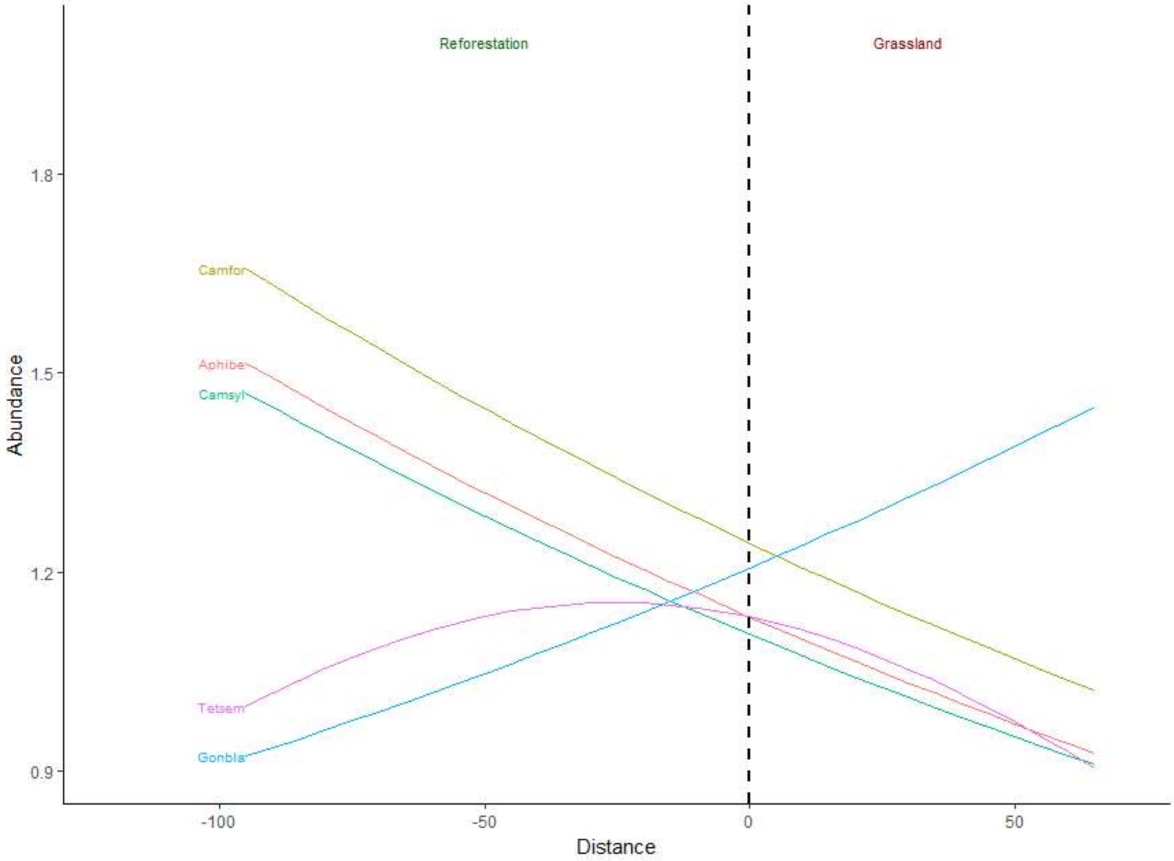


Figure 10. Year-round abundance of the different species of ants. Values predicted by the models (Table S1). Aphibe, *Aphaenogaster iberica*; Camfor, *Camponotus foreli*; Camsyl, *Camponotus sylvaticus*; Gonbla, *Goniomma blanci*; Tetsem, *Tetramorium semilaeve*.

Correlation between the differences in the environmental factors in the two habitat and in the ant assemblages

There were significant correlations between environmental variables and the ant assemblage variables that differed significantly in the two habitats (Figure 11, Table S2), as stated in the previous section. Ant species richness year-round and when most active in spring and summer was positively associated with the cover of *P. halepensis*, also species richness year-round and in summer was associated positively with the cover of stones. In autumn ant species richness was positively associated with the cover of rocks. Year-round ant species richness was negatively associated with *C. clusii* and a continuous cover of soil.

Total ant abundance year-round was positively associated with the cover of stones, as was total ant abundance in summer. As for ant species richness, seasonal total ant abundance in autumn was positively associated with the cover of rocks. In contrast, total ant abundance was negatively correlated year-round with a continuous cover of soil and in summer with the cover of *F. thymifolia*.

In respect to specific species, *G. blanci* year-round and spring abundances were positively associated with the cover of chamaephytes and with that of *F. thymifolia*. *Aphaenogaster iberica* year-round and summer abundances were positively associated with the cover of *P. halepensis*, like the abundance year-round and in spring of *C. foreli*. Also for *C. foreli* and the same seasons there was a negative association with the cover of *C. clusii*. *Camponotus sylvaticus*, however was positively associated with the cover of rocks in spring and negatively with a continuous cover of soil.

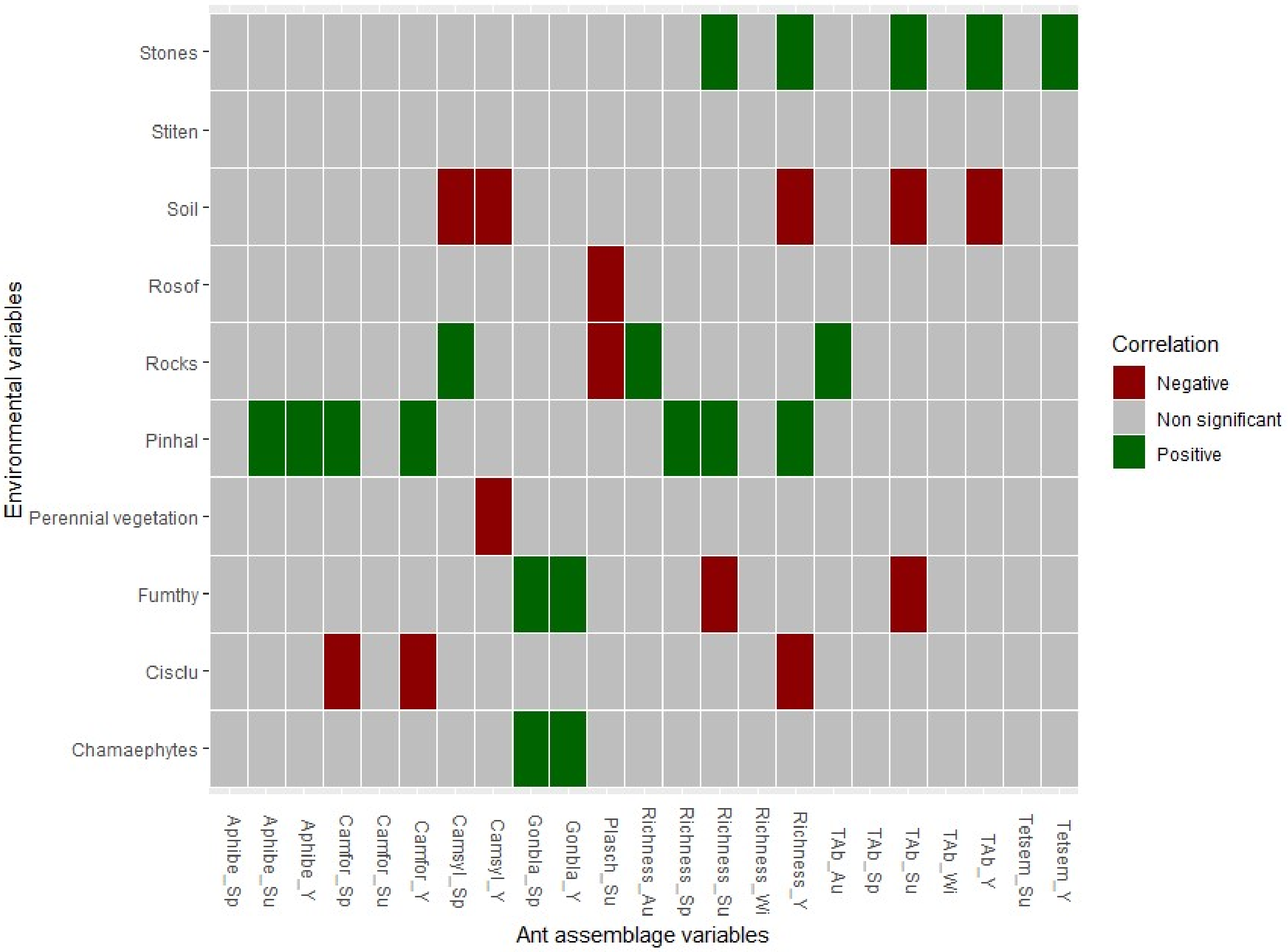


Figure 11. Significant correlations and their direction (positive/negative) between the environmental variables and those of the ant assemblage. Original Spearman's correlation coefficients and P values are in Table S2. Cisclu, *Cistus clusii*; Fumthy, *Fumana thymifolia*; Pinhal, *Pinus halepensis*; Rosof, *Rosmarinus officinalis*; Stiten, *Stipa tenacissima*; Tab, Total abundance; Aphibe, *Aphaenogaster iberica*; Camfor, *Camponotus foreli*; Camsyl, *Camponotus sylvaticus*; Gonbla, *Goniomma blanci*; Plasch, *Plagiolepis schmitzii*; Tetsem, *Tetramorium semilaeve*. Y, year-round; Sp, spring; Su, summer; Au, Autumn; Wi, winter.

NMDS axis 1 clearly separated *Crematogaster auberti* from the rest of the species, but not the pitfall trap catches by habitat (Figure 12). In contrast, axis 2 segregated the reforested area from grassland, although there was a considerable overlap between groups. Nevertheless, the Adonis test shows significant differences between pitfall trap catches in the reforested area and grassland (pseudo-F = 3.18, p = 0.013) but the partial R^2 of the habitat factor was low (5%). NMDS axis 1 was only significantly correlated with perennial plant richness (Table 3; positive correlation), while NMDS axis 2 was positively correlated with the cover of stones and *Pinus halepensis* and negatively with *Sideritis bourgeana*, chamaephytes and a continuous cover of soil.

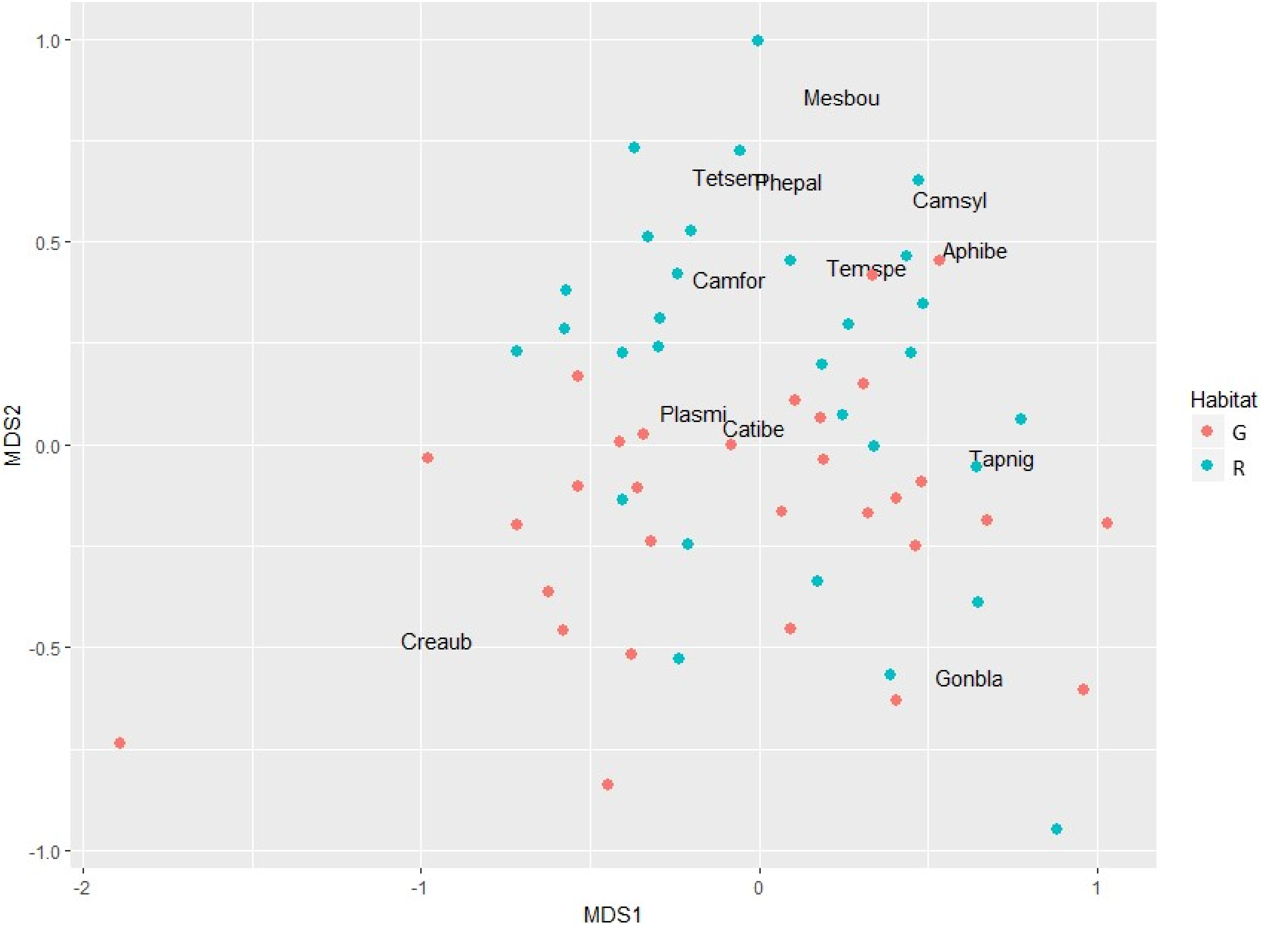


Figure 12. NMDS Representation of the NMDS analysis. Aphibe, *Aphaenogaster iberica*; Camfor, *Camponotus foreli*; Camsyl, *Camponotus sylvaticus*; Catibe, *Cataglyphis iberica*; Creaub, *Crematogaster auberti*; Gonbla, *Goniomma blanci*; Mesbou, *Messor bouvieri*; Phepal, *Pheidole pallidula*; Plasch, *Plagiolepis schmitzii*; Tapnig, *Tapinoma nigerrimum*; Temspe, *Temnothorax specularis*; Tetsem, *Tetramorium semilaeve*.

Table 3. NMDS Correlations between environmental variables and coordinates of samples (pitfall trap catches) in the NMDS space.

	NMDS Axis 1		NMDS Axis 2	
Variable	Spearman´s correlation	*p*	Spearman´s correlation	*p*
Perennial plant richness	0.336	0.009		
Chamaephyte cover			-0.308	0.010
Sideritis bourgeana cover			-0.290	0.025
Pinus halepensis cover			0.447	<0.001
Stone cover			0.462	<0.001
Cover of soil (no stones or rocks on soil surface)			-0.484	<0.001

Discussion

Environmental changes induced by reforestation

Vegetation and soil were disturbed by reforestation. We did not find a shift but mostly linear trends of different signs depending on the variable. Without doubt, this is related to the detailed spatial scale of the study, itself conditioned by the patchy design of the reforested area.

The disruption caused by mechanical soil preparation removed a large proportion of the dominant species of the grassland (*Stipa tenacissima*), which resulted in a reduction in its cover of > 60% in the inner part of the reforested area compared to that in the inner part of the grassland area (Figure 7). In our study area, the rate of emergence of seedlings of *S. tenacissima* is very low due to the high predation of seeds (by ants) and no germination on bare soil, with only germination occurring within tussocks of *S. tenacissima* (Barberá et al., 2006). Thus, the recolonization of reforested area by this species is very slow. The introduced *Pinus halepensis* developed poorly (Barberá et al., 2005) but in the inner part of reforested area its cover was similar to that of *S. tenacissima*. The abundance of the two subdominant shrubs differed in the two habitats. That of *Cistus clusii* and other Cistaceae was lower in the reforested habitat probably because their seed banks only become highly active after fires (Thanos et al., 1992). In contrast, *Rosmarinus officinalis* increased in the open spaces left by the removal of *S. tenacissima* during reforestation, as this species has an active seed bank and good colonization potential. In summary, reforestation greatly changed the structure of the grassland, reducing especially the cover of *S. tenacissima* and Cistaceae, which were the main taxa in the original grassland. On a more detailed scale the changes are gradual, not abrupt. Neither plant species richness nor the general physiognomy of the vegetation (vertical structure and overlaying) was significantly affected, as the poor growth of *P. halepensis* resulted in a shrub-like structure not very different from that of the original grassland.

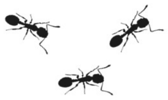
Soil surface was changed greatly by reforestation. The mechanical preparation of the site broke through the petrocalcic hardpan and brought large numbers of rocks to the surface of the soil. Within our study area, the surface of soil in the grassland characteristically consisted mainly of a continuous cover of soil with a few rocks on the surface and moderate abundance of stones. Cover of stones was highest in the ecotone. This can be probably explained by the disturbance caused by vehicles turning and manoeuvring between reforestation rows (pers. obs.). Consistent with this hypothesis is the lower cover of perennial plants in the ecotone.

Effect of reforestation on ant assemblages.

There was higher ant species richness (increase > 70% at the scale of transect × habitat accounting for the whole year and > 50% of pitfall catches at a distance of 60 m from the ecotone in the spring-summer season) and of total abundance by > 100% in the reforested area. Nevertheless, the multivariate analysis (Figure 12) indicates that the first trend in variation (axis 1) is unrelated to reforestation and is characterized by *C. auberti* differing from the other species in occurring in areas with a poor plant species richness. The second trend in variation indicates that the pitfall catches differ in the two habitats and, although there is overlap this may be a result of a gradual change in the environment between two habitats. The Adonis test was, however, significant. In summary, reforestation clearly changed the ant assemblage.

Previous studies on the transition from grassland to forest found a variety of effects: highest species richness at the ecotone (Downie et al., 1996; Pinheiro et al., 2010), increasing linear response in species richness from forest to grassland (Kotze & Samways, 2001; Yekwayo et al., 2016), or an increasing linear response in species richness from grassland to forest (Bieringer & Zulka, 2003), as in this study. It seems that the response of arthropods to changes in their habitat resulting from reforestation may be case- and taxon-specific.

Although patterns at the level of the ant assemblage were clear (specially for species richness and total abundance), on a species by species basis they are fuzzier. There are two possible reasons for this. The most abundant species (*Tapinoma nigerrimum*) may be a generalist not affected by changes induced by reforestation, and the other are scarce species for which random noise may obscure any effects. Nevertheless, two species were significantly more abundant in the grassland, four more abundant in the reforested area, and two in the ecotone. More species responded positively than negatively to reforestation. The underlying causes of this are mainly related to the changes in the vegetation and soil. Corley et al.’s (2006) study of the effects of pine afforestation on arid grasslands dominated by *Stipa* spp. on the Patagonian steppe, reveals that open forest



plantations have ant assemblages similar to those on native steppes. Other authors report that scattered presence of trees or invasion by shrubs of open grassland is associated with an increase in species richness and abundance of ants (Bestelmayer, 2005; Azcárate & Peco, 2012). This may be due to several factors. Reyes-López et al. (2003) report that some species are more common near trees than in open areas, suggesting that milder thermal environments may favour ants. However, a high density of trees on open grassland has an adverse effect on the ant assemblage (Reyes-López et al., 2003; Corley et al., 2006).

The results of this study are consistent with an improvement in the conditions for ants in grasslands with scattered shrubs and/or trees, as in our case. Year-round ant species richness and the abundances of two species of ants (*Camponotus foreli* and *Aphaenogaster iberica*) were positively correlated with the cover of *P. halepensis*, and the cover of this species is also correlated with axis 2, which segregates pitfall trap results by habitat. Trees are exclusive to the reforested area, but being widely spread and small they do not constitute a forest, but shrub land, with no more vegetation cover than the grassland. But, typically, for the same cover the biomass is much greater than that of shrubs and probably the microhabitats associated with trees are quite different from those associated with *S. tenacissima* and dominant shrubs. Not all the effects of the changes in vegetation were positive. *Goniomma blanci* was more abundant in grassland than in the reforested area. Furthermore, the abundance of this species was positively associated with *Fumana thymifolia* (a Cistaceae species) in spite of the low cover of this plant. *Goniomma kugleri*, another species of this genus on the Iberian Peninsula is specialized in harvesting the seeds of Cistaceae (Bastida et al., 2009), therefore it is very probable that *G. blanci* is similarly specialized and this would explain its lower abundance in the reforested area where the cover of Cistaceae is considerably less than in the grassland.

Food and nesting space are the most important resources for ants (Blüthgen & Feldhaar, 2010). A higher cover of stones and rocks provide more cool shelters for ant nests (Fernández-Escudero et al., 1993; Thomas, 2002; Robinson, 2008). Indeed, thermal properties of rocks benefit ants in two ways: by reducing high temperatures during hot weather, and by rapid and effective absorption of solar heat in cold weather, enabling better brood development (Dean and Turner, 1991; Thomas, 2002). Furthermore, rocks protect colonies against predators, and in addition the soil below these stones is moister and therefore easier to excavate (Dean & Turner, 1991). Reyes-López et al. (2003) report that in an open forest in Sierra Morena (Spain), the relationship between availability of stones for nesting and number of ant nests is positive. Dahms et al. (2010), studying semi-natural grasslands in Sweden, report a positive relationship between rock cover and ant species richness. Finally, Friedrich & Philpott (2009) suggest that intraspecific and interspecific competition may be less when there is an abundance of nest sites.

In the reforestation, the planting of pines was preceded by mechanical preparation of the site, which resulted in a major disturbance of the surface properties of the soil. The petrocalcic horizon in the soil was partially broken, fragmented and mixed with the surface horizon. This increased the frequency of stones and rocks and reduced the cover of soil. Total ant species richness and total ant abundance was positively correlated with the number of stones on the surface of the soil and the segregation by the NMDS analysis (axis 2) of pitfall trap catches by habitat was correlated with the cover of stones and soil. The abundance of *Camponotus sylvaticus*, which is known to shelter under stones/rocks, is negatively correlated with the cover of soil. More interestingly, the positive association of ant species richness and abundance with rock cover occurs in autumn but not year-round or in spring-summer, indicating a positive heating effect in cooler seasons.

The reforested area in this study is a more heterogeneous habitat, especially when jointly considering soil and vegetation and not just vegetation. Small-scale habitat complexity influences interspecific competition and resource use in ant communities, which results in differences among species that contribute to resource partitioning and permit subordinate species to avoid competition resulting in a greater diversity of ants (Luque & Reyes, 2007). Tews et al. (2004) introduce the concept of ‘keystone structures’ in terms of vegetation complexity as a distinct spatial structure, which provides resources, shelter or ‘goods and services’ crucial for other species. It would seem that both the abundance of stones on the surface of the soil and the small size of the pines in the system studied may match the concept of a keystone structure for ant assemblages.

Conclusions

The changes in the vegetation summarised by the decrease in the cover of *S. tenacissima* and Cistaceae, basic elements in grassland, and an increase in the cover of pine seems to provide more resources for the ant assemblage. The parallel disturbance of the soil seems to increase the availability of shelters. Altogether, these changes resulted in an increase in the richness and abundance of ants. Nevertheless, the positive effect of disturbing soil during reforestation is probably not the norm. Most mechanized reforestation in the Mediterranean was carried out on hillslopes and resulted in extensive erosion and large bare patches that are extremely slowly recolonized by vegetation (especially on escarpments under terraces), very different from the gentle slopes studied here.

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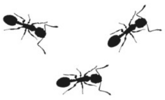

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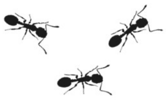

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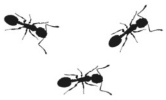

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Supplementary material

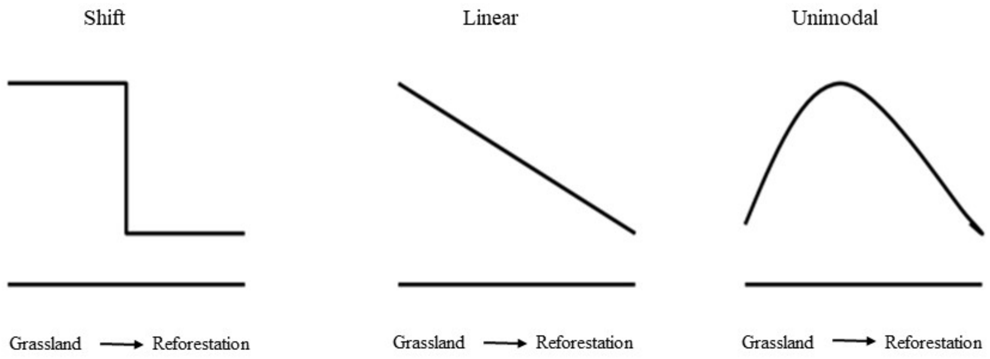


Figure S1. Possible models of response of a variable to the ecotone and/or changes between habitats.

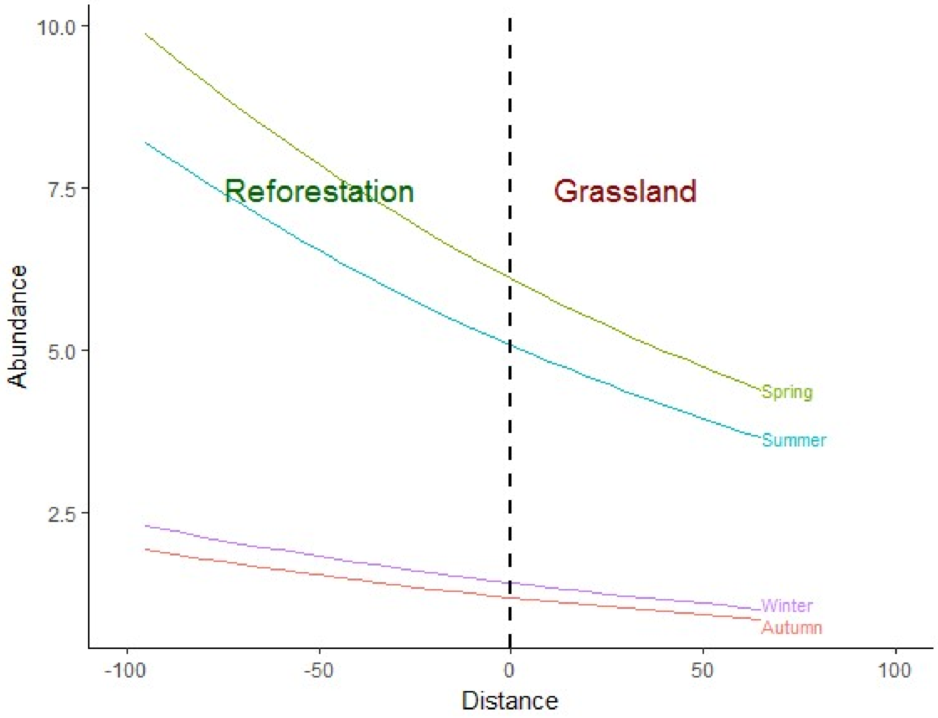
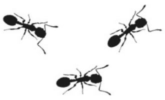


Figure S2. Total ant abundance. Expected values from models on Table S1.



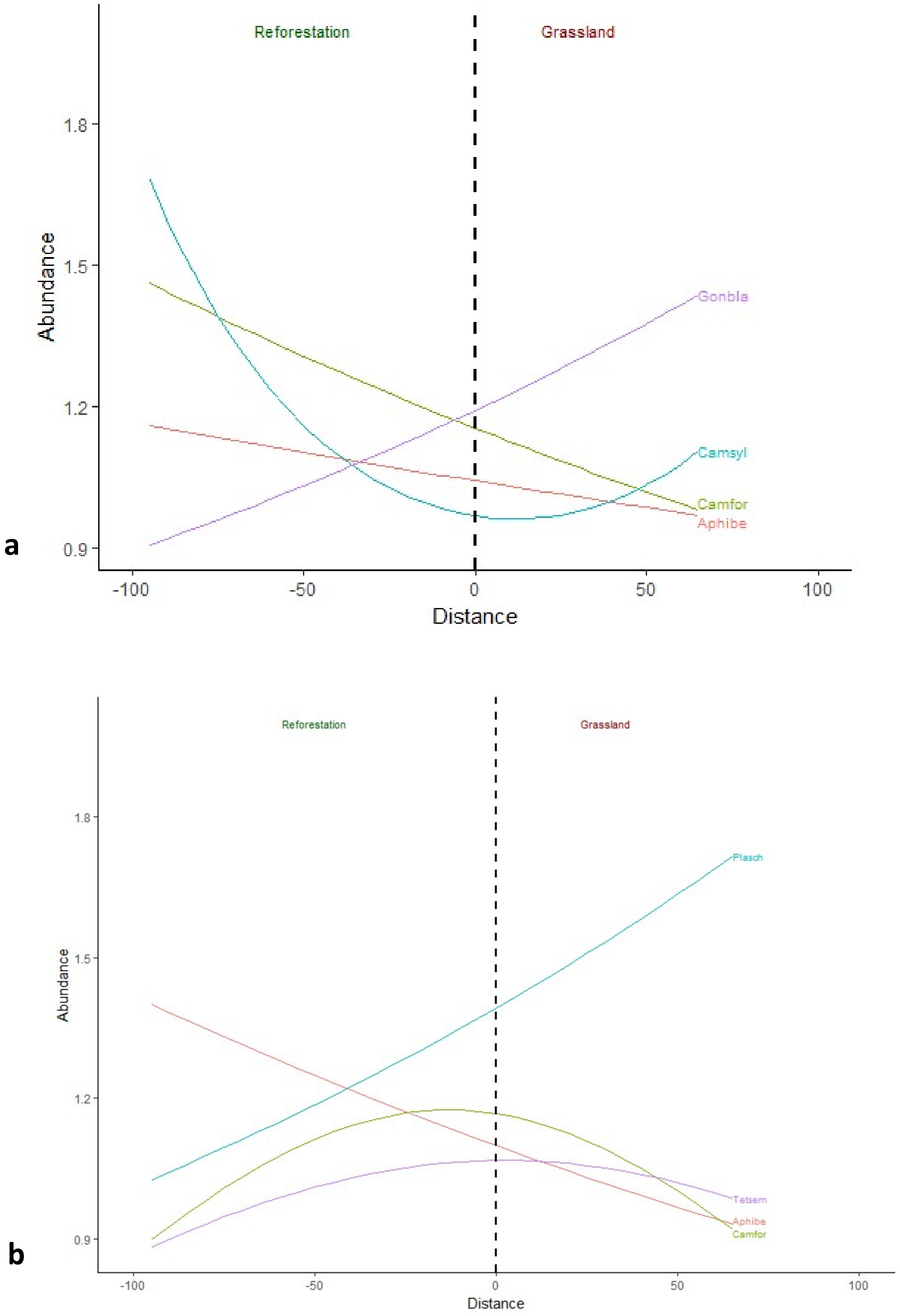
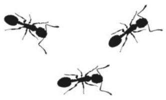


Figure S3. Ant species abundance. Expected values from models on Table S1. (a) Spring, (b) Summer. Aphibe, *Aphaenogaster iberica*; Camfor, *Camponotus foreli*; Camsyl, *Camponotus sylvaticus*; Gonbla, *Goniomma blanci*; Plasch, *Plagiolepis schmitzii*; Tetsem, *Tetramorium semilaeve*.

Table S1. Full structure and basic statistics of the parameters of the generalized linear mixed models that were statistically significant according to the procedures described in Material and methods. For each model the link function (transformation of the dependent variable) and the adequate error function is indicated. In the figures in the main text the link function is inverted, i.e., on the original scale of the dependent variable. Signed distance is the distance to the ecotone, with negative sign to the inner part of the reforested area and positive sign for that to the inner part of the grassland.

						Link function	***Error function***
SOIL VARIABLES							
Smooth soil						Logit	Binomial
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	0.08656	0.1396	2	0.62	0.5986		
Signed distance to ecotone	0.005765	0.002494	53	2.31	0.0247		
Stones						Logit	Binomial
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	-0.2271	0.1666	2	-1.36	0.306		
Signed distance to ecotone	-0.00098	0.002907	52	-0.34	0.7367		
Signed distance to ecotone^2	-0.00009	0.000042	52	-2.03	0.0472		
Rocks						Logit	Binomial
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	-3.4364	0.3914	2	-8.78	0.0127		
Signed distance to ecotone	-0.01381	0.006405	52	-2.16	0.0358		
Signed distance to ecotone^2	0.000236	0.000087	52	2.72	0.0087		
VEGETATION VARIABLES							
Cistus clusii						Logit	Binomial
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	-3.1201	0.158	2	-19.75	0.0026		
Signed distance to ecotone	0.00757	0.003849	53	1.97	0.0545		
Fumana thymifolia						Logit	Binomial
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	-7.1428	1.5162	2	-4.71	0.0422		
Signed distance to ecotone	0.06106	0.02562	53	2.38	0.0208		
Pinus halepensis						Logit	Binomial
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	-4.2097	0.5019	2	-8.39	0.0139		
Signed distance to ecotone	-0.08226	0.01956	52	-4.21	0.0001		
Signed distance to ecotone^2	-0.00061	0.000192	52	-3.17	0.0026		



Rosmarinus officinalis						Logit	Binomial
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	-2.0836	0.1214	2	-17.16	0.0034		
Signed distance to ecotone	-0.0061	0.002584	53	-2.36	0.022		
Stipa tenacissima						Logit	Binomial
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	-0.7936	0.2336	2	-3.4	0.0768		
Signed distance to ecotone	0.01034	0.00227	53	4.56	<0.0001		
Chamaephytes cover						Log	Poisson
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	-0.9466	0.366	2	-2.59	0.1226		
Signed distance to ecotone	0.01771	0.005334	53	3.32	0.0016		
Total vegetation cover						Logit	Binomial
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	0.0154	0.1798	2	0.09	0.9396		
Signed distance to ecotone	0.003515	0.002477	52	1.42	0.1618		
Signed distance to ecotone^2	0.000081	0.00004	52	2.01	0.0501		
ANT ASSEMBLAGE VARIABLES							
Total ant abundance							
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*	Log	Normal
Intercept	0.1828	0.1357	2	1.35	0.3102		
Signed distance to ecotone	-0.00505	0.001875	230	-2.69	0.0076		
May	1.6267	0.1306	230	12.45	<0.00001		
August	1.4429	0.1306	230	11.05	<0.00001		
November	0						
February	0.1715	0.1306	230	1.31	0.1906		
Ant richness (pitfall scale)						Log	Poisson
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	-1.5581	0.2857	2	-5.45	0.032		
Signed distance to ecotone	-0.00363	0.001786	230	-2.03	0.0432		
May	2.3909	0.2898	230	8.25	<0.00001		
August	2.2713	0.2913	230	7.8	<0.00001		
November	0						
February	0.7309	0.3376	230	1.31	0.0314		

ANT SPECIES ABUNDANCE							
Aphaenogaster iberica (year round)						Log	Normal
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	0.1239	0.04614	2	2.68	0.1153		
Signed distance to ecotone	-0.00307	0.001064	53	-2.88	0.0057		
Aphaenogaster iberica (spring)							
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*	Log	Normal
Intercept	0.04235	0.02188	2	1.94	0.1925		
Signed distance to ecotone	-0.00111	0.000504	53	-2.21	0.0317		
Aphaenogaster iberica (summer)							
Effect	Estimate	SE	DF	t Value	Pr > |t|	Log	Normal
Intercept	0.09404	0.04093	2	2.3	0.1484		
Signed distance to ecotone	-0.00255	0.000944	53	-2.71	0.0091		
Camponotus foreli (year round)							
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	0.2177	0.0493	2	4.42	0.0476	Log	Normal
Signed distance to ecotone	-0.00303	0.001083	53	-2.8	0.0072		
Camponotus foreli (summer)							
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*	Log	Normal
Intercept	0.1543	0.05477	2	2.82	0.1063		
Signed distance to ecotone	-0.00105	0.001025	52	-1.02	0.3121		
Signed distance to ecotone^2	-0.00004	0.000018	52	-2.07	0.0438		
Camponotus sylvaticus (year round)						Log	Normal
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	0.1003	0.03736	2	2.69	0.1152		
Signed distance to ecotone	-0.003	0.000861	53	-3.49	0.001		
Camponotus sylvaticus (spring)						Log	Normal
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	-0.03149	0.04515	2	-0.7	0.5577		
Signed distance to ecotone	-0.00115	0.000823	52	-1.39	0.1694		
Signed distance to ecotone^2	0.000049	0.000018	52	2.75	0.0082		
Goniomma blanci (year round)						Log	Normal
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	0.1873	0.06286	2	2.98	0.0966		
Signed distance to ecotone	0.00283	0.001332	53	2.13	0.0383		

Goniomma blanci (spring)						Log	Normal
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	0.1759	0.06221	2	2.83	0.1056		
Signed distance to ecotone	0.002875	0.001317	53	2.18	0.0334		
Plagiolepis schmitzii (summer)						Log	Normal
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	0.3315	0.06705	2	4.94	0.0386		
Signed distance to ecotone	0.003211	0.001541	53	2.08	0.0421		
Temnothorax racovitzai (year round)						Log	Normal
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	0.02044	0.01579	2	1.29	0.3249		
Signed distance to ecotone	-0.00077	0.000364	53	-2.11	0.0392		
Temnothorax racovitzai (summer)						Log	Normal
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	0.02044	0.01579	2	1.29	0.3249		
Signed distance to ecotone	-0.00077	0.000364	53	-2.11	0.0392		
Tetramorium semilaeve (year round)						Log	Normal
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	0.1242	0.05274	2	2.35	0.1427		
Signed distance to ecotone	-0.00151	0.000777	52	-1.94	0.0579		
Signed distance to ecotone^2	-0.00003	0.000016	52	-2.15	0.0359		
Tetramorium semilaeve (summer)						Log	Normal
Effect	*Estimate*	*SE*	*DF*	*t Value*	*Pr > |t|*		
Intercept	0.06498	0.04011	2	1.62	0.2466		
Distecotono	0.000093	0.000672	52	0.14	0.8899		
Distecotono2	-0.00002	9.36E-06	52	-2.36	0.022		

Table S2. Spearman's correlation coefficients and false discovery rate (FDR) between environmental variables and ant assemblage variables significantly changing between habitats. The threshold for FDR of Benjamini-Hochberg was set to 0.2 (see Methods). According this all the values of FDR <0.2 are considered statistically significant. Cisclu, *Cistus clusii*; Fumthy, *Fumana thymifolia*; Pinhal, *Pinus halepensis*; Rosof, *Rosmarinus officinalis*; Stiten, *Stipa tenacissima*; Tab, Total abundance; Aphibe, *Aphaenogaster iberica*; Camfor, *Camponotus foreli*; Camsyl, *Camponotus sylvaticus*; Gonbla, *Goniomma blanci*; Plasch, *Plagiolepis schmitzii*; Temrac, *Temnothorax racovitzai*; Tetsem, *Tetramorium semilaeve*

Spearman's correlation coefficient

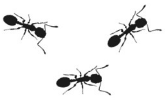
	TAb_Y	TAb_Su	TAb_W	TAb_Sp	TAb_Au	Aphibe_Y	Aphibe_Su	Aphibe_Sp
Chamaephytes	-0,08	-0,07	0,02	-0,05	-0,05	-0,08	-0,08	-0,05
Cisclu	-0,08	-0,08	-0,14	-0,05	-0,07	-0,04	-0,03	-0,11
Perennial vegetation	0,01	0,02	0,02	-0,01	-0,07	0,09	0,08	0,06
Fumthy	-0,05	-0,02	0,11	0,04	-0,02	-0,11	-0,07	-0,07
Stones	0,05	0,02	-0,02	0,12	0,00	0,00	-0,08	0,10
Pinhal	0,07	0,07	0,02	0,15	0,05	0,03	0,04	0,20
Rocks	0,07	-0,03	0,04	0,08	0,04	0,08	0,08	0,09
Rosof	-0,07	-0,07	-0,10	-0,06	0,03	0,10	0,09	0,12
Stiten	0,02	0,02	0,07	0,00	-0,06	-0,03	-0,03	-0,01
Cover of soil	-0,05	-0,05	0,08	-0,14	-0,11	-0,03	0,06	-0,14

FDR values

	TAb_Y	TAb_Su	TAb_W	TAb_Sp	TAb_Au	Aphibe_Y	Aphibe_Su	Aphibe_Sp
Chamaephytes	0,56	0,32	0,91	0,80	0,95	0,86	0,62	0,79
Cisclu	0,44	0,45	0,91	0,80	0,95	0,88	0,80	0,79
Perennial vegetation	0,92	0,89	0,91	0,98	0,95	0,86	0,62	0,79
Fumthy	0,82	0,06	0,91	0,88	0,95	0,86	0,62	0,79
Stones	0,17	0,06	0,91	0,80	0,98	1,00	0,62	0,79
Pinhal	0,33	0,58	0,91	0,80	0,95	0,10	0,10	0,79
Rocks	0,56	0,89	0,91	0,80	0,16	0,86	0,62	0,79
Rosof	0,56	0,45	0,91	0,80	0,95	0,86	0,62	0,79
Stiten	0,90	0,89	0,91	0,98	0,95	0,88	0,80	0,92
Cover of soil	0,17	0,18	0,91	0,80	0,59	0,88	0,80	0,79

Spearman's correlation coefficient

	Camfor_Y	Camfor_Su	Camfor_Sp	Camsyl_Y	Camsyl_Sp	Gonbla_Y	Gonbla_Sp	Plasmi_Su
Chamaephytes	-0,06	0,03	-0,09	-0,05	-0,06	0,04	0,07	0,07
Cisclu	-0,05	-0,09	-0,05	-0,06	-0,06	-0,02	0,01	-0,07
Perennial vegetation	-0,08	-0,09	-0,03	-0,06	-0,04	0,04	0,06	-0,02
Fumthy	-0,11	-0,08	-0,08	-0,05	-0,05	0,00	0,00	-0,02
Stones	0,06	0,10	0,05	0,06	0,05	-0,06	-0,07	0,04
Pinhal	0,06	0,08	0,06	0,06	0,05	-0,06	-0,06	-0,07
Rocks	0,05	-0,04	0,05	0,06	0,04	-0,04	-0,06	-0,07
Rosof	-0,01	-0,11	0,07	-0,05	-0,05	-0,06	-0,06	-0,07
Stiten	-0,10	-0,07	-0,06	-0,05	-0,05	0,06	0,07	0,06
Cover of soil	-0,09	-0,06	-0,08	-0,06	-0,05	0,05	0,06	0,05



FDR values

	Camfor_Y	Camfor_Su	Camfor_Sp	Camsyl_Y	Camsyl_Sp	Gonbla_Y	Gonbla_Sp	Plasmi_Su
Chamaephytes	0,65	0,85	0,48	0,57	0,30	0,14	0,28	0,47
Cisclu	0,17	0,68	0,19	0,31	0,42	0,86	0,90	0,56
Perennial vegetation	0,63	0,68	0,84	0,21	0,17	0,74	0,54	0,87
Fumthy	0,52	0,68	0,48	0,41	0,54	0,00	0,00	0,87
Stones	0,65	0,68	0,76	0,38	0,50	0,22	0,28	0,87
Pinhal	0,17	0,68	0,19	0,31	0,26	0,26	0,33	0,34
Rocks	0,65	0,85	0,76	0,31	0,17	0,74	0,54	0,22
Rosof	0,94	0,68	0,76	0,41	0,30	0,65	0,54	0,27
Stiten	0,63	0,68	0,76	0,22	0,50	0,65	0,43	0,72
Cover of soil	0,63	0,68	0,48	0,21	0,17	0,26	0,29	0,72

Spearman's correlation coefficient

	Richness_Su	Richness_Wi	Richness_Sp	Richness_Au	Richness_Y	Temrac_Y	Temrac_Su	Tetsem_Y
Chamaephytes	-0,09	0,02	-0,08	-0,04	-0,06	-0,07	-0,07	0,01
Cisclu	-0,10	-0,14	-0,06	-0,07	-0,03	-0,08	-0,08	0,00
Perennial vegetation	0,04	-0,01	0,01	-0,06	-0,05	0,06	0,06	-0,10
Fumthy	-0,04	0,07	0,01	-0,03	-0,07	-0,04	-0,04	-0,07
Stones	0,04	0,04	0,07	-0,01	0,02	0,06	0,06	0,09
Pinhal	0,04	0,04	0,04	0,05	0,00	0,03	0,03	0,09
Rocks	-0,04	0,07	0,07	0,04	0,05	0,08	0,08	0,10
Rosof	-0,07	-0,09	0,06	0,03	0,03	0,06	0,06	0,08
Stiten	-0,01	0,03	-0,05	-0,05	-0,07	-0,02	-0,02	-0,13
Cover of soil	-0,07	0,03	-0,06	-0,11	-0,01	-0,07	-0,07	-0,09

FDR values

	Richness_Su	Richness_Wi	Richness_Sp	Richness_Au	Richness_Y	Temrac_Y	Temrac_Su	Tetsem_Y
Chamaephytes	0,68	0,92	0,70	0,91	0,55	0,52	0,52	0,97
Cisclu	0,62	0,92	0,28	0,91	0,11	0,45	0,45	0,97
Perennial vegetation	0,99	0,96	0,98	0,91	0,67	0,52	0,52	0,69
Fumthy	0,14	0,92	0,98	0,91	0,49	0,69	0,69	0,69
Stones	0,14	0,92	0,70	0,94	0,05	0,52	0,52	0,37
Pinhal	0,14	0,92	0,10	0,91	0,01	0,10	0,10	0,69
Rocks	0,99	0,92	0,28	0,14	0,21	0,42	0,42	0,69
Rosof	0,91	0,92	0,78	0,91	0,77	0,27	0,27	0,69
Stiten	0,99	0,92	0,84	0,91	0,49	0,84	0,84	0,65
Cover of soil	0,29	0,92	0,28	0,60	0,04	0,48	0,48	0,37

Spearman's correlation coefficient

	Tetsem_Su
Chamaephytes	0,04
Cisclu	-0,02
Perennial vegetation	-0,07
Fumthy	-0,05
Stones	0,18
Pinhal	0,02
Rocks	-0,05
Rosof	0,06
Stiten	-0,13
Cover of soil	-0,12

FDR values

	Tetsem_Su
Chamaephytes	0,85
Cisclu	0,86
Perennial vegetation	0,83
Fumthy	0,83
Stones	0,83
Pinhal	0,86
Rocks	0,83
Rosof	0,83
Stiten	0,83
Cover of soil	0,83

Chapter 4

A New Ant Species, *Temnothorax ansei* sp.n. (Hymenoptera: Formicidae) from the Arid Environments of South-eastern Spain.

Photo credit for all the photos of Chapter 4: Michele Esposito, from www.AntWeb.org

A New Ant Species, *Temnothorax ansei* sp.n. (Hymenoptera: Formicidae) from the Arid Environments of South-eastern Spain.

Abstract

We describe here a new ant species in the genus *Temnothorax*, species group laurae, based on morphological evidence supported by other biological information. It has been discovered at two locations in southeast Spain: Pulpí (Almería) and Lorca (Murcia). This species is a member of the *laurae* species group based on the following characters: large eyes, long and erect hairs on alitrunk, petiole and postpetiole, rectangular head and metanotal groove. Other characters unique to new species are: dark brown in color, decumbent pilosity, and smooth and shiny head and pronotum. In addition, this new species is distinctly nocturnal in its activity patterns. Based on the morphological measurements of the queens and the workers, as well as our observations in the laboratory, we predict that this species has a dependent colony founding. An identification key to the Iberian Peninsula species of the *laurae* group is also provided.

Introduction

Temnothorax (Mayr, 1861), is a speciose genus of small and generally inconspicuous ant with a predominantly Holartic distribution. The most recent catalogue lists 380 valid species and 47 valid subspecies (Bolton, 2015). Colonies are typically small, often with less than 100 workers (Prebus, 2015). *Temnothorax* is divided into nine species groups (Cagniant & Espadaler, 1997). One of those is *laurae* species group, whose members are characterized by large eyes relative to the length of the head capsule (OI>30), postpetiole more or less trapezoidal in dorsal view (Prebus, 2015), long and erect hairs on alitrunk, petiole and postpetiole, rectangular head, and the presence of metanotal groove (Tinaut, 1994; Cagniant & Espadaler, 1997). They are rarely collected, probably because of their small colony size and nocturnal activity patterns; caused by hot and arid environment they inhabit (Tinaut, 1994; Cagniant & Espadaler, 1997).

In the Iberian Peninsula, there are five known species belonging to the *laurae* group: *Temnothorax blascoi* (Espadaler, 1996), *T. caesari* (Espadaler, 1997b), *T. crepuscularis* (Tinaut, 1994), *T. universitatis* (Espadaler, 1997a) and *T. naeviventris* (Santschi, 1910). Two of these are present in the Segura River Basin (south-east Spain): *T. blascoi* (Catarineu & Tinaut, 2012) and *T. universitatis* (see Table 2). Considering the difficulty of detecting individuals in this group, it is likely there are more undescribed species.

On July of 2014, in a pitfall transect, we collected two workers of a *Temnothorax* species, belonging to the *laurae* group, that we were unable to identify, at a location near Pulpí (Almería, Andalucía, Spain) 1.716W, 37.389N (decimal format). The same species was also collected in two pitfall transects in Lorca (Murcia, Spain) 1.774W, 37.670N and 1.774W, 37.685N (Figure 1).

Figure 1. Map of Spain with the location of the Segura River Basin outlined and the two localities with *Temnothorax ansei* sp.n. indicated with black squares

Using nocturnal baits, with tuna, cookies, and Wilson media, we managed to locate and excavate five nests at the Pulpí site obtaining five living colonies. We were able to keep three of these colonies in the laboratory until males and queens were acquired.

Material and methods

Digital color images were obtained by AntWeb and are available online (Fisher, 2002; http://www.antweb.org). Measurements were taken of 27 workers, eight queens and five males with a stereo microscope Leica S6D at a micrometer magnification of up to x80. Morphometric characters and indices are based on Seifert et al. (2014), Prebus (2015), and Seifert & Csósz (2015):

BI. Buschinger Index: SPST/ SPL.

CS.Cephalic size: the arithmetic mean of HL and HW

EL. Eye Length: maximum diameter of compound eye, including all structural visible ommatidia irrespective of the pigmentation status, measured in oblique lateral view.

HL. Head Length: maximum distance from the mid-point of the anterior clypeal margin to the mid-point of the posterior margin of head, measured in full-face view.

HW. Head Width: width of head directly behind the eyes, measured in full-face view.

OI. Ocular Index: EL/HW x 100.

SPL. Minimum distance between the center of the propodeal spiracle and the margin of subspinal excavation, measured with both end points positioned in the same focal level.

SPST. Distance between the center of the propodeal stigma and the spine tip, the stigma center refers to the midpoint defined by the outer cuticular ring but not to the center of the real stigma opening that may be positioned eccentrically.

TL. Tibia Length: maximum length of the tibia.

WL. Weber's Length: diagonal length of mesosoma in lateral view from the posteroventral margin of propodeal lobe to the anterior-most point of pronotal slope, excluding the neck.

The varying degrees of inclination of pubescence and pilosity are often of high diagnostic value throughout a broad spectrum of ant genera. In this context, we use the terms “erect”, “suberect”, “subdecumbent”, “decumbent”, and “appressed” following Wilson (1955).

Description

Temnothorax ansei **sp.n.**

Type material examined

Holotype: a worker from a location nearby Pulpí (Almería, Spain), 1.716W, 37.389N (decimal format), elevation 243 m, 23 July 2014, collected by C. Catarineu and labelled with the specimen code W01-10S501.

Paratypes: 26 workers, 10 queens and nine males with the same data as holotype; six workers from Lorca (Murcia, Spain; three workers from 1.774W, 37.670N, Elevation 591 m; three workers from 1.774W, 37.685N, Elevation 464 m). All the paratypes were collected by C. Catarineu from July 2014 to June 2015.

Repositories

The National Museum of Natural Sciences (MNCN, Madrid, Spain) holotype, two workers, one queen and one male (registration number C.T. 2730). The California Academy of Sciences, USA, two workers, one intermorph, one queen and one male (CASENT0763773, CASENT0763775, CASENT0919826, CASENT0919953 and CASENT 0919954). The Natural History Museum, London, U.K., two workers, one queen and one male. Museum d’Histoire Naturelle, Genève, Switzerland, two workers, one queen and one male (MHNG ENTO numbers 10129 to 10132). University of Granada, Spain, two workers, one queen and one male. Coll. Xavier Espadaler (Autonomous University of Barcelona, Spain) two workers, one queen and one male. Coll. Joaquín L. Reyes-López (University of Cordoba, Spain) eight workers, three queens, one intermorph and one male. Coll. Chema Catarineu (Murcia, Spain) 11 workers, five queen, one intermorph and three male.

Description of worker (Figures 2-4)

Measurements (in mm): HL 0.66–0.76, HW 0.50–0.59, CS 0.58–0.68, EL 0.16–0.24, WL 0.80–0.98, SPST 0.18–0.26, SPL 0.10–0.14, BI 1.60–2.50, TL 0.48–0.60; OI 29.55–44.19 (n=27 workers).

Head, mesosoma and gaster dark brown, mandibles, legs and antennae brownish yellow. Hairs on alitrunk, petiole and postpetiole long and erect (mean mesonotum hair length=104µm). Mandibles, legs, funiculi and scapes with short decumbent pubescence. Head, pronotum and gaster with short and sparse decumbent pubescence. Head rectangular with parallel sides. Occiput straight. Eyes large (EL/CS=0.26-0.39). Antennae 12-segmented, antennal scape reaching occiput. Mandibles with five teeth: one apical longer, one subapical of intermediate length and the three smaller and irregular. Antennal club three-segmented, funiculus concolorous. Head smooth and shiny with some longitudinal costulae between the frontal carinae present, costulae never reaching occiput. Malar area costulate and reticulate. Clypeus smooth and shiny, a median longitudinal carinae, and two lateral carinae present and conspicuous. Frontal triangle smooth and shiny. Alitrunk with metanotal groove. Pronotum smooth and shiny. Mesonotum and propodeum laterally weakly reticulate, smooth and shiny dorsally. Propodeal spines long (SPST/CS=0.29–0.41), integument between spines smooth and shiny. Petiole and postpetiole finely reticulate-rugulose laterally, smooth and shiny dorsally. Petiole triangular in profile, with rounded apex and with four long setae on postero-dorsal surface. Postpetiole rounded in profile, trapezoidal in dorsal view, wider than long, and wider than petiole, with six long setae. Gaster smooth and shiny.

Variability: propodeal spines can vary in size, apex of petiole varies from less to more rounded.

Figure 2. *Temnothorax ansei* sp.n. worker in lateral view.

Figure 3. *Temnothorax ansei* sp.n. worker in dorsal view.

Figure 4. Head of *Temnothorax ansei* sp.n. worker in frontal view.

Description of queen (Figures 5–7)

Measurements (in mm): HL 0.66–0.71, HW 0.51–0.58, CS 0.59–0.64, EL 0.19–0.24, WL 0.90–1.04, SPST 0.19–0.25, SPL 0.09–0.14, BI 1.45–2.29, TL 0.50–0.59, OI 34.88–41.86 (n=8 queens).

Size and color as in workers. Hairs on alitrunk, petiole and postpetiole long and erect (mean mesonotum hair length=78µm). Eyes large (EL/CS=0.31–0.37), ocelli well developed. Antennae 12-segmented. Antennal scape reaching occiput. Antennal club three-segmented, funiculus concolorous. Mesosoma only slightly bigger than in workers. Anterior edges of pronotum slightly visible from the dorsal view. Pronotum, scutum and scutellum smooth and shiny. Mesopleurae with a few lateral costulae. Propodeum with lateral costulae, smooth and shiny between the spines. Petiolar node triangular in profile, its apex more acute than in workers. Well developed spines but somewhat smaller than in workers (SPST/CS=0.32–0.39). Petiole, postpetiole and gaster as in workers. Transparent wings, with very reduced veins. Pterostigma transparent, light yellow.

Variability: propodeal spines can vary in size, apex of petiole varies from less to more rounded.

We have found two worker-queen intermorphs, one in the wild (Figures 8–10), and the other born in laboratory conditions.

Figure 5. *Temnothorax ansei* sp.n. queen in lateral view.

Figure 6. *Temnothorax ansei* sp.n. queen in dorsal view.

Figure 7. Head of *Temnothorax ansei* sp.n. queen in frontal view.

Figure 8. *Temnothorax ansei* sp.n. intermorphic individual in lateral view.

Figure 9. *Temnothorax ansei* sp.n. intermorphic individual in dorsal view.

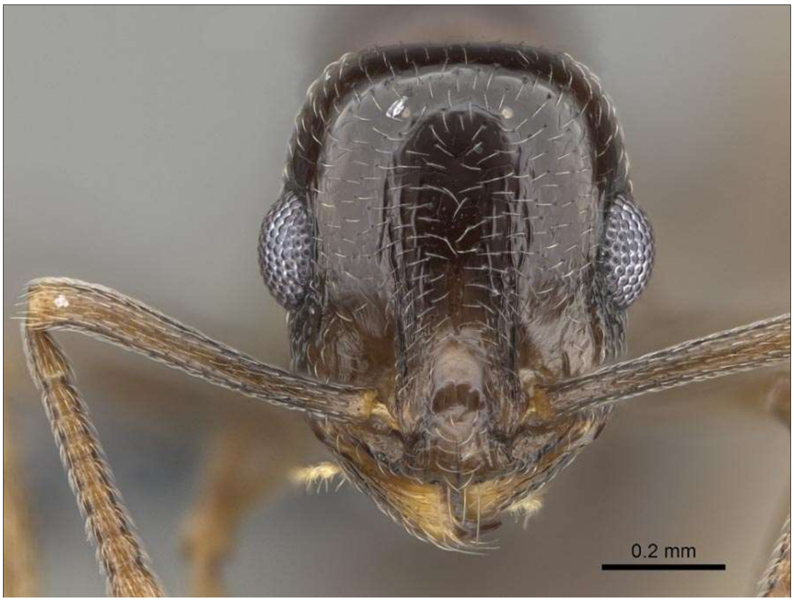


Figure 10. Head of *Temnothorax ansei* sp.n. intermorphic individual in frontal view.

Description of male (Figures 11–13)

Measurements (in mm): HL 0.49–0.58, HW 0.40–0.50, CS 0.44–0.54, EL 0.19–0.23, WL 0.98–1.08, TL 0.60–0.76, OI 45.00–50.00 (n= 5 males).

Head, alitrunk, petiole, postpetiole and gaster light brown; antennae and legs lighter. Hairs on alitrunk, petiole and postpetiole long and erect (mean mesonotum hair length=84µm). Oval-shaped head, smooth and shiny. Very large eyes (EL/CS=0.42–0.46), nearly half of head length, located in the lower half of face sides. Ocelli well developed. Antennae 13-segmented. Scape surpassing the occipital margin and as long as the first 8 segments of the funiculus. Antennal club four-segmented. Mandibles with one apical tooth, one subapical and 2–3 smaller. Mesopleaurae and propodeum smooth, with less prominent costulae than in worker and queen. Pronotum, prescutum, scutum and scutellum smooth and shiny. Notauli marked. Scutum and scutellum separated by a depression. Metanotum present, narrow and separated from scutellum and propodeum. Propodeum reticulated, with some lateral costulae and without spines, only with a very slight angle. Petiole low and with node rounded. Gaster smooth and shiny. Wings as queens.

Variability: some males have the propodeum rounded.

Figure 11. *Temnothorax ansei* sp.n. male in lateral view.

Figure 12. *Temnothorax ansei* sp.n. male in dorsal view.

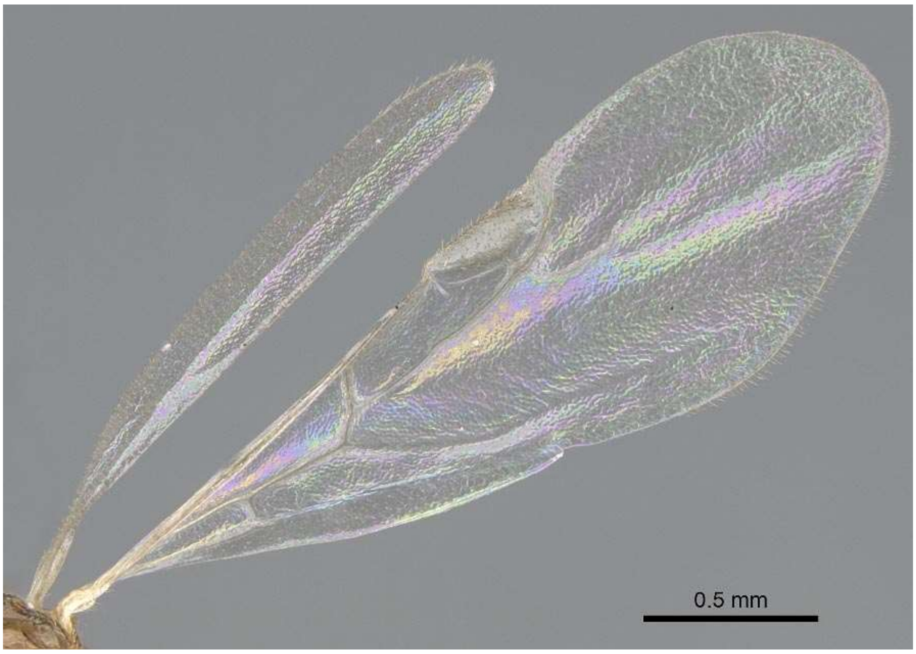
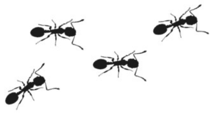


Figure 13. *Temnothorax ansei* sp.n. male wings.



Etymology

The species recognizes and honours the Asociación de Naturalistas del Sureste (ANSE), an organization rooted in the south-east of Spain since 1973 (ANSE, 2015). This is a naturalist and environmentalist, non-governmental organization which, by its efforts on nature research, awareness, and conservation activism, has earned the respect and support of generations of scientists and citizens. Most significantly, for the last five decades, ANSE has been a force of scientific and environmental activism promoting the formal recognition of the previously poorly-valued semiarid ecosystems of the south-east of Spain, where *T. ansei*, our newly discovered species, finds its home.

Biology

Foraging workers of *T. ansei* sp. n. were attracted to the baits at night, or sometimes during twilight (in June and July, the workers begin to forage from approximately about 19:00 GMT). After feeding for a few minutes, they carried a small cookie crumb to their nearby nest, although never more than one meter away. Through careful monitoring in a territory of about 230 m^2, we were able to locate five nests, each with a single entrance hole in the soil of approximately 1 mm in diameter. These nests were very hard to detect as they lacked any external signs or evidence of ant activity.

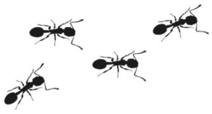
We excavated the five nests and found that the main chamber was about 15–20 cm deep. Colonies ranged from two to 52 workers. Two of them had two wingless queens (Table 1). Three of these colonies were relocated to our laboratory and have been maintained there under controlled conditions to date. The other two, an incipient colony with only two workers and other with 13, did not survive in captivity. Ants were fed with honey-water and small crickets and have produced during two years new males and females. These sexuals were born from eggs laid by the queen in the laboratory. All 19 queens born in captivity lost their wings in the first 1–4 weeks, 13 of them in the first three days, and then continued living normally in the nest, cooperating with the others. This behaviour has also been observed in other species of the group, for instance T. caesari (Espadaler, 1997b). Eggs, larvae and pupae were placed by the workers in one group on the floor of one chamber altogether.

Table 1. Nest excavations data for the new species *Temnothorax ansei* sp.n., discovered in 2014 in the south-east of Spain.

Nest	Excavation date	queen wingless	workers	queen winged	male
1	01/11/2014	1	52		1
2	23/05/2015	2	20	1	
3	13/06/2015	2	21		2
4	13/06/2015	1	13		5
5	20/06/2015	1	2		

Table 2. Ant species sympatric with *Temnothorax ansei* sp.n.

Species/ Locality	**Pulpí**	**Lorca S2**	**Lorca S3**
Aphaenogaster iberica Emery	X		X
Camponotus foreli Emery	X	X	X
Camponotus sylvaticus Olivier	X	X	X
Cardiocondyla batesi Forel			X
Cataglyphis iberica Emery		X	X
Crematogaster auberti Emery	X	X	X
Goniomma hispanicum André	X		
Goniomma kugleri Espadaler	X		X
Iberoformica subrufa Roger			X
Leptanilla sp	X		
Messor barbarous Linnaeus	X	X	
Messor bouvieri Bondroit	X	X	X
Oxyopomyrmex saulcy Emery			X
Pheidole pallidula Nylander	X		X
Plagiolepis schmitzii Forel		X	X
Solenopsis sp			X
Temnothorax cristinae Espadaler	X		
Temnothorax formosus Santschi			X
Temnothorax universitatis Espadaler			X
Temnothorax luteus Forel			X
Temnothorax niger Forel	X		
Temnothorax racovitzai Bondroit		X	
Temnothorax specularis Emery	X	X	X
Tetramorium semilaeve André	X	X	X



Ecology

Both collecting localities are semiarid habitats with sparse vegetation cover, dominated by *Stipa tenacissima* L. grassland at the Pulpí site and chamaephytes and little shrubs at the Lorca site. Altitudes are 243 m at Pulpí and 464–591 m at Lorca. The average annual rainfall is 272 and 313 mm at Pulpí and Lorca sites, respectively.

We found 25 ant species at the same habitat (Table 2), including *T. universitatis*, a species we consider, based on the morphology, to be the closest relative of *T. ansei* sp.n.

Discussion

Taxonomic position and diagnosis

This new species, *T. ansei* sp.n. clearly belongs to the *laurae* group, by showing characteristic features of this group such as large eyes in relation to the length of the head capsule (OI > 30). Additional traits consistent with the *laurae* group are head capsule elongated (CI < 85), postpetiole more or less trapezoidal in dorsal view and widest anterior to the midlength of the segment (Prebus, 2015). As many species within this group, *T. ansei* sp.n. is also found in arid environments.

Based on the morphology, the closest relative of *T. ansei* sp.n. in the *laurae* group is species *T. universitatis*. We can differentiate between those two species because *T. universitatis* worker is light brown or dirty yellow, with head less shiny, alitrunk dorsally opaque and pronotum laterally rugulose.

From the other Iberian species in the laurae group, the main differences are:

Temnothorax blascoi is smaller, light yellow, with dense pilosity all over the body and with long and thin setae similar to *Temnothorax recedens* Espadaler.

Temnothorax caesari has mesosoma olivaceus brown, less shinning and less quadrangular head. Dorsal striae in pronotum and mesonotum. Pilosity erect on head and gaster.

Temnothorax crepuscularis is yellow, with metanotal groove unmarked, abundant semi-erect setae on head and gaster, head and alitrunk rough, petiole and postpetiole dorsally rough.

Temnothorax naeviventris is light yellow, with head and alitrunk coarse and opaque, and with pilosity erect in head.

Based on our original findings and the prior contributions by other authors (Santschi, 1910; Tinaut, 1994; Espadaler, 1996, 1997a, 1997b), we propose the following dichotomous key to the Iberian *laurae* group species. This key is based on the external morphology of the workers:

Key to the worker caste of Iberian Temnothorax, laurae species group

1 Metanotal groove absent.. *T. crepuscularis*
- Metanotal groove present.. 2
2 Dense pilosity all over the body...3
- Sparse pilosity all over the body..5
3 Head and gaster dark brown; mesosoma olivaceus brown; propodeal spines shorter and blunt.. *T. caesari*
- Light yellow; propodeal spines long, thin and acute...4
4 Dorsal setae long, thin and curved ....*T. blascoi*
- Dorsal setae thick and blunt...*T. naeviventris*
5 Light brown or dirty yellow; pronotum laterally dull..........................*T. universitatis*
- Dark brown; pronotum laterally smooth and shiny.......................................*T. ansei*

Biological observations

Ants show two contrasting strategies of colony founding. In the Independent Colony Foundation (ICF), a queen can raise the first generation of brood alone. The ICF strategy frequently involves claustral founding where queens are characterized by voluminous wing muscles which can histolize after dispersal. These queens must also have high metabolic reserves. In contrast, Dependent Colony Foundation (DCF), is a strategy in which the queen is helped by some workers to create a new colony-budding or fission (Hölldobler & Wilson, 1990; Heinze & Tsuji, 1995; Peeters & Ito, 2001; Keller et al., 2014).

In a majority of species, mating and dispersals take place during a nuptial flight. There is always a trade off between the costs and benefits of flight. Wing reduction or simplified thoracic structures could be some of the steps associated with the shift in mating and dispersal strategies from nuptial flight and ICF to DCF (Tinaut & Heinze, 1992).

In the past, DCF has been linked with a range of ecological factors, for example habitat patchiness, nest site limitation, food limitation, competition, predation, climate, or nest site instability (Heinze & Tsuji, 1995; Cronin et al., 2013). Several studies suggested also a correlation between the loss of flight capability in sexually active females and the ecological conditions in arid and semi-arid environments (Tinaut & Heinze, 1992; Tinaut, 1994; Heinze & Tsuji, 1995). Unsurprisingly, at least 5 to 8 % of all ant species from Spain or Algeria produce queens incapable of dispersal and mating flights (Tinaut & Heinze, 1992).

In the polymorphic queen of *T. longispinosus* (Roger), dependent-founding queens are smaller than independent-founding queens and exhibited significantly lower flight activity. This reduced flight activity may facilitate returning to the natal nest after mating (Howard & Kennedy, 2007). Our data show that in *T. ansei* sp.n., queens are small and similar to the size of workers. The queen/worker volume has been related to nest founding strategies, independent or dependent (Stille, 1996). For *T. ansei*, this ratio is 1,91 (five workers and five queens from the same locality) and this fits with the dependent foundation (ratio 1.9–2.7), being very far from independent foundation range (ratio 5.1–9.1).

In *T. ansei* sp.n., we found that queens lost their wings within the nest. Espadaler (1997b), studying *T. caesari*, found that seven out of seven queens born in the laboratory lost their wings within two days, and without being fertilized. It is known that the queens that disperse by mating flights do not lose their wings inside the colony (Hölldobler & Wilson, 1990; Heinze & Tsuji, 1995), and the rapid loss of wings in females seems to indicate intranidal mating (Heinze & Tsuji, 1995). Furthermore, *T. ansei* sp.n. seems to be partially polygynous, both in the wild and in the laboratory. Two out of five nests excavated have had two wingless queens, and in the laboratory the three colonies nowadays have seven, six and two wingless queens respectively but this could be caused by laboratory conditions. Finally, we have found three worker-queen intermorphs (with our current data, the ratio intermorphs/workers is 3/32).

Our data shows that the *T. ansei* sp.n. colony founding strategy is likely to be Dependent Colony Foundation. Further research is needed to examine the mating behaviour and to confirm the colony founding strategy of this new species.

Acknowledgements

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General discussion

Previous page: *Crematogaster auberti* (photo credit: Chema Catarineu)

General Discussion

The ant myrmecofauna in the Segura River Basin, at this point, comprises 110 species belonging to 30 genera and five subfamilies (as detailed in Chapter I), but the actual species number is undoubtedly higher. Since the publication of the paper, which corresponds to Chapter I, this inventory has changed. On the one hand, we have found another four ant species and, on the other hand, *Camponotus figaro* Collingwood & Yarrow 1969 were synonymized under *Camponotus piceus* Leach 1825, in a recent taxonomic revision (Seifert, 2019). Therefore, our current check-list reach 113 ant species. However, it is clear that with a larger sampling effort, the study of hypogeal species, and the revision of some complex genera, the number of species will increase. One proof of our incomplete knowledge about our regional ant fauna is the discovery of a new ant species, *Temnothorax ansei* sp.n., described it in Chapter IV. Possibly, *T. ansei* and other species are suffering declines in their populations, in its geographic distribution or are affected by habitat fragmentation. Further research is needed to clarify whether there are ant threatened species in the Segura River Basin, and if so, what are its threatened categories.

At the regional scale, the biodiversity is the result of the evolutionary processes (speciation, extinction and dispersal processes; Swensson, 2011), modulated by the climatic and geological history (Wiens & Donoghue, 2004; Cavender-Bares et al., 2009; Lessard et al., 2011). In the ant species pool of our study area, there are different evolutionary and zoogeographic origins (two zoogeographical zones and 14 zoogeographical elements; see Chapter I), probably linked with the position of the study area between Africa and Europe; the complex geotectonic, paleogeographic, and paleoclimatic history during the last 7 My; the complex geomorphology; and the high climate and habitat diversity.

The climatic oscillations during the Pleistocene Ice Ages are known to have had an important influence on the zoogeographic history of Europe driving the repeated contraction/expansion of the zoogeographical ranges (Hewitt, 2011). We must bear in mind that the Iberian Peninsula was one of the most important refuges in Europe during the Pleistocene Ice Ages (Hewitt, 2000, 2001, 2011; Gómez & Lunt, 2007) and the Baetic ranges, where the Segura River Basin is located, was one of them (Carrión et al., 2003; Gómez & Lunt, 2007; Hewitt, 2011). After the last glacial maximum period (20–14 ky BP) some species have expanded their range northward from their Iberian refugia, or tracked suitable habitats higher up along elevation gradients within mountain ranges (Gómez & Lunt, 2007; Hewitt, 2001).

Two paleogeographical events allowed the exchange of terrestrial organisms between Africa and the Iberian Peninsula. The first event was the separation of parts of the Baetic region from the Iberian mainland and its connection by the south to Africa during the Baetic crisis 16–14 Mya (Veith et al., 2004). The second event was the closure of the Mediterranean-Atlantic connections due to tectonic processes during the Messinian

Salinity Crisis 5.96–5.33 Mya (Veith et al., 2004; Agustí et al., 2006; Hewitt, 2011; Gibert et al., 2013). During these events, ant species were also exchanged, and currently there are 43 species of the Segura Basin ants also present in the Maghreb (accounting for 41% of the recorded species).

At lower spatial scales, the different patterns of biodiversity depends greatly on the environmental filters (Ricklefs, 2004; Cavender-Bares et al., 2009; Swensson, 2011). In the Segura River Basin, we found a general environmental gradient from areas with high elevation, high precipitation, relatively well-vegetated and cool temperatures, to areas with the opposite characteristics (from the NW mountains to the SW-S-SE coasts). This general environmental gradient is an important force structuring ant diversity in our study area.

This general environmental gradient affects the ant species distribution, according to their zoogeographic origins, as we showed in Chapter I. There is a greater proportion of ant species from the mixed and deciduous forest zone in the high-mid altitudes in the Segura River Basin, where the climate is cooler, and more humid. The Euro-Caucasian and Euro-West-Siberian elements have a tendency to be more associated to forest with a higher precipitation, whilst the South Palearctic elements seem to be more associated to ecosystems more similar to the forest-steppe zone with intermediate precipitation. The proportion of ant species from the Mediterranean zone is greater in the lowland areas, and decreases with the altitude, possibly because they are more thermophilic species. More phylogenetical and zoogeographical research about the evolutionary relations of species and their elevational distribution will be important in determining vulnerability to climate change.

Ant species richness (SR) and ant abundance monotonically increase along the general environmental gradient from the lower to the higher areas of the Segura River Basin, as shown in Chapter II. Monotonic increase in ants SR is an uncommon pattern in altitudinal gradients, but has been cited in arid environments (Sanders et al., 2003), related with higher precipitation and primary production in higher areas. The altitude ranges studied in Chapters I–II is 0–1,410 m, and we cannot exclude that there exists a mid-elevation peak above the upper range of our study, taking into account that the summit of the mountains in this area reaches 2,000 m. It is necessary to investigate the SR patterns above altitude range covered in this thesis, and we are already working on it.

Temperature, within the studied range (0-1,410 m), does not seem to be the main driver underlying ant taxonomic diversity. Ants are termophilic species (Hölldobler & Wilson, 1990) and, in other studies, ant SR is positively correlated with temperature (Hawkins et al., 2003), while in the Segura River Basin, the relationship is the opposite. Precipitation and NVDI (normalized difference vegetation; an indicator of vegetation density) seem to be the more important drivers controlling ant taxonomic diversity. Precipitation, in semi-arid areas, is well known to have strong influence on primary production (Paknia & Pfeifer, 2014), and therefore, the semi-arid climate in the Segura River Basin implies that precipitation should be a very important driver for ant diversity, allowing higher primary production in the wetter areas. The higher NDVI indicate greater net primary productivity (Kerr & Ostrovsky, 2003) in the higher altitude areas. When primary productivity is higher, the availability of resources leads to an increase in ant biomass (Clarke & Gaston, 2006). The more-individual hypothesis (Srivastava & Lawton, 1998) predicts that more productive sites can support higher abundance within a community and thus, more species richness, as is the case in our study area.

Ant phylogenetic diversity (PD) tracks ant SR response to the general environmental gradient. Interestingly however, PD increases proportionally more on forests and the underlying cause seems to be that ant species of the Formicinae subfamily has a higher species richness in the forests than in the shrublands, while Myrmicinae SR is very similar in both habitats. Forest patches seems to act as refuges for Formicinae species as localities along the main environmental gradient gets warmer and dryer, where these forests are usually associated to favourable micro and mesoclimatic conditions like north-faced slopes.

There is a shifting pattern of phylogenetic structure along the basic environmental gradient, from phylogenetic overdispersion in the higher areas to phylogenetic clustering in the lower areas. Ant species are less closely related than expected in the higher altitude areas, and more closely related in the lower altitude areas. The transition zone occurs between 220–700 m altitude, 16–17ºC of mean temperature, and 250–400 mm of annual precipitation. Above this transition zone, we detected phylogenetic overdispersion, and under it, we detected phylogenetic clustering. Note that this overdispersion or clustering is referred to a regional standard, and it is strictly relative to the whole phylogenetic structure of the region. Nevertheless, the important point is the matching between trend from 'overdispersion' to 'clustering' and the environmental gradient.

We detected significant phylogenetic signals in the continuous functional traits, so we hypothesized that there is an important degree of niche conservatism in the Segura River Basin ants. This is consistent with Arnan et al. (2016), which found a high degree of niche conservatism evidence in European ant communities. The existence of niche conservatism, allows us to assume that interspecific competition could explain the phylogenetic overdispersion, and environmental filtering could explain the phylogenetic clustering.

Thus, we can state that the harsh conditions of aridity at low elevations areas constitute an environmental filter in our study area that underlies the general phylogenetically clustered structure, nuanced by limiting similarity at the transect scale on localities where environmental filter is more rigorous. The ant species are more closely related than expected by chance, and possibly share phylogenetically conserved traits that allow them to survive in areas with low precipitation, high temperatures and low primary productivity. Delsine et al. (2010) state that aridity could not be stressful enough to constitute an phylogenetic environmental filter for ant communities, bearing in mind that aridity-tolerance traits are widespread in the ant phylogeny, but our results show the opposite in our study case, covering a broad range of precipitation (203–746 mm).

In the higher, cooler and wetter environments of the Segura River Basin, interspecific competition seems to be more important shaping the overdispersed phylogenetic structure, assuming that related taxa compete more intensely and are less likely to coexist (Webb et al., 2002; Cavender-Bares et al., 2009).

Evolutionary and climatic history may also be an underlying cause for the reduced taxonomic and phylogenetic diversity in the lower lands in the Segura River Basin. The climatic oscillations during the Pleistocene Ice Ages had an important influence on the species zoogeography and, after the last glacial period some species tracked their optimal climate upwards, but at the lowest elevations did not exist species to replace those that moved upward (Gómez Lunt, 2007; Szewczyc & McCain, 2016). Other cause is, possibly, the higher level of anthropogenic changes in the low and mid lands than in the higher and better-preserved NW mountains of the Segura River Basin.

The relationships between the diversity indices and the environmental factors, and the effects of the aridity environmental filter in the Segura River Basin are more evident at larger scales because than at lower scales, as shown in Chapter II. At the local scale, species interactions (especially competition), and probably very local environmental factors have a major relevance as community drivers (Cavender-Bares et al., 2009). More research should be carried out with ants and other taxa to disentangle the complex biotic and abiotic factors that structure the different communities at the local scale.

For a better understanding about these local community drivers, In Chapter III, we conduct a local case-study about the effects of a reforestation with *Pinus halepensis* of a semiarid *Stipa tenacissima* grassland in the southern pediment of Sierra del Picarcho (Murcia) on the local ant assemblage. The disturbance caused by the mechanical pine reforestation on vegetation involved the decrease of *Stipa tenacissima* and *Cistaceae* cover and its substitution by pine cover. In this case, ant species richness and abundance increased largely from grassland to reforestation and more species have had a positive response to reforestation than a negative one. The underlying causes of this pattern are mainly related to the vegetation and soil changes and are associated with the increase of pine cover, most probably providing additional food resources to the ant assemblage as well as a higher sheltering facility provided by the increase of superficial and in-ground rock cover caused by the mechanical preparation of the terrain.

Further research is needed to investigate how communities are affected by the different local anthopogenic impacts. Unfortunately, in the Segura River Basin, we can find a wide range of them: deforestation, fire, uncontrolled urban development, expansion of intensive agriculture, inadequate reforestations, soil degradation or habitat fragmentation (Piñero et al., 2011).

The magnitude of the anthropogenic ecosystem changes has accelerated in the last decades and we are now facing a major threat, the Climate Change, which is already occurring and causing dramatic ecosystem changes with important effects on biodiversity (Pecl et al., 2017). The Climate Change effects are not well understood despite its great importance (Doblas-Miranda et al., 2017), but most climate models forecast substantial increases in temperature and declines in precipitation, reducing water availability in the whole Mediterranean Basin (Hoerling et al., 2012; Gupta et al., 2018) and evidences of the worsening conditions are already presented (Valdés-Abellán et al., 2017). We can expect that, in the years to come, the aridity environmental filter that we have detected in Chapter II, enhance its effect and spread his influence area, affecting not only ant communities but also other taxa and the whole ecosystems.

Management for biodiversity conservation requires monitoring ecosystem changes and their effects on the biocenosis, if we want to understand the ongoing ecosystem changes. For this task, ants could be crucial bioindicators in the coming years. We need more ecological studies, at different spatial scales, taking into consideration the three diversity components, Taxonomic, Functional and Phylogenetic, for a better understanding of the structuring factors of the ant communities, and other taxa, in the south east Iberian Peninsula.

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General conclusions

Previous page: *Colobopsis truncata* (photo credit: Alberto Sánchez Martín)

General conclusions

CHAPTER I. Zoogeography of the Ants (Hymenoptera: Formicidae) of the Segura River Basin.

1. We present the first checklist of the ant species of the Segura River Basin, with 110 species that belong to 30 genera. Twenty-two of these species have been recorded for the first time in this area.

2. Ant species were classified into zoogeographical classes and elements. The zoogeographical composition is dominated by the species of the Mediterranean zone (75.2%), followed by the mixed and deciduous forest zone (19.1%). The most important zoogeographic elements are: Iberian (20%), Holomediterranean (17.1%) and West-Mediterranean (13.3%). There are only six cosmopolitan species (5.71%).

3. There is a larger proportion of species from the mixed and deciduous forest zone in the high-mid altitudes in the Segura River Basin, where the climate is cooler, and more humid.

4. The Euro-Caucasian and Euro-West Siberian elements are more associated to forest with a higher precipitation, whilst the South Palearctic elements seem to be more associated to ecosystems more similar to the forest-steppe zone with intermediate precipitation.

5. The existence of different zoogeographic origins in the Segura River Basin is probably linked with: the position between Africa and Europe; the complex geotectonic, paleogeographic, and paleoclimatic history during the last 7 My; the complex geomorphology; and the high climate and habitat diversity.

CHAPTER II. Environmental filtering in a semiarid region underlies ant diversity gradients and phylogenetic structure.

1. There is a general environmental gradient in the Segura River Basin, which is an important force structuring ant diversity, from the higher, colder, wetter and better vegetated areas to the lower, hotter, dryer and poorly vegetated areas.

2. Precipitation seems to be the more important environmental factor controlling ant taxonomic diversity, allowing a higher primary production and ant diversity in the wetter areas.

3. Ant taxonomic diversity is highly correlated with phylogenetic diversity, but the correlation with functional diversity is weaker.

4. Two dominant subfamilies on the area respond differently to the main environmental gradient, Formicinae being much more sensitive than Myrmicinae. These differences in sensitivity are behind important changes on phylogenetic diversity and structure of the communities along the general environmental gradient.

5. Phylogenetic diversity structure is overdispersed towards the higher, colder, wetter areas and clustered towards the lower, hotter and dryer areas.

6. As we detected niche conservatism, we hypothesized that the harsh conditions of aridity in the low areas constitute an environmental filter clustering the phylogenetic structure, while the interspecific competition could be the main force shaping the overdispersed phylogenetic structure on the other extreme of the gradient.

7. The relationships between the ant diversity indices and the environmental factors, and the effects of the aridity environmental filter, are more evident at the coarser spatial scale because, but at the finer scale, species interactions (especially competition) and probably very local environmental factors have a major relevance as community drivers.

CHAPTER III. Effect of pine reforestation associated with soil disturbance on ant assemblages (Hymenoptera: Formicidae) in a semiarid steppe.

1. The disturbance of the vegetation caused by reforestation resulted in a decrease in the cover of *Stipa tenacissima* and Cistaceae and an increase in the cover of *Pinus halepensis.*

2. The mechanical preparation of the site also resulted in changes in the soil surface, with an increase in the cover of stones and rocks.

3. Ant species richness and abundance were larger in the reforestation than in the grassland and more species showed a positive than a negative response to reforestation.

4. The underlying causes of the increase in the richness and abundance of ants, in the reforested site, are mainly related to changes in vegetation and structure of the soil surface. The increase in the cover of pine, probably provided additional food resources, and the greater cover of stones and rocks provided more shelter for the ant assemblage.

CHAPTER IV. A New Ant Species, *Temnothorax ansei* sp.n. (Hymenoptera: Formicidae) from the Arid Environments of South-eastern Spain.

1. We describe a new ant species in the genus *Temnothorax*, species group *laurae*, based on morphological evidence supported by other biological information. *Temnothorax ansei* sp.n. is a member of the *laurae* species group based on the following characters: large eyes, long and erect hairs on alitrunk, petiole and postpetiole, rectangular head and metanotal groove. Other characters unique to new species are: dark brown in color, decumbent pilosity, and smooth and shiny head and pronotum. In addition, this new species is distinctly nocturnal in its activity patterns.

2. This new species has been discovered at two locations in southeastern Spain: Pulpí (Almería) and Lorca (Murcia), in semiarid habitats with sparse vegetation.

3. Based on the morphological measurements of the queens and the workers, as well as our observations in the laboratory, we predict that this species has a dependent colony founding.

4. We provide an identification key to the Iberian Peninsula species of the *laurae* species group.

Global change and conservation problems.

Over thousands of years, the Segura River Basin has suffered the effects of deforestation, fire and pastoralism. During the last decades, the area has suffered dramatic ecosystem changes caused by uncontrolled urban development, expansion of intensive agriculture, abandonment of extensive livestock, inadequate reforestations, soil degradation, habitat fragmentation, and a deficiency in the conservation management (Piñero et al., 2011). Climate change, in combination with other anthropogenic changes, is already leading and will cause ecosystem changes towards more arid states, biodiversity loss, and other unpredictable alterations (Doblas-Miranda et al., 2017).

In this thesis, we have detected an environmental filter, which is an important force structuring ant diversity, in the lower, hotter, dryer and poorly vegetated areas in the Segura River Basin. Climate change will reinforce this filter affecting not only the ant communities, but probably also the structure and functioning of terrestrial ecosystems. Furthermore, other anthropogenic changes, can also cause different ecological effects hardly predictable. We have observed that a reforestation can lead deep changes in the ant communities through unexpected mechanisms as the increase in stoniness.

Management for the biodiversity conservation require monitoring the ecosystem changes and their effects on the biocenosis. For this task, ants could be crucial bioindicators in the coming years.

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Acknowledgements

Why ants?

I suppose that all the naturalists, at some time, have shown interest in these amazing social insects and most of us have over the years, knelt down to see ants foraging, carrying food, hunting, or fighting. Therefore, it is not surprising that we have learnt how to identify some of the more conspicuous species. I have been an irretrievable naturalist since the day I was born, so I have always been interested in ants. This interest increased greatly about ten years ago when I had the opportunity to read “Journey to the ants: a story of scientific exploration”, a magnificent book written by Bert Hölldobler and Edward O. Wilson. The reading of this book impelled me to seek further information about ants in other books, papers or web pages. Coincidentally, I connected to an online forum called “La Marabunta” where I found out about the existence of the AIM (“Asociación Ibérica de Mirmecología”), which was on the point of hold the fifth Iberian Mirmecology Congress in Ronda (Málaga, south Spain). Without hesitating, I registered in that Congress called “Taxomara 2010”.

In the Ronda´s Congress, I met interesting people including myrmecologists professionals and amateurs. The experience was really positive and enriching, and the enthusiastic family comprising the AIM association provided me with their own passion for ants. In that unforgettable congress, Dr. Xavier Espadaler proposed to implement a training course on ant identification with only one student per course. I was one of the lucky ones and, during an intensive week in the Autonomous University of Barcelona, Dr. Xavier Espadaler taught me how to identify Iberian ants. Never had I learnt so much in such a short time. Therefore, I am very grateful to Dr. Espadaler, not only for that week, but for the numerous times I asked him by email about species identification difficulties. He always answered my questions providing me clear and valuable information.

I also contacted Dr. Alberto Tinaut, Professor of Zoology at the Granada University, who is another of the well-known experts in Iberian Mirmecology. I asked him for his collaboration in an investigation about the ants of the Región de Murcia, and he kindly accepted. I learnt a lot from him throughout that research period, and he helped me several times to solve my own doubts on ant taxonomy. Therefore, I am also very grateful to him for his valuable help and support.

Encouraged by this new passion, I decided to study for a PhD regarding the Segura River Basin ants. I started studying for a Master’s Degree at the Faculty of Biology of the University of Murcia. This was a very enjoyable time for me returning to my Faculty after so many years, and I should like to sincerely thank all the great teachers within different

subject areas for their effort and support, some of whom have been my friends for a very long time. Special thanks to Rosa Gómez Cerezo, for her cooperation and good advices.

Since the Ronda´s Congress, I attended a total of nine of the AIM annual meetings. I learnt a lot during such events, which included communications, field trips and ant identification in the laboratory. I want to thank AIM, as an association, and I extend my thanks to all the participants in the “Taxomaras”.

In 2016, the AIM, with the collaboration of Asociación de Naturalistas del Sureste (ANSE) and the Faculty of Biology of the University of Murcia, organized the XI Iberian Mirmecology Congress in Murcia. I want to thank the financial aid of Fundación Séneca and the valuable assistance of the professors Francisco Robledano Aymerich y Eulalia Clemente Espinosa.

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Special heartfelt thanks to my wife, Nuria, for her support, love and understanding. It has not been easy to harmonize my PhD with our jobs, and with the responsibilities of a family with three children of school-going age, but she always made it possible. She helped me with my field work and also with the English revisions. Thank you for being who you are. Finally, I want to thank my children Sofía, Gonzalo and Leo for accompanying me to the ant field sampling. I also want to apologise for not enjoying more time with them throughout the last years. I look forward to spending more time with all of them in the coming years.

Chema Catarineu, 20 May 2019