

RESEARCH

Subterranean Ants: The Case of *Aphaenogaster cardenai* (Hymenoptera: Formicidae)Vicente M. Ortuño,^{1,2} José D. Gilgado,¹ and Alberto Tinaut³¹Departamento de Ciencias de la Vida, Facultad de Biología, Ciencias Ambientales y Química, Universidad de Alcalá, E-28871, Alcalá de Henares, Madrid, Spain²Corresponding author, e-mail: vicente.ortuno@uah.es³Departamento de Zoología, Facultad de Ciencias, Universidad de Granada, E-18071, Granada, Spain

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ABSTRACT. Recently, a series of systematized studies of the Milieu Souterrain Superficiel (MSS) are being carried in several enclaves of the Iberian Peninsula, which have entailed the finding of the enigmatic ant *Aphaenogaster cardenai* Espadaler, 1981, hitherto considered as hypogean, in a mountain range far away from its known distribution area. Its ecological role and its possible area of distribution are discussed due to this finding, as well as its known morphology, distribution, habitat use, flight ability of the sexual forms, and moment of activity. This enabled reviewing and discussing the actual knowledge on the possible adaptations and exaptations of the Formicidae to the subterranean environments in wide sense and concretely to the MSS. According to all above, ants might adapt to the deepest hypogean environments by means of changes in their social structure, but without those changes, the MSS would be their last frontier in their process of colonization of hypogean environments.

RESUMEN. En la actualidad, una serie de estudios sistematizados en el Medio Subterráneo Superficial (MSS), se están llevando a cabo en diversos enclaves de la península Ibérica, lo que ha propiciado el descubrimiento de la enigmática hormiga *Aphaenogaster cardenai* Espadaler, 1981 en un macizo montañoso muy alejado de su área de distribución conocida. Esta especie ha sido considerada, hasta el momento, como una especie hipogea. Se discute su rol ecológico y su posible área de distribución real de acuerdo con este nuevo hallazgo, así como la morfología de las castas conocidas, corología, capacidad de vuelo de las formas sexuadas y su período de actividad. Esto ha permitido revisar y discutir el estado actual del conocimiento sobre las posibles adaptaciones y exaptaciones de los Formicidae a los ambientes subterráneos (sensu lato), y concretamente al MSS. De acuerdo con todo lo anterior, las hormigas podrían adaptarse a los ambientes hipogeos más profundos mediante la modificación de su estructura social, pero sin dichos cambios, el MSS sería su última frontera en su proceso de colonización de los ambientes hipogeos.

Key Words: hypogean ant, subterranean biology, shallow subterranean habitat, Iberian Peninsula

The habitat first described as Milieu Souterrain Superficiel and widely known as MSS (Fig. 1) (Juberthie et al. 1980; Juberthie 1981, 1983, 2000; Vailati 1988; Giachino and Vailati 2005, 2006; among others) is a hypogean environment generally formed by the fragmentation of the bedrock and accumulation of debris, which contains a wide network of small voids and fissures. It has been translated to English in different ways, e.g., Culver (2001) and Culver and White (2004) adopted “Mesocavernous” and “Mesovoid Shallow Substratum”, a terminology still used, to preserve the acronym MSS, well known and established in the literature (Hlaváč et al. 2006, López and Oromí 2010, Nitzu et al. 2010, Rendoš et al. 2012, among others). However, other authors use the more accurate translation “Superficial Subterranean Environment” (Camacho 1992), “Superficial Subterranean Habitat,” or “Shallow Subterranean Habitat” (SSH) (Howarth 1983, Culver et al. 2012) but suggest to preserve the acronym MSS (Giachino and Vailati, 2010). However, according to Pipan et al. (2011), the MSS would be only one type of SSH, and therefore, it would be confusing using SSH and MSS as synonyms. Other authors use “Superficial Underground Compartment” or SUC (Deltshev et al. 2011). Therefore, henceforth, we are maintaining the well-known acronym MSS to avoid confusion.

This environment has been long described as an optimum habitat for troglolithic forms, but it is also a suitable habitat for other facultative organisms (Gers 1992, Ortuño and Toribio 1994, Hernando et al. 1999). Studies that have been published in the Ibero-Balearic area are almost anecdotic (Hernando et al. 1999; Galán 2001, 2003; Galán and Nieto 2011), and the results have usually been presented in the form of a brief reference in faunistic works (Ortuño 1996: 194), in relation to the discovery of some new hypogean taxa (Fresneda and Hernando 1994, Fresneda et al. 1997, Toribio and Rodríguez 1997, Carabajal

et al. 1999, Fresneda and Escolà 2001, Ortuño and Toribio 2005, among others) or new localities for certain epigeal species (Ortuño and Toribio 1994).

Recent samplings in the Iberian Peninsula have revealed the presence of *Aphaenogaster cardenai* Espadaler, 1981 in a siliceous MSS of the Sierra de Guadarrama (Madrid, Spain) (Fig. 2).

A. cardenai had already been found in caves and in the MSS (sensu Juberthie 1983) in both dolomitic and siliceous areas of Andalusia and Extremadura (southern Iberian Peninsula) (Tinaut and Pérez 2011). The presence of this ant in the MSS has been confirmed from diverse and sporadic findings, and as a result, it has been considered a species that belongs mostly to the MSS (Tinaut and López 2001). It is also possible that, as Bernard (1968) proposed for other species of the same genus, it inhabits both the MSS and the fissure network of the bedrock (mesocaverns sensu Howarth 1983).

The finding reported here, hundreds of kilometers away from the localities where the species is known to inhabit, notably widens its distribution area (Fig. 2) and raises a series of questions about its biology, distribution and habitat use, as well as reflections on the possible hypogean nature of some ants, in general, and this Iberian ant in particular. But, what do we exactly mean by “hypogean”?

In the field of subterranean biology, the terms “subterranean,” “hypogean,” and “endogean” have been used with different meanings (Fig. 1) by different authors (see e.g., Racovitza 1907; Vandel 1964; Ginet and Decu 1977; Howarth 1983; Barr and Holsinger 1985; Bellés 1987; Camacho 1992; Gers 1992; Juberthie and Decu 1994; Casale et al. 1998; Juberthie 2000; Gilbert and Deharveng 2002; Sket 2004, 2008; Culver and White 2004; Trajano and Bichuette 2006; Romero 2009; Giachino and Vailati 2010; Moseley 2010; Ortuño and Gilgado 2010;

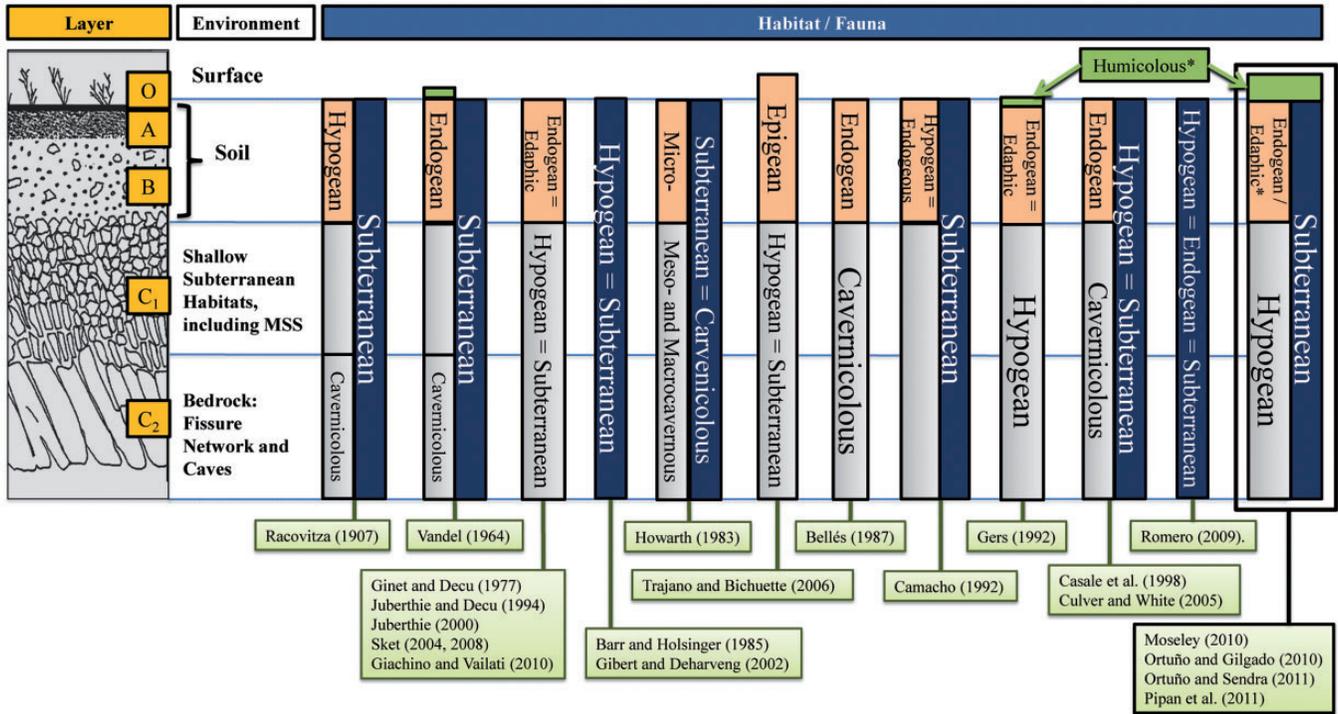


Fig. 1. Cross-section of the soil/subsoil layers, with the corresponding ecological environment and typology of the fauna, according to different authors. The black frame indicates the suggested criterion. The asterisk indicates that it is not clear whether all authors have the same criterion, because humicolous and edaphic are in a diffuse boundary among the surface and horizons O and A.

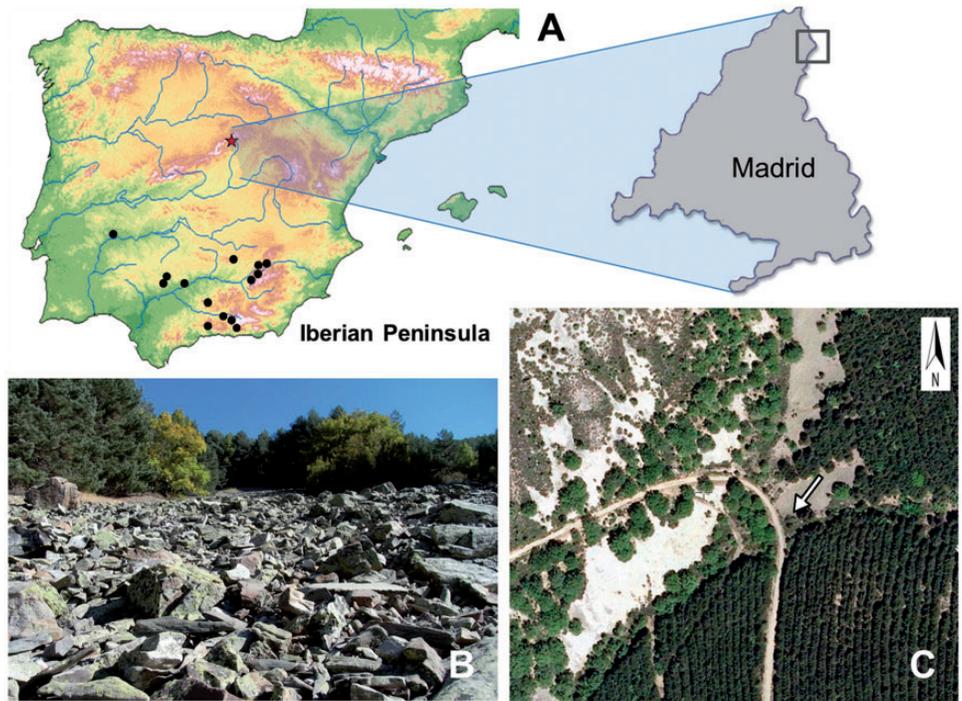


Fig. 2. Location area of the new population of *A. cardenai*. (A) Map with the known localities (black circle) and the new location (red star). (B) Image of the siliceous scree. (C) Location of the scree (satellite image obtained from the SIGPAC – M.M.A.M.R.M 2012).

Ortuño and Sendra 2011; Pipan et al. 2011). In the same way, myrmecologists have also used those terms with different meanings (see e.g.: Wheeler 1910, Bolton 1988, Roncin and Deharveng 2003, Passera and Aron 2005, Ryder Wilkie et al. 2007, Tinaut and Bensusan 2011).

Etymologically, both terms “Hypogean” and “Subterranean” have the same origin in greek and latin (Hypo = Sub = Below, under; and Geo = Terra = earth, ground) but also two correct possible meanings: 1) below the soil layer (i.e., below horizons A and B) and 2) below the surface (both within and below the horizons A and B). On the other hand, “Endogean” is the etymologically correct greek word (Endo = Inside) for the soil horizons A and B, but no author uses an equivalent latin expression (i.e. “Intraterranean”). Thus, the greek words “Hypogean” and “Endogean” would differentiate two different environments, whereas the latin word “Subterranean” would not. In this sense, we suggest following the criterion of considering both “Endogean” and “Hypogean” as “Subterranean” environments (Fig. 1), and “Epigeal” (Epi = Over) for surface habitats. Although those terms should be restricted to those species or populations that complete their life cycle underground (Ortuño and Gilgado 2010), the particular social division into castes of ants deserves especial attention. If the majority of the colony (the workers) spend their whole lifetime foraging in the “endogean” or “hypogean” environment, but the reproductive forms depend on the surface, they would have an “endogean” or “hypogean” strategy, respectively, even if those species or populations cannot be strictly considered endogean or hypogean as a whole.

Regarding the genus *Aphaenogaster* Mayr, 1853, there are three groups of species with different body designs that match those different lifestyles or strategies in the Mediterranean region: 1) “Epigeal” species: These species exhibit a dark-colored integument and have a slender appearance due to their elongated body and appendages (e.g., *Aphaenogaster iberica*) (Fig. 3A). 2) “Endogean” species: which are characterized by workers of small size, short appendages, microphthalmia, and slightly pigmented integument such as *Aphaenogaster dulcinea* (Fig. 3B), *Aphaenogaster subterranea*, and *Aphaenogaster subterranoidea*. 3) “Hypogean” species, a group represented by *A. cardenai* (Fig. 3C). This species is slender, has long appendages, and exhibits a body plan typical of the epigeal species; however, its amber color and very small eyes correspond with endogean species. This combination of characters seem to suggest a lifestyle adapted to hypogean environments, where the interstices and voids are bigger than in the soil layers (Fig. 1), and therefore where longer appendages are useful (Racovitza 1907: 411, Howarth 1983, Hüppop 2000, Ortuño and Gilgado 2010, Ortuño 2011).

The hypogean environment poses several difficulties for life and adaptation of the Formicidae (Tinaut and López 2001) in relation to their particular mode of social life and their morphological and biological diversification into castes. Wilson (1962) was the first author to discuss the difficulties for ants to be “cavernicolous,” considering their population densities, their mode of reproduction and the scarcity of trophic resources usually available in caves. He presented an interesting reasoning for the strategies that they could adopt to live in large underground spaces and suggested that ants could never really be troglotic because they are unable to maintain sufficiently large cave demes. He sums up stating that the adaptation of the ants to these environments should be associated with a relative increase in the number of queens and a decrease in the number of workers.

In this article, the relationship between *A. cardenai* and the MSS, the factors involved in its dispersal and the adaptations of the Formicidae to the hypogean environments will be discussed.

Materials and Methods

The locality where the ants were captured is a scree (Fig. 2) situated at 1,490 a.s.l., at the south-eastern orientation of the peaks “Cabeza Minga” and “Tornera” (Puebla de la Sierra, Madrid, Spain), UTM: 30TVL64. This area is included in the Network of Biosphere Reserves

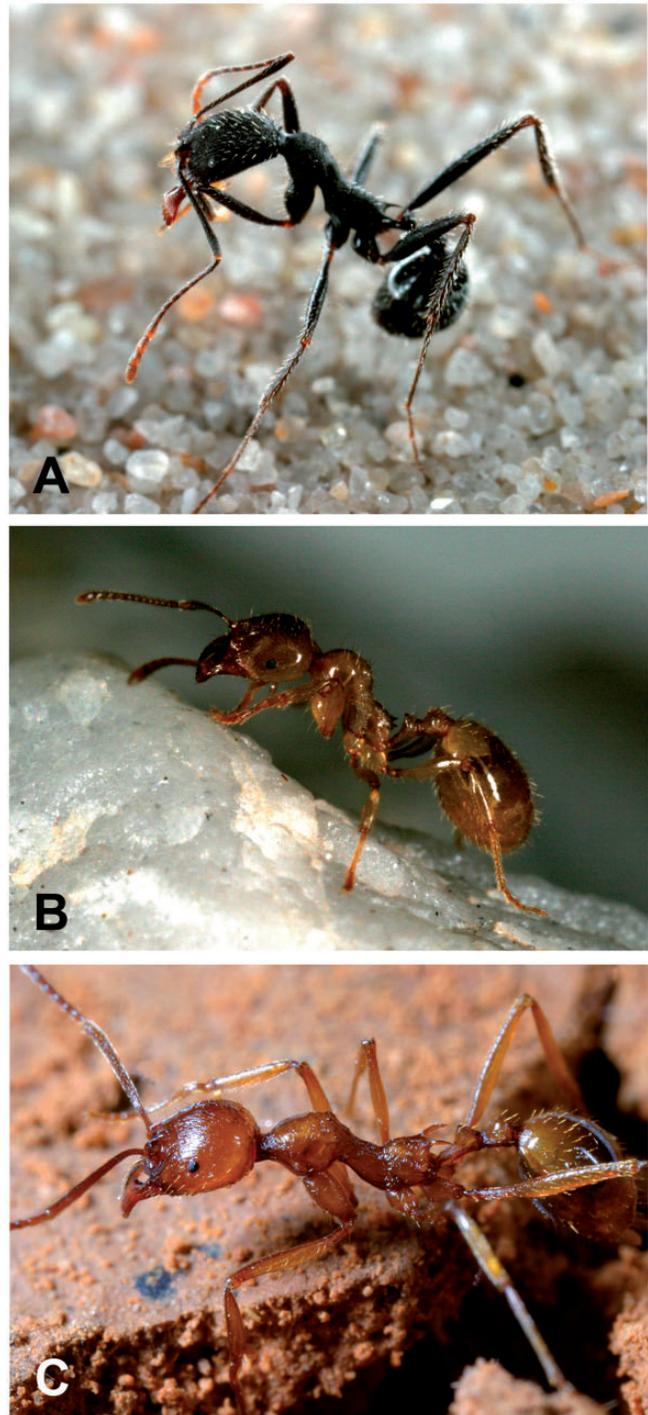


Fig. 3. Photographs of *Aphaenogaster*. (A) *A. iberica* showing an example of the morphotype of a member of the Formicidae with epigeal activity. (B) *A. dulcinea* showing an example of the morphotype of a member of the Formicidae with endogean activity. (C) *A. cardenai* showing an example of the morphotype of a member of the Formicidae with hypogean activity.

(Leralta 2010). The scree comprises the debris of Ordovician metamorphic rocks, mainly slaty schists and quartzites (Abati et al. 2009), and it is surrounded by an environment where sandstones and plutonic rocks such as granite are present. As a result, the vegetation is typical of siliceous substrates. The forests surrounding the scree contain *Quercus pyrenaica* Willd. with reforestation pines of the species *Pinus sylvestris*

L. The dominant species in the undergrowth are *Cistus ladanifer* L., *Lavandula stoechas* L., and *Erica arborea* L.

A pitfall trap was set at 90 cm deep in the ground, with brine as a conservator. It was baited with fermented cheese in a vial, as described by [Giachino and Vailati \(2010\)](#). The trap was installed for 4 mo and was collected on 30 June 2011. Specimens collected are deposited in the collection of Vicente M. Ortuño (University of Alcalá, Spain) and Alberto Tinaut (University of Granada, Spain), references 9,470 and 9,471.

Results

Eight *Aphaenogaster* worker specimens were collected in the subterranean pitfall trap. They were compared with specimens of *A. cardenai* from diverse Andalusian localities and identified with no doubt as the same species. The remaining fauna collected in the same trap were epigean species. These fauna included hygrobic species of the coleopteran Carabidae family like *Trechus schaufussi*, *Platyderus montanellus*, and *Nebria salina*. Also diptera of several families such as Phoridae and two species of *Megaselia*; a species of the family Hybotidae, probably of the genus *Drapetis*; and one species of Sciaridae. There were also other Formicidae, like *Lasius grandis*, *Camponotus pilicornis*, and *Camponotus cruentatus*. Additional fauna included *Nemobius interstitialis* of the Orthoptera Gryllidae; and finally, *Palpimanus gibbulus* of the Aranei Palpimanidae.

Discussion

Hypogean Environments and Subterranean Ants. The first question to consider is whether *A. cardenai* is a truly hypogean ant. Although a winged male of *A. cardenai* was captured in the epigean environment attracted by a light trap ([Tinaut 1985](#)), the workers have always been captured in a subterranean environment (in at least 12 localities), except for two specimens ([Reyes-López et al. 2008](#)). Following the ecological classification of [Sket \(2008\)](#) for cave fauna (troglo=cave), this species would not be a “troglobiont” and might be included in an equivalent category to the term “subtroglophile” (first proposed by [Ruffo 1957](#)). Thus, according to its eventual presence above the ground, the term “hypogean” should not be strictly applied to *A. cardenai*, even neither for any other hitherto known species of ant, although as stated above, the workers of this species would follow a “hypogean” strategy.

However, there are two species that might be strictly hypogean. The first one is the species *Hypoponera ragusai*, which has a similar demographic structure to that proposed by [Wilson \(1962\)](#) for a hypothetical cave adaptation but is also found outside of caves ([Tinaut 2001](#)). Because insects can have different cavernicolous habits, depending on latitude ([Bellés 1987](#)) it would be more accurate considering that this species comprises hypogean populations but it is not hypogean as a whole.

The second species is *Leptogenys khammouanensis*, discovered in deep zones of two caves in Laos ([Roncin and Deharveng 2003](#)). This species exhibits a set of morphological characters, which are convergent with those of other strictly hypogean species belonging to other groups of arthropods. However, confirmation is needed to discern whether this species of ant is strictly hypogean, given the lack of information about the sexual forms and the demographic structure of the colonies.

To understand why morphology alone is not enough to discern if a species of ant is strictly hypogean or not, it is necessary to introduce a brief discussion about this issue. It has been discussed that some typical adaptations of the troglolobiotic fauna in general, such as a reduction (or absence) of the eyes, elongated appendages, and depigmentation may not be strictly correlated with a hypogean way of life ([Desutter-Grandcolas 1997](#)). In the particular case of the ants, those characteristics are even relatively frequent in epigean ant species. Regarding the eye reduction or absence, according to [Tinaut and López \(2001\)](#),

the complex system of communication based on pheromones would compensate for a lack of vision as actually happens in eyeless epigean army ants and termites with no significantly longer appendages. However, because those groups of ants with reduced (or absent) eyes are not the evolutionary origin of most ant species with endogean or hypogean habits (except for the European Ponerinae), anophthalmia or eye reduction cannot be considered as a preadaptation to hypogean environments.

In a similar way, [Tinaut and López \(2001\)](#) indicated that the apterism of the sexual forms would be a hypogean adaptation in the Formicidae. Nevertheless, although this character could be favored in subterranean habitats, it does not have to be an exclusive adaptation to those environments. At this point, one can take again the example of *H. ragusai*, which shows epigean populations, but with apterous sexual forms ([Tinaut 2001](#)). Moreover, as other authors have already pointed out, apterism and brachypterism in ants could be explained due to other circumstances such as a change in the reproductive strategy typical of the heteromorphic colonies with the formation of new societies near the maternal nest ([Winter and Buschinger 1986](#), [Heinze 1989](#), [Heinze and Buschinger 1989](#)); a change in the dispersal strategy in islands or desert borders ([Bolton 1986](#)) or other biological or ecological factors unrelated to the individual as, e.g., the aridity of the environment ([Tinaut and Heinze 1992](#)). Perhaps, it would be more accurate to consider anophthalmia or eye reduction and apterism or brachypterism as exaptations to subterranean environments.

In contrast to the difficulties faced by ants in adapting to the deepest subterranean environments (most of the caves, potholes, and the network of cracks and fissures that are embedded in the bedrock), the greatest number of findings of *A. cardenai* have been made in superficial hypogean environments, including epithelial caves (close to the surface) and the MSS ([Reyes-López et al. 2008](#), [Tinaut and Pérez 2011](#)). This suggests that, although its presence in caves is not anecdotic, the MSS and the most shallow fissure network would provide the optimum conditions for the survival of this species. This preference for shallow hypogean habitats would concur with the arguments of [Wilson \(1962\)](#) in relation to the scarcity of resources in caves, who noted that this would preclude the stabilization of a high enough number of colonies. Both the MSS and the fissure network usually contain more organic matter than the deep hypogean environments ([Gers 1998](#)). This availability of nutrients would be a key factor in the formation of a high enough number of colonies, which would guarantee genetically healthy populations. Another important characteristic of the MSS that would make it a preferential habitat for *A. cardenai*, in contrast with deep hypogean environments, would be the easy access to the surface once the time for reproduction and dispersal arrived, since the sexual forms are winged.

The Distribution of *A. cardenai*. Another relevant topic with this new finding of *A. cardenai* is its expected distribution. Given the scarce information available on its distribution and habitat use, three issues should be taken into consideration in relation to the amplitude and the continuity of the area of distribution of this species: 1) the fact that sexual forms are able to fly; 2) the absence of intensive and systematized studies of the MSS, which leads to a lack of information about the faunistic communities that inhabit it; and 3) the possible bias in the study of this species because of the subterranean habits of the workers and the probably nocturnal habits of the sexual forms.

In relation to the winged sexual forms, it is known that the male *A. cardenai*, described by [Tinaut \(1985\)](#), has two pairs of functional wings and that these sexual forms must copulate after a nuptial flight. Although the female has not been described yet, a specimen was captured that very probably belongs to this species (A.T., personal observations). This specimen has a robust thorax with the responsible musculature of the beating of the two functional pairs of wings. As is the case with other species, the female is responsible for finding a new nest for the colony. Undoubtedly, this capability of flying would have enabled the species to disperse and increase its distribution area by

colonizing interconnected subterranean habitats and decreasing the risk of the consanguinity of the colonies.

The displacement capability through flight of *A. cardenai* remains unknown; however, it can be assumed that the real flight distance of *A. cardenai* does not exceed a few kilometers, according to the known data for other species belonging to the Formicidae (Markin et al. 1971, Vogt et al. 2000), which would be enough to colonize other new subterranean spaces even located in diverse mountain ranges. All the above supports the hypothesis that the distribution of *A. cardenai* must be considerably larger than is currently known. However, caves and potholes have been studied for more than a century in the Iberian Peninsula, and this species has not been observed in places other than Andalusia and Extremadura so far, (e.g., the Spanish Levant, Cantabric mountains or the Iberian System mountain ranges), suggesting that *A. cardenai* is absent in those regions, even in the MSS and in mesocaverns in contact with caves.

In relation to the second issue, i.e., the lack of intensive and systematized studies of the shallow subterranean environments, there is little information on the Iberian MSS, especially in nonkarstic areas. For this reason, the possibility of a connection between this new population and the other in the south of Iberian Peninsula cannot be rejected. Thus, intensive research of the Iberian MSSs might reveal new localities and shed light on its real distribution area.

The third issue is deduced from the capture of a winged male in a light trap (Tinaut 1985), in addition to the absence of other records of sexual forms of this species. This fact suggests that the nuptial flight of *A. cardenai* might take place in the night. Roncin and Deharveng (2003) suggested that this ant might have a nocturnal nature rather than a subterranean one. Although this hypothesis was based on observations of the behavior of other species of *Aphaenogaster* and not *A. cardenai*, it is possible that the workers might occasionally ascend in the night to the most superficial zones of the MSS or even to the surface. Two records of surface captures in pitfall traps provide support for this hypothesis, one on the shores of two small rivers in the Sierra Morena Mountain Range (Cordoba) (Reyes-López et al. 2008) near a place where this species had already been found digging a hole in the ground. However, in other locality where this species had been previously found, several groups of 20 pitfall traps were installed in the same zone, monthly during a year, but with no positive results with respect to *A. cardenai* (González Moliné et al. 1988). This study, in addition to other numerous faunistic studies with pitfall traps where this species has not been found, demonstrates that the epigeal activity of the workers must be minimal. However, in regard to the sexual forms, new records in light traps will surely appear, and they will contribute to improve our understanding of the distribution of this species.

It can be concluded that, regarding the species as a whole, *A. cardenai* could hardly be classified as hypogean because the sexual forms perform the nuptial flight outside the subterranean environment. Nevertheless, regarding the workers, which make up the majority of the colony, it seems clear that they are bound to the hypogean environment, especially to the MSS or to the shallow or superficial zones of the crack or fissure network, and therefore, they follow a hypogean strategy. These areas are probably its ecological optima. Thus, *A. cardenai* can be added to the list of arthropod taxa which are anecdotic in caves but prevalent in the MSS, its real biotope (Casale and Rondolini 1983, Juberthie 1983, Ortuño 1996, Fresneda et al. 1997, among others). This fact shows that the MSS is not only a transitional zone between the epigeal and deep hypogean environment but a particular habitat by itself (Juberthie 1983: 44; Galán 2001), and its further research will shed light on the biogeography of several species. Furthermore, it should be noted that the MSS is a habitat where epigeal ants are usually found foraging or exploring. Nevertheless, according to all above, the MSS and some epithelial (superficial) caves might be the deepest hypogean environment where ants can be adapted to live while they still maintain their status of a social insect with their typical population structure, reproduction, and

unique biological characteristics. On the other hand, it should be remembered that neither apterism (nor brachypterism) in sexual forms nor anophthalmia (nor eye reduction), depigmentation nor elongation of appendages in sexual forms or workers can be strictly considered characters that indicate an adaptation to hypogean environments for ants. Those characters should be considered exaptations that might be favorable in the conquest of subterranean environments. True adaptations of the ants to hypogean environments should be in consonance with changes in the population structure of the colony and their reproductive strategy (Wilson 1962).

In the light of the findings presented herein, we agree with Ryder Wilkie et al. (2007) who stated that ants with a tendency to subterranean life may in fact be the “final frontier” in the study of the biodiversity of the Formicidae.

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