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RESEARCH ARTICLE

# Substantial, and significant, expansion of ant hosts range for *Myrmicinosporidium* Hölldobler, 1933 (Fungi)

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**Abstract** Six new genera, three tribes and one subfamily are added to the list of known hosts for the enigmatic endoparasitic fungus *Myrmicinosporidium* Hölldobler. *Aphaenogaster senilis*, *Cataglyphis hispanica*, *Crematogaster auberti*, *Goniomma hispanicum*, *Messor barbarus*, *Tapinoma nigerrimum* and *Tapinoma simrothi* were collected from olive groves and detected as infected with spores of the fungus. *Pheidole pallidula* and *Tetramorium semilaeve* were also found to be infected. The finding of seven hosts (seven genera, three subfamilies) from a single olive grove is an evidence that the fungus has a phylogenetically wide host spectrum and is, therefore, a generalist microparasite. Portugal is also a new country for *Myrmicinosporidium*.

**Keywords** Endoparasitic fungus · Olive grove · Portugal · Dolichoderinae · New hosts

## Introduction

Schmid-Hempel (1998) noted a general lack of data of parasites and diseases in social insects. This is being currently addressed at theoretical level (Boomsma et al., 2005; Cremer et al., 2007) and supported with field data and experiments (Ugelvig and Cremer, 2007; Ugelvig et al., 2010; Reber and Chapuisat, 2011). A recent review of Holarctic entomoparasitic fungi on ants (Espadaler and Santamaria, 2012) also covered briefly an endoparasitic fungus, *Myrmicinosporidium.* However, subsequent work revealed an enormous extension of the host range of this fungus. A study of ants collected in pitfall traps in olive groves from Portugal showed new data that already rendered the previous study out of date. The subject is an enigmatic endoparasitic fungus, *Myrmicinosporidium durum* Hölldobler, whose lifecycle is only partly known (Sánchez-Peña et al., 1993) and that seems to be infrequently detected (Buschinger et al., 2004). The spores (diameter 47–61 µm) show a highly characteristic concave shape that is an artefact of alcohol fixation (Buschinger and Winter 1983); otherwise they are biconcave. Ant workers, queens and males may be infected, although behaviour seems to be only slightly impaired: infected workers are usually captured in pitfall traps, thus, foraging. Infected queens have also been captured in swarming (Buschinger et al., 2004). Those fungi seem to be close to Chytridiomycetes (Sánchez-Peña et al., 1993), although it remains to be studied where they belong within the fungi phylogeny.

We upgrade the previous knowledge with six new genera, three tribes, and one subfamily, which are added here to the list of known ant hosts. The fungus is also new for Portugal.

## Materials and methods

For a wider project seeking to explore the usefulness of arthropods as tools for assessing the impact of agricultural

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practices, pitfall traps were used to characterize the ant assemblages of olive groves subjected to different management schemes. The study was carried out in Baixo Alentejo, Portugal in eight olive groves using intensive organic (O; $n = 4$) low density (LD; $n = 2$) and intensive high density (HD; $n = 2$) management systems (Table 1). Sixteen pitfall traps per olive grove, opened 1 week per month, for 3 months (June, September, November), were used to characterize the ant assemblages of olive groves subjected to the three different management schemes. The large olive groves are surrounded by a matrix of arable land and *Quercus* forest fragments. The climate is continental Mediterranean, with 605 mm of annual rainfall. Mean monthly temperatures reach a high of 32.3 °C in July and a low of 5.4 °C in January.

Ants were identified under a dissection microscope with a cold light source, using standard references (Collingwood and Prince, 1998; Seifert, 1992) and compared with our own collections. During the identification process, spores of the fungus *Myrmicinosporidium* were immediately detected in the first sample, which contained *Tetramorium semilaeve* (André) and *Messor barbarus* (Linnaeus). Spores were directly seen inside the legs and the distended gaster of the yellowish *Tetramorium* and also in the extremely dark gaster of the otherwise normally coloured *Messor*. After this discovery, the gaster of all specimens from species with dark cuticles was punched with forceps. If infected, spores appeared immediately out of the gaster. Light yellow coloured ants were directly inspected for spores. Samples are preserved at the Polytechnic Institute of Beja.

## Results

*Myrmicinosporidium* infected workers were detected in 4 out of 8 olive groves, and in 16 samples out of 384. Those samples comprised 2,093 workers of which 79 had spores of the fungus (Table 2). Spores were detected only in June samples (8.VI.2011, leg. C. Gonçalves). No spores were

**Table 1** Characteristics of studied olive groves ($n = 8$) from Baixo Alentejo (Portugal)

| Management system | Irrigation | Pitfall traps with infected ants (from 48 per field) | Ants captured in all traps | Ants captured from traps with infected ants | Ants infected |
|---|---|---|---|---|---|
| Organic | Yes | 1 | 83 | 18 | 3 |
| Organic | Yes | 5 | 139 | 94 | 40 |
| Organic | No | 0 | 260 | 0 | 0 |
| Organic | No | 9 | 180 | 139 | 35 |
| LD | Yes | 0 | 300 | 0 | 0 |
| LD | Yes | 0 | 287 | 0 | 0 |
| HD | Yes | 0 | 620 | 0 | 0 |
| HD | Yes | 1 | 231 | 2 | 1 |

Distribution of ants captured and ants infected with spores of *Myrmicinosporidium*. See text for specific information. *Samples analyzed* 16 pitfall traps × 3 months/field × 8 fields = 384 samples

*LD* low density, *HD* high density

**Table 2** Ant species infected with *Myrmicinosporidium* spores, localities and number of infected workers, traps with infected workers and total number of workers captured in pitfall traps set at olive groves of Baixo Alentejo (Portugal)

| Ant species | Locality | # workers infected | # traps with infected workers | # captured workers in 48 traps |
|---|---|---|---|---|
| *Aphaenogaster senilis* Mayr[a] | Póvoa de São Miguel | 8 | 4 | 12 |
| | Moura | 10 | 4 | 46 |
| *Cataglyphis hispanica* (Emery)[a] | Póvoa de São Miguel | 1 | 1 | 21 |
| *Crematogaster auberti* Emery[a] | Póvoa de São Miguel | 3 | 3 | 4 |
| *Goniomma hispanicum* (André)[a] | Moura | 1 | 1 | 1 |
| *Messor barbarus* (Linnaeus)[a] | Serpa | 1 | 1 | 15 |
| | Póvoa de São Miguel | 6 | 1 | 6 |
| | Moura | 16 | 6 | 44 |
| *Tapinoma nigerrimum* (Nylander)[a] | Moura | 9 | 4 | 50 |
| *Tapinoma simrothi* Krausse[a] | Póvoa de São Miguel | 6 | 3 | 34 |
| *Tetramorium semilaeve* (André) | Serpa | 2 | 1 | 3 |
| | Póvoa de São Miguel | 9 | 4 | 9 |
| *Pheidole pallidula* (Nylander) | Póvoa de São Miguel | 7 | 3 | 8 |

[a] New host

detected in the ants captured in September ($n = 1,423$ workers) and November ($n = 1,431$ workers) using a similar trapping scheme. Prevalence in June: 3.7 % for total data, with much variability for each species. It is disputable whether specific prevalence should be defined as a percentage of infected ants within a trap or within a field. Further, we refrained from exploring this epidemiological characteristic.

Other species captured in the olive groves, but not detected as infected: *Camponotus pilicornis* Roger, *Camponotus foreli* Emery, *Camponotus micans* (Nylander), *Camponotus sylvaticus* (Olivier), *Camponotus lateralis*

**Table 3** Ant species hosts (35) known for *Myrmicinosporidium* Hölldobler (January 2012)

| SF | Tr | Species | Country | Reference |
|---|---|---|---|---|
| F | **For** | ***Cataglyphis hispanica*** (Emery) | Portugal | This work |
| F | Pl | *Nylanderia vividula* (Nylander) | USA | 10 |
| F | Pl | *Plagiolepis pygmaea* (Latreille) | Croatia; Italy; Spain | 11; 5 |
| F | Pl | *Plagiolepis vindobonensis* Lomnicki | Austria | 11 |
| F | Pl | *Plagiolepis taurica* Santschi | Hungary | 9 |
| M | Ph | ***Aphaenogaster senilis*** Mayr | Portugal | This work |
| M | Fo | *Chalepoxenus muellerianus* (Finzi) | Italy | 11 |
| M | **Cr** | ***Crematogaster auberti*** Emery | Portugal | This work |
| M | Ph | ***Goniomma hispanicum*** (André) | Portugal | This work |
| M | Ph | ***Messor barbarus*** (Linnaeus) | Portugal | This work |
| M | Ph | *Pheidole bicarinata* Mayr | USA | 10 |
| M | Ph | *Pheidole pallidula* (Nylander) | France; Spain | 3; 5 |
| M | Ph | *Pheidole tysoni* Forel | USA | 10 |
| M | Ph | *Pheidole williamsi* Wheeler | Ecuador | 4 |
| M | My | *Pogonomyrmex badius* (Latreille) | USA | 10 |
| M | My | *Pogonomyrmex barbatus* (F. Smith) | USA | 11 |
| M | Da | *Pyramica membranifera* (Emery) | USA | 10 |
| M | Fo | *Temnothorax affinis* (Mayr) | Croatia | 2 |
| M | Fo | *Temnothorax albipennis* (Curtis) | Italy | 11 |
| M | Fo | *Temnothorax angustulus* (Nylander) | Italy | 11 |
| M | Fo | *Temnothorax exilis* (Emery) | Italy | 11 |
| M | Fo | *Temnothorax lichtensteini* Bondroit | Spain | 3 |
| M | Fo | *Temnothorax nylanderi* (Förster) | Italy | 11 |
| M | Fo | *Temnothorax racovitzai* Bondroit | Spain; Italy | 3; 11 |
| M | Fo | *Temnothorax recedens* (Nylander) | Croatia; France | 11; 2 |
| M | Fo | *Temnothorax tuberum* (Fabricius) | Germany | 8 |
| M | Fo | *Temnothorax unifasciatus* (Latreille) | Croatia; France; Switzerland, Italy | 11; 2; 1 |
| M | Te | *Tetramorium caespitum* (Linnaeus) | Hungary | 9 |
| M | Te | *Tetramorium semilaeve* (André) | Spain | 5 |
| M | So | *Solenopsis carolinensis* Forel | USA | 10 |
| M | So | *Solenopsis fugax* (Latreille) | France; Germany; Hungary; Spain; Switzerland | 3; 6, 7; 9; 5; 2 |
| M | So | *Solenopsis invicta* Buren | USA | 10 |
| M | Te | *Strongylognathus caeciliae* Forel | Spain | 5 |
| **D** | **Ta** | ***Tapinoma nigerrimum*** (Nylander) | Portugal | This work |
| **D** | **Ta** | ***Tapinoma simrothi*** Krausse | Portugal | This work |

Subfamily and Tribe follow Bolton (2003). The bold font denotes new subfamily, tribe or host

*SF* subfamily, *Tr* tribe, *For* Formicini, *Pl* Plagiolepidini, *Ph* Pheidolini, *Fo* Formicoxenini, *Cr* Crematogastrini, *My* Myrmicini, *Te* Tetramoriini, *So* Solenopsidini, *Ta* Tapinomini

References: 1, Buschinger et al. (2004); 2, Buschinger and Winter (1983); 3, Espadaler (1982); 4, Espadaler (1997); 5, García and Espadaler (2010); 6, Hölldobler (1927); 7, Hölldobler (1929); 8, Hölldobler (1933); 9, Kanizsai (2010); 10, Pereira (2004); 11, Sánchez-Peña et al. (1993)

(Olivier), *Camponotus cruentatus* (Latreille), *Cardiocondyla batesii* Forel, *Crematogaster scutellaris* (Olivier), *Crematogaster sordidula* (Nylander), *Formica cunicularia* Latreille, *Formica subrufa* Roger, *Hypoponera eduardi* (Forel), *Lasius lasioides* (Emery), *Lasius grandis* Forel, *Plagiolepis pygmaea* (Latreille), *Plagiolepis schmitzii* Forel, *Tapinoma madeirense* Forel, *Tetramorium forte* Forel, *Solenopsis* sp.

## Discussion

The seven new hosts noted here raise the number of known hosts to 35 species, belonging to 16 genera and 10 tribes (Table 3). A new subfamily, Dolichoderinae, is added to the Formicinae and Myrmicinae as subfamilies, where *Myrmicinosporidium* has been detected. Therefore, up to now only the formicoid clade (Moreau et al., 2006; Brady et al., 2006) contains examples of infected ants.

Feeding habits of the newly detected infected ants are highly variable: from granivores (*Messor, Goniomma*) to scavengers (*Cataglyphis*), mainly nectarivores (*Crematogaster*) and omnivores (*Aphaenogaster*, *Tapinoma*). Thus, the distinct methods of acquisition and composition of the different foods implied in these varied feeding regimes do not seem to be crucial in the cycle of the fungus, suggesting that neither food nor feeding habits are characteristic to the infection process. At present, no arboricolous ants have been found to be infected.

As all specimens were collected in pitfall traps, we assume that workers were foraging seemingly unaffected. Selection against virulence should be particularly strong in ants, where most transmission events will be within the colony (Boomsma et al., 2005). Little is known about the biology and specificity of *Myrmicinosporidium*. Its presence in the Palearctic, the Nearctic and the Southern hemisphere, and differences in spore size (Pereira, 2004) allowed speculation regarding the conspecificity of all *Myrmicinosporidium* populations. Espadaler and Santamaria (2012) expressed doubts over the conspecificity of all cases of *Myrmicinosporidium*, because of the wide phylogenetic range of host species. Now, with the new data revealed here, those doubts seem to be premature. Instead, we now believe that the detection within the small physical limits of one Portuguese olive grove (Póvoa de São Miguel) of seven hosts, belonging to seven genera, from five tribes, and three subfamilies argues against possible specific differentiation of *Myrmicinosporidium* on different hosts. A similar situation was already noted within the limits of an organic citrus grove in Spain (Espadaler and Santamaria, 2012), where four genera belonging to four tribes from two subfamilies were found to be parasitized with *Myrmicinosporidium*. This supports the hypothesis that *Myrmicinosporidium* is indeed a single gene pool, a generalist microparasite, and not a mixture of differentiated host lineages. Finally, it is worth mentioning that these two sites are under organic management. This is an agricultural method that promotes abundance and diversity of soil fungi (Hole et al., 2005; Birkhofer et al., 2008). Of course, we accept that these two sites (one in Portugal, one in Spain) do not offer a high number of replicates and our argument is merely a suggestion that the soil conditions created by organic management may promote the presence of *Myrmicinosporidium* infected ants. Experimental rearing of the fungus through cross-feeding controlled colonies, as performed by Buschinger and Kleespies (1999) with the neogregarine *Mattesia geminata* Jouvenaz and Anthony, and molecular studies, are required to clarify the phylogenetic position of *Myrmicinosporidium*. Only a genetic analysis will allow to determine whether the different host ant species are infected by same clones or different strains or maybe even cryptic species of the fungus.

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