# First Record of *Myrmicinosporidium durum* (Fungi) parasitizing *Pheidole nodus* and *P. indica* (Hymenoptera: Formicidae) from Japan

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**Abstract** The fungus *Myrmicinosporidium durum* Hölldobler is reported from Iki Island, northern Kyushu, Japan, where it parasitizes the two ant species, *Pheidole nodus* and *P. indica*. This is a first report of the fungus in East Asia.

# Introduction

Myrmicinosporidium durum Hölldobler is an obligate endoparasitic fungus of ants, discovered almost a century ago (Hölldobler, 1933). The systematic position of the fungus is still unclear (Sanchez-Peña et al., 1993; Espadaler & Santamaria, 2012), but it is thought to be a member of the subphylum Entomophthoromycotina (Csata et al., 2013). The worker, queen and male castes of ants can all be infested by the fungus (Buschinger et al., 2004; García & Espadaler, 2010). It is possible to collect infested ants in pitfall traps, suggesting that the parasite does not alter the ants' behaviour (Buschinger et al., 2004). The infested ants are easily recognized by the spores inside their bodies (Sanchez-Peña et al., 1993; Pereira, 2004), and these spores become bowlshaped when dehydrated in alcohol (Buschinger et al., 2004). Nothing is known about the life cycle of this parasitic fungus or how it infects the host (Sanchez-Peña et al., 1993). The fungus parasites the red imported fire ants, Solenopsis invicta, but its potential use as a biological control agent is unknown (Pereira, 2004; Oi et al., 2015). We refer to the fungus as M. durum in this study, although the conspecificity of

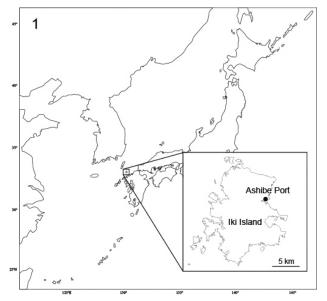


Fig. 1. Map of Iki Island, northern Kyushu, Japan, with collecting locality.

*Myrmicinosporidium* is uncertain due to its wide range of host species (Espadaler & Santamaria, 2012).

According to Gonçalves et al. (2012) and Peral et al. (2017), the fungus has been reported from Europe (e.g., Hölldobler, 1927; Sanchez-Peña et al., 1993; Buchinger et al., 2004; Lapeva-Gjonova, 2014; Giehr et al., 2015), western Asia (Csősz et al., 2012), the United States (Pereira, 2004), and the Galápagos Islands (Espadaler, 1997). Until now, 40 ant species have been identified as hosts for this parasite: Myrmicinae (33 species), Formicinae (5 species) and Dolichoderinae (2 species) (Gonçalves et al., 2012; Peral et al., 2017). Espadaler & Roig (2012) suggested that the fungus had a cosmopolitan or wide-ranging distribution, however, no previous record has been reported from East Asia. Here we report the first record for Myrmicinosporidium durum from Japan, where it was found parasitizing Pheidole nodus and P. indica ants.

### Materials and methods

The sample was collected with bait traps on 9th October 2019 at Ashibe Port (33°48'36"N 129°45'10"E) of Iki Island (Fig. 1), northern Kyushu, Japan. Iki Island, which has an area of 133.8 km², is located in the Genkai Sea. Harada *et al.* (2017) recorded 11 ant species from the Ashibe Port in their faunal surveys in northern Kyushu.

Infections were detected through the cuticle of the ants with a Leica M205C stereomicroscope. The scanning electron micrographs (SEM) were prepared with a JSM-5600LV scanning electron microscope. The samples were sputter coated with gold (JEOL Ion Sputter JFC-1100). The infested ant specimens were preserved in 80% ethanol and deposited at the Institute of Tropical Agriculture, Kyushu University, Japan.

# **Results and Discussion**

One minor worker of *Pheidole nodus* and one of *P. indica* were infested with spores of *Myrmicinosporidium durum* (Fig. 2A–D). *Pheidole nodus* and *P. indica* are new hosts for this endoparasitic fungus. The color of parasitized workers was like that of normal workers, although Espadaler & Roig (2012) found a light colored major worker of *P. pallidula* that was infested. The spores were abundant inside the head,

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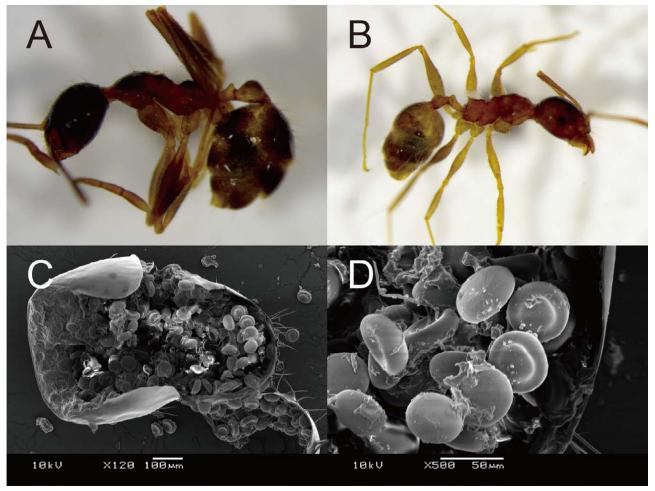


Fig. 2A–D. A, *Pheidole nodus* minor worker infested with spores of *Myrmicinosporidium durum*; B, *Pheidole indica* minor worker infested with spores of *Myrmicinosporidium durum*; C, SEM photographs of the gaster of *Pheidole nodus* dissected to show the spores of *Myrmicinosporidium durum*; D, SEM photographs of close-up view of the spores of *Myrmicinosporidium durum* inside the gaster of *Pheidole nodus*. The bowl-shaped depression is an artifact fixation in ethanol.

mesosoma and gaster, and some were also found in the petiole, postpetiole, coxae, femora and tibiae. Judging by the sizes of the spores, they did not seem to be in different stages of development. The spores measured 50.06  $\mu$ m  $\pm$  4.34 (mean  $\pm$  s.d.) (n = 20) (Fig. 2C–D).

In western Japan, *P. nodus* is one of the most common ants. It occurs and in habitats ranging from open land to woodland and makes its nests under the soil (Terayama *et al.*, 2014). *Pheidole indica*, common in southern Kyushu, occurs in rather dry habitats and also makes nests under the soil (Terayama *et al.*, 2014). This species was originally distributed over South and Southeast Asia but is now expanding its distribution to temperate regions all over the world (Sarnat *et al.*, 2015). The two *Pheidole* species infested are generally omnivorous, although feeding habits of the infested ants are highly variable (Gonçalves *et al.*, 2012).

Ant species from the genus *Pheidole* have been found hosting *M. durum* in several locations worldwide: *P. pallidula* in Europe (e.g., García & Espadaler, 2010; Espadaler & Roig, 2012); *P. williamsi* in the Galápagos Islands (Espadaler, 1997); and *P. tysoni* and *P. bicarinata* in the United States (Pereira, 2004). The literature shows that *Pheidole* ants acts as hosts throughout the world, and this body of research can contribute

to the systematic study of the fungus Myrmicinosporidium in the future.

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