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SHORT NOTE

The Effect of the Introduced Red Wood Ant *Formica paralugubris* on the Frequency of Ant Nests and First Plesiobiotic Association between *Myrmica ruginodis* (Nylander, 1846) and *Lasius flavus* (Fabricius, 1782)

Paride Balzani[1,2], Filippo Frizzi[1], Alberto Masoni[1], Giacomo Santini[1]

1-Department of Biology, University of Florence, Florence, Italy
2-Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, University of South Bohemia in Ceske Budejovice, Zátiší, Czech Republic.

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Corresponding author
Paride Balzani
Department of Biology
University of Florence
Via Madonna del Piano 6, 50019
Florence, Italy.
E-Mail: paride.balzani@unifi.it

Abstract

Red wood ants are ecologically dominant species that affect the composition of the invertebrate community. In the past century, one of these species, *Formica paralugubris* (Seifert 1996), was introduced outside its native area (the Alps) in the Apennines (Italy). Here we investigated the effect of an introduced population of *F. paralugubris* on the frequency of nests of other ant species in Abetone, Central Apennines. Ant nests were found only in the area unoccupied by *F. paralugubris*. In this area, we also found one pleometrotic association between queens of *Lasius flavus* (Fabricius, 1782) and two plesiobiotic associations, one between *Formica fusca* (Linnaeus, 1758) and *L. flavus* and the other one between *Myrmica ruginodis* (Nylander, 1846) and *L. flavus*. This latter is the first plesiobiotic relationship reported between these two species, the second reported for *M. ruginodis,* and the 10th for *L. flavus*. Additionally, this is one of the few plesiobiotic associations reported for the Mediterranean region and Southern Europe.

Red wood ants are ecologically dominant species (Johansson & Gibb, 2016) and recognized ecosystem engineers (Balzani et al., 2022a) that affect various taxa (Robinson et al., 2016). They also exclude other ant species from their area (Savolainen et al., 1989; Czechowski et al., 2013; Maák et al., 2021), thus reducing the available nesting sites for founding queens. Starting in the 50s of the past century, experimental introductions of different red wood ant species have been carried out in some European countries, such as Germany and Italy, to study the suitability of red wood ants as useful predators in the fight against forest pests (Gösswald, 1951; Pavan, 1959; Balzani et al. 2022b).

One of the most frequently employed species in these introductions was *Formica paralugubris* (Seifert, 1996), which was repeatedly transplanted from its original areas in the Alps to other sites of the Italian peninsula, where they were formerly absent (Pavan, 1959; Ronchetti & Groppali, 1995). Most introductions occurred in the Apennines mountains, where climatic conditions provided habitats suitable to this cold-climate species, thus allowing its successful establishment (Ronchetti et al., 1986; Groppali & Crudele, 2005; Masoni et al., 2019). These populations have considerably grown and acquired some invasivity features (Frizzi et al., 2018; Balzani et al., 2021), affecting various community components (Frizzi et al., 2020; Di Nuzzo et al., 2022).

Here we investigated whether the presence of the introduced red wood ant *F. paralugubris* in a Southern Europe montane forest affects the frequency of nests of the native ant species.

The study was carried out at Abetone (Central Italy; 44°08′50″ N, 10°40′24″ E), at 1200 m.a.s.l., which is a coniferous forest dominated by white fir (*Abies alba*) with

a scarce understory dominated by *Petasites sp*. Two areas were identified: one where *F. paralugubris* is present and one where the species is not present. Both areas presented the same environmental conditions (i.e. same altitude, habitat, vegetation type, exposition), thus excluding other potential confounding factors and representing an ideal site for such an investigation.

Two samplings were performed in July 2017 and August 2021. During each survey, for each area, four people walked four parallel transects (each 500 m long), looking under stones for ant nests. To standardize the search, 50 stones for each transect were checked. A few workers were collected from each colony for subsequent determination (using the identification keys by Czechowski et al., 2012 and Lebas et al., 2016), which was confirmed by a specialist. Determined specimens were deposited in the Ant collection of the Civic Museum of Natural History of Milano.

Ant nests were only found in the area non-occupied by *F. paralugubris*. A total of 38 nests were found (Table 1), two of which were plesiobiotic nests, with two species nesting under the same stone and whose colonies were separated by 2-3 centimeters. One plesiobiotic association was between *Formica fusca* (Linnaeus, 1758) and *Lasius flavus* (Fabricius, 1782), while the other was between *Myrmica ruginodis* (Nylander, 1846) and *L. flavus* (Figure 1). A pleometrotic association of eight founding queens of *Lasius flavus* was also found (Figure 2).

The introduced red wood ant *F. paralugubris* was found to exclude other ants from the area they occupy. This is in line with previous findings on other red wood ant species (Johansson & Gibb, 2016; Robinson et al., 2016) and another introduced population of this species (Frizzi et al. 2018). Additionally, we found one pleometrotic and two plesiobiotic associations in the area non-occupied by *F. paralugubris*.

Table 1. Ant colonies found in the study sites. The symbol + indicates a plesiobiotic association, while the symbol ¥ indicates a pleometrotic association.

Sampling year	Species	N
2017	*Myrmica ruginodis*	3
	Lasius flavus	2
	Myrmica ruginodis + *Lasius flavus*	1
2021	*Manica rubida*	2
	Myrmica sulcinodis	2
	Myrmica ruginodis	13
	Lasius flavus	10
	Lasius flavus queens ¥	8
	Formica fusca + *Lasius flavus*	1
	Lasius fuliginosus	3
	Camponotus ligniperda	1

While pleometrosis in queens of *L. flavus* and plesiobiosis between *F. fusca* and *L. flavus* was already reported (e.g. Waloff, 1957; Kanizsai et al., 2013), here we report for the first time a new plesiobiotic association between *Myrmica ruginodis* and *Lasius flavus*, contributing to the 58th plesiobiotic pair observed. This finding also represents one of the few plesiobiotic associations described in the Mediterranean region and, in general, Southern Europe (Donisthorpe, 1927).

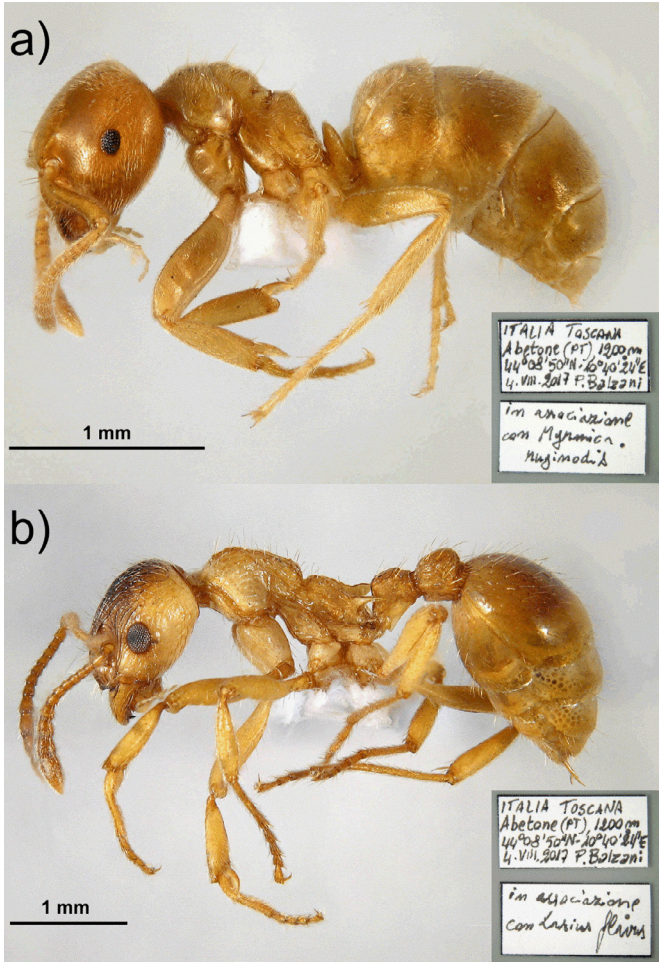

Fig 1. Specimens of a) *Lasius flavus* and b) *Myrmica ruginodis* from one plesiobiotic association. These specimens are deposited in the Ant collection of the Civic Museum of Natural History of Milano.

Plesiobiosis (term introduced by Wheeler, 1901) consists of the close proximity of colonies of different species (Czechowski, 2004). It is an association of heterospecific ant nests, "where colonies are established close together, usually under the same stone, but remain quite separate from one another" (Morley, 1946). More specifically, it is defined as "the occasional or regular nesting of heterospecific colonies of certain species in close proximity to each other without biological interdependence" (Kanizsai et al., 2013).

This association usually involves pairs of species differing in their morphology (body size) and behavior (foraging strategies or competitive ability) and, in most cases,

Fig 2. Pleometrotic queens of *Lasius flavus* found under one rock.

belonging to different genera at least (Hölldobler & Wilson, 1990). Indeed, because both species share the same nesting site and foraging area, interspecific competition may occur (Kanizsai et al., 2013). However, competition can be avoided if the two species have different habits and behavior. In line with this reasoning, the coexistence between *L. flavus* and *M. ruginodis* could be achieved through a combination of different biologies, feeding behavior, and activity patterns. While *L. flavus* is a hypogeic ant feeding underground, *M. ruginodis* is predominantly predacious relying on aboveground prey. Thus, competition among them could be easily avoided. Moreover, *M. ruginodis* was found to shift its foraging activity to night when other competitors were present (Vepsalainen & Savolainen, 1990). Nevertheless, this peaceful coexistence can be broken if the two colonies come in direct contact after the nest galleries break, underlying the precarious nature of this equilibrium (Kanizsai et al., 2013).

The onset of such an association has been attributed to the accidental close founding of the two species, which is maintained until the two colonies become too large and fighting occurs (Morley, 1946). However, some ecological constraints seem to facilitate this phenomenon, and there is evidence that it may be more frequent in habitats where available nesting sites are limited (Czechowski, 2004). Indeed, in such cases, interspecific competition could be more easily avoided than the intraspecific competition, provided that the species involved have different requirements and/or resource preferences, different activity periods, or foraging areas (Kanizsai et al., 2013). Consequently, plesiobiosis can be viewed as the result of a trade-off between the availability of nesting sites and interspecific competition (Czechowski, 2004).

In their review, Kanizsai et al. (2013) listed 48 plesiobiotic associations, with *Formica fusca* and *Lasius flavus* as the most frequent plesiobionts. This latter species has been found in association with species of the genus *Formica*, *Tetramorium* and (as an exception to the taxonomic divergence rule) other *Lasius* species (Kanizsai et al., 2013). Eight additional associations were found in Hungary during a long-term study (Gallé et al., 2014) and recently a new plesiobiotic association was found between *L. flavus* and *Formica lemani* in a heathland in Norway (Kvifte et al., 2017). Among all these, the most similar pair of plesiobiotic species previously described was between *L. flavus* and *M. scabrinodis*, reported in England (Morley, 1945).

Plesiobiosis is still a relatively understudied phenomenon, and information on this kind of interspecific relationship is still scarce, based on sporadic observations, and is biased toward the Northern latitudes (Kanizsai et al., 2013). Therefore, any additional information can improve our knowledge on this topic.

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Author contributions

P.B., F.F., A.M., and G.S. performed the sampling. P.B. wrote the first and the final drafts

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