A novel association between *Aphaenogaster subterranea* (Hymenoptera: Formicidae) and the nymphs of *Reptalus panzeri* (Hemiptera: Cixiidae)

GÁBOR LÓRINCZI

Department of Ecology, University of Szeged, Közép fasor 52, H-6726 Szeged, Hungary; e-mail: lorinczig@gmail.com


**Abstract.** The nymphs of the cixiid planthopper, *Reptalus panzeri* (Löw, 1883), were found cohabiting with the myrmicine ant *Aphaenogaster subterranea* (Latreille, 1798) in a sub-Mediterranean oak forest in mid-western Hungary. Except for a few cases, nymphs were recorded only in the nests of *A. subterranea*, or were recorded with foragers of this ant from neighbouring colonies. Adult planthoppers were also found in nests of *A. subterranea* in the emergence period. Neither field nor laboratory studies revealed direct or unambiguous interactions between this ant and the planthopper nymphs that would indicate a trophobiotic relationship, however the fact that nymphs do not try to escape from the ants, which do not attack the nymphs, indicates some kind of mutualistic relationship between them. The main food plant of *R. panzeri* nymphs in this habitat is likely to be the roots of manna ash (*Fraxinus ornus*).

**INTRODUCTION**

The association between ants and honeydew-producing hemipterans is well known and discussed extensively in the literature. In these interactions, tending ants harvest energy-rich honeydew and in return provide protection and other services that benefit the hemipterans (reviewed e.g., by Way, 1963; Buckley, 1987; Delabie, 2001). Ant-attendance of aphis, scale insects, mealybugs (Sternorrhyncha: Aphididae, Coccidae and Pseudococcidae) and treehoppers (Auchenorrhyncha: Membracidae) is the most common and is rare or uncertain in other hemipteran groups (Hölldobler & Wilson, 1990; Delabie, 2001). Within the Fulgoromorpha, relationships with ants are recorded for at least ten families (China & Fennah, 2001). Within the Fulgoromorpha, relationships with ants are recorded for at least ten families (China & Fennah, 2001), including Cixiidae, but these are poorly documented, except for the Tettigometridae (Bourgoin, 1997; Dejean & Bourgoin, 1998; Dejean et al., 2000; Lehouck et al., 2004). In ant-cixiid relationships it is mainly the ground-living nymphs that are ant attended (Myers, 1929; Mitjaev, 1967; Thompson et al., 1979; Thompson, 1984) and rarely the adult planthoppers (Holzinger, 2009). In the Central European fauna, the nymphs of species of *Reptalus* and *Pentastiridius* are found in association with ants (W.E. Holzinger, pers. com.), however, the species of ant or whether it is frequently associated with these planthoppers is unreported.

In the present paper, I report a close association between the myrmicine ant *Aphaenogaster subterranea* and the nymphs of the cixiid planthopper *Reptalus panzeri*. This was first discovered in 2010, when temporal changes in the density and spatial pattern of ant nests were studied from spring to autumn in two sub-Mediterranean woodland habitats (Lórinzci, in prep.). Though detailed records of this association were not made in that study, the nymphs of *R. panzeri* were observed cohabiting with host colonies throughout the season (from early April to late October) and appeared to occur only in the nests of *A. subterranea*. During the study presented here, I attempted to estimate the frequency of occurrence of planthopper nymphs in ant nests and make preliminary observations on the nature of this relationship.

**MATERIAL AND METHODS**

**Study species**

*Aphaenogaster subterranea* (Latreille, 1798) is a widely distributed myrmicine ant, which occurs in Central and Southern Europe, Moldova, Southern Ukraine, Crimea, Asia Minor and Caucasus (Kutter, 1977; Czechowski et al., 2002). It is a highly thermophilous species, inhabiting mostly moderately wet and warm deciduous forests and forest edges (Czechowski et al., 2002; Seifert, 2007), but also occurs in pine forests (Garrido et al., 2002; Lórinzci, 2008, 2011; Castracani et al., 2010) and occasionally in dry grasslands (Csósz et al., 2002; Dekoninck et al., 2007). In the area surveyed, *A. subterranea* is one of the most abundant species of ant and mainly nests under stones, in the soil, leaf litter and occasionally inside and/or under fallen branches (Lórinzci, 2011). Its colonies are monogynous and range in size from several hundred to several thousand workers (Schmid-Hempel & Crozier, 1999; Czechowski et al., 2002; Seifert, 2007). *A. subterranea* is an omnivorous ant and like other species in the genus *Aphaenogaster*, also uses tools to transfer liquid food back to the colony (Agbogba, 1985; Lórinzci, pers. obs.). Although species of *Aphaenogaster* are generally classified as opportunists using Andersen’s (1995, 1997) functional group scheme (e.g., King et al., 1998; Wike et al., 2010), *A. subterranea*, contrary to Castracani et al. ’s (2010) classification, is categorized as cryptic by Gómez et al. (2003).

*Reptalus panzeri* (Löw, 1883) is a cixiid planthopper, which occurs in Central and Southern Europe, England, Asia Minor and Caucasus, where it inhabits xerothermous habitats, especially sunny hillsides or plateaus up to 500 m (Holzinger et al., 2010).
2003). It is a polyphagous species with a wide variety of host plants, including trees, shrubs and also herbaceous plants (Nickel & Remane, 2002; Nickel, 2003; Jović et al., 2009; Bertin et al., 2010). Similar to other members of the family, the wax-tailed nymphs live underground, feeding on the roots of their host plants (see Figs 2–3). It has one generation per year and overwinters in the nymphal stage. Imagoes are active from mid-June to the beginning of August (Nickel & Remane, 2002; Holzinger et al., 2003; Jović et al., 2009). R. punzeri is of great economic importance because it is a potential vector of stolbur phytoplasma, a prokaryotic, phloem-restricted plant pathogen, which causes diseases in a number of economically important plants (Palermo et al., 2004; Riedle-Bauer et al., 2006; Jović et al., 2007, 2009).

Study area
This study was carried out in the oak forest on the south-facing slope of Péter Hill (Péter-hegy) (46°58‘33.54“N, 17°53‘34.62“E, altitude 207 m, dip angle 15–20°), which is located in the middle of the Balaton Uplands, in mid-western Hungary. The climate in this region is mild, with a mean annual temperature of 10–15°C and mean annual precipitation of 650–700 mm (Füleký et al., 2007). The type of forest in this area is a mosaic of karst shrub (Cotino-Quercetum pubescentis) and downy oak forest (Vicio sparsiflorae-Quercetum pubescentis) with downy oak (Quercus pubescent) and manna ash (Fraxinus ornus) being the dominant tree species (Borhidi, 2003). Detailed information about the vegetation in this area can be found in Debreczy (1973).

Field work
Ant nests were mapped and excavated in 25 (5 × 5) randomly placed plots of 3 × 3 m² on five occasions between the end of May and beginning of September 2011 (see Table 2). Every time an ant’s nest was found the following parameters were recorded: (1) the type of nesting site (i.e., under a stone, in the soil, leaf-litter or in fallen branches); (2) the estimated size of the colony (small, medium, large) on the basis of the number of workers/brood and the extent of the nest; (3) the presence, number, estimated size (small, medium, large) and exact location of individual planthopper nymphs inside the ant’s nest; (4) all the species of shrubs and trees close to the nest the roots of which could be present in the nests.

During nest mapping, I ignored such specific nesting sites as stones under, in the soil, leaf-litter or in fallen branches, where planthopper nymphs were unlikely to occur, so the nests of twig-nester/cavity-nester ant species (e.g., Temnothorax spp.) were undoubtedly under-represented in this study.

Planthoppers could be determined to species level only after adult males were found, since reliable identification of Reptalus species is based on the morphology of the male genitalia (Holzinger et al., 2003), however, it is now possible using DNA-based assays to identify nymphs and adult females (Bertin et al., 2010). Planthopper nymphs were kindly identified to family level and to species level by György Györfy (Department of Ecology, University of Szeged). Voucher specimens of ants were identified using the keys of Seifert (2007).

Laboratory work
Since detailed observations could not be carried out in the field as the ants were disturbed by opening their nest, a laboratory study was undertaken to determine the behavioural interactions between the two species. On three occasions fragments of colonies of A. subterranea and planthopper nymphs from nests were separately transferred to the laboratory. Within a few hours of collecting in the field nymphs were placed in Petri dishes with rootlets and nest material, and then workers of A. subterranea were added and the reactions of both species to one another was recorded.

RESULTS
Altogether, a total of 147 nests of 9 ant species were found during nest mapping. The most frequent species was A. subterranea, whose nests made up more than 50% of all nests found and were found in all the plots except one (Table 1). Like in nearly all the other species most of the nests of A. subterranea were found under stones.

Overall, a total of 110 planthopper nymphs were located in ant nests, 106 (96%) of which occurred in the nests of A. subterranea. Nymphs were only recorded in three cases with colonies of other species, two cases in the nests of Prenolepis nitens and one in a satellite nest of Lasius brunneus. It may be worthy of note, however, that within one-meter of the nests of these two species of ants there were nests of A. subterranea.

The mean number of nymphs in the 31 (40%) nests of A. subterranea was 3.42 (± 2.84 SD), the minimum number was 1 and the maximum 12. The ratios of nests with and without nymphs varied from 1 : 0.84 to 1 : 4 at the different times sampled (Table 2). Dead nymphs were

<table>
<thead>
<tr>
<th>Species</th>
<th>Relative abundance</th>
<th>Relative incidence</th>
<th>Type of nesting site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aphaenogaster subterranea</td>
<td>0.51</td>
<td>0.96</td>
<td>0.79</td>
</tr>
<tr>
<td>Prenolepis nitens</td>
<td>0.27</td>
<td>0.72</td>
<td>0.95</td>
</tr>
<tr>
<td>Lasius emarginatus</td>
<td>0.07</td>
<td>0.24</td>
<td>0.59</td>
</tr>
<tr>
<td>Lasius brunneus</td>
<td>0.05</td>
<td>0.20</td>
<td>0.625</td>
</tr>
<tr>
<td>Lasius citrinus</td>
<td>0.04</td>
<td>0.16</td>
<td>0.83</td>
</tr>
<tr>
<td>Ponera coarctata</td>
<td>0.03</td>
<td>0.16</td>
<td>1</td>
</tr>
<tr>
<td>Myrmecina graminicola</td>
<td>0.01</td>
<td>0.04</td>
<td>1</td>
</tr>
<tr>
<td>Temnothorax crassispinus</td>
<td>0.01</td>
<td>0.04</td>
<td>–</td>
</tr>
<tr>
<td>Temnothorax unifasciatus</td>
<td>0.01</td>
<td>0.04</td>
<td>–</td>
</tr>
</tbody>
</table>
found in three other *A. subterranea* nests and two nests contained only the characteristic waxy material produced by nymphs. The numbers of planthopper nymphs found in nests of colonies of *A. subterranea* of different sizes did not differ significantly (Kruskal-Wallis test, p = 0.18) (Fig. 1). Groups of nymphs usually consisted of individuals of different instars, although a greater proportion was made up of young instars in the samples collected after than, before the middle of July (Table 2).

Nymphs were found on six occasions outside ant nests, either singly or in pairs, never in groups, almost always accompanied by foragers of *A. subterranea* from neighbouring colonies even in the one of two cases when a nymph was found in a nest of *P. nitens*.

Inside the nests of *A. subterranea*, nymphs or groups of nymphs were either covered or not covered in waxy threads and located among groups of ant pupae, or, in the case of the nests under stones, on the undersurface of the stones (Fig. 3). Only in a few cases were nymphs found on roots in ant nests (Fig. 2). In the case of the largest group of 12 nymphs found during this study they were found aggregated aboveground in the leaf-litter, covered in waxy threads, directly beneath a fallen branch inhabited by a large colony of *A. subterranea*.

Most of the nests of *A. subterranea* with planthopper nymphs (65%) were found among roots, or close to manna ash (*Fraxinus ornus*) trees. This was also the host plant of the nymphs that were observed feeding on roots inside ant nests. In the remaining cases, the identity of the food plant could not be determined, however, it is likely that downy oak (*Quercus pubescens*), and perhaps European cornel (*Cornus mas*) and field maple (*Acer campestre*) are also host plants of the nymphs of *R. panzeri*.

Independently of the nest-mapping study, adult plant-hoppers were also observed on three occasions in the nests of *A. subterranea* at the end of June / beginning of July. Two of them were newly emerged and lightly pigmented tenernals, while the third was a fully developed adult that was ready to fly.

In the laboratory, no direct physical contact between ants and planthopper nymphs was recorded. Although ant workers did not attack the nymphs and nymphs did not attempt to escape (e.g., by jumping), but stayed still, they virtually ignored each other in most cases. There was only one case when one of the nymphs slightly raised and shook its abdomen in the presence of an ant worker, and emitted a droplet of honeydew, but the ant did not respond.

**DISCUSSION**

Within the Fulgoromorpha, ant-planthopper associations are documented mainly for the Tettigometridae (Bourgoin, 1997; Dejean & Bourgoin, 1998; Dejean et al., 2000; Lehouck et al., 2004), which account for almost three-quarters of the records of ant attendance in this sub-order. Previous studies on ant-cixiid relationships are rare, and most of them report only observations that might indicate the existence of possible associations (Myers, 1929; Thompson et al., 1979; Neece, 1980).

One of the reasons that so few ant-cixiid interactions are reported may be that cixiid planthoppers have characteristics not really favouring associations with ants, contrary to, e.g., tettigometrid species, which possess several morphological (e.g., no jumping apparatus, no wax plates, etc.) and behavioural adaptations (e.g., sessile behaviour, subsociality, etc.) to ant-attendance (Bourgoin, 1997).

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**TABLE 2. Presence-absence, total number and estimated sizes of *Reptalus panzeri* nymphs in the nests of *Aphaenogaster subterranea* on five dates during the course of a year.**

<table>
<thead>
<tr>
<th>Sampled on</th>
<th>Number of nests</th>
<th>Number and sizes of nymphs found in nests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>with nymphs</td>
<td>without nymphs</td>
</tr>
<tr>
<td>30 May</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>10 June</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>19 July</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>1 August</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>12 September</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>43</td>
</tr>
</tbody>
</table>
Fig. 2. A group of *Reptalus panzeri* nymphs on wax covered roots with workers of *Aphaenogaster subterranea*.

Fig. 3. A late instar nymph of *Reptalus panzeri* on the undersurface of an overturned stone that previously covered a nest of *Aphaenogaster subterranea*.
Nevertheless, durable ant-planthopper interactions may exist not only when hoppers are either sessile (non-jumping) or subsocial, but also when they are just unable to escape or forced to be gregarious (Bourgoin, 1997). Examples of the latter can be seen in the case of some epigeic delphacid species, which are found under ant shelters (Dejean et al., 1996) and of cixiid nymphs, some of which occur underground in ant nests (e.g., Mitjæv, 1967; Thompson, 1984). A special case is represented by some fulgoroid planthoppers with troglomorphies, i.e., morphological adaptations to subterranean or cavernicolous life, e.g., reduced eyes, wings and body pigmentation, presence of sensory hairs, etc. (China & Fennah, 1952; Hoch, 1990; Fletcher & Moir, 2009), which may have been facilitated by their associations with ants (Hoch et al., 2006).

According to Bourgoin (1997), four main types of ant-attendance can be distinguished within the Fulgoromorpha: (1) opportunistic or occasional attendance by ants that collect the honeydew casually dispersed by the planthoppers on the substrate; (2) underground attendance in ant nests; (3) aboveground attendance under natural shelters or shelters built by ants; (4) regular attendance by ants that antennate the planthoppers and collect honeydew directly from their anal openings. Similar to other reported cases of ant-cixiid associations, the relationship between A. subterranea and the nymphs of R. panzeri is a case of the second type of interaction, which is based only on cohabitation, without further evidence of trophobiotic interactions. At present, there is only one study that reports an advanced type of trophobiosis between ants and cixiids, involving, however, adult planthoppers, not subterranean nymphs (Holzinger, 2009).

It is suggested that the association reported here is facultative for A. subterranea as more nests were recorded without than with planthopper nymphs, although the exact ratio seems to be nearly 1 : 1 if the nests with only dead nymphs or traces of nymphs are also included. On the other hand, the association of R. panzeri with its ant partner appears to be obligatory as (1) most of the nymphs were found inside ant nests and (2) those nymphs found outside nests were almost in all cases accompanied by workers of A. subterranea from neighbouring colonies. Furthermore, although there is no direct evidence that planthoppers overwinter in ant nests, my previous and present results suggest that nymphs cohabit with ants from their first instar to adult emergence (see Table 2). The relationship also seems to be monospecific as the nymphs of R. panzeri were almost always exclusively associated with colonies or workers of A. subterranea. These are in contrast to many previous studies showing that the association of myrmecophilous leafhoppers and planthoppers with ants is mainly facultative, independently of how advanced the interactions are between them (Steiner et al., 2004; Pfeiffer & Linsenmair, 2007; Maravalhas & Morais, 2009). Even when they are obligatory ant-attended, hoppers associate most often with more than one ant species (Dejean et al., 2000; Moya-Raygoza & Nault 2000; Lehouck et al., 2004).

One can argue, nevertheless, that the almost exclusive preference of R. panzeri for nests of A. subterranea may be largely a result of the fact that this species of ant was the most abundant in this habitat during this study. However, according to previous studies carried out over a longer time-scale, P. nitens is a much more abundant species, which tends to expand its polydomous nest system in early spring and late autumn (Lörinczi, in prep.). Yet, contrary to this, in neither of these crucial periods were the nymphs of R. panzeri found in nests of P. nitens. Furthermore, the nymphs of R. panzeri have also been found in one particular mixed coniferous-deciduous forest in cohabitation with A. subterranea (Lörinczi, pers. obs.), suggesting that a close association between them is not limited to one habitat. Within the Cixiidae, a similar type of close relationship is reported between the formicine ant Nylanderia (= Paratrechina) arenivaga and the nymphs of Oecleus borealis in North-America (Thompson, 1984). In this case it is suggested that Oecleus nymphs might benefit from being able to feed on the roots of their host plant that are exposed by the excavations of the ants inside their nests and the ant from feeding on honeydew, although no trophobiotic interactions were observed.

Although no collecting of honeydew or direct physical contacts between the nymphs and ants were observed in this study, it is likely that there is some kind of mutualistic relationship between A. subterranea and R. panzeri, as (1) ant workers did not show predatory or agonistic behaviour towards planthopper nymphs; (2) nymphs, even though saltatory, did not attempt to escape from ants; (3) nymphs located outside of the nests of A. subterranea were generally visited by the foragers of this species, even when they were in the nests of other species of ant. Further investigations are needed, though, to prove that nymphs are not just tolerated inquilines in the nests of other species, even when they were in the nests of other species of ant. These are in contrast to many previous studies showing that the association of myrmecophilous leafhoppers and planthoppers with ants is mainly facultative, independently of how advanced the interactions are between them (Steiner et al., 2004; Pfeiffer & Linsenmair, 2007; Maravalhas & Morais, 2009). Even when they are obligatory ant-attended, hoppers associate most often with more than one ant species (Dejean et al., 2000; Moya-Raygoza & Nault 2000; Lehouck et al., 2004).

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