Myrmecological News | 20 | 37-42 | Vienna, September 2014

Discovery of a second mushroom harvesting ant (Hymenoptera: Formicidae) in Malayan tropical rainforests

Christoph VON BEEREN, Magdalena M. MAIR & Volker WITTE

Abstract

Ants have evolved an amazing variety of feeding habits to utilize diverse food sources. However, only one ant species, *Euprenolepis procera* (EMERY, 1900) (Formicidae: Formicinae), has been described as a specialist harvester of wild-growing mushrooms. Mushrooms are a very abundant food source in certain habitats, but utilizing them is expected to require specific adaptations. Here, we report the discovery of another, sympatric, and widespread mushroom harvesting ant, *Euprenolepis wittei* LAPOLLA, 2009. The similarity in nutritional niches of both species was expected to be accompanied by similarities in adaptive behavior and differences due to competitive avoidance. Similarities were found in mushroom acceptance and harvesting behavior: Both species harvested a variety of wild-growing mushrooms and formed characteristic mushroom piles inside the nest that were processed continuously by adult workers. *Euprenolepis procera* was apparently dominant at mushroom baits displacing the smaller, less numerous *E. wittei* foragers. However, interspecific competition for mushrooms is likely relaxed by differences in other niche dimensions, particularly the temporal activity pattern. The discovery of a second, widespread mushroom harvesting ant suggests that this life style is more common than previously thought, at least in Southeast Asia, and has implications for the ecology of tropical rainforests. Exploitation of the reproductive organs of fungi likely impacts spore development and distribution and thus affects the fungal community.

Key words: Social insects, ephemeral food, niche differentiation, sporocarp, fungivory, Malaysia, obligate mycophagist, fungal fruit body.

Myrmecol. News 20: 37-42 (online 24 March 2014)
ISSN 1994-4136 (print), ISSN 1997-3500 (online)

Received 8 August 2013; revision received 7 November 2013; accepted 18 November 2013
Subject Editor: Alexander S. Mikheyev

Christoph von Beeren, Department of Biology II, Ludwig-Maximilians University of Munich, Großhaderner Straße 2, 82152 Planegg-Martinsried, Germany; Laboratory of Insect Social Evolution, The Rockefeller University New York, 1230 York Avenue New York, NY 10065, USA.

Magdalena M. Mair & Volker Witte (contact author), Department of Biology II, Ludwig-Maximilians University of Munich, Großhaderner Straße 2, 82152 Planegg-Martinsried, Germany. E-mail: witte@bio.lmu.de

Introduction

Among social insects, ants are a group of outstanding diversity. With an estimated 20,000 species (WARD 2006), they show a great variety of feeding habits, among them predation, trophobiosis, seed harvesting, fungus farming, scavenging, and pollen consumption (HÖLLDOBLER & WILSON 1990, BARONI URBANI & DE ANDRADE 1997, CZECHOWSKI & al. 2008). An unusual dietary specialization was discovered a few years ago, i.e., the harvesting of wild-growing epigeous fungal fruit bodies, commonly called mushrooms (WITTE & MASCHWITZ 2008). So far, the formicine ant *Euprenolepis procera* (EMERY, 1900) has been the only known ant species whose natural diet is primarily based on a diverse spectrum of wild-growing mushrooms, a food source expected to require specific adaptations (CLARIDGE & TRAPPE 2005). Although fungus farming, the cultivation of mutualistic fungi, is well documented in attine ants (HÖLLDOBLER & WILSON 2011), harvesting of wild-growing fruit bodies remains an exception among ants, and has otherwise been rarely observed in leaf-cutter and carpenter ants (PFEIFFER & LINSENMAIR 2000, MUELLER & al. 2001, LECHNER & JOSENS 2012, MASIULIONIS & al. 2013, and references therein). Here, we describe the discovery of another wild-mushroom harvesting ant in the tropical rainforests of Malaysia, *Euprenolepis wittei* LAPOLLA, 2009 (Fig. 1). We report initial insights into the feeding habits and the basic biology of *E. wittei* and compare the two known mushroom harvesting *Euprenolepis* species. Occupying similar nutritional niches, we expected similar adaptations, but also differences in behavior due to niche differentiation.

Methods

Research was conducted at the Field Studies Centre of the University of Malaya in Ulu Gombak, Malaysia (03° 19.479' N, 101° 45.163' E, altitude 230 m), where *Euprenolepis wittei* was previously discovered. Since only fragmentary observations existed from the year 2008, this study aimed to collect more information on the biology of this new species systematically. Most observations presented in this article were made during a six-week field trip in

August and September 2009, which are supplemented with observations from September / October 2008 where applicable. Species identification followed the key in LAPOLLA (2009). In order to locate *E. wittei* colonies in the field, extensive mushroom baiting was carried out from 12 pm to 5 pm and 9 pm to 3 am. Pieces of fresh oyster mushroom (*Pleurotis* sp.) were deployed every 10 m on the forest floor along existing hiking trails ($N_{\text{baits}} = 164$). However, only eight *E. wittei* colonies were found and differently used for the following experiments.

Two of these colonies, one consisting of approximately 55 workers and another one of approximately 250 workers (each with a queen), were collected and kept in gypsum nests at the field station in order to observe the behavior of ants within the nest (for methods see WITTE & MASCHWITZ 2008). To obtain some basic information on the dietary spectrum of *Euprenolepis wittei*, small pieces (ca. 1 cm × 1 cm × 0.5 cm) of 19 randomly chosen, wild-growing mushroom fruit bodies were offered to four different colonies (two laboratory as well as two field colonies). Between four to seven different mushrooms were offered simultaneously in each trial. Laboratory colonies were previously starved for 24 h. Not all food items were tested with all colonies (for sample sizes of feeding experiment see Tab. 1). In addition, honey, a freshly killed cricket, and an opened fig from the forest floor were offered simultaneously, but separately from mushrooms. Material was regarded as a food source if it was accepted by at least one colony within a period of two hours, based on observations of ants either taking up liquid food directly or carrying food pieces to the nest.

Foraging activities of three field colonies were evaluated for 16 days by counting the ants at the nest entrances heading in- and outbound for a period of five minutes. Measurements were taken during day- (N = 78, 7 am to 6 pm) and nighttime (N = 199, 7 pm to 6 am). To evaluate colony sizes and nesting habits, all eight colonies were excavated and worker numbers were estimated. To assess both prevalence of *Euprenolepis wittei* at mushrooms and its resource utilization, fungal baits consisting of about 10 - 20 g of fresh oyster mushrooms (pieces of ca. 4 cm × 6 cm × 1 cm) bought at a local food store were placed randomly on the forest floor. The baits were checked between 11 pm and 3 am in order to note presence or absence of *Euprenolepis* ants. The number of mushroom-harvesting ants, defined as ants in direct contact with a given mushroom bait, was estimated hourly. After four hours, the percentage of consumed bait was estimated visually and noted. Furthermore, the beeline distance from bait to nest site was recorded. To assess *E. wittei*'s distribution in Malaysia, mushroom baits were additionally deployed in the same way in four other regions (Lentang: 3° 22.871' N, 101° 53.218' E, Bukit Rengit: 03° 35.779' N, 102° 10.814' E, Kuala Lompat: 03° 42.738' N, 102° 17.196' E, Endau Rompin: 02° 31.882' N, 103° 24.911' E). Presence or absence of *E. wittei* at baits was recorded.

Due to difficulties in locating cryptic *Euprenolepis wittei* colonies, sample sizes remained low. Hence, we analyzed data by using a PERMANOVA (version 1.0.5) based on 9,999 permutations, a sensitive, non-parametric test (ANDERSON & al. 2008) implemented in PRIMER 6 (version 6.1.15, Primer-E Ltd., Ivybridge, U.K.). Bray-Curtis similarities were calculated from single response variables. Foraging activity (number of workers) was compared between day and night. Furthermore, foraging distance, bait discovery time and bait depletion of *E. wittei* were compared with *E. procera*. The frequency of the two *Euprenolepis* species at baits was compared with Fisher's exact test, performed in PAST version 2.16 (HAMMER & al. 2001).

Results

Euprenolepis wittei occurred in all four sampled regions (number of baits harvested by *E. wittei* / number of baits deployed: Gombak: 9 / 164; Lentang: 3 / 38; Bukit Rengit 2 / 35; Kuala Lompat; 1 / 26; Endau Rompin 3 / 31). In feeding experiments at the main study site, *E. wittei* accepted 12 out of 19 randomly chosen, wild-growing fruit bodies as food (see Fig. 2 and Tab. 1). Honey, a dead cricket, and an opened fig from the forest floor were also readily accepted (for details see Tab. 1). Besides conducting experiments on the feeding habits of *E. wittei*, we occasionally observed them to harvest wild-growing mushrooms in the field (N = 7; C. von Beeren, M.M. Mair & V. Witte, Fig. 1a). Very similar to *E. procera*, *E. wittei* cut fungi into small pieces, which were then transported to the nests. Inside the laboratory nests, the pieces were arranged in piles (Fig. 1d). Workers licked the piles and mashed them continuously with their mandibles. Their gasters swelled noticeably indicating that fungal material was consumed.

Euprenolepis wittei showed no temporal foraging preference (within colony comparisons between diurnal and nocturnal activity: PERMANOVA, colony 1: $p = 0.301$, colony 2: $p = 0.462$, colony 3: $p = 0.208$; see Fig. 3). While *E. procera* preferred nesting inside preformed cavities (WITTE & MASCHWITZ 2008), all excavated *E. wittei* colonies (N = 8) had subterranean nest chambers at ca. 15 cm depth. Based on estimates of worker numbers, colony sizes were rather small, containing about 50 to 500 individuals (N = 8). One exceptionally large colony was observed in 2008 by VW to contain an estimated 3000 - 5000 workers. In each of the eight excavated colonies, only a single, wingless queen was found. During the observation period, two of the eight colonies migrated to a new nest site.

Compared to *Euprenolepis procera*, *E. wittei* was found less frequently on mushroom baits. Out of 164 baits placed in Ulu Gombak, only nine were harvested by *E. wittei*, while 64 were harvested by *E. procera* (Fisher's exact test, $p < 0.001$). In all cases where both species were found at a bait (13 cases, including observations in 2008), *E. procera* displaced *E. wittei* and took over the bait. The median foraging distance of 0.66 m in *E. wittei* (mean ± SD = 0.85 ± 0.44 m, median = 0.66, range = 0.45 - 1.75 m, N = 8) was significantly shorter than foraging distances of *E. procera* (data taken from WITTE & MASCHWITZ 2008: mean ± SD = 5.47 ± 4.63 m, median = 4.5 m, range: 1 - 25 m, N = 35; PERMANOVA, $p < 0.001$). Traffic flow on foraging trails was also lower in *E. wittei*, making it difficult to detect their nest sites (C. von Beeren, M.M. Mair & V. Witte, unpubl.). There was a trend indicating that the mean number of workers at baits was smaller in *E. wittei* (*E. wittei*: mean ± SD = 16.16 ± 9.99 workers, median = 18 workers, range: 5 - 38 workers, note the small sample size of N = 6, indicating a rather large effect size, baits taken over by *E. procera* not considered; *E. procera*: mean ± SD = 28.06 ± 16.63 workers, median = 27 workers, range: 1 - 76

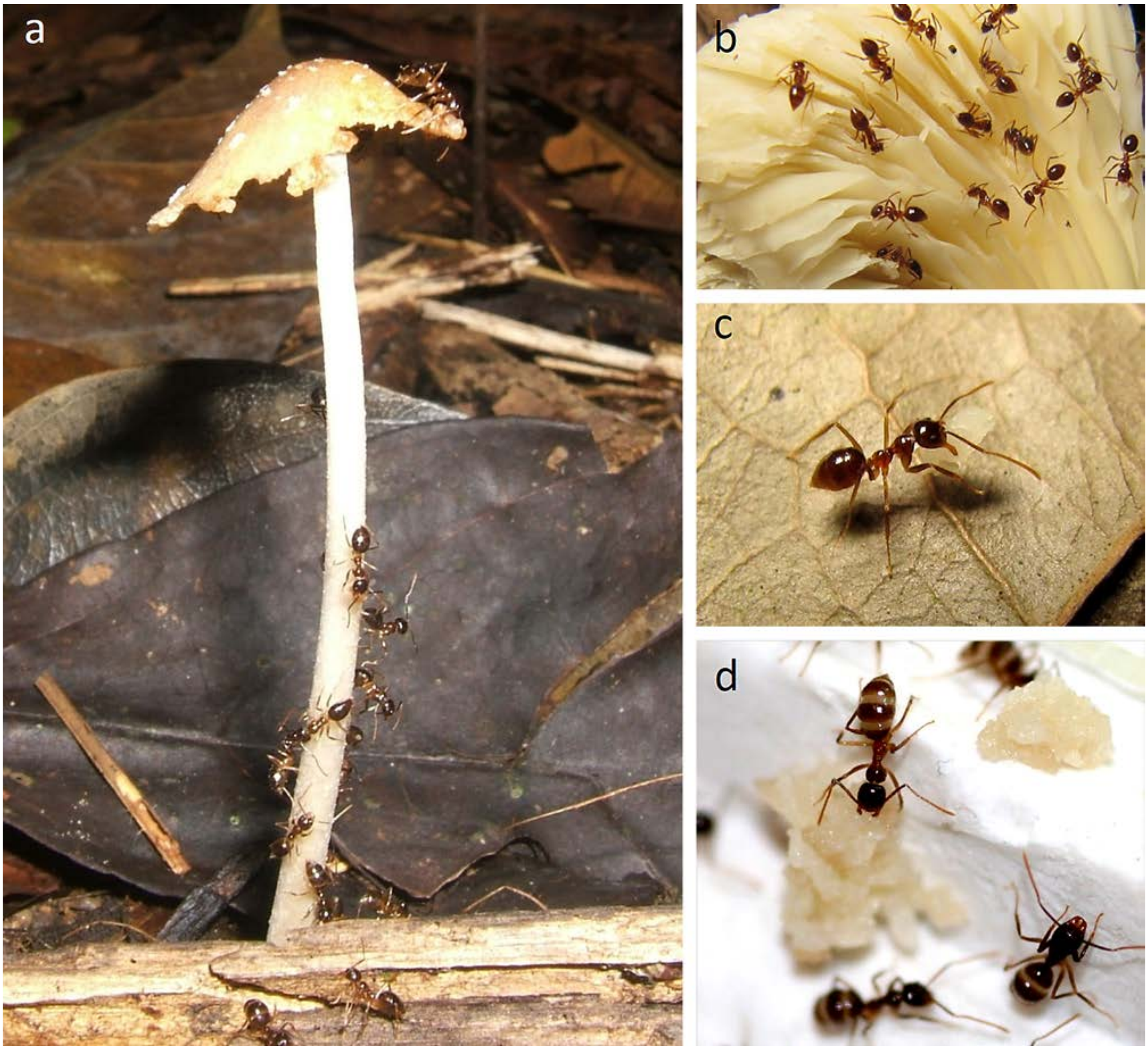

Fig. 1: Mushroom harvesting by the ant *Euprenolepis wittei*. (a) *E. wittei* was observed to harvest various wild-growing fungal fruit bodies. (b) Mushroom baits were used to evaluate the prevalence of ants and bait utilization. (c) Ants cut the fruit bodies into transportable pieces, which were subsequently transported to the nest by individual foragers. (d) In laboratory colonies, *E. wittei* built fungal piles within their nests, which were continuously processed by workers.

workers, N = 64; PERMANOVA, p = 0.065). *Euprenolepis wittei* consumed less fungal material than *E. procera* (*E. wittei*: mean ± SD = 17.50 ± 19.35 %, median = 12.5 % of bait depleted after 4 h, N = 6; *E. procera*: mean ± SD = 69.19 ± 38.69 %, median = 95 % of bait depleted after 4 h, N = 63; PERMANOVA, p = 0.006). The time for bait discovery did not differ between *Euprenolepis* species (*E. wittei*: mean ± SD = 2.22 ± 1.09 h, median = 2 h, range: 1 - 4 h, N = 9; *E. procera*: mean ± SD = 1.94 ±1.17 h, median = 1.5 h, range: 1 - 5 h, N = 64; PERMANOVA, p = 0.403).

Discussion

The present study provides the first insights into the biology of the ant *Euprenolepis wittei*. As in *E. procera*, feeding experiments revealed a rather wide fundamental food niche. In agreement with typical generalist feeding habits in ants (CARROLL & JANZEN 1973, HÖLLDOBLER & WILSON 1990), *E. wittei* accepted honey, dead insect and fruit as food. In the field, however, it was only observed feeding on wild-growing mushrooms, a variety of which it accepted in feeding experiments. Together with the special behavior of building mushroom piles in the nests, current evidence points to a dietary specialization on mushrooms very similar to *E. procera*. However, the realized niche of *E. wittei* in its natural environment, in particular the proportion and importance of mushrooms in its diet, remains to be investigated more thoroughly.

Utilizing mushrooms as a considerable part of the diet is expected to impose severe challenges (WITTE & MASCHWITZ 2008), and therefore likely requires appropriate adaptations. This may explain why obligate mycophagy is rare among ants and other animals (CLARIDGE & TRAPPE 2005). Fruit bodies potentially contain high values of nutrients (WALLIS & al. 2012), which are, however, difficult

Fig. 2: Fungal fruit bodies offered to *Euprenolepis wittei* in feeding experiments. Green dots represent fungi harvested by the ants, while red dots represent non-harvested fungi. Numbers depict fungal sample codes. Fungi were identified to the following taxa: 1. Tricholomataceae, 2. cf. *Mycena* (Mycenaceae), 3. cf. *Cookeina* (Sarcoscyphaceae), 4. Tricholomataceae, 5. - 7. unknown, 8. cf. *Favolaschia* (Mycenaceae), 9. cf. *Agaricus* (Agaricaceae), 10. cf. *Agaricus* (Agaricaceae), 11. unknown, 12. cf. *Lepiota* (Agaricaceae), 13. - 16. unknown, 17. cf. *Mycena* (Mycenaceae), 18. - 19. unknown.

to obtain as they are enclosed by chitinous cell walls, which are indigestible for most animals (Claridge & Trappe 2005). In this respect, the characteristic piling of mushroom pieces inside the nests, followed by continuous processing in both *Euprenolepis* species, likely facilitates the acquisition of nutrients. Interestingly, no comparable processing behavior of mushrooms has been described in ants occasionally harvesting wild mushrooms (Pfeiffer & Linsenmair 2000, Mueller & al. 2001, Lechner & Josens 2012, Masiulionis & al. 2013). We hypothesize that special digestive adaptations and / or endosymbiotic microorganisms may play an important role for efficient nutrient absorption in the two *Euprenolepis* species as demonstrated in other ants (Feldhaar & al. 2007, Poulsen & Sapountzis 2012). As two closely related *Euprenolepis* species share this unique feeding habit, the question arises whether their life

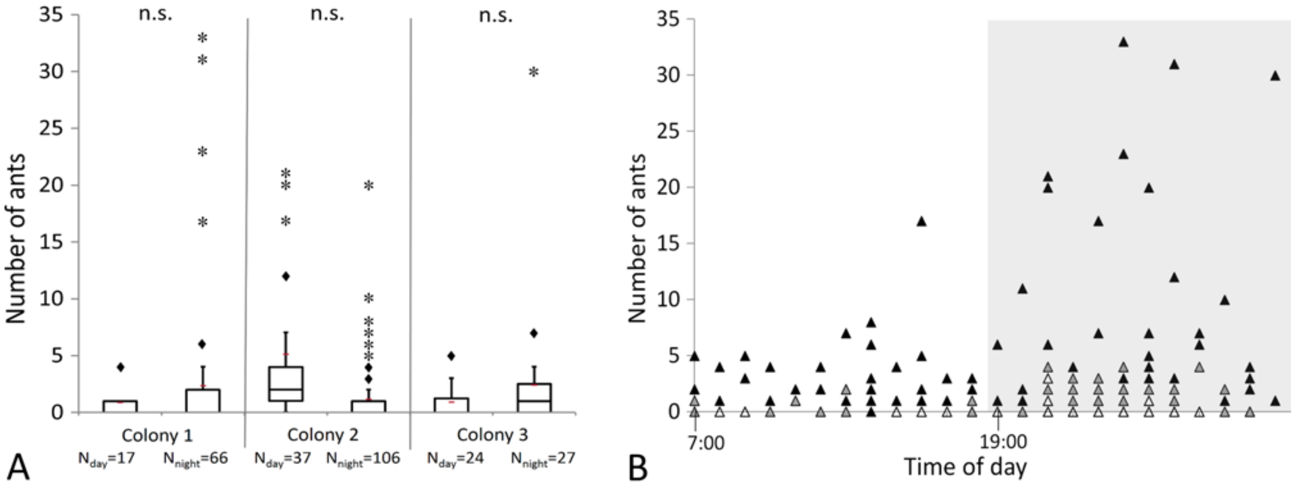

Fig. 3: Activity pattern of *Euprenolepis wittei* at nest entrances. The number of in- and outbound ants at nest entrances during five-minute observations of three nests. (A) Comparison between diurnal and nocturnal activities within colonies. For better visibility, one data point is not shown (colony 3, 12:00 o'clock, 50 ants); median (- = mean), quartiles (boxes), 10% and 90% percentiles (whiskers), and outliers (♦ = outlier, ∗ = extreme point) are shown. (B) Timeline of the daily activity pattern across colonies. Grey background shading indicates nocturnal activity. Black triangles depict one data point, grey triangles 2 - 4 data points, and white triangles more than 5 data points. n.s. = non-significant ($p \geq 0.05$).

style derived from a common mushroom harvesting ancestor. It is worth examining the basic natural history and phylogeny of other *Euprenolepis* ants in future studies, in particular because virtually nothing is known about their biology (Lapolla 2009).

Foraging on mushrooms can be understood as a strategy of avoiding interspecific competition with dominant ant species for more general diets (Witte & Maschwitz 2008). The question arises, how the two mushroom harvesting *Euprenolepis* species manage to coexist in the same habitat, in particular since *E. procera* was apparently dominant in direct competition at mushroom baits. Niche differentiation is one way to reduce interspecific competition in coexisting populations exploiting similar food niches (Gause 1934). Indeed, several behavioral differences were found between the two *Euprenolepis* species. While *E. procera* forages nocturnally (Witte & Maschwitz 2008), *E. wittei* showed no temporal activity preferences, possibly reducing interference competition with *E. procera*. Another difference between the two mushroom-harvesting ants is colony size. *Euprenolepis wittei* colonies are cryptic and small, containing mostly a few hundred workers and a single queen, while colonies of *E. procera* are larger (500 - 5,000 workers) and sometimes polygynous (Witte & Maschwitz 2008). Because smaller ant colonies are expected to need less food as indicated in fire ants (Macom & Porter 1995), lower nutritional needs in *E. wittei* might additionally relax competition with *E. procera* leading to a more cryptic life-style. Interspecific competition for mushrooms might also be relaxed by differing dependencies on that food source. Finally, *Euprenolepis* species differed in their nesting habits. *Euprenolepis procera* nests inside preformed cavities and shows no obvious nest construction (Witte & Maschwitz 2008). This opportunistic nesting habit, as well as the irregular foraging pattern and the observed migratory lifestyle, were suggested to be adaptations for efficiently exploiting ephemeral mushrooms (Witte & Maschwitz 2008). Interestingly, *E. wittei* shares the ir-

Tab. 1: Dietary spectrum of *Euprenolepis wittei*. Fungal species are numbered consecutively according to the fungal sampling code (see Fig. 2). Experiments on fungal food were conducted only in 2009. Observations on honey and cricket consumption were supplemented with data from 2008 (see Methods).

Food source	**Number of colonies tested**	**Colonies accepting food**
Honey	7	7
Cricket	6	6
Fig	3	2
Fungus 1	3	3
Fungus 2	3	2
Fungus 3	3	1
Fungus 4	4	1
Fungus 5	4	2
Fungus 6	3	3
Fungus 7	3	2
Fungus 8	3	3
Fungus 9	2	1
Fungus 10	2	2
Fungus 11	3	3
Fungus 12	3	3
Fungus 13	3	0
Fungus 14	3	0
Fungus 15	3	0
Fungus 16	3	0
Fungus 17	3	0
Fungus 18	2	0

regular foraging behavior with *E. procera* whereas nesting habit and migration frequency apparently differ. *Euprenolepis wittei* colonies were found in self-constructed soil nests and they migrated less frequently to new nest sites. Using a subterranean nest may avoid interspecific competition with *E. procera* for nesting sites and presumably decreases predation risk, as locating and accessing such nests is more difficult. However, it may be a disadvantage for efficient mushroom harvesting. In this context, it is worth studying whether the nomadic life habit of *E. procera* is indeed advantageous, as suggested by WITTE & MASCHWITZ (2008), in order to harvest ephemeral mushrooms. Taken together, *E. wittei* was found to share important characteristics with *E. procera*, particularly generalist mushroom utilization and the characteristic processing of mushroom material in the nest. These food niche similarities were accompanied by differences in other niche parameters, likely resulting from competition for the same diet.

Opposed to specialized mycophagy, opportunistic feeding on mushrooms is rather common among animals (CLARIDGE & TRAPPE 2005). Among ants, obligate fungivory of wild-growing mushrooms has so far only been described in the species *E. procera* (WITTE & MASCHWITZ 2008). It remains to be determined more thoroughly to what extent the closely related species *E. wittei* is an obligate fungivore. However, regardless of its dependency on fungal food, the discovery of another, widespread mushroom-harvesting ant in Malayan rainforests suggests that this life style is more common than previously thought. Large scale harvesting of the fruit bodies of fungi may impact fungal communities, which may have implications for the ecology of tropical rainforests in general, because fungi play key roles in these biomes (HYDE & al. 2005).

Acknowledgments

The authors thank Stefanie Weigl, Sofia Lizon à l'Allemand, Tomer Czaczkes, Sebastian Pohl, Ian Butler, and Hannah Fried-Petersen. Many thanks go to Prof. Reinhard Agerer for taxonomic classification of fungi. The authors are grateful for financial support from the DFG (Deutsche Forschungsgemeinschaft, Project WI 2646/7-1).

References

ANDERSON, M.J., GORLEY, R.N. & CLARKE, K.R. 2008: PERMANOVA+ for PRIMER: Guide to software and statistical methods. – PRIMER-E, Plymouth, UK.

BARONI URBANI, C. & DE ANDRADE, M.L. 1997: Pollen eating, storing, and spitting by ants. – Naturwissenschaften 84: 256-258.

CARROLL, C.R. & JANZEN, D.H. 1973: Ecology of foraging by ants. – Annual Review of Ecology and Systematics 4: 231-257.

CLARIDGE, A.W. & TRAPPE, J. 2005: Sporocarp mycophagy: nutritional, behavioral, evolutionary, and physiological aspects. In: DIGHTON, J., WHITE, J. & OUDERMANS, P. (Eds.): The fungal community: its organisation and role in the ecosystem. – CRS Press, Taylor & Francis Group, Boca Raton, FL, pp. 599-611.

CZECHOWSKI, W., MARKÓ, B. & RADCHENKO, A. 2008: Rubbish dumps reveal the diet of ant colonies: *Myrmica schencki* EM. and *Myrmica rubra* (L.) (Hymenoptera: Formicidae) as facultative pollen eaters. – Polish Journal of Ecology 56: 737-741.

FELDHAAR, H., STRAKA, J., KRISCHKE, M., BERTHOLD, K., STOLL, S., MUELLER, M.J. & GROSS, R. 2007: Nutritional upgrading for omnivorous carpenter ants by the endosymbiont *Blochmannia*. – BioMed Central Biology 5: 48.

GAUSE, G.F. 1934: The struggle for existence. – The Williams & Wilkins company, Baltimore, 161 pp.

HAMMER, Ø., HARPER, D.A.T. & RYAN, P.D. 2001: PAST: Palaeontological statistics software package for education and data analysis. – Palaeontologia Electronica 4: 9.

HÖLLDOBLER, B. & WILSON, E.O. 1990: The ants. – Harvard University Press, Cambridge, MA, 732 pp.

HÖLLDOBLER, B. & WILSON, E.O. 2011: The leafcutter ants: civilization by instinct. 1st edition. – W.W. Norton & Company Ltd., London, UK, 160 pp.

HYDE, K., CAI, L. & JEEWON, R. 2005: Tropical fungi. In: DIGHTON, J., WHITE, J. & OUDERMANS, P. (Eds.): The fungal community: its organisation and role in the ecosystem. – CRS Press, Taylor & Francis Group, Boca Raton, FL, pp. 93-116.

LAPOLLA, J.S. 2009: Taxonomic revision of the Southeast Asian ant genus *Euprenolepis*. – Zootaxa 2046: 1-25.

LECHNER, B.E. & JOSENS, R. 2012: Observation of leaf-cutting ants foraging on wild mushrooms. – Insectes Sociaux 59: 285-288.

MACOM, T.E. & PORTER, S.D. 1995: Food and energy requirements of laboratory fire ant colonies (Hymenoptera: Formicidae). – Environmental Entomology 24: 387-391.

MASIULIONIS, V.E., WEBER, R.W.S. & PAGNOCCA, F.C. 2013: Foraging of *Psilocybe* basidiocarps by the leaf-cutting ant *Acromyrmex lobicornis* in Santa Fé, Argentina. – SpringerPlus 2: 254.

MUELLER, U.G., SCHULTZ, T.R., CURRIE, C.R., ADAMS, R.M. & MALLOCH, D. 2001: The origin of the attine ant-fungus mutualism. – The Quarterly Review of Biology 76: 169-197.

PFEIFFER, M. & LINSENMAIR, K.E. 2000: Contributions to the life history of the Malaysian giant ant *Camponotus gigas* (Hymenoptera: Formicidae) . – Insectes Sociaux 47: 123-132.

POULSEN, M. & SAPOUNTZIS, P. 2012: Behind every great ant, there is a great gut. – Molecular Ecology 21: 2054-2057.

WALLIS, I.R., CLARIDGE, A.W. & TRAPPE, J.M. 2012: Nitrogen content, amino acid composition and digestibility of fungi from a nutritional perspective in animal mycophagy. – Fungal Biology 116: 590-602.

WARD, P.S. 2006: Ants. – Current Biology 16: R152-R155.

WITTE, V. & MASCHWITZ, U. 2008: Mushroom harvesting ants in the tropical rain forest. – Naturwissenschaften 95: 1477-1486.