

Novel blend of life history traits in an inquiline ant, *Temnothorax minutissimus*, with description of the male (Hymenoptera: Formicidae)

Alfred BUSCHINGER & Timothy A. LINKSVAYER

Abstract

Forty-five colonies of *Temnothorax curvispinosus* (MAYR, 1866) containing the rare inquiline ant *T. minutissimus* (SMITH, 1942) have been collected near Bloomington, Indiana, USA. The colonies were censused and some were kept in laboratory culture. Dealate and alate females of *T. curvispinosus* and *T. minutissimus* were dissected. The parasite females have a total of six ovarioles. Both, *T. minutissimus* and the host species, are facultatively polygynous, and the parasite is host-queen tolerant. Alate males were found in August and September. High numbers of alate and dealate, mated but not yet reproductive, young females of *T. minutissimus* were present in colonies collected in early spring, a feature which had been known as "Intranidal Mated Offspring Hibernation" (IMOH). Mated young queens of *T. minutissimus* seem to disperse in spring to invade host colonies. Apparently they are accepted quite easily by host colonies. Rearing of colonies collected in the early spring, or hibernated in the laboratory, yielded first a brood of sexuals of *T. curvispinosus*, and subsequently considerable numbers of gyne pupae of *T. minutissimus* appeared. Only very few males were produced (sex ratio about 0.1 ♂/♀). Intranidal mating attempts were observed, and newly mated young females were detected in colonies having reared gynes and males of *T. minutissimus*.

Life history of the species thus is a novel combination of traits found in different other parasitic ant species: Intranidal mating and IMOH as in a few European "degenerate slavemakers" of the genus *Myrmoxenus*, but the parasite is host-queen tolerant, as is the case in two of the three European inquiline species of *Leptothorax* (former *Doronomyrmex*). The development of the parasites after the host species sexuals is a novel trait.

The male of *T. minutissimus* is described. It is characterized by a reduced number of antennomeres (9-11 instead of 12), and a certain "morphological feminization".

Key words: *Temnothorax minutissimus*, *Temnothorax curvispinosus*, inquilinism, intranidal mating, female-biased sex ratio, IMOH, morphological feminization of male

Prof. Dr. Alfred Buschinger, Rossbergring 18, D-64354 Reinheim, Germany.
E-mail: hormigaleon.buschinger@t-online.de (contact author)

Timothy A. Linksvayer, Department of Biology, Indiana University, Bloomington, IN 47405, USA.

Introduction

"Social parasitism" occurs when a parasitic social insect species depends on another, usually closely related social insect species for at least one stage in colony development. A parasite-host relationship between two closely related species may frequently originate through intraspecific parasitism (e.g., BUSCHINGER 1990, BOURKE & FRANKS 1991, SAVOLAINEN & VEPSÄLÄINEN 2003). However, social parasitism in the broad sense also includes the "guest ants" that live together with only distantly related host species (e.g., the myrmicine genus *Formicoxenus* with the other myrmicine genera *Myrmica* and *Manica* or with the formicine genus *Formica*, respectively). Several major types of social parasitism in the strict sense have been described, including temporary social parasitism, dulosis, and inquilinism.

In temporary social parasitism, a young parasite queen penetrates a host nest, replaces the host queen, and rears her first brood with the aid of the remaining host workers. Later, after the natural death of the host workers, such colonies have numerous workers of the parasitic species, living similarly to independent species. A second group of social parasites practice dulosis, and are the slavemakers. Again, the young queen replaces the host colony queen(s), usually by force, and in some species the queen also kills or ejects all host workers, taking over only the host colony brood from which her first "slaves" emerge. Slavemaker workers also develop, but they are specialized on slave raiding and cannot forage or rear the slavemaker's broods. Instead, they attack neighboring nests of the host species from where

they retrieve worker pupae, thus replenishing the stock of slaves in the slavemaker colony.

The third group of social parasites, the "inquilines", are species that usually have lost their own worker caste. Mated young queens, by one or the other technique, join the host colonies in which they typically coexist with the host species queen(s). A few inquiline species are dubbed "host queen intolerant" because they are never found in queenright host nests.

A surprising wealth of morphological and behavioral particularities has been found in such social parasites, and nearly each species, or at least each genus of parasitic ants exhibits its own blend of techniques to ensure its survival and to exploit the social characteristics of its respective host species. Some species, instead of mating during a nuptial flight, mate inside the mother nests and thus are adelphogamous. Hibernation of dealate, mated, young queens is observed in some of these species making the colonies seemingly polygynous during parts of the annual cycle. Wingless, workerlike females may occur, and the sex ratio may be considerably female-biased due to reduced male production. Both host and inquiline species may be monogynous or facultatively polygynous, etc.; for details, particularly referring to the various hypotheses on the evolution of social parasitism, see BOURKE & FRANKS (1991), BUSCHINGER (1986a, 1990, 2002) and HÖLLDOBLER & WILSON (1990).

In this paper, we will investigate a poorly known inquiline species of which we recently were able to gather a number of colonies from the field, *Temnothorax* (= *Leptothorax*, subgenus *Myrafant*) *minutissimus* (SMITH, 1942). Our experiments and observations suggest that this species exhibits a novel blend of life history traits.

Temnothorax minutissimus has been described by SMITH (1942) after four females from the collections of the United States National Museum of Natural History, Washington DC, USA (USNM). Because the females were in the same series with several workers of *T. curvispinosus* (MAYR, 1866), the author suggested that the new species is an inquiline of the latter.

Until recently, practically nothing new has been published on the species' life history. HEINZE (1989) mentions preliminary enzyme-electrophoretic results suggesting a close relationship of *T. minutissimus*, *T. duloticus* (WESSON, 1937) and *Protomognathus americanus* (EMERY, 1895) to their hosts. *Temnothorax duloticus* and *P. americanus* are slavemaker species that both enslave *T. curvispinosus* and in addition a few other species of *Temnothorax*. A couple of websites refer to new records and provide a few more biological observations of *T. minutissimus*.

Besides the original collection from Washington D.C. (SMITH 1942), *T. curvispinosus* colonies with



Fig. 1: Part of a *Temnothorax curvispinosus* colony with workers and a large queen, and a few alate gynes of the inquiline, *Temnothorax minutissimus*.

T. minutissimus have also been collected in Michigan (ALLOWAY undated), West Virginia (S. Foitzik, pers. comm.), New York (COVER 1996) and Ohio (HERBERS 2004). Apparently, multiple gynes of *T. minutissimus* can be found in a host species colony, but only the "socially dominant" female lays eggs, and this individual spends most of its time riding on the back of the host queen (ALLOWAY undated). Males of *T. minutissimus* apparently are produced in low numbers (HERBERS 2004). In this paper, we report collection data and further observations from 45 colonies of *T. curvispinosus* with *T. minutissimus*, and we provide an as yet lacking description of the male of *T. minutissimus*.

Material and methods

A total of 45 *T. curvispinosus* colonies with *T. minutissimus* were collected from June 2002 to August 2004 (Tab. 1) from the Griffy Nature Preserve and the adjacent Indiana University Griffy Woods Research and Teaching Preserve, near Bloomington, Indiana, USA. For laboratory rearing, proven techniques were applied as described by BUSCHINGER (1995). Dissections of females were made according to BUSCHINGER (1968) and ALLOWAY & al. (1982). Laboratory colonies were checked once a week, except for the time of hibernation. Since we lacked *T. minutissimus* colonies of similar size and composition in large numbers, the laboratory studies were designed in a way that a maximum of information could be gained from each single colony. Therefore the fate of a number of colonies is described in detail (Appendix).

Results

Temnothorax minutissimus is a rare workerless parasite of *T. curvispinosus*. For example, among 556 and 610 colonies of *T. curvispinosus* collected for different studies in 2003 and 2004, only 18 (3.3 %) and 14 (2.3 %), respectively, were parasitized by *T.*

Tab. 1: Details of colonies of *Temnothorax minutissimus* when collected, and production in laboratory culture. NA is entered when initial collection data is not available. w = workers; Lv = larvae; p = pupae; *P. am.* = *Protomognathus americanus*.

Coll. date	Colony #	<i>T. curvisp.</i> ♀♀/ w/ Lv	<i>T. minut.</i> ♀♀ Dealate/ alate	<i>T. minut.</i> ♂♂	Notes	<i>T. minut.</i> Production (♀♂)
26 Jun 02	251	1/ 41/ 45	ca 3 / 0	0	See Appendix	18 / 1
26 Jun 02	267	2/ 41/ 67	ca 2 / 0	0		
30 Sep 02	302	1/ 18/ 10	1/ 1	1		
30 Sep 02	308	0/ 17/ 10	5/ 0	0		
30 Sep 02	317	1/ 76/ 11	0/ 2	0		
30 Sep 02	321	1/ 35/ 7	0/ 6	0		
30 Sep 02	326	0/ 32/ 7	3 / 4	0	See Appendix	0 / 0
15 Mar 03	519	1/ 228/ 56	11/ 8	0	See Appendix	65 / 6
15 Mar 03	521	1/ 76/ 57	3/ 3	0	See Appendix	6 / 1
10 Apr 03	883	1/ 34/ 33	19/ 9	0	See Appendix	5 / 1
10 Apr 03	884	1/ 30/ 20	42/ 45	0	See Appendix	4 / 0
10 Apr 03	891	1/ 65/ 11	8/ 0	0		
10 Apr 03	898	4/ 161/ 121	1/ 0	0		
10 Apr 03	900	0/ 75/ 40	1/ 0	0	+ 1 w <i>P. am.</i>	
10 Apr 03	907	1/ 38/ 20	30/ 21	0		
12 Apr 03	966	1/ 53/ 15	3/ 2	0		
13 Apr 03	1034	1/ 43/ 25	1/ 0	0		
13 Apr 03	1038	0/ 166/ 159	11/ 0	0	+ 1 ♀, 12 w <i>P. am.</i>	
31 Mar 03	1047	3/ 154/ 61	8/ 0	0		
31 May 03	1086	0/ 10/ 13	1/ 0	0		
31 May 03	1221	1/ 26/ 15	1/ 0	0		
31 May 03	1256	1/ 62/ 31	1/ 0	0	See Appendix	
31 May 03	1260	1/ 64/ 42	8/ 0	0		
31 May 03	1265	0/ 24/ 14	1/ 0	0		
31 May 03	1268	2/ 5/ 9	1/ 0	0	See Appendix	0 / 0
16 Mar 04	2004	3/ 28/ 14	2/ 0	0		0 / 1
25 Mar 04	2116	1/ 28/ NA	0/ 1	0		0 / 0
25 Mar 04	2131	1/ 25/ 23	7/ 2	0		0 / 0
25 Mar 04	2153	1/ 39/ NA	15/ 0	0		1 / 1
25 Mar 04	2165	2/ NA/ NA	1/ 0	0		0 / 0
11 Apr 04	2441	1/ 12/ 8	0/ 0	0		4 / 1
11 Apr 04	2443	1/ 40/ 28	12/ 1	0		15 / 2
11 Apr 04	2445	1/ 11/ 16	2/ 0	0		0 / 0
11 Apr 04	2449	1/ 12/ 13	2/ 0	0		2 / 0
18 Apr 04	2551	0/ 26/ 11	9/ 1	0		1 / 0
18 Apr 04	2552	2/ 27/ 32	4/ 0	0		3 / 0
18 Apr 04	2572	2/ NA/ NA	1/ 0	0		3 / 0
18 Apr 04	2604	0/ NA/ NA	1/ 0	0		1 / 1
18 Apr 04	2608	2/ 58/ 50	1/ 0	0		1 / 0
22 Aug 04	3536	1/ 34/ 13	6/ 9 (p)	5 (2 p)		
22 Aug 04	3537	1/ 23/ 3	1/ 0	0		
22 Aug 04	3538	1/ 17/ 16	2/ 3 (p.)	0		
22 Aug 04	3539	4/ 28/ 25	2/ 1	0		
22 Aug 04	3540	0/ 74/ 61	4/ 16 (2 p)	4 (1 p)	1 ♀, 21 w <i>P. am.</i>	
22 Aug 04	3541	1/ 36/ 17	4/ 9 (5 p)	4 (1 p)		



Fig. 2: Males (left in lateral view; right: heads in frontal view); from top to bottom: the inquiline, *Temnothorax minutissimus*; the host species, *Temnothorax curvispinosus*; the slavemaker, *Protomognathus americanus*; and the slavemaker, *Temnothorax duloticus*.

minutissimus. No workers belonging to the parasite species could be detected. The 45 colonies with *T. minutissimus* were collected at three nearby sites but were absent from many other surrounding areas, suggesting a patchy distribution. Most of these colonies were collected in open areas near the bases of large oak trees.

The original composition of the field-collected colonies is summarized in Tab. 1. Alate females were found in colonies collected in August – September and March – April, but not in May – June (no collections were made in July). There were usually only one or a few dealate *T. minutissimus* females, but in some colonies there were numerous dealate females and occasionally numerous alate *T. minutissimus* females as well (Fig. 1).

In the last column of Tab. 1, the laboratory production of the colonies is listed. By far not all colonies produced *T. minutissimus* offspring, or did so in only very low numbers. Just a few colonies reared a higher number of parasite females. Remarkably, extremely few males of *T. minutissimus* were found, both in the field-collected colonies (27 males, including male pupae vs. 441 females, including dealate and alate females and female pupae) and among the progeny of the laboratory colonies (14 ♂♂ vs. 129 ♀♀ = 0.11 ♂/♀). In the six colonies collected in August 2004, males were relatively more common and the sex ratio was 13 ♂♂ vs. 57 ♀♀ = 0.23 ♂/♀.

The exact number of parasite progeny could not be recorded in all lab-reared colonies, and the numbers are minimum values because specimens sometimes died and were consumed by their nestmates before a census could be made.

A brief history of the events in several particularly interesting and/or prolific colonies is presented in the Appendix.

A couple of marginal observations may be reported here: Queens of *T. minutissimus* (i.e. the reproductive specimens) not only were seen several times to crawl on top of the *T. curvispinosus* queens, sometimes licking them, but on three occasions a queen of *T. minutissimus* was seen to devour an egg (probably one laid by a host queen; however, it was not possible to differentiate between the eggs of host and parasite because both are of equal size and shape).

Two young, mated females of *T. minutissimus* that were introduced into a nest in which all females of *T. minutissimus* and the queen of *T. curvispinosus* had been dissected, were seen licking host workers (# 519).

Three colonies (# 900, # 1038 and # 3540) comprised both dealate and/or alate females of *T. minutissimus* as well as specimens of the slavemaking ant, *Protomognathus americanus*. Whereas in one colony only a single worker of *P. americanus* was found, the other two comprised both workers and a queen each of this slavemaker species.

Dissection of > 80 females of *T. minutissimus* revealed that they invariably had six ovarioles. In mated females the spermathecae usually were completely filled with sperm, though in a few instances (# 251, # 326) very few sperm was found, down to an estimated 10 % of the "normal" amount.

Finally, on a couple of occasions we observed a male of *T. minutissimus* mounting females in the nest and trying to mate. However, a true coupling was not observed.

Description of the male of *Temnothorax minutissimus*

Three males have been deposited in the United States National Museum of Natural History, Washington DC, USA.

Male: Total length ca. 2.15 mm, thorax length 0.76 mm, head width 0.45 mm, scape length 0.24 mm.

Particular characters: Sculpture of head punctate (as in queen); antennal scape comparatively longer than in male of *T. curvispinosus*; pronotum slowly ascending in comparison with steep ascend in *T. curvispinosus*; petioles shorter and stouter than in *T. curvispinosus*; propodeum with or (rarely) without pair of short, acute spines; compound eyes markedly smaller than in males of *T. curvispinosus* (Fig. 2 and Tab. 2).

Antennomeres: In *T. minutissimus* males reduced to 9 - 10 - 11 antennomeres (variable), as compared to *T. curvispinosus* males which have the usual 12 antennomeres.

Color: dark greyish-brownish (as compared to light yellow-brownish, pale males of *T. curvispinosus*).

Differential diagnosis: For differences to the male of the host species see above. Compared with the males of other (potential) parasites that may be encountered together with *T. curvispinosus*, the male of the slave-maker *Protomognathus americanus* (Fig. 2) is larger, has larger compound eyes, smaller mandibles, and lacks any propodeal spines. The male of the slave-making *T. duloticus* (Fig. 2) also is larger, has larger compound eyes, and has epinotal spines with a wider base, hence dentiform in shape. The male of *T. minutissimus* as yet is unique among the N-American Formicoxenini in having a reduced number of antennomeres (less than 12, sometimes as low as 9).

Discussion

Temnothorax minutissimus as yet has been found exclusively within colonies of the widespread and common independent species *T. curvispinosus*. Our observations confirm that *T. minutissimus* is an obligatory inquiline of this species. As most (if not all) inquiline ant species, *T. minutissimus* is strongly host specific, parasitizing exclusively *T. curvispinosus* (cf. HÖLLDOBLER & WILSON 1990). Slavemakers, on the

Tab. 2: Sizes and indices of males of *T. minutissimus*, *T. curvispinosus*, *T. duloticus* and *P. americanus*. HW = head width incl. eyes; TL = Length of thorax; total L = length of whole specimen; Eye D = largest diameter of compound eye; SL = scape length. All measurements in μm .

Species	HW	TL	Total L	Eye D	SL	Index SL : HW	Index TL : Eye D
<i>T. minutissimus</i> 1	460	780	2160	130	250		
<i>T. minutissimus</i> 2	440	760	2200	124	220		
<i>T. minutissimus</i> 3	460	760	2080	128	240		
Mean <i>T. minutissimus</i>	- 453 -	- 766.6 -	- 2147 -	- 127.3 -	- 236.6 -	0.52	6.02
<i>T. curvispinosus</i> 1	640	1120	2800	280	200		
<i>T. curvispinosus</i> 2	620	1060	2840	270	180		
<i>T. curvispinosus</i> 3	630	1140	2800	260	200		
Mean <i>T. curvispinosus</i>	- 630 -	- 1107 -	- 2813 -	- 270 -	- 193.3 -	0.30	4.1
<i>T. duloticus</i> 1	620	1040	2600	240	230	0.37	4.33
<i>P. americanus</i> 1	640	1080	2320	230	280	0.43	4.7

other hand, usually enslave more than one host species, which also applies for *Protomognathus americanus* enslaving the host species of *T. minutissimus* as well as *T. ambiguus* (EMERY, 1895) and *T. longispinosus* (ROGER, 1863), species that as yet have never been found parasitized by any inquiline species (BUSCHINGER 1990). *Temnothorax duloticus* has been found with *T. curvispinosus* and *T. longispinosus*.

Temnothorax minutissimus may live both in monogynous and polygynous colonies of the host species, and may itself be monogynous or slightly polygynous (Appendix, col. # 1268, # 884). Polygyny means that more than one female of either species are reproductive, functional queens. As yet, however, no colony with multiple queens of both host and parasite has been found. Facultative polygyny both of host and parasite species is not unusual among inquilines. It has been observed, among others, in *Leptothorax* (= *Doronomyrmex*) *kutteri* (BUSCHINGER, 1966), an inquiline of *L. acervorum* (FABRICIUS, 1793) (BUSCHINGER 1966), and in *Plagiolepis xene* STÄRCKE, 1936 and *P. grassei* LE MASNE, 1956, both inquilines of *P. pygmaea* (LATREILLE, 1798) (PASSERA 1969).

Since both host and parasite queens coexist within one host nest, *T. minutissimus* must be considered a "host-queen tolerant inquiline" (BOURKE & FRANKS 1991). Its life history with respect to queen numbers perfectly fits the suggestions of BOURKE & FRANKS (1991) and BUSCHINGER (1990). In both papers a condition of (facultative) polygyny in an independent species is considered a likely precondition for sympatric speciation and evolution of inquiline ants which consequently should be closely related to the respective host species.

In several instances clear evidence of intranidal mating has been found in *T. minutissimus* (Appendix, col. # 251, # 521, # 884), though except for vigorous mating attempts in the nests, mating itself could

not be seen. Mating hence takes place among sisters and brothers (or at least close relatives in polygynous colonies), and the males must be polygamous. Due to the sometimes very small number of males it nevertheless may happen that some of the gynes do not receive enough sperm to completely fill up their spermathecae (Appendix, col. # 251, # 326). Intranidal mating is not unusual in inquiline ants (HÖLDOBLER & WILSON 1990) or in "degenerate slavemakers" of the genus *Myrmoxenus* (BUSCHINGER 1986a).

Evidently the mated dealate (but sometimes also alate) females of *T. minutissimus* remain in the mother nests over winter. Considerable numbers have been found in colonies collected in the spring (Tab. 1). This behavior has been described also to occur in several species of the genus *Myrmoxenus*, another group of slavemakers and "degenerate slavemakers" of *Temnothorax* species in Europe (BUSCHINGER 1986a). In the European independent *T. gredosi* this feature had been dubbed "IMOH", Intranidal Mated Offspring Hibernation (BUSCHINGER 2002).

In the spring the mated young queens of *T. minutissimus* have the tendency to leave from the mother colonies. Colony foundation thus apparently occurs after hibernation. Put into formicaries containing unparasitized host colonies young mated females of *T. minutissimus* can be accepted in host species colonies both in spring and in fall. Their trials to penetrate the host nest may result either in an uncomplicated acceptance or, in other instances, in the eviction of the parasites by host colony workers (Appendix, col. # 519, # 521, # 883, # 1256). According to our lab and field observations we suggest that females of *T. minutissimus* penetrate the host colonies in spring. Since most mated females at that time are already dealate, and since probably the alate ones only rarely, if at all, will use their wings, this mode of dispersion on foot cannot be very effective in colonizing distant host nests. Dispersal on foot pro-

bably explains the patchy distribution of the parasite within larger host species populations. More data on frequencies and circumstances of penetration vs. eviction are desirable, though.

As far as is known, sexuals in independent *Temnothorax* species develop from hibernated larvae, i.e. as "slow brood" in the sense of Brian (cf. HÖLLEBLER & WILSON 1990: 352, A.B. unpubl. data). In the parasitic *T. minutissimus*, on the contrary, we found evidence of a rapid brood development of the sexuals (Appendix, col. # 251, # 519, # 521). However, colony # 251, after a second hibernation in absence of a queen of *T. minutissimus*, produced numerous females of *T. minutissimus* from hibernated slow brood. In several European species of *Myrmoxenus* also, in particular the workerless "degenerate slavemakers" preferably produce rapid brood sexuals (BUSCHINGER 1986a). In three colonies (Appendix, col. # 251, # 519, # 883) the sexuals of *T. minutissimus* appeared after the colonies had reared host species females earlier in the same season. This feature deserves more attention since it apparently has never been observed in other parasitic ants as yet. Usually either the sexuals of host and parasite are reared simultaneously, or exclusively parasite sexuals are produced.

Apparently only very few males of *T. minutissimus* are reared in the parasite colonies. The numerical sex ratio was estimated to be close to 0.1 ♂/♀. It may well be that *T. minutissimus* colonies under natural conditions produce more males or less females, respectively, than colonies in laboratory culture. The sex ratio of the colonies from August 2004 (13 ♂♂ and 57 ♀♀) thus may be more representative of conditions in nature. More field data will be necessary. In any case, the strongly female-biased sex ratio again corresponds quite well to the low male frequencies of European species of *Myrmoxenus* with intranidal mating and inbreeding (BUSCHINGER 1986a).

In three colonies comprising *T. minutissimus*, specimens of the slavemaker, *Protomognathus americanus*, were found in addition (col. # 900, # 1038, # 3540), together with workers of *T. curvispinosus*. In all three colonies a host species queen was lacking. In # 900 only one worker of *P. americanus* was found, whereas # 1038 and # 3540 contained a queen and several workers of the slavemaker species. It may be suspected that the latter two were *P. americanus* colonies that had raided *T. curvispinosus* colonies parasitized by *T. minutissimus*, and that the *T. minutissimus* specimens had developed from the pillaged brood. A similar explanation had been suggested by BUSCHINGER (1971) for colonies of the European slavemaker, *Harpagoxenus sublaevis* (NYLANDER, 1849), where a few colonies also contained *Leptothorax* (= *Doronomyrmex*) *kutteri*. *Leptothorax kutteri* is a workerless inquiline of *Leptothorax acervorum* which is the main host species of *H. sublaevis* as well. Co-

existence of two parasitic species in one colony of their common host species has been recorded also for *Plagiolepis pygmaea* and its two inquilines, *P. xene* and *P. grassei* (LE MASNE 1956). It had been termed "double parasitism". The instance of *T. minutissimus* and *P. americanus*, however, probably does not deserve this term: Other than perhaps is the case with the *Plagiolepis* parasites, it may be questioned that *T. minutissimus* has a chance to reproduce within a colony of the slavemaker *P. americanus*.

As had been reported by ALLOWAY (undated), we also saw females of *T. minutissimus* sometimes riding on top of a host species queen. Usually these were parasite queens with a swollen gaster, hence reproductive. So this behavior probably is not a sign of "dominance" as had been suggested by ALLOWAY (undated), but rather may be necessary for the parasite queen to obtain some special food, be it from the host queen's surface, or from workers of *T. curvispinosus* feeding their own queen, or even regurgitated by the host queen herself.

In "guest ants" of the genus *Formicoxenus* a similar "riding" on the body of their hosts, *Formica* and *Myrmica* spp., respectively, has been recorded. This behavior evidently is a kind of appeasement that helps the guests in soliciting food from the hosts. This has been directly observed both in North American *Formicoxenus quebecensis* FRANCOEUR, 1985 with *Myrmica* spp. (FRANCOEUR & al. 1985) and in European *F. nitidulus* (NYLANDER, 1846) and its *Formica* hosts (BUSCHINGER 1986b). According to an alternative or probably additional suggestion of LENOIR & al. (1997) such "riding" may help the parasites of genus *Formicoxenus* to acquire the host species' colony odor. For *T. minutissimus* an experimental study will be necessary to support one or the other explanation, or perhaps both.

The male of *T. minutissimus* which is described here for the first time, exhibits characters that fit well to intranidal mating: The compound eyes are markedly smaller than in the swarming host species, and the thorax is smaller and narrower, indicating a smaller mass of flight muscles. Nevertheless, the useless wings have been retained in both, males and gynes. This, however, is also true for the nest-mating species of *Myrmoxenus* mentioned above.

An intriguing feature of the males of *T. minutissimus* is their morphological similarity to the females: Propodeal spines, small compound eyes, rounded head, reduced number of antennomeres, short and stout petioles. According to KUSNEZOV (1956) this "feminization" of males seems to be a more common feature in parasitic ant species, though it does not always occur in inquiline species, and it seems to be absent in temporary parasitic as well as in dulotic groups. We rather suspect some relation to intranidal mating and inbreeding. "Feminization" in this context means a reduction of morphological attri-

butes characteristic of ordinary ant males, and a tendency to become more similar to the gyne habitus, whereas functionally the specimens remain males.

In conclusion, *T. minutissimus* is a species with a blend of characters as yet unknown among the workerless inquiline ants. It is tiny in comparison to its host species and all other species among the genus *Temnothorax* (formerly = *Leptothorax* subgenus *Myrafant*). Its life history is characterized by a permanent, host-queen tolerant inquilinism, lack of a worker caste, strongly female-biased sex ratio, intranidal mating, IMOH and dispersal of young queens on foot in the spring. The rare males exhibit a certain morphological feminization. With these preliminary results and the basic knowledge of the colony life cycle it should be feasible to produce a sufficient number of colonies for series of experiments, e.g., on colony foundation and on the effects of *T. minutissimus* on productivity and population dynamics of its host.

Acknowledgements

We would like to acknowledge the editors and two anonymous reviewers for helpful comments, and financial support to TL from the Indiana University Teaching and Research Preserve Grant Program.

Zusammenfassung

Fünfundvierzig Kolonien von *Temnothorax curvispinosus* mit der arbeiterinnenlosen, parasitischen Ameise *T. minutissimus* wurden in der Nähe von Bloomington, Indiana, USA, gesammelt. Die Völker wurden ausgezählt und einige davon wurden im Labor gehalten. Geflügelte und entflügelte Weibchen von *T. curvispinosus* und *T. minutissimus* wurden seziiert. Die Parasiten-Weibchen haben stets insgesamt sechs Ovariolen. Sowohl *T. minutissimus* als auch die Wirtsart sind fakultativ polygyn. *T. minutissimus* toleriert die Anwesenheit der Wirtskönigin (-nen).

Geflügelte Männchen wurden im August bzw. September angetroffen. Im Frühjahr waren geflügelte und ungeflügelte, aber noch nicht reproduktive Weibchen in großer Zahl in den Nestern, ein als "Intranidal Mated Offspring Hibernation" (IMOH), intranidale Überwinterung begatteter Jungweibchen, bekanntes Verhalten. Offenbar wandern junge *T. minutissimus*-Weibchen erst im Frühjahr aus, um in Wirtskolonien einzudringen. Anscheinend werden sie relativ leicht von Wirtskolonien akzeptiert.

Kolonien, die im Frühjahr gesammelt wurden bzw. im Labor überwintert hatten, produzierten zunächst eine Brut von *T. curvispinosus*-Geschlechtstieren, anschließend entstanden *T. minutissimus*-Weibchenpuppen in beträchtlicher Zahl. Nur sehr wenige Männchen wurden aufgezogen (Geschlechterverhältnis nahe 0.1 ♂/♀). Begattungsversuche innerhalb der Nester wurden beobachtet, und in Kolonien, die sowohl Männchen als auch Weibchen produziert hatten, wurden frisch begattete Weibchen nachgewiesen.

Der Lebenszyklus von *T. minutissimus* repräsentiert damit eine neuartige Kombination von Merkmalen, die von verschiedenen anderen Sozialparasiten bekannt sind: Begattung im Nest und IMOH, so wie bei einigen europäischen "degenerierten Sklavenhaltern" der Gattung *Myrmoxenus*. Anders als bei diesen jedoch toleriert die Parasitenart im Falle von *T. minutissimus* die Wirtskönigin(-nen), so wie das bei zwei der drei europäischen Inquilinenarten der Gattung *Leptothorax* (früher *Doronomyrmex*) der Fall ist. Die Entwicklung der Parasiten nach der Geschlechteraufzucht des Wirtsvolkes ist ein neuartiges Merkmal.

Das Männchen von *T. minutissimus* wird beschrieben. Es ist durch eine Reduktion der Fühlergliedzahl gekennzeichnet (9 - 11 anstatt 12), sowie durch eine gewisse morphologische Verweiblichung.

References

- ALLOWAY, T.M. undated: <<http://www.erin.utoronto.ca/~w3psy/people/AllowayHomePage/Minutissimus.html>>, retrieved on 7 November 2004.
- ALLOWAY, T.M., BUSCHINGER, A., TALBOT, M., STEWART, R. & THOMAS, C. 1982: Polygyny and polydomy in three North American species of the ant genus *Leptothorax* MAYR (Hymenoptera: Formicidae). – *Psyche* 89: 249-274.
- BOURKE, A.F. & FRANKS, N.R. 1991: Alternative adaptations, sympatric speciation and the evolution of parasitic, inquiline ants. – *Biological Journal of the Linnean Society* 43: 157-178.
- BUSCHINGER, A. 1966 ("1965"): *Leptothorax (Mychothorax) kutteri* n.sp., eine sozialparasitische Ameise (Hymenoptera, Formicidae). – *Insectes Sociaux* 12: 327-334.
- BUSCHINGER, A. 1968: Mono- und Polygynie bei Arten der Gattung *Leptothorax* MAYR (Hymenoptera Formicidae). – *Insectes Sociaux* 15: 217-226.
- BUSCHINGER, A. 1971: Zur Verbreitung und Lebensweise sozialparasitischer Ameisen des Schweizer Wallis (Hym., Formicidae). – *Zoologischer Anzeiger* 186: 47-59.
- BUSCHINGER, A. 1986a: Evolution, speciation, and inbreeding in the parasitic ant genus *Epimyrma* (Hymenoptera, Formicidae). – *Journal of Evolutionary Biology* 2: 265-283.
- BUSCHINGER, A. 1986b: Evolution of social parasitism in ants. – *Trends in Ecology and Evolution* 1: 155-160.
- BUSCHINGER, A. 1990: Sympatric speciation and radiative evolution of socially parasitic ants - Heretic hypotheses and their factual background. – *Zeitschrift für zoologische Systematik und Evolutionsforschung* 28: 241-260.
- BUSCHINGER, A. 1995: Life history of the parasitic ant, *Epimyrma bernardi* ESPADALER, 1982. – *Spixiana* 18: 75-81.

- BUSCHINGER, A. 2002: Intranidal Mated Offspring Hibernation (IMOH) in the Ant species *Leptothorax gredosi* (Hymenoptera: Formicidae: Formicoxenini). – *Entomologia Generalis* 26: 1-8.
- COVER, S. 1996: The Ants of Central Park, Long Island, and Southern New England, USA <http://research.amnh.org/entomology/social_insects/ants/centralp.html>, retrieved on 7 November 2004.
- FRANCOEUR, A., LOISELLE, R. & BUSCHINGER, A. 1985: Biosystématique de la tribu Leptothoracini (Formicidae, Hymenoptera). 1 – Le genre *Formicoxenus* dans la région holarctique. – *Naturaliste Canadien* 112: 343-403.
- HEINZE, J. 1989: A biochemical approach toward the systematics of the *Leptothorax "muscorum"* group in North America (Hymenoptera: Formicidae). – *Biochemical Systematics and Ecology* 17: 595-601.
- HERBERS, J. 2004: <<http://www.biosci.ohio-state.edu/~herbers/Temnothorax%20minutissimus.htm>>, retrieved on 7 November 2004.
- HÖLLDOBLER, B. & WILSON, E.O. 1990: The Ants. – Harvard University Press, Cambridge, Mass., 732 pp.
- KUSNEZOV, N. 1956: Der Sexualdimorphismus bei den Ameisen. – *Zeitschrift für wissenschaftliche Zoologie* 159: 319-347.
- LE MASNE, G. 1956: Recherches sur les fourmis parasites. Le parasitisme social double. – *Comptes rendues des Séances de l'Académie des Sciences (Paris)* 243: 1243-1246.
- LENOIR, A., MALOSSE, C. & YAMAOKA, R. 1997: Chemical mimicry between parasitic ants of the genus *Formicoxenus* and their host *Myrmica* (Hymenoptera, Formicidae). – *Biochemical Systematics and Ecology* 25: 379-389.
- PASSERA, L. 1969: Interactions et fécondité des reines de *Plagiolepis pygmaea* Latr. et des ses parasites sociaux *P. grassei* LE MASNE et PASSERA et *P. xene* ST. (Hym. Formicidae). – *Insectes Sociaux* 16: 179-194.
- SAVOLAINEN, R. & VEPSÄLÄINEN, K. 2003: Sympatric speciation through intraspecific social parasitism. – *Proceedings of the National Academy of Science USA* 100: 7169-7174.
- SMITH, M.R. 1942: A new, apparently parasitic ant. – *Proceedings of the Entomological Society of Washington* 44: 59-61.

Appendix

Details are provided here for a number of colonies that were kept in laboratory culture, or were particularly interesting for one or the other aspect. Dissections of females were made according to BUSCHINGER (1968) and ALLOWAY & al. (1982). As in these papers, "A" means a mated, egg-laying female, "b" is a newly mated, not yet laying individual, "c" is unmated; "C" is a virgin, but egg-laying female. The interpretation of the results is summarized (separated by a dash and in brackets) for each colony, or for single annual cycles of a colony, for easier reference in the Results and Discussion chapters.

Colony # 251 (coll. 26 June 2002) :The exact number of original dealate females of *T. minutissimus* was not recorded but there were at least three. In the first laboratory summer, at least 20 young females of *T. minutissimus* were reared (plus probably a male that was responsible for their insemination, see below). After a first laboratory hibernation (6 January to 21 April 2003), with only two surviving larvae, many of the dealate females were crawling around outside the nest chamber. When dissected, seven were not inseminated, but five had sperm in the spermathecae, though in comparatively low amount (ca 10 - 20 - 50 % of the normal sperm mass). None of the dissected females had been egg-laying (A-♀). Three females remained in the nest, together with the host queen.

About 8 weeks later, the first *T. minutissimus* female pupae were recorded. One female of *T. minutissimus* was observed laying an egg (it was of the same size as the host species eggs), and twice a female was seen "riding" on top of the host queen, vigorously licking her. A total of four females and one male of *T. minutissimus* were reared in this season. – (Rapid brood sexuals, since only two larvae had hibernated; spermathecae may be insufficiently filled).

In fall 2003, only four females of *T. minutissimus* had remained, and all were dissected. All four had sperm in their spermathecae, two were "b" (recently mated, hence mated in the nest, spermathecae full but ovaries short as in virgins), and two had somewhat longer ovarioles, apparently had been egg-laying already. – (Intranidal mating).

A second hibernation followed (24 October 2003 - 29 March 2004), with the host queen but no *T. minutissimus* specimens remaining in the nest.

Until 27 May a total of 41 females of *T. curvispinosus* were reared that began to leave from the nest for swarming. At that time, pupae of *T. minutissimus* were first recorded. A total of 18 females of *T. minutissimus* but no males were reared. By August 2004, nevertheless all the females had lost their wings. – (Sexuals of *T. minutissimus* are reared within the same season but after the host species sexuals. Sexuals of *T. minutissimus* can develop from hibernated "slow brood").

Colony # 326 (coll. 30 September 2002): All seven females of *T. minutissimus* (3 dealate, 4 alate) were dissected on 6 January 2003 (1 A; 6 b. Two b-♀♀ had only about 10 to 20 % of the regular sperm mass in their sperma-

thecae). – (Mated young females remain in the mother colony over winter; perhaps due to low male number some gynes may be insufficiently inseminated).

Colony # 519 (coll. 15 March 2003): After a first laboratory summer the colony contained one queen of *T. curvispinosus* and > 10 females of *T. minutissimus*. On 22 September 2003 all remaining females of *T. minutissimus* were dissected ("A 7 bCc", one mated, egg-laying queen, 7 newly mated females, one virgin but egg-laying female, and one virgin female). The female of *T. curvispinosus* has been a reproductive queen (A).

Two young females of *T. minutissimus* were introduced (from col. # 884) on 9 October 2003. The colony unexpectedly reared numerous females of *T. curvispinosus* in fall conditions.

It was hibernated from 21 November 2003 until 3 March 2004 (three female pupae of *T. curvispinosus* seemingly had survived the winter, but disappeared during the first two weeks in spring conditions). The two young females of *T. minutissimus* (from # 884) had survived and grew fat.

The colony again reared numerous females of *T. curvispinosus* between 8 May and 2 June 2004. Then some female pupae of *T. minutissimus* appeared. Until 15 August 2004, a total of 65 females and 6 males of *T. minutissimus* were reared. – (Rearing of *T. minutissimus* after host sexuals; low sex ratio; probably rapid brood sexuals; mated females of *T. minutissimus* can be accepted in fall, though in already formerly parasitized colony).

Colony # 521 (coll. 15 March 2003): On 24 September 2003, after a first laboratory summer, all three remaining females of *T. minutissimus* were dissected. One was newly mated (b), two were in a state between b and A, just developing fertility and probably having already laid a few eggs.

Two females of *T. minutissimus* were put into the fornicary (from col. # 884), but were soon evicted.

After a hibernation (7 November 2003 to 3 March 2004), without adults of *T. minutissimus*, three other females of *T. minutissimus* (from col. # 883, hibernated in their mother nest) were successively added. Two were quickly evicted, but one remained in the nest and apparently reproduced there.

The colony reared numerous host species females, and when they began to leave for swarming, one male pupa and 6 female pupae of *T. minutissimus* appeared.

By 25 June 2004, one male and three females of *T. minutissimus* were recorded. When dissected, all three females proved newly mated (b). – (Intranidal mating; females of *T. minutissimus* in fall ejected from foreign host colony, in spring accepted; probably rapid brood sexuals; low male production).

Colony # 883 (coll. 10 April 2003): At the end of a first laboratory summer, on 18 September 2003, the colony comprised one queen of *T. curvispinosus* and numerous females, female pupae, and one male pupa of *T. minutissimus*. The colony was hibernated from 31 October 2003 to 3 March 2004. The host queen and about 10 females of *T. minutissimus* survived the winter.

In spring, females of *T. minutissimus* began to leave from the nest. Three were introduced into col. # 521, of which one apparently succeeded. One female was put into col. # 1034 where it was accepted, but died after seven weeks without having reproduced.

The colony reared a few females of the host species, then 5 females of *T. minutissimus*, and no males. – (IMOH; hibernated young females tend to leave for colony foundation in the spring; host species sexuals reared before the parasite sexuals).

Colony # 884 (coll. 10 April 2003): At the end of the first laboratory summer the colony on 18 September 2003 comprised a host queen, ca. 30 females and 4 pupae of *T. minutissimus*. On 24 September 2003 and 9 October 2003 a total of 26 females of *T. minutissimus* were dissected. Two females that appeared "fat" were "A", mated and egg-laying queens. 23 females were "b", newly mated, one was "c", virgin. Four females were used for colony foundation experiments (two in col. # 519 where they succeeded, two in col. # 521 where they failed). One female of *T. minutissimus* remained in the colony.

After a hibernation from 21 November 2003 to 3 March 2004, the remaining female of *T. minutissimus* grew fat. Numerous host workers were reared but no females of *T. curvispinosus*. By July 2004 a total of 4 females of *T. minutissimus* had been reared. – (Polygyny of *T. minutissimus*; intranidal mating).

Colony # 1256 (coll. 31 May 2003): After a first laboratory summer and a hibernation the colony contained no female of *T. minutissimus* but a queen of *T. curvispinosus* that was soon executed by its own workers. On 23 April 2004 one queen of *T. minutissimus* (if mated?; from # 884) was introduced. It survived until 2 July 2004 (for 9 weeks). The colony reared ca. 20 gynes *T. curvispinosus* and numerous males. – (Introducing female of *T. minutissimus* in spring succeeded).

Colony # 1268 (coll. 31 May 2003): On 28 July 2003 all females were dissected. Both, the two females of *T. curvispinosus* and the female of *T. minutissimus*, were mated and fully reproductive queens. – (Polygyny of host colony).