



RESEARCH ARTICLE - ANTS

Two Cleptoparasitic Ant Crickets (Orthoptera: Myrmecophilidae) that Share Similar Host Ant Species Differentiate Their Habitat Use in Areas of Sympatry in Japan

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Abstract

Myrmecophilus crickets (Myrmecophilidae, Orthoptera) are typical ant guests. In Japan, about 10 species are recognized on the basis of morphological and molecular phylogenetic frameworks. We focused on two of these species, *M. kinomurai* and *M. kubotai*, and compared their host and habitat use. Previous work based on a limited sampling effort suggested that these two species share some ant species as hosts, but that their habitat preferences (open versus shaded) differ. Here, on the basis of exhaustive sampling across Japan, we confirmed that *M. kinomurai* and *M. kubotai* do not differ in their host ant preferences: both prefer formicine ants as hosts. As for habitat preferences, *M. kubotai* occurred significantly more often in open habitats than in shaded ones ($P < 0.05$). In contrast, *M. kinomurai* showed no habitat preference in areas where *M. kubotai* did not occur. However, *M. kinomurai* showed an obvious preference for shaded environments in areas of sympatry with its potential competitor *M. kubotai*. This pattern suggests that interspecific competition between *M. kinomurai* and *M. Kubotai* is a factor causing habitat differentiation in areas of sympatry.

Introduction

Habitat segregation may enable potentially competing organisms to coexist in a given region (Schoener, 1974). Habitat segregation has been well-studied in species pairs of various taxa, including herbivorous mammals (e.g. Main & Coblents, 1990; Conradt et al., 1999), birds (e.g. Lynch et al., 1985), and aquatic organisms (e.g. Hearn, 1987; Leibold, 1991). However, such segregation can be interpreted as a result of either interspecific competition (e.g. Chiba, 1996) or differences in habitat preference (e.g. Steen et al., 2014).

There are only a few cricket taxa that are myrmecophilous and *Myrmecophilus* (Orthoptera: Myrmecophilidae) is one of them (Kistner, 1982; Ingrisich, 1995). These ant crickets live in ant nests and exploit debris, ant eggs and larvae, and other food resources in diverse ways (Wheeler, 1900; Wasmann, 1901; Hölldobler, 1947; Henderson & Akre, 1986; Sakai & Terayama, 1995; Akino et al., 1996; Komatsu et al., 2009). Some *Myrmecophilus* species mimic the ant colony's chemistry by acquiring cuticular hydrocarbons from the ants via physical contact,

causing the ants to recognize them as nest mates (Henderson & Akre, 1986; Sakai & Terayama, 1995; Akino et al., 1996).

In Japan, at least 10 species of *Myrmecophilus* have been recognized on the basis of body surface structures, and each of these species has been collected from nests of specific ant species (Maruyama, 2004). By using molecular phylogenetic methods, Komatsu et al. (2008) detected seven well-supported mtDNA lineages in Japanese *Myrmecophilus*; these lineages do not completely agree with the morphological taxonomy. Komatsu et al. (2008) were able to group the lineages into at least two categories on the basis of their host specificity: specialists, which are commensally associated with a few ant species, and generalists, which are commensally associated with many ant species or genera. Additionally, each mtDNA lineage appeared to show a habitat use preference (Komatsu et al., 2008). In this study, we investigated host and habitat use by the two most commonly occurring species, *M. kinomurai* (mtDNA lineage D+G of Komatsu et al., 2008) and *M. kubotai* (lineage E+F of Komatsu et al., 2008), on the basis of exhaustive sampling across Japan.



Myrmecophilus kubotai is found only in Honshu and Shikoku, whereas *M. kinomurai* is distributed widely from Hokkaido to Kyushu. Thus, there is partial overlap in their distribution areas (Maruyama, 2006). Komatsu et al. (2008) investigated the host and habitat preferences of these two cricket species by random sampling in Honshu, where they coexist, and reported that both *M. kubotai* and *M. kinomurai* most frequently use formicine ants as host, and one particular formicine ant species, *Lasius japonicus*, was used by both cricket species (Komatsu et al., 2008). Moreover, Komatsu et al. (2008) reported that the collected *M. kubotai* samples were from open habitats such as grassland or wasteland whereas the collected *M. kinomurai* samples were from shaded habitats such as forests. These results may indicate that *M. kubotai* and *M. kinomurai* are in a competitive relationship for adequate host ant species and that they differentiate their habitat to avoid encountering each other. However, because Komatsu et al. (2008) sampled these two cricket species mainly in Honshu, where they coexist, they could not determine whether their habitat preferences would differ between the areas of coexistence (Honshu and Shikoku) and areas inhabited by only one (*M. kinomurai*) of the two species (Hokkaido and Kyushu).

In this study, we conducted exhaustive sampling, mainly in Honshu, where these two cricket species coexist, but also in Hokkaido and Kyushu, where *M. kubotai* does not occur, and examined their habitat use and how it differed in different areas. We then compared host-ant and habitat use between the two cricket species.

Materials and Methods

Collection of samples

Sampling was conducted from Hokkaido to Kyushu, Japan, in 2004-2008 (Appendix 1). Adult or nymph *Myrmecophilus* crickets were collected from randomly-selected ant nests. We collected as many crickets as possible by excavating the nest if it was subterranean or by spraying insect rejectant (repellent to keep mosquitoes out) into the nest if it was arboreal. The collected cricket samples were immediately preserved in absolute ethanol. When we collected samples, we recorded the habitat type (open versus shaded) where the host ant nests were located. If there were some sort of masking objects (e.g., trees) around the nest entrance (within 10m in radius), we determined the habitat as shaded. If not so, we determined the habitat as open.

The collected cricket samples were identified by using field-emission scanning microscopy (JEOL, JSM-6390) or mtDNA sequencing (see below). The specimens were digitally micrographed without coating. Voucher specimens are deposited in the Institute of Tropical Agriculture, Kyushu University, Fukuoka, Japan. Some of the samples used in this study were also used by Komatsu et al. (2008, 2010).

DNA analysis

We used mitochondrial sequences of collected samples to distinguish the target lineages of *M. kinomurai* and *M. kubotai* (see below). Komatsu et al. (2008, 2010) used the cytochrome *b* (*cytb*) gene for molecular phylogenetic analysis. However, the primers for that gene region did not successfully amplify mtDNA in many of the *M. kinomurai* samples. Therefore, we used the 16S ribosomal RNA (16S rRNA) gene, which has a lower substitution rate and has been used in studies of several orthopteran insects (e.g. Allegrucci et al., 2005; Lu & Huang, 2006).

DNA was extracted from the hind legs of the crickets by using a DNeasy Blood & Tissue Kit (QUIAGEN); the other body parts were preserved for morphological identification. A 511-bp fragment of the mitochondrial 16S ribosomal RNA (16S rRNA) gene corresponding to positions 12887-13398 in the *Drosophila yakuba* mtDNA genome was amplified by polymerase chain reaction (PCR) with the primers 16Sbr (5'-CCG GTC TGA ACT CAG ATC ACG T -3') and 16Sar (5'-CGC CTG TTT AAC AAA AAC AT -3') (Simon et al., 1994) and using the following temperature profile: 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 90 s. After amplification, the PCR products were purified using a QIAquick PCR purification kit (QUIAGEN). Cycle sequencing reactions were performed with BigDye Terminator Ver 1.1 Cycle Sequencing kit on an ABI 3100 automated sequencer.

We used the obtained 16S rRNA dataset and MEGA4 software (Tamura et al., 2007) to reconstruct the phylogeny by the neighbor-joining (NJ) method. Our previous phylogenetic analysis based on the *cytb* gene revealed two cryptic lineages in both of the morphospecies *M. kinomurai* and *M. kubotai* (lineages D and G in *M. kinomurai* and lineages E and F in *M. kubotai*; Komatsu et al., 2008, 2010), and we recognized a similar split lineage in our analysis of the 16S rRNA gene. However, one 16S rRNA lineage of *M. kinomurai*, corresponding to lineage D of Komatsu et al. (2008), was quite rare and was collected from a fairly limited area. In addition, one 16S rRNA lineage of *M. kubotai*, corresponding to lineage F of Komatsu et al. (2008), almost exclusively used the myrmicine ant, *Tetramorium tsushimae*, as host. Therefore, we excluded samples of these lineages (corresponding to lineages D and F of Komatsu et al., 2008) from the analysis.

Statistics

Details of the sampled ant crickets (host ant species, collected habitats, etc.) are given in Appendix 1. We used one-way chi-squared test to compare the frequency of occurrence of *M. kinomurai* and *M. kubotai* in formicine ant nests relative to other ant subfamilies. We arbitrarily divided Japan into six occurrence areas: Hokkaido, Northern Honshu (Tohoku), Eastern Honshu (Kanto and Chubu), Western Honshu (Kansai and Chugoku), Shikoku, and Kyushu.

We next sorted the areas into two categories: coexistence areas (Eastern Honshu, Western Honshu and Shikoku) where both *M. kinomurai* and *M. kubotai* distributed and non-coexistence areas (Hokkaido, Northern Honshu, and Kyushu) where only *M. kinomurai* distributed. Within each of the two categorized area, the preference of the two cricket species for open or shaded habitats was determined by using a one-way chi-squared test. For example, the proportion of *M. kinomurai* individuals collected from open habitat among all collected *M. kinomurai* individuals (observed proportion in open habitat) in a given area was compared to the proportion of investigated ant nests in open habitats among the total investigated ant nests (expected proportion in open habitat). If the crickets inhabited the open habitat significantly more often than expected, we judged that they “preferred” that habitat. The statistical analyses were performed with the R software package (ver. 2.3.1; R Development Core Team, 2005).

Results and Discussion

We surveyed a total of 1250 ant colonies representing 69 species (Appendix 2). These 69 species were distributed among ant subfamilies as follows: Amblyoponinae (AM), 1 species (2 colonies); Formicinae (FO), 31 species (699 colonies); Dolichoderinae (DO), 2 species (17 colonies); Myrmicinae (MY), 24 species (459 colonies); Ponerinae (PO), 8 species (69 colonies); and Proceratiinae (PR), 3 species (4 colonies). In addition, one *M. kubotai* cricket individual was collected from a termite nest, and six individuals (including one each of *M. kinomurai* and *M. kubotai*) were collected from outside ant nests (e.g. on the ground) (Appendix 2).

Host specificity

We collected a total of 880 *Myrmecophilus* individuals (Appendix 2) of the five species, *M. gigas*, *M. kinomurai*, *M.*

kubotai, *M. sapporensis*, and *M. tertamorii*. For *M. kinomurai* and *M. kubotai*, most individuals were collected from formicine ant nests (*M. kinomurai* vs. *M. kubotai*, %, AM: 0 vs. 0, FO: 93 vs. 88, DO: 0 vs. 0, MY: 5 vs. 8, PO: 0 vs. 0, PR: 0 vs. 0). In addition, one *M. kubotai* cricket individual was collected from a termite nest, and six individuals (including one each of *M. kinomurai* and *M. kubotai*) were collected from outside ant nests (e.g. on the ground) (Appendix 2). Thus, we confirmed the previous finding of Komatsu et al. (2008) that *M. kinomurai* and *M. kubotai* preferred Formicine ants as hosts. We detected no significant difference in preference toward Formicinae between *M. kinomurai* and *M. kubotai* (Chi-squared test for FO and MY, $P = 0.54$). Further, the most-preferred host ant species was the same (*Lasius japonicus*) between these two cricket species (*M. kinomurai*, 25 of 58 hosts; *M. kubotai* 24 of 52 hosts). *Myrmecophilus kinomurai* was collected in all six occurrence areas of Japan, but *M. kubotai* was collected only in Eastern Honshu, Western Honshu, and Shikoku (not in Hokkaido, Northern Honshu, or Kyushu).

Phylogenetic analysis

The DNA lineages of the *M. kinomurai* and *M. kubotai* samples were determined by NJ analysis of a 530-bp sequence of the mitochondrial 16S rRNA gene. Of the 110 analyzed samples, 58 belonged to *M. kinomurai* (corresponding to lineage G of Komatsu et al., 2008) and 52 belonged to *M. kubotai* (corresponding to lineage E of Komatsu et al., 2008).

Habitat preferences

In areas of sympatry with the closely related *M. kinomurai* (i.e. coexistence areas), *M. kubotai* occurred significantly more often in open habitats, whereas *M. kinomurai* showed an obvious preference for shaded environments (Table 1, Fig 1). In contrast, where *M. kubotai*

Table 1. Habitat specificity of the ant crickets determined by chi-squared test for biased habitat preferences of the ant crickets in those areas where only *M. kinomurai* occurred and where both crickets were distributed. If the crickets inhabited open (or shaded) habitats in a significantly higher proportion than the expected proportion (i.e., the proportion of that habitat among total available ant nests), they were judged to “prefer” that habitat. See text for details. ++, $P < 0.01$; ns, not significant. O, open habitat; S: shaded habitat.

Sampling area	Species (No. of samples)	Environment (No. of samples)	Expected proportion (No. of investigated ant nests in that habitat / total investigated ant nests)	Observed proportion (No. of crickets collected from that habitat / total collected crickets)	<i>P</i>
Non-coexistence area	<i>M. kinomurai</i> (27)	Open (19)	250/393	19/27	ns
		Shaded (8)	143/393	8/27	
	<i>M. kubotai</i> (0)	Open (0)	-	-	-
		Shaded (0)	-	-	-
Coexistence area	<i>M. kinomurai</i> (31)	Open (7)	487/857	7/31	++(S) ++(O)
		Shaded (24)	370/857	24/31	
	<i>M. kubotai</i> (52)	Open (43)	487/857	43/52	
		Shaded (9)	370/857	9/52	

did not occur, *M. kinomurai* did not show an obvious preference for either habitat type (Table 1, Fig 1). These results suggest that interspecific competition between *M. kinomurai* and *M. kubotai* is a factor causing *M. kinomurai* to shift its habitat use in areas of sympatry. This observed habitat shift of *M. kinomurai* in areas of sympatry with its potential competitor *M. kubotai* can be interpreted in two ways. One possible interpretation is that the primary host ant species shows a habitat difference between coexistence areas (e.g. western Honshu) and non-coexistence areas (e.g. Hokkaido). In this study, many *M. kinomurai* individuals were collected from nests of *Lasius japonicus* (Appendix 2). The studied nests of *L. japonicus* were found mainly in open habitats both in coexistence areas (number of nests in open vs. shaded habitat, 99 vs. 35) and non-coexistence areas (99 vs. 10) although the habitat preference *M. kinomurai* was different between the areas (Chi-squared test, $P < 0.01$). Therefore, it is unlikely that the observed habitat difference of *M. kinomurai* between the two types of areas reflected a habitat difference of the primary host ant species between them.

A second possible interpretation is that in the coexistence areas *M. kinomurai* shifts to a host ant species inhabiting open habitats rather than shaded habitats because of interspecific competition in those areas. In fact, the host ant species used by *M. kinomurai* differed significantly between the coexistence areas and non-coexistence areas (co-existence areas vs. non-coexistence areas, number of samples: Aj, 1 vs. 0; Cj, 1 vs. 2; Co, 0 vs. 1; Fj, 1 vs. 2; Fs, 1 vs. 0; Fy, 1 vs. 0; Lc, 1 vs. 0; Lf, 5 vs. 3; Lj, 6 vs. 19; Lni, 4 vs. 0; Lsp, 5 vs. 0; Lu, 1 vs. 0; Pl, 1 vs. 0; Ppu, 1 vs. 0; Tt, 1 vs. 0; out, 1 vs. 0; Chi-squared test; $P = 0.03$; see Appendix 1 for host ant species codes). In coexistence areas, *M. kinomurai* tended not to use *L. japonicus*, an openland dweller (as mentioned above); instead, it used shaded habitat dwellers such as *L. nipponensis* and *L. spathepus*. Moreover, most *M. kinomurai* were collected from shaded environments in coexistence areas (Fig 1). Therefore, in the coexistence areas *M. kinomurai* may shift to a host ant species preferring shaded environments.

Divergence of habitat use resulting from interspecific competition between sympatric species has been reported before (i.e., a form of ecological character displacement; Chiba, 1996).

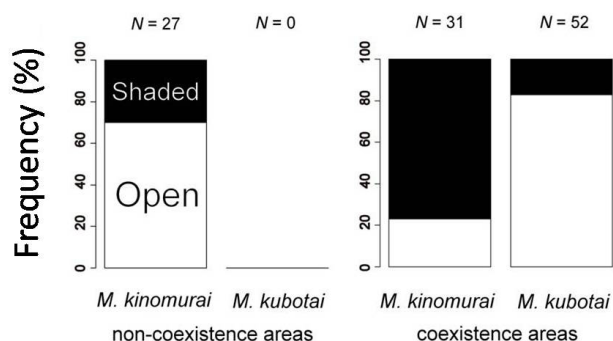


Fig 1. Habitat preferences of *M. kinomurai* and *M. kubotai* in coexistence and non-coexistence areas of Japan.

Because *M. kinomurai* and *M. kubotai* are dominant species and share the same host ant taxon (Formicinae), it is plausible that there is competitive interaction between the two species. Because *Myrmecophilus* crickets use basically the same resource, the co-occurrence of more than one *Myrmecophilus* species within the same ant nest may threaten the survival of one of the cricket species. Thus, *M. kinomurai* may shift its habitat by shifting its host ant species from an open habitat-dwelling ant species to a shaded habitat-dwelling species in areas in which it coexists with *M. kubotai*.

Such avoidance may have another advantage. At least in Japanese *Myrmecophilus*, the morphological differentiation of genital characters among species is too minor to be used for taxonomic differentiation (Maruyama, 2006), which suggests that different species of Japanese *Myrmecophilus* can potentially mate with each other. Therefore, differentiation of habitat or host use, or both, may function as a premating isolation mechanism in this genus (i.e., a form of reproductive character displacement). The nature of the ecological or reproductive competition between *M. kinomurai* and *M. kubotai* remains unknown. The adverse effects of interactions between these species on their fitness and survival need to be investigated by examining interspecific behavioral interactions and their effect on fertility and survival experimentally, for example, by artificially introducing both species into a single host ant colony.

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Appendix 1. Overview of the sampled specimens of *M. kinomurai* (mtDNA cytb lineage G) and *M. kubotai* (mtDNA cytb lineage E) (habitat preferences are summarized in Fig. 1) and their 16S GenBank accession numbers. Host ant species codes: Cj, *Camponotus japonicus*; Co, *Camponotus obscuripes*; Fj, *Formica japonica*; Fs, *Formica sanguinea*; Fy, *Formica yessensis*; Lc, *Lasius capitatus*; Lf, *Lasius flavus*; Lfu, *Lasius fuji*; Lj, *Lasius japonicus*; Lni, *Lasius nipponensis*; Lsa, *Lasius sakagami*; Lsp, *Lasius spathepus*; Lu, *Lasius umbratus*; Mj, *Myrmica jessensis*; Mk, *Myrmica kotokui*; Pl, *Polyrhachis lamellidens*; Ppu, *Pristomyrmex punctatus*; Ps, *Polyergus samurai*; Trs, *Reticulitermes speratus*; Tt, *Tetramorium tsushimae*; Aj, *Aphaenogaster japonica*; Out, collected from outside an ant nest. O, open habitat; S, shaded habitat; -, no data. *, *M. kinomurai* mtDNA lineage D; **, *M. kubotai* mtDNA lineage F.

Species	Sample No.	Host ant	Locality (italics indicate non-coexistence areas)	Habitat	Date (yy.mm.dd)	Collector	Altitude (m)	Accession No.
<i>M. kinomurai</i>	198b	Fj	<i>Asamushionsen, Aomori, Aomori</i>	S	05.04.29	Komatsu T	112	AB818261
<i>M. kinomurai</i>	h99a	Lj	<i>Kita, Sapporo, Hokkaido</i>	S	06.09.08	Komatsu T	15	AB818257
<i>M. kinomurai</i>	h99b	Lj	<i>Kita, Sapporo, Hokkaido</i>	S	06.09.08	Komatsu T	15	AB818258
<i>M. kinomurai</i>	h84	Lj	<i>Takadomari, Fukagawa, Hokkaido</i>	S	06.09.06	Komatsu T	100	AB818259
<i>M. kinomurai</i>	h85	Lj	<i>Takadomari, Fukagawa, Hokkaido</i>	S	06.09.06	Komatsu T	100	AB818260
<i>M. kinomurai</i>	h30a	Lj	<i>Toyooka, Hidaka, Hokkaido</i>	S	06.08.29	Komatsu T	250	AB818262
<i>M. kinomurai</i>	1027	Lj	<i>Kamiagata, Tsushima, Nagasaki</i>	S	08.04.26	Komatsu T	50	AB818291
<i>M. kinomurai</i>	1054	Lj	<i>Kamiagata, Tsushima, Nagasaki</i>	S	08.04.27	Komatsu T	50	AB818292
<i>M. kinomurai</i>	200a	Cj	<i>Sannai, Aomori, Aomori</i>	O	05.06.19	Komatsu T	21.5	AB818245
<i>M. kinomurai</i>	201	Cj	<i>Sannai, Aomori, Aomori</i>	O	05.06.19	Komatsu T	21.5	AB818246
<i>M. kinomurai</i>	256a	Co	<i>Hitachinai, Ani, Akita</i>	O	05.08.26	Komatsu T	250	AB818256
<i>M. kinomurai</i>	h18	Fj	<i>Toya-ko, Sobetsu, Hokkaido</i>	O	04.08.28	Komatsu T	250	AB818254
<i>M. kinomurai</i>	h97a	Lf	<i>Kita, Sapporo, Hokkaido</i>	O	06.09.08	Komatsu T	15	AB818243
<i>M. kinomurai</i>	h58a	Lf	<i>Pinnesiri, Nakatonbetsu, Hokkaido</i>	O	06.09.04	Komatsu T	63	AB818249
<i>M. kinomurai</i>	h58c	Lf	<i>Pinnesiri, Nakatonbetsu, Hokkaido</i>	O	06.09.04	Komatsu T	63	AB818250
<i>M. kinomurai</i>	202	Lj	<i>Takanozaki, Imabetsu, Aomori</i>	O	05.06.21	Komatsu T	0	AB818239
<i>M. kinomurai</i>	h17a	Lj	<i>Minami-Utsu, Date, Hokkaido</i>	O	06.08.28	Komatsu T	5	AB818240
<i>M. kinomurai</i>	h17b	Lj	<i>Minami-Utsu, Date, Hokkaido</i>	O	06.08.28	Komatsu T	5	AB818241
<i>M. kinomurai</i>	h17c	Lj	<i>Minami-Utsu, Date, Hokkaido</i>	O	06.08.28	Komatsu T	5	AB818242
<i>M. kinomurai</i>	h96	Lj	<i>Kita, Sapporo, Hokkaido</i>	O	06.09.08	Komatsu T	15	AB818244
<i>M. kinomurai</i>	175	Lj	<i>Sannai, Aomori, Aomori</i>	O	04.08.12	Komatsu T	22	AB818247
<i>M. kinomurai</i>	176	Lj	<i>Sannai, Aomori, Aomori</i>	O	04.08.13	Komatsu T	22	AB818248
<i>M. kinomurai</i>	h59g	Lj	<i>Pinnesiri, Nakatonbetsu, Hokkaido</i>	O	06.09.04	Komatsu T	63	AB818251
<i>M. kinomurai</i>	h59h	Lj	<i>Pinnesiri, Nakatonbetsu, Hokkaido</i>	O	06.09.04	Komatsu T	63	AB818252
<i>M. kinomurai</i>	180b	Lj	<i>Tappi, Minmaya, Aomori</i>	O	04.08.21	Komatsu T	80	AB818253
<i>M. kinomurai</i>	h20a	Lj	<i>Toya-ko, Sobetsu, Hokkaido</i>	O	04.08.28	Komatsu T	250	AB818255
<i>M. kinomurai</i>	177a	Lj	<i>Hakkouda, Aomori, Aomori</i>	O	04.08.14	Komatsu T	1300	AB818263
<i>M. kinomurai</i>	182	Aj	<i>Satoyamabe, Matsumoto, Nagano</i>	S	04.09.13	Komatsu T	650	AB818277

Appendix 1. Overview of the sampled specimens of *M. kinomurai* (mtDNA cytb lineage G) and *M. kubotai* (mtDNA cytb lineage E) (habitat preferences are summarized in Fig. 1) and their 16S GenBank accession numbers. Host ant species codes: Cj, *Camponotus japonicus*; Co, *Camponotus obscuripes*; Fj, *Formica japonica*; Fs, *Formica sanguinea*; Fy, *Formica yessensis*; Lc, *Lasius capitatus*; Lf, *Lasius flavus*; Lfu, *Lasius fiji*; Lj, *Lasius nipponensis*; Lsa, *Lasius sakagami*; Lsp, *Lasius spathepus*; Lu, *Lasius umbratus*; Mj, *Myrmica jessensis*; Mk, *Myrmica kotokui*; Pl, *Polyrhachis lamellidens*; Ppu, *Pristomyrmex punctatus*; Ps, *Polyergus samurai*; Trs, *Reticulitermes speratus*; Tt, *Tetramorium tsushimae*; Aj, *Aphaenogaster japonica*; Out, collected from outside an ant nest. O, open habitat; S, shaded habitat; -, no data. *, *M. kinomurai* mtDNA lineage D; **, *M. kubotai* mtDNA lineage F. (Continuation)

Species	Sample No.	Host ant	Locality (italics indicate non-coexistence areas)	Habitat	Date (yy.mm.dd)	Collector	Altitude (m)	Accession No.
<i>M. kinomurai</i>	12	Fj	Sandani, Takayama, Gifu	S	04.06.27	Maruyama M	1100	AB818288
<i>M. kinomurai</i>	106	Fs	Satoyamabe, Matsumoto, Nagano	S	04.05.24	Komatsu T	650	AB818276
<i>M. kinomurai</i>	271	Lc	Satoyamabe, Matsumoto, Nagano	S	06.07.07	Komatsu T	630	AB818274
<i>M. kinomurai</i>	44	Cj	Satoyamabe, Matsumoto, Nagano	S	04.04.17	Komatsu T	650	AB818275
<i>M. kinomurai</i>	s50a	Lf	Kamihabarara, Ono, Fukui	S	08.09.16	Koshiyama Y	900	AB818278
<i>M. kinomurai</i>	s50b	Lf	Kamihabarara, Ono, Fukui	S	08.09.16	Koshiyama Y	900	AB818279
<i>M. kinomurai</i>	s50c	Lf	Kamihabarara, Ono, Fukui	S	08.09.16	Koshiyama Y	900	AB818280
<i>M. kinomurai</i>	30	Lj	Miyamotohigashikata, Nagaoka, Niigata	S	04.04.11	Komatsu T	60	AB818264
<i>M. kinomurai</i>	171b	Lj	Higashikawade, Azumino, Nagano	S	04.06.30	Komatsu T	620	AB818273
<i>M. kinomurai</i>	203	Lj	Iriyamabe, Matsumoto, Nagano	S	05.07.01	Komatsu T	1400	AB818290
<i>M. kinomurai</i>	136b	Lni	Arigasaki, Matsumoto, Nagano	S	04.06.03	Komatsu T	585	AB818268
<i>M. kinomurai</i>	136d	Lni	Arigasaki, Matsumoto, Nagano	S	04.06.03	Komatsu T	585	AB818269
<i>M. kinomurai</i>	167	Lni	Arigasaki, Matsumoto, Nagano	S	04.06.24	Komatsu T	585	AB818270
<i>M. kinomurai</i>	172	Lni	Arigasaki, Matsumoto, Nagano	S	04.07.01	Komatsu T	585	AB818271
<i>M. kinomurai</i>	s21a	Lsp	Imamura, Neo, Gifu	S	04.05.08	Maruyama M	204	AB818266
<i>M. kinomurai</i>	s21d	Lsp	Imamura, Neo, Gifu	S	04.05.08	Maruyama M	204	AB818267
<i>M. kinomurai</i>	s12d	Lsp	Togo, Oki, Shimane	S	04.08.02	Komatsu T	62	AB818293
<i>M. kinomurai</i>	s43a	Lsp	Tenno-zan, Oyamazaki, Kyoto	S	08.09.05	Ito T	270	AB818294
<i>M. kinomurai</i>	s43b	Lsp	Tenno-zan, Oyamazaki, Kyoto	S	08.09.05	Ito T	270	AB818295
<i>M. kinomurai</i>	199b	Lu	Iriyamabe, Matsumoto, Nagano	S	05.06.06	Komatsu T	1400	AB818289
<i>M. kinomurai</i>	s3b	Pl	Hatogaya, Shirakawa, Gifu	S	05.05.25	Maruyama M	600	AB818272
<i>M. kinomurai</i>	s39	Ppu	Kuchikamogawa, Shimanto, Kouchi	S	08.03.16	Befu T	300	AB818296
<i>M. kinomurai</i>	s51	out	Nakaokubo, Toyama, Toyama	S	08.10.21	Koshiyama Y	82	AB818265
<i>M. kinomurai</i>	455a	Fy	Nagasaka, Sakae, Nagano	O	07.05.04	Komatsu T	1000	AB818281
<i>M. kinomurai</i>	832b	Lf	Okuhidaonsen, Takayama, Gifu	O	07.08.17	Komatsu T	1250	AB818283
<i>M. kinomurai</i>	151	Lf	Iriyamabe, Matsumoto, Nagano	O	04.06.13	Komatsu T	1400	AB818287
<i>M. kinomurai</i>	458	Lj	Nagasaka, Sakae, Nagano	O	07.05.04	Komatsu T	1000	AB818282
<i>M. kinomurai</i>	206a	Lj	Hijirikogen, Omimura, Nagano	O	05.07.13	Komatsu T	1260	AB818284

Appendix 1. Overview of the sampled specimens of *M. kinomurai* (mtDNA cytb lineage G) and *M. kubotai* (mtDNA cytb lineage E) (habitat preferences are summarized in Fig. 1) and their 16S GenBank accession numbers. Host ant species codes: Cj, *Camponotus japonicus*; Co, *Camponotus obscuripes*; Fj, *Formica japonica*; Fs, *Formica sanguinea*; Fy, *Formica yessensis*; Lc, *Lasius capitatus*; Lf, *Lasius flavus*; Lfu, *Lasius fuji*; Lj, *Lasius japonicus*; Lni, *Lasius nipponensis*; Lsa, *Lasius sakagami*; Lsp, *Lasius spathepus*; Lu, *Lasius umbratus*; Mj, *Myrmica jessensis*; Mk, *Myrmica kotokui*; Pl, *Polyrhachis lamellidens*; Pp, *Pristomyrmex punctatus*; Ps, *Polyergus samurai*; Trs, *Reticulitermes speratus*; Tt, *Tetramorium tsushimae*; Aj, *Aphaenogaster japonica*; Out, collected from outside an ant nest. O, open habitat; S, shaded habitat; -, no data. *, *M. kinomurai* mtDNA lineage D; **, *M. kubotai* mtDNA lineage F. (Continuation)

Species	Sample No.	Host ant	Locality (italics indicate non-coexistence areas)	Habitat	Date (yy.mm.dd)	Collector	Altitude (m)	Accession No.
<i>M. kinomurai</i>	115	Lj	Iriyamabe, Matsumoto, Nagano	O	04.05.29	Komatsu T	1400	AB818286
<i>M. kinomurai</i>	207	Tt	Hijirikogen, Omimura, Nagano	O	05.07.13	Komatsu T	1260	AB818285
<i>M. kubotai</i>	440	Fj	Ueno, Taito, Tokyo	S	07.04.21	Komatsu T	10	AB818327
<i>M. kubotai</i>	1263c	Lfu	Yata, Mishima, Shizuoka	S	08.08.12	Komatsu T	15	AB818328
<i>M. kubotai</i>	1263d	Lfu	Yata, Mishima, Shizuoka	S	08.08.12	Komatsu T	15	AB818329
<i>M. kubotai</i>	s40	Lj	Hattori-Ryokuchi, Toyonaka, Osaka	S	08.05.30	Ichikawa A	26	AB818344
<i>M. kubotai</i>	s42a	Lj	Akashi-Kouen, Akashi, Hyogo	S	08.09.06	Ichikawa A	28	AB818345
<i>M. kubotai</i>	s37a	Lj	Chishima, Osaka, Osaka	S	08.05.11	Ichikawa A	33	AB818346
<i>M. kubotai</i>	s38	Lj	Chishima, Osaka, Osaka	S	08.05.11	Ichikawa A	33	AB818347
<i>M. kubotai</i>	658	Lj	Aido, Jinzekikogen, Hiroshima	S	07.06.05	Komatsu T	525	AB818348
<i>M. kubotai</i>	183b	Lni	Arigasaki, Matsumoto, Nagano	S	04.09.22	Komatsu T	585	AB818330
<i>M. kubotai</i>	362a	Cj	Awashima, Numazu, Shizuoka	O	05.10.20	Komatsu T	0	AB818297
<i>M. kubotai</i>	362b	Cj	Awashima, Numazu, Shizuoka	O	05.10.20	Komatsu T	0	AB818298
<i>M. kubotai</i>	52	Cj	Takibe, Azumino, Nagano	O	04.04.21	Komatsu T	571	AB818313
<i>M. kubotai</i>	168	Cj	Asahi, Matsumoto, Nagano	O	04.06.25	Komatsu T	620	AB818318
<i>M. kubotai</i>	914a	Cj	Nada, Kobe, Hyogo	O	07.09.16	Komatsu T	250	AB818339
<i>M. kubotai</i>	60	Fj	Shimokanuki, Numazu, Shizuoka	O	04.04.23	Komatsu T	0	AB818301
<i>M. kubotai</i>	s27	Fj	Ishioka, Ibaraki	O	-	Tsuneoka Y	100	AB818306
<i>M. kubotai</i>	241a	Fj	Asahi, Matsumoto, Nagano	O	05.09.30	Komatsu T	620	AB818314
<i>M. kubotai</i>	241b	Fj	Asahi, Matsumoto, Nagano	O	05.09.30	Komatsu T	620	AB818315
<i>M. kubotai</i>	97b	Fj	Asahi, Matsumoto, Nagano	O	04.05.18	Komatsu T	620	AB818316
<i>M. kubotai</i>	97d	Fj	Asahi, Matsumoto, Nagano	O	04.05.18	Komatsu T	620	AB818317

Appendix 2. Overview of ant nests surveyed and numbers of ant crickets found.

Ant Subfamily (No. of species)	Genus	Species	No. of crickets found (Total nests surveyed)	No. of <i>M. kinomurai</i> found	No. of <i>M. kubotai</i> found
Amblyoponinae (1)	<i>Amblyopone</i>	<i>A. silvestrii</i>	0 (2)	0	0
Formicinae (31)	<i>Acropyga</i>	<i>A. nipponensis</i>	0 (3)	0	0
		<i>Camponotus</i>	<i>C. devestivus</i>	0 (3)	0
		<i>C. japonicus</i>	13 (43)	3	5
		<i>C. kiusiuensis</i>	0 (2)	0	0
		<i>C. nawai</i>	0 (1)	0	0
		<i>C. nipponicus</i>	0 (1)	0	0
		<i>C. obscuripes</i>	3 (4)	1	0
		<i>C. vitiosus</i>	0 (4)	0	0
	<i>Formica</i>	<i>F. fukaii</i>	0 (1)	0	0
		<i>F. hayashi</i>	13 (40)	0	0
		<i>F. japonica</i>	206 (115)	3	9
		<i>F. lemani</i>	0 (15)	0	0
		<i>F. sanguinea</i>	5 (2)	1	0
	<i>Lasius</i>	<i>F. yessensis</i>	4 (8)	1	0
		<i>L. capitatus</i>	3 (3)	1	1
		<i>L. flavus</i>	56 (46)	8	0
		<i>L. fuji</i>	6 (6)	0	2
		<i>L. hayashi</i>	1 (22)	0	0
		<i>L. japonicus</i>	221 (243)	25	24
		<i>L. nipponensis</i>	13 (5)	4	3
		<i>L. orientalis</i>	5 (2)	0	0
<i>L. productus</i>		0 (1)	0	0	
<i>L. sakagamii</i>		5 (15)	0	2	
<i>Lasius</i>	<i>L. spathepus</i>	18 (9)	5	0	
	<i>L. talpa</i>	0 (3)	0	0	
	<i>L. umbratus</i>	2 (7)	1	0	
	<i>Nylanderia</i>	<i>N. flavipes</i>	0 (87)	0	0
		<i>N. sakurae</i>	0 (5)	0	0
<i>Plagiolepis</i>	<i>P. flavescens</i>	0 (1)	0	0	
<i>Polyrhachis</i>	<i>P. lamellidens</i>	3 (1)	1	0	
<i>Prenolepis</i>	<i>P. sp.</i>	0 (1)	0	0	
Dolichoderinae (2)	<i>Ochetellus</i>	<i>O. glaber</i>	0 (15)	0	0
	<i>Technomyrmex</i>	<i>T. gibbosus</i>	0 (2)	0	0

Appendix 2. Overview of ant nests surveyed and numbers of ant crickets found (Continuation).

Ant Subfamily (No. of species)	Genus	Species	No. of crickets found (Total nests surveyed)	No. of <i>M. kinomurai</i> found	No. of <i>M. kubotai</i> found
Myrmicinae (24)	<i>Aphaenogaster</i>	<i>A. japonica</i>	4 (23)	1	0
	<i>Cardiocondyla</i>	<i>C. kagutsuchi</i>	0 (1)	0	0
	<i>Crematogaster</i>	<i>C. matsumurai</i>	0 (4)	0	0
		<i>C. osakensis</i>	0 (21)	0	0
		<i>C. vagula</i>	0 (7)	0	0
	<i>Myrmica</i>	<i>M. jessensis</i>	1 (1)	0	1
	<i>Myrmica</i>	<i>M. kotokui</i>	6 (38)	0	1
		<i>M. taediosa</i>	0 (2)	0	0
	<i>Pheidole</i>	<i>P. fervida</i>	1 (51)	0	0
		<i>P. noda</i>	0 (26)	0	0
		<i>P. pieli</i>	0 (5)	0	0
	<i>Pristomyrmex</i>	<i>P. punctatus</i>	2 (36)	1	0
	<i>Pyramica</i>	<i>P. benten</i>	0 (4)	0	0
		<i>P. hexamera</i>	0 (1)	0	0
		<i>P. membranifera</i>	0 (1)	0	0
	<i>Solenopsis</i>	<i>S. japonica</i>	0 (9)	0	0
	<i>Monomorium</i>	<i>M. intrudens</i>	0 (4)	0	0
	<i>Carebara</i>	<i>C. yamatonis</i>	0 (2)	0	0
	<i>Strumigenys</i>	<i>S. lewisi</i>	0 (25)	0	0
	<i>Temnothorax</i>	<i>T. spinosior</i>	0 (2)	0	0
	<i>Tetramorium</i>	<i>T. bicarinatum</i>	0 (3)	0	0
		<i>T. tsushimae</i>	281 (159)	1	2
	<i>Vollenhovia</i>	<i>V. emeryi</i>	0 (10)	0	0
	Ponerinae (8)	<i>Cryptopone</i>	<i>C. sauteri</i>	0 (8)	0
<i>Hypoponera</i>		<i>H. nubatama</i>	0 (6)	0	0
		<i>H. sauteri</i>	0 (1)	0	0
<i>Pachycondyla</i>		<i>P. chinensis</i>	0 (35)	0	0
<i>Pachycondyla</i>		<i>P. javana</i>	0 (5)	0	0
		<i>P. pilosior</i>	0 (11)	0	0
<i>Ponera</i>	<i>P. japonica</i>	0 (1)	0	0	
	<i>P. scabra</i>	0 (2)	0	0	
Proceratiinae (3)	<i>Proceratium</i>	<i>P. itoi</i>	0 (1)	0	0
		<i>P. japonicum</i>	0 (1)	0	0
		<i>P. watasei</i>	0 (2)	0	0
Termite (1)	<i>Reticulitermes</i>	<i>R. speratus</i>	1	0	1
Outside nest			6	1	1
Total			880 (1250)	58	52