

Morphological characteristics reflect food sources and degree of host ant specificity in four *Myrmecophilus* crickets

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Abstract *Myrmecophilus* crickets are well-known inquilines that live and obtain food resources in ant nests. In Japanese *Myrmecophilus* species various degrees of host specificity are reflected in behavioral differences among species. For example, extremely specialized species perform trophallaxis with their host ant species, whereas generalist species may steal food from their hosts without any intimate contact. We examined behavioral variations among four *Myrmecophilus* species that use different hosts and show different degrees of specificity, and we also compared morphological traits such as mandible shape and hind leg length among the species. The morphometric analyses showed that an extreme host-specialist species had less complex and largely non-functional mandibles, reflecting its dependence on trophallaxis with its host ant species. In contrast, extreme host-generalist and/or moderate specialist species, which directly eat solid foods, had more complex and functional mandibles which they use to cut and crush their foods, such as insect carcasses and ant larvae. The extreme host-specialist species

had shorter hind legs than the extreme host-generalist. This may reflect that it suffers few attacks from the host ants. Our results show that in *Myrmecophilus* food sources shape behavioral interactions with host ant species and correlate with morphological characteristics.

Keywords Food habit · Interaction · Myrmecophily · Orthoptera · Specialization

Introduction

Myrmecophilus Berthold, 1827 (Myrmecophilidae) is a genus of crickets that exhibit myrmecophily, that is, they are inquilines that live inside ant nests in association with the ants (Savi 1819; Kistner 1979, 1982; Maruyama 2004). All known *Myrmecophilus* species exhibit myrmecophily (Ingrisch 1995; Maruyama 2004, 2006). Most species are scavengers that acquire food resources such as ant eggs and larvae and nest debris in the ant nests (Wheeler 1900; Hölldobler 1947; Sakai and Terayama 1995; Komatsu et al. 2009), but some species perform trophallaxis with their host ants. Although these crickets live within ant nests in all life stages, *Myrmecophilus* individuals often leave one ant nest and invade nearby nests (Wheeler 1900, 1910, 1928; Maruyama 2006). To enable their integration into an ant society from which non-nest mates are excluded, some *Myrmecophilus* species acquire cuticular hydrocarbons from the body surfaces of the ants and use them as chemical camouflage (Akino et al. 1996).

Myrmecophilus taxonomy has been confused because superficial characteristics such as coloration, body shape and size, and the presence of leg spurs have been used to distinguish species (Maruyama 2004). In Japan, three or four species are recognized on the basis of ambiguous

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characteristics such as body color and size (Sakai and Terayama 1995; Maruyama 2004). In addition to these taxonomic problems, the host ant specificity of *Myrmecophilus* species remains unclear. In the past, all species were thought to be host generalists, and the host range of each cricket species was believed to include more than 30 ant species spanning various subfamilies (Sakai and Terayama 1995). Opportunistic host use by North American and European *Myrmecophilus* species has caused them to be regarded as generalist parasites that switch their host species from smaller to larger as they grow (Wheeler 1900; Schimmer 1909; Hölldobler 1947; Baccetti 1967; Bernard 1968).

Recently, however, Maruyama (2004) used traits such as body shape and the density of body hair to distinguish ten Japanese *Myrmecophilus* species, and the evidence suggests that these crickets use different ant species as hosts, with varying degrees of specialization. In addition, molecular phylogenetic studies based on mitochondrial DNA support the conclusion that each *Myrmecophilus* species (mtDNA lineage) has preferences for specific ant taxa (Komatsu et al. 2008, 2010). By using both morphological and molecular phylogenetic frameworks, it is possible to discriminate among Japanese *Myrmecophilus* species and to evaluate differences in host specificity among the species. Komatsu et al. (2009) showed that two *Myrmecophilus* species, both of which live in the Ryukyu Islands and share one host ant species, are differentiated by their food sources and degree of host dependency. They found that *M. albicinctus* Chopard 1924 is an extreme host-specialist species; it was collected exclusively from the nests of *Anoplolepis gracilipes* (Smith, 1857) and it displayed close behavioral relationships, such as trophallaxis, with the ants. In contrast, *M. formosanus* Shiraki 1930 is an extreme host-generalist that uses various host ant species across subfamilies; this species did not display any physical intimacy with the ants but consumes ant larvae and dead insects (Maruyama 2004; Komatsu et al. 2008). Komatsu et al. (2010) observed similarly distinctive behaviors in two mtDNA lineages of *M. kubotai* Maruyama, 2004, on Honshu Island that have different host preferences. One lineage is a host specialist that prefers the ant species *Tetramorium tsushimae* Emery, 1925 and is frequently fed liquid foods by the ants, whereas the other lineage is a host generalist that uses any of several ant species within a single subfamily, mainly feeds itself, and eats solid foods (Komatsu et al. 2008, 2010).

These studies led to the conclusion that different Japanese *Myrmecophilus* species eat different foods and display distinct feeding behaviors, depending on their degree of host specificity (Maruyama 2004; Komatsu et al. 2008, 2010). These observations suggest that different species may have evolved not only behavioral but also morphological adaptations according to their ant-association strategy. For example, we hypothesized that species displaying a high degree

of host specificity and showing intimate contact with ant workers including trophallaxis might have smaller mandibles with a simplified morphology. *Myrmecophilus* hind leg morphology correlates with mandibular morphology and with degree of host specificity. *M. formosanus* is a host-generalist species that apparently avoids being attacked by the ants by escaping quickly (Komatsu et al. 2009), behavior facilitated by hind leg structure. Such species may not integrate into the ant colony by using chemical mimicry; rather, they may use strong hind legs to jump away from attacking ants (Wheeler 1900; Henderson and Akre 1986). Host-generalist *Myrmecophilus* species might be expected to have stronger hind legs in proportion to body size.

In this study, we conducted morphological measurements of the mandibles and hind legs of four Japanese *Myrmecophilus* species to investigate (1) whether food sources and mandibular morphology were related, and (2) whether in more host-generalist species, the hind legs were more developed.

Materials and methods

Study species

Four Japanese *Myrmecophilus* species were used in this study. *Myrmecophilus albicinctus* is an extreme specialist ant parasite that uses only a single ant host species, *Anoplolepis gracilipes* (Komatsu et al. 2009). In laboratory experiments of this species only trophallaxis of host ants was recognized as its feeding tactics (Komatsu et al. 2009). *Myrmecophilus tetramorii* Ichikawa 2001 is a moderate specialist ant parasite that uses only two ant species, most frequently *Tetramorium tsushimae*, a myrmicine (Komatsu et al. 2013). It can eat solid foods like *M. formosanus* (Komatsu et al. 2013), as described below. *Myrmecophilus kubotai* Maruyama, 2004, is a moderate generalist parasite that uses 10 ant species, mainly within subfamily Formicinae (Komatsu et al. 2008, 2010). It can eat both solid foods and liquid foods via trophallaxis (Komatsu et al. 2010). *Myrmecophilus formosanus* is an extreme generalist ant parasite that eats solid foods and uses various ant taxa from several subfamilies (Komatsu et al. 2009).

Komatsu et al. (2008, 2010) have identified two phylogenetic host races within *M. kubotai*; one uses several species of formicine ants as hosts, and the other uses *T. tsushimae* exclusively. In this study, we used the former race of *M. kubotai* as representative of a generalist parasite that can use several host species, even though all of the potential host species belong to a single subfamily. The identification of “generalist” *M. kubotai* and “specialist” by morphology is difficult at the moment, so we used the molecular approaches

of Komatsu et al. (2008, 2010) and extracted DNA from hind legs of the specimens to identify race.

Sampling

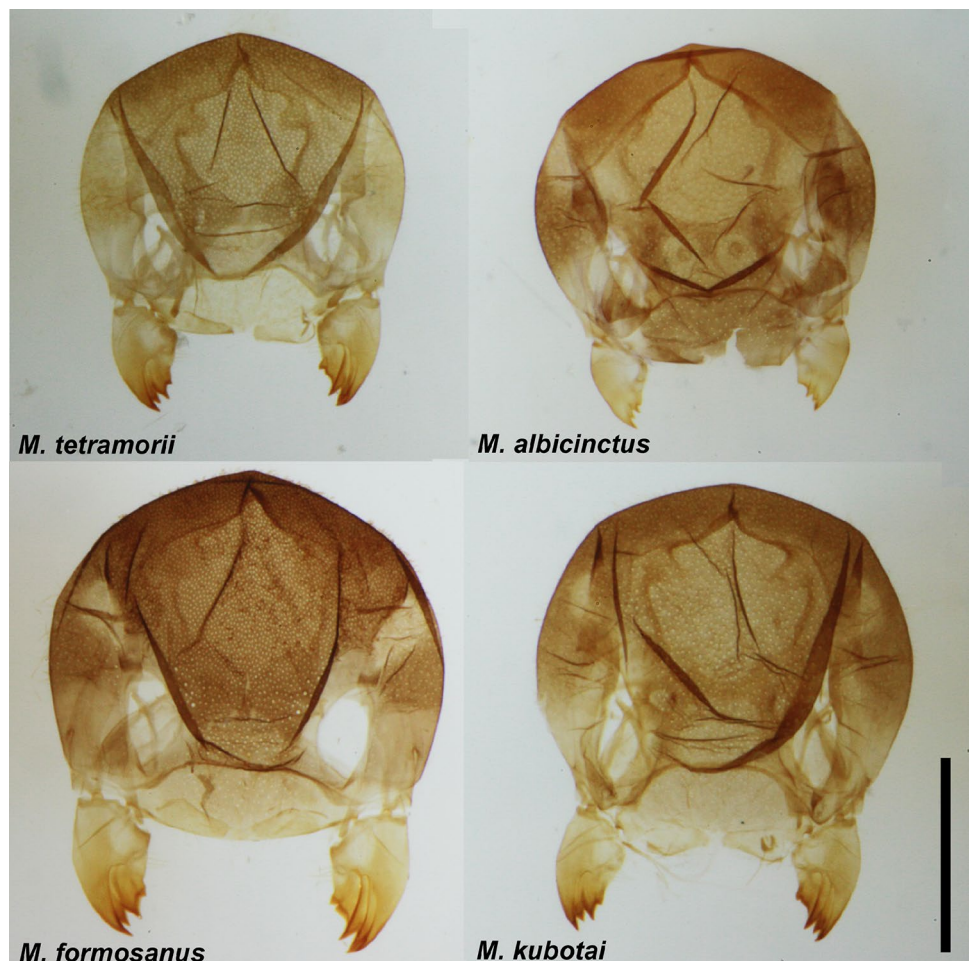
From 2007 to 2015, *M. albicinctus* and *M. formosanus* were sampled in several regions of the Ryukyu Islands (Okinawa and Iriomote Islands) and *M. kubotai* and *M. tetramorii* were sampled on Honshu Island (at Matsumoto, Nagano Prefecture, and Ishioka, Ibaraki Prefecture). We collected specimens of *Myrmecophilus* crickets in secondary forests, agricultural fields, and urban lands. As described by Komatsu et al. (2009), we searched for ant nests by turning over stones and breaking apart decayed logs. Whenever crickets were found in an ant nest, we collected as many individuals as possible and preserved them in absolute alcohol for subsequent analysis.

Measurement

We surveyed nests of total 100 ant species and collected more than 300 specimens of four *Myrmecophilus* species:

M. albicinctus from *Anoplolepis gracilipes*, *M. formosanus* from *A. gracilipes*, *Diacamma* sp. and some species of *Nylanderia* (formerly *Paratrechina*) and *Pheidole*, *M. kubotai* from *Camponotus japonicus*, *Formica japonica*, Some species of *Lasius*, two species of *Myrmica*, and *Tetramorium tsushimae*, *M. tetramorii* from *T. tsushimae* and *F. japonica*, respectively. We selected and measured total 78 adult specimens of *Myrmecophilus* crickets (24 of *M. albicinctus*, 14 of *M. formosanus*, 21 of *M. kubotai*, and 19 of *M. tetramorii*, respectively). Before measurement, we cleared and mounted all specimen's heads of the four *Myrmecophilus* species and prepared slide mount samples of each individual (Fig. 1) following the method of Kozarzhenskaya (1986). We then captured digitized images of the slide-mounted samples with a digital camera (EOS Kiss X3; Canon, Tokyo, Japan) mounted on a binocular microscope (40× magnification). We subsequently measured morphological traits on the digital images using Photo Measure software (Kenis Ltd., Osaka, Japan). To observe the mandibles clearly, we removed the labium and maxillae from the heads of all specimens before preparing slide mounts. Using the methods of Koshikawa et al. (2002), Tsuchiya et al. (2008),

Fig. 1 Slide-mounted samples of the head capsule of the four *Myrmecophilus* species. Scale bar = 0.5 mm



Judge and Bonanno (2008), and Neoh and Lee (2009), we measured body length (length from top of head to anus), head length (length of head to side base of mandibles) and width, and the lengths of 12 mandible parts (a–l, Fig. 2), together with the lengths of hind leg parts (femur, tibia, and tarsus) (Table 1). For leg length, we measured one side of hind leg (mainly right side) for each specimen because the collected crickets in the field often lacked one side of hind leg.

Statistical analyses

For statistical analysis we chose characters and focused on body length, maximum mandible length $[(a + g)/2]$ and tooth length $(d + h + i + j)$ to compare mandible shape among the species, which have different absolute body sizes. For leg length, the hind legs of some specimens of each species lacked tarsi. We omitted those measurements from the analysis and used the sum of the femur and tibia lengths as leg length. We evaluated body length, leg length/body length, mandible length/body length, tooth length/mandible length using Kruskal–Wallis test. In cases where the Kruskal–Wallis test was significant, we used Wilcoxon paired comparison test for multiple comparison between *Myrmecophilus* species.

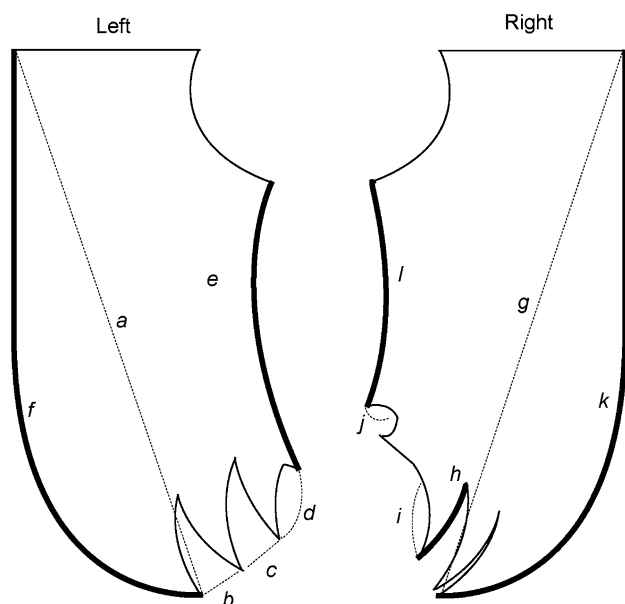


Fig. 2 Diagrams of *Myrmecophilus* cricket mandibles showing the measured parts: a, maximum length of left mandible; b, distance between apex and first marginal tooth of left mandible; c, distance between first and second marginal tooth of left mandible; d, distance between second marginal tooth and molar prominence of left mandible; e + f, marginal length of left mandible; g, maximum length of right mandible; h, anterior cutting edge of second marginal tooth; i, posterior cutting edge of second marginal tooth; j, anterior cutting edge of third marginal tooth; k + l, marginal length of right mandible

All statistical analyses were performed with the JMP version 9.0 software package (SAS Institute, Inc., Cary, NC).

Results

The mean values of body length, leg lengths, mandible length/body length, tooth length/mandible length, and leg length/body length in four *Myrmecophilus* species are presented in Table 2. Among the four species, only in *M. albicinctus* did all examined specimens lack the right mandible cutting edge (j in Fig. 1).

Body size

There are significant differences among the four species (Kruskal–Wallis test, $\chi^2 = 16.09$, $df = 3$, $P = 0.001$). *M. kubotai* was significantly larger than *M. tetramorii* (Wilcoxon signed rank tests, $P < 0.01$) and showed no significant difference with *M. formosanus* and *M. albicinctus* (Wilcoxon signed rank tests: $P > 0.05$). *M. formosanus* was significantly larger than *M. tetramorii* (Wilcoxon signed rank tests, $P = 0.002$) and showed no significant difference with *M. albicinctus* (Wilcoxon signed rank tests, $P > 0.05$). *M. albicinctus* was significantly larger than *M. tetramorii* (Wilcoxon signed rank tests, $P = 0.003$).

Leg length/body length

There are significant differences among the four species (Fig. 3A; Kruskal–Wallis Test, $\chi^2 = 20.55$, $df = 3$, $P < 0.001$). *M. kubotai* was significantly larger than *M. albicinctus* (Wilcoxon signed rank tests, $P = 0.002$) and showed no significant difference with *M. formosanus* and *M. tetramorii* (Wilcoxon signed rank tests, $P > 0.05$). *M. formosanus* was significantly larger than *M. albicinctus* and *M. tetramorii* (Wilcoxon signed rank tests, $P = 0.001$ and $P < 0.001$, respectively). *M. albicinctus* showed no significant difference with *M. tetramorii* (Wilcoxon signed rank tests, $P = 0.126$).

Mandible length/body length

There are no significant difference among the four species (Kruskal–Wallis Test, $\chi^2 = 7.72$, $df = 3$, $P = 0.052$).

Tooth length/mandible length

There are significant differences among the four species (Fig. 3B; Kruskal–Wallis Test, $\chi^2 = 59.58$, $df = 3$, $P < 0.001$). *M. kubotai* was significantly larger than *M. albicinctus* (Wilcoxon signed rank tests, $P < 0.001$) and smaller than *M. formosanus* and *M. tetramorii* (Wilcoxon signed rank

Table 1 Measured body length, head length (and width), the lengths of 12 mandible parts, together with the lengths of hind leg parts

Species	Individual	Marginal length						Teeth length						Head width		Teeth number	Body length
		f	e	k	l	b	c	d	h	i	j	Right	Left				
<i>M. formosanus</i>	1	0.353	0.109	0.385	0.113	0.03	0.032	0.065	0.041	0.038	0.016	0.859	4	4	3.591		
	0119minami	0.392	0.103	0.376	0.141	0.031	0.04	0.063	0.047	0.048	0.018	0.953	4	4	2.628		
	774	0.468	0.172	0.368	0.165	0.032	0.041	0.067	0.049	0.051	0.019	1.046	4	4	3.117		
	860b	0.4	0.116	0.4	0.15	0.024	0.039	0.058	0.044	0.039	0.017	0.975	4	4	2.205		
	869a	0.402	0.15	0.397	0.144	0.032	0.033	0.06	0.048	0.042	0.017	0.924	4	4	2.487		
	869b	0.402	0.157	0.394	0.153	0.032	0.034	0.069	0.06	0.052	0.015	0.971	4	4	2.425		
	921	0.394	0.146	0.398	0.146	0.028	0.039	0.054	0.043	0.036	0.019	0.961	4	4	2.895		
	924c	0.416	0.168	0.423	0.158	0.031	0.038	0.065	0.05	0.037	0.021	0.96	4	4	3.586		
	924d	0.415	0.163	0.413	0.148	0.029	0.035	0.054	0.05	0.045	0.019	0.995	4	4	3.085		
	d1	0.423	0.172	0.411	0.157	0.032	0.036	0.058	0.045	0.047	0.02	1.048	4	4	3.646		
	d2	0.452	0.178	0.448	0.151	0.031	0.038	0.063	0.046	0.046	0.019	1.008	4	4	3.012		
	hon20a	0.464	0.15	0.428	0.156	0.03	0.038	0.061	0.05	0.042	0.016	0.941	4	4	2.826		
	hon20b	0.4	0.163	0.426	0.142	0.028	0.04	0.057	0.042	0.038	0.017	0.988	4	4	2.642		
	s36	0.402	0.13	0.416	0.157	0.025	0.031	0.063	0.046	0.044	0.018	0.981	4	4	2.766		
	Average	0.413	0.148	0.406	0.149	0.03	0.037	0.061	0.047	0.043	0.018	0.972	-	-	2.922		
<i>M. tetramorii</i>	1	0.344	0.134	0.4	0.134	0.024	0.027	0.045	0.041	0.041	0.02	0.83	4	4	2.462		
	10	0.319	0.146	0.339	0.127	0.022	0.023	0.037	0.041	0.039	0.018	0.921	4	4	1.894		
	11	0.372	0.157	0.365	0.132	0.03	0.027	0.056	0.047	0.043	0.017	0.885	4	4	2.037		
	12	0.335	0.112	0.333	0.104	0.032	0.034	0.045	0.043	0.04	0.016	0.846	4	4	2.754		
	13	0.306	0.12	0.32	0.115	0.029	0.026	0.04	0.04	0.037	0.021	0.905	4	4	2.89		
	14	0.35	0.154	0.362	0.145	0.03	0.026	0.051	0.04	0.044	0.021	0.913	4	4	2.679		
	15	0.349	0.14	0.351	0.143	0.025	0.03	0.046	0.041	0.039	0.016	0.927	4	4	2.748		
	0120sat01	0.327	0.133	0.326	0.126	0.021	0.029	0.047	0.042	0.04	0.015	0.879	4	4	2.576		
	0120sat02	0.328	0.156	0.327	0.097	0.024	0.031	0.046	0.034	0.044	0.012	0.835	4	4	2.316		
	16	0.34	0.142	0.328	0.122	0.024	0.031	0.041	0.036	0.041	0.015	0.882	4	4	2.536		
	17	0.342	0.143	0.335	0.125	0.025	0.027	0.043	0.045	0.045	0.013	0.868	4	4	2.186		
	2	0.334	0.138	0.347	0.127	0.024	0.025	0.042	0.046	0.044	0.017	0.864	4	4	2.025		
	3	0.273	0.107	0.285	0.116	0.021	0.018	0.032	0.028	0.034	0.009	0.795	4	4	1.929		
	4	0.38	0.158	0.384	0.139	0.03	0.03	0.046	0.047	0.043	0.015	0.951	4	4	2.754		
	5	0.354	0.156	0.368	0.142	0.029	0.027	0.044	0.045	0.043	0.015	0.918	4	4	2.38		
6	0.366	0.164	0.374	0.16	0.029	0.033	0.051	0.046	0.043	0.018	0.937	4	4	3.199			
7	0.279	0.103	0.277	0.1	0.026	0.026	0.029	0.03	0.033	0.009	0.814	4	4	1.977			
8	0.373	0.15	0.377	0.145	0.03	0.03	0.05	0.044	0.042	0.016	0.98	4	4	2.113			
9	0.343	0.146	0.364	0.153	0.032	0.026	0.053	0.048	0.047	0.017	0.895	4	4	2.276			
<i>M. tetramorii</i>																	

Table 1 (continued)

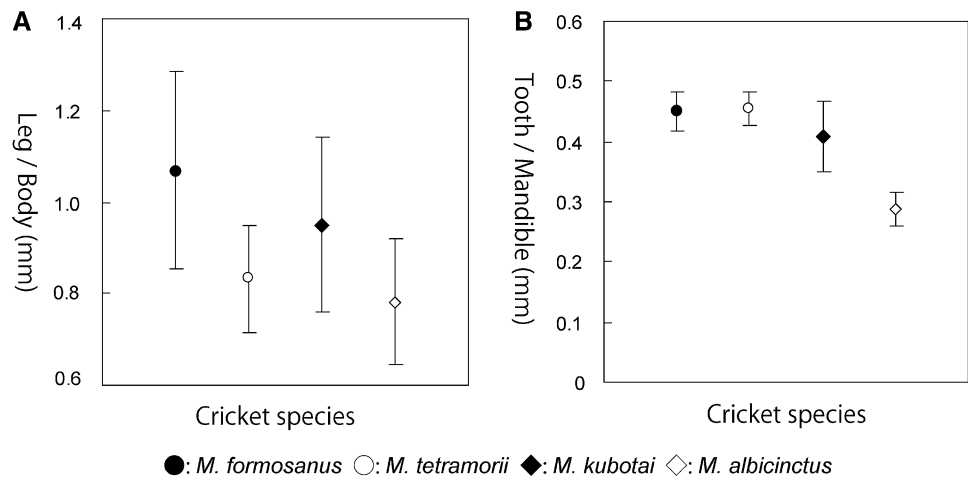
Species	Individual	Marginal length						Distance between teeth						Teeth length			Head width		Teeth number		Body length
		f	e	k	l	b	c	d	h	i	j	Right	Left								
Average		0.338	0.14	0.345	0.129	0.027	0.028	0.044	0.041	0.041	0.016	0.887	-	-	2.407						
<i>M. kubotai</i>																					
	11	0.37	0.156	0.372	0.135	0.032	0.027	0.048	0.037	0.042	0.09	1.016	4	4	2.652						
	110	0.414	0.179	0.415	0.136	0.031	0.029	0.049	0.04	0.044	0.007	1.054	4	4	3.386						
	116	0.36	0.189	0.313	0.142	0.029	0.031	0.049	0.041	0.044	0.007	0.982	4	4	2.681						
	117a	0.423	0.159	0.43	0.137	0.031	0.029	0.056	0.042	0.049	0.011	1.041	4	4	3.348						
	117b	0.435	0.154	0.434	0.156	0.038	0.029	0.06	0.033	0.036	0.01	1.084	4	4	2.406						
	118	0.414	0.154	0.388	0.132	0.033	0.032	0.042	0.038	0.038	0.011	1.176	4	4	3.828						
	119	0.403	0.176	0.423	0.148	0.023	0.033	0.058	0.047	0.05	0.013	1.103	4	4	3.383						
	12	0.356	0.139	0.334	0.136	0.029	0.03	0.043	0.04	0.04	0.007	0.948	4	4	2.22						
	13	0.361	0.139	0.358	0.122	0.032	0.024	0.051	0.041	0.038	0.012	0.992	4	4	3.143						
	141	0.426	0.165	0.442	0.166	0.029	0.033	0.057	0.042	0.044	0.008	0.991	4	4	3.183						
	145	0.404	0.149	0.4	0.158	0.032	0.03	0.049	0.04	0.042	0.01	1.066	4	4	2.839						
149a		0.383	0.145	0.389	0.142	0.031	0.029	0.056	0.04	0.039	0.007	1.028	4	4	2.265						
149b		0.352	0.154	0.314	0.15	0.028	0.025	0.045	0.034	0.038	0.007	0.997	4	4	2.507						
149c		0.373	0.148	0.37	0.141	0.031	0.031	0.044	0.035	0.038	0.01	0.946	4	4	2.391						
15		0.346	0.155	0.361	0.129	0.034	0.026	0.045	0.03	0.03	0.009	1.005	4	4	2.841						
16		0.362	0.15	0.361	0.153	0.03	0.024	0.049	0.033	0.043	0.007	0.957	4	4	2.383						
165		0.437	0.174	0.458	0.151	0.33	0.039	0.053	0.045	0.054	0.014	1.219	4	4	3.443						
p14a		0.383	0.168	0.386	0.137	0.03	0.026	0.049	0.04	0.048	0.01	1.089	4	4	3.709						
p14b		0.41	0.166	0.403	0.162	0.027	0.03	0.059	0.044	0.049	0.011	1.036	4	4	3.656						
p14c		0.39	0.152	0.412	0.156	0.028	0.032	0.055	0.044	0.048	0.006	1.075	4	4	3.51						
p14d		0.378	0.152	0.392	0.143	0.028	0.028	0.051	0.041	0.045	0.007	0.971	4	4	3.627						
Average		0.39	0.158	0.388	0.144	0.044	0.029	0.051	0.039	0.043	0.013	1.037	-	-	3.019						
<i>M. albicinctus</i>																					
	10	0.258	0.138	0.278	0.102	0.026	0.022	0.024	0.019	0.028	0	0.837	4	3	2.651						
	11	0.288	0.14	0.279	0.109	0.034	0.021	0.022	0.028	0.031	0	0.899	4	3	3.166						
	12	0.31	0.12	0.302	0.12	0.037	0.02	0.024	0.022	0.03	0	0.841	4	3	2.461						
	13	0.308	0.15	0.292	0.101	0.029	0.021	0.024	0.022	0.029	0	0.968	4	3	3.791						
746a		0.273	0.117	0.281	0.099	0.035	0.015	0.025	0.018	0.027	0	0.906	4	3	2.889						
829a		0.289	0.125	0.278	0.14	0.029	0.02	0.025	0.026	0.029	0	0.833	4	3	3.111						
829b		0.281	0.128	0.27	0.107	0.028	0.021	0.024	0.025	0.026	0	0.883	4	3	3.171						

Table 1 (continued)

Species	Individual	Marginal length						Distance between teeth						Teeth length			Head width		Teeth number		Body length
		f	e	k	l	b	c	d	h	i	j	Right	Left								
<i>M. albicinctus</i>	829c	0.282	0.124	0.383	0.085	0.031	0.021	0.03	0.018	0.021	0	0.897	4	3	3.205						
	829d	0.277	0.13	0.274	0.118	0.036	0.018	0.023	0.024	0.029	0	0.905	4	3	2.906						
	830	0.26	0.114	0.252	0.088	0.029	0.023	0.028	0.022	0.025	0	0.904	4	3	2.816						
	896a	0.275	0.103	0.253	0.094	0.032	0.016	0.021	0.017	0.022	0	0.828	4	3	2.966						
	896b	0.292	0.12	0.286	0.148	0.035	0.021	0.022	0.02	0.026	0	0.91	4	3	3.224						
	9	0.282	0.119	0.272	0.1	0.026	0.022	0.02	0.017	0.026	0	0.867	4	3	2.793						
	951b	0.381	0.114	0.263	0.109	0.032	0.02	0.023	0.019	0.025	0	0.779	4	3	2.834						
	964a	0.3	0.126	0.281	0.11	0.032	0.021	0.03	0.028	0.029	0	0.992	4	3	2.163						
	Omotoa	0.283	0.117	0.254	0.113	0.032	0.018	0.026	0.028	0.028	0	0.721	4	3	2.157						
	omotob	0.292	0.166	0.254	0.087	0.025	0.02	0.019	0.016	0.02	0	0.91	4	3	2.963						
	omotoc	0.277	0.127	0.28	0.107	0.029	0.02	0.023	0.022	0.026	0	0.844	4	3	2.631						
	SiroobikumeA	0.279	0.127	0.26	0.1	0.035	0.02	0.025	0.025	0.03	0	0.881	4	3	2.652						
	SiroobikumeB	0.259	0.128	0.238	0.078	0.029	0.019	0.027	0.025	0.03	0	0.859	4	3	2.517						
	SiroobikumeC	0.274	0.124	0.265	0.1	0.034	0.021	0.026	0.023	0.029	0	0.852	4	3	3.058						
	SirooreA	0.27	0.108	0.25	0.1	0.024	0.026	0.024	0.022	0.028	0	0.907	4	3	2.397						
SirooreB	0.27	0.111	0.26	0.1	0.037	0.02	0.027	0.021	0.025	0	0.854	4	3	2.55							
SirooreC	0.26	0.115	0.26	0.11	0.032	0.019	0.026	0.02	0.026	0	0.83	4	3	2.079							
Average	0.28	0.125	0.27	0.11	0.031	0.02	0.025	0.022	0.027	0	0.871	-	-	2.798							

Table 2 Mean length \pm SD of body, leg, mandible and tooth (mm), together with relative size of body parts in four *Myrmecophilus* species

Species	Sample size	Body length	Leg length	Mandible length	Tooth length	Mand/body	Tooth/mand	Leg/body
<i>M. formosanus</i>	14	2.922 \pm 0.450	3.053 \pm 0.438	0.377 \pm 0.015	0.170 \pm 0.011	0.131 \pm 0.018	0.450 \pm 0.032	1.069 \pm 0.215
<i>M. tetramorii</i>	19	2.407 \pm 0.370	1.978 \pm 0.252	0.313 \pm 0.025	0.143 \pm 0.016	0.132 \pm 0.018	0.454 \pm 0.028	0.833 \pm 0.117
<i>M. kubotai</i>	21	3.019 \pm 0.534	2.801 \pm 0.387	0.358 \pm 0.026	0.147 \pm 0.021	0.122 \pm 0.019	0.409 \pm 0.058	0.950 \pm 0.191
<i>M. albicinctus</i>	24	2.798 \pm 0.399	2.151 \pm 0.263	0.256 \pm 0.011	0.073 \pm 0.007	0.093 \pm 0.013	0.287 \pm 0.028	0.782 \pm 0.136

Fig. 3 Leg length/ body length (a) and tooth length/mandible length (b) of the four *Myrmecophilus* species

tests, $P < 0.001$). *M. formosanus* was significantly larger than *M. albicinctus* (Wilcoxon signed rank tests, $P < 0.001$) and showed no significant difference from *M. tetramorii* (Wilcoxon signed rank tests, $P = 0.478$). *M. albicinctus* was significantly smaller than *M. tetramorii* (Wilcoxon signed rank tests, $P < 0.001$).

Discussion

Differentiation of mandibular morphology among *Myrmecophilus* species

This study reveals that the food sources and mandible morphology are correlated for four *Myrmecophilus* species. In general, an insect mandible consists of the incisor, which is used for cutting, and the molar region, which is used for grinding (Chapman 1995). All of the Japanese *Myrmecophilus* crickets examined in this study had well-developed incisors, and three species (except for *M. albicinctus*) had in addition a cutting edge near the molar region on the side of the mandible (Fig. 2, j).

Behavioral observations have shown that *M. formosanus*, *M. kubotai*, and *M. tetramorii* feed themselves solid foods (Komatsu et al. 2009, 2010, 2013). In contrast, *M. albicinctus*, which lacks the cutting edge on the right mandible, consumes liquid foods obtained from the host ants by trophallaxis. The behavioral differences are reflected in

the mandibular morphology. Relative mandible lengths for body length did not differ among the four *Myrmecophilus* species. However, relative tooth lengths of *M. albicinctus* were significantly smaller of the other species; in particular, it is notable that relative tooth length of *M. albicinctus* was much smaller than that of *M. tetramorii*, the other host specialist. This suggests that simplification of mandibular morphology evolved in *M. albicinctus* as a reflection of their specific feeding habit. It is interesting that *M. albicinctus* has retained cutting edges in the incisor region on both sides of the mandible. Although this species feeds mainly on liquid foods regurgitated by ants, its mandible retains functionality for the consumption of solid foods. The dependence of *M. albicinctus* on trophallaxis was first observed in the laboratory by Komatsu et al. (2009). However, preliminary laboratory observations suggest that although *M. albicinctus* does not eat *A. gracilipes* eggs, it sucks out their contents after first cutting the egg surface with its mandible (T. Shimada and T. Komatsu, unpublished). This food habit may explain the retention of a functional cutting incisor by *M. albicinctus*.

Myrmecophilus tetramorii, which we consider to be a host specialist because it uses only two different hosts, is not behaviorally integrated into its host colony (Komatsu et al. 2013). In addition, in contrast to *M. albicinctus*, it shows larger tooth length and has a more complicated mandibular shape. This may be evidence that *M. tetramorii* usually feeds itself solid foods. A restricted

host range may not always be accompanied by intimate contact with the host ant species and corresponding morphological specialization.

Tooth length of *M. formosanus* was significantly larger than *M. kubotai* even though both are host generalists. Previous studies have suggested that *M. formosanus* has specialized feeding behavior for eating only solid foods while *M. kubotai* eats not only solid foods but also liquid foods (Komatsu et al. 2009, 2010). In fact, the food sources of *M. formosanus* resemble those of *M. tetramorii* (Komatsu et al. 2009, 2013); it is not surprising that tooth length for these two species showed no significant difference. This reflects the fact that both of species have complicated tooth shapes relative to the mandible length.

Differences in hind leg length among *Myrmecophilus* species

In comparisons of the ratios of mean leg lengths to body lengths, *M. formosanus*, the extreme host generalist, had the largest values among the four *Myrmecophilus* species, and *Myrmecophilus kubotai*, the moderate host generalist, had the second largest values. *Myrmecophilus formosanus* does not interact intimately with its host ants and frequently displays escape behavior (Komatsu et al. 2009), so the notable development in its hind legs may reflect the food sources of this non-integrated *Myrmecophilus* species. *Myrmecophilus kubotai* may also be subject to attacks by ants (Komatsu et al. 2010). In contrast, *M. albicinctus* and *M. tetramorii* had smaller legs relative to the other two generalist species. Interestingly, *M. tetramorii* had smaller values than *M. albicinctus*. The result is more difficult to explain because *M. tetramorii* has non-integrated habits inside the ant colony but it is highly host-specific to *T. tsushimae*. Under normal circumstances, we expected that *M. albicinctus*, the extreme host specialist, would show the smallest leg length among four *Myrmecophilus* species owing to its behavioral intimacy with a single host ant species. It appears to have less need to escape quickly from the ants. It is unclear why *M. tetramorii* has the smallest legs, but its main hosts are species of genus *Tetramorium*; these ants typically move relatively slowly (Fiedler 1990; Javier and Xim 1994), and they are tiny, especially *T. tsushimae*. As a result, *M. tetramorii* has a low probability of being captured by the host ants, so it may not need to escape quickly. In contrast, *A. gracilipes*, the specific host of *M. albicinctus*, moves quickly so *M. albicinctus* may make ready to rare and unexpected attack of ants. In previous laboratory experiments, quite a few individuals of *M. albicinctus* received aggressive reactions from ants (Komatsu et al. 2009).

Morphological diversification within *Myrmecophilus*

As discussed here, *Myrmecophilus* species show various degrees of specialization with regard to both behavioral ecology and morphology. One factor driving the diversification in this genus may be ecological diversity across host ants. For example, *A. gracilipes*, the specific host of *M. albicinctus*, is an omnivorous feeder but it prefers liquids containing sugar, such as homopteran honeydew (Reimer et al. 1990; Lach 2003). It is possible that the mandibles of *M. albicinctus* evolved to a shape suitable for feeding on liquids via trophallaxis from the host ants. The more developed mandibles of *M. formosanus* likely reflect its non-liquid food sources. Moreover, its main hosts are carnivorous ponerine ants, most of which do not practice trophallaxis (Hölldobler 1985).

Competition among congeners for food resources may also be a factor driving morphological diversification in *Myrmecophilus*. For example, *T. tsushimae*, the main host of *M. tetramorii*, is parasitized specifically by a lineage of *M. kubotai* (Komatsu et al. 2008; Komatsu 2013). In contrast to *M. tetramorii*, the *M. kubotai* lineage displays quite intimate behaviors toward its host and is fed by ants via trophallaxis feeding (Komatsu et al. 2010). This *M. kubotai* lineage often coexists with *M. tetramorii* within the same *T. tsushimae* nest, but competition between *M. tetramorii* and *M. kubotai* for food resources do not occur as their feeding strategies and microhabitats differ (Komatsu 2013, 2014). Our results support the hypothesis that each *Myrmecophilus* species has evolved morphological adaptations and feeding strategies to fit its host-associated lifestyle.

As in these Japanese species, the degree of host specificity in *Myrmecophilus* crickets worldwide may vary. It is often difficult, however, to judge the degree of host specificity from laboratory observations of a few living individuals. By measuring the mandibular shape of a *Myrmecophilus* species in the field, we may be able to predict its food sources and the degree of behavioral intimacy that it has with its host species, even though its behavior cannot be observed directly.

This study showed that morphological diversification is related to behavioral specialization in congeneric myrmecophilous crickets. Some other myrmecophilous insect genera include species that show considerable differentiation of behavior and food sources, such as *Phengaris* (formerly *Maculinea*) and *Spindasis* butterflies (Yamaguchi 1988; Pierce 1995; Veenakumari et al. 1997; Als et al. 2004; Fiedler 2006). The morphological differentiation we identified among *Myrmecophilus* species suggests that in other myrmecophilous taxa, a similar diversification of morphology may exist among congeneric species related to the degree of host species and behavioral specialization.

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