
Regular Article

Symbiotic protist communities in the termite *Coptotermes formosanus* in Japan and a comparison of community structures between workers and soldiers

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SUMMARY

Termites harbor a host species-specific symbiotic community composed of excavate protist species in their digestive tracts. Although termite-protist symbiosis is well known, our knowledge concerning the variations in symbiont species compositions and variations in community structures among individuals, colonies and host castes is very limited. Here we collected 11 colonies of the termite *Coptotermes formosanus* in the Western Japan Archipelago and investigated the variations in the protist species compositions. We investigated the symbiont community structures in three host colonies, and for the first time, variations of the community structures were compared between worker and soldier castes. Three parabasal species, *Pseudotrichonympha grassii*, *Holomastigotoides hartmanni* and *Spirotrichonympha leidyi*, were observed in all examined host individuals in the 11 colonies. One small trichomonadid species was found in hosts obtained from Iriomote Island and Okinawa Island. The mean symbiont community size in the workers was 30–50 times larger than that of the soldiers, and the host caste had a significant effect on the protist community structure. A principal component analysis revealed that the community structure was significantly more variable in the soldiers compared to the workers. Differences in food quality and community size between these termite castes probably affect the protist community structure and its variations.

Key words: Symbiosis, Parabasalia, Oxymonadida, Protist community

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INTRODUCTION

The symbiosis between host termites and protist symbionts living in the host hindgut is the most well known type of mutualism mediated by wood degradation (Cleveland, 1923; Inoue et al., 2000; Brugerolle and Radek, 2006; Brune and Ohkuma, 2011; Ohkuma and Brune, 2011). The anaerobic symbionts are assigned to two excavate groups, Parabasalia and Preaxostyleta (Adl et al., 2012), and usually a termite individual possesses more than one symbiont species in its hindgut making up a symbiont community. The symbiont species composition in a host termite shows strong host-specificity at the species-to-species level (Kirby, 1934; Honigberg, 1970; Inoue et al., 2000; Kitade, 2004; Kitade et al., 2012). However, sometimes a fraction of termite individuals in a colony or, rarely, a whole colony lacks a particular protist species (Honigberg, 1970). Except for a few host genera (*Reticulitermes*: Mannesmann, 1974; Kitade and Matsumoto, 1993; Cook and Gold, 1998, 1999; Lewis and Forschler, 2004; *Coptotermes*: Yoshimura et al., 1994; *Hodotermopsis*: Kitade et al., 2012), variations in symbiont species compositions between individuals or among field colonies have not been investigated. In addition, the existing knowledge of the variations in symbiont community structures — i.e., the ratios of the numbers of individuals of each symbiont species — is very limited, although it is important for the study of the communities from the evolutionary viewpoint.

Termites are well known as a social insect group, and a colony of most termite species consists of many individuals, including a king and a queen as the “reproductive caste” and their offspring, workers, and soldiers as the “neuter caste”. Although most taxonomic and ecological studies of termite protists have been conducted using the symbionts in workers (which are available in high numbers), earlier studies noted that termite soldiers also possess hindgut protists

(Cleveland, 1925; Honigberg, 1970). Most of the studies that focused on the differences in the symbiont species compositions and/or community structures between workers and other castes have been carried out for *Reticulitermes* host species (Cook and Gold, 1998; Lewis and Forschler, 2004; Shimada et al., 2013).

The workers, the major decomposers of wood material in a termite colony, possess well-developed, swollen hindgut rumens, whereas the soldiers specialize in colony defense; their hindgut volumes are smaller than those of the workers. The hindgut protists in the soldiers are thus expected to be much less abundant than in the workers, although these two castes share identical microenvironments in a colony. Comparisons of the symbiont community structures and its variations among the workers and soldiers from different colonies and localities will therefore help us identify the factors (e.g., host caste or microenvironment) that affect the protist community structures. However, for the termite symbiotic protists, comparative analysis of the community structure variations has not been carried out.

Here we investigated the variations in symbiont species compositions and community structures of the termite species *Coptotermes*

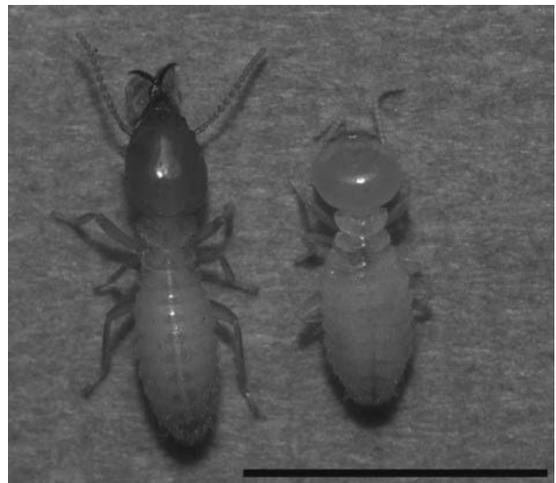


Fig. 1. Soldier (left) and worker (right) of *C. formosanus*. Scale bar = 5 mm.

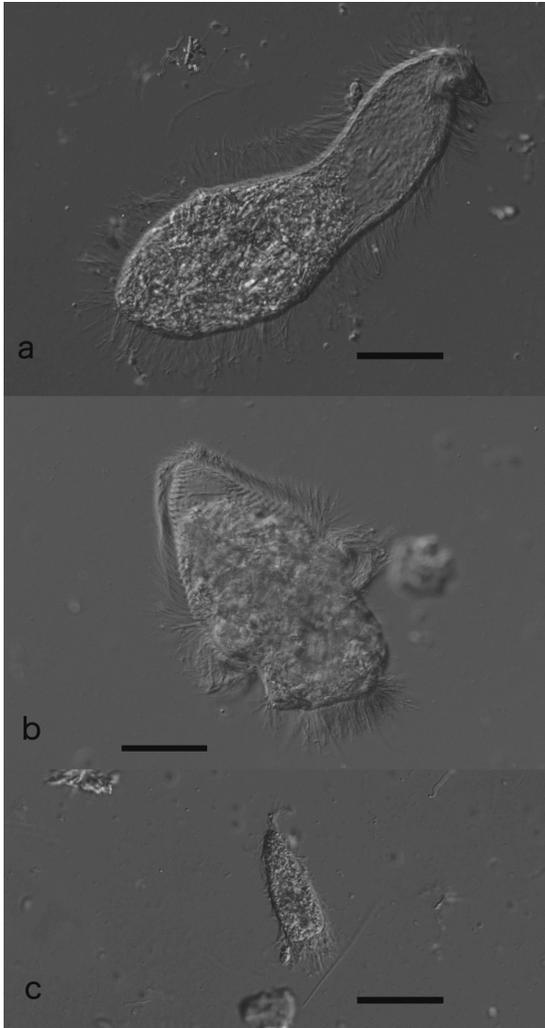


Fig. 2. Three symbiotic protist species of *C. formosanus*. a: *Pseudotriconympha grassii*, b: *Holomastigotoides hartmanni*, c: *Spirotriconympha leidy*. Scale bars = 50 μ m.

formosanus (Fig. 1), a notorious pest species found throughout East Asia including the southern and western parts of the Japan Archipelago. A mature colony of this species reaches 1,000,000 in number (Morimoto, 2000). Koidzumi (1921) described three symbiont species in *C. formosanus*: *Pseudotriconympha grassii*, *Holomastigotoides hartmanni*, and *Spirotriconympha leidy* (Fig. 2) assigned in the class Trichonymphea in the phylum Parabasalia (Cepicka et al., 2010). Lai et al. (1983) investigated the protist species compositions of

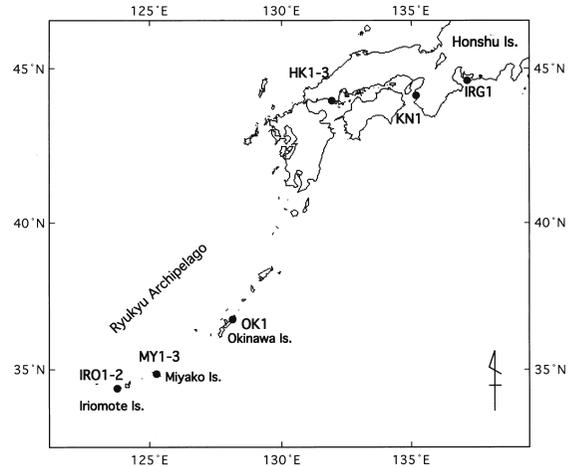


Fig. 3. Sampling localities of *C. formosanus* nests in this study.

this species using multiple castes from a single colony. However, their method of microscopic observation without a coverslip was not proper for the specific identification, and their results need to be reconfirmed. Yoshimura et al. (1994) have reported seasonal variations of the symbiont communities using laboratory and field colonies. In the present study, we first examined variations in the symbiont species compositions among individuals, colonies and localities of *C. formosanus* using worker termites from six localities in Japan. Then, using three host colonies, we investigated community structures and its variations to compare them between the worker and soldier castes and among different colonies.

MATERIALS AND METHODS

Sample Collection and Observation of the Protist Community

We collected 11 partial host termite colonies in six localities in the Japan Archipelago (Fig. 3): one colony from Cape Irago, Aichi Prefecture (IRG1) in October 2012, one from Kainan, Wakayama Prefecture (KN1) in October 1995, three colonies from Hikari, Yamaguchi Prefecture (HK1–3) in September 2012, one from Okinawa Island

(OK1) in May 1995, three from Miyako Island (MY1–3) in June 1995, and two from Iriomote Island (IRO1–2) in September 2011. The partial colonies were brought back to the laboratory with nest wood and maintained individually in plastic boxes at 22–25°C until the investigation of the termites' intestinal protists.

Before investigating the protist composition, we prepared and observed protargol-stained specimens of the intestinal contents following the method by Kitade et al. (1997). The morphology of the specimens and living materials were carefully compared in order to identify the symbiont species in the living state.

To investigate the protist species composition, we determined the presence or absence of the above three protist species in five worker termites from each of the 11 termite colonies. The examined workers were in fifth- or later instars. Other protist species were also checked if present. For the examination, we pulled out the intestine of the living insect with forceps, dissected it in a drop of 0.45% NaCl solution, and suspended the intestinal contents. The intestinal fluid was then put onto a glass slide, and living protists were observed with a differential interference microscope (BX-50, Olympus, Tokyo). The investigations were carried out within 20 days after the collection of the colonies.

The community structures of the protists in workers and soldiers were investigated using a host colony collected in Cape Irago and Hikari (IRG1, HK1 and 2) within 10 days after the collection. To examine the symbionts in a worker termite, the gut contents of a worker were suspended in 30 µl of 0.45% NaCl solution. The fluid was dispensed onto five Thoma counting chambers, and we counted the protists in 3.6 µl, 3.6 µl, and 0.125 µl of the solution for *P. grassii*, *H. hartmanni*, and *S. leidyi*, respectively. To examine the symbionts in soldiers, the gut contents were suspended in 20 µl of NaCl solution, and we counted the three protist species in 3.6 µl of the solution.

Statistical Analyses

We analyzed the factors affecting the symbiont community size in a termite by a Poisson regression analysis, using the data of the host colonies HK1, HK2 and IRG1. Host castes (worker, soldier) and colonies were assigned as explanatory factors. Similarly, to evaluate the factors (castes and colonies) affecting the population ratio of the symbiont species, arcsin-transformed population ratio data were subjected to a two-way multivariate analysis of variance (MANOVA). Similarity patterns of the protist community structure between the host individuals were assessed by a principal component analysis (PCA) using arcsin-transformed protist population ratio data of the three above-named host colonies. The first principal component (PC1) scores were then subjected to *F*-tests to compare the degrees of community structure variation between the castes and the colonies. All of the analyses and tests were carried out with R ver. 2.9.2 (The R project for statistical computing, <http://www.r-project.org/>). A function “princomp2” provided by S. Aoki (<http://aoki2.si.gunma-u.ac.jp/R/pca.html>) was used for the PCA.

RESULTS

The three parabasal species reported by Koidzumi (1921), *P. grassii*, *H. hartmanni* and *S. leidyi*, were observed in all host individuals examined in all 11 colonies (Table 1). One small parabasal species in the order Trichomonadida was found in host individuals from Iriomote Island and Okinawa Island; the occurrence rates of this species were not high (0.25 to 0.75), compared to the three species mentioned above (1.0 for all examined colonies) and the other parabasal symbionts reported from lower termites (Kitade and Matsumoto, 1993; Kitade et al., 2012). The population size of the trichomonadid species in the host individuals was also small (less than 100, Kitade, O., unpublished data). The taxonomic status of this species should be studied in the future.

Table 1. No. of host workers that harbored each symbiotic protist species (N = 5)

Host Colony	Protist Species			
	<i>P. grassii</i>	<i>H. hartmanni</i>	<i>S. leidyi</i>	Trichomonadidae sp.
IRG1	5	5	5	0
KN1	5	5	5	0
HK1	5	5	5	0
HK2	5	5	5	0
HK3	5	5	5	0
OK1	5	5	5	2
MY1	5	5	5	0
MY2	5	5	5	0
MY3	5	5	5	0
IRO1	5	5	5	1
IRO2	5	5	5	4

All three of the described parabasalid species were found in termites from the three colonies in which the symbiont community structure was investigated. The occurrence rates of these species in worker termites were 1.0 for all of the examined colonies, whereas one soldier each in colonies HK2 and IRG1 lacked *P. grassii*, and one in colony HK2 lacked both *P. grassii* and *S. leidyi*. The arithmetic mean symbiont community sizes of the workers in each colony ranged from 8207 to 9207, which was approx. 30–50 times larger than the mean symbiont community sizes of the soldiers (range 156 to 229; Table 2). The Poisson regression analysis revealed that both host caste ($z = -31.70$, $P < 0.001$) and the host colony (HK1-

HK2: $z = 8.10$, $P < 0.001$; HK1-IRG1: $z = 11.13$, $P < 0.001$) had a significant effect on the community size.

In the worker termites, *S. leidyi* was the most dominant in number, followed by *H. hartmanni* and then *P. grassii* in all 11 colonies (Table 2). The population size difference between the latter two species was small, and at the host individual level, *P. grassii* was more abundant than *H. hartmanni* in 4 of the 15 workers examined. In the soldiers, the relative abundance of *S. leidyi* was markedly low, and the relative abundance of the three symbiont species varied among individuals. The relative abundance of the three species was significantly affected by the host caste (two-way MANOVA, $F = 28.28$, $df = 1$, $P < 0.001$), but it was not affected by the host colony ($F = 0.97$, $df = 2$, $P = 0.45$) or the interaction between caste and colony ($F = 0.65$, $df = 2$, $P = 0.69$).

The 1st and 2nd principal components (PC1 and PC2) obtained from the PCA based on the relative abundance data of the three protist species explained 68.9% and 31.1% of the total variance, respectively. The scatterplot of the PC scores of each termite individual shows clear aggregations of workers (Fig. 4), indicating that the workers had similar symbiont community structures. In contrast, the variation in PC scores was larger in the soldiers, showing greater variations in the symbiont community structures. Individuals in the same

Table 2. Mean no. (SE) of estimated symbiotic protist species in workers (N = 5) and soldiers (N = 5) of three host colonies

Host Caste / Colony	Protist Species			Total
	<i>P. grassii</i>	<i>H. hartmanni</i>	<i>S. leidyi</i>	
Worker				
IRG1	563 (74)	580 (82)	8,060 (1,420)	9,210 (1,310)
HK1	495 (197)	532 (130)	7,870 (2,050)	8,900 (2,200)
HK2	193 (37)	382 (117)	7,630 (1,470)	8,210 (1,520)
Soldier				
IRG1	74 (43)	142 (63)	82 (16)	299 (100)
HK1	31 (15)	83 (64)	64 (42)	179 (59)
HK2	41 (6)	33 (9)	133 (87)	207 (85)

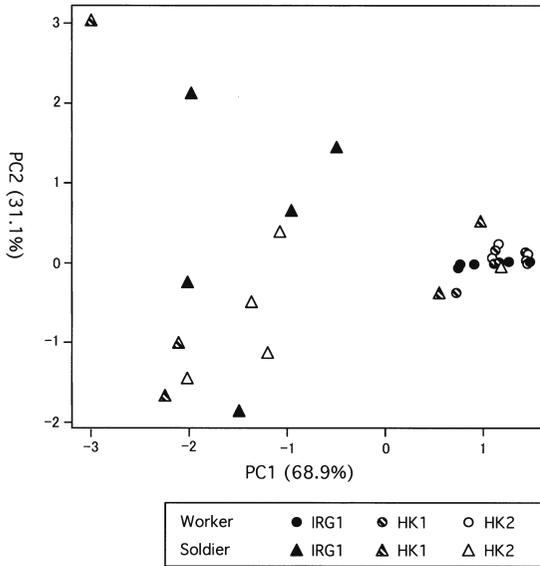


Fig. 4. Scatterplot of principal component scores (PC1 and 2) of each termite individuals based on the protist community structures. Different symbols and colors indicate castes (worker and soldier) and colonies (IRG1 and HK1-2).

colony did not exhibit a clear tendency to aggregate. These results correspond well to the results of the MANOVA. An F -test showed that the variation in the soldier PC1 scores was significantly larger than that of the workers ($F = 0.10$, $df = 14$, $P < 0.001$). Host colony did not have a significant effect on the variances of PC1 scores (F -test with Bonferroni correction, HK1-HK2, $F = 0.63$, $df = 9$, $P > 0.05$; HK1-IRG1, $F = 1.17$, $df = 9$, $P > 0.05$; HK2-IRG1, $F = 1.85$, $df = 9$, $P > 0.05$).

DISCUSSION

The high occurrence rates of the three described symbiotic protist species in *C. formosanus* workers observed in the present study are similar to those of hindgut protists of other East Asian termite taxa, e.g., *Reticulitermes* spp. (Kitade et al., 1993) and *Hodotermopsis sjostedti* (Kitade et al., 2012), and perhaps with those of lower termites in general. The unusual, lower infection rate of the other tiny trichomonadid

species, together with its small population size, suggest that this species is an opportunistic parasite and does not take part in wood degradation.

Except for this species, the symbiont species composition of the worker hindgut community identified in the present study is identical to that of the *C. formosanus* in Taiwan (Koidzumi, 1921) and Miyazaki, Japan (Yoshimura et al., 1993, 1994). The host-species specificity is the general trend of termite protist communities (Honigberg, 1970; Inoue et al., 2000). Symbiont fauna composed of species belonging to *Pseudotrichonympha*, *Holomastigotoides*, and *Spirotrichonympha* are a characteristic of *Coptotermes* hosts (Yamin, 1979; Kitade and Matsumoto, 1998) and *Pseudotrichonympha*, at least, was inherited from the common ancestor of the host family Rhinotermitidae (Noda et al., 2007).

The community size in the worker termites estimated for the three colonies (IRG1, HK1 and 2) did not show remarkable differences from that of three field colonies in Miyazaki (mean 7,280, range 3,750–11,140, $n = 15$ individuals) investigated by Yoshimura et al. (1994). The population size of *H. hartmanni* (382–580) was considerably smaller than that of the Miyazaki colonies (mean 2,160, range 1,160–3,350, $n = 15$ individuals), possibly because our investigation was carried out from late October to late November, while the latter data were collected year-round except in mid-winter. In laboratory colonies of the termite *Reticulitermes speratus*, the breeding temperature affected the symbiont community structures (Takayanagi, M. and Nemoto, M., unpublished data).

In *Reticulitermes* hosts, the relative symbiont species abundance was similar between workers and soldiers (Cook and Gold 1998; Lewis and Forschler 2004). Here we first indicated clear differences of the symbiont community structures between these castes of *C. formosanus*. The results of the MANOVA indicated that the differences in host-symbiont relationships between these castes

had stronger effects on the community structure than the differences in non-biological physical environments between the colonies. It is likely that the host caste affects not only the carrying capacity for the populations of community members but also the quality of their microhabitats. Workers can take undegraded wood fragments into their guts by masticating the wood and digesting it, but the soldiers depend on partially degraded wood received from nestmate workers as their food (Cleveland 1925; Cook and Gold 1998), through proctodeal and stomodeal trophallaxis. These differences in food sources would be expected to change the community structure of the hindgut symbionts.

Yoshimura et al. (1993) showed that *P. grassii* disappeared quickly when *C. formosanus* workers were fed low-molecular-weight cellulose. The absence of this symbiont species in three of the 15 soldiers examined in our study may be the result of a shortage of undegraded cellulose in the soldiers' food. To test this idea, comparisons should be conducted of the symbiont community structures between young and old workers, which possess hindgut lumens with different sizes although they have similar food sources.

To the best of our knowledge, this study is the first to reveal that the variation in protist species compositions and community structures are much larger in termite soldiers than workers. The generality of this finding should be further tested using multiple host species. The difference in the community stability between castes, which share the same physical environment in a host nest, may reflect the community size, i.e., the population size of each component species. In general, an organism with a small population size is likely to be extinguished (MacArthur and Wilson, 1967). Our recent study (Kitade et al., 2012) showed that the variations in species compositions of symbiotic protist communities were much smaller in a host termite with a larger body size and symbiont community size, *Hodotermopsis sjostedti*, than in

host termites with smaller body and community sizes, *Reticulitermes* spp., even though the two host groups are distributed sympatrically.

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