

## Characteristics of the Symbiotic Flagellate Composition within the Termite Family Rhinotermitidae (Isoptera)

OSAMU KITADE<sup>1\*</sup> and TADAO MATSUMOTO<sup>2</sup>

<sup>1</sup>Natural History Laboratory, Faculty of Science, Ibaraki University, 2-1-1 Bunkyo, Mito, Ibaraki 310-8512, Japan. Tel. +81-29-228-8375, Fax. +81-29-228-8404; <sup>2</sup>Department of Biology, Graduate School of Arts and Sciences, University of Tokyo, 3-8-1 Komaba, Meguro, Tokyo 153-8902, Japan. Tel. +81-3-5454-6628, Fax. +81-3-5454-4322

Received June 5, 1997; Accepted November 16, 1997

### Abstract

All lower termites contain a symbiotic flagellate community in their hindguts. Yamin (1979) listed the symbiotic protistan composition of 31 species that belong to the family Rhinotermitidae. In this study, the symbiont composition of additional 23 Rhinotermitid termite species were investigated. The flagellate genera *Spirotrichonympha*, *Pseudotrichonympha* and *Holomastigotoides* are prevalent among Rhinotermitid hosts. Members of the genus *Reticulitermes* lack the latter two flagellate genera, but possess the most diverse flagellate community of the Rhinotermitids, being the only genus to contain flagellates of the order Oxymonadida. A one to one host-species to symbiont-species relationship is found within the genera *Parrhinotermes* and *Termitogeton*.

Keywords: Termite, Rhinotermitidae, symbiotic flagellates

Presented at the Second International Congress of Symbiosis, April 13-18, 1997, Woods Hole, MA

\*The author to whom correspondence should be sent.

## 1. Introduction

All lower termites possess flagellate symbionts in the swollen part of their hindguts (termed 'paunch'). These flagellates play an important part in the digestion of cellulose and may contribute up to 40% of the host wet weight (Honigberg, 1970; Breznak and Brune, 1994).

All flagellate symbionts found in lower termites belong to the three protistan orders Trichomonadida, Hypermastigida and Oxymonadida. Most host species possess more than one species of flagellate. Yamin (1979) summarized data from 229 papers and presented a list of symbiotic flagellates from hosts representing all 6 recognised families of lower termites. According to the list, 434 symbiont species were described from a total of 205 lower termite species. Individual termite taxa usually contain a specific flagellate species composition. However, some flagellate species are found in more than one host species. Some authors suggested that symbiont composition reflects phylogeny of the host termites (Kirby, 1937; Honigberg, 1970; Kitade and Matsumoto, 1993a,b). There have also been claims that symbionts can be transferred horizontally via interspecies aggression. Thorne (1990, 1991) presented evidence that this mode of transfer could explain the origin of flagellate symbionts in the wood-feeding cockroach genus *Cryptocercus*. Aggression between different termite species is a well documented phenomenon (Thorne and Haverty, 1991), however transfer of symbionts via necrophagy followed by hindgut colonization has yet to be demonstrated.

Worldwide, the lower termite family Rhinotermitidae contains 14 genera (Krishna 1970). Yamin (1979) listed the flagellate composition of 31 Rhinotermitid species belonging to 7 genera of this family. We investigated the symbiont composition of an additional 25 Rhinotermitid species, including members of one genus, *Parrhinotermes*, not included in Yamin's study.

## 2. Materials and Methods

Termite species investigated in this study and their localities are shown in Table 1. More than one colony of each species was examined wherever possible. Worker (or pseudergate) gut contents were placed directly on glass slides or fixed with Schaudin's fluid and impregnated with protalgol stain (Honigberg and Davenport, 1951) immediately after the termite collection. Symbionts were observed by Nomarski differential interference or normal light microscopy and identified to the generic level and species level where possible, by comparison with original flagellate descriptions (see Yamin, 1979 and references therein).

Table 1. Termite species investigated in this study. Number of investigated termite colonies are shown in the brackets.

Host species	Localities
<i>Reticulitermes kanmonensis</i> Takematsu (13)	Onoda, Yamaguchi Pref., Japan
<i>R. amamianus</i> (Morimoto) (11)	Amamioshima Is., Japan
<i>R. miyatakei</i> (Morimoto) (9)	Amamioshima Is., Japan
<i>R. okinawanus</i> (Morimoto) (9)	Okinawa Is., Japan
<i>R. yaeyamanus</i> (Morimoto) (19)	Ishigakijima Is., Japan
<i>R. flaviceps</i> (Morimoto) (5)	Taiwan Is., R.O.C.
<i>R. guangzhouensis</i> Ping (1)	Guangzhou, China
<i>R. sp. 1</i> (1)	Beijing, China
<i>R. sp. 2</i> (6)	Hongkong Is., Hongkong
<i>R. sp. 3</i> (6)	Taipei, Hongkong
<i>R. sp. 4</i> (2)	Taipei, Hongkong
<i>Heterotermes tenuior</i> (Haviland) (3)	Borneo Is., Malaysia
<i>H. sp. 1</i> (2)	Altagracia, Venezuela
<i>H. sp. 2</i> (1)	Queensland, Australia
<i>H. sp. 3</i> (1)	Queensland, Australia
<i>Coptotermes sp. 1</i> (2)	Altagracia, Venezuela
<i>C. curvignathus</i> Holmgren (1)	Borneo Is., Malaysia
<i>C. sp. 2</i> (2)	Borneo Is., Malaysia
<i>Schedorhinotermes sp. 1</i> (1)	Queensland, Australia
<i>S. sarawakensis</i> (Holmgren) (2)	Borneo Is., Malaysia
<i>S. sp. 2</i> (3)	Borneo Is., Malaysia
<i>Parrhinotermes queenslandicus</i> Mjoberg (2)	Queensland, Australia
<i>P. aequalis</i> (Haviland) (3)	Borneo Is., Malaysia
<i>Prorhinotermes japonicus</i> (Holmgren) (1)	Ranyu Is., R.O.C.
<i>Termitogeton planus</i> (Haviland) (4)	Borneo Is., Malaysia

### 3. Results and Discussion

Table 2 shows the protistan genera found in each Rhinotermitid species investigated in this study. A total of 13 symbiont genera (including subgenera) were identified from 7 Rhinotermitid genera. It should be noted that within symbiont genera, species can differ between some host termite species. In individual termite species examined here, a specific flagellate composition (to the species level) was always found when more than one colony was examined. Similar symbiont genera are found within species of a genus. *Reticulitermes*

Table 2. Symbiont genus composition of Rhinotermitid species investigated in this study

Rhinotermitid species	Flagellate genera (subgenera)				Order Hypermastigida										
	Di	Py	St	He	Tr	Genus	A*	Hoto	Mi	Sptr	Sp	Ho	Ps	Te	Trph
	Order Oxymonadida				Order Trichomonadida										
<i>Reticulitermes kanmonensis</i>	+	+	-	+	-	-	-	-	+	-	+	+	-	+	+
<i>R. amamianus</i>	+	+	-	+	-	-	-	-	+	-	+	+	-	+	+
<i>R. miyatakei</i>	+	+	-	+	-	-	-	-	+	-	+	+	-	+	+
<i>R. okinawanus</i>	+	+	-	+	-	-	-	-	+	-	+	+	-	+	+
<i>R. yaeyamanus</i>	+	+	-	+	-	-	-	-	+	-	+	+	-	+	+
<i>R. flaviceps</i>	+	+	-	+	-	-	-	-	+	-	+	+	-	+	+
<i>R. guangzhouensis</i>	+	+	-	+	-	-	-	-	+	-	+	+	-	+	+
<i>R. sp. 1</i>	+	+	-	+	+	-	-	-	+	-	+	+	-	+	+
<i>R. sp. 2</i>	+	+	-	+	-	-	-	-	+	-	+	+	-	+	+
<i>R. sp. 3</i>	+	+	-	+	-	-	-	-	+	-	+	+	-	+	+
<i>R. sp. 4</i>	+	+	-	+	-	-	-	-	+	-	+	+	-	+	+
<i>Heterotermes tenuior</i>	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-
<i>H. sp. 1</i>	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-
<i>H. sp. 2</i>	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-
<i>H. sp. 3</i>	-	-	-	+	-	-	-	+	-	+	-	-	+	-	-
<i>Coptotermes</i> sp.	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-
<i>C. curvignathus</i>	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-
<i>C. sp.</i>	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-
<i>Schedorhinotermes</i> sp. 1	-	-	+	+	-	-	-	+	-	+	+	-	-	-	-
<i>S. sarawakensis</i>	-	-	-	+	+	-	-	-	+	-	+	-	-	-	-
<i>S. sp. 2</i>	-	-	-	+	+	-	-	-	+	-	+	-	-	-	-
<i>Parrhinotermes queenslandicus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>P. aequalis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Prorhinotermes japonicus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Termitogeton planus</i>	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-

\*A genus in the family Joeniidae, related to *Joenopsis*. Di = *Dinenympha*, Py = *Pyrsonympha*, St = *Stephanonympha*, He = *Hexamastix*, Tr = *Trichomonas*, Hoto = *Holomastigotoides*, Mi = *Microjoenia*, Sptr = *Spirotrichonympha*, Sp = *Spironympha*, Ho = *Holomastigotes*, Ps = *Pseudotrachonympha*, Te = *Teranympha*, Trph = *Trichonympha*.

Table 3. Summary of symbiont genus composition of Rhinotermitid genera. Data from this study and Yamin (1979) are incorporated

Flagellate genera (subgenera)	Rhinotermitid Reticuli- termes	Hetero- termes	Copto- termes	Schedorhino- termes	Parrhino- termes	Psammo- termes	Prorhino- termes	Termito- geton
Order Oxymonadida								
<i>Dinenympha</i>	+	-	-	-	-	-	-	-
<i>Pyronympha</i>	+	-	-	-	-	-	-	-
Order Trichomonadida								
<i>Hexamastix</i>	+	-	-	+	-	-	-	-
<i>Monocercomonas</i>	+	-	-	-	-	-	-	-
<i>Tricerocomitus</i>	-	-	+	-	-	-	-	-
<i>Stephanonympha</i>	-	-	+	+	-	-	-	-
<i>Trichomonas</i>	+	+	-	+	-	-	-	-
Order Hypermastigida								
Genus <i>A</i>	-	-	-	-	-	-	+	-
<i>Holomastigotoides</i>	-	+	+	+	-	+	+	-
<i>Microjoenia</i>	+	-	-	+	-	-	-	-
<i>Spirotrichonympha</i>	+	+	+	+	-	+	+	-
<i>Spironympha</i>	+	-	-	+	-	-	-	-
<i>Holomastigotes</i>	+	-	-	-	-	-	-	-
<i>Pseudotriconympha</i>	-	+	+	+	+	+	+	+
<i>Teronympha</i>	+	-	-	-	-	-	-	-
<i>Trichonympha</i>	+	+	-	-	-	+	-	-

species examined always contained the flagellate genera *Dinenympha*, *Pyrsonympha*, *Microjoenia*, *Holomastigotes* and *Trichonympha*, and the majority of taxa contained *Hexamastix*, *Spirotrichonympha* and *Teranympha*. The latter symbiont genus has only been reported in *Reticulitermes* collected from Asian countries, and is not found in representatives from Europe and America (Yamin, 1979; Kitade and Matsumoto, 1993b). *Reticulitermes* and *Heterotermes* are thought to be phylogenetically close due to similarities in morphology, and comprise the subfamily Heterotermitinae (Krishna, 1979). However, symbiont composition does not agree with the apparent affinity between these groups. Rather, *Heterotermes* and *Coptotermes* (the sole genus of the subfamily Coptotermitinae) species share common symbiont compositions, both harbouring the protistan genera *Holomastigotoides*, *Spirotrichonympha* and *Pseudotriconympha*. The three major symbiont genera found in *Schedorhinotermes* species are *Microjoenia*, *Spirotrichonympha* and *Pseudotriconympha*. Species of the Rhinotermitid genera *Parrhinotermes* and *Termitogeton* contained only one type of flagellate species of the genus *Pseudotriconympha*. This phenomenon does not occur in any other lower termite genus examined thus far (Yamin, 1979).

A summary of symbiont composition in Rhinotermitidae incorporating data from this study and that of Yamin (1979) is given in Table 3. It is clear that symbiont species from the genera *Pseudotriconympha*, *Holomastigotoides* and *Spirotrichonympha* are the most prevalent among Rhinotermitid taxa. In the genera *Heterotermes* and *Coptotermes*, reports of symbionts other than from the aforementioned genera are only fragmentary. In *Schedorhinotermes* species, *Holomastigotoides* has never been reported, but additional genera to *Pseudotriconympha* and *Spirotrichonympha*, such as *Microjoenia* and *Spirotrichonympha* are commonly found in the hindgut fauna. *Reticulitermes* species generally have the most diverse symbiotic communities, harbouring 7–8 protistan genera in their hindguts, but interestingly lack *Holomastigotoides* and *Pseudotriconympha*, which are thought to be major components of the cellulose dissimilation process in *Coptotermes formosanus* (Yoshimura, 1993).

The presence of strikingly different protistan compositions within Rhinotermitid taxa, and particularly between genera which are apparently phylogenetically close (*Reticulitermes* and *Heterotermes*), raises obvious questions about how such a situation arose. Is it possible that these deviations in symbiont composition came about via interspecies aggression, perhaps between taxa of different lower termite families? Or, can these compositions be explained by occasional divergence and/or loss of symbiont species, occurring within hosts which are also undergoing speciation? We are currently undertaking an investigation of phylogenetic relationships between Rhinotermitid species using mitochondrial DNA sequence data. We hope this

will help to identify patterns of symbiont gain and loss in the evolution of this lower termite family.

### Acknowledgments

We express our appreciation to Dr. M. Maryati, Dr. Y.-I. Chu and Mr. J.-S. Wang for providing the materials and support of the study. We also wish to thank Mr. N. Lo for his thoughtful comments on early drafts of the manuscript and for correcting English. This research was supported by a grant from JSPS Research fellowships for Young Scientists to O.K. and by Grant-in-Aid for Scientific Research No. 90106609 to T.M. from the Ministry of Education, Science, Sports and Culture, Japan.

### REFERENCES

- Breznak, J.A. and Brune, A. 1994 Role of microorganisms in the digestion of lignocellulose by termites. *Annual Review of Entomology* **39**: 453-487.
- Honigberg, B.M. 1970. Protozoa associated with termites and their role in digestion. In: *The Biology of Termites*. vol. II. K. Krishna and F.M. Weesner, eds., Academic Press, London, pp. 1-36.
- Honigberg, B.M. and Davenport, H.A. 1951. Staining flagellate protozoa by various silver-protein compounds. *Stain Technology* **29**: 241-246.
- Kirby, H. 1937. Host-parasite relations in the distribution of protozoa in termites. *University of California Publications in Zoology* **41**: 189-212.
- Kitade, O. and Matsumoto, T. 1993a. Cluster analysis on symbiotic protistan fauna of termites and a wood-feeding cockroach *Cryptocercus*. In: *Endocytobiology*, V.S. Sato, M. Ishida and H. Ishikawa eds., Tübingen University Press, Tübingen, Germany, pp. 155-159.
- Kitade, O. and Matsumoto, T. 1993b. Symbiotic protistan faunae of *Reticulitermes* (Isoptera: Rhinotermitidae) in the Japan Archipelago. *Sociobiology* **23**: 135-153.
- Krishna, K. 1970. Taxonomy, phylogeny, and distribution of termites. In: *The Biology of Termites*. vol. II, K. Krishna and F.M. Weesner, eds., Academic Press, London, pp. 127-152.
- Thorne, B.L. 1990. A case for ancestral transfer of symbionts between cockroaches and termites. *Proceedings of the Royal Society of London, B* **241**: 37-41.
- Thorne, B.L. 1991. Ancestral transfer of symbionts between cockroaches and termites: an alternative hypothesis. *Proceedings of the Royal Society of London, B* **246**: 191-195.
- Thorne, B.L. and Haverty, M.I. 1991. A review of intracolony, intraspecific, and interspecific agonism in termites. *Sociobiology* **19**: 115-145.
- Yamin, M.A. 1979. Flagellates of the orders Trichomonadida Kirby, Oxymonadida Grassé, and Hypermastigida Grassi & Foà reported from lower termites (Isoptera families Mastotermitidae, Kalotermitidae, Hodotermitidae, Termopsidae, Rhinotermitidae, and

- Serritermitidae) and from the wood-feeding roach *Cryptocercus* (Dictioptera: Cryptocercidae). *Sociobiology* 4: 1-119.
- Yoshimura, T., Azuma, J., Tsunoda, K., and Takahashi, M. 1993. Cellulose metabolism of the symbiotic protozoa in termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) II. Selective defaunation of protozoa and its effect on cellulose metabolism. *Mokuzai Gakkaisi* 39: 221-226.