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# MITES AND NEMATODES ASSOCIATED WITH THREE SUBTERRANEAN TERMITE SPECIES (ISOPTERA: RHINOTERMITIDAE)

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#### ABSTRACT

Mites and nematodes associated with three subterranean termite species, Reticulitermes flavipes (Kollar), Reticulitermes virginicus (Banks), and Coptotermes formosanus Shiraki were studied. Mites belonging to 8 families were found associated with the three termite species. Australhypopus sp. (Acari: Acaridae) was the most common mite on R. flavipes and R. virginicus. Histiostoma formosana Phillipsen and Coppel (Acari: Acaridae) was the dominant mite species living on C. formosanus. Nematode, Rhabditis sp. (Rhabditida: Rhabditidae) was found in the three termite species examined. Nematodes did not cause termite mortality or abnormal behavior. Percentages of R. flavipes, R. virginicus, and C. formosanus parasitized by nematodes were 67.9, 38.8, and 3.3%, respectively. The nematodes were found mainly in the termite heads (85.8% in R. flavipes and R. virginicus). The abundance of mites varied with colonies and termite species. Australhypopus sp. occurred in large numbers when injured or dead termites are present, or when moisture of the rearing medium is low in R. flavipes and R. virginicus colonies. Histiostoma formosana and Cosmoglyphus absoloni Samšiňák occurred in large numbers in C. formosanus colonies. Australhypopus sp. was tested against R. flavipes in the laboratory. It did not cause significant termite mortality at a rate of 10 mites/termite. From a biological point of view, mites investigated were not good candidates for controlling termites.

 $\label{thm:continuous} Key Words: Mites, nematodes, subterranean termites, \textit{Coptotermes formosanus, Reticulitermes flavipes, Reticulitermes virginicus}$ 

#### RESUMEN

Se estudiaron tres especies de termitas subterráneas, Reticulitermes flavipes (Kollar), Reticulitermes virginicus Banks) and Coptotermes formosanus (Shiraki), las cuales se encontraron asociadas con nematados y acaros. Ocho familias de acaros se encontraron asociados con las tres especies de termitas. El acaro mas común fue Australhypopus sp. (Acari: Acaridae) el cual se presentó en las termites R. flavipes and R. virginicus. Histiostoma formosana Phillipsen and Coppel (Acari: Acaridae) se lo encontró como especie dominante viviendo sobre C. formosanus. Un nemátodo Rhabditis sp. (Rhabditida: Rhabditidae) se encontró en las tres especies examinadas. Los nematodos no causaron una mortalidad en las termites ni un comportamiento anormal sobre ellas. R. flavipes, R. virginicus, and C. formosanus se encontraron parasitadas por nematodos cuyos porcentajes fueron 67.9, 38.8, and 3.3%, respectivamente. Los nematodos se encontraron principalmente en la cabeza de las termitas (85.8% in R. flavipes and R. virginicus). La abundancia de los acaros variaron dependiendo de la colonia y especie de termitas. En colonias de R. flavipes y R. virginicus el acaro Australhypopus sp. apareceó en mayor cantidad si hay presencia de termitas heridas o muertas, o cuando el contenido de humedad del medio de cría es bajo. Histiostoma formosana y Cosmoglyphus absoloni Samsinak se presentó en mayor cantidad en colonias de C. formosanus. Australhypopus sp. fue probado contra R. falvipes bajo condiciones de laboratorio. En una relacion de 10 acaros/termitas, la mortalidad de termitas no fue significante. Desde el punto de vista biológico, los acaros investigados no fueron buenos candidatos para el control de termitas.

Translation provided by author.

Many organisms are associated with termites (Kistner 1969). Their relationships with termites may be parasitic (e.g., phorid flies), mutualistic

(e.g., protozoa), phoretic (e.g., mites), predatory (e.g., ants), or commensal (e.g., mites, termitiphile beetles).

Among the organisms associated with termites, the most numerous and least studied are the mites (Acari) (Eickwort 1990). Mites are commonly seen in termite colonies (Becker 1969, Samšiňák 1964, Phillipsen & Coppel 1977a,b, Costa-Leonardo & Soares 1993). Some mites are only incidentally found in termite nests, while others are obligate associates (Samšiňák 1964). Generally, most mites associated with termites were considered saprophagous or phoretic. These mites do not have any significant effect on the health of their termite hosts in nature. Few mites feed on termites. Some, such as Acotyledon formosani Phillipsen and Coppel, a species that should be assigned to the genus Australhypopus, are abundant in weak termite colonies and cause death (Phillipsen & Coppel 1977a). The phoretic instar or deutonymph of A. formosani appeared to negatively affect a large laboratory colony of Coptotermes formosanus Shiraki by fastening primarily to termite heads and mouthparts, thereby impeding normal feeding (Phillipsen & Coppel 1977a). Conversely, termite-associated mites may benefit the termites by scavenging on other arthropods or fungi (Eickwort 1990). Despite the abundant and diverse mite fauna existing with termites, little is known for their diversity, biology, ecology, and the nature of their associations.

Termites have been recorded as being parasitized by various species of nematodes (Table 1). Some of these nematodes caused mortality to termite hosts in laboratory observations (Merrill & Ford 1916, Pemberton 1928). Mermis sp. and Neosteinernema longicurvicauda Nguyen and Smart may kill their termite hosts upon emergence (Ruttledge 1925, Nguyen & Smart 1994). However, their rate of parasitism apparently is very low and has been recorded only by the above authors. There is little information on the abundance of species of nematodes associated with termites.

In the U.S., subterranean termites cause serious damage to homeowners each year (Su 1994). Reticulitermes flavipes (Kollar), Reticulitermes

virginicus (Banks), and *C. formosanus* are among the most widely distributed and destructive subterranean termites (Snyder 1954, Su & Scheffrahn 1988). Scientists have been searching for environmentally compatible and sustainable control methods for these termites.

In this study, we investigated the diversity and abundance of mites and nematodes associated with three subterranean termite species: *R. flavipes*, *R. virginicus*, and *C. formosanus*. We examined the nature of their association with termites and the possibility of using these mites and nematodes as biological control agents.

#### MATERIALS AND METHODS

#### Collection and Maintenance of Termites

Reticulitermes flavipes and R. virginicus were collected in mixed forests in Washington county, and in loblolly pine (Pinus taeda L.) forests in Pearl River and Harrison counties in Mississippi. They were kept in cylindrical plastic containers (15.5 cm diameter, 4 cm deep) with 1-2 cm deep vermiculite and sand (1:1 by volume). Corrugated cardboard and/or pine wood blocks were added as food. One hundred to 2,000 individuals were collected from each termite colony. At least 15 colonies of each species were collected from each of the three counties during the period of October, 1998-August, 2000. Samples from ten C. formosanus colonies were collected from pine wood bait buried near trees in a university campus in the city of Guangzhou, China, in August 1999. One sample of C. formosanus colony was collected from a pine stump in the city of Cenxi, Guangxi, China. Two *C. formosanus* samples were collected from the cardboard bait buried in a city park in New Orleans, Louisiana on 15 June 1999 and 17 August 1999, respectively. Each C. formosanus sample had over 2,000 individuals. The termite colonies were kept for a maximum period of 120 d from field collection date at room temperature (21-25°C) in the laboratory.

TABLE 1. NEMATODES ASSOCIATED WITH TERMITES.

Nematode species	Termite host	Relationship	References
Mikoletzkya aerivora (Cobb)	Reticulitermes lucifugus	Occurs in head glands; host becomes sluggish	Merrill & Ford 1916
Rhabditis janeti Duthier	R. flavipes	Feeding on decaying matter	Banks & Snyder 1920
Rhabditis sp.	Neotermes, Reticulitermes, Capritermes, Coptotermes, Macrotermes, Microceroter- mes, Rhinotermes, Termes	Parasitic	Pemberton 1928
Mermis sp.	Cornitermes, Thoracotermes	Parasitic; kills host	Ruttledge 1925
$Neosteinernema \ longicurvicauda \ Nguyen \ and \ Smart$	R. flavipes	Parasitic; kills host	Nguyen & Smart 1994

Biology, Abundance, and Distribution of Mites and Nematodes Associated with Termites

Termites (number varied depending on purpose of the observation) from each colony were checked for mites and nematodes under a dissecting microscope (70-400×). To examine the density of Laelaptonyssus n.sp. (Laelaptonyssidae), 300 termites (workers and soldiers) were examined from each of the 3 colonies sampled from Washington County (total n = 900). The Termites were kept in the laboratory up to 30 d before examination. We assumed that the mites and nematodes associated with termites remained on/inside the termite body after the termites were transferred to the laboratory. General collections of mites were made by putting termites in 70% ethanol and immediately examining the location and number of mites. The mites dislodged slowly from termite body once submerged in 70% ethanol. To check for nematodes, termites were dissected with two No. 3 insect pins and the specimens washed with a drop of water so the nematodes could be seen in suspension. White traps were used to extract adult nematodes from termites (Kaya & Stock 1997). Ten white traps were made. Each trap consisted a  $90 \times 15$  cm petri dish and a 5 cm diameter moist filter paper disk resting on a platform. In each white trap, 10 R. flavipes (from Washington Co.) freshly killed by fine point forceps were put on the filter paper. The traps were placed in a moist large plastic container and then maintained at 25°C in an incubator. After 10 days, tap water was added to the petri dish and the existence of nematodes was examined.

The temporal changes in abundance of mites and nematodes were examined from one laboratory reared R. flavipes colony. The colony (about 5,000 individuals) was collected in a forest in Washington Co., Mississippi and maintained in a plastic box  $(31 \times 24 \times 11 \text{ cm})$  with 5 cm deep sand and pieces of corrugated cardboard. Twenty to 120 workers were examined weekly. We also checked weekly for presence of adult nematodes in the rearing medium and around dead termites using a dissecting microscope.

To examine the relationship between the abundance of mites and nematodes, the same individuals of *R. flavipes* and *R. virginicus* were examined for both mites and nematodes. They were first checked using a dissecting microscope for mite densities, then they were put on a glass slide and dissected to check for presence of nematodes. Nematodes and mites from *C. formosanus* were examined from different individuals. Termites had been kept in the laboratory for 60-80 d at examination date since collection.

Different parts of the termite body, e.g., head, thorax, and abdomen, were examined separately for nematodes. Termites from three *R. flavipes* colonies (total of 39 termites) and four *R. virgini*-

cus colonies (total of 85 termites) were examined. Voucher specimens have been deposited in collections of the Stoneville Research Quarantine Facility, USDA Agricultural Research Service, Stoneville, MS. and Museum of Zoology, Michigan State University, Ann Arbor, Michigan.

Effect of Australhypopus sp. on R. flavipes

The most abundant mite in *Reticulitermes* colonies was an undescribed species in the genus Australhypopus (Acaridae). The only species currently placed in this genus is the type-species, A. flagellifer Fain and Friend, described from deutonymphs collected from feces of a numbat, Myrmecobius fasciatus Waterhouse, a termitophagous marsupial, in Western Australia (Fain & Friend 1984). Examination of type material by B. O'Connor indicates that three other termitophilous acarid species should also be placed in this genus: Acotyledon formosani Phillipsen and Coppel, A. lishimei Samšiňák, and Tyroglyphus viduus Berlese and Leonardi. A systematic revision of this genus is in preparation by B. O'Connor and A. Huang (University of Michigan).

In our laboratory colonies of both species of Reticulitermes, when dead termites were present there were enormous numbers of *Australhypopus* sp. This mite was propagated by mixing dead termites (killed by freezing) with healthy termites. The termites were then placed in cylindrical plastic containers (15.5 cm diameter, 4 cm deep) with 1 cm deep vermiculite and sand as rearing medium and corrugated cardboard as food. Termites were kept at room temperature (21-25°C). Large numbers of adults and nymphs of *Australhypopus* sp. were present 15 d later and were harvested by brushing them from the inside surface of the container into a small round plastic container (5.0 cm diameter, 3.5 cm deep) filled with 0.01% Triton X-100 (wetting agent) (Sigma-Aldrich, Inc.) fluid. Mite density was determined. Mites (in feeding stages) were then transferred to  $100 \times 15$  mm petri dishes with 40 healthy termite workers per dish and a corrugated cardboard disk at rate of 5 and 10 mites per worker (200 and 400 mites per dish, respectively) using a 5 ml pipet. A total of 5 ml solution was added to each dish. The mites evenly dispersed soon after being transferred to the dishes. The experiment was a completely random design with each treatment rate was replicated three times. The control dishes received only 0.01% Triton X-100 solution. All of the replicates were from the same termite colony. Dishes were kept at 26°C, 85% RH in a dark chamber. Observations for termite mortality were made every 7 d for 5 wk.

Statistical Analysis

Mortality data were arcsine of the square root transformed and analyzed using repeated measures Analysis of Variance (ANOVA) (Littell et al. 1996). Analysis was performed using PROC MIXED in SAS software (SAS Institute 1999).

#### RESULTS

Biology, Distribution, and Abundance of Mites Associated with Termites

Examination and dissection of *R. flavipes*, *R. virginicus*, and *C. formosanus* colonies showed that the mite fauna associated with the three termite species was quite diverse (Table 2). The mites were found mostly on the termite heads, although some were also found on the thoraces, abdomens, and legs.

Australhypopus sp. (Acaridae), the most common mite species found on both *R. flavipes* and *R. virginicus*, has a distinct phoretic stage, the deutonymph (termed "hypopus" in older literature), in addition to the trophic stages. The

deutonymphs were most often seen attaching to termite heads and mouthparts. They do not have feeding mouthparts, and therefore cannot feed on termites. Once the termites die from injury or other causes such as disease, deutonymphs soon (as early as 24 h after host death) transform into tritonymphs, one of the trophic stages, and feed on the dead termite body. Adult *Australhypopus* sp. emerged as early as 1 d after emergence of tritonymphs. Adults lay eggs near dead termites. The eggs were laid singly or in batches of 2 to 30. The eggs hatched after 1-3 d. High mite densities were found in weak termite colonies, which were characterized by individuals showing lower body weight and flatter abdomens.

Enormous numbers of *Australhypopus* sp. deutonymphs were found on the inside wall of the containers holding *R. flavipes* colonies in the laboratory. The head and legs of *R. flavipes* workers were found covered by deutonymphs of *Australhypopus* sp. Thousands of dead *Australhypopus* 

TABLE 2. MITES FOUND ASSOCIATED WITH THREE SUBTERRANEAN TERMITE SPECIES.

Mite species	Termite host	Location	Relationship with termites
Histiostomatidae			
Histiostoma formosani	C. formosanus	New Orleans, Guangzhou, Cenxi	Phoretic; on termite head and legs
Histiostoma sp.	R. flavipes, R. virginicus, and C. formosanus	Washington, Pearl River, Harrison, New Orleans, Cenxi, Guangzhou	Phoretic; bacteriophagous; on termite head and legs
Acaridae			
$Australhypopus \ { m sp.}$	R. flavipes, R. virginicus, and C. formosanus	Washington, Pearl River, Harrison, New Orleans, Guangzhou, Cenxi	Phoretic; mostly on termite head
Cosmoglyphus absoloni (Samšiňák)	C. formosanus	Cenxi, Guangzhou	Phoretic; mostly on termite head
Schwiebea spp.	R. flavipes, R. virginicus, and C. formosanus	Washington, Pearl River, Harrison, New Orleans, Guangzhou	Opportunistic; on termite body and in rearing medium
Laelapidae			
$Hypoaspis\ miles\ (Berlese)$	R. flavipes, R. virginicus	Washington	Opportunistic; in rearing medium
Laelaptonyssidae			
Laelaptonyssus n.sp.	R. flavipes	Washington, Pearl River	Phoretic; feeding habit unknown; on termite body
Digmasellidae			
$Dendrolaelaps \ { m sp.}$	R. flavipes	Washington	Opportunistic; omnivorous; in rearing medium
Microdispidae			
Unknown genus	R. flavipes	Washington	Phoretic; in rearing medium
Pygmephoridae			
${\bf Near}\ Unguidispus$	R. flavipes	Washington	Phoretic; in rearing medium
Scutacaridae			
Unknown genus	R. flavipes, C. formosanus	Guangzhou	Opportunistic; on termite body and in rearing medium

sp. deutonymphs also could be found immediately outside of the termite rearing containers. Two factors were found closely related with high mite populations: the existence of injured or dead termites; and very low moisture content in the rearing medium.

Schwiebea sp. and Histiostoma sp. also were commonly seen on R. flavipes and R. virginicus, but did not occur as often as *Australhypopus* sp. Laelaptonyssus n.sp. (Laelaptonyssidae) (Krantz 2001) was found in 5 of the R. flavipes colonies of the 30-40 colonies examined. This is a rare species and apparently lives only on termites. Numbers were always low. Among three R. flavipes colonies that had this mite, the mite density ranged from 1.3 to 17.3 per hundred termites (mean = 9.2%, n = 900). Up to three *Laelaptonys*sus n.sp. were seen on one termite. They were mostly seen on the dorsal or lateral sides of the termite abdomen, and moved rapidly when disturbed. This mite died soon after the termite host died.

On C. formosanus, the dominant mite species were *Histiostoma formosana* Phillipsen and Coppel and Cosmoglyphus absoloni (Samšiňák). Histiostoma formosana deutonymphs were phoretic and were mostly found on termite tarsi and tibia, whereas deutonymphs of *C. absoloni* normally attached to the termite head. When H. formosana populations were high (≥15 per termite), they could be seen on the whole body. These two species appeared in great numbers (∃20 per termite) in weak, laboratory-reared colonies. *Histiostoma* sp., Australhypopus sp., and Schwiebea sp. also were common in C. formosanus colonies held in the laboratory. Laelaptonyssus chinensis Samšiňák, a rarely observed species that lives only on termites, was found in one colony collected from Cenxi, Guangxi, China.

### Distribution and Abundance of Nematodes in Termites

Nematodes were often seen inside the termite body. The only nematode observed was Rhabditis sp. (Rhabditida: Rhabditidae). Among the three termite species, nematodes were most common in R. flavipes and least common in C. formosanus. The maximum number of nematodes per termite also was different (Table 3). We did not find the adult form of the nematodes either by observation of the termites externally or by extracting nematodes from termites using the white trap method.

The distribution of nematodes in three body parts was similar in R. flavipes and R. virginicus (Fig. 1). Most of the nematodes were found in termite heads with an average of 85.8% of the total occurrences in R. flavipes and R. virginicus. Only 7.0% and 7.2% of the nematodes were found in the thorax and abdomen, respectively. Due to the low count, distribution of nematodes in different *C. formosanus* body parts could not be described.

Table 3. Abundance of mites and nematodes associated with three subterranean termite species.

Termite species	No. of colonies examined	Total number of termite workers examined	$\begin{array}{c} Percentage \ of \\ termites \ with \ mites \\ (Mean \pm SE) \end{array}$	Percentage of termites with nematodes (Mean ± SE)	Maximum no. of nematodes in a termite	No. of mites per termite (Mean $\pm$ SE)	No. of nematodes per termite $(Mean \pm SE)$
Reticulitermes flavipes	9	120	$28.6 \pm 10.8$	$67.9 \pm 27.7$	13	$0.4 \pm 0.2$	$2.3 \pm 0.9$
R. virginicus	7	137	$28.6\pm15.1$	$38.8\pm14.7$	8	$0.4\pm0.2$	$1.3\pm0.5$
Coptotermes formosanus	12	009	$NA^{1}$	$3.3 \pm 2.1$	က	$0.8 \pm 0.3$	$0.05\pm0.03$

Data were not collected

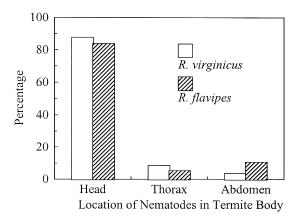
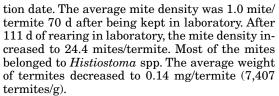


Fig. 1. Distribution of Rhabditis sp. in termite body.

Temporal Changes in Mite and Nematode Populations

Mite and nematode populations varied not only between termite colonies, but also changed over time. One R. flavipes colony (about 5,000 individuals) was observed in the laboratory for three months (Fig. 2). The density of mites was 1.1 (n = 47) per termite when termites were collected from the field. The mite densities dropped and fluctuated to 0.1-0.3 mites per termite when held in the laboratory. The percentage of termites infested with mites varied between 10 and 32.2% (n = 20 to 120). Average nematode numbers per termite also fluctuated between 0.8 and 2.5. The percentages of termites with nematodes varied between 60-100% (n = 20) from 4 examination dates.

The *C. formosanus* colonies collected from China showed a dramatic increase in mite population and decrease in body weight and termite numbers. Five colonies were observed for their mite populations and body weight. The weight of *C. formosanus* (4% soldiers) was measured as 3.58  $\pm$  0.01 mg/termite (279  $\pm$  0.6 termites/g) at collec-



High mite density could be lowered if termites are kept in appropriate conditions. We observed a colony of R. flavipes (with over 5,000 individuals) maintained in a 10-gallon aquarium ( $51 \times 25.5 \times 30.5$  cm). The container was filled with 2 cm deep vermiculite and 8 cm deep of southern pine wooden stakes. The inside walls of the aquarium were covered with enormous numbers of Austral-hypopus sp. deutonymphs 2-3 wk after termites were transferred from the field. This was caused by the existence of injured termites. Mites disappeared from the inner walls of the container later. This suggests that there was a cycle of reproduction of mites on the dead termites, followed by a population crash from the lack of dead termites.

#### Relationship between Mites and Termites

Although a high density of *Australhypopus* sp. was observed on R. flavipes and R. virginicus, it was not observed to cause significant mortality to termites in the laboratory. The adults and tritonymphs were observed feeding only on dead termites. Termite mortality was not significantly higher when mites were added to the termite colony (F = 1.53; df = 2, 6; P = 0.29) (Fig. 3). Termite individuals with high numbers of mites (10 mites per termite) were capable of living for many weeks. Therefore, the presence of large numbers of Australhypopus sp. did not appear to cause significant health problems for termite colonies. The mite species C. absoloni from termite colonies collected in China showed a similar relationship with *C. formosanus*.

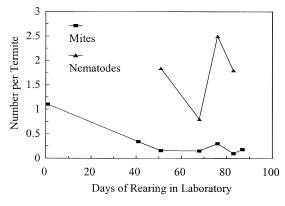


Fig. 2. Temporal changes in mite and nematode populations in a *Reticulitermes flavipes* colony reared in laboratory.

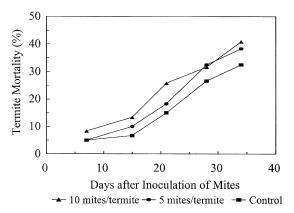


Fig. 3. Effect of  $Australhypopus\ {
m sp.}$  on  $Reticulitermes\ flavipes$ .

Relationship between Rhabditis sp. and Termites

The presence of nematodes in the three termite species did not cause any abnormal morphology or behavior of the termites. We often observed nematodes attached to the mouthparts of healthy *R. flavipes* and *R. virginicus* when they were immersed in 70% ethyl alcohol. This demonstrates that the nematodes were located close to the immediate opening of termites' mouthparts. Dead *R. flavipes*, *R. virginicus*, and *C. formosanus* placed in rearing containers and on white traps did not yield nematodes. No nematodes were found in dead termites when dissected in water.

#### DISCUSSION

This study provided information on the biology, abundance, and mite and nematode fauna associated with three species of subterranean termites. Information on taxonomy and biology of the mites associated with termites is still very limited and needs to be further clarified. From a biological control point of view, the mites investigated were not good candidates for controlling termites. They had little effect on termites even at high densities. Grace (1997) also reported no discernible effect of phoretic mites on survival and feeding of R. flavipes. The presence of Australhypopus sp. may even benefit the termites. We observed that Australhypopus sp. population increased in density when R. flavipes and R. virginicus died from treatment with entomopathogenic nematodes or pathogens. The mites consumed the dead termites that were killed by nematodes or pathogenic fungi. As a result, the nematodes and fungus could not initiate secondary infections in termites. Therefore, the remaining termites can be expected to survive after initial infection with pathogens or nematodes. We also observed Australhypopus sp. preying upon entomopathogenic nematodes in the laboratory.

Australhypopus sp. and Histiostoma spp. are similar to "Acotyledon" formosani in that they both have feeding and non-feeding life stages. They both have the potential to develop large numbers and were more abundant in weak and disturbed termite colonies.

In this study, Australhypopus sp. did not cause any apparent harm to the large termite group observed in the laboratory. The mite numbers increased greatly soon after disturbance to termites and collapsed later. Phillipsen & Coppel (1977a) stated that "Acotyledon" formosani was harmful to C. formosanus. We speculate that huge numbers of deutonymphs may simply overwhelm small groups of termites. "Acotyledon" formosani was closely related with Australhypopus sp. We suspect that they have similar relationships with termite hosts.

The rates of nematode parasitism among the three termite species were quite different. Copto-

termes formosanus had the lowest percentage of parasitization and lowest maximum number of nematodes per termite, but more studies are needed to clarify their biology. Pemberton (1925) did not find any nematodes from C. formosanus collected in Honolulu, Hawaii. However, our results suggest that careful examination of large numbers of individuals may reveal nematodes in C. formosanus individuals. We did not find the parasitic nematode, Neosteinernema longicurvicauda, reported by Nguyen & Smart (1994). This nematode is host-specific and kills the host upon emergence (Nguyen & Smart 1994). It could be a potentially useful agent for control of R. flavipes. More survey is warranted to search for useful nematodes to control subterranean termites.

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