Termite nest evolution fostered social parasitism by termitophilous rove beetles

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Colonies of social insects contain large amounts of resources often exploited by specialized social parasites. Although some termite species host numerous parasitic arthropod species, called termitophiles, others host none. The reason for this large variability remains unknown. Here, we report that the evolution of termitophily in rove beetles is linked to termite nesting strategies. We compared one-piece nesters, whose entire colony life is completed within a single wood piece, to foraging species, which exploit multiple physically separated food sources. Our epidemiological model predicts that characteristics related to foraging (e.g., extended colony longevity and frequent interactions with other colonies) increase the probability of parasitism by termitophiles. We tested our prediction using literature data. We found that foraging species are more likely to host termitophilous rove beetles than one-piece nesters: 99.6% of known termitophilous species were associated with foraging termites, whereas 0.4% were associated with one-piece nesters. Notably, the few one-piece nesting species hosting termitophiles were those having foraging potential and access to soil. Our phylogenetic analyses confirmed that termitophily primarily evolved with foraging termites. These results highlight that the evolution of complex termite societies fostered social parasitism, explaining why some species have more social parasites than others.

KEY WORDS: nest, phylogenetic comparative analysis, sis model, social evolution, social parasitism.

Two social insect lineages, ants and termites, are among the most abundant terrestrial animals in the world (Wilson 1971; Tuma et al. 2020). They form social groups building nests, some well-protected structures isolated from the outer environment and containing abundant resources. A wide variety of arthropods have independently evolved to be integrated into the nests of social insects and exploit the resources they contain (Kistner 1982). These specialized social parasites are "guests," distinct from other forms of social parasites or kleptoparasites of social insects by their high level of integration in the colony (Breed et al. 2012; Breed 2020). They are called myrmecophiles (ant as a host) or termitophiles (termite as a host) and perform a large part of their life cycle within the nest of social insects and have often developed convergent behavioral and morphological adaptations (Kistner 1982; Parker 2016). The evolution of social parasitism did not occur randomly across the tree of life.

Some animal lineages evolved social parasitism more frequently than others, presumably because their ancestors possessed morphological and ecological preadaptations to successfully invade social insect colonies (Parker 2016). Similarly, some termite and ant species host numerous species of social parasites, whereas the nests of others are free of any social parasites (Kistner 1969; Fiedler 2001; Geiselhardt et al. 2007). Although previous studies suggested that termite species with large colonies are more likely to host social parasites (Wilson 1971; Kistner 1979; Päivinen et al. 2003), the other factors promoting the evolution of social parasitism remain poorly understood.

Termites include more than 3000 described species (Krishna et al. 2013) with variable social structures and abilities to build nests (Emerson 1938; Chouvenc et al. 2021). The nest structure is critical to the evolution of social parasitism because nests serve as the habitat of termitophiles. Termite species can be assigned

to one of three nesting types based on their food and nest resources: one-piece nesters, multiple-piece nesters, and separate-piece nesters (Abe 1987; Mizumoto and Bourguignon 2020). One-piece nesters complete their colony life cycle within a single piece of wood serving as nest and food resources. Multiple-piece nesters expand their colonies across distinct wood pieces interconnected by a network of underground tunnels. And separate-piece nesters build central nests, physically separated from their food resources. Multiple-piece nesters and separate-piece nesters are different from one-piece nesters in that they forage outside their nest to find and exploit new resources (Korb 2008). Based on this characteristic, we refer to species of multiple-piece nesters and separate-piece nesters as "foraging species." The colonies of foraging species potentially have a longer life span than that of one-piece nesting species because their nest longevity is not limited by the resources present in a single wood piece (Korb 2008), providing a more stable environment for social parasites. Furthermore, the movements of termite colony members among physically separated nest/food sites are expected to increase the opportunity of infection. Thus, we predict an association between the evolution of foraging species and termitophily.

The distinction between one-piece nesters and foragers is blurred by the existence of species with intermediate nesting behaviors. Some species classified as one-piece nesters have the potential to come out of their nesting piece of wood to colonize neighboring wood items (Bourguignon et al. 2016; Mizumoto and Bourguignon 2020) or to access soil particles as nutrient resources (Mullins et al. 2021). All foraging species of termites have access to soil (Chouvenc et al. 2021), providing additional opportunities for termite colonies to be infected by termitophiles. Notably, the few species of one-piece nesting termites hosting termitophilous species in their colonies are known to have access to soil. We predict an association between access to soil in termites and termitophily.

In this study, we focused on the evolution of termitophily in the rove beetle subfamily Aleocharinae (Coleoptera, Staphylinidae), which includes most termitophilous species among arthropods (Seevers 1957; Kistner 1982). We used a mathematical model to show that termite colony traits related to nesting type, including colony longevity and foraging abilities, affect the propensity of parasitization by termitophiles. Next, we compiled literature information on 670 termitophilous rove beetle species and their termite host species, which belonged to a total of 265 termite genera. Using this dataset, we perform phylogenetic comparative analyses and demonstrate that the evolution of termite foraging is the precondition to the evolution of termitophily.

Model

New termite colonies are usually established during the swarming season by a pair of alate reproductives. Because termitophilous rove beetles do not disperse with alate reproductives (Emerson 1955; Kistner 1969; Maruyama et al. 2012), they are transmitted horizontally among colonies. Thus, newly established colonies are free of termitophiles. Following these assumptions, the dynamics of susceptible colonies (S) and infected colonies (I) can be described by the differential equations:

$$\frac{dS}{dt} = -\alpha SI + r_1 (S + I) - r_2 S, \text{ and}$$

$$\frac{dI}{dt} = \alpha SI - r_2 I,$$

where α is the colony's infection rate, r_1 is the new colony foundation rate, and r_2 is the colony death rate (Fig. 1a). These equations are identical to that of the susceptible-infected-susceptible (SIS) epidemiological model, which describes the infection dynamics of diseases lacking immunity (Hethcote 1989). In stable populations, colony foundation rate (r_1) is balanced by colony death rate (r_2), and the two rates equate to colony replacement rate (r), where $r = r_1 = r_2$. As colonies are either susceptible colonies (S) or infected colonies (I), $S = 1 - I$, the above equations can be written as

$$\frac{dI}{dt} = (\alpha - r) I - \alpha I^2.$$

The solution to this equation is obtained as follows (Hethcote 1989):

$$\lim_{t \to \infty} I(t) = 0 \, (\alpha \leq r),$$

$$\lim_{t \to \infty} I(t) = 1 - \frac{r}{\alpha} \, (\alpha > r).$$

These equations indicate that the proportion of colonies infected by termitophiles depends on the relationship between infection rate (α) and colony replacement rate (r), as shown in Figure 1c.

As one-piece nesting termites complete their colony life cycle within a single piece of dead wood, nest longevity partially depends on wood reserves (Fig. 1b). The exhaustion of wood induces the differentiation of individuals into alates attempting to found new colonies (Korb and Lenz 2004; Korb 2008). In this case, the colony replacement rate (r) is large and strongly linked to the regeneration of dead wood resources. In addition, the infection rate (α) is small in one-piece nesters as their colonies are enclosed within single pieces of wood with few interactions with the outside environment (Fig. 1b). On the other hand, the colony longevity of foraging species is not limited by the exhaustion of
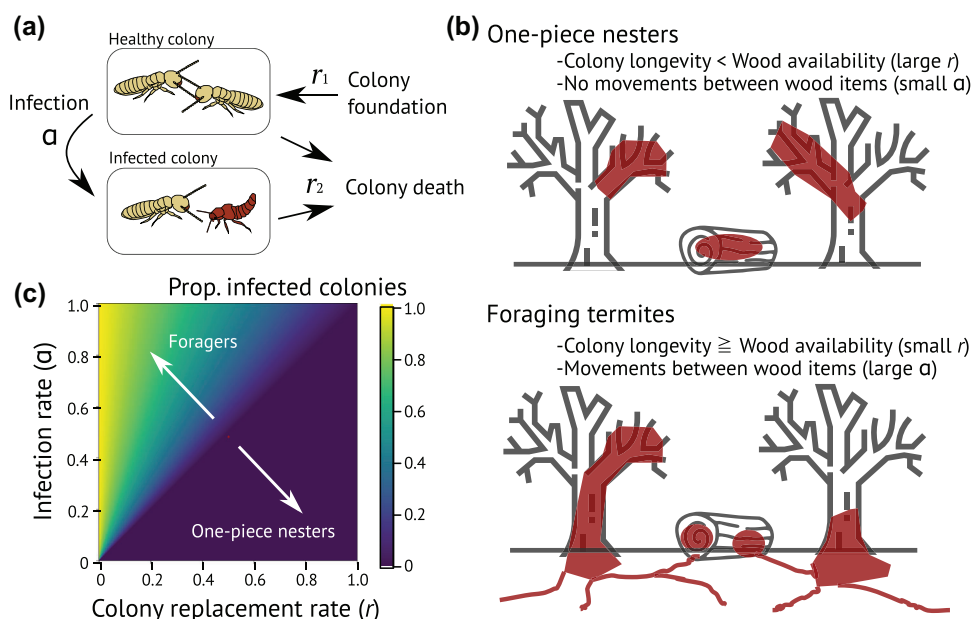
Figure 1. Framework of a susceptible-infected-susceptible (SIS) model applied to social parasitism and termite nesting behavior. (a) A simple epidemiological model for termitophile parasitization. Newly founded termite colonies are free of termitophiles, which are acquired through horizontal transfers among colonies. At equilibrium, colony foundation rate r_1 equals colony dead rate r_2. (b) The two representative termite nesting behaviors, one-piece nesters and foraging termites. Foraging termites exploit multiple wood items using a network of underground tunnels or aboveground shelter tubes. (c) The effect of infection rate and colony replacement rate on termitophilous parasitization success.

nesting wood as they can exploit new wood pieces and construct nests (Fig. 1b). As a result, their colony replacement rate (r) is expected to be lower than that of one-piece nesters. Furthermore, access to soil, foraging, and nest relocation events inevitably involve interactions with the environment outside the colony, leading to a larger infection rate (α) (Fig. 1b). Therefore, the ecological characteristics linked to one-piece nesting strategies (large r and small α) are not favorable to termitophilous rove beetles, whereas foraging species (small r and large α) are more likely to host social parasites (Fig. 1c).

Methods

DATA COLLECTION

We assembled a dataset comprising records for 670 species of termitophilous Aleocharinae rove beetles associated with 265 termite genera. The dataset was derived from 77 published papers (Data S1). We counted the number of described termitophilous rove beetle species associated with each termite genus. In addition to literature data, we considered two undescribed species of termitophilous rove beetles that we collected in the nests of *Apilitermes* and *Globitermes*. These two termite genera were previously unknown to host termitophilous rove beetles. We excluded rove beetle species belonging to the subtribe Athetina (tribe Athetini) and Myrmedoniina (tribe Lomechusini), as they

generally live outside termite nests and are often generalist predators instead of specialized termitophilous beetles. We also excluded rove beetle species recorded as associated with more than one termite genus. Termitophilous rove beetles generally have species-specific relationships with their host termites (Kistner 1969), and multiple host records are likely accidental (e.g., collection from logs inhabited by several termite species). The complete list of termitophilous rove beetles and their termite hosts used in this study is available in Data S2 and Data S3.

We classified termite genera into one-piece nesters and foragers (multiple-piece nesters + separate-piece nesters) as determined in Abe (1987). One-piece nesters include *Zootermopsis*, all genera of Stolotermitidae, Stylotermitidae, and Serritermitidae, almost all genera of Kalotermitidae, and the rhinotermitids *Prorhinotermes* and *Termitogeton* (Mizumoto and Bourguignon 2020). Foragers include *Mastotermes*, *Hodotermopsis*, all Hodotermitidae, *Paraneotermes*, most genera of Rhinotermitidae, and all genera of Termitidae (Mizumoto and Bourguignon 2020). Although *Hodotermopsis* was previously considered as a one-piece nester (Abe 1987; Mizumoto and Bourguignon 2020), we classified the genus as a multiple-piece nester as advised by Kitade et al. (2012).

Among the one-piece nesters described above, some species are capable of foraging and have access to the soil, even though their foraging abilities are limited in comparison to that of

foraging species. We tested whether access to the soil by termites is potentially linked to termitophily. Based on previous literature surveys (Bucek et al. 2021), we considered the kalotermitids *Postelectrotermes* and *Neotermes* as being able to tunnel through the soil. We also treated *Prorhinotermes* in Rhinotermitidae as having access to the soil as they have been referred to as subterranean termites (Scheffrahn et al. 2003). We recorded the number of described termite species for each genus using the termite database (Constantino 2016).

PHYLOGENY

We used the genus-level time-calibrated Bayesian phylogenetic tree of termites reconstructed by Mizumoto and Bourguignon (2021). This phylogenetic tree included 137 extant genera and 39 extinct species or genera and was inferred from previously published complete mitochondrial genome data (see Data S3 in Mizumoto and Bourguignon 2021) combined with a data matrix including 111 morphological characters published by Engel et al. (2016). The tree topology was largely consistent with other termite phylogenetic trees reconstructed using mitochondrial genome (Bourguignon et al. 2015, 2017) and transcriptome data (Bucek et al. 2019). Note that this phylogeny randomly selected one representative species for every genus, and thus the polyphyletic genera, such as *Nasutitermes*, were represented as monophyletic groups occupying singular phylogenetic positions. We pruned extinct species from the phylogenetic tree because no information concerning the presence of termitophiles associated with extinct termite species is available, except for the recently described Burmese amber fossil *Cretotrichopsenius* (Jiang et al. 2021). The final tree is represented in Figure S1.

PHYLOGENETIC COMPARATIVE ANALYSES

All analyses were performed using R version 4.0.1 (R Core Team 2020). We used the 137 termite genera included in the phylogeny and the information on the rove beetles they host. We performed the analyses twice: once on the entire dataset, including 137 termite genera, and once on a reduced dataset from which poorly studied genera were removed. The reduced dataset included 71 termite genera. Poorly studied termite genera were removed because the actual number of termitophilous rove beetles associated with these genera is unclear due to a lack of research effort. We used the number of published papers for each termite genus as a proxy for research effort and removed termite genera having fewer than 181 Google Scholar hits (50% quantile). The results obtained with the reduced dataset and the full dataset were congruent (Figs. 2 and S1).

To test whether termitophile presence is associated with termite nesting type, we used a phylogenetic generalized linear model (PGLM) implemented in the "phyloglm" function (logistic_MPLE method, 1000 independent bootstrap replicates) of the

package *phylolm* (Rettenmeyer et al. 2011). The presence of termitophiles associated with each termite genus was treated as a binomial response variable (0, absent; 1, present), and the nesting behavior was treated as a fixed effect (foragers or one-piece nesters). We also investigated the relationship between nesting behavior and the number of termitophile species per termite species in each genus, using the phylogenetic generalized least squares (PGLS) implemented in the function pgls() of the package caper_1.0.1 (Orme 2018).

We tested whether colony size is a predictor of the presence of termitophiles. We compiled a dataset of estimated colony size including 122 termite species belonging to 53 genera (Data S4). Most colony size records were previously summarized in two articles (Lepage and Darlington 2000; Porter and Hawkins 2001) (Data S4). We used maximum colony size as a representative value of colony size because mean and median values of colony size are potentially biased by the inclusion of incipient colonies (Porter and Hawkins 2001). When maximum colony size was not available, we used mean values. Then, we calculated the mean colony size for every genus using species values. The analyses were performed on the natural log values of the colony size. We carried out PGLMs and PGLS as described above.

We also explored the correlation between termitophile presence and termite nesting behavior and access to the soil using the Pagel test implemented in the "fitPagel" function of the package *phytools* (Revell 2012). Pagel test is a correlation test for binary traits proposed by Pagel (1994), which corrects for phylogenetic nonindependence of data points to test whether two discrete traits are correlated. Specifically, the "fitPagel" function calculates the log-likelihood of two models in which termitophile presence was dependent or independent on nesting strategies. We compared the Akaike Information Criterion (AIC) of these two models.

SAMPLING EFFORT

We investigated whether research effort correlates with the number of recorded termitophiles. We tested whether the number of termitophiles increases with research effort across genera. We examined the relationship between the number of Google Scholar search hits received by every termite genus and the presence of termitophiles using generalized linear models (GLM) with binomial errors. The number of hits was treated as a fixed effect. A likelihood ratio test was used to assess the statistical significance of the explanatory variable (type II test). One-piece nesters and foragers were analyzed separately. For this analysis, we removed the termite genus "*Termes*" as most Google Scholar hits for this word were related to the French word "termes," meaning "terms."

We investigated whether the species richness of termite genera correlates with the presence of termitophiles. We used GLM with binomial errors, where the number of termite species for each genus was treated as a fixed effect. We also examined the
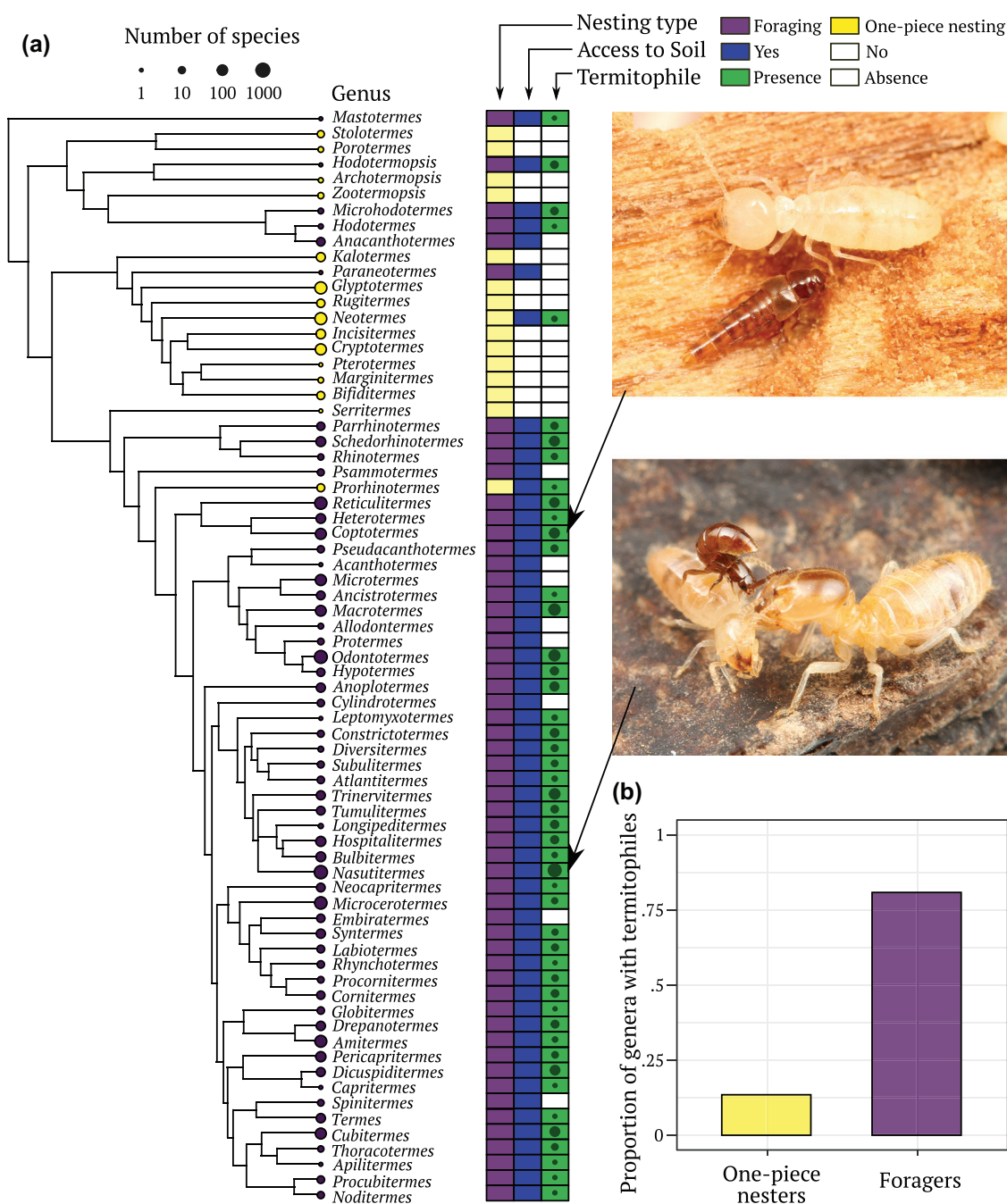
Figure 2. Relationship between termite nesting behavior and association with termitophilous rove beetles. **(a)** Phylogeny of termites alongside termite nesting type, access to the soil, and recorded association with termitophilous rove beetles. The sizes and colors of the circles on the tips of the termite phylogeny represent the number of described species and the nesting type for every termite genus, respectively. The sizes of the green circles in the boxes below the heading "termitophile" indicate the number of described termitophilous rove beetle species associated with each termite genus. The two photos show *Coptotermes formosanus* with *Sinophilus yukoae* (above) and *Nasutitermes corniger* with *Abroteles beaumonti* (bellow). **(b)** Proportion of one-piece nesting and foraging termite genera associated with termitophilous rove beetles.

relationship between the number of termite species and the number of termitophilous rove beetle species recorded across termite genera using linear models (LM).

Results

Out of the 670 described termitophilous rove beetle species, 667 species are associated with foraging termite species (99.6%) and
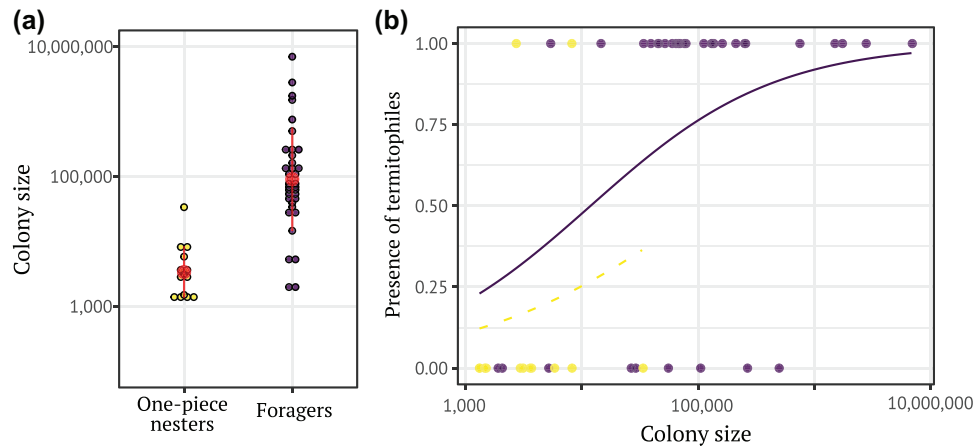
Figure 3. Relationships between termite colony size and termitophile presence in one-piece nesting and foraging termite genera. **(a)** Comparison of the colony size between one-piece nesters and foragers. Red dots and bars indicate mean ± standard deviation. **(b)** Relationship between colony size and termitophile presence. The solid purple line is the regression curve calculated with PGLM in foragers, indicating a significant weak positive relationship ($P = 0.045$). The dashed yellow line is the regression curve calculated for one-piece nesters indicating no significant relationship ($P = 0.546$).

three species are associated with one-piece nesters (0.4%). Given that 18.7% of termite species are one-piece nesters (567 in 3039 species), termitophilous rove beetles appear to be preferentially associated with foraging termite species. All the species of one-piece nesters associated with termitophiles have access to the soil (*Neotermes* and *Prorhinotermes*). Thus, all termitophiles are associated with termites having access to the soil. After accounting for phylogenetic relatedness among termite genera, we found that foraging termite genera are significantly more likely to host termitophilous rove beetles than one-piece nesting termite genera (PGLM; estimate ± SE = 3.183 ± 0.794, $z = 4.008$, 95% CI = 1.874–5.226, $P < 0.001$; Fig. 2). Similarly, the Pagel test showed that nesting strategies are correlated with termitophile presence. We found that a model in which termitophile presence was dependent on nesting strategies better described the evolutionary process than a model in which both parameters were independent (independent: AIC = 118.587, dependent: AIC = 112.694, likelihood-ratio test: $P = 0.008$; Fig. 2). The same was true for the relationship between termitophile presence and access to the soil by termite hosts (independent: AIC = 114.335, dependent: AIC = 99.203, likelihood-ratio test: $P < 0.001$; Fig. 2).

The three species of termitophilous rove beetles associated with one-piece nesters belong to *Prorhinopsenus* (Trichopseniini) (Data S2 and S3). *Prorhinopsenius* was also found in one colony of the foraging termite genus *Mastotermes* (Kistner 1998). Previous studies hypothesized several host transfers to explain the association of *Prorhinopsenius* with several unrelated termite hosts (Roisin and Pasteels 1993; Kistner 1998) or that *Prorhinopsenius* evolved termitophily early on and was associated with the ancestor of modern termites (Kistner 1998). In any case, the number of independent colonization events in one-piece nesters is at

most three, including two events in *Neotermes* and one event in *Prorhinotermes*. In contrast, the diversity of termitophilous rove beetles associated with foraging termites is much greater, including 190 genera classified into 19 tribes (Data S3). We compared the number of termitophilous species found in one-piece nesters and foragers and found that foragers hosted on average more termitophilous species than one-piece nesters (PGLS; $F_1 = 4.619$, $P = 0.035$).

Termite colony size also affected the presence of termitophiles. In the analysis performed on combined one-piece nesters and foraging termites, colony size was strongly associated with the presence of termitophiles (PGLM; estimate ± SE = 1.628 ± 0.477, $z = 3.410$, 95% CI = 0.884–2.561, $P < 0.001$). However, colony size could be a confounding variable of nesting types because foraging termites had a larger colony size than one-piece nesters (PGLS; $F_1 = 17.904$, $P < 0.001$; Fig. 3a). Therefore, we tested the effect of colony size on the presence of termitophiles for one-piece nesters and foraging termites separately. We found that colony size weekly predicted the presence of termitophiles in foragers (PGLM; estimate ± SE = 1.265 ± 0.630, $z = 2.007$, 95% CI = 0.292-2.449, $P = 0.045$; Fig. 3b), whereas no correlation was observed between the two variables in one-piece nesters (PGLM; estimate ± SE = 1.009 ± 1.670, $z = 0.604$, 95% CI = -0.825 to 2.806, $P = 0.546$; Fig. 3b).

We tested whether the research effort received by termite genera correlates with termitophile records. We found that termitophilous rove beetles were more commonly described from well-studied foraging termite genera (GLM, $\chi^2_1 = 73.989$, $P < 0.001$; Fig. S2). However, this trend was not observed for one-piece nesting termites (GLM, $\chi^2_1 = 0.863$, $P = 0.353$; Fig. S2). Similarly, termitophilous rove beetles were more commonly recorded

in species-rich genera than in species-poor genera for foraging termites (GLM, $\chi^2_1 = 50.034$, $P < 0.001$; Fig. S3a), whereas no significant association was found for one-piece nesters (GLM, $\chi^2_1 = 2.996$, $P = 0.083$; Fig. S3a). The number of described termitophilous rove beetle species per termite genus positively correlated with the species richness of termite genera (Fig. S3b). However, the slopes differed between nesting strategies. More than 30% of foraging termite species hosted termitophile species on average (estimate $\pm$ SE $= 0.386 \pm 0.022$, $F_1 = 301.24$, $P < 0.001$; Fig. S3b), whereas less than 1% of one-piece nester species hosted termitophile species (estimate $\pm$ SE $= 0.006 \pm 0.002$, $F_1 = 10.146$, $P = 0.004$; Fig. S3b).

Discussion

We show that termitophilous rove beetles are predominantly associated with foraging termite genera and are rare in the colonies of one-piece nesters. Among one-piece nesters, the two genera known to be associated with termitophilous rove beetles have access to the soil and the ability to forage out of their nests. Our epidemiological model suggests that foraging termites have more termitophilous rove beetles because of their longer colony longevity and because they frequently venture outside their nests. These two ecological traits could explain the observations that some species of termites are associated with many social parasites, whereas others appear to host no social parasites. The same model may also be applied to ants. Like termites, ants also host a variable number of social parasites, or myrmecophiles, with some ant species hosting no myrmecophiles, whereas others are associated with many. Notably, the richest myrmecophile communities are found in army ant colonies (Rettenmeyer et al. 2011), which, unlike other ant species, produce new colonies by budding and have an open nest (Kronauer 2020). Army ant ecology implies a small colony replacement rate (r) and a large infection rate (α), both of which facilitate social parasitism by myrmecophiles (Fig. 1c). Overall, our results indicate that the evolution of social parasitism depends on the host's nesting biology.

Foraging abilities do not guarantee the presence of termitophiles, indicating that other factors play a role in the evolution of social parasitism. Colony size is an important factor determining the occurrence of termitophiles (Wilson 1971; Kistner 1979; Päivinen et al. 2003) because larger colonies represent greater resources and potentially include more niches for termitophiles to occupy. The colonies of foraging termites can expand across multiple nest sites and thus often exceed the size of the colonies of one-piece nesters (Fig. 3a). We found that the presence of termitophiles was not correlated to colony size in one-piece nesters, whereas termitophiles were more commonly found in foraging genera with large colonies than in those with small colonies (Fig. 3b). Therefore, large colony size facilitates

the evolution of termitophily only in foraging termites, possibly because foragers expand their colony range over single piece of wood according to the colony size growth, providing more diverse niches that can sustain a variety of termitophilous species. However, these results must be considered preliminary because colony sizes have been measured for a limited number of termite genera. Additional studies are needed to evaluate the role of colony size on termitophile evolution.

Our model predicts that a lower colony replacement rate, or longer colony longevity, will increase the termitophile infection probability. Termite colony longevity has been measured for a handful of species. For example, the time until colony maturation was estimated to be 2 years for *Kalotermes*, 4 years in *Insicitermes* and *Zootermopsis*, and 6 years for *Neotermes* (Nutting 1969). All these genera are one-piece nesters, and among them, only *Neotermes*, which has the longest maturation time, hosts termitophile rove beetles (Fig. 2a).

Many species of termitophilous rove beetles are still awaiting formal description. This is especially true for those associated with poorly studied termite genera. Therefore, the absence of known termitophilous species associated with a given termite genus is often questionable for the possible existence of undescribed species, a common problem of presence–absence datasets (e.g., Fielding and Bell 1997). However, our results are robust to potential bias. First, our analyses with and without poorly studied termite genera yielded similar results (Figs. 2 and S1). Second, should all termite genera host termitophilous rove beetles, it is expected that the probability of finding termitophiles in a given genus increases with research effort. We observed this relationship in foraging termites, indicating the existence of many unknown termitophilous rove beetle species. Yet, we found no correlation between research effort and the presence of termitophilous rove beetles in one-piece nesters, suggesting that one-piece nesters truly lack termitophiles (Fig. S2). Furthermore, the two one-piece nesting termites with records of associated termitophiles, *Neotermes* and *Prorhinotermes*, have the potential to forage and engage in multiple-piece nesting (Waterhouse and Norris 1993; Rupf and Roisin 2008; Mizumoto and Bourguignon 2020). Our results highlight a strong association between foraging and the presence of termitophiles.

Social parasites can provide ecological information about their host species, such as the putative native distribution of invasive termite species (Maruyama et al. 2012). The evolutionary history of termite nesting behavior remains controversial. It is still unclear whether the most recent common ancestor of modern termites was a one-piece nester (Inward et al. 2007) or engaged in multiple-piece nesting (Bourguignon et al. 2016; Mizumoto and Bourguignon 2020). Because behavior rarely leaves fossil traces, the nesting behavior of extinct termite species must be inferred using indirect evidence. In this study, we demonstrate a strong

link between the presence of termitophiles and termite foraging behavior. Termitophilous rove beetles have been found in amber inclusions (Seevers 1971; Kistner 1998; Yamamoto et al. 2016; Cai et al. 2017), the oldest of which are from the 99-million-year-old Burmese amber (Yamamoto et al. 2016; Cai et al. 2017; Jiang et al. 2021), highlighting the antiquity of the association between termites and termitophilous beetles. The early association between termites and termitophilous beetles during the mid-Cretaceous implies that termites evolved foraging early on, possibly soon after they evolved eusociality. The discovery of older fossils of termitophiles, ideally with host information, as was the case for the recent description of *Cretotrichopsenius* (Jiang et al. 2021), could provide strong support to the idea that the most recent common ancestor of modern termites had access to soil and was able to forage outside its nest.

In conclusion, we show that nest expansion, foraging, and access to the soil in termites promoted the evolution of termitophily in rove beetles by increasing infection opportunities and decreasing colony replacement rates. Thus, the development of advanced societies provided room for social parasitism. Our study examined the evolution of termitophily from the perspective of the termite host and is complementary to the alternative approach from the perspective of parasite species. Future studies resolving the phylogenetic relationships among termitophilous rove beetles are required to unveil the history of their colonization process, including gain/loss events throughout termite radiation, co-cladogenesis, and horizontal transfer across host species. Furthermore, more sampling efforts across other lineages of termitophilous arthropods, such as Diptera, Thysanura, Neuroptera, and other Coleoptera, are needed to reconstruct the whole evolutionary history of social parasitism in termites.

AUTHOR CONTRIBUTIONS
NM and TK conceptualized the idea of the study and curated the data. NM performed formal analysis and wrote the original draft. TK performed investigation. NM designed the methodology. TB and TK provided resources. TB reviewed and edited (major) the manuscript. TK reviewed and edited (minor) the manuscript.

DATA ARCHIVING
Data that support the findings of this study are available in Dryad (https://doi.org/10.5061/dryad.6t1g1jx19). The link for review is https://datadryad.org/stash/share/X8UQ33mqq-Q0sZOD3MTKc2fZpzi1G-tjOJKhNfC1wFY.

Also, Data S1–S5 are uploaded at Dryad and Zenodo, following the advice of the journal editor: https://datadryad.org/stash/dataset/doi:10.5061/dryad.6t1g1jx19; https://zenodo.org/record/5972842#.Yh4EbOhBw2w; https://zenodo.org/record/6053977#.Yh4EbuhBw2w.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES
Abe, T. 1987. Evolution of life types in termites. Pp. 125–148 *in* S. Kawano, J. Connell, and T. Hidaka, eds. Evolution and coadaptation in biotic communities. Univ. of Tokyo Press, Tokyo.

Bourguignon, T., N. Lo, S. L. Cameron, J. Sobotnik, Y. Hayashi, S. Shigenobu, D. Watanabe, Y. Roisin, T. Miura, and T. A. Evans. 2015. The evolutionary history of termites as inferred from 66 mitochondrial genomes. Mol. Biol. Evol. 32:406–421.

Bourguignon, T., R. A. Chisholm, and T. A. Evans. 2016. The termite worker phenotype evolved as a dispersal strategy for fertile wingless individuals before eusociality. Am. Nat. 187:372–387.

Bourguignon, T., N. Lo, J. Sobotnik, S. Y. W. Ho, N. Iqbal, E. Coissac, M. Lee, M. M. Jendryka, D. Sillam-Dussès, B. Krizkova, et al. 2017. Mitochondrial phylogenomics resolves the global spread of higher termites, ecosystem engineers of the tropics. Mol. Biol. Evol. 34:589–597.

Breed, M. D. 2020. The importance of words: Revising the social insect lexicon. Insectes Soc. 67:459–461.

Breed, M. D., C. Cook, and M. O. Krasnec. 2012. Cleptobiosis in social insects. Psyche 2012:484765.

Bucek, A., J. Šobotník, S. He, M. Shi, D. P. McMahon, E. C. Holmes, Y. Roisin, N. Lo, and T. Bourguignon. 2019. Evolution of termite symbiosis informed by transcriptome-based phylogenies. Curr. Biol. 29:3728.e4–3734.e4.

Bucek, A., M. Wang, J. Sobotník, D. Sillam-Dussès, N. Mizumoto, P. Stiblik, C. Clitheroe, T. Lu, P. J. J. Gonzalez, A. Mohagan, et al. 2021. Transoceanic voyages of "drywood" termites (Isoptera: Kalotermitidae) inferred from extant and extinct species. Molecular Biology and Evolution and in press, https://doi.org/10.1101/2021.09.24.461667.

Cai, C., D. Huang, A. F. Newton, K. T. Eldredge, and M. S. Engel. 2017. Early evolution of specialized termitophily in cretaceous rove beetles. Curr. Biol. 27:1229–1235.

Chouvenc, T., J. Šobotník, M. S. Engel, and T. Bourguignon. 2021. Termite evolution: mutualistic associations, key innovations, and the rise of Termitidae. Cell. Mol. Life Sci. 78:2749–2769.

Constantino, R. 2016. Termite database.

Emerson, E. A. 1938. Termite nests: a study of the phylogeny of behavior. Ecol. Monogr. 8:247–284.

———. 1955. Geographical origins and dispersions of termite genera. Fieldiana Zool. 37:465–521.

Engel, M. S., P. Barden, M. L. Riccio, and D. A. Grimaldi. 2016. Morphologically specialized termite castes and advanced sociality in the early cretaceous. Curr. Biol. 26:522–530.

Fiedler, K. 2001. Ants that associate with Lycaeninae butterfly larvae: diversity, ecology and biogeography. Divers. Distrib. 7:45–60.

Fielding, A. H., and J. F. Bell. 1997. A review of methods for the assessment of prediction errors in conservation presence/absence models. Environ. Conserv. 24:38–49.

Geiselhardt, S. F., K. Peschke, and P. Nagel. 2007. A review of myrmecophily in ant nest beetles (Coleoptera: Carabidae: Paussinae): linking early observations with recent findings. Naturwissenschaften 94:871–894.

Hethcote, H. W. 1989. Three basic epidemiological models. Pp. 119–144 *in* S. A. Levin, T. G. Hallam, and L. J. Gross, eds. Applied mathematical ecology. Springer, Berlin.

Inward, D. J. G., A. P. Vogler, and P. Eggleton. 2007. A comprehensive phylogenetic analysis of termites (Isoptera) illuminates key aspects of their evolutionary biology. Mol. Phylogenet. Evol. 44:953–967.

Jiang, R.-X., H.-R. Zhang, K. T. Eldredge, X.-B. Song, Y.-D. Li, E. Tihelka, D. Huang, S. Wang, M. S. Engel, and C.-Y. Cai. 2021. Further evidence of Cretaceous termitophily: description of new termite hosts of the trichopseniine Cretotrichopsenius (Coleoptera: Staphylinidae), with emendations to the classification of lower termites (Isoptera). Palaeoentomology https://doi.org/10.11646/PALAEOENTOMOLOGY.4.4.13.

Kistner, D. H. 1969. The biology of termitophiles. Pp. 525–557 *in* K. Krishna and F. M. Weesner, eds. Biology of termites. Academic Press, New York.

Kistner, D. H. 1979. Social and evolutionary significance of social insect symbionts. Pp. 339–413 *in* H. R. Herman, ed. Social insects. Academic Press, New York.

Kistner, D. H. 1982. The social insects' bestiary. Pp. 2–244 *in* H. R. Herman, ed. Social insects. Academic Press, New York

Kistner, D. H. 1998. New species of termitophilous Trichopseniinae (Coleoptera: Staphylinidae) found with Mastotermes darwiniensis in Australia and in Dominican Amber. Sociobiology 31:51–76.

Kitade, O., Y. Hayashi, and K. Takatsuto. 2012. Variation and diversity of symbiotic protist composition in the damp-wood termite *Hodotermopsis sjoestedti*. Japanese J. Protozool. 45:29–36.

Korb, J. 2008. The ecology of social evolution in termites. Pp. 151–174 *in* J. Korb and J. Heinze, eds. Ecology of social evolution. Springer, Berlin.

Korb, J., and M. Lenz. 2004. Reproductive decision-making in the termite, *Cryptotermes secundus* (Kalotermitidae), under variable food conditions. Behav. Ecol. 15:390–395.

Krishna, K., D. A. Grimaldi, V. Krishna, and M. S. Engel. 2013. Treatise on the Isoptera of the world: vol 1, Introduction. Bull. Am. Museum Nat. Hist. 377:1–200.

Kronauer, D. J. C. 2020. Army ants: nature's ultimate social hunters. Harvard University Press, Cambridge, MA.

Lepage, M., and J. P. E. C. Darlington. 2000. Population dynamics of termites. Pp. 333–361 *in* T. Abe, D. E. Bignell, and M. Higashi, eds. Termites: evolution, sociality, symbioses, ecology. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Maruyama, M., T. Kanao, and R. Iwata. 2012. Discovery of two aleocharine staphylinid species (Coleoptera) associated with *Coptotermes formosanus* (Isoptera: Rhinotermitidae) from Central Japan, with a review of the possible natural distribution of *C. formosanus* in Japan and surrounding. Sociobiology 59:605–616.

Mizumoto, N., and T. Bourguignon. 2020. Modern termites inherited the potential of collective construction from their common ancestor. Ecol. Evol. 10:6775–6784.

———. 2021. The evolution of body size in termites. Proc. R. Soc. B Biol. Sci. 288:20211458.

Nutting, W. L. 1969. Flight and colony foundation. Pp. 233–282 *in* K. Krishna and F. M. Weesner, eds. Biology of termites. Academic Press, New York.

Orme, D. 2018. The caper package: comparative analyses in phylogenetics and evolution in R.

Pagel, M. 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. Proc. R. Soc. B Biol. Sci. 255:37–45.

Päivinen, J., P. Ahlroth, V. Kaitala, J. S. Kotiaho, J. Suhonen, and T. Virola. 2003. Species richness and regional distribution of myrmecophilous beetles. Oecologia 134:587–595.

Parker, J. 2016. Myrmecophily in beetles (Coleoptera): evolutionary patterns and biological mechanisms. Myrmecol. News 22:65–108.

Porter, E. E., and B. A. Hawkins. 2001. Latitudinal gradients in colony size for social insects: termites and ants show different patterns. Am. Nat. 157:97–106.

R Core Team. 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org/.

Mullins, A., T. Chouvenc, and N. Y. Su. 2021. Soil organic matter is essential for colony growth in subterranean termites. Sci. Rep. 11:21252.

Rettenmeyer, C. W., M. E. Rettenmeyer, J. Joseph, and S. M. Berghoff. 2011. The largest animal association centered on one species: the army ant eciton burchellii and its more than 300 associates. Insectes Soc. 58:281–292.

Revell, L. J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol. Evol. 3:217–223.

Roisin, Y., and J. Pasteels. 1993. Prorhinopsenius neotermitis sp. n. (Coleoptera, Staphylinidae, Trichopseniinae), guest of Neotermes (Isoptera, Kalotermitidae) in Papua New Guinea. Entomologie 63:145–150.

Rupf, T., and Y. Roisin. 2008. Coming out of the woods: do termites need a specialized worker caste to search for new food sources? Naturwissenschaften 95:811–819.

Scheffrahn, R. H., N.-Y. Su, B. Cabrera, and W. Kern. 2003. Cuban Subterranean Termite (proposed), Florida Dampwood Termite (old unofficial name), *Prorhinotermes simplex* (Hagen) (Insecta: Isoptera: Rhinotermitidae). Univ. Florida EENY 282:1–3.

Seevers, C. H. 1957. A monograph on the termitophilous Staphylinidae (Coleoptera). Fieldiana, Zool. 40:1–334.

———. 1971. Fossil Staphylinidae in Tertiary Mexican amber (Coleoptera). Univ. Calif. Publ. Entomol. 63:77–86.

Tuma, J., P. Eggleton, and T. M. Fayle. 2020. Ant-termite interactions: an important but under-explored ecological linkage. Biol. Rev. 95:555–572.

Tung Ho, L. S., and C. Ané. 2014. A linear-time algorithm for gaussian and non-gaussian trait evolution models. Syst. Biol. 63:397–408.

Waterhouse, D. F., and K. R. Norris. 1993. Biological control: pacific prospects - supplement 2. Australian Centre for International Agricultural Research, Canberra, Australia.

Wilson, E. O. 1971. The insect societies. Harvard Univ. Press, Cambridge, MA.

Yamamoto, S., M. Maruyama, and J. Parker. 2016. Evidence for social parasitism of early insect societies by Cretaceous rove beetles. Nat. Commun. 7:1–9.

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Supplemental materials
DataS1-S5