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Subterranean termite open-air foraging and tolerance to desiccation: Comparative water relation of two sympatric *Macrotermes* spp. (Blattodea: Termitidae)☆

Jian Hu [a,1], Kok-Boon Neoh [a,1], Arthur G. Appel [b], Chow-Yang Lee [a,*]

[a] Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia
[b] Department of Entomology and Plant Pathology, 301 Funchess Hall, Auburn University, AL 36849-5413, USA

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ABSTRACT

The foraging patterns of termites are strongly related to physiological limits in overcoming desiccation stress. In this study, we examined moisture preferences and physiological characteristics of *Macrotermes carbonarius* (Hagen) and *M. gilvus* (Hagen) as both exhibit conspicuous patterns of foraging activity. Despite both species showing no significant differences in calculated cuticular permeability, and percentage of total body water, they differed greatly in rate of water loss and surface area to volume ratio. For example, *M. carbonarius* which had a lower surface area to volume ratio (29.26–53.66) showed lower rate of water loss and percentage of total body water loss. This also resulted in higher LT_{50} when exposed to extreme conditions (≈2% RH). However, contrasting observations were made in *M. gilvus* that has smaller size with higher surface area to volume ratio of 40.28–69.75. It is likely that the standard equation for calculating insect surface areas is inadequate for these termite species. The trend was further supported by the result of a moisture preference bioassay that indicated *M. carbonarius* had a broader range of moisture preference (between 5% and 20%) than *M. gilvus* which had a relatively narrow moisture preference (only 20%). These results explain why *M. carbonarius* can tolerate desiccation stress for a longer period foraging above-ground in the open air; while *M. gilvus* only forages below ground or concealed within foraging mud tubes.

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1. Introduction

The ability to tolerate desiccation is crucial in terrestrial animals. The capability of individuals to inhabit xeric environments is usually linked to physiological specializations or certain adaptive strategies that may have evolved to reduce body water loss (Hadley, 1994).

Similarly, desiccation stress plays a pivotal factor in termites' biology and ecology by manipulating population dynamics, colony foundation, and feeding and foraging activity (Haagsma and Rust, 1995; Abe et al., 2000). Though termites are highly susceptible to desiccation, desiccation stress can be overcome in several ways: (1) the possession of water sacs in the termite's labial gland. The water stored in the water sacs is used to compensate water loss during desiccation stress (Grube and Rudolph, 1999). Also, a recent study on *Reticulitermes flavipes* (Kollar) documented that these termites use their water sacs to displace water to drier areas by transferring water to a food source (Gallagher and Jones, 2010). (2) Reduced rate of desiccation (lower cuticular permeability) allows termites to inhabit xeric environments. Shelton and Grace (2003) reported that *Coptotermes gestroi* (Wasmann) has a lower cuticular permeability compared with *C. formosanus* Shiraki. In all likelihood, this reflects greater arid adaptation, since the former is widely distributed in the tropics. Similarly, *Reticulitermes tibialis* Banks that showed relatively low water loss rate, were found mainly inhabiting in more arid environments (Collins and Richards, 1963). The rate of water loss of drywood termites is always lower than those of moisture-dependent termites, such as dampwood termites and subterranean termites (Collins and Richards, 1966). (3) Possessing a well developed waterproofing cuticle. Collins and Richards (1963) documented that unlike other *Reticulitermes* spp., *R. tibialis* secondary reproductives possess a thick cement layer on the cuticle that might serve as an additional waterproofing barrier.

Macrotermes carbonarius (Hagen) and *M. gilvus* (Hagen) belong to the Macrotermitinae and are widely distributed in Southeast Asia. They are to some extent, of economic importance, particularly in agricultural crops (Lee, 2002), as perimeter pest species in buildings and structures in the urban environment (Lee et al., 2007). The Macrotermitinae exhibit pronounced caste dimorphism in steriles (i.e., two sizes of workers and soldiers) (Engel and Krishna, 2004). Despite their congeneric relationship, *M. carbonarius* and *M. gilvus* differ conspicuously in foraging behavior. For example, *M. carbonarius* forages above-ground in open air with extensive trails. This species was observed to forage in the open for over 17 h (1800 to 1100 the next morning) under a range of 70% to 86% RH atmospheric relative humidity (J. Hu, unpublished data). Furthermore, *M. carbonarius* emerges from exit holes in their mounds and extensively forages in

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* Corresponding author. Tel.: +60 194225999; fax: +60 4 656 5125.
E-mail address: chowyang@usm.my (C.-Y. Lee).

[1] Denotes equal contribution.

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the open, above-ground on pavement trails that may reach up to a distance of 23 m (Inoue et al., 2001). In contrast, *M. gilvus* forages below ground, concealed within mud foraging tubes (Acda, 2004), but may sometimes, forage a short distance (≈20 cm)in the open; a similar foraging system has been observed in *M. michaelseni* (Sugio, 1995).

There are a number of publications on the foraging activity and behavior of open-air foraging termites [e.g., *Longipeditermes longipes* (Haviland) (Miura and Matsumoto, 1998b), *Hospitalitermes hospitalis* (Haviland) (Jones and Gathorne-Hardy, 1995), *H. medioflavus* (Haviland) (Miura and Matsumoto, 1995), *H. umbrinus* (Haviland) (Collins, 1979), *H. rufus* (Haviland) (Miura and Matsumoto, 1998a), *Constrictotermes cyphergaster* (Silvestri) (Moura et al., 2006), *Lacessititermes* sp. (Roonwal, 1970), and *M. carbonarius* (Sugio, 1995)]. However, studies on the physiological limits and capabilities in overcoming desiccation stress during above-ground foraging are still lacking.

M. carbonarius and *M. gilvus* were selected for this study because their foraging behavior differs even though they are sympatric, co-inhabit the same terrestrial environment, and share a similar food source (Neoh and Lee, 2009). To understand the adaptations necessary to support the different foraging strategies displayed by *M. carbonarius* and *M. gilvus*, we first set up a series of moisture preference tests. Second, we compared the cuticular permeability, ratios of body surface area to volume, water loss rate, total body water loss, and the time of termite mortality under desiccating conditions (LT_{50} and LT_{95}) between the species. The concept that 'insects that inhabit arid environments generally have lower cuticular permeability than those of inhabiting humid habitats' has been widely used to explain many insect responses to desiccation stress (Appel et al., 1983; Lighton and Feener, 1989; Zachariassen, 1996; Hull-Sanders et al., 2003; Bazinet et al., 2010). Thus, we hypothesized that the cuticular permeability of *M. carbonarius* is likely lower compared to that in *M. gilvus* (i.e., *M. carbonarius* is more resistant to desiccation) to allow them the long period of above-ground foraging activities.

2. Materials and methods

2.1. Study site

The study was conducted on Penang Island, located on the northwestern coast of peninsular Malaysia. Penang experiences an equatorial climate that is uniformly warm (temperature: 26 to 35 °C) and humid (relative humidity: 70 to 90%) throughout the year. Rainfall is evenly distributed throughout the year, although January–February and June–July are considered the driest periods of the year (see http://www.met.gov.my/index.php?lang=english).

2.2. Insect sampling

M. carbonarius and *M. gilvus* were collected from Universiti Sains Malaysia, Minden campus, Penang, Malaysia (5″21°N, 100″18°E). Varying sizes of nests (*M. carbonarius*, height: 38–48 cm, width 91–134 cm; *M. gilvus*, height: 13–65 cm, width: 42–125 cm) were selected in this study. We opened the nest by removing the outer layer of soil and randomly collected the fungus combs that housed the termites. Termites were then brought to the laboratory. Termites were carefully isolated from the fungus combs and detached nest materials. In all instances, we only used freshly collected termites for testing.

2.3. Moisture preferences assay

Two colonies of *M. carbonarius* and *M. gilvus* were tested. The test arena consisted of one center container and six peripheral plastic containers (diameter: 9.5 cm; height: 5.5 cm). The center container was designated as a termite introducing container and connected with 10.5 cm lengths of Tygon tubing (outer diameter: 1.2 cm; inner diameter: 1.0 cm) to the six peripheral containers. Six peripheral containers were interconnected with 9 cm lengths of Tygon tubings. Each container contained 50 g sand with different levels of moisture, i.e., 0, 5, 10, 15, 20 and 25% (wt: wt). Each treatment had one of each of the moisture treatments arranged randomly (by drawing lots). Each moisture treatment also had 0.5 g fungus comb as a food source; the central container only had dry sand and provided no food. Three hundred termites with 135 minor workers, 135 major workers, 15 minor soldiers and 15 major soldiers based on the caste ratios of natural field population (Lee and Lee, 2011) were introduced in the center of the container. The treatment was maintained in the dark at 26.4 ± 0.2 °C and 63.2 ± 0.6% RH. The experiments were terminated at day 7 as termites were found to undergo their routine (e.g., tunneling and wall construction) in the preferred containers. Tygon tubing was blocked with stoppers and the number of termites in each container with different moisture levels was recorded. Percentages of termites distributed in each container were calculated as live termite in each moisture treatment divided by the total number of live termites in the entire arena. Tunnel formations were scanned using a flat bed scanner (CanonCanoScan LiDE20, Canon Inc., China) and measured. Two field colonies of each species were collected and the test was replicated 7 times for each colony.

2.4. Water loss and cuticular permeability analysis

Percentage total body water content, mass loss over time, and cuticular permeability were determined gravimetrically as described by Appel and Tanley (1999), and Shelton and Grace (2003) with slight modifications. Three major soldiers and six minor workers, major workers, and minor soldiers were selected randomly from three colonies of both species. All termites were used within 1 h of collection. Only intact termites were used. The experiment was replicated 7 times for both species. We used an 11-L glass desiccator containing 1 kg of anhydrous $CaSO_4$ (Fisons Scientific Apparatus, Leicestershire, UK) (the desiccant was dried at 200 °C for at least 1 h) that maintained the RH in the desiccator at approximately 0–2% and resulted in saturation deficit of 29.764 mm Hg at 29 ± 1 °C. Six individuals of each caste (except major soldiers, n = 2) were placed in 22 mL glass vials. Fresh masses were weighted to the nearest 0.01 mg with a digital analytical balance (Sartorius Extended ED2245, Sartorius AG, Germany) prior to experiment. After that, the vials containing termites were placed in the desiccators. Mass losses and mortality of termites were recorded at selected time intervals (2, 4, 6, 8, 10, 12 and 24 h) (Appel, 1993; Shelton and Grace, 2003). Following the 24 h mass determination, the termites were transferred to an oven and held at 55 °C for 72 h (dried to constant mass), and then re-weighed to obtain their mass. To test whether there were differences between freshly collected and laboratory-maintained termites, the water loss and cuticular permeability test were also conducted on termites that maintained in an incubator (Medcenter Einrichtungen GmbH, München, Germany) at 30 ± 1 °C and 95% RH for a week.

Based on the two-compartment mass loss model, water loss during the first 2 h of desiccation represents the cuticular permeability (CP) of an insect (Appel et al., 1983; Mazer and Appel, 2001). Thus, in this study, CP values were generated using the mass loss (water loss) during the first 2 h as μg of H_2O lost per surface area (cm^2) per unit time (h) per saturation deficit (mm Hg) (Edney, 1977). Surface areas of termites were estimated using Meeh's (1897) formula: $S = 12\,M^{2/3}$, and Haagsma et al.'s (1996) formula: $S = 0.0886 + 26.85\,M - 214.21\,M^2$, where S = body surface area (cm^2) and M = initial mass (g). A simple correlation between CP value and fresh mass was analyzed to test the reliability of the CP values. We found that CP values using Meeh (1897) and Haagsma et al. (1996) surface area model estimates were not significantly related to initial mass for all castes ($P > 0.05$). This indicated that surface area estimation model did not bias CP values. Water loss rate was calculated using the weight loss between the initial and 2 h masses (mg) as mg of H_2O lost per initial mass (g) per unit time (h).

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Percentage of total body water was calculated as %TBW = [(initial mass − dry mass)/initial mass] × 100% (Shelton and Grace, 2003). Mean hourly mass loss and %TBW were used to calculate mass and %TBW loss. Ratios of surface area to volume were calculated by the model of Meeh (1897) surface area divided by fresh mass.

2.5. Statistical analysis

Statistical analyses were performed using Statistix 9.0 (Analytical Software, 2008). Percentages of termites distributed in different moisture levels and percentages of total body water loss were arcsine square root transformed prior to analysis. Body mass, CP value, and the transformed values were analyzed using analysis of variances (ANOVA) followed by Tukey's HSD tests at $\alpha = 0.05$ to test for the differences between castes. In an attempt to remove the effects of body size, body water content, and water loss rates were analyzed using One-way analysis of covariance (ANCOVA) with fresh mass of termites as the covariate (Packard and Boardman, 1999). The mortality of termites was also pooled and subjected to probit analysis using POLO-PC (LeOra Software, 1997) to generate LT_{50} and LT_{95} value [the time (h) of termite mortality under desiccation condition].

3. Results

3.1. Moisture preferences assay

Moisture significantly affected the distribution of *M. gilvus* ($F = 7.114$, d.f. = 5, $P < 0.01$). A post hoc test revealed that most *M. gilvus* (67.31 ± 6.24%, mean ± SE) aggregated in the 20% moisture container (Fig. 1a). The result was further supported by presence of higher tunneling activity (a mean tunnel distance of 22.3 ± 3.0 cm, in the 20% moisture container compared with other moisture treatment containers). In contrast, no significant difference was found in the containers with moisture level of 0%, 5%, 10%, 15% and 25%. At the end of the experiment, *M. gilvus* from colonies A and B showed 44.90 ± 4.47% and 55.39 ± 1.96% (mean ± SE) of survival rate, respectively. We also found that the carcasses of *M. gilvus* were distributed evenly in all containers and completely walled-off with sand.

M. carbonarius, however, was evenly distributed in 5%, 10%, 15%, and 20% moisture level areas (Fig. 1b); while the number of termites in the containers with 0% and 25% moisture was significantly less than the other treatments ($F = 37.802$, d.f. = 5, $P < 0.01$). Unlike *M. gilvus*, *M. carbonarius* often foraged on the surface of sand. Almost no tunnels were built. Survival rate of *M. carbonarius* from colony A and colony B were 82.64 ± 1.29% and 87.71 ± 1.64%, respectively. Survival of *M. carbonarius* was significantly greater than that of *M. gilvus* ($F = 136.90$, d.f. = 1, $P < 0.01$). Carcasses of *M. carbonarius* were often cut into pieces and partially walled-off with dry sand in the containers of moisture level 0% or in the Tygon tubings.

3.2. Water loss and cuticular permeability analysis

Fresh mass of *M. gilvus* and *M. carbonarius* ranged from 5.14 ± 0.14 to 26.49 ± 0.31 mg and 11.25 ± 0.22 to 69.17 ± 1.00 mg, respectively (Table 1). For both species, post hoc tests demonstrated that major soldiers had the greatest fresh body mass compared to those of minor workers, major workers, and minor soldiers; while minor workers had the least fresh body mass. Overall, *M. carbonarius* had greater body mass than *M. gilvus*. These results may also imply that the body water content of *M. carbonarius* was significantly higher than *M. gilvus* (minor worker: $F = 136.919$, d.f. = 1, $P < 0.05$; major worker: $F = 142.294$, d.f. = 1, $P < 0.05$; minor soldier: $F = 111.047$, d.f. = 1, $P < 0.05$; major soldier: $F = 42.637$, d.f. = 1, $P < 0.05$) (Table 1). Body water content for *M. gilvus* ranged 4.08 ± 0.09 to 19.67 ± 0.30 mg and 9.11 ± 0.17 to 52.92 ± 0.81 mg for *M. carbonarius*. There were significant differences in body water content between castes of both *M. gilvus*

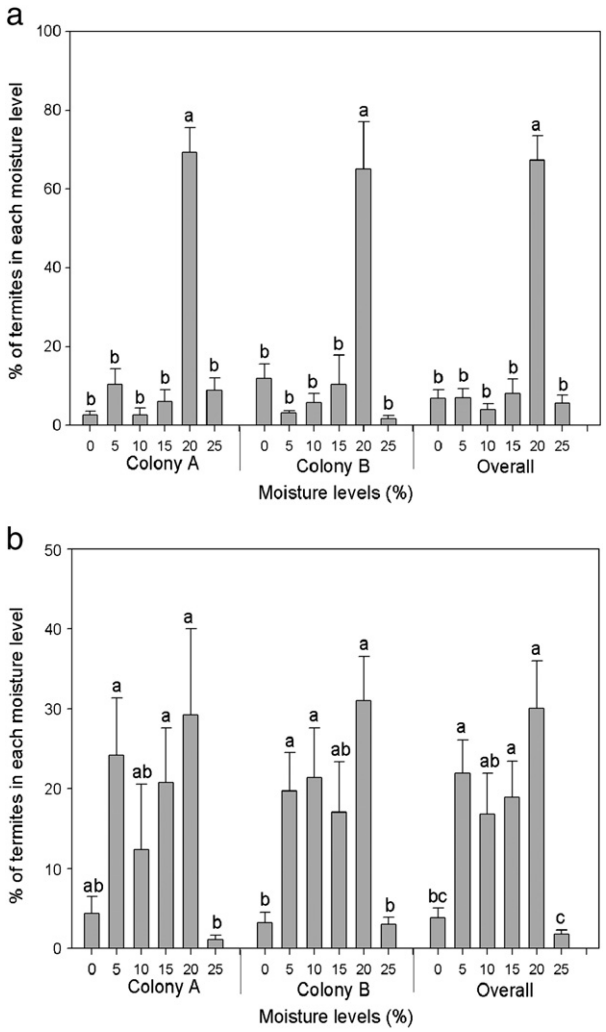

Fig. 1. Mean percentage (±SE) of (a) *M. gilvus* and (b) *M. carbonarius* distributes in container with different moisture level. Different letters denote significant differences between distributions of termites in different moisture level on the day 7.

($F = 1291.01$, d.f. = 3, $P < 0.01$) and *M. carbonarius* ($F = 1155.37$, d.f. = 3, $P < 0.01$). Within species, the largest difference occurred between minor workers and major soldiers.

Cuticular permeability values [using Meeh's (1897)] ranged from 14.96 ± 0.81 to 51.51 ± 0.93 μg cm^{-2} h^{-1} mm Hg^{-1} for *M. gilvus* and from 14.74 ± 0.97 to 49.52 ± 2.15 μg cm^{-2} h^{-1} mm Hg^{-1} for *M. carbonarius* (Table 1). There were significant differences between castes in both species (*M. gilvus*: $F = 285.15$, d.f. = 3, $P < 0.001$; *M. carbonarius*: $F = 67.97$, d.f. = 3, $P < 0.001$), in which, minor soldier had the lowest cuticular permeability value followed by major soldiers, minor workers, and major workers. Post hoc tests revealed that there were no significant differences between species of the corresponding caste. Similar trend in terms of levels of significance among castes and species were also observed for the values generated using Haagsma et al.'s (1996) surface area estimation formula when compared with Meeh's (1897) formula.

Ratios of surface area to volume ranged from 40.28 ± 0.16 to 69.75 ± 0.63 for *M. gilvus* and from 29.26 ± 0.14 to 53.66 ± 0.37 for *M. carbonarius* (Table 1). Ratios of surface area to volume of minor workers in both species were significantly greater than those in major workers, minor soldiers, and major soldiers. Overall, a post hoc test showed that the ratios of surface area to volume in *M. carbonarius* were significantly lower than *M. gilvus*. Water loss rate ranged from 29.72 ± 0.96 to 97.80 ± 1.81 mg g^{-1} h^{-1} for *M. gilvus* and from 17.60 ± 1.21 to 72.11 ± 2.16 mg g^{-1} h^{-1} for *M. carbonarius*

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Table 1
Fresh mass, body water content, ratios of surface area to volume, cuticular permeability values, water loss rate, and percentage of total body water (mean ± SE) of *M. gilvus* and *M. carbonarius*.

Physiological parameters	Species	Castes			
		Minor workers (n = 112)	Major workers (n = 112)	Minor soldiers (n = 112)	Major soldiers (n = 56)
Fresh mass (mg)	*M. gilvus*	5.14 ± 0.14 a(a)	9.30 ± 0.32 a(b)	5.69 ± 0.12 a(a)	26.49 ± 0.31 a(c)
	M. carbonarius	11.25 ± 0.22 b(a)	24.91 ± 0.71 b(b)	23.49 ± 0.49 b(b)	69.17 ± 1.00 b(c)
[a]Body water content (mg)	*M. gilvus*	4.08 ± 0.09 a(a)	7.55 ± 0.24 a(b)	4.50 ± 0.08 a(a)	19.67 ± 0.30 a(c)
	M. carbonarius	9.11 ± 0.17 b(a)	20.73 ± 0.53 b(b)	19.25 ± 0.52 b(c)	52.92 ± 0.81 b(d)
Cuticular permeability (μg H_2O cm^{-2} h^{-1} mm Hg^{-1})	*M. gilvus* (M)	47.62 ± 0.78 a(a)	51.51 ± 0.93 a(b)	14.96 ± 0.45 a(c)	28.97 ± 1.54 a(d)
	M. carbonarius (M)	47.49 ± 1.34 a(a)	49.52 ± 2.15 a(a)	14.74 ± 0.97 a(b)	27.86 ± 2.98 a(c)
	M. gilvus (H)	76.87 ± 1.27 a(a)	85.36 ± 1.54 a(b)	24.37 ± 0.73 a(c)	47.57 ± 2.52 a(d)
	M. carbonarius (H)	78.69 ± 2.22 a(a)	81.29 ± 3.54 a(a)	24.16 ± 1.59 a(b)	61.64 ± 6.64 a(c)
Ratios of surface area to volume	*M. gilvus*	69.75 ± 0.63 a(a)	57.37 ± 0.67 a(c)	67.35 ± 0.46 a(b)	40.28 ± 0.16 a(d)
	M. carbonarius	53.66 ± 0.37 b(a)	41.25 ± 0.42 b(b)	41.98 ± 0.29 b(b)	29.26 ± 0.14 b(c)
[a]Water loss rate (mg g^{-1} h^{-1})	*M. gilvus*	97.80 ± 1.81 a(a)	87.21 ± 2.27 a(b)	29.72 ± 0.96 a(c)	34.45 ± 1.90 a(ab)
	M. carbonarius	72.11 ± 2.16 b(a)	58.51 ± 2.49 a(ab)	17.60 ± 1.21 b(c)	23.00 ± 2.43 a(ac)
Total body water (%)	*M. gilvus*	79.60 ± 0.58 a(ab)	81.30 ± 0.40 a(a)	79.21 ± 0.40 a(b)	74.21 ± 0.47 a(c)
	M. carbonarius	79.87 ± 0.35 a(ab)	82.72 ± 0.72 a(a)	81.39 ± 0.86 b(ab)	76.20 ± 0.36 b(c)

Means in the same column followed by different letters are significantly different ($P<0.05$, Student *t*-test).
Means in the same row followed by different letters in parentheses are significantly different ($P<0.05$, ANOVA, Tukey's HSD).
(M) denotes surface area estimation using Meeh's (1897) formula.
(H) denotes surface area estimation using Haagsma et al.'s (1996) formula.
[a] Parameters that were analyzed using ANCOVA and separated by least significant difference with fresh weight as a covariate.

(Table 1). For both species, minor soldier had the lowest water loss rate; conversely, minor worker had the largest water loss rate (*M. gilvus*: $F = 81.562$, d.f. = 3, $P<0.05$; *M. carbonarius*: $F = 21.077$, d.f. = 3, $P<0.05$). Between species, minor worker ($F = 29.967$, d.f. = 1, $P<0.05$) and minor soldier ($F = 11.141$, d.f. = 1, $P<0.05$) of *M. carbonarius* commonly had a significantly lower water loss rate than *M. gilvus*.

%TBW ranged from 74.21 ± 0.47 to 81.30 ± 0.40% and 76.20 ± 0.36 to 82.72 ± 0.72% for *M. gilvus* and *M. carbonarius*, respectively (Table 1). There were significant difference within *M. gilvus* ($F = 42.378$, d.f. = 3, $P<0.05$) and *M. carbonarius* ($F = 21.028$, d.f. = 3, $P<0.05$) and major soldier %TBW was significantly lower in both species. Between species, the %TBW of minor soldiers ($T = 2.289$, d.f. = 40, $P<0.05$) and major soldiers ($T = 3.371$, d.f. = 40, $P<0.05$) of *M. carbonarius* was greater than *M. gilvus*.

Percentage of total body water loss of four castes for both species against desiccation time was modeled using a power function of the form $y = a(1 - e^{(-bx)})$, where y = percentage of total body water loss, x = desiccation time (h), a = y-axis intercept and b = rate constant (SigmaPlot, 2006) (Table 2). For both species, %TBW loss by caste increased curvilinearly with desiccation time (Fig. 2), with workers generally losing a greater percentage of mass than those of the soldiers of both species. Post-hoc tests showed that workers generally lost greater percentage of water than in soldiers and minor soldier had the lowest %TBW loss among the castes after first 2 h but there were no significant difference when compared to major soldiers (Fig. 2a and b). After 24 h, mean %TBW loss in *M. gilvus* was 93.71 ± 0.51% for minor worker, 92.19 ± 0.79% for major workers, 97.08 ± 0.68% for minor soldiers and 95.71 ± 1.21% for major soldiers (Fig. 2a). In *M. carbonarius*, mean %TBW loss in *M. carbonarius* was 77.85 ± 0.56% for minor worker, 81.79 ± 0.84% for major workers, 51.72 ± 2.83% for minor soldiers and 56.15 ± 2.83% for major soldiers (Fig. 2b). There was a significant difference in %TBW after 24 h between species by caste indicating that *M. carbonarius* termites lost less %TBW for each caste compared with *M. gilvus*.

Overall, data obtained from freshly collected termites and those after being maintained in the laboratory condition for a week showed mixed result. In most instances, the values increased drastically. For example, in *M. gilvus*, CP values increased 13%–183% among castes in 1-wk old termites compared with the freshly collected termites. In *M. carbonarius*, CP values increased 33.78%–58.41% for major workers and minor soldier (Table 3).

High mortality was recorded for *M. gilvus* after 12 h (89.8%); while in *M. carbonarius* there was approximately 37.8% mortality rate (all castes) after 12 h. Furthermore, 52.3% of minor soldiers still survived after being exposed to 29 ± 1 °C and ≈0–2% RH for 24 h. In *M. gilvus*, minor workers and major workers survived for 5.3 h, whereas LT_{50} values were significantly higher for minor soldiers (10.1 h) and major soldiers (8.4 h), respectively. The survival rate of *M. carbonarius* is similar to that showed by *M. gilvus* with regard to castes difference (LT_{50} values ranging between 12.7 and 23.9 h for all castes) (Table 4). It is worth noting that LT_{50} and LT_{95} of soldiers of both species were always greater than those in workers. Between species, *M. carbonarius* represented a 1.8–2.4-fold higher desiccation tolerance as compared to *M. gilvus* considering LT_{50} of all castes and 2.0–2.5-fold higher for LT_{95} of all castes.

Table 2
Power function regression coefficients (mean ± SE) for percentage of total body water loss over time for four castes of *M. gilvus* and *M. carbonarius*, $y = a(1 - e^{(-bx)})$, where $0 \le X \le 24$.

Species	Caste	*a*	*B*	*F*	*P*	R^2
M. gilvus	Minor workers	96.14 ± 3.16	0.13 ± 0.01	1075.93	<0.0001	0.997
	Major workers	96.79 ± 1.09	0.12 ± 0.00	10182.68	<0.0001	0.999
	Minor soldiers	274.36 ± 81.20	0.02 ± 0.01	874.25	<0.0001	0.997
	Major soldiers	187.49 ± 18.50	0.03 ± 0.00	2549.55	<0.0001	0.999
M. carbonarius	Minor workers	86.92 ± 3.29	0.09 ± 0.01	1415.37	<0.0001	0.998
	Major workers	101.27 ± 2.10	0.07 ± 0.00	8499.48	<0.0001	0.999
	[a]Minor soldiers	2.08 ± 0.04	0.00 ± 0.00	16348.60	<0.0001	0.999
	Major soldiers	133.02 ± 2.65	0.02 ± 0.00	113761.30	<0.0001	0.999

[a] Percentage of total body loss of minor soldiers for *M. carbonarius* against desiccation time were modeled using a polynomial quadratic of the $y = c + ax + b(x^2)$, $c = 0.04 \pm 0.18$.

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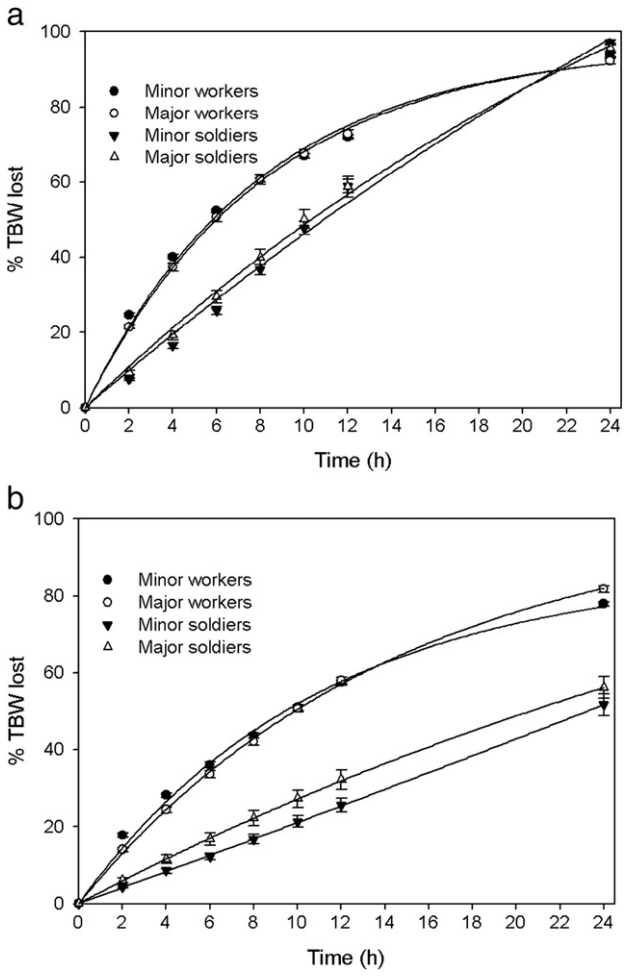

Fig. 2. Mean percentage of Total body water loss (±SE) over time of freshly collected termites of (a) *M. gilvus* and (b) *M. carbonarius* at 29.0 ± 1.0 °C and 0–2% RH.

4. Discussion

Termites collected from field colonies and kept in laboratory conditions over even a short period are always found to have reduced vigor (Su and La Fage, 1984). This indirectly may result in changes of physiology and levels of biological molecules in termites (Arquette et al., 2006a). Arquette et al. (2006a) found that body water content of *R. flavipes* decreased 1.9–4.3%. This may reflected the inconsistent results of the earlier data sets of *C. formosanus* and *R. flavipes* termite obtained by varying data collection at certain day intervals (Sponsler and Appel, 1990; Shelton and Grace, 2003). Similarly, our data showed that there were significant differences in most of the parameters tested between freshly collected samples and samples that maintained in incubator for one week (Table 3). In this study, we improved the method using termites that were freshly collected from the nests and were subjected to water loss and cuticular permeability analysis within an hour after the collection. This might be the best approach to representing levels of biological molecules and desiccation tolerances in natural conditions. In addition, the temperature under experimental condition (29 °C) that differed from ambient temperature under field conditions (26 to 35 °C) may also affect the physiological parameters tested.

In our bioassay, the results of moisture preferences indicated that *M. gilvus* had narrow moisture level preference (20%) in which similar to the moisture content of the habitacle zone (mean = 22.64 ± 4.38%) (Foo et al., 2011), whereas *M. carbonarius* had a wide range of moisture level preference (5%–20%). Indirectly, this resulted in high mortality in *M. gilvus* case partly due to desiccation stress. It is also important to note that *M. carbonarius* usually congregated at two or three out of the four preferred moisture containers (except 0% and 25%) and therefore resulted in high level of variability in spatial distribution across replicates (thus high standard error was obtained in *M. carbonarius*) (Fig. 1). It is also possible that the dead carcasses may be "repellent" and deter the termites from entering areas with many carcasses as had been shown in many termiticide efficacy tests (e.g., Su, 2005). This phenomenon might affect the termite distribution in the experimental set up in due course. However, in this experiment, we found that the carcasses were properly handled. For example, *M. carbonarius* often carried the carcasses to the least preferred containers or tubes connected to the container (i.e., 0% moisture). Seemingly, those areas were designated as "cemetery". Though *M. gilvus* workers were found to distribute the carcasses evenly in the containers, the carcasses were well walled-off.

Another two Southeast Asian subterranean termite species, *Microcerotermes crassus* Snyder and *C. gestroi*, construct tunnels and show similar moisture preference at 20% (Wong and Lee, 2010), the same case as *M. gilvus*. In the present study, *M. carbonarius* and *M. gilvus* showed similar percentage of total body water, although they differed greatly in fresh mass. Our results also revealed that *M. gilvus* died at a critical point of between 59.73% and 78.30% of their total body water; *M. carbonarius* died as the water loss threshold reached 63.44% to 72.03% (Table 2 and Table 4). These fit into the higher end of the range of water loss tolerance in insect, which is 17–89% of the total body water (Hadley, 1994). From these results we can conclude that both species share similar water loss tolerance thresholds. In a study of African termites, *Macrotermes bellicosus* (Smeathman) and

Table 3
Fresh mass, body water content, cuticular permeability value, water loss rate, and percentage of total body water (mean ± SE) of *M. gilvus* and *M. carbonarius* after being maintained for one week in the laboratory.

Physiological parameters	Species	Castes			
		Minor workers (n = 10)	Major workers (n = 13)	Minor soldiers (n = 13)	Major soldiers (n = 10)
Fresh mass (mg)	*M. gilvus*	4.34 ± 0.10[a]	7.39 ± 0.18[a]	6.12 ± 0.14[a]	23.93 ± 0.72[a]
	M. carbonarius	8.80 ± 0.39[a]	16.37 ± 0.74[a]	19.84 ± 1.04[a]	59.25 ± 1.96[a]
Body water content (mg)	*M. gilvus*	3.41 ± 0.11[a]	5.67 ± 0.16[a]	4.78 ± 0.10	17.37 ± 0.55[a]
	M. carbonarius	6.78 ± 0.21[a]	12.99 ± 0.54[a]	15.57 ± 0.81[a]	44.73 ± 1.56[a]
Cuticular permeability (μg H_2O cm^{-2} h^{-1} mm Hg^{-1})	*M. gilvus* (M)	53.81 ± 6.59[a]	71.38 ± 2.58[a]	42.41 ± 4.93[a]	43.96 ± 5.98[a]
	M. carbonarius (M)	37.92 ± 2.00[a]	66.25 ± 3.42[a]	23.35 ± 1.79[a]	22.17 ± 1.59
Water loss rate (mg g^{-1} h^{-1})	*M. gilvus*	125.77 ± 14.91[a]	140.34 ± 5.03[a]	88.15 ± 8.87[a]	58.34 ± 6.30[a]
	M. carbonarius	71.21 ± 4.70	100.18 ± 5.45[a]	33.92 ± 3.82[a]	21.88 ± 1.71
Total body water (%)	*M. gilvus*	78.65 ± 1.40	76.06 ± 0.63[a]	77.75 ± 0.41	73.23 ± 1.02[a]
	M. carbonarius	77.74 ± 1.33	79.55 ± 1.57	78.69 ± 1.22[a]	75.46 ± 0.38

(M) denotes surface area estimation using Meeh's (1897) formula.
[a] The values are significantly different from those in freshly collected termites (Table 1) ($P<0.05$, Student *t*-Test).

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Table 4
LT_{50} and LT_{95} value of four castes of *M. gilvus* and *M. carbonarius* when exposed to 29 ± 1 °C and ≈0–2% RH.

Species	Caste	n	LT_{50}(h) (95% Confidence limits)	LT_{95}(h) (95% Confidence limits)	Mortality (12 h) (%)	Mortality (24 h) (%)
M. gilvus	Minor workers	112	5.3(5.1–5.5)	7.7(7.3–8.3)	100.0	100
	Major workers	112	5.3(5.1–5.5)	8.0(7.6–8.7)	100.0	100
	Minor soldiers	112	10.1(9.7–10.5)	16.8(15.3–18.9)	69.6	100
	Major soldiers	56	8.4(7.8–8.9)	14.3(12.8–16.9)	89.8	100
M. carbonarius	Minor workers	112	12.7(12.1–13.4)	19.6(17.7–22.5)	41.1	100
	Major workers	112	11.1(10.6–11.6)	16.1(14.8–18.4)	64.9	100
	Minor soldiers	112	23.9(20.8–29.3)	60.1(44.7–96.9)	13.8	47.7
	Major soldiers	56	15.1(13.7–17.0)	32.4(26.6–43.7)	31.3	83.3

Trinervitermes geminates (Wasmann) died after losing more than 50% and 60% of their initial weight (Abushama, 1974).

Though we have no complete data on respiratory water loss, CP values here may only slightly overestimate true CP. Cuticular water loss represents the major route of water loss in termites. For example, in a study of drywood termite *Incisitermes minor* (Hagen) showed that the cuticular water loss in pseudergates accounted for 93.5% of the total daily water in which 20-fold higher than respiratory water loss at 25 °C (Shelton and Appel, 2000). Furthermore, study on water loss traces in *I. minor* pseudergates (Shelton and Appel, 2000) and *R. flavipes* alates (Shelton and Appel, 2001b) revealed that the peaks of water loss did not fluctuate in line with CO_2 release. Thus, respiratory contributes minimally to water loss (Shelton and Appel, 2001a). A number of authors have used the concept that was outlined in the Introduction to explain the geographical distribution and life traits of insect species (Appel et al., 1983; Mack and Appel, 1986; Shelton and Appel, 2000; Hull-Sanders et al., 2003). They suggested that xeric-adapted arthropods have relatively lower CP values; in other words, arthropods can conserve a greater amount of water and may tolerate a drier environment conditions. However, in the present study, we found no significant differences in CP values between the two species (Table 1). Similar observations were also made by Mazer and Appel (2001) who found little evidence of a relationship between the desiccation tolerance and cuticular permeability in five Heliconiinae longwing butterflies.

Epicuticular lipid plays a pivotal role in insect desiccation resistance. For example, high water loss rate was recorded in epicuticular lipid-extracted arboreal and terrestrial ants (Hood and Tschinkel, 1990). This is also true for many cockroach species, such as German cockroach (Appel and Tanley, 1999) and smokybrown cockroach (Appel et al., 1986). In the present study, we do not rule out the possible involvement of epicuticular lipid in conserving water. In addition, other factors that may influence the water trait in termites include glycogen. It is well known that oxidation of glycogen provides metabolic water during desiccation stress in insects. In *Drosophila melanogaster* Meigen, high levels of glycogen were found in desiccation resistance-group of flies (Gibbs et al., 1997; Folk et al., 2001). Sawabe and Mogi (1999) reported that the vitality of *Aedes* mosquitoes (i.e., *Aedes aegypti* (L.), *Ae. albopictus* (Skuse), and *Ae. paullusi* (Stone & Farmer)) under desiccation condition was significantly associated with the high lipid and glycogen levels. To date, glycogen relating desiccation resistance in termites had been rarely studied. Arquette and Forschler (2006b) proposed that the high glycogen level in *R. flavipes* might be due to seasonality (i.e., termite activity). In a study of laboratory-cultured *R. flavipes*, the level of glycogen content of workers was not correlated with the water trait in workers (the amount of glycogen fluctuated despite the workers' body water content depleting through time) (Arquette et al., 2006a).

Besides, the body water balance of termite is also maintained through water uptake complement with the possession of water sacs in the termite's labial gland. Sieber and Kokwaro (1982) reported that *Macrotermes michaelseni* (Sjöstedt) workers took up approximately large water amount of 16% of their fresh weight after being desiccated for 15 h. The water was initially stored in the foregut and was then transferred to the water sacs. Grube and Rudolph (1999) found that the content of the water sacs in *R. flavipes* was used to complement termite body water imbalance when body water loss was up to 59.6%. Thus, it is likely that *M. carbonarius* workers filled up their water sacs enabling them to sustain a long period of above-ground foraging activity as demonstrated by *Hodotermes mossambicus* (Hagen) (Mitchell et al., 1993). More intensive study of this feature is required.

Nonetheless, the present study showed distinct differences in water loss rates and %TBW loss among species (Table 1). Even though both species have similar rates of water loss per unit surface (similar in CP values), we found that the ratio of surface area to volume of the two *Macrotermes* tested seems to be in inverse proportion to the fresh mass (volume). This resulted in a low surface area to volume ratio in *M. carbonarius* and less water to be vaporized from the insect cuticle relative to their total water mass. In contrast, *M. gilvus* apparently registered high water loss rate and %TBW loss due to high surface area to volume ratio (Table 1). It is likely that the standard equation for calculating insect surface areas is inadequate for these termite species. A standard equation assumes both proportional increases in surface areas with increasing body mass and equivalent permeability over the entire body surface. The CP value generated using an alternate surface area estimation formula (Haagsma et al., 1996) also showed similar trend (i.e., the levels of significant differences between castes in both species, and between species of the corresponding caste) when compared to CP values generated using Meeh's (1897) formula. Nevertheless, the CP values generated using Haagsma et al.'s (1996) formula should be viewed with caution, since it is only applicable for termites that body mass range from 1 to 50 mg (weight of major soldiers of *M. carbonarius*: 69.17 ± 1.00 mg) (Shelton and Appel, 2001b). Thus, a surface area estimation formula specifically for large termite species, such as *Macrotermes* spp. clearly needs to be developed.

Nevertheless, the present results imply that *M. carbonarius* accompanied by the feature of higher body water content has longer time period to tolerate water loss than *M. gilvus* before which the amount of water loss reaches the critical point. This situation explained that why *M. carbonarius* is adaptable to the long-period above-ground foraging activity in conjunction with the finding of moisture preference assay that outlined in this study. Hood and Tschinkel (1990) compared the arboreal and terrestrial ants and claimed that the desiccation tolerance increased with the body sizes. Similarly, Cohen and Pinto (1977) also reported that two blister beetles, *Megetra cancellata* (Brandt and Erichson) and *Pleuropasta reticulate* Van Dyke, though, both showed similar CP values, the former that larger in size only lose 2.2% body weight per hour while the latter lost 6.1% per hour.

5. Summary and conclusion

Insects improve their physiological abilities in order to encounter desiccation stress in three general ways (1) limiting water loss rate, (2) increase the amount of water that can be lost before death, and

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(3) increase body water content, or in various combinations (Bazinet et al., 2010). However, in our study, there were no significant differences observed in cuticular permeability, and percentage of total body water among species, suggesting that those parameters were not their physiological limitations for encountering desiccation stress during above ground foraging. In fact, the present results show that the size of these particular species matters more than other physiological parameters tested. In other words, the surface area to volume ratio contributes greatly to the water loss rate and percentage of body water loss in both sympatric species. Therefore the standard equation for calculating insect surface areas is inadequate for these termite species. This also explained that why *M. carbonarius* with a lower surface area to volume ratio can tolerate desiccation for longer in the open during foraging activity; while *M. gilvus* only able to forage below ground or concealed within mud foraging tubes.

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