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On the Chemical Disguise of a Physogastric Termitophilous Rove Beetle

CS ROSA^{1,2}, PF CRISTALDO^{1,3}, DF FLORENCIO^{1,4}, A MARINS¹, ER LIMA¹, O DESOUZA¹

1 - Pós-graduação em Entomologia, Departamento de Entomologia, Universidade Federal de Viçosa, Minas Gerais, Brazil

2 - Universidade Federal do Triângulo Mineiro, Iturama-MG, Brazil

3 - Universidade Federal de Sergipe, São Cristóvão-SE, Brazil

4 - Departamento de Agrotecnologia e Ciências Sociais, Universidade Federal Rural do Semi-Árido, Mossoró-RN, Brazil

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Corresponding author

Paulo Felipe Cristaldo

Universidade Federal de Sergipe

São Cristóvão-SE, Brasil.

E-Mail: pfellipec@gmail.com

Abstract

Inter-specific symbiotic links are often reinforced by morphological, physiological, or behavioral trait modification undergone by the associated species. In some cases, such as in physogastric termitophile staphylinids, such modifications do facilitate the social interaction. Here we inspect chemical traits of the physogastric staphylinid *Corotoca melantho* (Insecta: Coleoptera) and its termite host *Constrictotermes cyphergaster* (Insecta: Blattodea: Isoptera), aiming to verify whether staphylinids resemble their host. First, we compared CHC profiles of hosts and guests within and among termitaria, to gather evidence on the origin of such profiles in guests. Then, we examined nitrogen and carbon isotopic signatures of these cohabitants to inspect whether chemical disguise is achieved by predation of host workers by staphylinids. Beetles presented CHC more similar to the CHC of their cohabiting termites than to (i) their conspecifics and (ii) termites from another nest, thereby favouring the hypothesis on CHC acquisition by guests. Isotopic signatures revealed that such similarities could not be majorly determined by share nutrition between these cohabitants. In general, our results evidenced that chemical disguise in termitophiles may function as a strategy for social integration in morphological mimics.

Introduction

Species interactions are seen as the main force driving the diversification and organization of life (Thompson, 1999). Some of these interactions are so close that the involved species undergo morphological, physiological, or behavioral trait modification which ease their association (Kneip et al., 2007; Muscatine & Porter, 1977). This is particularly common for the invaders of social insect nests, such as the rove beetles of the subfamily Aleocharinae (Staphylinidae), a group holding a disproportionate number of lineages specialized as cohabitants of social insect colonies (Kistner, 1982). An interesting question, still unsolved, is “why have aleocharines, as opposed to almost any other insect group, been so successful at invading colonies of social insects?” (Yamamoto et al., 2016). It is being argued by Parker (2016) that preadaptation such as a predatory diet, physically or chemically defensive morphology and small body size would have acted in synergy to allow myrmecophily and termitophily in this group.

Specifically for termitophilic aleocharines, physically defensive morphologies have been reported as important facilitators of their social integration. These can be recognized in two types of body plan: a limuloid and a disguising form Kistner (1982). In the limuloid (horseshoe crab) body form, expansions in the lateral margins of the body conceal vulnerable appendages, protecting them from host attacks. In the disguising form, a swollen thorax or –more commonly– a grotesquely expanded abdomen provides striking resemblance to the host (Cunha et al., 2015) hence avoiding detection of the beetle as a non-nestmate. Recent studies (Kanao et al., 2016; Yamamoto et al., 2016) on the evolution of the limuloid body of the Aleocharinae have shed revealing light on the reasons for the success of this subfamily as social parasites. As for physogastry, we still need more efforts to give continuity to the seminal works by Seevers (Seevers, 1957) and Kistner (Kistner, 1979, 1982).



Physogastry in rove beetles is notable for its apparent termitophilic role: morphometrical congruence between the hypertrophic abdomens of hosts and guests (Cunha et al., 2015) can be reasonably interpreted as tactile (or “Wasmannian mimicry” *sensu* Rettenmeyer, 1970). In fact, morphological similarity has been long considered a disguising trait allowing termitophiles to pass host worker’s inspection, as these latter cannot distinguish mimics from models based on their body constitution and surface structure (Sands & Lamb, 1975). Succeeding inspection means that the termitophile is recognized as a nestmate and this would not only avoid aggression but could also ease allogrooming and trophallactic exchanges with their host (Kistner, 1979). More extremely, such a disguise could help termitophiles to approach and prey upon termites, their brood, and their eggs.

The integrity of social insect colonies, however, does not seem to majorly rely on morphological inspection (in fact, a lot of social insect symbionts do not resemble their hosts). Rather, a sophisticated recognition system, based on chemical cues (Nash & Boomsma, 2008) notably from cuticular hydrocarbons (CHC) (Blomquist & Bagnères, 2010), helps detecting alien species. CHC allow colony members to distinguish nestmates from non-nestmates through comparison of their own CHC template. Morphological disguise, therefore, should work properly when in consonance with some form of chemical disguise.

A range of chemical disguise strategies can be used by invaders, such as (i) biosynthesize disguising CHC profiles (*‘innate chemical mimicry’*), (ii) biosynthesize some specific CHC compounds of their host and acquiring other disguising compounds after getting in touch with termites and/or their nest walls (*‘acquired chemical mimicry’*) and (iii) displaying no or a weak quantity of recognition cues allowing them to keep unnoticed into the host colonies (*‘chemical insignificance’*). In addition to these strategies, host can not able to detect the mimic as a different organism (*‘chemical crypsis’*) and host can detect and recognize invaders as an uninteresting organism (*‘chemical masquerade’*) (for more details see von Beeren et al., 2012).

Here we investigated the chemical disguise strategy used by *Corotoca melantho* Shjødte, 1853, one of the most striking physogastric Staphylinidae: Aleocharinae beetles, which is an obligatory termitophile of *Constrictotermes cyphergaster* (Silvestre, 1901) termites and has previously been observed engaging physical contacts with their host (Grassé, 1986). We start off by comparing CHC profiles of hosts and guests, within and among termitaria. If guests acquire CHC, one should expect their profiles to match colony-specific CHC profiles of termites. Alternatively, if guests only biosynthesize disguising CHC, the similarity between beetles and termites would be most obvious at the species- rather than at the colony-level. Finally, we also examine nitrogen and carbon isotopic signatures of these cohabitants, to inspect whether chemical disguise is acquired by predation of termite workers by staphylinid beetles.

Material and Methods

Ethics statement. All necessary permits were obtained for the described study, which complied with all relevant regulations of Brazil. This includes collecting and transportation permits from IBAMA (The Brazilian Institute for the Environment and Renewable Natural Resources), and permission from EMBRAPA (The Brazilian Enterprise for Agricultural Research) to conduct the study on their site. Tacit approval from the Brazilian Federal Government is implied by hiring the authors as Scientific Researchers. No protected species were sampled.

Terms definition. The terms “termitarium” (plural: termitaria), “mound”, or “nest” denote the physical structure built by termites. “Colony” denotes the assemblage of termite individuals living and cooperating intra-specifically within a nest.

“Coexistence” and “cohabitation” are used as synonyms and refer to the simultaneous occurrence of termites and other arthropods within a given termitarium, without implication of reciprocal positive or negative influences. These non-termite cohabitants are referred to as “termitophiles”, that is, “[...] arthropods which live in the same nests as their host or have some other obligatory relationship with them” (Kistner, 1979). The terms “guest”, “resident”, and “intruder” may also be used to refer to termitophiles simply because they establish themselves –not necessarily by force– in nests which had not been built by/for them in the first place.

Study system: the termite host and its termitophile.

Constrictotermes cyphergaster (Blattodea: Isoptera: Termitidae: Nasutitermitinae) is a common termite species in Brazilian savannas (“Cerrado”) (Mathews, 1977) and dry scrub (“Caatinga”) (Vasconcellos et al., 2007). Nests of this species are frequently built on trees (DeSouza et al., 2016; Moura et al., 2006; Vasconcellos et al., 2007), harbouring many organisms, including other termite species –so called “inquilines”– and a number of termitophiles, mainly Staphylinidae (Insecta: Coleoptera) (Cunha et al., 2003; Mathews, 1977; Seevers, 1957). Among these latter, *Corotoca melantho* Shjødte, 1853 (Staphylinidae: Aleocharinae: Corotocini) is the most frequent and abundant obligatory cohabitant.

In the studied site, this termitophile has been recorded in 83% of all *C. cyphergaster* nests as long as they are bigger than 2.2 litres (Cristaldo et al., 2012). Under natural conditions, *Corotoca melantho* is reported to live in close contact with their termite host with no aggressive behavior from soldiers and workers of host colonies (Costa & Vanin, 2010; Cunha et al., 2015). Stomodaeal trophallaxis is assumed (but not explicitly observed) to occur based on their unusual mouthparts: differently from other Aleocharinae, members of the Corotocini tribe have the mentum fused to the submentum forming a shield-like sclerite (Figure 18q in Seevers, 1957). This stationary condition of the mentum has led Seevers (*op. cit*) to suppose that these beetles receive liquid aliment from their hosts. Apart from records (Grassé, 1986) on termites licking

their cohabiting *C. melantho*, additional explicit information on the specific behavior of this termitophile is not available in the literature.

Termitophile staphylinids (but not free-living or myrmecophile ones), often present hypertrophic abdomen (*i.e.*, “physogastric”) folded over the thorax, making them visually similar to their host. Among them, the Aleocharinae: Corotocini comprise the most pronounced cases (Fig 1) and among Corotocini, *C. melantho*’s physogastry is so marked as to make it morphometrically indistinguishable from its host (Cunha et al., 2015). This pronounced abdominal hypertrophy is compatible with their ovoviviparity.

Study area and sampling

C. cyphergaster nests ($N=11$) were collected in Brazilian savanna (“Cerrado”), near the town of Sete Lagoas (19°27’ S, 44°14’ W), state of Minas Gerais, South-eastern Brazil. In the study area, altitude varies from 800 to 900 m above sea level and climate can be characterized as equatorial with dry winter (Aw) according to Köppen’s classification. Epigeous and arboreal nests with no signal of damage were sampled in an area of 114 m². Collections were performed in July 2008 and May 2011.

In the laboratory, nests were completely dissected and inspected to collect, using entomological soft forceps, a sample of individuals (soldiers and workers) from termite colonies and all termitophilous Staphylinidae. Specimens to be used for identification were preserved in 80% alcohol, labelled, and subsequently identified to the lowest possible taxonomic level. Termite hosts were identified to species following Mathews (1977) and by comparison with the samples in the Termite Section of the Entomological Museum of the Federal University of Viçosa (MEUV), where voucher specimens were deposited (# UFV8735, UFV8736, UFV8737). The Staphylinidae were sent to a specialist for identification. Specimens used for chemical analyses (CHC and stable isotopes) were prepared as described below. Each type of analysis always involved both staphylinids and termites from the same nest, but not all nests provided enough material for all analyses. Hence, out of the 11 nests studied, four provided enough guest-host pairs for CHC and isotope analyses, five nests provided material just for CHC analyses and three nests provided material for isotope analyses only. In summary, staphylinids and termites were probed in nine nests for CHC and in seven nests for isotopes.

Cuticular hydrocarbons: extraction and analysis. In order to assess the degree to which CHC profiles of *C. melantho* resembled those of its termite hosts, individuals of each of species were washed in solvent and extracts thereby obtained where then analysed by gas chromatography coupled with mass spectrometry (GC–MS). For CHC extraction, initially one sample composed of 10 termite workers and another sample composed of 10 beetles from each nest were stored separately in glass vials, and then freeze-killed. Thereafter,

samples were submerged in 100 µL of *n*-hexane (HPLC grade) for 10 min, following the procedure described by Haverty et al. (1996) and Marten et al. (2009). CHC extractions were conducted without agitation of the glass vials. Extracts were then transferred to a fresh vial and the solvent was allowed to evaporate at room temperature. Extracts were redissolved with 10 µL of *n*-hexane per sample. Extractions for each of the cohabiting species from a single nest were carried out independently, to avoid CHC mixing. GC-MS analyses were performed using a GCMS-QP2010 SE Shimadzu equipped with Rtx[®]-5MS, with samples (1 µL) injected under 220°C, in splitless mode. A fused silica column (30 m - 0.25 mm ID; 0.25 µm) was used for separation. Helium was used as a carrier gas at a constant flow rate of 0.87 mL/min. The temperature program was: 130°C to 290°C at 7°C/min and held for 15 min. Scans of mass spectrometer were performed from 40 to 500 m/z using the electron impact mode (70 eV). Samples were injected in no particular order, *i.e.* randomly. The peaks were classified based on their mass spectra and non-isothermal Kovats retention indices (KI) (using definition of van Den Dool & Dec. Kratz, 1963) evaluated on the basis of *n*-alkane series (Carlson et al., 1998).

Stable isotopes analysis. In order to inspect trophic relationships between guests and hosts, we analysed the nitrogen and carbon stable isotope concentrations of both *C. melantho* beetles and their host *C. cyphergaster* termite workers. Such isotopic concentrations, here also referred to as “isotopic signatures”, were obtained by measuring ¹³C/¹²C and ¹⁵N/¹⁴N ratios (hereafter δ¹³C and δ¹⁵N, respectively) from the bodies of the beetles and termite individuals under study. Termite workers of each species in the termitaria were sorted, when possible, into 10 subsamples, each with a sufficient number of individuals to obtain a dry biomass of 1.5 µg for full-body isotopic analysis. This allowed to characterize these species by a set of δ¹³C and δ¹⁵N pairs obtained from several individuals from the same colony, this set circumscribing a bi-dimensional space in a Cartesian plot whose axes represent the concentration of such isotopes in the bodies of the individuals.

The δ¹³C and δ¹⁵N ratios in the body of an organism reflect the food consumed and assimilated during its lifespan (DeNiro & Epstein, 1978; Eggers & Jones, 2000). Full-body isotopic analysis has been recently used to infer the diet (Florencio et al., 2013) and the trophic relationships (De Visser et al., 2008) of termitaria cohabitants. Such inferences are possible because, while δ¹³C changes little as carbon moves through the food web, δ¹⁵N enriched by 3–4‰ each trophic step (Post, 2002). Hence, two organisms positioned at two adjacent trophic steps (*e.g.*, a consumer and its prey or its donor of exudates) will show roughly no change in their δ¹³C body content but will differ by 3‰ in δ¹⁵N. Such differences in δ¹⁵N accumulate across trophic levels (Tayassu et al., 1997). We have, hence, used δ¹³C and δ¹⁵N ratios to infer whether diets of *C. melantho* beetles could be based on termite-derived materials.

Samples for stable isotope analysis were prepared according to Florencio et al. (2011) (field procedure) and Florencio et al. (2013) (lab procedure). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ concentrations were measured using an Isotope Ratio Mass Spectrometer (IRMS, ANCA-GLS 20-20, SerCon, UK) from the Stable Isotopes Laboratory at Federal University of Viçosa (UFV). The instrument's analytical precision was estimated to be $\pm 0.1\text{‰}$ for carbon and $\pm 0.2\text{‰}$ for nitrogen. The natural abundance of the heavy $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ relative to the light ^{12}C and ^{14}N , typically corresponding to the rare and abundant isotopes, is expressed as per thousand (‰) deviation from an international standard (belemnite of the Pee Dee Formation in South Carolina, USA (PDB) for carbon and atmospheric nitrogen (air) for nitrogen).

Statistical analyses

All statistical analyses were conducted in the R statistical computing environment (R Development Core Team, 2015).

Bray-Curtis dissimilarities in CHCs profiles between staphylinids and their termite host were compared using multivariate analyses with the “*vegan*” package (Oksanen et al., 2015). Dissimilarities were calculated for quantitative (proportion of the compound relative to the total amount of compounds in a run) and qualitative (presence/absence of the compound) data. For quantitative data, squared-root transformation and standardization was applied before calculating Bray-Curtis dissimilarities. Compounds whose peaks contributed to less than 1% of the total area of peaks in a run were not used in the quantitative nor in the qualitative analysis. Non-metric multidimensional scaling (NMDS) was used to plot dissimilarities among samples. Permutational multivariate analysis of variance (PERMANOVA), performed

via “*adonis*” routine of the “*vegan*” package based on the Bray-Curtis dissimilarity and 999 permutations, was conducted to test the existence of significant variations in the CHCs profiles between species and among nests, similarly to what was done by Proffit et al. (2011) for CHC profiles of moths ovipositing in tomato leaves.

Isotopic diet limits were statistically defined as Bayesian standard ellipses plotted around pairs of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ points representative of termites' or beetles' diet space, such ellipses being to bivariate data as standard deviation is to univariate data. Because these ellipses define the statistical limits for the dimensions of each diet, non-overlapping ellipses indicate statistically distinct diet spaces. Ellipses and associated metrics were calculated using the “*siber*” routines Jackson et al. (2011) from the “*sir*” Parnell et al. (2010) package.

Results

A total of 199 *C. melantho* beetles (Fig 1) were collected from the *C. cyphergaster* nests. The host colonies contained between three to 36 of these beetles, with an average of 19.9 ± 1.4 (mean \pm SE). Besides *C. melantho*, three other Staphylinidae species were also found living in some but not all of these nests: *Termitocola silvestrii* (Wasmann 1902) (Aleocharinae: Termitonannini), *Spirachtha eurymedusa* Schiødte, 1853 (Aleocharinae: Corotocini) and *Termitoiceus* sp. (Aleocharinae: Corotocini), all of which occurring in lower abundance than *C. melantho*.

CHC profiles. In total, we identified 67 GC peaks in the beetles and their termite host species (Supplementary Material - Table SM01), corresponding to 14 to 20 compounds in *C. melantho* beetles and 15 to 23 compounds

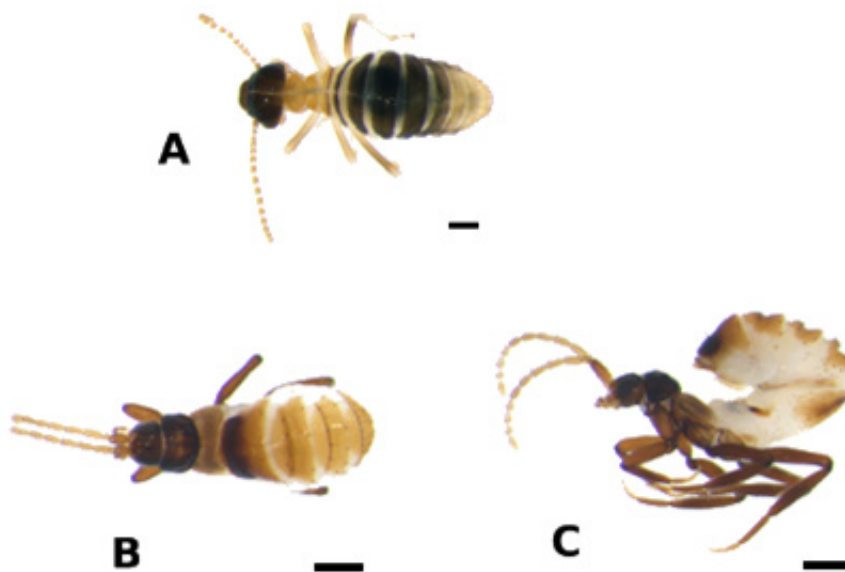


Fig 1. Morphological congruence between *Corotoca melantho* beetles and their termite host, *Constrictotermes cyphergaster*. (A) Dorsal view of termite worker, (B) dorsal view and (C) lateral view of termitophile beetle. Scale bar = 0.5 mm.

in their termite hosts. These profiles were rather congruent between guests and hosts (Fig 2), such that NMDS ordination did not reveal any clear distinction between them, in both quantitative (Fig 3A) and qualitative analysis (Fig 3B).

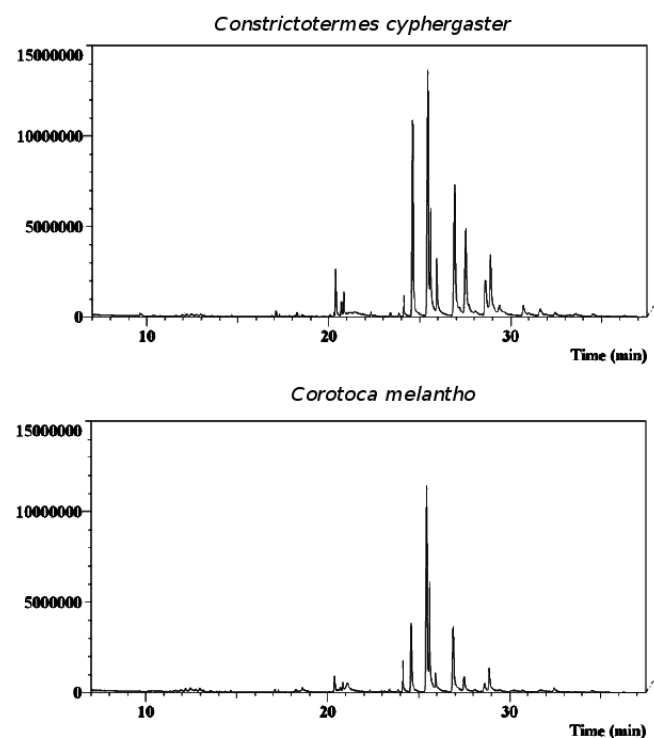


Fig 2. Typical cuticular hydrocarbon profiles detected for *Constrictotermes cyphergaster* (Blattodea: Isoptera: Termitidae) and their termitophile *Corotoca melantho* (Coleoptera: Staphylinidae) inhabiting a single nest (n14) in a Cerrado environment at the municipality of Sete Lagoas, MG, South-eastern Brazil.

That is, CHC profiles of beetles and their termite host species are similar chemical composition (the presence of compounds) and the relative abundance of compounds.

Beetles presented CHC more similar to the CHC of their cohabiting termites than to (i) their conspecifics from another nest and (ii) termites from another nest. This pattern was consistent for both quantitative and qualitative CHC analyses Table 1.

Permutational analysis of variance confirmed the above patterns (Table 2): differences in CHC profiles between termitophiles and termites could not be attributed to their specific identity ($p=0.124$ for compound relative abundance and $p=0.141$ for compounds presence). Rather, these differences were explained only by their nest of origin ($p=0.001$ for compound relative abundance and $p=0.007$ for compound presence), with termitophiles differing less from their heterospecific cohabitants than from their con-specifics found in distinct nests.

Table 1. Bray-Curtis dissimilarities (mean \pm se) between cuticular hydrocarbon (CHC) profiles of (i) termitophile staphylinids *Corotoca melantho* and their cohabiting host termites *Constrictotermes cyphergaster*, (ii) these same beetle and termite species inhabiting distinct termitaria, and (iii) con-specific staphylinids inhabiting distinct termitaria. Dissimilarities were calculated based on the presence/absence (“Qualitative”) or the relative abundance (“Quantitative”) of compounds in the CHC profiles.

Comparison	Qualitative	Quantitative
<i>C. melantho</i> \times <i>C. cyphergaster</i> same nest	0.40 \pm 0.08	0.54 \pm 0.08
<i>C. melantho</i> \times <i>C. cyphergaster</i> different nest	0.55 \pm 0.02	0.72 \pm 0.02
<i>C. melantho</i> \times <i>C. different nest</i>	0.53 \pm 0.02	0.70 \pm 0.03

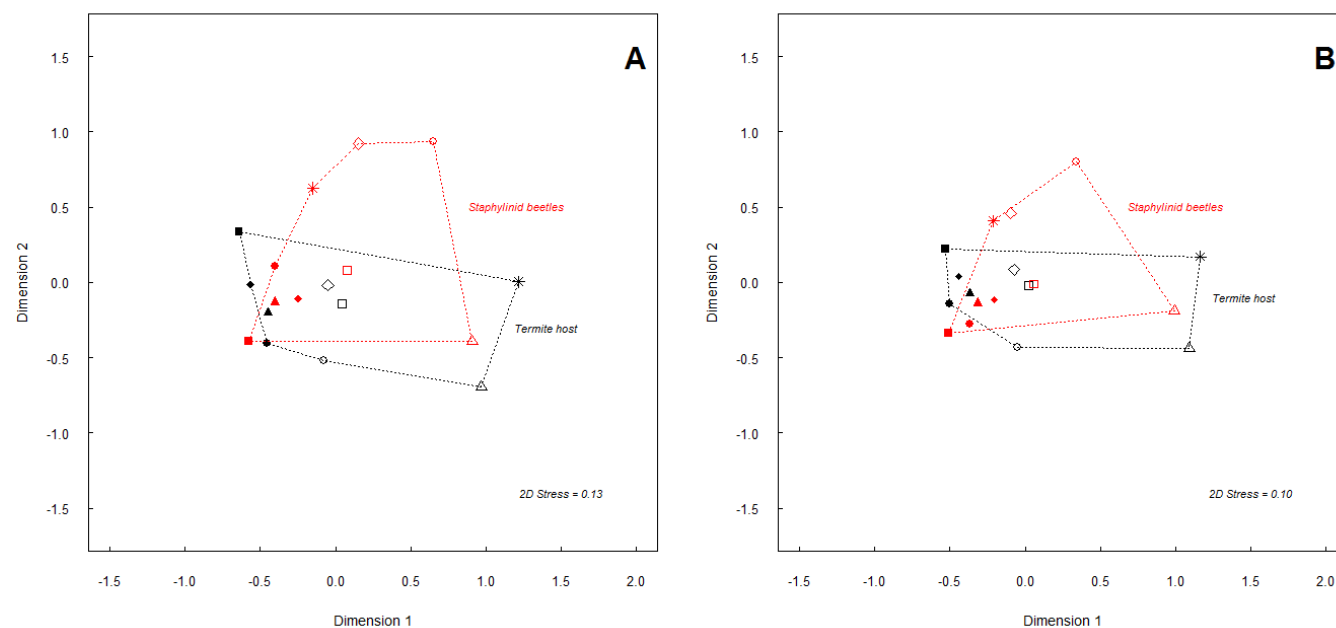


Fig 3. The chemical congruence between cuticular hydrocarbon profiles from staphylinid beetles, *Corotoca melantho*, and their termite host, *Constrictotermes cyphergaster*, as revealed by non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities. (A) Dissimilarities estimated using the relative abundance of compounds in the CHC profiles. (B) Dissimilarities estimated using presence/absence of compounds in the CHC profiles. Red symbols: staphylinid beetles; Black symbols: termite host. Similar symbols indicate beetles and termite sampled in the same nest.

Isotopic signatures placed cohabitant termitophiles and termite workers apart in diet spaces (Fig 4). $\delta^{13}\text{C}$ ratios indicated that termitophiles and termite workers did not feed nor did they assimilate from the same carbon source. Accordingly, $\delta^{15}\text{N}$ ratios indicated that staphylinids fed either

on highly decomposed materials or preyed upon a wide variety of prey, as suggested for termitophile and myrmecophile staphylinids by Costa-Lima (1952, p. 315). Either of these diets, however, cannot be said to derive from termites, as $\delta^{13}\text{C}$ ratios are not coincidental among guests and hosts.

Table 2. Permutational Multivariate Analysis of Variance using Bray-Curtis distance matrices under “*vegan*” package. Bray-Curtis dissimilarities were calculated based on the presence/absence (“qualitative”) or the relative abundance (“quantitative”) of compounds in the CHC profiles.

	Df	Sums of Sqs	Mean Sqs	<i>Pseudo-F</i>	R	Pr(>F)	
Presence of compounds							
nest	8	1.75898	0.21987	2.3490	0.65566	0.001	***
species	1	0.17495	0.17495	1.8691	0.06521	0.141	
Residuals	8	0.74883	0.09360		0.27913		
Total	17	2.68277			1.00000		
Relative abundance of compounds							
nest	8	3.0172	0.37716	2.3243	0.65947	0.001	***
species	1	0.2599	0.25988	1.6015	0.05680	0.124	
Residuals	8	1.2982	0.16227		0.28373		
Total	17	4.5753			1.00000		

Discussion

Adaptations to integrate into colonies of ants and termites abound in staphylinid beetles (Kistner, 1982). Among these, *C. melantho* would stand as archetypical termitophiles (Seevers, 1957) on top of their striking physogastry (see Fig 1 and section “Introduction”), these beetles are obligatory cohabitants of *C. cyphergaster* termites, reproduce by ovoviviparity within these hosts’ termitaria, and present wings reduced to membranous pads hence limiting mobility and favouring a confined lifestyle. Our findings extend to chemical disguise the list of *C. melantho* adaptations to termitophily. Previous work has been showed that *C. melantho* also have morphological disguise (Cunha et al, 2015).

Chemical disguise provided by CHC profiles built on similar host-specific compounds have been reported as the primary mechanism of invertebrate integration into nests of ants (Akino et al., 1999; Elgar & Allan, 2006; Elmes et al., 1999; Guillem et al., 2014; Schönrogge et al., 2008), bees (Kather et al., 2015), and wasps (Van Oystaeyen et al., 2015). As for termites, extreme congruence at the species level in guest-host CHC profiles has been already detected for limuloid Staphylinidae (Howard et al., 1980). Here, we found CHC profiles of *C. melantho* to bear close resemblance to their hosts’ profiles (Figs 2 and 3), but this similarity, rather than broadly defined at the species level, was established at the level of the colony (Tables 1 and 2). Such a result supports the notion that these termitophiles would acquire CHC compounds after invade their host nests (‘*acquired chemical mimicry*’ *sensu* Von-Beeren et al. (2012)). In the ‘*acquired chemical mimicry*’, invaders can (i) biosynthesize some specific recognition cues of their host and, after invasion, acquired other recognition

cues after getting in touch with termites or their nests or (ii) acquire disguising recognition cues only after getting in touch with termites or their nest. This consists in a major distinction between limuloid and physogastric termitophilic rove beetles as, in the former, mimetic CHC can only be biosynthesized (Howard et al., 1980) (*i.e.*, ‘innate chemical mimicry’). Such a scenario is entirely in accordance with previous records on the chemical invisibility of obligate myrmecophiles (Lenoir et al., 2001) and termitophile Aleocharinae beetles (Sands & Lamb, 1975) from the same Corotocini tribe as *C. melantho* here reported. These are known to profit from being “chemically insignificant” (odourless) to enter the host colony, subsequently acquiring the *gestalt* colony odour by physical contact. The likelihood of such distinct CHC origins in limuloid *versus* physogastric termitophiles is reinforced by their opposing rank at the continuum of degrees of interactions between guests and hosts (Lenoir et al., 2001): while limuloids avoid contacts, physogastrics engage constant interactions with their hosts (Kistner, 1982).

It is plausible to suspect a major role of physogastry in facilitating such acquisition by promoting intimate (tactile, feeding) interactions between guests and hosts, such that chemical and physical traits act synergistically to allow termitophily, as posited by Parker (2016). After all, CHC compounds can be transferred interspecifically by interindividual physical and feeding interactions (Elgar & Allan, 2004; Liang & Silverman, 2000). Results from our isotopic signatures analysis (Fig 4) revealed that these staphylinids and termites were positioned apart, mainly, in $\delta^{15}\text{N}$ axes. In six out of seven studied nests, staphylinids were located from two to seven trophic steps above their hosts, indicating highly variable and in many cases highly decomposed diets.

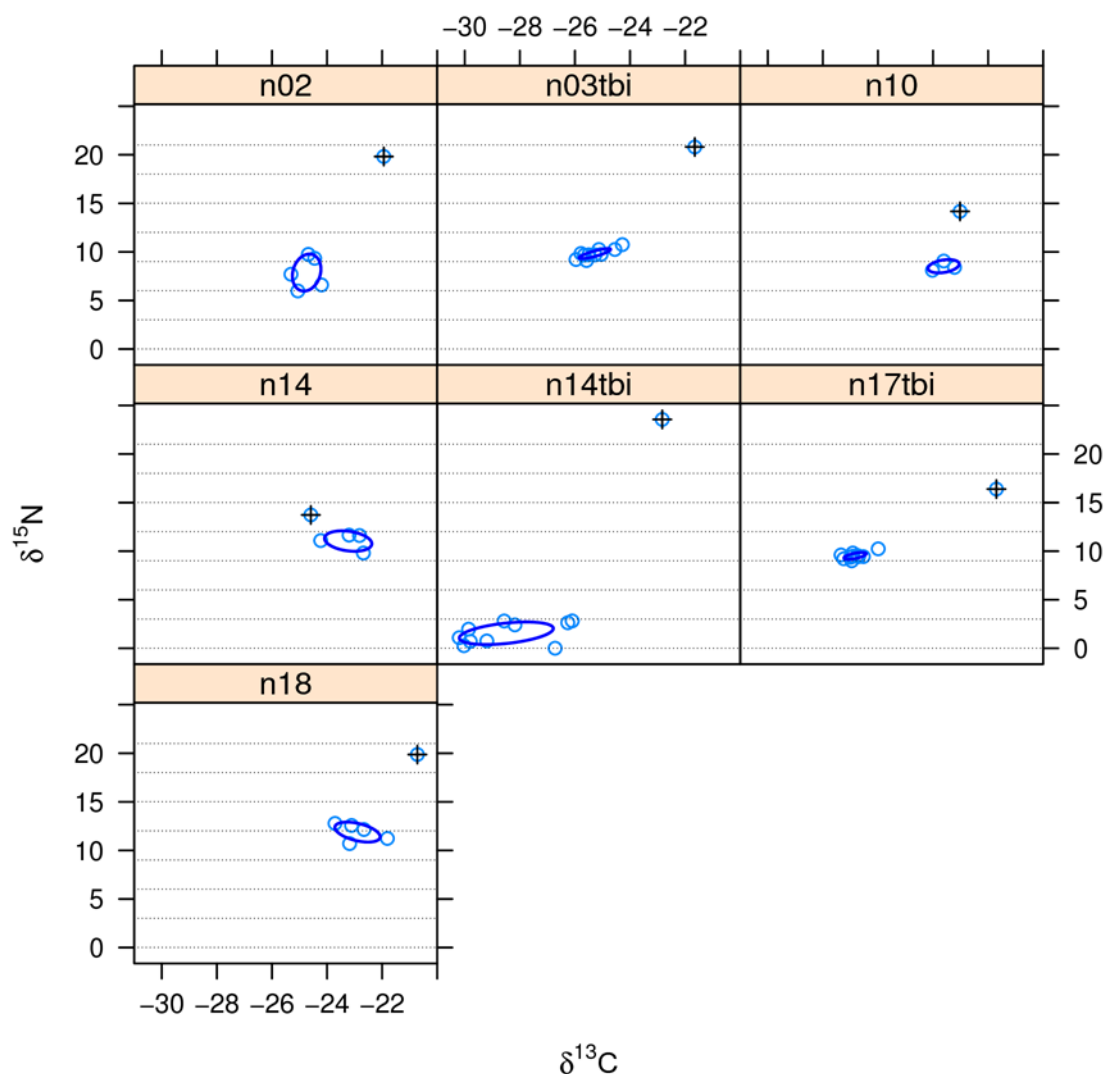


Fig 4. Isotopic signatures of termites and staphylinid beetles coexisting in termitaria, delimited by Bayesian standard ellipses. Each panel represents a single termitarium. Axes represent the ratio of stable carbon and nitrogen isotopes in termite bodies. Each dot refers to a sample weighing ≥ 1.5 mg from either a group of termite workers (hollowed dots) or staphylinids (cross-marked dots). Termites are *Constrictotermes cyphergaster* and beetles are *Corotoca melantho*. Dotted lines parallel to x-axis define 3 intervals thought to correspond to trophically distinct positions.

In a single case guests were only one trophic step above hosts (Fig 4, nest “n14”), indicating direct ingestion of termite via predation. The high variation in $\delta^{15}\text{N}$ axis between beetles and host is somehow stranger. However, such high variations may have occurred due to the methodological procedures in the isotopic analyses (*i.e.* analysis with gut contents, which can have plant or fungi material) or by predation of life stages not measure by us (*i.e.*, larve and/or eggs).

Two other non-dietary and non-exclusive routes seem more likely: (i) tactile interactions (*e.g.* allogrooming) between guests and hosts or (ii) direct rubbing by the guests on internal nest lining. These routes remain hypothetical, as the evaluation of their relative importance to the acquisition of hosts’ CHC by guests is outside the scope of this paper. In the event that interindividual tactile interactions are proven relevant for mimetic CHC transfer in this system, then Parker’s hypothesis (Parker, 2016) on the synergistic action between physical and

chemical termitophilic traits gains additional support. Future studies must investigate trophallaxis behavior between guest and host, in order to clarify this unrevealed association.

In summary, evidence indicates that chemical (present work) and morphological (Cunha et al., 2015) disguises in termitophiles may function as a strategy for social integration in *C. cyphergaster* colonies.

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Supplementary Material

<http://periodicos.uefs.br/index.php/sociobiology/rt/suppFiles/1942/0>
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