

**THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION**

**Section 1**

**Biology**

**Diversity of hindgut symbiotic flagellate protist communities of the European subterranean termite in Portugal**

Sónia Duarte, Tânia Nobre<sup>3</sup>, Marta Duarte<sup>1</sup>, Paulo A.V. Borges<sup>2</sup>, Lina Nunes<sup>1,2</sup>

<sup>1</sup> LNEC, National Laboratory for Civil Engineering, Structures Dep., Av. do Brasil, 101, 1700-066, Lisbon, Portugal

<sup>2</sup> cE3c, Centre for Ecology, Evolution and Environmental Changes/Azorean Biodiversity Group and University of the Azores, Department of Agricultural Sciences, 9700-042 Angra do Heroísmo, Terceira Island, Azores, Portugal

<sup>3</sup> Molecular Biology Laboratory, ICAAM – Institute of Mediterranean Agricultural and Environmental Sciences, University of Évora, Núcleo da Mitra, Évora, Portugal

Paper prepared for the 47<sup>th</sup> IRG Annual Meeting  
Lisbon, Portugal  
15-19 May 2016

**Disclaimer**

The opinions expressed in this document are those of the author(s) and are not necessarily the opinions or policy of the IRG Organization.

**IRG SECRETARIAT**  
**Box 5609**  
**SE-114 86 Stockholm**  
**Sweden**  
**[www.irg-wp.com](http://www.irg-wp.com)**

# Diversity of hindgut symbiotic flagellate protist communities of the European subterranean termite in Portugal

Sónia Duarte<sup>1,2</sup>, Tânia Nobre<sup>3</sup>, Marta Duarte<sup>1</sup>, Paulo A.V. Borges<sup>2</sup>, Lina Nunes<sup>1,2</sup>

<sup>1</sup> LNEC, National Laboratory for Civil Engineering, Structures Dep., Av. do Brasil, 101, 1700-066, Lisbon, Portugal

<sup>2</sup> cE3c, Centre for Ecology, Evolution and Environmental Changes/Azorean Biodiversity Group and University of the Azores, Department of Agricultural Sciences, 9700-042 Angra do Heroísmo, Terceira Island, Azores, Portugal

<sup>3</sup> Molecular Biology Laboratory, ICAAM – Institute of Mediterranean Agricultural and Environmental Sciences, University of Évora, Núcleo da Mitra, Évora, Portugal

## ABSTRACT

The flagellate protist communities are an important part of the termite, as they lead the lignocellulose digestion. Termites (*Reticulitermes grassei*) were sampled from forest and urban environments in mainland Portugal where they are native and in Faial Island, Azores (invasive populations). Termites' gut contents was analysed morphologically and the diversity of the flagellate protist community evaluated based on morphotypes. From the two Azorean invasive populations we were able to identify 12 different morphotypes whereas some of the populations in the mainland had as few as 6. Indeed, on the fourteen native populations the number of flagellate protists morphotypes ranged between 6 and 12. Shannon Wiener diversity index was used to calculate a variation partitioning between geographical and local variables.

Our results suggest the existence of a core group of flagellate protists, probably performing key steps in the lignocellulose digestion. However, these communities may be more diversified and factors linked with the geographic location are likely a key influence of the flagellate protist communities analysed. In the invasive urban termite populations the high flagellate protist communities' diversity, as well as the similarity between the two populations captured, may indicate a switch of *R. grassei* foraging and social habits in the invasive termite populations. Local conditions also influenced the flagellate protist communities, although not so markedly as geographic location. In this study, native termite colonies from urban environments showed the less diverse flagellate protist communities.

**Keywords:** subterranean termite, flagellate protist communities, hindgut symbiont

## 1. INTRODUCTION

Lower termites account for 80% of the economically important species (Nobre and Nunes 2007, Rust and Su 2012). The number of invasive termite species has increased above 50% since 1969 (Evans *et al.* 2013), a fact which may be related to the globalisation of trade, among other factors, such as climate change - which will probably contribute to the increase of termite species range of distribution to places where their presence was previously limited by climatic factors (Su and Scheffrahn 2000, Lee and Chon 2011, Evans *et al.* 2013, Guerreiro *et al.* 2014) - or the growth of human population. In 2010, the global economic impact of invasive termites was

estimated at 40 billion dollars, and subterranean termites accounted for 80% of this impact, i.e. approximately 32 billion dollars (Rust and Su 2012).

Subterranean termites established a tripartite symbiotic system, including termite, flagellate protists and prokaryotes, which work together to achieve efficient lignocellulose degradation. Flagellate protists have an important role in that mechanism, as they ferment cellulose to acetate, the main energy source of the termite host (Yamin 1980; Yoshimura *et al.* 1996; Hongoh 2011). The objective of this work was to evaluate the possible changes in symbiotic flagellate protist communities living inside the subterranean termite *Reticulitermes grassei* Clément in relation to different geographical locations (including termites' native or invasive character) and local conditions (urban or natural forest environments).

## **2. EXPERIMENTAL METHODS**

### **2.1 Termites**

Subterranean termites belonging to the species *R. grassei* were captured in sixteen different locations of Portugal mainland. Seven districts from North to South were sampled and from each district one colony from an urban environment and one colony from a natural forest environment were captured. In the Faial Island of the Azores Archipelago, two urban invasive populations were captured (Fig. 1, Table 1).

### **2.2 Flagellate protists**

From each location, twenty worker termites in the 4<sup>th</sup> instar were evaluated in terms of protists diversity and abundance by direct observation under a microscope, and for flagellate protists quantification a haemocytometer was used as described previously (Duarte *et al.* 2016). Flagellate protists were identified according with species, or other major taxa, descriptions (Leidy 1877; Brugerolle and Lee 2000; Brugerolle 2006; Brugerolle and Bordereau 2006; Lewis and Forschler 2006).

### **2.3 Geographical and local variables**

An analysis of variation partitioning was done using R, for investigating the contribution of geographical and local conditions in explaining the differences on the flagellate protist communities observed among the termite colonies observed. The variables were divided into: 1) geographical location, consisting in a dataset constructed from the square order combinations of the xy coordinates of each location, centred on their means and divided by their standard deviation (Borcard *et al.* 1992, Grand and Cushman 2004); and 2) local conditions, consisting in the characterization of each local conditions when termites were captured, as the type of environment – urban or natural; the wood substrate – grabbed in houses, backyards, public gardens inside cities, or forests; and the type of wood substrate – fallen branches, pallet or timber – timber beam or door frame). The Shannon Wiener biodiversity index, as it is considered to be a measure of community complexity (Magurran 2003), was calculated in order to perform the variation partitioning analysis. Redundancy analysis (RDA) and partial RDA were used to perform the variation partitioning.

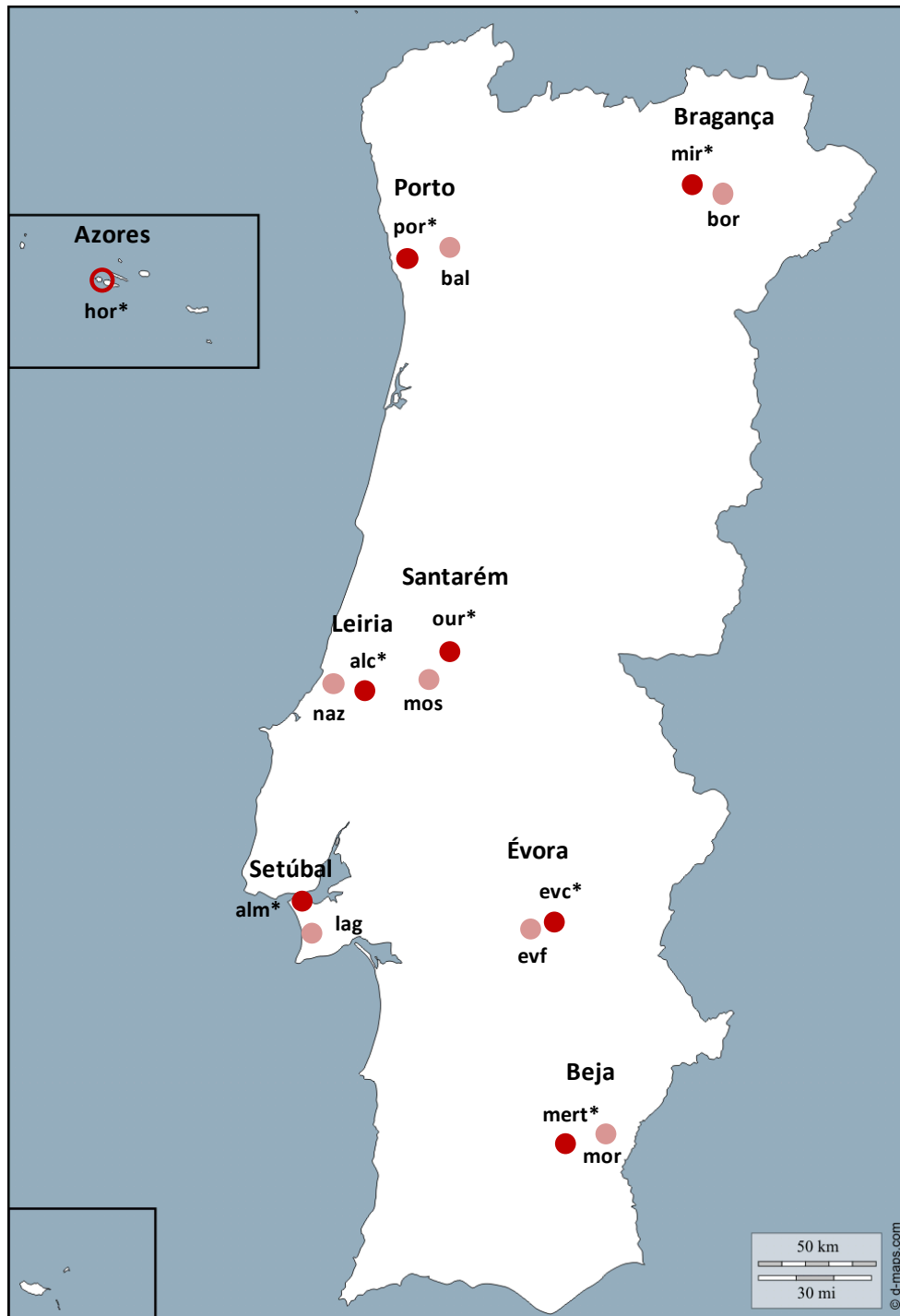


Figure 1: Map with location of the termite colonies captured for this study. The two invasive colonies from the Azorean islands, and the colonies from seven districts of Portugal mainland, for each district a colony from urban environment (\*) and one colony from a natural environment were captured. alc: Alcobaça, alm: Almada, bal: Baltar, bor: Bornes, evc: Évora city; evf: Évora forest; hor: Horta, lag: Lagoa de Albufeira (Sesimbra), mert: Mértola, mir: Mirandela, mor: Moreanes, naz: Nazaré, our: Ourém, mos: Porto de Mós, por: Porto.

Table 1: Characterization of the location and termite substrates where the colonies of *R. grassei* were sampled.

District	Location		Environment	Termite substrate	
	City	Code		Type	Substrate
Azores	Horta	hor1*	Urban	backyard	pallet board
	Horta	hor2*	Urban	house	timber beam
Bragança	Mirandela	mir*	Urban	public garden	fallen branch
	Bornes	bor	Forest	trees	fallen branch
Porto	Porto	por*	Urban	public garden	fallen branch
	Baltar	bal	Forest	trees	fallen branch
Santarém	Ourém	our*	Urban	public garden	fallen branch
	Porto de Mós	mos	Forest	trees	fallen branch
Leiria	Alcobaça	alc*	Urban	trees	fallen branch
	Nazaré	naz	Forest	trees	fallen branch
Setúbal	Almada	alm*	Urban	public garden	fallen branch
	Sesimbra	ses	Forest	trees	fallen branch
Évora	Évora (city)	evc*	Urban	backyard	pallet board
	Évora (forest)	evf	Forest	trees	fallen branch
Beja	Mértola	mert*	Urban	house	door frame
	Moreanes	mor	Forest	trees	fallen branch

## 2.4 Urban vs. natural environment

For comparing termite colonies' flagellate protist communities from urban and natural environments in each district of Portugal mainland, a Wilcoxon signed rank test was performed in R (2013), assuming that the null hypothesis would be the flagellate protist communities from termite colonies captured in urban and natural environment are equal.

## 3. RESULTS AND DISCUSSION

### 3.1 Flagellate protist communities

The flagellate protists were preliminarily identified based on morphological characters and separated into 12 morphotypes (Table 2). The flagellate protist communities' relative abundances showed differences among all termite colonies sampled (Fig. 2).

Table 2: Flagellate protists identification to morphotypes based on morphological characters.

Phylum	Class	Order	Family	Genus	Species
n18	Parabasalia				
n1	Trypanosomatida	Trichonymphida	Trichonymphidae	<i>Trichonympha</i>	<i>T. agilis</i>
n17			Trichonymphidae		
n9	Spirotrichonympha	Spirotrichonymphida	Holomastigotoididae	<i>Spirotrichonympha</i>	<i>S. flagellata</i>
n4				<i>Holomastigotes</i>	<i>H. elongatum</i>
n8				<i>Microjoenia</i>	<i>M. hexamitoides</i>
n13	Trichomonadea	Honigbergiellida	Tricercomitidae	<i>Hexamastix</i>	
n6	Hypotrichomonadea	Hypotrichomonadida	Hypotrichomonadidae		
n2	Preaxostyla		Oxymonadida	<i>Pyrrsonympha</i> sp.1	
n7				<i>Pyrrsonympha</i> sp.2	
n3				<i>Dinenympha</i>	<i>D. gracilis</i>
n5					<i>D. fimbriata</i>

### 3.2 Geographical and local variables

The model including all explanatory variables significantly explained 87.9% of the variation observed within the flagellate protist communities ( $p=0.016$ ); local conditions explained 27.3% and 10.5% after partialling out the geographic coordinates effect, while the geographical coordinates explained 77.4% and 60.6% after partialling out the local conditions effects. The interaction between these two groups of variables explained 16.8% of the variation observed.

These results show a strong effect of the geographical coordinates on the flagellate protist communities for the termite colonies observed within this study, although local conditions also play an important role.

*Reticulitermes grassei* has a marked geographical pattern regarding its colony structure along its range of distribution (Kutnik *et al.* 2004, Nobre *et al.* 2008, Vargo *et al.* 2013, Bankhead-Dronnet *et al.* 2015). A change in the colony structure may alter the termite foraging and social habits, which might influence directly the flagellate protist communities. *R. grassei* colonies tend to be mainly closed and mutually aggressive in its southern range of distribution (south of Iberian Peninsula), while in the northern range (north of Iberian Peninsula and southwestern coast of France) the colonies tend to be mainly extended, and less aggressive (Vargo *et al.* 2013, Bankhead-Dronnet *et al.* 2015). The model of extended, less aggressive colonies encourages the individuals to contact with each other, and horizontal transfer of gut fluids promotes the exchange of hindgut symbionts among a wider population of termites.

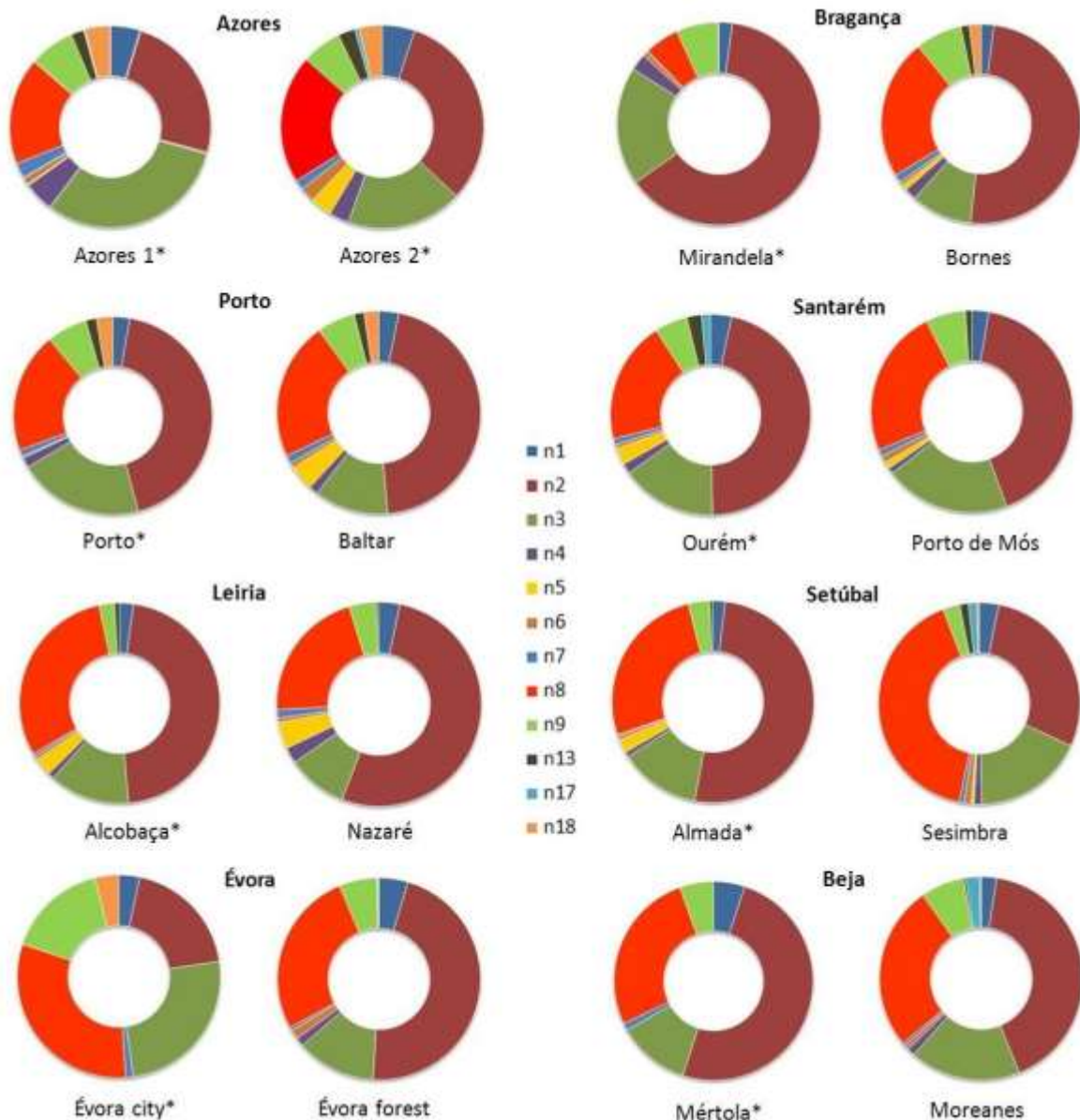


Figure 2: Flagellate protist communities' relative abundance for termites captured in urban (\*) and natural environment within each district of Portugal mainland, and also in the Azorean urban invasive populations.

The strong geographical effect observed is certainly linked to the two urban invasive populations of *R. grassei* observed in Horta, Faial Island, which were markedly different from the mainland populations by its diversity, despite being urban colonies. Therefore we hypothesize that in Horta *R. grassei* should be organized into a uniclonal system, or as an extended family with multiple reproductives. This adaptive behaviour or tendency was already stated for other invasive termites, for example, *Reticulitermes flavipes* (Kollar) (Dronnet *et al.* 2005) and *Reticulitermes urbis* Bagnères, Uva et Clément (Leniaud *et al.* 2010). This hypothesis would explain the higher diversity and the strong similarity of both populations observed in Faial Island. In this study *Dinenympha fimbriata* Kirby (n5) was absent in the termite colonies from the southern Portugal

area, and this may be related with factors as, for example, the specific environmental conditions that limit the distribution of this species.

### 3.3 Urban and natural environment

The three least diverse communities belong to termites captured inside cities: the first was captured on carpentry timber (Mértola city); the second was captured in a pallet board in a backyard (Évora city); and the third was captured in a public garden (Mirandela city). All the districts, except for Leiria ( $p=0.098$ ) and Setúbal ( $p=0.120$ ), showed significant differences among the flagellate protist communities of urban and natural environments captured termites ( $p<0.05$ ; Bragança:  $p=0.002$ ; Porto:  $p<0.001$ ; Santarém:  $p=0.003$ ; Évora:  $p<0.001$ ; Beja:  $p<0.001$ ). Generally, the urban termite populations seem to have a less complex community of flagellate protists represented by less species. The different organization of the colonies observed and the possible different strategies of foraging on termites living in urban and natural environments, are both dependent on environmental conditions, such as temperature, soil moisture or food availability (Vargo *et al.* 2013).

## 4. CONCLUSIONS

The flagellate protist communities are an integrant part of the termite, as they are crucial to lignocellulose digestion and are able to adapt to the different conditions and needs of their hosts. Our results indicate the existence of a core group of flagellate protists, probably performing key steps in the lignocellulose digestion. However, these communities may be more diversified, according with factors as geographic location - the example of distribution of *D. fimbriata* or the putative exchange of foraging and social habits in invasive termite populations - and local conditions. In this study, termite colonies from urban environments showed less diverse flagellate protist communities.

## 5. REFERENCES

- Bankhead-Dronnet, S, Perdereau, E, Kutnik, M, Dupont, S, Bagnères, A G (2015) Spatial structuring of the population genetics of a European subterranean termite species. *Ecology and Evolution*, **5**(15), 3090-3102.
- Borcard, D, Legendre, P, Drapeau, P (1992): Partialling out the spatial component of ecological variation. *Ecology*, **73**(3), 1045-1055.
- Brugerolle, G (2006): Comparative cytological study of four species in the genera *Holomastigotes* and *Uteronympha* n. comb. (Holomastigotidae, Parabasalia), symbiotic flagellates of termites. *Journal of Eukaryotic Microbiology*, **53**(4), 246-259.
- Brugerolle, G, Lee, J J (2000): Order Oxymonadida. Phylum Parabasalia. In: *The illustrated guide to the Protozoa*, volume II, ed. Lee, J J, Leedale, G F, Bradbury, P. Society of Protozoologists Kansas, USA. pp 1186-1250.
- Brugerolle, G, Bordereau, C (2006): Immunological and ultrastructural characterization of spirotrichonymphid flagellates from *Reticulitermes grassei* and *R. flavipes* (syn. *R. santonensis*),



with special reference to *Spirotrichonympha*, *Spirotrichonympha* and *Microjoenia*. *Organisms Diversity & Evolution*, **6**, 109-123.

Dronnet, S, Chapuisat, M, Vargo, E L, Lohou, C, Bagnères, A G (2005): Genetic analysis of the breeding system of an invasive subterranean termite, *Reticulitermes santonensis*, in urban and natural habitats. *Molecular Ecology*, **14**(5), 1311-1320.

Duarte, S, Duarte, M, Borges, P A V, Nunes, L (2016): Dietary-driven variation effects on the symbiotic flagellate protist communities of the subterranean termite *Reticulitermes grassei* Clément. *Journal of Applied Entomology* (Accepted).

Evans, T A, Forschler, B T, Grace, J K (2013): Biology of invasive termites: a worldwide review. *Annual Review of Entomology*, **58**, 455-474.

Grand, J, Cushman, S A (2004): A multi-scale analysis of species-environment relationships: Breeding birds in a pitch pine-scrub oak (*Pinus rigida-Quercus ilicifolia*) community. *Biological Conservation*, **112**(3), 307-317.

Guerreiro, O, Cardoso, P, Ferreira, J M, Ferreira, M T, Borges, P A V (2014): Potential distribution and cost estimation of the damage caused by *Cryptotermes brevis* (Isoptera: Kalotermitidae) in the Azores. *Journal of Economic Entomology*, **107**(4), 1554-1562.

Hongoh, Y (2011): Toward the functional analysis of uncultivable, symbiotic microorganisms in the termite gut. *Cellular and Molecular Life Sciences*, **68**, 1311-1325.

Kutnik, M, Uva, P, Brinkworth, L, Bagnères, A G (2004): Phylogeography of two European *Reticulitermes* (Isoptera) species: the Iberian refugium. *Molecular Ecology*, **13**(10): 3099-3113.

Lee, S H, Chon, T S (2011): Effects of climate change on subterranean termite territory size: a simulation study. *Journal of Insect Science*, **11**, 80. Available from: <http://jinsectscience.oxfordjournals.org/content/11/1/80>

Leidy, J (1877): On intestinal parasites of *Termes flavipes*. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **29**, 146-149.

Leniaud, L, Dedeine, F, Pichon, A, Dupont, S, Bagnères, A G (2010): Geographical distribution, genetic diversity and social organization of a new European termite, *Reticulitermes urbis* (Isoptera: Rhinotermitidae). *Biological Invasions*, **12**, 1389-1402.

Lewis, J L, Forschler, B T (2006): A nondichotomous key to protist species identification of *Reticulitermes* (Isoptera: Rhinotermitidae). *Annals of the Entomological Society of America*, **99**(6), 1028-1033.

Magurran, A E (2003): Measuring biological diversity. Blackwell Publishing, London.

- Nobre, T, Nunes, L (2007): Non-traditional approaches to subterranean termite control in buildings. *Wood Material Science & Engineering*, **3-4**, 147-156.
- Nobre, T, Nunes, L, Bignell, D E (2008): Colony interactions in *Reticulitermes grassei* population assessed by molecular genetic methods. *Insectes Sociaux*, **55**, 66-73.
- R Core Team (2014): R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rust, M, Su, N-Y (2012): Managing social insects of urban importance. *Annual Review of Entomology*, **57**, 355-375.
- Su, N-Y, Scheffrahn, R H (2000): Termites as pests of buildings. In: *Termites: Evolution, Sociality, Symbioses, Ecology*, ed. Abe, T, Bignell, D E, Higashi, M. The Netherlands, Kluwer Academic Publishers, Dordrecht. pp 437-453.
- Vargo, E L, Leniaud, L, Swoboda, L E, Diamond, S E, Weiser, M D, Miller, D M, Bagnères, A G (2013): Clinal variation in colony breeding structure and level of inbreeding in *Reticulitermes grassei* and *R. flavipes*. *Molecular Ecology*, **22**(5), 1447-1462.
- Yamin, M A (1980): Cellulose metabolism by the termite flagellate *Trichomitopsis termopsidis*. *Applied and Environmental Microbiology*, **39**(4), 859-863.
- Yoshimura, T, Fujino, T, Tsunoda K, Takahashi, M (1996): Ingestion and decomposition of wood and cellulose by the protozoa in the hindgut of *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) as evidenced by polarizing and transmission electron microscopy. *Holzforschung*, **50**, 99-104.