Symbiotic flagellate protists as a target for subterranean termite control strategies

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Doutora em Ciências Agrárias

"*Life did not take over the globe by combat,*

but by networking."

Lynn Margulis and Dorion Sagan (1986),

Microcosmos: Four Billion Years of

Evolution from Our Microbial Ancestors,

[pp. 27 of 1997 edition from University of California Press]

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Abstract

Reticulitermes grassei, is a native Iberian subterranean termite and significant economic pest of wood in service, widespread in the mainland of Portugal and invasive in Faial Island (Azores). The thesis reports research designed to: 1) optimise termite in-ground long-term monitoring stations; 2) characterise the symbiotic flagellate protist community; 3) determine whether the protist community shows geographical and situational variation (between rural and urban settings, and between indigenous and invasive populations); and 4) assess the response of the protist community to different diets and to candidate repellent or toxic supplements added to the diets.

1) Differing designs and configurations of in-ground monitoring stations and their cellulosic matrices were evaluated in terms of mass loss, moisture content variation and termite presence, both in Portugal and the USA. Spatial coordinates and monitoring station environmental variables were factored into the analyses. Optimization of termite in-ground monitoring stations was found to be most dependent on correct evaluation of termite feeding preferences, resistance to decay of the bait matrix and bait matrix design. These criteria may vary with geographical location and environmental constraints.

2) All the termites captured within mainland Portugal and Faial Island were confirmed by a genetic method to belong to the species *R. grassei*, with the origin of the latter probably being the Atlantic north coast of the Iberian Peninsula. Twelve morphotypes of flagellate protists were identified living inside this termite's hindgut, distinguished using different microscopical techniques. Of this group, six morphotypes were always present in all termite populations sampled across Portugal (mainland and island invasive): n1, n2, n3, n7, n8, n9; forming a core group, accompanied by other secondary facultative symbionts. In the core group, molecular methods still under development identified morphotype n1 as *Trichonympha* sp. and n8 as belonging to the Order Spirotrichonymphida (probably *Microjoenia hexamitoides*).

3) Termites from rural environments showed more diverse and abundant protists. The Azorean invasive termite colonies also had more diverse protist communities than originally predicted from theory, a result that points to the fact that these termites are probably organized into a single multiple nest-cooperating colony.

4) Flagellate protists showed a rapid response to dietary changes, with depletion of the community both for starving and cellulose-only feeding termites. Termites feeding on thermally treated wood also showed a significantly different flagellate protist community. Morphotypes n2 (*Pyrsonympha* sp.) and n8 (*Microjoenia hexamitoides*), the most abundant in the hindgut of *R. grassei*, seem to play a major and complementary role in the maintenance of hindgut protist equilibrium.

It is hypothesized that flexibile nutritional symbiosis equilibrium of flagellates assists defence of the termite holobiont to dietary changes and also to potentially harmful xenobiotic substances. The same flexibility may permit adaptation to external or internal conditions as might arise when new habitats are being colonized invasively.

This thesis sets baseline knowledge about flagellate protists in *R. grassei* which will inform future innovative termite control strategies. Biotechnological application of this knowledge is also in prospect.

Future research in this area should include more detailed analyses of protist community structure (and refinement of rapid identification techniques). The effects of biogeography, climate change and habitat disturbance on the evolution of and biotic interactions within the prokaryotic gut community should also be investigated.

Keywords: flagellate protists, hindgut symbiotic fauna, invasive species, lignocellulose digestion, *Reticulitermes grassei*, subterranean termite, termite monitoring stations.

Resumo

Reticulitermes grassei, é uma térmita subterrânea autóctone da Península Ibérica e uma praga importante da madeira em serviço, está distribuída por todo o território de Portugal continental e é invasora na ilha do Faial (Arquipélago dos Açores). A investigação patente nesta tese foi delineada para atingir os seguintes objetivos: 1) a necessidade de otimização de estações de monitorização de térmitas subterrâneas de forma a apoiar a realização de estudos a longo prazo; 2) a necessidade de responder ao desafio de Lineu e identificar e descrever a comunidade de protistas flagelados simbióticos associados à térmita *R. grassei*; 3) determinar se a comunidade de protistas flagelados apresenta variação geográfica e circunstancial (através da comparação de térmitas de ambientes rurais e urbanos, bem como térmitas autóctones e térmitas invasoras); 4) avaliar resposta da comunidade de protistas a diferentes dietas e substâncias potencialmente repelentes ou tóxicas adicionadas ao alimento das térmitas.

1) Diferentes configurações e designs de estações subterrâneas de monitorização da atividade de térmitas e de matrizes celulósicas foram avaliadas quanto à perda de massa, variação do teor de água e presença de térmitas, paralelamente em Portugal e nos EUA. As coordenadas geográficas e as variáveis ambientais foram tidas em conta na análise efetuada. A otimização das estações de monitorização de térmitas subterrâneas mostrou estar relacionada em maior grau com: a correta avaliação das preferências alimentares da espécie de térmita em questão, a capacidade de resistência à degradação da matriz celulósica utilizada, e o melhor design da estação de monitorização e respetiva matriz celulósica. Estes critérios de avaliação podem ser diferir de acordo com a localização geográfica e as condições ambientais.

2) Todas as térmitas capturadas em Portugal Continental e na ilha do Faial pertencem à espécie *R. grassei*, e a origem da população invasora terá provavelmente sido a zona norte da costa atlântica da Península Ibérica. O resultado mais saliente foi a identificação de doze morfotipos de protistas flagelados simbiontes no intestino da térmita, para tal usando-se diversas técnicas de microscopia. Deste grupo de protistas, seis morfotipos estavam presentes em todas as populações de térmitas observadas em

Portugal (no território continental e na ilha do Faial): n1, n2, n3, n7, n8 e n9; formando um grupo principal, acompanhado por outros morfotipos que foram considerados como simbiontes secundários facultativos. Deste grupo principal, foram identificados, através de métodos moleculares que estão a ser otimizados, o morfotipo n1 como *Trichonympha* sp. e o morfotipo n8 como pertencente à ordem Spirotrichonymphida (provavelmente a espécie *Microjoenia hexamitoides*).

3) Térmitas provenientes de ambientes rurais mostraram uma tendência para acomodarem uma comunidade de protistas mais diversa e abundante. No entanto, e contrariamente ao que seria de prever, a comunidade de protistas flagelados da população de térmitas invasoras nos Açores possui uma elevada diversidade. Este resultado de certo modo surpreendente aponta para a hipótese de estas térmitas estarem socialmente organizadas num sistema colaborativo de uma colónia única com múltiplos reprodutores.

4) Os protistas flagelados demonstraram uma rápida adaptação às alterações alimentares das térmitas, resultando por exemplo, numa comunidade de protistas menos variada e numerosa em térmitas em jejum e térmitas alimentadas apenas com celulose. Térmitas alimentadas com madeira termicamente modificada demonstraram uma comunidade de protistas significativamente diferente de térmitas alimentadas com a mesma espécie de madeira sem tratamento térmico. Os morfotipos n2 (*Pyrsonympha* sp.) e n8 (*Microjoenia hexamitoides*), os mais abundantes no intestino de *R. grassei*, e parecem desempenhar um papel importante e complementar na manutenção do funcionamento e equilíbrio da zona posterior do intestino da térmita.

Conclui-se que, possivelmente, o equilíbrio dinâmico desta simbiose nutricional parece garantir uma ampla gama de ferramentas ao holobionte (térmita e seus simbiontes) relativamente à exposição a substâncias potencialmente nocivas e a alterações gerais na dieta, e eventualmente também a mudanças nas condições externas e internas da térmita, como por exemplo, a adaptação a novas áreas invadidas ou a condições ambientais variáveis e adversas.

Esta tese estabelece uma base ao conhecimento sobre os protistas flagelados simbióticos com *R. grassei*, que contribuirá para investigação futura nesta área, por

exemplo, no desenvolvimento de novas estratégias de controlo de térmitas. Outra aplicação possível a este conhecimento é na biotecnológica.

O roteiro para a investigação futura nesta área deverá responder às questões levantadas durante este trabalho e incluir tópicos relacionados com a estrutura da comunidade de protistas flagelados (e otimização das técnicas de identificação), bem como as suas interações biogeográficas, evolutivas e bióticas com bactérias. O impacto das alterações climáticas e a resiliência das térmitas e seus simbiontes a alterações deverão também ser incluídos nesse roteiro.

Palavras-chave: protistas flagelados, fauna simbiótica da zona posterior do intestino, digestão de compostos lenhocelulósicos, *Reticulitermes grassei*, térmita subterrânea, estações subterrâneas de monitorização de atividade de térmitas.

List of chapters, publications, and author contributions

To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text.

This thesis includes a version of four original papers published in indexed peer reviewed journals and two unpublished publications in preparation. The inclusion of co-authors reflects the fact that the work resulted from active collaboration between researchers and acknowledges input into team-based research. All the work presented was conducted in the National Laboratory for Civil Engineering *campus*, part of Chapter 3 was conducted at NIBIO (Norwegian Institute for Bioeconomy Research), Norway and in the Faculty of Sciences of Lisbon University.

General introduction – S. Duarte wrote the text, which was revised by the supervisors.

Chapter 1 – A version of Chapter 1 has been published [**Duarte, S., Taylor, A.M., Lloyd, J.D., Duarte, M. & Nunes, L. 2016. Optimization of termite in-ground monitoring stations: An evaluation trial. *Maderas. Ciencia y Tecnología,* 18(1), 191-206**]. S. Duarte was involved in data collection and responsible for data analysis, as well as for writing the entire draft version of the paper and revising it according to co-authors comments. A.M. Taylor and J.D. Lloyd were involved in concept formation and data collection; both also contributed to manuscript reviewing. M. Duarte was involved in data collection. L. Nunes was the supervisory author on this study and was involved throughout it in concept formation and manuscript reviewing.

Chapter 2 - A version of Chapter 2 has been published **[Duarte, S., Nunes, L., Borges, P.A.V., Fossdal, C.G. & Nobre, T. 2017. Living inside termites: an overview of symbiotic interactions, with emphasis on flagellate protists. *Arquipelago. Life and Marine Sciences*, 34, 21-42.**] S. Duarte wrote the text, which was revised by all the authors. T. Nobre was the supervisory author on this study and was involved throughout it in concept formation and manuscript reviewing.

Chapter 3 – A version of the molecular part of Chapter 3 is being prepared for publication [**Duarte, S., Nobre, T., Silva, S.E., Paulo, O.S., Borges, P.A.V, & Nunes, L. Molecular identification and phylogeny of two flagellate protist symbionts of the subterranean termite *Reticulitermes grassei* Clément**]. S. Duarte was responsible for all major areas of concept formation, data collection and analysis, as well as for writing the entire draft version of the paper and revising it according to co-authors comments. T. Nobre was involved in concept formation, data analysis, methodology adjustment and manuscript reviewing. S.E. Silva was involved in the laboratory work and data analysis and contributed to manuscript reviewing. O.S. Paulo and P.A.V. Borges contributed to manuscript reviewing. L. Nunes will be the supervisory author on this study and was involved throughout it in data collection, concept formation and manuscript reviewing.

Chapter 4 - A version of Chapter 4 has been published [**Duarte, S., Nobre, T., Borges, P.A.V. & Nunes, L. (2018) Symbiotic flagellate protists as undercover drivers of adaptation and invasiveness of the subterranean termite *Reticulitermes grassei* Clément. *Ecology and Evolution*, 1-12**]. S. Duarte was responsible for all major areas of concept formation, data collection and analysis, as well as for writing the entire draft version of the paper and revising it according to co-authors comments. P.A.V. Borges and T. Nobre were involved in the concept formation and contributed to manuscript reviewing. L. Nunes was the supervisory author on this study and was involved throughout it in concept formation and manuscript reviewing.

Chapter 5 - A version of Chapter 5 has been published [**Duarte, S., Duarte, M., Borges P.A.V. & Nunes, L. 2017. Dietary driven variation effects on the symbiotic flagellate protists communities of the subterranean termite *Reticulitermes grassei* Clément. *Journal of Applied Entomology,* 141(4), 300-307**]. S. Duarte was responsible for all major areas of concept formation, data collection and analysis, as well as for writing the entire draft version of the paper and revising it according to co-authors comments. M. Duarte contributed to the data collection and laboratory work. P.A.V. Borges contributed to data analysis and reviewing of the manuscript. L. Nunes was the

supervisory author on this study and was involved throughout it in concept formation and manuscript reviewing.

Chapter 6 - A version of Chapter 6 is being prepared for publication [**Duarte, S., Nobre, T., Borges, P.A.V. & Nunes, L. Dynamic shifts on the symbiotic flagellate protists community of subterranean termites in response to different substances.**]. S. Duarte was responsible for all major areas of concept formation, data collection and analysis, as well as for writing the entire draft version of the paper and revising it according to co-authors comments. T. Nobre was involved in the concept formation and contributed to manuscript reviewing. P.A.V. Borges contributed to manuscript reviewing. L. Nunes will be the supervisory author on this study and was involved throughout it in concept formation and manuscript reviewing.

General discussion - S. Duarte wrote the text, which was revised by the supervisors.