Arquipelago - Life and Marine Sciences ISSN: 0873-4704

Living inside termites: an overview of symbiotic interactions, with emphasis on flagellate protists

Sónia Duarte, L. Nunes, P.A.V. Borges, C.G. Fossdal & T. Nobre

Duarte, S., L. Nunes, P.A.V. Borges, C.G. Fossdal & T. Nobre 2017. Living inside termites: an overview of symbiotic interactions, with emphasis on flagellate protists. *Arquipelago*. Life and Marine Sciences 34: 21-43.

To degrade lignocellulose efficiently, lower termites rely on their digestive tract's specific features (i.e., physiological properties and enzymes) and on the network of symbiotic fauna harboured in their hindgut. This complex ecosystem, has different levels of symbiosis, and is a result of diverse co-evolutionary events and the singular social behaviour of termites. The partnership between termites and flagellate protists, together with prokaryotes, has been very successful because of their co-adaptative ability and efficacy in resolving the needs of the involved organisms: this tripartite symbiosis may have reached a physiologically stable, though dynamic, evolutionary equilibrium. The diversity of flagellate protists fauna associated with lower termites could be explained by a division of labour to accomplish the intricate process of lignocellulose digestion, and the ability to disrupt this function has potential use for termite control. Multi-level symbiosis strategy processes, or the cellulolytic capacity of flagellate protists, may lead to innovative pathways for other research areas with potential spin-offs for industrial and commercial use.

Key words: flagellate protists, hindgut symbiotic fauna, lignocellulose digestion, subterranean termites

Sónia Duarte[1,2] (email:sduarte@lnec.pt), Lina Nunes[1,2], Paulo A.V. Borges[2], Carl G. Fossdal[3] & Tânia Nobre[4], [1]LNEC, National Laboratory for Civil Engineering, Structures Department, Av. do Brasil, 101, PT-1700-066, Lisbon, Portugal, [2]cE3c, Centre for Ecology, Evolution and Environmental Changes / Azorean Biodiversity Group and University of the Azores, Faculty of Agrarian and Environmental Sciences, PT- 9700–042 Angra do Heroísmo, [3]NIBIO Norwegian Institute for Bioeconomy Research, Division for Biotechnology and Plant Health, Forest Health, NO-1431 Ås, Norway, ICAAM – Institute of Mediterranean Agricultural and Environmental Sciences, Laboratory of Entomology, University of Évora, Évora, Portugal.

INTRODUCTION

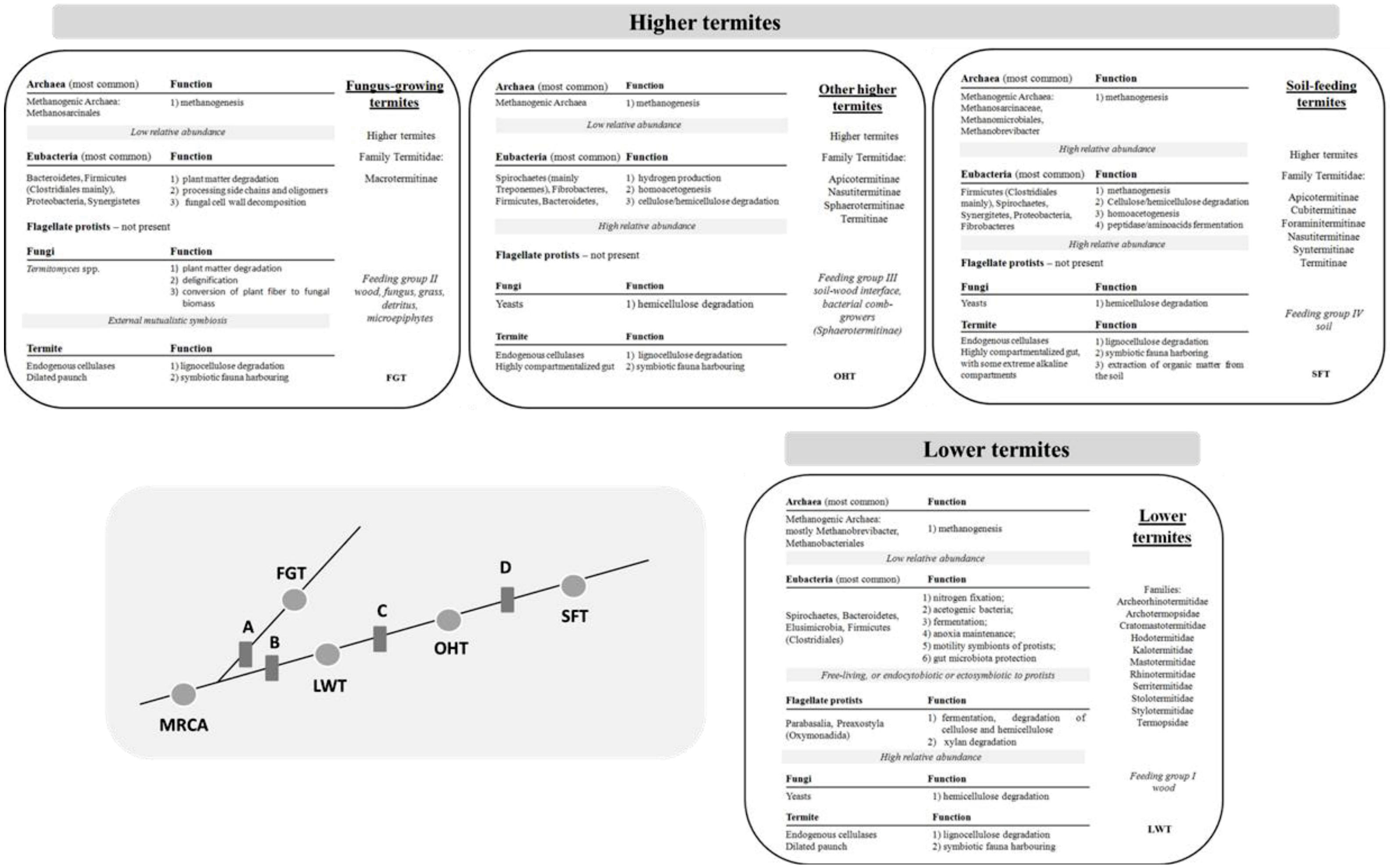
Termites are social insects closely related to cockroaches, from which they evolved (Lo et al. 2000; Inward et al. 2007a; Engel et al. 2009) and are denominated as Polyneoptera a monophyletic group including the cockroaches, Dermaptera, Plecoptera, Orthoptera, Embioptera,Phasmatodea, Mantophasmatodea, Grylloblattodea, Mantodea, and Zoraptera. More than 3,000 species of termites have been described globally, but their areas of high diversity are located in the tropics, particularly in Africa, South America and Asia (Krishna et al. 2013). Termites can be informally divided into two groups: lower (all families but Termitidae) and higher termites (Termitidae), based on the presence or absence of flagellate protists in their hindgut, respectively, and also on different feeding and nesting habits, and different intestinal compartmentalisation (Eggleton 2011; Hongoh 2011; Krishna et al. 2013). These insects are abundant in many terrestrial ecosystems, particularly in the tropics where they are a dominant invertebrate group that heavily contributes to the lignocellulose decomposition process – thus have been called 'ecosystem engineers' (Eggleton 2011; Palin et al. 2011). Termites also have a major role in diverse ecosystem functions, such as nutrients and organic matter cycling and redistribution, soil fertility promotion, generation and regulation of soil biodiversity and ecosystem restoration (Zimmermann et al. 1982; Bignell & Eggleton 2000; Sugimoto et al. 2000; Jouquet et al. 2011). They are major contributors to the ecologic stability of their habitats. By preparing different substrates, like wood or leaf-litter, into forms easily accessed by microorganisms, termites play a major role as ecosystem conditioners (Lawton et al. 1996). Termites are able to degrade lignocellulose efficiently (e.g. Ohkuma 2008; Husseneder 2010; Watanabe & Tokuda 2010) and their feeding habits span a gradient from sound wood to other lignocellulosic plant materials with different humification gradients, such as plant litter or soil (Sleaford et al. 1996). This niche differentiation has allowed termites to promote an impact on the global terrestrial carbon cycle, exceeding the cumulative decomposition roles of other arthropods (Bignell et al. 1997).

Efficient lignocellulose utilisation as a food source by termites is possible because of the establishment of endo- and ectosymbiosis, including microorganisms of all major taxa. These symbionts are Archaea and Bacteria, and protists: unicellular eukaryotes belonging to two separate lineages, the parabasalids and the oxymonads (Fig.1) (Bignell 2000; Bignell & Eggleton 2000; Brune & Ohkuma 2011; Adl et al. 2012; Brune 2013). Ectosymbiosis has evolved in the fungus-growing termites (Macrotermitinae) which cultivate a basidiomycete fungus (*Termitomyces* spp.) (e.g. Nobre & Aanen 2012), whereas the majority of the other termites rely solely on endosymbiosis. The fauna harboured inside the hindgut assists the termite host with energy metabolism, nitrogen and vitamin supply and also additional defence mechanisms (Salem et al. 2014, Peterson et al. 2015, Zheng et al. 2015).

Higher termites, account for nearly 75% of Isoptera species richness and yet belong to a single family, Termitidae. These termites have retained their bacterial symbionts, but lack the protozoan gut symbionts. They have various feeding habits, with clear separation of feeding and nesting sites, and exhibit a highly compartmentalised intestine (except for Macrotermitinae and Sphaerotermitinae). Dissimilarly, lower termites feed strictly on lignocellulose and are aided by hindgut symbionts during the digestion process; they are considered to be an ancestral branch of termites which comprises 11 families (Krishna et al. 2013). Lower termites have a dilated section of the anterior hindgut (the paunch) where the bulk of symbiotic microbiota is harboured. Most lower termites nest and feed within the same wood resource. With potential impact on within-nest endosymbiont transmission, lower termites rely on regurgitation of crop contents and saliva (stomodeal trophallaxis) as well as proctodeal trophallaxis, involving anus-to-mouth exchanges of hindgut fluids, to pass food and gut contents to nest mates, whereas higher termites rely mainly on stomodeal trophallaxis (Eggleton 2011; Shimada et al. 2013; Mirabito & Rosengaus 2016). Proctodeal trophallaxis fosters the social, nutritional and symbiotic fauna interactions among lower termites belonging to the same colony, probably playing a key role in the integration of the information of these different environments (Nalepa 2015). Trophallaxis may be horizontal, among nestmates, or vertical, among parents and offspring.

LOWER TERMITES GLOBAL IMPACT

Because of their feeding habits and preferences, lower termites have an important ecological impact on diverse ecosystems, but are also considered to be structural, agricultural and forestry pests, as they attack cultivated plants and forest nurseries (Rouland-Lefèvre 2011). Lower termites account for 80% of the economically important species known to cause major problems in artificial constructions (Nobre & Nunes 2007; Rust & Su 2012). There is concern that the number of invasive termite species has increased more than 50% since 1969 (Evans et al. 2013), which may be related to the globalisation of trade. In 2010, the global economic impact of termites was estimated at 35.6 billion euros, and subterranean termites accounted for 80% of this figure, i.e. approximately 24 billion euros (Rust & Su 2012).



Higher termites

Fungus-growing termites

Higher termites

Family Termitidae:

Macrotermitinae

Archaea (most common)	**Function**
Methanogenic Archaea: Methanosarcinales	1) methanogenesis

Low relative abundance

Eubacteria (most common)	**Function**
Bacteroidetes, Firmicutes (Clostridiales mainly), Proteobacteria, Synergistetes	1) plant matter degradation 2) processing side chains and oligomers 3) fungal cell wall decomposition

Flagellate protists – not present

Fungi	**Function**
Termitomyces spp.	1) plant matter degradation 2) delignification 3) conversion of plant fiber to fungal biomass

External mutualistic symbiosis

Termite	**Function**
Endogenous cellulases Dilated paunch	1) lignocellulose degradation 2) symbiotic fauna harbouring

Feeding group II
wood, fungus, grass, detritus, microepiphytes

FGT

Other higher termites

Higher termites

Family Termitidae:

Apicotermitinae
Nasutitermitinae
Sphaerotermitinae
Termitinae

Archaea (most common)	**Function**
Methanogenic Archaea	1) methanogenesis

Low relative abundance

Eubacteria (most common)	**Function**
Spirochaetes (mainly Treponemes), Fibrobacteres, Firmicutes, Bacteroidetes,	1) hydrogen production 2) homoacetogenesis 3) cellulose/hemicellulose degradation

High relative abundance

Flagellate protists – not present

Fungi	**Function**
Yeasts	1) hemicellulose degradation

Termite	**Function**
Endogenous cellulases Highly compartmentalized gut	1) lignocellulose degradation 2) symbiotic fauna harbouring

Feeding group III
soil-wood interface, bacterial comb-growers (Sphaerotermitinae)

OHT

Soil-feeding termites

Higher termites

Family Termitidae:

Apicotermitinae
Cubitermitinae
Foraminitermitinae
Nasutitermitinae
Syntermitinae
Termitinae

Archaea (most common)	**Function**
Methanogenic Archaea: Methanosarcinaceae, Methanomicrobiales, Methanobrevibacter	1) methanogenesis

High relative abundance

Eubacteria (most common)	**Function**
Firmicutes (Clostridiales mainly), Spirochaetes, Synergitetes, Proteobacteria, Fibrobacteres	1) methanogenesis 2) Cellulose/hemicellulose degradation 3) homoacetogenesis 4) peptidase/aminoacids fermentation

High relative abundance

Flagellate protists – not present

Fungi	**Function**
Yeasts	1) hemicellulose degradation

Termite	**Function**
Endogenous cellulases Highly compartmentalized gut, with some extreme alkaline compartments	1) lignocellulose degradation 2) symbiotic fauna harboring 3) extraction of organic matter from the soil

Feeding group IV
soil

SFT

Lower termites

Lower termites

Families:
Archeorhinotermitidae
Archotermopsidae
Cratomastotermitidae
Hodotermitidae
Kalotermitidae
Mastotermitidae
Rhinotermitidae
Serritermitidae
Stolotermitidae
Stylotermitidae
Termopsidae

Archaea (most common)	**Function**
Methanogenic Archaea: mostly Methanobrevibacter, Methanobacteriales	1) methanogenesis

Low relative abundance

Eubacteria (most common)	**Function**
Spirochaetes, Bacteroidetes, Elusimicrobia, Firmicutes (Clostridiales)	1) nitrogen fixation; 2) acetogenic bacteria; 3) fermentation; 4) anoxia maintenance; 5) motility symbionts of protists; 6) gut microbiota protection

Free-living, or endocytobiotic or ectosymbiotic to protists

Flagellate protists	**Function**
Parabasalia, Preaxostyla (Oxymonadida)	1) fermentation, degradation of cellulose and hemicellulose 2) xylan degradation

High relative abundance

Fungi	**Function**
Yeasts	1) hemicellulose degradation

Termite	**Function**
Endogenous cellulases Dilated paunch	1) lignocellulose degradation 2) symbiotic fauna harbouring

Feeding group I
wood

LWT

Fig. 1. Possible evolutionary trajectory, from a most recent common ancestor of the cockroach type, of the simplified feeding groups of termites (LWT – Lower termites; FGT – Fungus-growing termites; OHT – Other higher termites; SFT – Soil-feeding termites) based on their symbiotic fauna [based on: Donovan et al. 2001, Eggleton & Tayasu 2001, Inward et al. 2007b, Bujang et al. 2014; Otani et al. 2014]. A= acquisition of externalised gut through the establishment of a mutualistic external symbiosis with basidiomycete fungi; B=acquisition of flagellate protists; C=loss of flagellate protists; D=acquisition of strict soil-feeding habits. Some subfamilies have representatives in more than one feeding group.

As the human population increases, production, trade and use of wooden structures and bio-products susceptible to termite infestation increases, potentially increasing the spread of termite pest species. Warmer seasons and changes in precipitation patterns due to climate change are expected to influence termite territory size and distribution. For example, the known 27 species of invasive termite species are likely to increase their ranges (Su & Scheffrahn 2000; Evans et al. 2013), favouring termite populations in places where their presence was previously limited by these factors (Lal 2004; Peterson 2010; Lee & Chon 2011; Guerreiro et al. 2014). There is thus a need to develop efficient preventive and control methods for avoiding possible future termite pests' outbreaks.

Subterranean termite control strategies are studied and applied worldwide and mainly rely on the use of chemical or physical barriers, wood treatment with insecticides or wood modification by acetylation, furfurylation or other techniques, and subterranean termite population control using baits. Few of the primary issues in termite control are the need to efficiently kill the entire colony and the durable protection of the materials. Difficulties arise due to the cryptic and diffuse nature of rhinotermitid pest termite species, which forage either in extensive underground galleries, build nests hidden underneath the soil surface, or live in small colonies inside the wood they infest. Subterranean termite control has relied on the use of persistent, broad spectrum insecticides applied to the soil beneath structures. Therefore, in the last few decades, although much remains to be investigated, integrated pest management strategies (IPM) have been favoured, such as local spot treatment of infested timber and population control through the use of baits and insect growth regulators (like chitin synthesis inhibitors) (Evans & Iqbal 2014).

If the biology and ecology of the pest species are considered, management strategies can potentially become more specific, and therefore also potentially more sustainable. Further understanding of how termites feed and obtain nutrients and grow as a colony will assist in developing greatly needed innovative termitecontrol methods.

LIGNOCELLULOSE DIGESTION

FEEDING SUBSTRATE

Wood is a natural material composed of three main types of components: cellulose (framework substance), hemicellulose (matrix substance present between cellulose microfibrils) and lignin (incrusting substance for cell wall solidification).

For the digestion of these main components, several enzymes are needed; some of those enzymes are not yet identified in lower termites, such as exoglucanases or hemicellulases, and enzymes present inside the flagellate protists, enabling the provision of these cellulases during lignocellulose digestion (Hongoh 2011). Cellulases are enzymes which have the ability to produce sugars from crystalline cellulose (Slaytor 1992). Cellulose and hemicellulose are thus degraded to sugars, which are then processed into acetate, hydrogen and carbon dioxide. These products may be used directly by the termite or may interact with other nutrients processed inside termite hindgut. Acetate is used as the main energy source of the termite (Breznak 1982).

DIGESTIVE TRACT

Termites' ability to efficiently digest cellulose relies not only on chemical features (cellulolytic enzymes), but also on the digestive tract's physiological properties. The termite digestive tract is composed of different parts: mouth, salivary glands, foregut, midgut and hindgut (Fig. 2; salivary glands not shown in this figure), and each part has a specific function in terms of lignocellulose breakdown. The lignocellulose breakdown starts in the mouth, with the use of solid and hardened mandibles to chew the wooden substrates; the crop and the proventriculus are additional organs situated in the foregut, which are responsible for further milling and filtering of the ingested wood particles (Watanabe & Tokuda 2010; Brune & Ohkuma 2011). Indeed this physical conditioning of the food is crucial for efficient digestion as it results in proper cleavage of the substrate and thus facilitates the access of cellulolytic enzymes

The hindgut harbours a rich symbiotic fauna, which was thought to be parasitic in the early years of research. Only after Cleveland (1923) was clear that termites devoid of hindgut symbionts were unable to digest lignocelluloses fully, suggesting that the relation between host termite and the hindgut fauna was nutritional symbiosis. Since then, the evidence of flagellate protists' contribution to lignocellulose digestion, through their cellulolytic enzymes, has been demonstrated and is widely accepted (e.g. Yamin & Trager 1979; Yamin 1980; Slaytor 1992; Yoshimura et al. 1996; Inoue et al. 1997; Scharf et al. 2011a; Xie et al. 2012; Tsukagoshi et al. 2014).

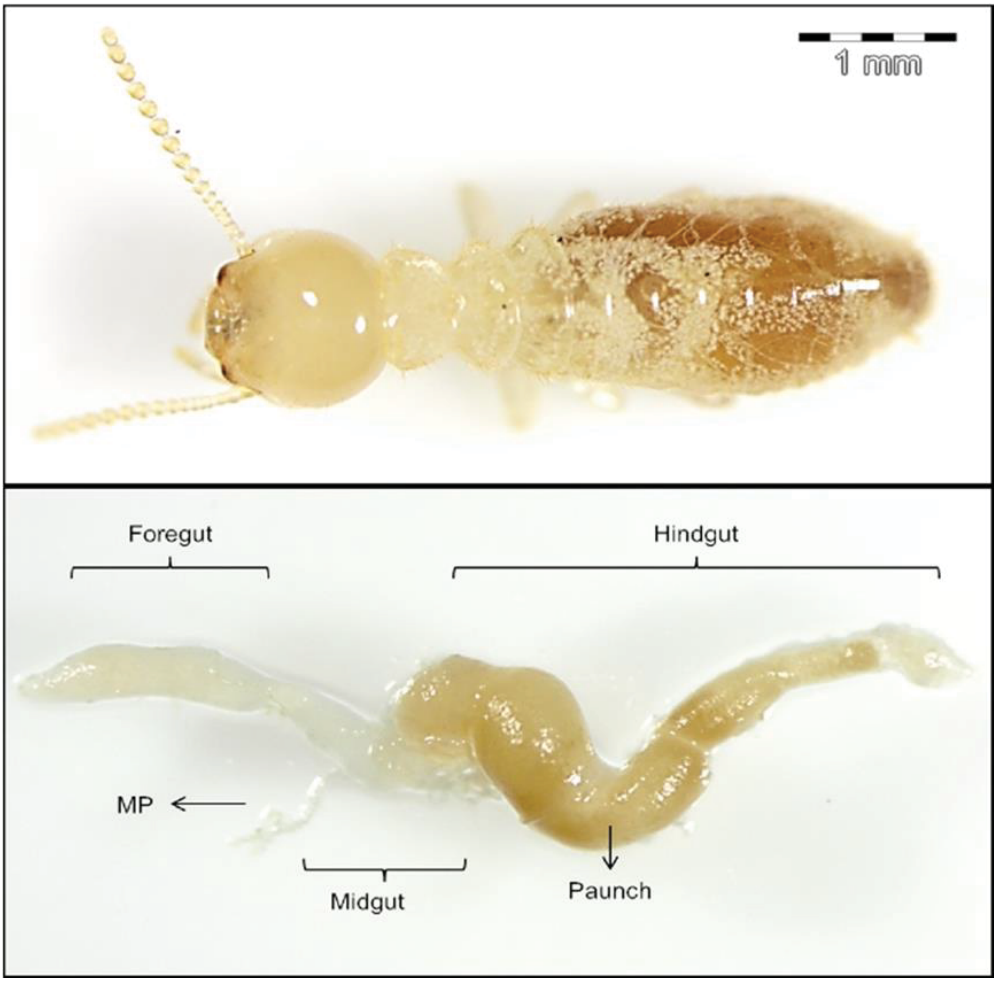


Fig. 2. Photo showing a worker and the extracted gut with different parts of the subterranean termite *Reticulitermes grassei* Clément gut: Foregut; Midgut, including the Malpighian tubules (MP) at the posterior end of the midgut; Hindgut

The enlarged hindgut is a key structure for lower termites' ability to digest lignocellulose efficiently, as it concentrates major chemical action on cellulose, with a dilated paunch harbouring the majority of symbiotic fauna. Though fairly simple in lower termites, the anterior and posterior paunch, and the anterior and posterior colon are sequentially structured for digestion, each serving as defined micro-niches in terms of gradients of oxygen, hydrogen and pH, created by a combination of host and hindgut symbiont activities (Brune & Friedrich 2000), creating difficulties for researchers to reproduce the physical-chemical conditions of the hindgut within the laboratory. Radial concentrations of oxygen and hydrogen showed a peripheral zone of the hindgut where oxygen is available, and enabling the survival of both aerobic or facultative aerobic microorganisms, whereas in the hindgut centre, an anaerobic environment is established, with a zone with high hydrogen concentration, resulting from the activity of flagellate protists, which release hydrogen, carbon dioxide and acetate and are anaerobic (Brune et al. 1995). The hydrogen and carbon dioxide produced is then used by prokaryotes for methanogenesis or acetogenesis, whereas acetate may be used by the termite host (Brune 1998, 2013). Furthermore, to harbour anaerobic flagellate protists, the termite hindgut functions as an oxygen sink and this gas needs to be consumed (Brune et al. 1995). Therefore, flagellate protists are often associated with methanogenic bacteria and other facultative or strict aerobic hindgut microbiota which consume the oxygen, maintaining an anoxic environment in some parts of the paunch (Brune 1998; Brune & Friedrich 2000).

Additionally, endogenous cellulases in lower termites have been identified and are harboured mainly in the salivary glands, playing an important role in the lignocellulose degradation process (Slaytor 1992; Watanabe et al. 1998; Scharf & Tartar 2008; Scharf et al. 2011a; König et al. 2013; Peterson et al. 2015). The lignocellulose digestion is probably the result of synergistic action of both termites and their symbionts; though the degree of nutritional mutualism has some times been questioned (Scharf et al. 2011a; Scharf 2015). Recent metatranscryptomic analysis of termite hindgut content has indeed confirmed that the lignocellulolytic system has a tripartite origin: protists, bacteria and termites (Xie et al. 2012; Peterson et al. 2015).

MULTI-LEVEL SYMBIOSIS

TERMITE AND ITS SYMBIOTIC FAUNA AS A HOLOBIONT

Symbiosis was defined by Anton de Bary in 1879 as the ‘living together of different species’; however, this definition does not describe the complex nature of the relation between termites and their gut symbiotic fauna. The highly complex ecosystem with different levels of symbiosis inside termite guts is the result of complex and diverse evolutionary events and also of the singular social behaviour of termites. This relationship certainly goes beyond the Anton de Bary concept of symbiosis. Besides the hindgut symbiotic microbiota, the termite colony has also been considered as an organism, since the basic functions are clearly divided in its different parts: reproduction and dispersion (queens, kings and alates), construction, feeding and tending (workers), active defence (soldiers) and protection, homoeostasis and fortification (nest) (Eggleton 2011). The gut symbiotic fauna may be directly involved not only in feeding functions, but also in tending, defence, and homoeostasis (Matsuura 2001; Ugelvig & Cremer 2012; Chouvenc et al. 2013; Sen et al. 2015). The symbiotic fauna is probably also involved in shaping the termite social behaviour. For example, recently it was shown that lower termites exhibit different undertaking behaviour towards conspecific (necrophagy) or congeneric (burial behaviour) termite corpses. This behaviour was interpreted as a defence mechanism together with a cost mitigation strategy (Sun et al. 2013). Another possible advantage of this behaviour may be the protection of the hindgut symbiotic fauna, avoiding the introduction of new elements into the hindgut fauna by the ingestion of corpses of termites belonging to other species. The term holobiont has been accepted to refer to the host and its microbiota as a whole unit able to live, develop, survive and evolve together (Rosenberg & Zilber-Rosenberg 2013; Scharf 2015). The cooperation between hosts and their microbiota results in a positive contribution to the fitness of the association, providing an increased ability to adapt more rapidly to changing conditions (Rosenberg & Zilber-Rosenberg 2013). The capacity to tackle imposed changes and stresses results from the synergy of combined capacities. Furthermore, social insects such as termites are robust in resisting genetic diversity losses during phenomena such as introductions or other sources of low population genetic bottlenecks (Ugelvig & Cremer 2012). From this perspective, it is clear that fitness changes in the termite colony can be induced at different levels.

Unarguably, the symbiotic association between lower termites and their hindgut symbionts has advantages for both, since the termites are able to receive contributions to their main energy supply resulting from lignocellulose digestion, and hindgut symbionts have shelter, protection and food, supplied by the termite host (Radek 1999; Noda et al. 2009; Brune 2013; Tamschick & Radek 2013). Probably, the nature of the symbiotic fauna, and the relationships between and amongst them, are driven by host social behaviours and shifts in feeding habits. However, some have argued that the symbiotic microbes lead to the development of social habits in wood-feeding insects (termites and cockroaches) (e.g. Nalepa et al. 2001), so the paradox remains. Nevertheless, symbionts may impose important selective pressures on their hosts, as well as diet preferences or feeding habits (Rosengaus et al. 2011; Xiang et al. 2012; King et al. 2013).

The tripartite symbiosis between termite host, flagellate protists, and prokaryotes responsible for lignocellulose degradation translates into a unique symbiotic community, which is probably under strong co-evolutionary pressure. Indeed, the majority of gut microbiota are considered to be autochthonous symbionts likely to have co-evolved with their termite host species (Hongoh et al. 2005; Noda et al. 2007; Tai et al. 2015). The invasion of new habitats or the proximity of different termite species does not seem to influence the gut symbiotic fauna structure of termite species, which tends to maintain its integrity in terms of diversity of symbiont species (Kitade 2004; Hongoh et al. 2005; Husseneder et al. 2010; Boucias et al. 2013). Within a termite colony, a rather species-specific gut symbiotic fauna is expected, as termites rely on horizontal transmission of hindgut symbionts to recover the hindgut symbiotic community, since, when they moult, symbionts are also discarded. The recovery is done by proctodeal or stomodeal trophallaxis. This within-nest symbiont transmission was observed in a genomic and metagenomic study of a fungus-growing termite (Poulsen et al. 2014). The obligatory vertical mode of transmission of the gut symbiotic fauna to the next generation probably determines the gut symbiotic structure associated with termite species colonies, leading thus to higher levels of host-symbiont specificity (Hongoh et al. 2005; Noda et al. 2007; Husseneder 2010).

Some evidences of co-evolution between flagellate protists and their host termites rely on phylogenetic analyses which show a clear co-diversification pattern, although factors such as stochastic, dietary and ecological effects are also important in the long term evolution of symbiont communities (Tai et al. 2015). Parabasalid protists seem to be strongly influenced by host phylogeny, and the symbiotic bacteria communities seem to be more influenced by dispersal and environmental acquisition (Tai et al. 2015). Within the parabasalids, the Cristamonadea class seems to be strongly associated with the Kalotermitidae family, whereas the Spirotrichonymphea class is linked with the Rhinotermitidae termite family (Tai et al. 2015).

In contrast, flagellate protists belonging to the genus *Trichonympha* are widely distributed and were detected in six different termite families and also inside the wood-feeding cockroaches belonging to the genus *Cryptocercus*. This supports the hypothesis that *Trichonympha* spp. symbionts were acquired by the most recent common ancestor of termites and wood-feeding *Cryptocercus* spp. cockroaches (Inward et al. 2007a; Carpenter et al. 2009; Ikeda-Ohtsubo & Brune 2009; Tai et al. 2015). It is important to highlight, however, that some authors found a greater cryptic diversity of *Trichonympha* species inside one host, using molecular analyses, than the diversity predicted by use of morphological analyses only (James et al. 2012; Tai et al. 2013). This suggests that termite hindgut diversity estimations could be biased because of the underestimation of symbiont diversity, especially considering that *Trichonympha* spp. are among the largest species of flagellate protists living inside termites (James et al. 2012; Tai et al. 2013).

Intracellular bacterial symbionts of termite hindgut flagellates belonging to Endomicrobia (phylum Termite Group 1) tend to form a unique phylogenetic lineage with either their flagellate protist host species or their termite host, suggesting co-speciation events (Ikeda-Ohtsubo et al. 2007; Tai et al. 2015). Co-speciation between *Bacteroidales* endosymbionts and their host protists was demonstrated in 13 out of 14 taxa of the protist genus *Pseudotrichonympha*, and in turn these protist species showed an almost complete co-speciation with host termite species belonging to the Rhinotermitidae (Noda et al. 2007). In a recent study of the 'Endomicrobia' associated with *Trichonympha* genus, strict host specificity was concluded (Zheng et al. 2015). In contrast, oxymonads' and parabasalids' 'Endomicrobia' symbionts are rather similar between flagellate protist hosts, suggesting that they are horizontally transmitted among different flagellate protist species living inside the same termite hindgut, accounting for the high levels of symbiont transfer between flagellate protist hosts and thus lacking (or having reduced levels) of host-symbiont specificity (Ikeda-Ohtsubo et al. 2007; Ikeda-Ohtsubo & Brune 2009).

An obligatory ectosymbiont of the flagellate protist *Devescovina* spp. has been identified, suggesting that this highly specific relationship evolved as a consequence of strong metabolic interactions (Desai et al. 2010).

At another level of this symbiotic network, the adaptation of morphological characters of both host protists and ecto- and endosymbiotic bacteria has been observed (Noda et al. 2007, 2009). Specialised cell-surface features which facilitate bacterial attachment indicate a close integration of these ectosymbionts in the protist metabolism, and corroborate the co-evolution hypothesis on their symbiotic relationships (Radek et al. 1992; Dolan et al. 2000).

HINDGUT PROKARYOTES

Most of the hindgut prokaryotes are bacteria, whereas the Archaea represent a low percentage of hindgut symbionts. The major groups of bacteria usually identified in lower termite hindgut are: *Spirochaetes, Bacteroidetes, Elusimicrobia* and *Firmicutes* (Clostridiales) (Stingl et al. 2005; Boucias et al. 2013; Brune 2013). The termite hindgut has proven to be a rich source of novel organisms, including new findings for science at high taxonomic levels (Brune 1998; Ohkuma 2003; Boucias et al. 2013, Sato et al. 2014; Tai et al. 2015).

The maintenance of an anoxic environment in some parts of the paunch is thought to be just one of the many putative roles of the symbiotic bacteria. These bacteria exhibit different levels of association: free-living in the hindgut (symbiotic with the insect host), directly associated with flagellate protists (either as endo- or as ectosymbionts) or associated with the gut wall (Tamschick & Radek 2013). The tasks of symbiotic bacteria (prokaryotes) and their roles are not completely understood and many species remain undescribed (for a review on this issue see Brune 2013). Brune (2013) defined different types of hindgut prokaryotes including: lignocellulolytic bacteria, bacteria involved in oxygen reduction reactions, fermentation bacteria, bacteria responsible for hydrogen metabolism and nitrogen-fixing bacteria.

Recently, cultivation-independent studies helped to identify and catalogue hindgut prokaryotes in relation to their function inside the termite hindgut, including some evidences on their role in lignocellulose digestion (Warnecke et al. 2007; Boucias et al. 2013; He et al. 2013; King et al. 2013; Peterson et al. 2015). Advances in sequencing and its accessibility raise expectations regarding future unveiling of the role of bacteria inside the termite hindgut.

FLAGELLATE PROTISTS

Flagellate protists are part of the unicellular eukaryotes belonging to two separate lineages: the order Oxymonadida (Phylum Preaxostyla) and the Phylum Parabasalia (Brugerolle 1991; Moriya et al. 1998; Čepička et al. 2010; Adl et al. 2012). In spite of the difficulties in laboratory cultivation of most of these organisms, their taxonomy was initially based on morphological characters (e.g. Brugerolle 1991; Cavalier-Smith 1993). With culture-independent techniques it has become possible to reconstruct the phylogeny of some groups of flagellate protists, and some studies are based on both morphological and molecular data (e.g. Carpenter et al. 2010; Čepička et al. 2010).

Table 1. Key diagnostic characters of the major groups of symbiotic flagellate protists living inside lower termites' intestine belonging to the phyla Parabasalia and Preaxostyla (order Oxymonadida) (adapted from: Brugerolle & Lee 2000; Čepička et al. 2010; Adl et al. 2012; Radek et al. 2014).

					Flagellar apparatus/Mastigont system			Cytoskeletal arrangement			
	Closed mitosis	Golgi body	Hydrogenosomes	Nuclei	Flagellar system	Basal bodies arrangement	Microtubular fibers/sheets	Axostyle type	Undulating membrane	Costa type	Comb-like structure
Parabasalia	external spindle	+	+		Basically: 4 flagella	Basically: 4 basal bodies, 3 anterior; 1 recurrent	Pelta - covers the anterior part of the cell				
Class Hypotrichomonadea		+	+		4	biramous parabasal body		cone-like, usually stout	lamelliform	A	+
Class Trichomonadea		+	+		2 - 6	single karyomastigont		cone-like or reduced	lamelliform, if present	B, if present	-
Class Tritrichomonadea		+	+	1 or 2	0 - 5	single karyomastigont	pelta-axostyle complex may be reduced or absent	tube or cone-like, if present	rail-form, if present	A, if present	+/ -
Class Cristamonadea		+	+	1 or more	2 to thousands per mastigont	single to multiple mastigonts	often spiralised or ramified axostyle	Stout rod or reduced	transformed into cresta (in some)	A, reduced or absent	+ (ancestral) or absent
Class Trichonymphea		+	+	1	hundreds to thousands	cell body divided into anterior rostrum and post-rostral area		thin and numerous, do not protude outside the cell	-	-	-
Class Spirotrichonymphea		+	+	1		kinetosomes arranged into spiraled rows		tube-like and stout; multiple thin bands; or reduced	-	-	-
Preaxostyla	internal spindle										
Order Oxymonadida		-	-	1 or more	4, or a multiple of 4, flagella arranged in pairs	2 to several karyomastigonts	Preaxostylar lamina linking the 2 basal body pairs; Pelta (caps the nucleus)	crystalline axostyle; microtubular rod extending through the entire length of the cell	-	-	-

The oxymonads do not have energy-generating organelles or a highly developed intracellular membrane systems, like dictyosomes, and live mostly inside insects. Oxymonads may be motile or attached to termite hindgut walls. The most common oxymonads living inside termite hindgut belong to the genera *Dinenympha*, *Pyrsonympha* or *Oxymonas* (Brugerolle & Radek 2006). In contrast to the oxymonads, the parabasalids have anaerobic energy-producing organelles (hydrogenosomes) and a characteristic parabasal apparatus, which is comprised of dictyosomes associated with parabasal fibres. Parabasalids may be large and are highly motile, with four or more flagella. These protists live mostly inside termites and cockroaches as symbionts, but some species may be parasites or commensals of vertebrate hosts (Čepička et al. 2010). Table 1 summarises information on flagellate key diagnostic characters (additional information on the flagellate protists' internal structures is also available in Table 1 of the supplementary material). Flagellate protists are strictly anaerobic and ferment cellulose to acetate (Yamin 1980; Yoshimura et al. 1996; Hongoh 2011). Acetate is also important as a precursor for the synthesis of other products as amino acids or cuticular hydrocarbons (Breznak 1982). Carbon dioxide and hydrogen, two other products of cellulose fermentation, are used by the anaerobic bacteria mainly to produce acetate through reductive acetogenesis (Hongoh 2011).

Protists represent the majority of termite hindgut microorganisms (Katzin & Kirby 1939; Inoue et al. 1997; König et al. 2013). The general uniqueness of these protists to termites and to wood-feeding cockroaches belonging to the genus *Cryptocercus* highlights the origin of termites from a cockroach-like ancestor, corroborated by molecular phylogenetic data (Inward et al. 2007a). The mode of transmission of protists and the close relationships developed between host and symbionts in terms of metabolic interactions and needs probably account for the developed specificity (Kitade et al. 2012). Based on recent attempts to infer termite family relationships (Engel et al. 2009; Bourguignon et al. 2015), and according to available data on flagellate protist classes (Parabasalia) and order (Preaxostyla) identified to date, a gradual tendency towards diversification of the symbiotic community inside the termite hosts is evident. However, the symbiotic community has been researched only in a small number of termite species and rather asymmetrically, with a preponderance in some families. Plotting the number of flagellate protist groups per termite host family, it is possible to see an increase in diversity along host evolutionary pathway until the point at which termites completely lose their flagellate protists and are able to switch to a more diversified diet (family Termitidae) (Fig. 3).

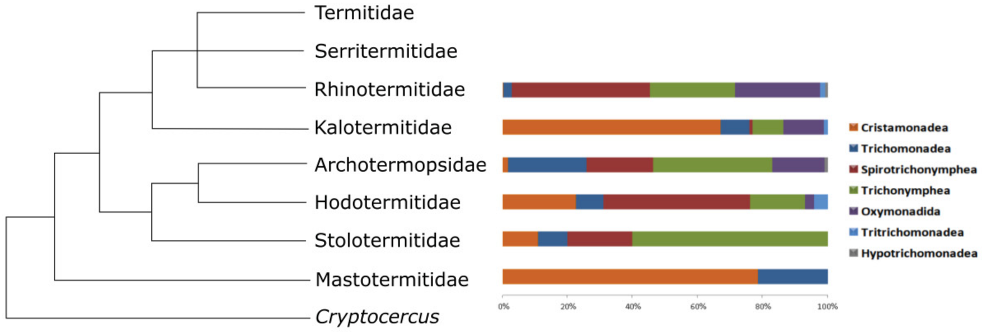


Fig. 3. Simplified scheme of phylogenetic relationships within termites (based on Engel et al. 2009 and Bourguignon et al. 2015; see these authors for further details) and the number of flagellate protist species belonging to classes (Parabasalia) and order (Preaxostyla) identified to date inside termites.

The Rhinotermitidae family is not an exception, and a similar pattern is observed when the subfamilies are analysed in terms of flagellate protist diversity: the basal subfamilies (Rhinotermitinae, Prorhinotermitinae, Psammotermitinae, Termitogetoninae) have three or fewer flagellate protist groups, whereas Coptotermitinae and Heterotermitinae, considered to be the sister groups of the family Termitidae (Bourguignon et al. 2015), exhibit a higher taxonomic diversity (four to six) of flagellate protists groups.

Different types of events may have occurred during termite diversification, e.g., the external uptake of symbionts or the horizontal transmission of symbionts within different termite species which were foraging the same area and resources. The diversification of termites towards an optimisation of the digestive process, depending on the environmental conditions and type of resources exploited by the termites, are important factors to explain the flagellate protist communities associated with certain groups of termites. Further insights into the evolutionary pathways and constraints driving the relationships among termites and their symbiotic flagellate protist communities are needed. This may shed light on the mechanisms and driving forces which determine the establishment of symbiotic relationships among different organisms and give rise to holobiotic forms of life.

Many flagellate protists are considered species-specific, such as *Pyrsonympha vertens* Leidy and *Joenia annectens* Grassi that have only been identified inside the hindgut of *Reticulitermes flavipes* (Kollar) and *Kalotermes flavicollis* (Fabricius), respectively. Whereas *Holomastigotes elongatum* (Grassi) seems to be a species with less host-specificity, as it was identified inside 12 different termite species belonging to three different families (Archotermopsidae, Hodotermitidae and Rhinotermitidae). However, this finding may be questionable as these protists' identification relied only on morphological characters evaluated by different authors (Harper et al. 2009). In some termite species, such as *Coptotermes heimi* (Wasmann), *Heterotermes indicola* (Wasmann), *Cryptotermes havilandi* (Sjöstedt) or *R. flavipes* more than 20 different flagellate protists have been identified to date (*C. heimi*, *H. indicola* and *C. havilandi*: Yamin 1979 and references therein, Desai et al. 2010; *R. flavipes*: Leidy 1877; Mello 1920; Cleveland 1923; Breznak & Pankratz 1977; Mauldin et al. 1977; Bloodgood et al. 1975; Mauldin et al. 1981; Lelis 1992; Cook & Gold 1998; Stingl & Brune 2003, Stingl et al. 2005; Brugerolle 2006, Brugerolle & Bordereau 2006, Lewis & Forschler 2006; Hu 2008; Lewis & Forschler 2010; Hu et al. 2011, Tamschick & Radek 2013; and references therein: Kudo 1939; Ghidini 1942; Yamin 1979; Grassé 1982). However, other termite species may have a less diverse flagellate protist community, such as *Incisitermes snyderi* (Light), with only three identified species of flagellate protists (Dolan et al. 2000; Gerbod et al. 2002; Harper et al. 2009). As an example of a diverse flagellate protist community, inside the European subterranean termite from Portugal, *Reticulitermes grassei* Clément, 12 different morphotypes were identified based on morphological traits. The European subterranean termite *R. grassei* (Rhinotermitidae) is native of the Iberian Peninsula, and also present in the Atlantic coast of France (Kutnik et al. 2004). One invasive population of *R. grassei* was identified in the UK (Jenkins et al. 2001), and another in Faial Island of the Azores (Ferreira et al. 2013). The flagellate community dynamics after an invasive event may be of major interest for understanding the mechanisms of adaptation to new environments of subterranean termites. The flagellate protist community from *R. grassei* is presumed to be dominated by *Pyrsonympha* sp. (42.4%), *Microjoenia hexamitoides* Grassi (13.4%), *Dinenympha gracilis* Leidy (10.4%), and *Spirotrichonympha flagellata* Grassi (5.4%); *Trichonympha agilis* Leidy (4.3%), *Hexamastix* sp. (4.0%) and *Holomastigotes elongatum* Grassi (3.3%) were also represented in all termites observed (Duarte et al. 2016).

Flagellate protist identification errors owed to over- or underestimation of the flagellate protist community living inside a termite hindgut are common. The misidentification of the different life cycle stages of one flagellate protist species as a different species is an example of overestimation of flagellate protist diversity. Underestimation errors may also occur because of: 1) manipulation constraints; 2) lack of identification power of the DNA markers used; 3) the frequent concentration of research efforts on the larger species, which are more easily analysed morphologically, overlooking the smaller ones, and 4) misidentification of different similar species as an only species through morphological analysis (Harper et al. 2009; James et al. 2013; Tai et al. 2013). Nonetheless, recent advances in genomic and metagenomic techniques are leading to higher quality and affordable community sequence strategies. As a consequence, metagenomic data on gut community of different termite host species are increasing. However, the lack of correspondence of operational taxonomic units obtained with metagenomic analyses with known flagellate protist species is a drawback that requires further taxonomic research efforts. A resilient bottleneck still remains regarding suitable genetic markers that are taxonomically and/or functionally valid and have enough discrimination power regarding downstream data analyses.

The diversity of flagellate protist fauna associated with lower termites could be explained by a strong division of the labour required to accomplish the intricate process of lignocellulose digestion; a species or group of species acts in specific phases of this process (Yoshimura et al. 1996; Inoue et al. 1997; Todaka et al. 2007; Raychoudhury et al. 2013).

POTENTIAL SPIN-OFFS

BIOTECHNOLOGY APPLICATIONS

Lignocellulose is one of the most abundant renewable types of biomass available on earth, and cellulose-based biofuels are considered to be a sustainable alternative to reduce our dependence on fossil fuels (Ragauskas et al. 2006; Yang & Wyman 2008). However, the industrial processing of lignocellulose conversion to energy needs to be adjusted in order to reduce costs and improve energy production and consequently compete with fossil fuels. For example, current biological conversion of cellulosic biomass for bioethanol production is based on bacterial and fungal cellulolytic systems (Sun & Scharf 2010). The most expensive part of bioethanol production is the pretreatment (Ragauskas et al. 2006; Yang & Wyman 2008). The information on the identification and characterisation of endogenous and symbiotic genes and enzymes from an effective natural bioreactor, such as subterranean termite gut, is relevant for the improvement of industrial processes (Helle et al. 2004; Brune 2007; Yang & Wyman 2008; Scharf & Boucias 2010; Tsukagoshi et al. 2014). Cellulolytic enzymes and genes from termites and their symbiotic fauna are potential candidates for integrating, and consequently refining, bioethanol production technologies, by the identification of relevant catalysts and/or by the discovery of potential recombinant enzymes which enable the maximum efficiency of the processes; it is also possible to use mutagenesis in order to functionally improve enzymes (e.g. Helle et al. 2004; Scharf & Tartar 2008; Husseneder 2010; Scharf & Boucias 2010; Zhang et al. 2010; König et al. 2013).

Other technical processes could benefit from deep knowledge of termite and symbiont interactions inside termite gut. For example, the breakdown of lignocellulose into shorter structural elements can form the chemical building blocks for the production of new synthetic materials (Ragauskas et al. 2006). The intestinal tract of termites is a rich source of glycanolytic microorganisms, which may be used for other applications, such as the prevention of slime production and other undesirable side-products during vinification (Blättel et al. 2011) or for bioremediation purposes, because of their ability to degrade toxic substances (Ke et al. 2012). Furthermore, it may also be possible to use termite symbionts for nitrogen fixation in soil fertilisation (Husseneder 2010; Du et al. 2012; Thong-On et al. 2012).

NEXT-GENERATION TERMITICIDES

Termite gut microbiota and respective cellulosic activity may be a strategic target for designing molecular-based bio-pesticides for termite control (Zhang et al. 2010). The effectiveness of the potential biological control agents previously studied has been compromised because of the symbiotic hindgut fauna, which has a protective role regarding novel and potentially harmful microorganisms, of the termite immune system and hygienic behaviour, such as grooming activities and burying and isolation of dead termites (Chouvenc & Su 2012; Sun et al. 2013). A recent study on the synergistic effects of using a nicotinoid and a pathogenic agent showed the potential of this mixture to disrupt termite social behaviour and cause deleterious effects on the colonies. One of the major effects of this treatment was the decrease of the flagellate protist populations living inside the termites (Sen et al. 2015). Knowledge on lignocellulose digestion processes may allow the definition of potential targets for novel termite control strategies based on an alternative mode of action approach. The development of next-generation termiticides, targeting cellulolytic activities encoding genes, of endogenous or symbiont origin, with RNA interference techniques has proven to be possible (Zhou et al. 2008; Itakura et al. 2009; Scharf et al. 2011b; for a review of RNA interference advances in termites and/or symbiotic protists see Scharf 2015). For example, a high-dose stranded RNA force feeding trial led to the silencing of two termite genes, one of them involved in cellulose digestion, the other in caste differentiation, and this led to an increase in mortality in the experimental population (Zhou et al. 2008).

Paratransgenesis represents a target-specific strategy, which relies on the manipulation of genetically engineered natural symbionts (gut bacteria) which will act as a Trojan horse, as they are capable of surviving inside termite gut while carrying and expressing toxins which are then spread throughout the colony by social interactions (Douglas 2007; Husseneder et al. 2010; Rangberg et al. 2012, Sethi et al. 2014). The conjugation of this technique with a ligand-lytic peptide, which will enable the design of specific ligands for flagellate protists, has already proven to be effective against lower termite pests (Husseneder et al. 2010; Sethi et al. 2014). Further applications of this technology may involve the control of other insect pests which harbour flagellate protists and/or may act as vectors of protists; also, the technique could be refined to develop drugs against disease-causing protists (Sethi et al. 2014).

IMMUNITY AGENTS ROLE AND OTHER APPLICATIONS

Termite gut symbiotic microbiota is an active part of the efficient immunity system of termites (Chouvenc et al. 2013). Studies on free-living heterotrophic protists in water discovered their important role in eliminating viruses by feeding on them (Deng et al. 2014). Some flagellate protists inside termites may prey on bacteria by ingestion (e.g. Noda et al. 2009); this process may also imply eventual feeding on viruses. Possibly some protists assume the same role inside termites, and even inside other animals, acting as potential elimination agents of viruses or other pathogenic agents.

OVERVIEW

Our attitude towards the described organism, whether for searching for more effective control strategies or for determining its correct use as a biotechnology model, should shift from the individual termite and its gut microbiota as separate entities towards a more holistic approach considering this holobiont as an independent, evolutionary and functional unit. By adding an ecological and environmental axis to this holobiotic approach, we will be better able to integrate protists' diversity and ecology, contributing to further applications such as: 1) understanding the co-evolution mechanisms that lead to the establishment of this highly efficient natural bioreactor and its consequent ability to convert lignocellulose into energy sources (Tai et al. 2015); 2) the possible adjustment of diverse technical industrial processes such as a biorefinery (Scharf & Tartar 2008; Scharf 2015); and 3) the application of novel strategies for a more sustainable termite control in urban environments (Husseneder 2010; Scharf 2015).

ACKNOWLEDGEMENTS

SD's work was supported by the Portuguese Foundation for Science and Technology (FCT) (SFRH/BD/84920/2012). TN was supported by a Marie Curie fellowship (FP7-PEOPLE-2012-CIG Project Reference 321725) and by FCT (SFRH/BCC/52187/2013). This work was also supported by the project 'Control of the Termite *Reticulitermes flavipes* in Praia da Vitória (Terceira)' (2014-2017) funded by CMPV and DRA of the Azores. The authors wish to thank to the anonymous reviewers which contributed to the improvement of this manuscript with their comments.

REFERENCES

Adl, S.M., A.G.B. Simpson, C.E. Lane, J. Lukes, D. Bass, S.S. Bowser, M.W. Brown, F. Burki, M. Dunthorn, V. Hampl, A. Heiss, M. Hoppenrath, E. Lara, L. Le Gall, D.H. Lynn, H. McManus, E. Mitchell, S.E. Mozley-Standridge, L.W. Parfrey, J. Pawlowski, S. Rueckert, L. Shadwick, C.L. Schoch & F.W. Spiegel 2012. The revised classification of Eukaryotes. *Journal of Eukaryotic Microbiology* 59(5): 429-493.

Bignell, D.E. 2000. Introduction to symbiosis. Pp. 189-208 in: Abe, T., D.E. Bignell & M. Higashi (Eds). *Termites: Evolution, Sociality, Symbioses, Ecology.* The Netherlands, Kluwer Academic Publishers, Dordrecht. 466 pp.

Bignell, D.E. & P. Eggleton 2000. Termites in ecosystems. Pp. 363-387 in: Abe, T., D.E. Bignell & M. Higashi (Eds). *Termites: Evolution, Sociality, Symbioses, Ecology.* The Netherlands, Kluwer Academic Publishers, Dordrecht. 466 pp.

Bignell, D.E., P. Eggleton, L. Nunes & K.L. Thomas 1997. Termites as mediators of carbon fluxes in tropical forests: Budgets for carbon dioxide and methane emissions. Pp. 109-133 in: Watt, A.D., N.E. Stork & M.D. Hunter (Eds*). Forests and Insects.* London, UK, Chapman & Hall. 406 pp.

Blättel, V., M. Larisika, P. Pfeiffer, C. Nowak, A. Eich, J. Eckelt & H. König 2011. β-1,3-glucanase from *Delftia tsuruhatensis* Strain MV01 and its potential application in vinification. *Applied and Environmental Microbiology* 77(3): 983-990.

Bloodgood, R., K.R. Miller, T. Fitzharris & J. Mcintosh 1975. The ultrastructure of *Pyrsonympha* and its associated microorganisms. *Journal of Morphology* 143(1): 77-105.

Boucias, D.G., Y. Cai, Y. Sun, V.U. Lietze, R. Sen, R. Raychoudhury & M.E. Scharf 2013. The hindgut lumen prokaryotic microbiota of the termite *Reticulitermes flavipes* and its responses to dietary lignocellulose composition. *Molecular Ecology* 22: 1836-1853.

Bourguignon, T., N. Lo, S.L. Cameron, J. Sobotník, Y. Hayashi, S. Shigenobu, D. Watanabe, Y. Roisin, T. Miura & T.A. Evans 2015. The evolutionary history of termites as inferred from 66 mitochondrial genomes. *Molecular Biology and Evolution* 32(2): 406-421.

Breznak, J.A. 1982. Intestinal microbiota of termites and other xylophagous insects. *Annual Review of Microbiology* 36: 323-343.

Breznak, J. & S. Pankratz 1977. In situ morphology of the gut microbiota of wood-eating termites *Reticulitermes flavipes* and *Coptotermes formosanus*. *Applied and Environmental Microbiology* 33(2): 406-426.

Brugerolle, G. 1991. Flagellar and cytoskeletal systems in amitochondrial flagellates: Archamoeba, Metamonada and Parabasala. *Protoplasma* 164: 70-90.

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. & J.J. Lee 2000. Order Oxymonadida. Phylum Parabasalia. Pp. 1186-1250 in: Lee, J.J., G.F. Leedale & P. Bradbury (Eds). *The illustrated guide to the Protozoa*, volume II. Kansas, USA, Society of Protozoologists. 1432pp.

Brugerolle, G. & C. Bordereau 2006. Immunological and ultrastructural characterization of spirotrichonymphid flagellates from *Reticulitermes grassei* and *R. flavipes* (syn. *R. santonensis*), with special reference to *Spirotrichonympha*, *Spironympha* and *Microjoenia*. *Organisms Diversity & Evolution* 6: 109-123.

Brugerolle, G. & R. Radek 2006. Symbiotic protozoa of termites. Pp. 243-269 in: König, H. & A. Varma (Eds). *Intestinal microorganisms of soil invertebrates.* Germany, Springer-Verlag Berlin Heidelberg. 483 pp.

Brune, A. 1998. Termite guts: world's smallest bioreactors. *Trends in Biotechnology* 16(1): 16-21.

Brune, A. 2007. Woodworker's digest. *Nature* 450: 487-488.

Brune, A. 2013. Symbiotic associations between termites and prokaryotes. Pp. 545-577 in:

Rosenberg, E., E. DeLong, E. Stackebrandt, S. Lory & F. Thompson (Eds). *The Prokaryotes – Prokaryotic biology and symbiotic associations*. Germany, Springer-Verlag Berlin Heidelberg. 607 pp.

Brune, A. & M. Friedrich 2000. Microecology of the termite gut: structure and function on a microscale. *Current Opinion in Microbiology* 3: 263-269.

Brune, A. & M. Ohkuma 2011. Role of termite gut microbiota in symbiotic digestion. Pp. 439-475 in: Bignell, D.E., Y. Roisin & N. Lo (Eds*). Biology termites: a modern synthesis.* of London, New York, Springer Science+Business Media B.V. 576 pp.

Brune, A., D. Emerson & J.A. Breznak 1995. The termite gut microflora as an oxygen sink: Microelectrode determination of oxygen and pH gradients in guts of lower and higher termites. *Applied and Environmental Microbiology* 61(7): 2681-2687.

Bujang, N.S., Harrison, N.A. & N.-Y. Su 2014. A phylogenetic study of endo-beta-1,4-glucanase in higher termites. *Insectes Sociaux* 61: 20-40.

Carpenter, K.J, L. Chow & P.J. Keeling 2009. Morphology, phylogeny, and diversity of *Trichonympha* (Parabasalia: Hypermastigida) of the wood-feeding cockroach *Cryptocercus punctulatus*. *Journal of Eukaryotic Microbiology* 56(4): 305-313.

Carpenter, K.J., A. Horak & P.J. Keeling 2010. Phylogenetic position and morphology of Spirotrichosomidae (Parabasalia): New evidence from *Leptospironympha* of *Cryptocercus punctulatus. Protist* 161: 122-132.

Cavalier-Smith, T. 1993. Kingdom Protozoa and its 18 phyla. *Microbiology Reviews* 57(4): 953-994.

Čepička, I., V. Hampl & J. Kulda 2010. Critical taxonomic revision of parabasalids with description of one new genus and three new species. *Protist* 161: 400-433.

Chouvenc, T. & N.-Y. Su 2012 When subterranean termites challenge the rules of fungal epizootics. PLoS ONE 7(3): e34484. Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0034484

Chouvenc, T., C.A. Efstathion, M.L. Elliot & N.-Y. Su 2013. Extended disease resistance emerging from the faecal nest of a subterranean termite. *Proceedings of the Royal Society* B: Biological Sciences 280: 20131885. Available from: http://rspb.royalsocietypublishing.org/content/280/1770/20131885

Cleveland, L. 1923. Symbiosis between termites and their intestinal protozoa. *Proceedings of the National Academy of Sciences of the United States of America* 9(12): 424-428.

Cook, T.J. & R.E. Gold 1998. Organization of the symbiotic flagellate community in three castes of the eastern subterranean termite, *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *Sociobiology* 1(1): 25-39.

Deng, L., S. Krauss, J. Feitchmayer, R. Hofmann, H. Arndt & C. Griebler 2014. Grazing of heterotrophic flagellates on viruses is driven by feeding behavior. *Environmental Microbiology Reports* 6(4): 325-330.

Desai, M.S., J.F. Strassert, K. Meuser, H. Hertel, W. Ikeda-Ohtsubo, R. Radek & A. Brune 2010. Strict cospeciation of devescovinid flagellates and *Bacteroidales* ectosymbionts in the gut of dry-wood termites (Kalotermitidae). *Environmental Microbiology* 12(8): 2120-2132.

Dolan, M.F., A.W. Wier & L. Margulis 2000. Budding and asymmetric reproduction of a trichomonad with as many as 1000 nuclei in karyomastigonts: *Metacoronympha* from *Incisitermes*. *Acta Protozoologica* 39: 275-280.

Donovan, S.E., P. Eggleton & D.E. Bignell 2001. Gut content analysis and a new feeding group classification of termites. *Ecological Entomology* 26: 356-366.

Douglas, A.E. 2007. Symbiotic microorganisms: untapped resources for insect pest control. *Trends in Biotechnology* 25(8): 338-342.

Du, X., X. Li, Y. Wang, J. Peng, H. Hong & H. Yang 2012. Phylogenetic diversity of nitrogen fixation genes in the intestinal tract of *Reticulitermes chinensis* Snyder. *Current Microbiology* 65: 547-551.

Duarte, S., M. Duarte, P.A.V. Borges & L. Nunes 2016. Dietary-driven variation effects on the symbiotic flagellate protist communities of the subterranean termite *Reticulitermes grassei* Clément. *Journal of Applied Entomology*. DOI:10.1111/jen.12331

Eggleton, P. 2011. An introduction to termites: biology, taxonomy and functional morphology. Pp. 1-26 in: Bignell, D.E., Y. Roisin & N. Lo (Eds*). Biology of termites: A modern synthesis.* London, New York: Springer Science+Business Media BV. 576 pp.

Eggleton, P. & I. Tayasu 2001. Feeding groups, lifetypes and the global ecology of termites. *Ecological Research* 16: 941-960.

Engel, M.S., D.A. Grimaldi & K. Krishna 2009. Termites (Isoptera): Their phylogeny, classification, and rise to ecological dominance. *American Museum Novitates* 3650: 1-27.

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

Evans, T.A. & N. Iqbal 2014. Termite (order Blattodea, infraorder Isoptera) baiting 20 years after commercial release. *Pest Management Science* Sep 22. Available from: http://onlinelibrary.wiley.com/doi/10.1002/ps.3913/abstract

Ferreira, M.T., P.A.V. Borges, L. Nunes, T.G. Myles, O. Guerreiro & R.H. Scheffrahn 2013. Termites (Isoptera) in the Azores: an overview of the four invasive species currently present in the archipelago. *Arquipelago*.Life and Marine Sciences 30: 39-55.

Gerbod, D., C. Nöel, M.F. Dolan, V.P. Edgecomb, O. Kitade, S. Noda, F. Dufernez, M. Ohkuma, T. Kudo, M. Capron, M.L. Sogin & E. Viscogliosi 2002. Molecular phylogeny of parabasalids inferred from small subunit rRNA sequences, with emphasis on Devescovinidae and Calonymphidae (Trichomonadea*). Molecular Phylogenetics and Evolution* 25: 545-556.

Ghidini, G.M. 1942. Studi sulle termiti: 11º - Le *Trichonympha* di *Reticulitermes lucifugus* Rossi. *Rivista di Biologia Coloniale* 5: 19-40.

Grassé, P.-P. 1982. *Termitologia. Anatomie, Physiologie, Biologie, Systématique des termites. Tome I: Anatomie, Physiologie, Reproduction*. Paris, France, Fondation Singer-Polignac, Masson. 676 pp.

Guerreiro, O., P. Cardoso, J.M. Ferreira, M.T. Ferreira & P.A.V. Borges 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.

Harper, J.T., G.H. Gile, E.R. James, K.J. Carpenter & P.J. Keeling 2009. The inadequacy of morphology for species and genus delineation in microbial eukaryotes: an example from the parabasalian termite symbiont *Coronympha*. *PLoS ONE* 4(8): e6577. Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0006577

He, S., N. Ivanova, E. Kirton, M. Allgaier, C. Bergin, R.H. Scheffrahn, N. Kyrpides, F. Warnecke, S. Tringe & P. Hugenholtz 2013. Comparative metagenomic and metatranscriptomic analysis of hindgut paunch microbiota in wood- and dung-feeding higher termites. *PLoS ONE* 8(4): e61126. Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0061126

Helle, S.S., A. Murray, J. Lam, D.R. Cameron & S.J.B. Duff 2004. Xylose fermentation by genetically modified *Saccharomyces cerevisiae* 259ST in spent sulfite liquor. *Bioresource Technology* 92: 163-171.

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

Hongoh, Y., P. Deevong, T. Inoue, S. Moriya, S. Trakulnaleamsai, M. Ohkuma, C. Vongkaluang, N. Noparatnaraporn & T. Kudo 2005. Intra- and interspecific comparisons of bacterial diversity and community structure support coevolution of gut microbiota and termite host. *Applied and Environmental Microbiology* 71(11): 6590-6599.

Honigberg, B.M., C.F.T. Maltern & W.A. Daniel 1971. Fine Structure of the Mastigont System in *Trichomas foetus* (Riedmüller). *Eukaryotic Microbiology* 18(2):183-198.

Hu, X.P. 2008. Starvation-associated mortality, cannibalism, body weight, and intestinal symbiotic protist profile of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Pp. 365-371 in: Robinson, W.H. & D. Bajomi (Eds). *Proceedings of the Sixth International Conference on Urban Pests*. Budapest, Hungary, OOK-Press Kft. 477 pp.

Hu, X.P., D. Song & X. Gao 2011. Biological changes in the Eastern subterranean termite, *Reticulitermes flavipes* (Isoptera, Rhinotermitidae) and its protozoa profile following starvation. *Insectes Sociaux* 58: 39-45.

Husseneder, C. 2010. Symbiosis in subterranean termites: A review of insights from molecular studies. *Environmental Entomology* 39(2): 378-388.

Husseneder, C., A. Sethi, L. Foil & J. Delatte 2010. Testing protozoacidal activity of ligand-lytic peptides against termite gut protozoa in vitro (Protozoa culture) and in vivo (Microinjection into termite hindgut). *Journal of Visualized Experiments* 46: e2190. Available from: http://www.jove.com/video/2190/testing-protozoacidal-activity-ligand-lytic-peptides-against-termite

Ikeda-Ohtsubo, W. & A. Brune 2009. Cospeciation of termite gut flagellates and their bacterial endosymbionts: *Trichonympha* species and '*Candidatus Endomicrobium trichonymphae'*. *Molecular Ecology* 18: 332-342.

Ikeda-Ohtsubo, W., M. Desai, U. Stingl & A. Brune 2007. Phylogenetic diversity of 'Endomicrobia' and their specific affiliation with termite gut flagellates. *Microbiology* 153: 3458-3465.

Inoue, T., K. Murashima, J.I. Azuma, A. Sugimoto & M. Slaytor 1997. Cellulose and xylan utilisation in the lower termite *Reticulitermes speratus. Journal of Insect Physiology* 43: 235-242.

Inward, D., G. Beccaloni & P. Eggleton 2007a. Death of an order: a comprehensive molecular phylogenetic study confirms that termites are eusocial cockroaches. *Biology Letters* 3: 331-335.

Inward, D., A.P. Vogler & P. Eggleton 2007b. A comprehensive phylogenetic analysis of termites (Isoptera) illuminates key aspects of their evolutionary biology. *Molecular Phylogenetics and Evolution* 44: 953-967.

Itakura, S., S. Murayama, Y. Kamata, H. Tanaka & A. Enoki 2009. RNA interference in symbiotic protists of the termite *Coptotermes formosanus* (Isoptera: Rhinotermitidae) through ingestion of siRNA by the host termite. *Sociobiology* 54(1): 77-87.

James, E.R., V. Tai, R.H. Scheffrahn & P.J. Keeling 2012. *Trichonympha burlesquei* n. sp. from *Reticulitermes virginicus* and evidence against a cosmopolitan distribution of *Trichonympha agilis* in many termite hosts. *International Journal of Systematic and Evolutionary Microbiology* 63(10): 3873-3876.

James, E.R., N. Okamoto, F. Burki, R.H. Scheffrahn & P.J. Keeling 2013. *Cthulhu macrofasciculumque* n. g., n. sp. and *Cthylla microfasciculumque* n.g., n. sp., a newly identified lineage of parabasalian termite symbionts. *PLoS ONE* 8(3): e58509. Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0058509

Jenkins, T.M., R.E. Dean, R. Verkerk & B.T. Forschler 2001. Phylogenetic analyses of two mitochondrial genes and one nuclear intron region illuminate European subterranean termite (Isoptera: Rhinotermitidae) gene flow, taxonomy, and introduction dynamics. *Molecular Phylogenetics and Evolution* 20(2): 286-293.

Jouquet, P., S. Traoré, C. Choosai, C. Hartmann & D.E. Bignell 2011. Influence of termites on ecosystem functioning. Ecosystem services provided by termites. *European Journal of Soil Biology* 47: 215-222.

Katzin, L. & H. Kirby 1939. The relative weights of termites and their protozoa. *Journal of Parasitology* 5: 444-445.

Ke, J., D. Singh & S.-L. Chen 2012. Metabolism of polycyclic aromatic hydrocarbons by the wood-feeding termite *Coptotermes formosanus* (Shiraki). *Journal of Agricultural and Food Chemistry* 60: 1788-1797.

King, J.H.P., N.M. Mahadi, J.C.F. Bong, K.H. Ong & O. Hassan 2013. Bacterial microbiome of *Coptotermes curvignathus* (Isoptera: Rhinotermitidae) reflects the coevolution of species and dietary pattern. *Insect Science* 21(5): 584-596.

Kitade, O. 2004. Comparison of symbiotic flagellate faunae between termites and a wood-feeding cockroach of the genus *Cryptocercus. Microbes and Environments* 19(3): 215-220.

Kitade, O., Y. Hayashi, K. Takatsuto & T. Matsumoto, 2012. Variation and diversity of symbiotic protist composition in the damp-wood termite *Hodotermopsis sjoestedti. Japanese Journal of Protozoology* 45(1,2): 29-36.

König, H., L. Li & J. Fröhlich 2013. The cellulolytic system of the termite gut. *Applied Microbiology and Biotechnology* 97: 7943-7962.

Krishna, K., D.A. Grimaldi, V. Krishna & M.S. Engel 2013. Treatise on the Isoptera of the world - Introduction. *Bulletin of the American Museum of Natural History* 377(1): 1-202.

Kudo, R.R. 1939. *Protozoology.* 5th Ed. USA, Charles C. Thomas Publisher. 716 pp.

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

Lal, R. 2004. Soil carbon sequestration to mitigate climate change. *Geoderma* 123: 1-22.

Lawton, J.H., D.E. Bignell, G.F. Bloemers, P. Eggleton & M.E. Hodda 1996. Carbon flux and diversity of nematodes and termites in Cameroon forest soils. *Biodiversity and Conservation* 5: 261-273.

Lee, S.H. & T.S. Chon 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.

Lelis, A. 1992. The loss of intestinal flagellates in termites exposed to the juvenile hormone analogue (JHA) - methoprene. *Material und Organismen* 27(3): 172-178.

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

Lewis, J.L. & B.T. Forschler 2010. Impact of five commercial baits containing chitin synthesis inhibitors on the protist community in *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *Environmental Entomology* 39(1): 96-104.

Lo, N., G. Tokuda, H. Watanabe, H. Rose, M. Slaytor, K. Maekawa, C. Bandi & H. Noda 2000. Evidence from multiple gene sequences indicates that termites evolved from wood-feeding cockroaches. *Current Biology* 10: 801-804.

Matsuura, K. 2001. Nestmate recognition mediated by intestinal bacteria in a termite, *Reticulitermes speratus*. *Oikos* 92: 20-26.

Mauldin, J.K., F.L. Carter & N.M. Rich 1981. Protozoan populations of *Reticulitermes flavipes* exposed to heartwood blocks of 21 American species. *Material und Organismen* 16(1): 15-28.

Mauldin, J.K., N. Rich & D. Cook 1977. Cellulose catabolism and lipid synthesis by normally and abnormally faunated termites *Reticulitermes flavipes. Insect Biochemistry* 7: 27-31.

Mello, F. 1920. Considération sur les Trychonymphides de l'intestin de l'*Archotermopsis wroughtoni* Desn., étudiés par le Dr. A. Imms. *Bulletin de la Société Portugaise des Sciences Naturelles* Tome VIII: 189-198.Moriya, S., M. Ohkuma & T. Kudo 1998. Phylogenetic position of symbiotic protist *Dinenympha exilis* in the hindgut of the termite *Reticulitermes speratus* inferred from the protein phylogeny of elongation factor 1α. *Gene* 210: 221-227.

Nalepa, C.A. 2015. Origin of termite eusociality: trophallaxis integrates the social, nutritional, and microbial environments. *Ecological Entomology* 40: 323-335.

Nalepa, C.A., D.E. Bignell & C. Bandi 2001. Detritivory, coprophagy, and the evolution of digestive mutualisms in Dictyoptera. *Insectes Sociaux* 48: 194-201.

Nobre, T. & L. Nunes 2007. Non-traditional approaches to subterranean termite control in buildings. *Wood Material Science & Engineering* 3-4: 147-156.

Nobre, T. & D.K. Aanen 2012. Fungiculture or termite husbandry? The ruminant hypothesis. *Insects* 3: 307-323.

Noda, S., Y. Hongoh, T. Sato & M. Ohkuma, 2009. Complex coevolutionary history of symbiotic *Bacteroidales* bacteria of various protists in the gut of termites. BMC *Evolutionary Biology* 9: 158. Available from: http://bmcevolbiol.biomedcentral.com/articles/10.1186/1471-2148-9-158

Noda, S., O. Kitade, T. Inoue, M. Kawai, M. Kanuka, K. Hiroshima, M. Kanuka, K. Hiroshima, Y. Hongoh, R. Constantino, V. Uys, J. Zhong, T. Kudo & M. Ohkuma 2007. Cospeciation in the triplex symbiosis of the termite gut protists (*Pseudotrichonympha* spp.), their hosts, and their bacterial endosymbionts. *Molecular Ecology* 16: 1257-1266.

Ohkuma, M. 2003. Termite symbiotic systems: efficient bio-recycling of lignocellulose. *Applied Microbiology and Biotechnology* 61: 1-9.

Ohkuma, M. 2008. Symbioses of flagellates and prokaryotes in the gut of lower termites. *Trends in Microbiology* 16(7): 345-352.

Otani, S., A. Mikaelyan, T. Nobre, L.H. Hansen, N.A. Koné, S.J. Sorensen, D.K. Aanen, J.J. Boomsma, A. Brune & M. Poulsen 2014. Identifying the core gut microbiome of fungus-growing termites. *Molecular Ecology* 23: 4631-4644.

Palin, O.F., P. Eggleton, Y. Malhi, C.A.J. Girardin, A. Rozas-Dávila & C.L. Parr 2011. Termite diversity along an Amazon-Andes elevation gradient, Peru. *Biotropica* 43(1): 100-107.

Peterson, C.J. 2010. Termites and climate change: Here, there and everywhere? *EARTH* - The Science Behind the Headlines. Available from: http://www.earthmagazine.org/article/termites-and-climate-change-here-there-and-everywhere

Peterson, B.F., H.L. Stewart & M.E. Scharf 2015. Quantification of symbiotic contributions to lower termite lignocellulose digestion using antimicrobial treatments. *Insect Biochemistry and Molecular Biology* 59: 80-88.

Poulsen, M., H.F. Hu, C. Li, Z.S. Chen, L.H. Xu, S. Otani, S. Nygaard, T. Nobre, S. Klaubauf, V. Lombard, P.M. Schindler, F. Hauser, H. Pan, Z. Yang, A. Sonnenberg, Z.W. de Beer, Y. Zhang, M.J. Wingfield, B. Henrissat, C. Grimmelikhuijzen, R.P. de Vries, J. Korb, D.K. Aanen, J. Wang, J.J. Boomsma & G. Zhang 2014. Complementary symbiont contributions to plant decomposition in a fungus-farming termite. *Proceedings of the National Academy of Sciences of the United States of America* 111(40): 14500-14505.

Radek, R. 1999. Flagellates, bacteria, and fungi associated with termites: diversity and function in nutrition - a review. *Ecotropica* 5: 183-196.

Radek, R., K. Hausmann & A. Breunig 1992. Ectobiotic and endobiotic bacteria associated with the termite flagellate *Joenia annectens*. *Acta Protozoologica* 31: 93-107.

Radek, R., J.F.H. Strassert, J. Krüger, K. Meuser, R.H. Scheffrahn & A. Brune 2014. Phylogeny and ultrastructure of *Oxymonas jouteli*, a rostellum-free species, and *Opisthomitus longiflagellatus* sp.nov., oxymonadid flagellates from the gut of *Neotermes jouteli. Protist* 165: 384-399.

Ragauskas, A.J., C.K. Williams, B.H. Davison, G. Britovsek, J. Cairney, C.A. Eckert, W.J. Frederick Jr., J.P. Hallett, D.J. Leak, C.L. Liotta, J.R. Mielenz, R. Murphy, R. Templer & T. Tschaplinski 2006. The path forward for biofuels and biomaterials. *Science* 311(5760): 484-489.

Rangberg, A., D.B. Diep, K. Rudi & G.V. Amdam 2012. Paratransgenesis: An approach to improve colony health and molecular insight in honey bees (*Apis mellifera*)? *Integrative and Comparative Biology* 52(1): 89-99.

Raychoudhury, R., R. Sen, Y. Cal, Y. Sun, V.U. Lietze, D.G. Boucias & M.E. Scharf 2013. Comparative metatranscriptomic signatures of wood and paper feeding in the gut of termite *Reticulitermes flavipes* (Isoptera: Rhinotermitidae*). Insect Molecular Biology* 22(2): 155-171.

Rosenberg, E. & I. Zilber-Rosenberg 2013. Role of microorganisms in adaptation, development, and evolution of animals and plants: the hologenome concept. Pp. 347-358 in: Rosenberg E. & I. Zilber-Rosenberg (Eds). *The prokaryotes – Prokaryotic biology and symbiotic associations*. Switzerland, Springer International Publishing. 607 pp.

Rosengaus, R.B., C.N. Zecher, K.F. Schultheis, R.M. Brucker & S.R. Bordenstein 2011. Disruption of the termite gut microbiota and its prolonged consequences for fitness. *Applied and Environmental Microbiology* 77(13): 4303-4312.

Rouland-Lefèvre, C. 2011. Termites as pests of agriculture. Pp. 499-517 in: Bignell, D.E., Y. Roisin & N. Lo (Eds). *Biology of termites: A modern synthesis.* London, New York, Springer Science+Business Media B.V. 576 pp.

Rust, M. & N.-Y. Su 2012. Managing social insects of urban importance. *Annual Review of Entomology* 57: 355-375.

Salem, H., E. Bauer, A.S. Strauss, H. Vogel, M. Marz & M. Kaltenpoth 2014. Vitamin supplementation by gut symbionts ensures metabolic homeostasis in an insect host. *Proceedings of the Royal Society of London B: Biological Sciences* 281: 20141838. Available from: http://rspb.royalsocietypublishing.org/content/281/1796/20141838

Sato, T., H. Kuwahara, K. Fujita, S. Noda, K. Kihara, A. Yamada, M. Ohkuma & Y. Hongoh 2014. Intranuclear verrucomicrobial symbionts and evidence of lateral gene transfer to the host protist in the termite gut. *The ISME Journal* 8: 1008-1019.

Scharf, M.E. 2015. Termites as targets and models for biotechnology. *Annual Review of Entomology* 60: 77-102.

Scharf, M.E. & A. Tartar 2008. Termite digestomes as sources for novel lignocellulases. *Biofuels, Bioproducts and Biorefining* 2(6): 540-552.

Scharf, M.E. & D.G. Boucias 2010. Potential of termite-based biomass pre-treatment strategies for use in bioethanol production. *Insect Science* 17: 166-174.

Scharf, M.E., Z. Karl, A. Sethi & D.G. Boucias 2011a. Multiple levels of sinergistic collaboration in termite lignocellulose digestion. *PloS ONE* 6(7): e21709. Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0021709

Scharf, M.E., X. Zhou, F. Oi, M.M. Wheeler, M.R. Tarver & M.R. Coy 2011b. Regulating termite colony development and decreasing the fitness of a termite colony, with a food source comprises feeding double stranded RNA corresponding to hexamerin-1 (Hex-1) or Hex-2 genes to the termites in the colony. *US Patent US7968525-B1*.

Sen, R., R. Raychoudhury, Y. Cai, Y. Sun, V.-U. Lietze, B.F. Peterson, M.E. Scharf & D.G. Boucias 2015. Molecular signatures of nicotinoid-pathogen synergy in the termite gut. *PLoS ONE* 10(4): e0123391. Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0123391

Sethi, A., J. Delatte, L. Foil & C. Husseneder 2014. Protozoicidal trojan-horse: use of a ligand-lytic peptide for selective destruction of symbiotic protozoa within termite guts. *PLoS ONE* 9(9): e106199. Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0106199

Shimada, K., N. Lo, O. Kitade, A. Wakui & K. Maekawa 2013. Cellulolytic protist numbers rise and fall dramatically in termite queens and kings during colony foundation. *Eukaryotic Cell* 12(4): 545-550.

Slaytor, M. 1992. Cellulose digestion in termites and cockroaches: What role do symbionts play? *Comparative Biochemistry and Physiology* 103(4): 775-784.

Sleaford, F., D.E. Bignell & P. Eggleton 1996. A pilot analysis of gut contents in termites from the Mbalmayo Forest Reserve, Cameroon. *Ecological Entomology* 21: 279-288.

Stingl, U. & A. Brune 2003. Phylogenetic diversity and whole-cell hybridization of oxymonad flagellates from the hindgut of the wood-feeding lower termite *Reticulitermes flavipes. Protist* 154: 147-155.

Stingl, U., R. Radek, H. Yang & A. Brune 2005. "Endomicrobia": Cytoplasmatic symbionts of termite gut protozoa form a separate phylum of prokaryotes. *Applied and Environmental Microbiology* 71(3): 1473-1479.

Su, N.-Y. & R.H. Scheffrahn 2000. Termites as pests of buildings. Pp. 437-453 in: Abe, T., D.E. Bignell & M. Higashi (Eds). *Termites: Evolution, Sociality, Symbioses, Ecology.* The Netherlands, Kluwer Academic Publishers, Dordrecht. 466 pp.

Sugimoto, A., D.E. Bignell & J.A. Macdonald 2000. Global impact of termites on the carbon cycle and atmospheric trace gases. Pp. 409-435 in: Abe, T., D.E. Bignell & M. Higashi (Eds). *Termites: Evolution, Sociality, Symbioses, Ecology*. The Netherlands, Kluwer Academic Publishers, Dordrecht. 466 pp.

Sun, J.-Z. & M.E. Scharf 2010. Exploring and integrating cellulolytic systems of insects to advance biofuel technology. *Insect Science* 17: 163-165.

Sun, Q., K.F. Haynes & X. Zhou 2013. Differential undertaking response of a lower termite to congeneric and conspecific corpses. *Scientific Reports* 3: 1650. Available from: http://www.nature.com/articles/srep01650

Tai, V., E.R. James, S. Perlman & P.J. Keeling 2013. Single-cell DNA barcoding using sequences from the small subunit rRNA and Internal Transcribed Spacer region identifies new species of *Trichonympha* and *Trichomitopsis* from the hindgut of the termite *Zootermopsis angusticollis. PLoS ONE* 8(3): e58728. Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0058728

Tai, V., E.R. James, C.A. Nalepa, R.H. Scheffrahn, S.J. Perlman & P.J. Keeling 2015. The role of host phylogeny varies in shaping microbial diversity in the hindguts of lower termites. *Applied and Environmental Microbiology* 81: 1059-1070.

Tamschick, S. & R. Radek 2013. Colonization of termite hindgut walls by oxymonad flagellates and prokaryotes in *Incisitermes tabogae*, *I. marginipennis* and *Reticulitermes flavipes. European Journal of Protistology* 49: 1-14.

Thong-On, A., K. Suzuki, S. Noda, J.-I. Inoue, S. Kajiwara & M. Ohkuma 2012. Isolation and characterization of anaerobic bacteria for symbiotic recycling of uric acid nitrogen in the gut of various termites. *Microbes and Environments* 27(2): 186-192.

Todaka, N., S. Moriya, K. Saita, T. Hondo, I. Kiuchi, H. Takasu, M. Ohkuma, C. Piero, Y. Hayashizaki & T. Kudo 2007. Environmental cDNA analysis of the genes involved in lignocellulose digestion in the symbiotic protist community of *Reticulitermes speratus. FEMS Microbiology Ecology* 59: 592-599.

Tsukagoshi, H., A. Nakamura, T. Ishida, M. Otagiri, S. Moriya, M. Samejima, K. Igarashi, K. Kitamoto & M. Arioka 2014. The GH26 β-mannanase RsMan26H from a symbiotic protist of the termite *Reticulitermes speratus* is an endo-processive mannobiohydrolase: Heterologous expression and characterization. *Biochemical and Biophysical Research Communications* 452: 520-525.

Ugelvig, L.V. & S. Cremer 2012. Effects of social immunity and unicoloniality on host-parasite interactions in invasive insect societies*. Functional Ecology* 26: 1300-1312.

Warnecke, F., P. Luginbühl, N. Ivanova, M. Ghassemian, T.H. Richardson, J.T. Stege, M. Cayouette, A.C. McHardy, G. Djordjevic, N. Aboushadi, R. Sorek, S.G. Tringe, M. Podar, H.G. Martin, V. Kunin, D. Dalevi, J. Madejska, E. Kirton, D. Platt, E. Szeto, A. Salamov, K. Barry, N. Mikhailova, N.C. Kyrpides, E.G. Matson, E.A. Ottesen, X. Zhang, M. Hernández, C. Murillo, L.G. Acosta, I. Rigoutsos, G. Tamayo, B.D. Green, C. Chang, E.M. Rubin, E.J. Mathur, D.E. Robertson, P. Hugenholtz & J.R. Leadbetter 2007. Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature* 450: 560-569.

Watanabe, H. & G. Tokuda 2010. Cellulolytic systems in insects. *Annual Review of Entomology* 55: 609-632.

Watanabe, H., H. Noda, G. Tokuda & N. Lo 1998. A cellulase gene of termite origin. *Nature* 394: 330-331.

Xiang, H., L. Xie, J. Zhang, Y.-H. Long, N. Liu, Y.-P. Huang & Q. Wang 2012. Intracolonial differences in gut bacterial community between worker and soldier castes of *Coptotermes formosanus. Insect Science* 19: 86-95.

Xie, L., L. Zhang, Y. Zhong, N. Liu, Y. Long, S. Wang, X. Zhou, Z. Zhou, Y. Huang & Q. Wang 2012. Profiling the metatranscriptome of the protistan community in *Coptotermes* *formosanus* with emphasis on the lignocellulolyctic system. *Genomics* 99: 246-255.

Yamin, M.A. 1979. Flagellates of the orders Trichomonadida Kirby, Oxymonadida Grassé, and Hypermastigida Grassi & Foà reported from lower termites (Isoptera families Mastotermitidae, Kalotermitidae, Hodotermitidae, Termopsidae, Rhinotermitidae, and Serritermitidae) and from the wood-feeding roach *Cryptocercus* (Dictyoptera: Cryptocercidae). *Sociobiology* 4: 5-119.

Yamin, M.A. 1980. Cellulose metabolism by the termite flagellate *Trichomitopsis termopsidis. Applied and Environmental Microbiology* 39(4): 859-863.

Yamin, M.A. & W. Trager 1979. Cellulolytic activity of an axenically-cultivated termite flagellate, *Trichomitopsis termopsidis. Journal of General Microbiology* 113: 417-420.

Yang, B. & C.E. Wyman 2008. Pretreatment: the key to unlocking low-cost cellulosic ethanol. *Biofuels, Bioproducts and Biorefining* 2: 26-40.

Yoshimura, T., T. Fujino, K. Tsunoda & M. Takahashi 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.

Zhang, D., A.R. Lax, J.M. Bland, J. Yu, N. Fedorova & W.C. Nierman 2010. Hydrolysis of filter-paper cellulose to glucose by two recombinant endogenous glycosyl hydrolases of *Coptotermes formosanus. Insect Science* 17: 245-252.

Zheng, H., C. Dietrich, C.L. Thompson, K. Meuser & A. Brune 2015. Population structure of *Endomicrobia* in single host cells of termite gut flagellates (*Trichonympha* spp.). *Microbes and Environments* 30(1): 92-98.

Zhou, X., M.M. Wheeler, F.M. Oi & M.E. Scharf 2008. RNA interference in the termite *Reticulitermes flavipes* through ingestion of double-stranded RNA. *Insect Biochemistry and Molecular Biology* 38: 805-815.

Zimmermann, P.R., J.P. Greenberg, S.O. Wandinga & P.J. Crutzen 1982. Termites: A potentially large source of atmospheric methane, carbon dioxide and molecular hydrogen. *Science* 218(5): 563-565. *6.*

Received 21 Sep 2016. Accepted 26 Sep 2016.

ANNEX 1 – SUPPLEMENTARY MATERIAL

Supplementary Table 1.

Description of the internal structures and body of the flagellate protists living inside lower termites and belonging to the phyla Parabasalia and Preaxostyla (order Oxymonadida). Adapted from: Honigberg et al.(1971); Brugerolle & Lee(2000); Brugerolle & Radek(2006); Čepička et al. (2010); Radek et al. (2014).

Structure	Description/Location
Centrioles	pair of organelles that form the core of the centrosome located near to the nucleus
Centrosome	structure which function as a microtubulus organizing centre located near to the nucleus
Basal bodies or kinetosomes	modified centrioles that give rise to cilia and flagella below the cell membrane
Mastigont or kinetid	kinetosome(s) with its cytoskeletal root system below the cell membrane
Karyomastigont	mastigont plus its own associated nucleus
Axostyle	microtubular structure which forms the cell axis, made of sheets of microtubules spiralized, or lying in parallel layers (oxymonads), to a hollow or filled tube or cone
Pelta	second microtubular sheet covering the anterior end of the cell
Recurrent flagellum (RF)	backwardly directed flagellum, running posteriorly over the body of the flagellate in a loose or attached state; when attached it often becomes part of an undulating membrane
Undulating membrane	RF adherent to a projection of the cytoplasmatic membrane (typical arrangement), or the projection may also be the flagellum itself adjacent to the cytoplasmatic membrane
Costa	non-microtubular striated fiber, type A or B according with the pattern of striation underlain the undulating membrane specific for Parabasalia
Cresta	fibrillar, noncontractile structure, with subtriangular shape located below the basal portion of the trailing flagellum characteristic of devescovinid flagellates
Parabasal body	modified Golgi dictyosome characteristic of the Parabasalia
Parabasal apparatus	complex consisting of a parabasal bodies attached to striated fibers
Rostrum	the apical end or tip of a protozoan body, when its shape is that of a beak is some other sort of distinctive protuberance in that area of the body anterior part of the cell
Flagellar apparatus	basal bodies of the flagella and their connected roots
Holdfast	any structure by which a given organism can attach, temporary or permanently, to some living or inanimate substrate
Infrakinetosomal body (IFK)	large, dense structure below the basal body complex
Suprakinetosomal body	crescent-shaped structure anterior to, and connecting, with kinetosome no.2 associated with IFK
Comb-like structure	periodic structure, comb-shaped extends between costa and IFK