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Symbiogenesis: the holobiont as a unit of evolution

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Summary. Symbiogenesis is the result of the permanent coexistence of various bionts to form the holobiont (namely, the host and its microbiota). The holobiome is the sum total of the component genomes in a eukaryotic organism; it comprises the genome of an individual member of a given taxon (the host genome) and the microbiome (the genomes of the symbiotic microbiota). The latter is made up of the genes of a variety of microbial communities that persist over time and are not eliminated by natural selection. Therefore, the holobiome can also be considered as the genomic reflection of the complex network of symbiotic interactions that link an individual member of a given taxon with its associated microbiome. Eukaryotic individuals can be analyzed as coevolved, tightly integrated, prokaryotic communities; in this view, natural selection acts on the holobiont as if it were an integrated unit. The best studied holobionts are those that emerged from symbioses involving insects. The presence of symbiotic associations throughout most of the evolutionary history of insects suggests that they were a driving force in the diversification of this group. Support for the evolutionary importance of symbiogenesis comes from the observation that the gradual passage from an ancestral to a descendant species by the accumulation of random mutations has not been demonstrated in the field, nor in the laboratory, nor in the fossil record. Instead, symbiogenesis expands the view of the point-mutation-only as the unique mechanisms of evolution and offers an explanation for the discontinuities in the fossil record ("punctuated equilibrium"). As such, it challenges conventional paradigms in biology. This review describes the relationships between xylophagous insects and their microbiota in an attempt to understand the characteristics that have determined bacterial fidelity over generations and throughout evolutionary history. [**Int Microbiol** 2013; 16(3):133-143]

Keywords: symbiogenesis · symbiosis · holobiont · holobiome · microbiota · microbiome · coevolution

Introduction

Symbiosis was an essential evolutionary mechanism in the origin of the eukaryotic cell [33,34]. The first living beings were prokaryotes. In fact, bacteria are the only organisms that are not dependent on others for their survival; on the contrary, they are necessary for the survival of other living beings. The mechanism that made it possible for a group of randomly gathered compounds to become autopoietic—that is, to form a distinct entity separated from the environment by a boundary and able to both maintain itself actively and autoreplicate [19,20,36]—has yet to be identified. The autopoietic unit, whether a bacterial biont (minimal autopoietic unit) or a holobiont (integrated biont organisms, i.e., animals or plants, with all of their associated microbiota), is capable of self-maintenance by sensing the environment and is able to adapt to new circumstances. Complex autopoietic units acquire novel properties when the assembly of their components results in higher functional-structural complexity (Fig. 1). However,

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Fig. 1. *Endosymbiosis: Homage to Lynn Margulis*, a painting by Shoshanah Dubiner. A six-foot wide reproduction of the painting occupies a hallway in the Morrill Science Center at the University of Massachusetts-Amherst, MA, USA where Lynn Margulis was a Distinguished Professor from 1988 until her death in 2011. (Image courtesy of the artist [http://www.cybermuse.com].)

autopoiesis alone, while necessary, is not a sufficient condition for life. Rather, living organisms constantly interact with their habitats, selectively taking up compounds from their surroundings to meet their particular needs but also excreting metabolic products and thus modifying their environment.

The term "symbiosis" was coined by Heinrich Anton de Bary (1831–1888) to describe the living together of "differently named organisms" [12]. Symbiosis is a long-term physical association of two or more partners, and symbiotic relationships can only occur under certain environmental conditions. In endosymbiosis, a topological condition, one partner lives inside the other. Symbiogenesis refers to the appearance of new morphologies, tissues, metabolic pathways, behaviors, or other recognizable evolutionary novelties in holobionts. The term was first introduced into the literature by the Russian Konstantin Sergeivich Mereschkovsky (1855–1921) and is equivalent to "symbionticism" or "microsymbiotic complexes", both of which were independently coined by the Swedish-American Ivan Emanuel Wallin (1883−1969) [56].

Evolutionary biologists have viewed mutations within individual genes as the major source of phenotypic variation. Mutations lead to adaptation through natural selection and ultimately generate diversity among species. Symbiogenesis, however, is another mechanism able to drive evolutionary innovation, as the holobiont is better adapted to the environment than its individual components. Joshua Lederberg (1925–2008) defined the holobiont as "the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space:" [28]. As such, the holobiome, i.e., the assembly of genetic information contributed by the animal or plant and its associated microbiota, is an essential aspect of the evolving holobiont. It is an essential life-changing force that has resulted in a complex coordinated coevolution of life forms.

Symbiogenesis could provide support for the theory of punctuated equilibrium proposed by Niles Eldredge and Stephen J. Gould [16], based on their observation that according to the fossil record evolution is largely static but then acts suddenly and often dramatically during brief (in geological terms) intervals of time, as evidenced by the periodic large changes in fossil registration. In evolving holobionts, symbiogenesis confers cellular-tissue, and organ-level developmental and morphological complexity [37,47]. Accordingly, a unified theory of evolution can be considered

in which the nuclear genome (of the eukaryotic component) and the microbiome (the genetic donation of the symbiotic microorganisms) are the interacting components that give rise to new species and varieties thereof.

Support for this view comes from the following: First, microbial symbionts are universally present in eukaryotes, and prokaryotic microorganisms are widespread in all environments on Earth. Consistent with their ecological ubiquity, many prokaryotic species establish close and in many cases persistent relationships with members of a wide range of eukaryotic taxa [20,60]. Second, hosts typically have strong specificity for microbial symbionts and their functions. Third, symbiotic relationships have enhanced the limited metabolic networks of most eukaryotes by contributing several prokaryotic metabolic capabilities, such as methanogenesis, chemolithoautotrophy, nitrogen assimilation, and essential-nutrient anabolism [17]. Also, many prokaryotes defend their symbiotic partners against natural enemies and promote their adaptation to specific ecological conditions [45,46]. Others may have parasitic or pathogenic effects on their hosts, causing attenuated host fitness and aberrations in reproduction [58]. Finally, host immune genes evolve rapidly in response to microbial symbionts and as a gene family are frequently involved in hybrid incompatibilities [4,5].

This review examines the characteristics that determine bacterial fidelity to certain groups of animals over generations and throughout evolution, by examining the relationships between two xylophagous insects (namely, the termite *Reticulitermes grassei*, and the social cockroach *Cryptocercus punctulatus*) and their microbiota (Fig. 2).

The role of hindgut microbes: the case of Dictyoptera

Insects account for most of the species-richness of the animal clade. Within this group, termites (Isoptera), cockroaches, and mantids form a well-established lineage of insects, the Dictyoptera. In termites, the omnivorous family Termitidae comprises 80 % of all termite species, and the six remaining wood-eating families (Mastotermitidae, Kalotermitidae, Hodotermitidae, Termopsidae, Rhinotermitidae and Serritermitidae), often misleadingly called "lower termites," the remaining 20 %. Termites can be considered as "social cockroaches," with the family Cryptocercidae as their closest relative. Thus, "lower" termites share with wood-feeding cockroaches (family Cryptocercidae; Blattaria, Dictyoptera) the unusual ability to efective degradation of lignocellulosic plant materials [2]. Depending on the species, the food preferences of termites range from wood to leaves, humus, detritus, and herbivore dung. By degrading lignin, cellulose, and hemicelluloses to fermentable carbohydrates, the diverse populations of prokaryotes and flagellated protists that inhabit the intestines of all wood-feeding lower termites are indispensable to their respective hosts. In the Termitidae, whose digestive system typically lacks protists, intestinal cellulolytic activity is due either to prokaryotic microbiota or to fungi, grown by the termites themselves or endogenous to the termite intestine [42].

In most insects, mating is the only "social behavior" as females abandon their eggs after depositing them. Consequently, opportunities for the direct transfer of gut microbes between members of the same species are for the

Fig. 2. Termites (left) and xylophagous cockroaches (right) mantained in laboratory conditions (Photographs by M. Berlanga and R. Duro.)

most part limited, unlike, for example, mammals and birds, which have prolonged parent–offspring connections [17]. However, several insect species, including cockroaches, termites, ants, and some wasps and bees, show gregarious or social behaviors. Among social insects, termites, which undergo incomplete metamorphosis, display a diversified caste polyphenism. Termite workers, soldiers, reproductive adults, and undifferentiated immature forms cooperate within their communities in an integrated manner. Each caste plays a significant role within the colony. Reproductive adults maintain the population; soldiers protect the colony from invaders; workers (the most numerous life stage in a colony) build and maintain the galleries, take care of the larvae, and feed the other colony members. Workers transfer food stomodeally (by regurgitation) and/or proctodeally (by excretion with the hindgut contents) (Fig. 3). Both oral trophallaxis and coprophagy allow the direct or indirect transmission of microorganisms and thus promote the coevolution of specialized host-dependent symbionts [1,22,35]. Proctodeal trophallaxis (feeding) is also the means by which microorganisms are vertically transmitted from workers to other individuals of the colony. In many insects, the proctodeal part of the intestine, i.e., the hindgut, is shed during ecdysis.

Consequently, the re-establishment of the gut microbiota of newly molted termite workers and soldiers depends on the contributions of fellow workers [3]. While young soldiers, before developing large mandibles, can chew wood, adult soldiers, owing to their large mandibles, cannot. Nevertheless, adults are able to digest proctodeal wood particles that have already passed through the gut of workers and are thus partially digested. Therefore, in wood-eating termites, the gut of soldiers, like that of workers, harbors protists and bacteria throughout the insect life cycle [3]. Note that an unexplained finding is that the genus *Wolbachia* is totally absent from workers but rather abundant in soldiers [3]. Based on the 16S rRNA gene phylogenies of *Wolbachia*, there are eight major clades (A–H). Clades A and B include most of the parasitic *Wolbachia* found in arthropods. Clades C and D include the majority of the mutualistic *Wolbachia* present in filarial nematodes, and clades E–H include *Wolbachia* from various arthropods. For clades E–H, the effects on the host are currently unknown; clade F is notable in that its members infect arthropods—especially termites—, but also nematodes. Phylotypes obtained from soldiers of *R. grassei* were affiliated with clade F [3]. *Wolbachia* species are associated with four distinct reproductive phenotypes in a wide range of Arthropoda: feminization, parthenogenesis, male killing, and cytoplasmic incompatibility. However, nothing is known about possible phenotypes linked to *Wolbachia* in Isoptera [58].

Examination of the protist population dynamics in the species *Reticulitermes speratus* during colony foundation has shown that protist numbers increase dramatically in termite queens and kings during the first 50 days of the colony foundation, but then begin to decrease by day 100, finally having disappeared by day 400. Ultimately, both kings and queens lose their protists entirely and become completely dependent upon their offspring for feeding. Protists are abundant in soldiers from mature colonies but absent in neotenics. This probably reflects the feeding of soldiers by workers via proctodeal trophallaxis and of reproductive members of the colony via stomodeal trophallaxis [50].

Termites and the *Cryptocercus* gut microbiota

The basic structure of the digestive tract is similar across insects, although a diversity of modifications associated with adaptation to different feeding modes can be found. The insect gut has three primary regions, foregut, midgut, and hindgut [17]; the foregut and hindgut originate from the ectoderm and the midgut from the endoderm. The Malpighian tubules comprising the excretory system in some insects and other animals extend into the body cavity and absorb wastes, such as uric acid, which are sent to the anterior hindgut, from which this system developmentally derives [17,43]. Many herbivorous insects have a tubular hindgut with several dilated compartments that harbor a dense gut microbiota. In these dilated compartments, or "fermentation chambers," the prolonged residence time of food allows its degradation by microbial symbionts, a situation analogous to that in the rumen or colon of mammals. With their ability to digest the major structural polymers of plant cell walls (cellulose and hemicelluloses), microorganisms are an important supplement of the digestive capacities of their hosts. The fermentation products, mainly acetate and other short-chain fatty acids, are reabsorbed by the insect and contribute substantially to its nutrition [42,43]. Termite guts are axially and radially structured habitats containing many chemical microniches, reflecting the combination of host and microbial activities [7]. All insect guts are surrounded by tissues aerated by the insect's tracheal system. Oxygen penetrates the peripheral hindgut contents to a depth of up to 150–200 μm below the epithelium. The removal of oxygen by the respiratory activity of the gut microbiota creates a microoxic periphery around an anoxic center [6]. In the case of termites, whose abdomen can be less than 1 mm wide, the redox potential forms an extreme
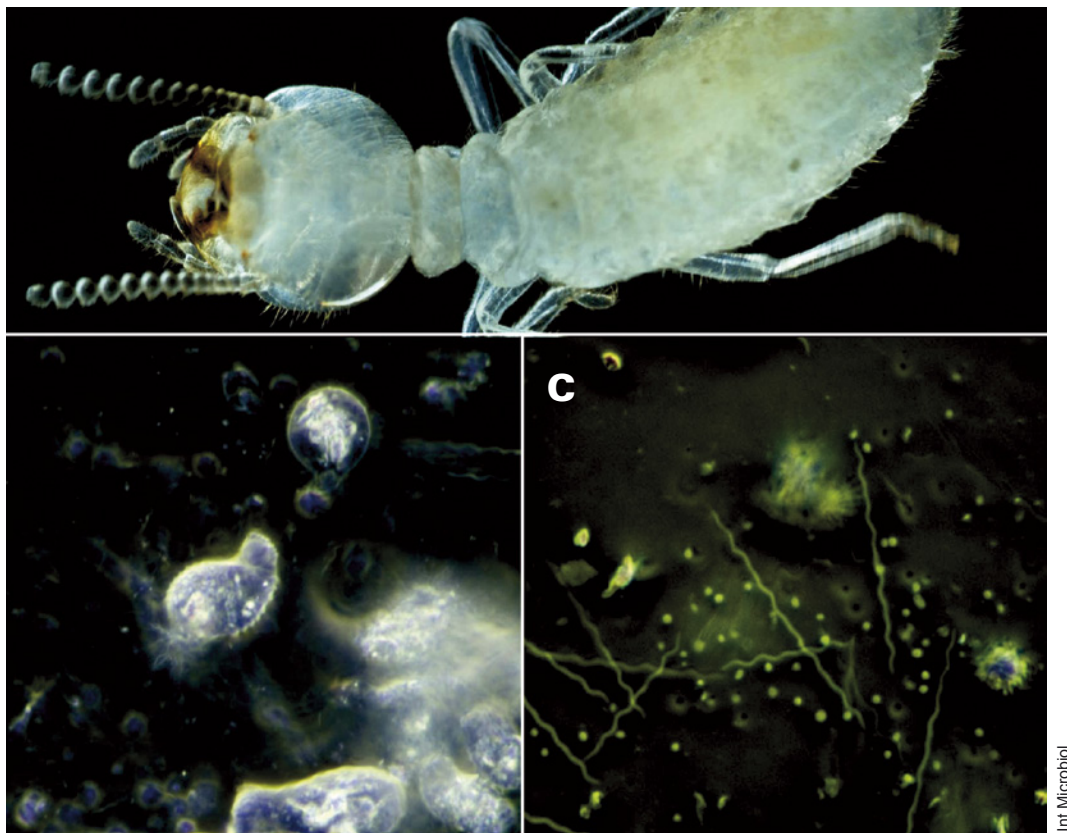
Fig. 3. (**A**) External appearance of the termite *Reticulitermes grassei*, soldier caste. (**B,C**) Microbiota from the whole-hindgut of a worker caste individual: (**B**) Protists and (**C**) spirochetes. (Photographs by R. Duro.)

gradient, ranging from *ca.* $E_h = +840$ mV to $E_h = –420$ mV over a few dozen micrometers (and returning to atmospheric conditions over the same tiny length).

The diversity of the hindgut microbiota of termites depends on several factors, including the variety of specialized structures present in the gut, the effect of pH, the sharp redox gradient, and the type of food ingested [9,14]. An indigenous biota is one that is present in all individuals, colonizes the gut habitat, and is maintained in "stable" climax communities. The microbiota detected in the guts of lower termites and *Cryptocercus* greatly differ from the microbiota of the environment (e.g., soil), and the anoxic and extremely low E_h conditions are in dramatic contrast to the oxic and positive E_h that surrounds these insects [2,3,14].

The hindgut bacteria of wood-feeding lower termites and cockroaches belong to several phyla, including Proteobacteria, Bacteroidetes, Firmicutes, Actinomycetes, Spirochetes, Verrucomicrobia, and Actinobacteria, as detected by 16S rRNA [17]. Of these, spirochetes may account for up to 50 % of all prokaryotes in the hindguts of some lower termites (Table 1, Fig. 4). Spirochete populations provide nitrogen, carbon sources, and electron donors to other resident microbial populations and to the host [27,32,57]. As specific symbionts that have coevolved with their respective termite hosts, spirochetes are stably harbored by several species and closely associated with members of the same termite family [1]. Termites preserved in amber provide direct paleontological evidence for the stable relationship between termites and their intestinal symbionts (protists and spirochetes) throughout at least 20 million years [59]. This ancient coevolution of unique and diverse spirochetes with xylophagous social insects explains the essential function contributed by these microbial symbionts for wood digestion.

Protists: unique inhabitants in termites and *Cryptocercus* hindguts

At least 15 % of all existing protist species depend on a symbiotic way of life; indeed, the symbiotic membership of nine separate protistan phyla (in four different kingdoms) is virtually 100 % [10]. Protists found in the digestive tract of

**Table 1.** Phyla composition (%) of the gut microbiota in several xylophagous insects*

Phylum	Termites				Cockroaches			Wood-feeding beetle
	Coptotermes formosanus	*Reticulitermes speratus*	*Reticulitermes grassei*	*Nasutitermes* spp.	*Cryptocercus punctulatus*	*C. punctulatus* (fasting)	*C. punctulatus* (dead)	*Anoplophora glabripennis*
Actinobacteria	2.0	5.3	4.0	1.5	6.6	10.5	45.8	31.0
Firmicutes (Clostridia-Bacilli)	10.8	39.5	12.0	7.0	32.1	10.6	29.2	27.3
Firmicutes (Mollicutes)	4.8	2.6			2.2			
Acidobacteria								0.1
Bacteroidetes	72.4	12.0	13.0	4.0	25.5	15.7		8.6
Fibrobacteres				5.0				
Planctomycetes	0.8	1.5						
Proteobacteria	1.6	13.4	18.0	4.0	20.7	42.2	25.0	32.0
Spirochetes	6.0	23.0	25.5	58.0	5.0	5.3		
Synergistetes	0.8				3.2	10.5		
TG1		2.0	18.0		2.2			
TG2			3.0					
TG3				17.5				
TM7			4.5			5.2		0.4
Verrucomicrobia	0.8	0.7	2.0		2.5			0.6
Other phyla				3.0				

*Data from *Coptotermes formosanus* (Rhinotermitidae) are based on Shinzato et al. [51]. Data from *Reticulitermes speratus* (Rhinotermitidae) are based on Hongoh et al. [21] and Nakajima et al. [38]. Data from *Reticulitermes grassei* (Rhinotermitidae) are based on Berlanga et al. [3]. Data from *Nasutitermes* spp. (Termitidae) are based on Köler et al. [26]. Data from *Cryptocercus punctulatus* (two different physiological states) are based on Berlanga et al. [2]. Data from *Anoplophora glabripennis* are based on Scully et al. [49].

termites and *Cryptocercus* cockroaches belong to the orders Trichomonadida, Cristamonadida, Hypermastigida, and Oxymonadida [15]. Hypermastigids are unique in nature, as they are found only in lower termites and *Cryptocercus* [8]. In fact, *Cryptocercus* cockroaches retain more diverse flagellate species than any extant termite species [15], with a single gut containing approximately 10^3–10^5 protistan cells/ml, accounting for at least 90 % of the volume of the hindgut. Each wood-feeding termite species harbors specific protists and hosts between 1 and 20 morphologically distinguishable species [3,31]. Yet, while termites support a characteristic community of gut protists, many protist species are not necessarily restricted to one termite species. Furthermore, many protist species are simultaneously associated with

different bacterial ectosymbionts, such as Spirochaetes, Bacteroidetes, and/or Synergistetes [43].

Ectosymbiotic spirochetes attach to the cell surfaces of protists in the termite gut, forming complex coevolutionary relationships (Fig. 5). A single protist cell usually sustains multiple spirochete species, and different protist genera share the same spirochete species [2,39]. Bacteroidetes are also involved in associations with a wide variety of gut protist species, as either intracellular endosymbionts or surface-attached ectosymbionts [40]. The close relationships of the ectosymbionts between related protist species suggest that the symbionts were acquired before the diversification of their protist hosts [13]. The Bacteroidetes ectosymbiont 'Candidatus Symbiothrix' is distributed among various
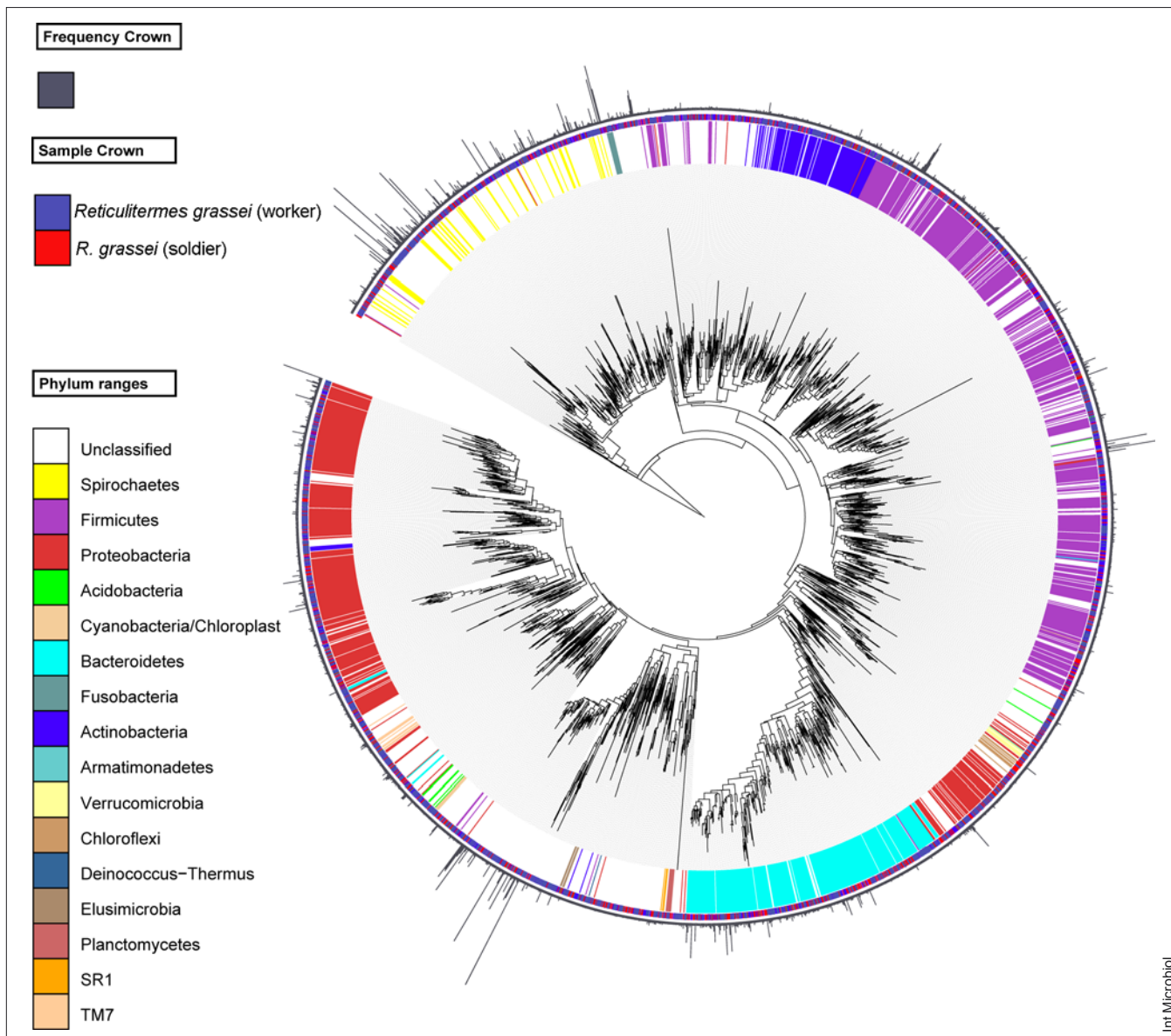
Fig. 4. Phylogenetic tree of the bacteria associated with the termite *Reticulitermes grassei*, worker and soldier castes, and their GenBank relatives of the most prevalent OTUs (with a distance threshold of 0.03), as generated by 454-pyrosequencing. Phylogenetic tree obtained by Interactive Tree of Life, a web-based tool [http://itol.embl.de], based on the guidelines by Letunic and Bork [30].

termites that harbor the protist *Dinenympha*. Another example of cospeciation is the endosymbiont Bacteroidetes from the protist *Pseudotrichonympha* [2,41].

'Endomicrobia' represent a special case in the coevolution of symbiont microorganisms, forming a separate line of descent in the bacterial tree and belonging to the termite group 1 (TG1) phylum. They are host-specific intracellular symbionts of termites and *Cryptocercus* gut flagellates [2, 24], with 'Endomicrobia' sequences from each flagellate host representing distinct phylotypes. Accordingly, the diversity of 'Endomicrobia' is thought to reflect the diversity of

their flagellate hosts [53]. Thus, 'Endomicrobia' phylotypes associated with *Trichonympha* species constitute a monophyletic group that is phylogenetically distinct from the phylotypes recovered from all other flagellates. The specific lineage of 'Endomicrobia' harbored by *Trichonympha* flagellates has been inherited by vertical transmission from their common ancestor [25]. The complete genome sequence of 'Endomicrobia' endosymbionts suggests that their association with their host flagellates stems from their ability to provide amino acids and cofactors [25]. The study of protists from the hindguts of both lower termites and the wood-feeding cockroach *Cryptocercus*
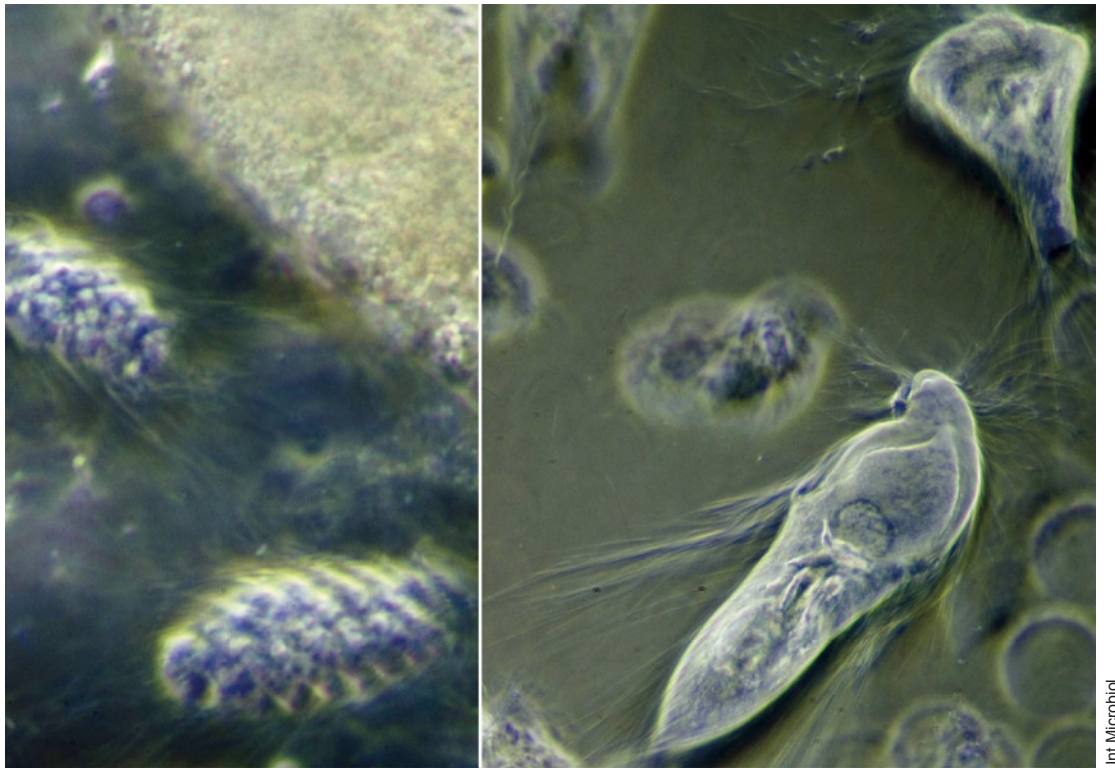
Fig. 5. Protists from the termite *Reticulitermes grassei*. (Photographs by R. Duro.)

provides evidence that bacteria–protists associations are very ancient, as are microbial–insect associations [24,44].

The colonization of prokaryotes on protist cells often results in an unusual ultrastructure of the junctional complexes [11,43,52]. Motility symbioses between prokaryotes and protist bionts have been reported between the flagellate *Mixotricha paradoxa* (Cristamonadida), found in the gut of the termite *Mastotermes darwiniensis*, and its ectosymbiotic spirochetes, and between the flagellate *Caduceia versatilis* (Cristamonadida), which inhabits the gut of the termite *Cryptotermes cavifrons*, and its ectosymbiotic bacteroidales [23]. However, the primary functions of symbiotic bacteria in protists are still unkown. Nonetheless, holobiont survival depends on both the maintenance of tripartite interactions (protist–bacteria–wood-feeding insect) and the preservation of the respective holobiome for the offspring is ensured by natural selection in a given environment.

Obligate endosymbiosis

Bacterial endosymbionts are found in many insect orders and their presence provides a molecular clock for estimating divergence times among taxa [18]. The endosymbiotic bacteria of cockroaches (including *Cryptocercus*) and lower termites (e.g., *Mastotermes darwiniensis*) are harbored within specialized cells—the bacteriocytes of the fat body—that are transferred vertically through the eggs. In fact, there is a close relationship between the endosymbionts of *Mastotermes* and those of *Cryptocercus*. One such endosymbiont is *Blattabacterium*, which based, on the analysis of its 16S rDNA genes, belongs to the class Flavobacteria in the phylum Bacteroidetes [29].

From the metabolic point of view, uric acid is a major nitrogenous waste product excreted by animals, including terrestrial insects, birds, and certain reptiles. Because of its poor solubility in water (only 60 mg/l at 20°C), uric acid, a non-toxic solid, is excreted to minimize water loss. However, in addition to being a nitrogenous waste, uric acid is apparently utilized as a nitrogen source or metabolic reserve in some insects, particularly those existing on a nitrogen-poor diet [54]. In the termite gut, uric acid degradation is an anaerobic process carried out primarily by gut bacteria [48,55]. The members of *Blattabacterium* that inhabit the fat bodies of most cockroaches are thought to participate in uric acid degradation, nitrogen assimilation, and nutrient provisioning. Genomic analysis and metabolic reconstruction indicate that *Blattabacterium*, despite lacking recognizable

uricolytic enzymes, is able to recycle nitrogen from urea and ammonia (both of which are uric acid degradation products) into glutamate by using the enzymes urease and glutamate dehydrogenase [48]. The genome of *Blattabacterium cuenoti*, whether from the termite *Mastotermes darwiniensis* or the social wood-feeding cockroach *Cryptocercus punctulatus*, lacks most of the pathways for the synthesis of essential amino acids found in the genomes of relatives of this bacterium isolated from non-wood-feeding hosts. This deficit is compensated by the presence of other members of the complex gut microbiota, which provide their host with all essential amino acids. In both *C. punctulatus* and *M. darwiniensis*, the hindgut microbiota are passed on to their offspring [55]. Previous claims suggesting that *Blattabacterium* is capable of uricolysis are not supported by genomic evidence, raising the question why these bacteria are located in such close proximity to uric acid deposits in fat bodies. A possible, albeit still speculative explanation is that a uricolytic *Blattabacterium* ancestor, in the course of genome reduction, may have lost its uricase, transferring this function to uric acid degraders among the gut microbiota.

Final considerations

The examples cited in this brief review illustrate the ubiquity of combinatorial modes of evolutionary innovation. A major change in evolution took place with the appearance of eukaryotic cells, which contained a nucleus and several organelles. Independent prokaryotes might have been spared elimination by interacting with other bionts to provide them with useful functions. Over time, these prokaryotic cells probably adjusted their reproductive needs such that they coevolved with other bionts, becoming deeply interdependent communities of microorganisms that eventually formed a holobiont, the functional equivalent of a single organism [33,34]. Extant symbioses likewise combine the development potential of two or more genomes. Selection pressures on the associates lead them to interact strongly and eventually to exploit niches where the presence of other life forms is ruled out, e.g., in extreme environmental conditions or where important nutrients are lacking. Symbiogenesis is a theory of speciation more comprehensive than that suggested by neo-Darwinian tenants: accretions of single-gene mutations in a given nucleus are strongly enhanced by the remarkable competitive advantages of holobionts. Symbiogenesis induces cyclical morphogenesis, which is an underestimated mode of evolutionary innovation. From our anthropocentric vision, it

may seem to us that the evolutionary pathway leading from the Cambrian explosion to humans was a major step in the history of life. Nevertheless, since the origin of nucleated cells—the latest major evolutionary step—evolution has only produced different variations of the same essential type of organisms, i.e., eukaryotes.

Competing interests. None declared.

References

1. Berlanga M, Paster BJ, Guerrero R (2007) Coevolution of symbiotic spirochete diversity in lower termites. Int Microbiol 10:133-139
2. Berlanga M, Paster BJ, Guerrero R (2009) The taxophysiological paradox: changes in the intestinal microbiota of the xylophagous cockroach *Cryptocercus punctulatus* depending on the physiological state of the host. Int Microbiol 12:227-236
3. Berlanga M, Paster BJ, Grandcolas P, Guerrero R (2011) Comparison of the gut microbiota from soldier and worker castes of the termite *Reticulitermes grassei*. Int Microbiol 14:83-93
4. Brucker RM, Bordenstein SR (2012) Speciation by symbiosis. Trends Ecol Evol 27:443-451
5. Brucker RM, Bordenstein SR (2013) The hologenomic basis of speciation: gut bacteria cause hybrid lethality in the genus *Nasonia*. Science 341:667-669
6. Brune A, Emerson D, Breznak JA (1995) The termite gut microflora as an oxygen sink: microelectrode determination of oxygen and pH gradients in guts of lower and higher termites. Appl Environ Microbiol 61:2681-2687
7. Brune A, Friedrich M (2000) Microecology of the termite gut: structure and function on a microscale. Curr Opinion Microbiol 3:263-269
8. Carpenter KJ, Horak A, Chow L, Keeling PJ (2011) Symbiosis, morphology, and phylogeny of Hoplonymphidae (Parabasalia) of the wood-feeding roach *Cryptocercus punctulatus*. J Eukaryot Microbiol 58:426-436
9. Colman DR, Toolson EC, Takacs-Vesbach CD (2012) Do diet and taxonomy influence insect gut bacterial communities? Mol Ecol 21: 5124-5137
10. Corliss JO (2004) Why the world needs protists! J Eukaryot Microbiol 51:8-22
11. D'Ambrosio U, Dolan M, Wier AM, Margulis L (1999) Devescovinid trichomonad with axostyle-based rotary motor ("Rubberneckia"): taxonomic assignment as *Caduceia versatilis* sp. nov. Eur J Protistol 35:327-337
12. De Bary A (1879) De la symbiose. Revue Internationale des Sciences III, pp 301-309. (*Die Erscheinung der Symbiose. Vortrag gehalten auf der Versammlung Deutscher Naturforscher und Aerzte zu Cassel*, K.J. Trübner, Strassburg.)
13. Desai MS, Strassert JFH, Meuser K, Hertel H, Ikeda-Ohtsubo W, Radek R, Brune A (2009) Strict cospeciation of devescovinid flagellates and

Bacteroidales ectosymbionts in the gut of dry-wood termites (Kalotermitidae). Environ Microbiol 12:2120-2132

14. Dillon RJ, Dillon VM (2004) The gut bacteria of insects: non-pathogenic interactions. Annu Rev Entomol 49:71-92

15. Dolan MF (2001) Speciation of termite gut protists: the role of bacterial symbionts. Int Microbiol 4:203-208

16. Eldredge N, Gould SJ (1977) Punctuated equilibria: the tempo and mode of evolution reconsidered. Paleobiology 3:115-151

17. Engel P, Moran NA (2013) The gut microbiota of insects – diversity in structure and function. FEMS Microbiol Rev, doi: 10.1111/1574-6976. 1202

18. Gil R, Belda E, Gosalbes MJ, et al. (2008) Massive presence of insertion sequences in the genome of SOPE, the primary endosymbiont of the rice weevil *Sitophilus oryzae*. Int Microbiol 11:41-48

19. Guerrero R (1998) Crucial crises in biology: life in the deep biosphere. Int Microbiol 1:285-294

20. Guerrero R, Berlanga M (2006) Life's unity and flexibility: the ecological link. Int Microbiol 9:225-235

21. Hongoh Y, Ohkuma M, Kudo T (2003) Molecular analysis of bacterial microbiota in the gut of the termite *Reticulitermes speratus* (Isoptera; Rhinotermitidae). FEMS Microbiol Ecol 44:231-242

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

23. Hongoh Y, Sato T, Noda S, Ui S, Kudo T, Ohkuma M (2007) *Candidatus* Symbiothrix dinenymphae: bristle-like *Bacteroidales* ectosymbionts of termite gut protists. Environ Microbiol 9:2631-2635

24. Hongoh Y, Sharma VK, Prakash T, Noda S, Taylor TD, Kudo T, Sakaki Y, Toyoda A, Hattori M, Ohkuma M (2008) Complete genome of the uncultured Termite Group 1 bacteria in a single host protist cell. Proc Natl Acad Sci USA 105:5555-5560

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

26. Köhler T, Dietrich C, Scheffrahn RH, Brune A (2012) High-resolution analysis of gut environment and bacterial microbiota reveals functional compartmentation of the gut in wood-feeding higher termites (*Nasutitermes* spp.) Appl Environ Microbiol 78:4691-4701

27. Leadbetter JR, Schmidt TM, Graber JR, Breznak JA (1999) Acetogenesis from H_2 plus CO_2 by spirochetes from termite guts. Science 283:686-689

28. Lederberg J, McCray AT (2001) 'Ome Sweet 'Omics—a genealogical treasury of words. Scientist 15:8

29. Letunic I, Bork P (2011) Interactive Tree of Life v2. Outline annotation and display of phylogenetic trees made easy. Nucleic Acids Res 39: W475-W478

30. Lewis JL, Forschler BT (2004) Protist communities from four castes and three species of *Reticulitermes* (Isoptera: Rhinotermitidae). Ann Entomol Soc Am 97:1242-1251

31. Lilburn TG, Kim KS, Ostrom NE, Byzek KR, Leadbetter JR, Breznak JA (2001) Nitrogen fixation by symbiotic and free-living spirochetes. Science 292:2495-2498

32. López-Sánchez MJ, Neef A, Patiño-Navarrete R, Navarro L, Jiménez R, Latorre A, Moya A (2009) *Blattabacteria*, the endosymbionts of cockroaches, have small genome sizes and high genome copy numbers. Environ Microbiol 10:3417-3422

33. Margulis L, Dolan MF, Guerrero R (2000) The chimeric eukaryote: Origin of the nucleus from the karyomastigont in amitochondriate protists. Proc Natl Acad Sci USA 97:6954-6959

34. Margulis L, Chapman M, Guerrero R, Hall J (2006) The last eukaryotic common ancestor (LECA): acquisition of cytoskeletal motility from aerotolerant spirochetes in the Proterozoic Eon. Proc Natl Acad Sci USA 103:13080-13085

35. Martinson VG, Moy J, Moran NA (2012) Establishment of characteristic gut bacteria during development of the honeybee worker. Appl Environ Microbiol 78:2830-2840

36. Maturana RH, Varela FJ (1972) Autopoiesis and cognition. In: Cohen RS, Wartofsky MW (eds) The realization of the living. Boston Studies in the Philosophy of Science. Vol 42. D. Reidel, Dordrecht, the Netherlands

37. McFall-Nagai M (2002) Unseen forces: The influence of bacteria on animal development. Develop Biol 242:1-14

38. Nakajima H, Hongoh Y, Usami R, Kudo T, Ohkuma M (2005) Spatial distribution of bacterial phylotypes in the gut of the termite *Reticulitermes speratus* and the bacterial community colonizing the gut epithelium. FEMS Microbiol Ecol 54:247-255

39. Noda S, Ohkuma M, Yamada A, Hongoh Y, Kudo T (2003) Phylogenetic position and in situ identification of ectosymbiotic spirochetes on protists in the termite gut. Appl Environ Microbiol 69:625-633

40. Noda S, Inoue T, Hongoh Y, et al. (2006) Identification and characterization of ectosymbionts of distinct lineages in *Bacteroidales* attached to flagellated protists in the gut of termites and a wood-feeding cockroach. Environ Microbiol 8:11-20

41. Noda S, Kitade O, Inoue T, et al. (2007) Cospeciation in the triplex symbiosis of termite gut protists (*Pseudotriconympha* spp.), their hosts, and their bacterial endosymbionts. Molec Ecol 16:1257-1266

42. Ohkuma M (2003) Termite symbiotic systems: efficient bio-recycling of lignocellulose. Appl Microbiol Biotech 61:1-9

43. Ohkuma M (2008) Symbioses of flagellates and prokaryotes in the gut of lower termites. Trends Microbiol 16:345-352

44. Ohkuma M, Noda S, Hongoh Y, Nalepa CA, Inoue T (2009) Inheritance and diversification of symbiotic trichonymphid flagellates from a common ancestor of termites and the cockroach *Cryptocercus*. Proc Biol Sci 276: 239-245

45. Oliver KM, Russell JA, Moran NA, Hunter MS (2003) Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. Proc Natl Acad Sci USA 100:1803-1807

46. Oliver KM, Degnan PH, Burke GR, Moran NA (2010) Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. Annu Rev Entomol 55:247-266

47. Ruby EG (2008) Symbiotic conversations are revealed under genetic interrogation. Nat Rev Microbiol 6:752-762

48. Sabree ZL, Kambhampati S, Moran NA (2009) Nitrogen recycling and nutricional provisioning by *Blattabacterium*, the cockroach endosymbiont. Proc Natl Acad Sci USA 106:19521-19526

49. Scully ED, Geib SM, Hoover K, et al. (2013) Metagenomic profiling reveals lignocellulose degrading system in a microbial community associated with a wood-feeding beetle. PLoS ONE 8:e73827

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

51. Shinzato N, Muramatsu M, Matsui T, Watanabe Y (2005) Molecular phylogenetic diversity of the bacterial community in the gut of the termite *Coptotermes formosanus*. Biosci Biotechnol Biochem 69:1145-1155

52. Stingl U, Maass A, Radek R, Brune A (2004) Symbionts of the gut flagellate *Staurojoenina* sp. from *Neotermes cubanus* represent a novel, termite-associated lineage of *Bacteroidales*: description of "*Candidatus* Vestibaculum illigatum". Microbiology 150:2229-2235

53. Stingl U, Radek R, Yang H, Brune A (2005) "*Endomicrobia*": Cyto-plasmatic symbionts of termite gut protozoa form a separate phylum of prokaryotes. Appl Environ Microbiol 71:1473-1479

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

55. Tokuda G, Elbourne LDH, Kinjo Y, et al. (2013) Maintenance of essential amino acid synthesis pathways in the *Blattabacterium cuenoti* symbiont of a wood-feeding cockroach. Biol Lett 9:20121153

56. Wallin IE (1927) Symbionticism and the origin of species. Williams & Wilkins, Baltimore, MD, USA

57. Warnecke F, Luginbühl P, Ivanova N, et al. (2007) Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. Nature 450:560-565

58. Werren JH, Baldo L, Clark ME (2008) *Wolbachia*: master manipulators of invertebrate biology. Nat Rev Microbiol 6:741-751

59. Wier A, Dolan M, Grimaldi D, Guerrero R, Wagensberg J, Margulis L (2002) Spirochete and protist symbionts of a termite (*Mastotermes electrodominicus*) in Miocene amber. Proc Natl Acad Sci USA 99:1410-1413

60. Zilber-Rosenberg I, Rosenberg E (2008) Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. FEMS Microbiol Rev 32:723-735

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