



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2018; 6(5): 421-425

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Received: 06-07-2018

Accepted: 07-08-2018

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## Symbionts associated with insect digestive system and their role in insect nutrition

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### Abstract

Insect guts are the most suitable breeding habitat for microbial colonization. There is a wide range of degree of dependence between insects and gut microbiota for basic functions. Insect digestive tracts differ significantly in morphology and physicochemical properties and that greatly influence microbial community structure. Midgut of lepidopteran larvae show extreme alkalinity (pH as high as 11–12, and alkaline conditions work better for their digestive enzymes. Exceptional condition is present in case of termites, with pH ranging from 5 to > 12 in the compartmentalized guts of some soil-feeding species. Lack of dependable transmission is the only obstacle to the evolution of intimate associations between gut microorganisms and host individuals. Social insects, such as termites, ants, and bees, are exceptions as they are provided with specialized beneficial functions in nutrition. There is still a large vacant place in information about insect gut communities. The extent of these roles is still unclear and awaits further studies.

**Keywords:** Symbionts associated, digestive system, their role, insect nutrition

### Introduction

Globally insects are the most diverse and abundant animal clade, on the basis of numbers of species, ecological habits, and biomass [3]. The evolutionary success of insects are highly attributable to their relationships with beneficial gut microbial communities which contribute critically in digestion of recalcitrant food components, govern mating and reproductive systems, protection from parasites, aid in intra and inter specific communication and increasing its efficiency as disease vectors [24, 52, 54]. Gut symbionts are commonly associated with insects feeding on wood or other lignified plant materials. The mutualistic association of insects with gut microflora span from the cultivation of fungal gardens to close association with symbiotic flagellates or prokaryotic bacteria housed within mycetomes or bacteriocytes of the insect fat body [26]. Mutualistic associations with gut microbial communities have great implications in insect nutrition and the focus of this review paper is on microbial symbionts that colonize the insect digestive tract and are directly associated with insect nutrition.

### Symbionts

A symbiont is an organism that is very closely associated with another, usually larger organism that is called host. It can live in or on or sometimes very near to its host. Symbionts are of two categories.

1. **Ectosymbiont:** An ectosymbiont is an organism that lives outside of its host cell.
2. **Endosymbiont:** An endosymbiont is an organism that lives inside of its host cell.

Examples of symbionts: Symbionts mainly comprises of bacteria, fungi, flagellates, protozoa like micro organisms.

**Examples of symbionts associated with insect digestive system:** Several different types of symbionts are present in insect gut. Likewise a variety of bacterial phyla are commonly present in insect guts, including Gammaproteobacteria, Alphaproteobacteria, Betaproteobacteria, Bacteroidetes, Firmicutes including Lactobacillus and Bacillus species, Clostridia, Actinomycetes, Spirochetes, Verrucomicrobia, Actinobacteria, and others [20]. Some protozoa and fungi also associate with the insect's digestive system such as in lower termites and other wood feeding insects.

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### Stability of the insect gut as a microbial habitat

From the perspective of microbial colonization, insect guts often present unstable habitats. Insects molt numerous times during larval development, shedding the exoskeletal lining of the foregut and hindgut each time and thus severely disrupting or eliminating any attached bacterial populations. The midgut produces and repeatedly sheds the peritrophic matrix and along with it associated microorganisms, most of which do not cross into the space adjacent to midgut epithelial cells. In holometabolous insects with distinct larval, pupal, and adult stages, there is a radical remodeling of the gut and other organs at metamorphosis, with the elimination of the entire larval gut and contents as a meconium that is enveloped in the peritrophic matrix of the pupal stage. However, many insect guts display specialized crypts or paunches that promote microbial persistence and insects do not molt once they reach the adult stage, so following the final molt, the foregut or hindgut wall provides a stable surface for colonization. Insects including cockroaches, termites, ants, and some wasps and bees, show gregarious or social behavior, including oral trophallaxis or coprophagy, which can enable direct or indirect social transmission, thus promoting the evolution of specialized host-dependent symbionts [31, 33, 50]. These types of adaptations for transmission to progeny or colony members give evolutionary advantages of maintaining a consistent microbiota.

### Physical conditions in insect guts

Physico-chemical conditions in the lumen of different gut compartments influence microbial colonization, and these can display extreme variation in both pH and oxygen availability. The pH of the lumen is actively regulated and often diverges from that of the hemolymph (pH nearly 7). Midguts of lepidopteran larvae show extreme alkalinity, with pH as high as 11–12 [1, 16, 28, 30]. The pH of lepidopteran guts is correlated with feeding on tannin-rich leaves and has been interpreted as an adaptation that lowers the binding of dietary protein with ingested tannins, improving nutrient availability [6], but it also has major consequences for microbial communities as it excludes most bacteria. In insect guts with large microbial communities, microbial metabolism actively shapes conditions within the lumen of different gut compartments. For example, in detritus feeding larvae of the scarab beetle *Pachnoda ephippiata*, microbial fermentation products including acetate, formate, and lactate are abundant in both midgut and hindgut, although profiles differ between the two compartments [47]. A study of the pH along the gut axis in *P. ephippiata* showed regular, pronounced variation, with values near 8 in the anterior midgut, rising to > 10 in the center of the midgut, and dropping to 7 in the hindgut [47] where microbial densities are highest [15]. In contrast, the gut lumens of some nonholometabolous insects often show less extreme pH gradients [1]. Termites are an exception, with pH ranging from 5 to > 12 in the compartmentalized guts of some soil-feeding species [12, 42]. The extreme alkalinity in some compartments of termite guts does not entirely prevent microbial colonization but instead supports the growth of specialized alkaline-tolerant symbiotic bacteria from Firmicutes, *Clostridium*, and Planctomycetes [7, 43]. Guts of termites have been characterized most extensively. Termites evolved from cockroach ancestors and have the most elaborate known gut communities of any insects.

### Examples of highly specialized gut bacteria

The wide range in intimacy and continuity of associations of insects with gut microorganisms is illustrated within the Heteroptera (order Hemiptera), which includes diverse insects with sucking mouthparts that feed on plant or animal fluids [41]. Many plant-feeding heteropteran species have midguts with caecae or crypts that house populations of symbiotic bacteria. At one extreme, these gut symbionts can be strictly heritable and approach intracellular symbionts or organelles in their level of specialization. The best-studied example is *Ishikawaella capsulata*, which lives in specialized crypts in guts of the stinkbug species *Megacopta punctatissima* (family: Plataspidae) [29]. *Ishikawaella capsulata* has all of the hallmarks of an obligate bacteriocyte-associated nutritional symbiont. [34, 57]. While *I. Capsulate* resides in the gut lumen and is thus not intracellular or transmitted within eggs, it achieves highly efficient vertical transmission: ovipositioning females defecate to produce a specialized symbiotic capsule on the outside of the egg case, and juveniles immediately ingest the capsule following hatching [34]. Many other heteropterans also possess bacterial symbionts, often in specialized midgut caecae. However, some heteropterans rely on environmental acquisition of a specific symbiont strain every generation, implying that the host gut selects the appropriate bacterial strains from a range of ingested organisms. For example, the bean bug, *Riptortus pedestris* (Heteroptera: Alydidae), acquires a specific *Burkholderia* symbiont orally every generation, and the symbiont forms dense colonies in midgut crypts [40]. A representative of another group of plant-feeding Heteroptera, *Nezara viridula* (Heteroptera: Pentatomidae), was also found to house a specific symbiont in gut crypts and to acquire the symbiont environmentally each generation [62] suggesting that environmental transmission is not always incompatible with high specificity of a symbiotic relationship. Grain weevils (genus *Sitophilus*) contain true endosymbionts that are transmitted through eggs and that live in cytosol of foregut cells of larvae and migrate to midgut epithelial cells in adults, apparently using bacterial type III secretion systems for cellular invasion [23].

### Digestive symbionts in insects other than termites

The most prominent examples are Scarabaeids and Tipulids, which have cellulolytic and hemicellulolytic bacteria attached to brush-like chitinous structures. The guts of omnivorous cockroaches contain microbiota of bacteria and methanogenic archaea endosymbionts in their hindguts. In the hindgut of *Acheta domesticus*, the density of microorganisms is even higher than that in termites, and there are brush-like supports for the attachment of bacteria that resemble those in scarab beetle larvae. It is very likely that insects other than termites access protein and recycle nitrogen via digestion by microbial symbionts. Proctodeal feeding is a form of social behavior that is restricted to the termites and the wood-feeding cockroach, *C. punctulatus*, but theoretically any consumption of feces would also allow access to the microbial protein. However, establishment and maintenance of a specific gut microbiota, as evidenced in the case of termites by many instances of cospeciation between host and symbionts, is facilitated by vertical transfer among parent and offspring. In contrast to the symbioses between insects and their intracellular bacteria, this is probably not accomplished by ovarial transmission, but by coprophagy or proctodeal trophallaxis.

### Role of symbionts in digestion process of termites

The best-studied nutritional gut mutualisms are those found in the hindguts of termites. The lower termite species, exclusively comprise wood-feeders, while the higher termite species include wood-, litter-, grass-, soil-, and lichen-feeders [37, 49]. Each termite species harbours a highly specific microbial gut community consisting of several hundreds of microorganisms including bacteria, archaea and protists [32]. These microorganisms play a dual mutualistic role for their host. First, they contribute to lignocellulose digestion and produce high levels of acetate, which represents the main carbon source for their host [4, 8, 10, 17, 27, 35, 58, 72, 73]. Second, they provide their host with nitrogen, which is typically deficient in decomposing plant materials [5, 9]. The main part of lignocellulose digestion is carried out by the specialized gut community present in the hindgut of termites [36, 56, 67, 70, 71]. In lower termites, lignocellulose digestion is mostly accomplished by protists [18, 19]. Species of the genus *Treponema*, dominating the hindgut of both lower and higher termite species, seem to be responsible for most of the acetogenic activity. Higher termites typically lack protists in their guts. The cellulolytic activity of bacteria within specific gut segments contributes critically to lignocellulose degradation in the hindgut of higher termites [42, 72]. Cellulolytic activity was found in the posterior proctodeal segments, which are densely populated by bacteria [70, 71]. Metagenomic and proteomic analysis of these regions revealed a high abundance of bacterial genes and proteins involved in cellulose degradation, acetogenesis and nitrogen fixation [11, 72].

### Transfer of symbionts

Specialized gut symbionts that are maintained through vertical transmission are found in social or gregarious insects, including social bees and termites. In honey bees (*Apis mellifera*), bacterial symbionts confined to the hindguts of adults are acquired in the first few days following emergence of adults from the pupal stage, through social interactions with other adult worker bees in the colony [50]. Honey bee gut inhabitants belong to a small number of distinctive lineages found only in honey bees and also in other *Apis* species and in *Bombus* species (bumble bees), which are also social and which are closely related to honey bees [44, 45, 48, 50]. Thus, vertical transmission through sociality may facilitate host-symbiont coevolution and emergence of a distinctive gut community. Ant species, all of which are social, also show a number of specialized gut bacteria and associated morphological modifications of the gut [13, 14] [21, 64, 65]. Termite gut communities are more complex, usually containing hundreds of species or phylotypes [31, 59]. Transmission appears to occur primarily through coprophagy or proctodeal trophallaxis within colonies. Different hindgut compartments house different bacterial communities. The extent of direct transfer of gut bacteria between conspecific hosts in nonsocial insects is unclear. Gregarious insects such as cockroaches and crickets, although lacking parental care and sociality, can transmit bacteria by defecating and feeding in a common area. In a study of gut microbiota of two termites, a social wood roach, and a solitary cockroach (*Periplaneta americana*), the three social species had guts dominated by specialized communities of symbionts, including bacteria and protozoans, whereas gut communities of the nonsocial *P. americana* were dominated by bacterial species common in the environment [66]. If this pattern were upheld in future studies, it would

imply a dominant role of sociality in the evolution of characteristic gut microbiota in insects. On the other hand, even in solitary insects with nonoverlapping generations, females could potentially transmit bacteria to progeny simply by defecating in the vicinity of eggs and having their gut bacteria ingested by their progeny. For this transmission route to be effective, larvae and adults would both need to host the same bacterial types, and bacteria would need to persist for some time in the environment.

### Conclusion

Insect guts, in general, display a large diversity in their morphology, physico-chemical properties and food content. These factors contribute to the broad array of different community structures and shape the gut microbiota of insects. Insects exhibit a wide range in their degree of dependence on gut microbiota, with extremes represented by some sap feeding insects, which have little or no gut microbiota but depend on intracellular symbionts for nutrients, and by termites, which greatly depend on the complex gut communities, that are essential for digesting food and producing nutrition. In addition, social insects have evolved specific mechanisms for bacterial transfer to progeny such as egg-smearing or egg capsules. Gut microorganisms are critical to the nutrition, physiology, immune responses, and pathogen resistance of many species. In future, we will likely learn much more about how insects discriminate between mutualistic gut microorganisms and harmful pathogens. Such insights will help in efforts to manipulate gut microorganisms of insects to control damaging insect species or to protect beneficial ones, including pollinators.

### Acknowledgement

Both the authors have contributed equally and have no conflict of interest. Authors are grateful to Dr. Krishna Karmakar for providing necessary advice to carry out the review work.

### References

1. Appel HM, Martin MM. Gut redox conditions in herbivorous lepidopteran larvae. *Journal of Chemical Ecology*. 1990; 16:3277-3290.
2. Barbehenn RV, Martin MM. Permeability of the peritrophic envelopes of herbivorous insects to dextran sulfate: a test of the polyanion exclusion hypothesis. *Journal of Insect Physiology*. 1997; 43:243-249.
3. Basset Y, Cizek L, Cuenoud P. Arthropod diversity in a tropical forest. *Science*. 2012; 338: 1481-1484.
4. Bignell DE, Eggleton P, Nunes L, Thomas KL. Termites as mediators of carbon fluxes in tropical forests: budgets for carbon dioxide and methane emissions. *Forests and Insects* (Watt AD, Stork NE & Hunter MD, eds). 1997, 109-134.
5. Benemann JR. Nitrogen fixation in termites. *Science*. 1973; 181:164-165.
6. Berenbaum M. Adaptive significance of midgut pH in larval Lepidoptera. *The American Naturalist*. 1980; 115:138-146.
7. Bignell DE. Morphology, physiology, biochemistry and functional design of the termite gut: an evolutionary wonderland. *Biology of Termites: A Modern Synthesis* (Bignell DE, Roisin Y & Lo N, eds). Springer, Dordrecht, 2010, 375-412.
8. Bignell DE, Eggleton P, Nunes L, Thomas KL. Termites

- as mediators of carbon fluxes in tropical forests: budgets for carbon dioxide and methane emissions. *Forests and Insects* (Watt AD, Stork NE & Hunter MD, eds). Chapman & Hall, London, 1997, 109-134.
9. Breznak JA, Brill WJ, Mertins JW, Coppel HC. Nitrogen fixation in termites. *Nature*. 1973; 244:577-580.
  10. Breznak JA, Switzer JM. Acetate synthesis from H<sub>2</sub> plus CO<sub>2</sub> by termite gut microbes. *Applied and Environmental Microbiology*. 1986; 52:623-630.
  11. Burnum KE, Callister SJ, Nicora CD, Purvine SO, Hugenholtz P, Warnecke F *et al.* Proteome insights into the symbiotic relationship between a captive colony of *Nasutitermes corniger* and its hindgut microbiome. *ISME Journal*. 2010; 5:161-164.
  12. Brune A, Ohkuma M. Role of the termite gut microbiota in symbiotic digestion. *Biology of Termites: A Modern Synthesis* (Bignell DE, Roisin Y & Lo N, eds). Springer, Dordrecht, 2010, 439-475.
  13. Bution ML, Caetano FH. Ileum of the Cephalotes ants: a specialized structure to harbor symbionts microorganisms. *Micron*. 2008; 39:897-909.
  14. Caetano FH, Bution ML, Zara FJ. First report of endocytobionts in the digestive tract of ponerine ants. *Micron*. 2009; 40:194-197.
  15. Cazemier AE, Hackstein JHP, Op den Camp HJM, Rosenberg J, van der Drift C. Bacteria in the intestinal tract of different species of arthropods. *Microbial Ecology*. 1997; 33:189-197.
  16. Chapman RF, Simpson SJ, Douglas AE. *The Insects: Structure, and Function*, 5th edn. Cambridge University Press, Cambridge, 2013.
  17. Chouaia B, Rossi P, Epis S. Delayed larval development in *Anopheles* mosquitoes deprived of Asaia bacterial symbionts. *BMC Microbiology*. 2012, 12.
  18. Cleveland LR. Symbiosis between termites and their intestinal protozoa. *Proceedings of National Academy Sciences USA*. 1923; 9:424-428.
  19. Cleveland LR. The physiological and symbiotic relationships between the intestinal protozoa of termites and their host, with special reference to *Reticulitermes flavipes* Kollar. *Biological Bulletin*. 1924; 46:203-227.
  20. Colman DR, Toolson EC, Takacs-Vesbach CD. Do diet and taxonomy influence insect gut bacterial communities? *Molecular Ecology*. 2012; 21:5124-5137.
  21. Cook SC, Davidson DW. Nutritional and functional biology of exudate-feeding ants. *Entomologia Experimentalis et Applicata*. 2006; 118:1-10.
  22. Cruden DL, Markovetz AJ. Microbial ecology of the cockroach gut. *Annual Review of Microbiology*. 1987; 41:617-643.
  23. Dale C, Plague GR, Wang B, Ochman H, Moran NA. Type III secretion systems and the evolution of mutualistic endosymbiosis. *Proceedings of National Academy Sciences USA*. 2002; 99:12397-12402.
  24. Edwards M, Jacobs-Lorena M. Permeability and disruption of the peritrophic matrix and caecal membrane from *Aedes aegypti* and *Anopheles gambiae* mosquito larvae. *Journal of Insect Physiology*. 2000; 46:1313-1320.
  25. Ferreira C, Capella AN, Sitnik R, Terra WR. Properties of the digestive enzymes and the permeability of the peritrophic membrane of *Spodoptera frugiperda* (Lepidoptera) larvae. *Comparative Biochemistry and Physiology Part A Physiology*. 1994; 107:631-640.
  26. Fierer N, Strickland MS, Liptzin D, Bradford MA, Cleveland CC. Global patterns in belowground communities. *Ecology Letters*. 2009; 12:1238-1249.
  27. Fox-Dobbs K, Doak DF, Brody AK, Palmer TM. Termites create spatial structure and govern ecosystem function by affecting N<sub>2</sub> fixation in an East African savanna. *Ecology*. 2010; 91:1296-1307.
  28. Fujita A, Hojo M, Aoyagi T, Hayashi Y, Arakawa G, Tokuda G *et al.* Details of the digestive system in the midgut of *Coptotermes formosanus* Shiraki. *Journal of Wood Science*. 2010; 56:222-226.
  29. Fukatsu T, Hosokawa T. Capsule-transmitted gut symbiotic bacterium of the Japanese common plataspid stinkbug, *Megacopta punctatissima*. *Applied and Environmental Microbiology*. 2002; 68:389-396.
  30. Harrison JF. Insect acid-base physiology. *Annual Review of Entomology*. 2001; 46:221-250.
  31. Hongoh Y, Deevong P, Inoue T, Moriya S, Trakulnaleamsai S, Ohkuma M *et al.* Intra and interspecific comparisons of bacterial diversity and community structure support coevolution of gut microbiota and termite host. *Applied and Environmental Microbiology*. 2005; 71:6590-6599.
  32. Hongoh Y. Diversity and genomes of uncultured microbial symbionts in the termite gut. *Bioscience Biotechnology and Biochemistry*. 2010; 74:1145-1151.
  33. Hongoh Y, Ekpornprasit L, Inoue T, Moriya S, Trakulnaleamsai S, Ohkuma M *et al.* Intracolony variation of bacterial gut microbiota among castes and ages in the fungus-growing termite *Macrotermes gilvus*. *Molecular Ecology*. 2006; 15:505-516.
  34. Hosokawa T, Kikuchi Y, Nikoh N, Shimada M, Fukatsu T. Strict host-symbiont cospeciation and reductive genome evolution in insect gut bacteria. *PLoS One*. 2006; 4:e337.
  35. Hungate RE. Quantitative analyses on the cellulose fermentation by termite protozoa. *Annals of Entomological Society of America*. 1943; 36:730-739.
  36. Inoue T, Murashima K, Azuma JI, Sugimoto A, Slaytor M. Cellulose and xylan utilisation in the lower termite *Reticulitermes speratus*. *Journal of Insect Science*. 1997; 43:235-242.
  37. Inward DJG, Vogler AP, Eggleton P. A comprehensive phylogenetic analysis of termites (Isoptera) illuminates key aspects of their evolutionary biology. *Molecular Phylogenetics and Evolution*. 2007; 44:953-967.
  38. Kaufman MG, Klug MJ, Merritt RW. Growth and food utilization parameters of germ-free house crickets, *Acheta domesticus*. *Journal of Insect Physiology*. 1989; 35:957-967.
  39. Kashima T, Nakamura T, Tojo S. Uric acid recycling in the hindgut of the shield bug *Parastrachia japonicas* (Hemiptera: Parastrachiidae) during diapause. *Journal of Insect Physiology*. 2006; 52:816-825.
  40. Kikuchi Y, Meng XY, Fukatsu T. Gut symbiotic bacteria of the genus *Burkholderia* in the broad-headed bugs *Riptortus clavatus* and *Leptocoris chinensis* (Heteroptera: Alydidae). *Applied and Environmental Microbiology*. 2005; 71:4035-4043.
  41. Kuechler SM, Renz P, Dettner K, Kehl S. Diversity of symbiotic organs and bacterial endosymbionts of lygaeoid bugs of the families Blissidae and Lygaeidae (Hemiptera: Heteroptera: Lygaeoidea). *Applied Environmental Microbiology*. 2012; 78:2648-2659.

42. Köhler T, Dietrich C, Scheffrahn RH, Brune A. High resolution analysis of gut environment and bacterial microbiota reveals functional compartmentation of the gut in wood-feeding higher termites (*Nasutitermes* spp.). *Applied Environmental Microbiology*. 2012; 78:4691-4701.
43. Köhler T, Stingl U, Meuser K, Brune A. Novel lineages of Planctomycetes densely colonize the alkaline gut of soil-feeding termites (*Cubitermes* spp.). *Environmental Microbiology*. 2008; 10:1260-1270.
44. Koch H, Schmid-Hempel P. Bacterial communities in central European bumblebees: low diversity and high specificity. *Microbial Ecology*. 2011; 62:121-133.
45. Koch H, Abrol DP, Li J, Schmid-Hempel P. Diversity and evolutionary patterns of bacterial gut associates of corbiculate bees. *Molecular Ecology*. 2013; 22:2028-2044.
46. Lehane MJ. Peritrophic matrix structure and function. *Annual Review of Entomology*. 1997; 42:525-550.
47. Lemke T, Stingl U, Egert M, Friedrich MW, Brune A. Physicochemical conditions and microbial activities in the highly alkaline gut of the humus-feeding larva of *Pachnoda ephippiata* (Coleoptera: Scarabaeidae). *Applied Environmental Microbiology*. 2003; 69:6650-658.
48. Li J, Qin H, Wu J, Sadd BM, Wang X, Evans JD *et al.* The Prevalence of parasites and pathogens in Asian honeybees *Apis cerana* in China. *PLoS ONE*. 2012; 7: e47955.
49. Lo N, Engel MS, Cameron S. Save Isoptera. *Biological Letters*. 2007; 3:562-563.
50. Martinson VG, Danforth BN, Minckley RL, Rueppell O, Tingek S, Moran NA. A simple and distinctive microbiota associated with honey bees and bumble bees. *Molecular Ecology*. 2011; 20:619-628.
51. Martinson VG, Moy J, Moran NA. Establishment of characteristic gut bacteria during development of the honeybee worker. *Applied Environmental Microbiology*. 2012; 78:2830-2840.
52. McMeniman CJ, Lane RV, Cass BN, Fong AWC, Sidhu M, Wang Y-F *et al.* Stable introduction of a life shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. *Science*. 2009; 323:141-144.
53. Lehane MJ. Peritrophic matrix structure and function. *Annual Review of Entomology*. 1997; 42:525-550.
54. Moll RM, Romoser WS, Modrakowski MC, Moncayo AC, Lerdthusnee K. Meconial peritrophic membranes and the fate of midgut bacteria during mosquito (Diptera: Culicidae) metamorphosis. *Journal of Medical Entomology*. 2001; 38:29-32.
55. Nardi JB, Bee CM. Regenerative cells and the architecture of beetle midgut epithelia. *Journal of Morphology*. 2012; 273:1010-1020
56. Nakashima K, Watanabe H, Saitoh H, Tokuda G, Azuma JI. Dual cellulose-digesting system of the wood-feeding termite, *Coptotermes formosanus* Shiraki. *Insect Biochemistry and Molecular Biology*. 2002; 32:777-784.
57. Nikoh N, Hosokawa T, Oshima K, Hattori M, Fukatsu T. Reductive evolution of bacterial genome in insect gut environment. *Genome Biology and Evolution*. 2011; 3:702-714.
58. Odelson DA, Breznak JA. Volatile fatty acid production by the hindgut microbiota of xylophagous Termites. *Applied Environmental Microbiology*. 1983; 45:1602-1613.
59. Ohkuma M, Brune A. Diversity, structure, and evolution of the termite gut microbial community. *Biology of Termites: A Modern Synthesis* (Bignell DE, Roisin Y & Lo N, eds). Springer, Dordrecht, 2010, 413-438.
60. Peters W, Wiese B. Permeability of the peritrophic membranes of some Diptera to labelled dextrans. *Journal of Insect Physiology*. 1986; 32:43-49.
61. Potrikus CJ, Breznak JA. Gut bacteria recycle uric acid nitrogen in termites: a strategy for nutrient conservation. *Proceedings of National Academy Sciences USA*. 1981; 78:4601-4605.
62. Prado SS, Rubinoff D, Almeida RPP. Vertical transmission of a pentatomid caeca-associated symbiont. *Annals of Entomological Society of America*. 2006; 99:577-585.
63. Ricci I, Valzano M, Ulissi U, Epis S, Cappelli A, Favia G. Symbiotic control of mosquito borne disease. *Pathogens and Global Health*. 2012; 106:380-385.
64. Roche RK, Wheeler DE. Morphological specializations of the digestive tract of *Zacryptocerus rohweri* (Hymenoptera: Formicidae). *Journal of Morphology*. 1999; 234:253-262.
65. Russell JA, Moreau CS, Goldman-Huertas B, Fujiwara M, Lohman DJ, Pierce NE. Bacterial gut symbionts are tightly linked with the evolution of herbivory in ants. *Proceedings of National Academy Sciences USA*. 2009; 106:21236-21241.
66. Sabree ZL, Huang CY, Arakawa G, Tokuda G, Lo N, Watanabe H, *et al.* Genome shrinkage and loss of nutrient-providing potential in the obligate symbiont of the primitive termite *Mastotermes darwiniensis*. *Applied Environmental Microbiology*. 2012b; 78:204-210.
67. Slaytor M. Cellulose digestion in termites and cockroaches: what role do symbionts play? *Comparative Biochemistry and Physiology B*. 1992; 103:775-784.
68. Shao L, Devenport M, Jacobs-Lorena M. The peritrophic matrix of hematophagous insects. *Archives of Insect Biochemistry and Physiology*. 2001; 47:119-125.
69. Spence KD, Kawata MY. Permeability characteristics of the peritrophic membranes of *Manduca sexta* larvae. *Journal of Insect Physiology*. 1993; 39:785-790.
70. Tokuda G, Lo N, Watanabe H. Marked variations in patterns of cellulase activity against crystalline- vs. carboxymethyl-cellulose in the digestive systems of diverse, wood-feeding termites. *Physiological Entomology*. 2005; 30:372-380.
71. Tokuda G, Watanabe H. Hidden cellulases in termites: revision of an old hypothesis. *Biological Letters*. 2007; 3:336-339.
72. Warnecke F, Luginbuhl P, Ivanova N *et al.* Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature*. 2007; 450:560-565.
73. Yamin MA. Cellulose metabolism by the flagellate trichonympha from a termite is independent of endosymbiotic bacteria. *Science*. 1981; 211:58-59.
74. Zurek L, Keddie BA. Contribution of the colon and colonic bacterial flora to metabolism and development of the American cockroach *Periplaneta americana* L. *Journal of Insect Physiology*. 1996; 42:743-748.