

Death of an order: a comprehensive molecular phylogenetic study confirms that termites are eusocial cockroaches

Daegan Inward[†], George Beccaloni
and Paul Eggleton*

Soil Biodiversity Group, Department of Entomology,
The Natural History Museum, London SW7 5BD, UK

*Author for correspondence (p.eggleton@nhm.ac.uk).

[†]Present address: Forest Research, Alice Holt Lodge, Farnham,
Surrey GU10 4LH, UK

Termites are instantly recognizable mound-builders and house-eaters: their complex social lifestyles have made them incredibly successful throughout the tropics. Although known as ‘white ants’, they are not ants and their relationships with other insects remain unclear. Our molecular phylogenetic analyses, the most comprehensive yet attempted, show that termites are social cockroaches, no longer meriting being classified as a separate order (Isoptera) from the cockroaches (Blattodea). Instead, we propose that they should be treated as a family (Termitidae) of cockroaches. It is surprising to find that a group of wood-feeding cockroaches has evolved full sociality, as other ecologically dominant fully social insects (e.g. ants, social bees and social wasps) have evolved from solitary predatory wasps.

Keywords: insect systematics; phylogenetics;
social evolution; classification

1. INTRODUCTION

Termites, cockroaches and mantids form a well-established lineage, the Dictyoptera, uniquely defined by having a perforation in the tentorium (the internal skeletal part of the head) and enclosing their eggs within a specialized case (ootheca). Within the Dictyoptera, there is agreement that both termites and mantids are monophyletic groups. However, hypotheses of relationships among the three groups have provoked controversy ever since the finding that the woodroach *Cryptocercus* shares several groups of symbiotic gut flagellates with early branching termites (Cleveland *et al.* 1934). This, together with morphological similarity between some termites' nymphs (pseudergates) and *Cryptocercus* nymphs, suggested a close phylogenetic relationship between the two groups (McKitterick 1964). However, some researchers have challenged this hypothesis by showing that (i) gut flagellates could have been passed from termites to *Cryptocercus* early in the history of the groups (Thorne 1990) and (ii) in phylogenetic studies (Thorne & Carpenter 1992; Kambhampati 1995), albeit with

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2007.0102> or via <http://www.journals.royalsoc.ac.uk>.

sparse taxon sampling, termites did not group with *Cryptocercus* or indeed nest within cockroaches. However, these results have been contested and recent phylogenetic studies (Lo *et al.* 2003; Terry & Whiting 2005), again, unfortunately, with sparse taxon sampling, have supported the original hypothesis of a monophyletic termites + cockroaches.

Two questions must be answered to resolve the phylogenetic position of termites. *Are termites cockroaches?* And if they are, *what is the sister group of the termites within the cockroaches?* No previous study has answered these questions unambiguously, as none of them have sufficiently comprehensive taxon sampling or completely adequate character information. Here, we provide definitive answers by sampling, for the first time, a fully representative set of Dictyoptera species and sequencing and analysing five gene loci.

2. MATERIAL AND METHODS

We sampled 107 Dictyoptera (in-group) species along with 11 out-groups. This included five of the 15 mantid families, all six cockroach families as well as 22 of the 29 cockroach subfamilies and all termite families and subfamilies. We used five gene loci (two mitochondrial: *12S*, and *cytochrome oxidase II*; and three nuclear: *28S*, *18S* and *histone 3*), which gave us approximately 4900 aligned base pairs. We estimated substitution models for each gene (Posada & Crandall 1998) and subsequently employed a Bayesian analysis (Ronquist & Huelsenbeck 2003) on the combined dataset to estimate tree topology and posterior probabilities for each node and for nodes not recovered in a majority of the trees. We also undertook a maximum parsimony (MP) analysis on the same aligned dataset. Full details of the methods are in the electronic supplementary material.

3. RESULTS AND DISCUSSION

Our Bayesian consensus tree (figure 1) answers both the questions: termites nest within the cockroaches and *Cryptocercus* is the sister group of the termites. Additionally, it shows termite + *Cryptocercus* clade as sister to Blattidae, and that combined clade as sister to Blattellidae + Blaberidae ('Blaberoidea' in figure 1). Polyphagidae + Nocticolidae ('Polyphagoidea') are then sister to all the other cockroaches (including the termites) and the mantids are sister to the cockroaches. Most of these relationships have 100% posterior probabilities, meaning that none of the 2501 sampled trees in the Bayesian analysis recover any other relationship. The probability of termites falling outside the cockroaches, using our dataset, is therefore extremely small. Alternative topologies within the Dictyoptera were statistically very unlikely (table 1). None of these alternative topologies affect our findings of a blattid + *Cryptocercus* + termite clade. The maximum parsimony analysis gives essentially the same tree topology as the Bayesian analysis, with strong support for the key nodes (see electronic supplementary material, appendices).

How do these results compare with the earlier analyses of Dictyoptera? This is difficult to assess for many of the studies because they have not included termites, or, what in retrospect is more misleading, have used termites as out-groups, therefore specifically excluded them *a priori* from nesting within the cockroaches. However, considering only studies where all the Dictyoptera have been included unconstrained, most recent studies support our findings (table 2), although not all these studies are independent: a number used some of the same genes as we have. The most comprehensive of the studies in table 2 (Klass & Meier 2006), however, has the

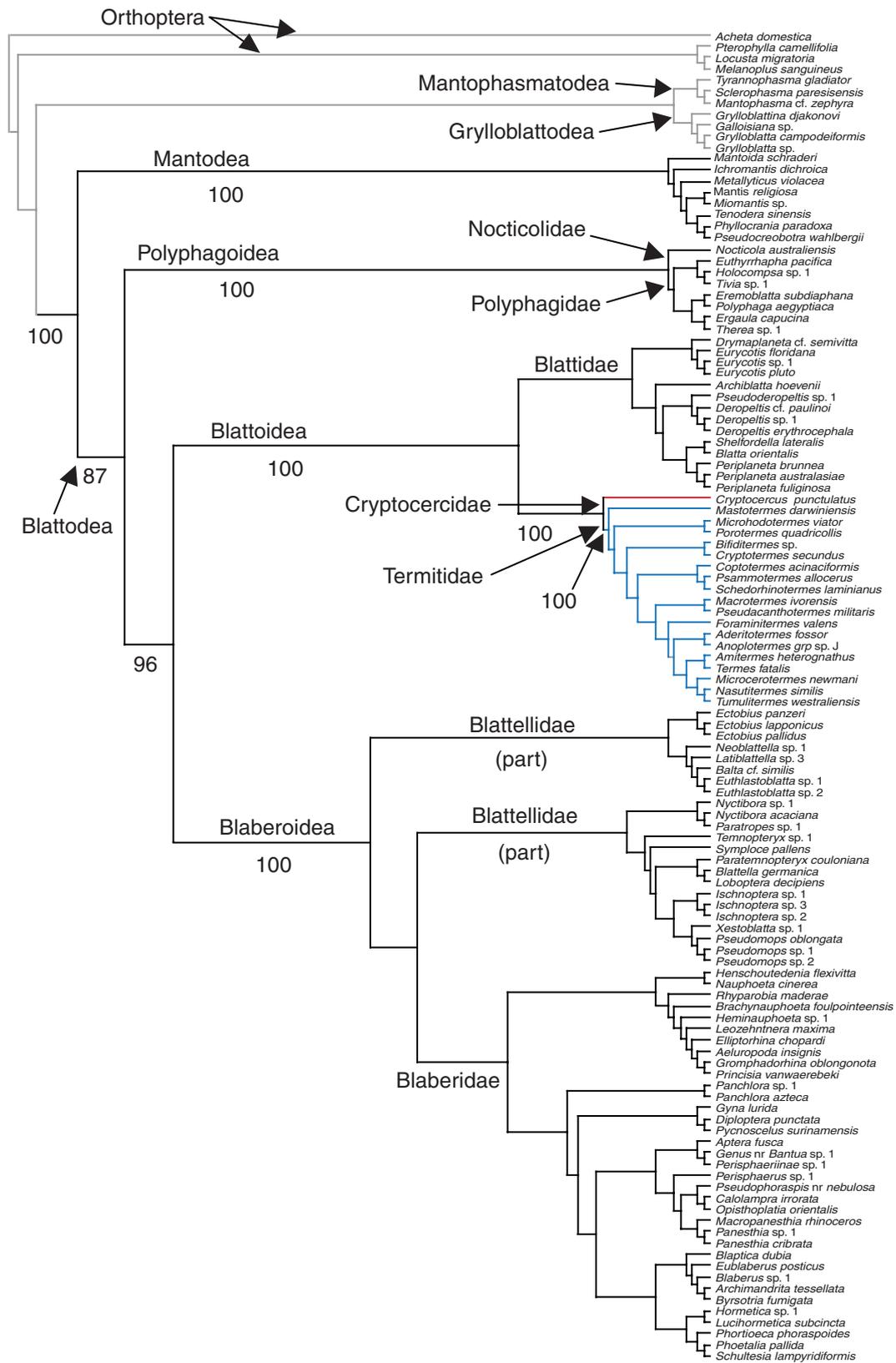


Figure 1. Topology of Bayesian majority rules consensus tree of 2501 trees. Red branch indicates position of *Cryptocercus*, blue branches indicate termite lineage. Numbers under the branches indicate posterior probabilities (i.e. the proportion of the 2501 sampled trees that contain the node) for key nodes. Names of major clades (e.g. superfamilies) are provisional.

advantage that it uses a completely independent morphological dataset and, although it differs in some basal parts of the tree (tables 1 and 2), it shows a strong level of support for a sister group relationship between termites and *Cryptocercus* and finds that this clade is nested within the cockroaches.

Even given this growing consensus, however, most previous researchers appear to have had little problem with accepting that termites could both be nested within the cockroaches and that termites (a clade) could still be considered as an order separate from the cockroaches (a grade). At the same time, however, most

Table 1. Posterior probabilities (post p) and S–H tests (Diff – ln L) for alternative phylogenetic hypotheses when compared with our preferred tree. (Post p , posterior probabilities for nodes in Bayesian analysis. Probabilities for Shimodaira–Hasegawa (S–H) test: *** $p < 0.001$, ** $p < 0.001–0.05$, * $p < 0.05–0.1$. Key to abbreviations: *Cryptoc*, *Cryptocercus*; polyp, Polyphagoidea.)

topology	post p	diff – ln L (S–H tests)
termites sister to all other Dictyoptera	<0.0004	–211.49***
termites sister to Blattodea	<0.0004	–190.48***
termites + <i>Cryptoc</i> + polyp + Blattodea	0.052	–53.8*
Blattoidea sister to all other Dictyoptera	0.002	–60.84*
Mantodea + polyp monophyletic	0.062	–46.7*
termites + polyp monophyletic (Klass & Meier 2006)	<0.0004	–83.92**

Table 2. Summary of recent phylogenetic hypotheses concerning termites. (Endog, endoglucanase; H3, histone 3; *, Taxon sampling too scanty to allow a clear hypothesis of position of termites within the Blattodea; T in B, termites nested within Blattodea?; T/C sister, termites and *Cryptocercus* sister groups? Papers discuss Blattodea relationships in isolation are not included here. No. of roaches, Number of cockroach (minus termites) families/subfamilies/genera/species in the various studies.)

character set	reference	T in B?	no. of roaches	T/C sister?
12S, 28S, 18S, COII, H3	this study	yes	6/22/65/81	yes
16S, 12S	Kambhampati (1995)	no	4/12/24/32	no*
morphology	Thorne & Carpenter (1992)	no	not species level	no*
morphology	Deitz et al. (2003)	yes (or sister)	not species level	yes
oothecae	Nalepa & Lenz (2000)	yes	no phylogeny	not fully discussed
endog, 18S	Lo et al. (2000)	yes	5/4/5/6	yes*
18S, 12S, 16S, COII	Lo et al. (2003)	yes	5/4/6/9	yes*
18S, 28S, H3	Terry & Whiting (2005)	yes	4/?/?/6	yes*
morphology	Klass & Meier (2006)	yes	5/13/20/21	yes

systematists now generally believe that formal taxonomic groupings should be monophyletic (Benton 2000). Therefore, we are led logically to the conclusion that the presently recognized order Blattodea is not an acceptable taxon if it does not include the termites, as it does not contain an ancestor and all its descendants. The finding that the termites are nested within the cockroaches causes a classificatory problem that we believe can best be resolved by changing the taxonomic rank of the termites. We propose that the presently recognized order Isoptera should no longer be used and that the species presently included in Isoptera should be classified within the family Termitidae as part of the order Blattodea within the superorder Dictyoptera. This means that the existing termite taxa need to be downgraded by one taxonomic rank (i.e. families become subfamilies, subfamilies become tribes; see electronic supplementary material), but would otherwise remain unchanged in species composition.

This result may appear surprising to many people who are aware that termites have apparently very different life history and social behaviours from cockroaches. However, it is scarcely unparalleled. Ants, social wasps and bees are also generally strikingly different in many aspects of their biologies from their closest solitary relatives. The evolution of sociality clearly has the propensity to change the nature of clades fundamentally, such that just four families of eusocial insects (Formicidae, Vespidae, Apidae and Termitidae) have come to dominate vital ecosystem processes (predation, pollination and decomposition; Grimaldi & Engel 2005).

Our findings allow the pathway to eusociality in termites to be reconstructed with more certainty (figure 2) and they generally support recent hypotheses based on nutritional and microbiological arguments (Nalepa et al. 2001). Termites have evolved from omnivorous cockroach ancestors with a diploid reproductive system that form their oothecae internally and exhibit different degrees of intraspecific coprophagy (faeces eating) and gregariousness. These last two characteristics have allowed specifically co-evolved gut symbioses to evolve, as facilitative coprophagy by conspecifics allows the transmission of a stable microbial assemblage from generation to generation. The key evolutionary shift appears to be the acquisition of mutualistic cellulolytic flagellates in the ancestor of termites and *Cryptocercus* that allowed the cockroaches to become wood feeding (although this shift is not only found in the termite + *Cryptocercus* clade within cockroaches, Brugerolle et al. 2003). Offspring of these wood-feeding cockroaches required lengthy parental contact to allow flagellate transfer between generations by proctodeal trophallaxis (nutrient transfer from the anus of one individual to the mouth of another; Nalepa et al. 2001). The subsequent shift to eusociality in the termites has involved reduction and eventual loss of the oothecae, as protection from desiccation is unnecessary inside a nest with a controlled internal climate. There has also been a trend towards monogamy, with progressive anatomical simplification of termite sperm (Baccetti et al. 1981) and reduction in complexity of the male genitalia (Klass et al. 2000), presumably due to lowered sperm competition.

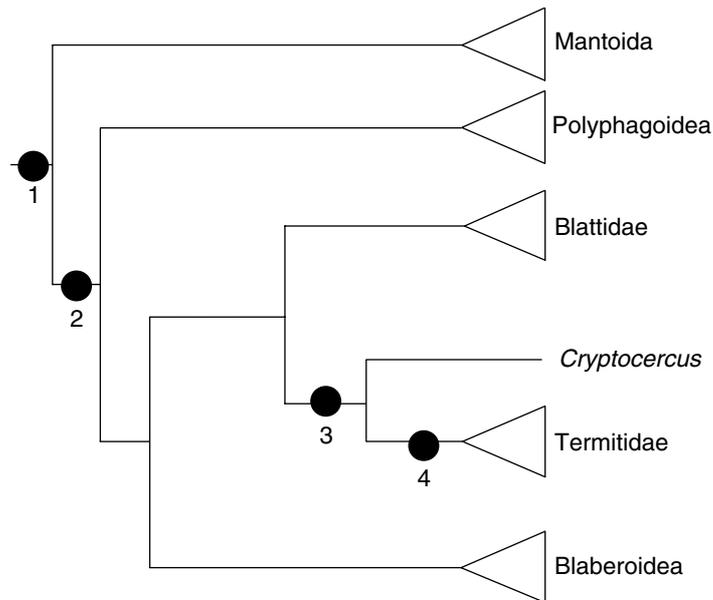


Figure 2. Simplified phylogenetic tree showing major evolutionary transitions within ancestral forms leading to the termites. 1, ootheca formed from secretions of the asymmetrical accessory glands; 2, ootheca formed internally in vestibulum, keel pointing upwards until deposited; 3, wood-feeding, unique hypermastigid and oxymonad gut flagellate assemblages, biparental care, proctodeal trophallaxis; and 4, male genitalia bilaterally symmetrical and much reduced, sperm immotile or weakly motile, true soldier caste, overlapping generations with reproductive division of labour ('eusociality').

In addition, establishment of permanent family groups (colonies) has led to the evolution of sterile worker and soldier castes in response to the need for foragers, alloparental care, nest builders and colony defenders (Higashi *et al.* 2000).

Our reconstruction emphasizes the strikingly different routes of Hymenoptera and Dictyoptera to eusociality. Ants, arguably the closest biological analogues of termites, have evolved from multiply provisioning predatory wasps (Wilson & Holldobler 2005) with a haplo-diploid reproductive system, phylogenetic and life-history characteristics far removed from those found in cockroaches. Any general theory explaining the evolution of insect eusociality must take these profound evolutionary differences fully into account.

We thank all colleagues who donated material (see electronic supplementary material, appendix 7) and Alfried Vögler for advice on molecular phylogenetics. This research was funded by a UK Leverhulme grant to P.E. and by an NHM Entomology DRF grant to P.E. and G.B.

- Baccetti, B., Dallai, R. & Callaini, G. 1981 The spermatozoon of arthropoda. 32. *Zootermopsis nevadensis* and isopteran sperm phylogeny. *Int. J. Inver. Rep.* **3**, 87–99.
- Benton, M. J. 2000 Stems, nodes, crown clades, and rank-free lists: is Linnaeus dead? *Biol. Rev.* **75**, 633–648.
- Brugerolle, G., Silva Neto, I. D., Pellens, R. & Grandcolas, P. 2003 Electron microscopic identification of the intestinal protozoan flagellates of the xylophagous cockroach *Parasphaeria boleiriana* from Brazil. *Parasitol. Res.* **90**, 249–256.
- Cleveland, L. R., Hall, S. K., Sanders, E. P. & Collier, J. 1934 The wood feeding roach *Cryptocercus*, its protozoa, and the symbiosis between protozoa and roach. *Mem. Am. Acad. Arts Sci.* **17**, 185–382.
- Deitz, L. L., Nalepa, C. & Klass, K. D. 2003 Phylogeny of the Dictyoptera re-examined (Insecta). *Entomologische Abhandlungen (Dresden)* **61**, 69–91.

- Grimaldi, D. A. & Engel, M. S. 2005 *Evolution of the insects*. Cambridge, UK; New York, NY: Cambridge University Press.
- Higashi, M., Yamamura, N. & Abe, T. 2000 Theories on the sociality of termites. In *Termites: evolution, sociality, symbioses, ecology* (eds T. Abe, D. E. Bignell & M. Higashi), pp. 212–223. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Kambhampati, S. 1995 A phylogeny of cockroaches and related insects based on DNA sequence of mitochondrial ribosomal RNA genes. *Proc. Natl Acad. Sci. USA* **92**, 2017–2020. (doi:10.1073/pnas.92.6.2017)
- Klass, K. & Meier, R. 2006 A phylogenetic analysis of Dictyoptera (Insecta) based on morphological characters. *Entomologische Abhandlungen* **63**, 3–50.
- Klass, K. D., Thorne, B. L. & Lenz, M. 2000 The male postabdomen of *Stoloterms inopinus*: a termite with unusually well-developed external genitalia (Dictyoptera: Isoptera: Stolotermitinae). *Acta Zool.* **81**, 121–130. (doi:10.1046/j.1463-6395.2000.00045.x)
- Lo, N., Tokuda, G., Watanabe, H., Rose, H., Slaytor, M., Maekawa, K., Bandi, C. & Noda, H. 2000 Evidence from multiple gene sequences indicates that termites evolved from wood-feeding cockroaches. *Curr. Biol.* **10**, 801–804. (doi:10.1016/S0960-9822(00)00561-3)
- Lo, N., Bandi, C., Watanabe, H., Nalepa, C. & Beninati, T. 2003 Evidence for cladogenesis between diverse dictyopteran lineages and their intracellular endosymbionts. *Mol. Biol. Evol.* **20**, 907–913. (doi:10.1093/molbev/msg097)
- McKitterick, F. 1964 A contribution to the understanding of cockroach-termite affinities. *Ann. Entomol. Soc. Am.* **58**, 18–22.
- Nalepa, C. A. & Lenz, M. 2000 The ootheca of *Mastotermes darwiniensis* Frogatt (Isoptera: Mastotermitidae): homology with cockroaches oothecae. *Proc. R. Soc. B* **267**, 1809–1813. (doi:10.1098/rspb.2000.1214)
- Nalepa, C. A., Bignell, D. E. & Bandi, C. 2001 Detritivory, coprophagy, and the evolution of digestive mutualisms in Dictyoptera. *Insectes Sociaux* **48**, 194–201. (doi:10.1007/PL00001767)

- Posada, D. & Crandall, K. A. 1998 MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818. (doi:10.1093/bioinformatics/14.9.817)
- Ronquist, F. & Huelsenbeck, J. P. 2003 MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574. (doi:10.1093/bioinformatics/btg180)
- Terry, M. D. & Whiting, M. F. 2005 Mantophasmatodea and phylogeny of the lower neopterous insects. *Cladistics* **21**, 240–257. (doi:10.1111/j.1096-0031.2005.00062.x)
- Thorne, B. L. 1990 A case for ancestral transfer of symbionts between cockroaches and termites. *Proc. R. Soc. B* **241**, 37–41. (doi:10.1098/rspb.1990.0062)
- Thorne, B. L. & Carpenter, J. M. 1992 Phylogeny of the Dictyoptera. *Syst. Entomol.* **17**, 253–268.
- Wilson, E. O. & Holldobler, B. 2005 The rise of the ants: a phylogenetic and ecological explanation. *Proc. Natl Acad. Sci. USA* **102**, 7411–7414. (doi:10.1073/pnas.0502264102)