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Identifying possible sister groups of Cryptocercidae + Isoptera: A combined molecular and morphological phylogeny of Dictyoptera

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ABSTRACT

Termites (Isoptera) offer an alternative model for the development of eusociality which is not dependent on a high degree of relatedness as found between sisters in hymenopterans (bees, wasps, ants). Recent phylogenetic studies have established that termites belong within the cockroaches as sister to the subsocial Cryptocercidae. Cryptocercidae shares several important traits with termites, thus we need to understand the phylogenetic position of Cryptocercidae + Isoptera to determine how these traits evolved. However, placement of Cryptocercidae + Isoptera is still uncertain. We used both molecular (12S, 16S, COII, 18S, 28S, H3) and morphological characters to reconstruct the phylogeny of Dictyoptera. We included all previously suggested sister groups of Cryptocercidae + Isoptera as well as taxa which might represent additional major cockroach lineages. We used Bayes factors to test different sister groups for Cryptocercidae + Isoptera and assessed character support for the consensus tree based on morphological characters and COII amino acid data. We used the molecular data and fossil calibration to estimate divergence times. We found the most likely sister groups of Cryptocercidae + Isoptera to be Tryonicidae, *Anaplecta* or Tryonicidae + *Anaplecta*. *Anaplecta* has never previously been suggested as sister group or even close to Cryptocercidae + Isoptera, but was formerly placed in Blaberoidea as sister to the remaining taxa. Topological tests firmly supported our new placement of *Anaplecta*. We discuss the morphological characters (e.g. retractable genitalic hook) that have contributed to the previous placement of *Anaplecta* in Blaberoidea as well as the factors that might have contributed to a parallel development of genitalic features in *Anaplecta* and Blaberoidea. Cryptocercidae + Isoptera is placed in a clade with Tryonicidae, *Anaplecta* and possibly Lamproblattidae. Based on this, we suggest that wood-feeding, and the resultant need to conserve nitrogen, may have been an important factor in the development of termite eusociality. Nocticolidae was placed as sister group to *Latindia* + *Paralatindia* (both Corydiidae), this clade was in turn placed as sister group to the remaining Corydiidae. The Nocticolidae + Corydiidae clade is supported by both morphological and COII amino acid changes. Our divergence time estimates placed the split between Mantodea and Blattodea at 273 mya (middle Permian) and the splits between the major blattodean lineages no later than 200 mya (end of Triassic).

1. Introduction

The termites (Isoptera[1]) offer an alternative model for the development of eusociality compared with the eusocial hymenopterans (bees, wasps and ants). The eusociality of termites differs from that in hymenopterans in several key aspects: In hymenopterans, all non-reproductives (workers etc.) are females whereas termites have non-reproductives of both sexes. In social Hymenoptera, brood care is only performed by females (queens or workers), while in termite colonies, both sexes (queen and king, workers of both sexes) care for the brood. Hymenopterans have haplodiploid sex-determination, which results in full sisters being more closely related (75%) than mother and offspring (50%); this is thought to be crucial in the repeated evolution of eusociality in Hymenoptera (Grimaldi and Engel, 2005). In termites, however, both sexes are diploid (White, 1976): full siblings share 50% of their genes. Termites thus offer an excellent model for development of eusociality in the absence of a haplodiploid sex-determination system (Boomsma, 2009).

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[1] Termites are formally classified as Termitoidae, an epifamily (rank between family and superfamily). Due to the greater name recognition of Isoptera, we here use the latter as an informal name.

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To determine how termite eusociality originated, we need to identify the starting point, which requires knowing the sister group of Isoptera. Recent phylogenetic work has established that termites belong within the cockroaches as sister to Cryptocercidae (Cameron et al., 2012; Djernæs et al., 2012; Inward et al., 2007; Klass, 1995; Klass and Meier, 2006; Lo et al., 2000, 2003, 2007; Murienne, 2009; Pellens et al., 2007; Ware et al., 2008). However, the subsocial Cryptocercidae (one genus, *Cryptocercus*) shares several behavioural traits with termites that are relevant in the context of sociality: biparental care, anal trophallaxis and wood-feeding, and they have a gut fauna similar to that found in most of the major lineages of the termites (presence of a variety of specific oxymonadid and hypermastigid gut flagellates) (Klass et al., 2008). As these characteristics had already been acquired by the ancestor of Cryptocercidae + Isoptera, it is necessary to know the sister group of this clade to determine how these traits evolved.

However, the placement of Cryptocercidae + Isoptera is still uncertain: suggested sister groups include Corydiidae[2] (Cameron et al., 2012), Corydiidae + Lamproblattidae (Klass, 1995; Klass and Meier, 2006), Corydiidae + Nocticolidae (Djernæs et al., 2012), Blattidae (Inward et al., 2007; Lo et al., 2003; Pellens et al., 2007), Tryonicidae (Murienne, 2009), Blattidae + Tryonicidae (Ware et al., 2008), and all other Dictyoptera (Lo et al., 2007). None of the suggested sister groups are known to share more similarities in lifestyle with Cryptocercidae + Isoptera than the others; for instance, representatives of all of them have been found in rotting wood (Bell et al., 2007).

This phylogenetic uncertainty exists despite a large number of studies of the relationships between the previously proposed major taxonomic units of Dictyoptera (Mantodea, Corydiidae, Nocticolidae, Blattidae, Ectobiidae,[3] Blaberidae, Cryptocercidae and Isoptera), both morphological and molecular (morphological: Grandcolas, 1996; Klass, 1995, 1997; Klass and Meier, 2006; molecular: e.g. Djernæs et al., 2012; Inward et al., 2007; Lo et al., 2000, 2003, 2007; Murienne, 2009; combined: Ware et al., 2008). The limited consensus to emerge from these studies has confirmed the position of Mantodea (mantids) as sister to the remaining Dictyoptera, placed Blaberidae within Ectobiidae (entire clade referred to as Blaberoidea), placed Cryptocercidae and Isoptera as sister groups (Isoptera thus subordinate within Blattodea) and placed Corydiidae and Nocticolidae as sister groups (referred to as Corydioidea), although Nocticolidae as sister group to Mantodea has alternatively been suggested by Lo et al. (2007). In addition, these studies identified the species-poor Lamproblattidae and Tryonicidae, both formerly assigned to Blattidae, as major lineages. Thus the currently recognised major lineages are Mantodea, Blattidae, Blaberoidea, Lamproblattidae, Tryonicidae and Cryptocercidae + Isoptera, and Corydioidea is strongly suggested as a major lineage. Apart from the basal dichotomy between Mantodea and the remaining lineages (=Blattodea) nearly every study has suggested different relationships between these groups (only Klass, 1995, 1997, and Klass and Meier, 2006 as well as Lo et al., 2000, and 2003 agreed with each other). Please refer to Ware et al. (2008) and Djernæs et al. (2012) for a more comprehensive review of studies of dictyopteran phylogeny.

The majority of studies of dictyopteran phylogeny have been either molecular or morphological. The only exception is Ware et al. (2008) using already published data, which resulted in a large proportion of missing data, and which had no targeted taxon sampling. Here we search for the sister group of Cryptocercidae + Isoptera using targeted taxon sampling and by generating both morphological and molecular data. In addition to the recognised major lineages, we include taxa that might represent unrecognised major lineages such as the genus *Anaplecta* (placed in Blaberoidea as sister to the remaining taxa) and the genus *Duchailluia* (placed in Blattidae, but having quite aberrant male genitalia). We use the phylogeny to illuminate the factors which may have favoured the development of subsociality in the ancestor of Cryptocercidae + Isoptera.

It is widely accepted that the major lineages of Dictyoptera diverged in the Jurassic (200–146 mya) (e.g. Grimaldi and Engel, 2005). However, termite fossils belonging to extant families are known from early Cretaceous (130 mya) (Thorne et al., 2000) and divergence time analyses of termites placed the split between Cryptocercidae and Isoptera in the early Triassic (231–243 mya) (Ware et al., 2010). Combined with the subordinate position of Isoptera within Blattodea this poses a quandary. The fossil evidence alone (Engel et al., 2007; Thorne et al., 2000) would require a rapid diversification of both stem and crown group Blattodea and the early termites (both stem and crown group) if these divergences should occur no earlier than the Jurassic. Ware et al.'s (2010) estimates would even require that the splits between the major lineages in Blattodea occurred no later than the Permian. We will address this problem using a fossil-calibrated relaxed molecular clock.

2. Materials and methods

2.1. Molecular data

The taxon sample consists of 57 dictyopteran taxa (ingroup) and 18 outgroup taxa (complete list of taxa in Table S1). The outgroup sampling includes at least two representatives for each polyneopteran order except Zoraptera (no representatives included) as well as representatives for Ephemeroptera and Odonata. The dictyopteran taxon sampling is focused on inclusion of representatives of all major lineages of Dictyoptera as well as taxa that might prove to be previously undetected major lineages. The molecular data set consists of six genes: the mitochondrial 12S (~390 nucleotides, nt), 16S (~430 nt), COII[4] (~730 nt), and the nuclear 28S (~600 nt), 18S (~1830 nt), H3 (~330 nt); the total length of the aligned molecular data set is 5584 nt. GenBank sequences were used when available (Table S1) and comprise a large proportion of our data set; these were mainly from previous Blattodea-focused work done by our team (Inward et al., 2007; Djernæs et al., 2012) and from previous work on Mantodea (Svenson and Whiting, 2009). New sequences, mostly concerning crucial cockroach taxa, were produced using standard methods, see Supplementary material for details. GenBank numbers are listed in Table S1. For three outgroup taxa, sequences from related species were combined as not all sequences were available for a single species. In these instances, the combination taxon was given the name of the lowest taxonomic unit containing all sequences (i.e. *Gryllus*, Mantophasmatidae and Oligotomidae). Combination taxa: *Aposthonia japonica* + *Oligotoma nigra* + *Oligotoma saundersii* called Oligotomidae; *Mantophasma* cf. *zephyrum* + *Mantophasma zephyrum* + *Sclerophasma paresisense* called Mantophasmatidae; *Gryllus campestris* + *Gryllus assimilis* + *Gryllus pennsylvanicus* + *Gryllus bimaculatus* called *Gryllus*. All new sequences were checked for contamination using unrestricted BLAST searches, and NJ trees were produced based on the alignment of each sequenced fragment to check for internal contamination and incorrectly identified GenBank sequences. Alignments were produced in MAFFT 7.017b (Katoh et al., 2005; Katoh and Toh, 2008; http://mafft.cbrc.jp/alignment/server/) using the Q-INS-i algorithm, but for H3, in which case the G-INS-i algorithm was used due to the lack of

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[2] Corydiidae was until recently called Polyphagidae, see Beccaloni and Eggleton (2011).

[3] Ectobiidae was until recently called Blattellidae, see Beccaloni and Eggleton (2011).

[4] Including parts of tRNA-Leu (5′ end) and tRNA-Lys (3′ end).