

# Supplementary Material

## to

### An integrative phylogenomic approach illuminates the evolutionary history of cockroaches and termites (Blattodea)

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## Note on taxon names

In several files and figures, some species names may be different compared to the text here and the main paper due to identification updates and corrections. Valid names are found in the main text and Table S1. It concerns the following names:

Name in analysed files (outdated)	Correct and valid name	Explanation
<i>Ischnoptera</i> sp.	<i>Ischnoptera deropeltiformis</i>	Correct species name updated
<i>Eurycotis floridiana</i> , <i>Eurycotis decipiens</i>	<i>Eurycotis floridana</i>	Typo corrected and wrong species name corrected that was partially used by accident
<i>Cryptocercus</i> sp.	<i>Cryptocercus wrighti</i>	Correct species name updated
<i>Lamproblatta albipalpa</i>	<i>Lamproblatta albipalpus</i>	Typo corrected
<i>Gratidia madagassa</i>	<i>Antongilia madagassa</i>	Correct genus name updated
<i>Timema christinae</i>	<i>Timema cristinae</i>	Typo corrected
<i>Blatella germanica</i>	<i>Blattella germanica</i>	Typo corrected
<i>Princisia vanwaerebecki</i>	<i>Princisia vanwaerebeki</i>	Typo corrected

## S1 Datasets and molecular analyses

### S1.1 Molecular dataset generation

#### *Taxon sampling, sequencing and assembly*

The dataset comprised 66 taxa in total, including sequence data from 45 Blattodea and 21 outgroup taxa. These outgroup taxa represent all major Polyneoptera lineages, including grasshoppers, crickets and allies (Orthoptera), stoneflies (Plecoptera), earwigs (Dermaptera), mantids (Mantodea), stick and leaf insects (Phasmatodea), heelwalkers (Mantophasmatodea), ice crawlers (Grylloblattodea), webspinners (Embiopoda), and ground lice (Zoraptera) (Table S1).

Data was derived from transcriptomes, except for the termite *Zootermopsis nevadensis* for which we used the official gene set derived from a whole genome project. Specimens were either preserved in liquid nitrogen, then kept frozen at -35°C or directly grounded in RNAlater and kept at 4°C until further processing. Details on all samples used for sequencing are provided on NCBI. RNA extraction and cDNA library preparation, transcriptome sequencing, and *de novo* assembly were conducted at the Beijing Genomics Institute (BGI) Shenzhen and are described in detail in Peters et al. (2017). All samples except for one were sequenced on HiSeq 2000 platform with 150 bp paired-end (PE) reads, generating approximately 2.5Gb of raw data each. Due to limited RNA inputs (< 1 ug), we used the TruSeq mRNA library Pre Kit (Illumina, Ca. USA) for *Diploptera punctata* and sequenced it with 90 PE reads. Details about procedure of RNA extraction, library preparation and Illumina sequencing are described in Peters et al. (2017).

Raw reads were assembled using the assembler SOAPdenovo-Trans -SOAPdenovo-Trans-31kmer (Xie et al., 2014) as described in Peters et al. (2017). Subsequent quality assessment including check and removal of contaminants as well as submission to NCBI Sequence Read Archive (SRA) and the Transcriptome Shotgun Assembly (TSA) database were conducted as described in Peters et al. (2017). For details on the number of contigs before and after contamination check, see Table S2. Transcriptome data published with this study are deposited in GenBank NCBI under the 1KITE umbrella project Bioproject ID 183205 (Table S1).

#### *Identification of orthologous sequences*

For the identification of orthologous transcripts we first generated a custom-made ortholog set from the public database OrthoDB 8 (<http://cegg.unige.ch/orthodb8>) (Kriventseva et al., 2015). The ortholog set was especially designed for Polyneoptera taxa. Therefore, we selected only those genes, which were inferred as single copy in the genomes of each of reference species (copy number = 1, other taxa included in the clade “Hexapoda” were set to an unknown number of copies “?”). We choose as reference species (official gene sets from whole genome projects available) *Ephemera danica*, *Ladona fulva* (both unpublished and access granted by the i5K community, in particular Stephen Richards, Bernhard Misof and Panagotis Provataris), *Zootermopsis nevadensis* (Terrapon et al., 2014), and *Rhodnius prolixus* (Mesquita et al., 2016). Cleaned versions (longest isoforms only and Selenocysteine replaced by X) of all

four official gene sets on amino-acid level as used in OrthoDB were kindly provided by Robert Waterhouse. Given the above set of reference species and with the hierarchical orthology reference node in the phylogenetic tree set to the clade comprising all insects (=Hexapoda), OrthoDB 8 specified 3,247 protein-coding genes as single-copy (orthologous sequence clusters for the four reference species). We downloaded corresponding files on transcript level (cds) respectively (see Table S3.1). Sequence headers were adjusted accordingly on transcript and amino-acid level. In addition, we removed sequences only occurring at amino-acid or cds level with scripts provided with the Orthograph package (Petersen et al., 2017). Using Orthograph (v.0.5.4), an ortholog set database was build based on the full official gene sets and respective orthologs. This ortholog set was subsequently used for orthology inference for all included species. Ortholog table, and official gene sets are available on DRYAD (Supplementary File “S1.1\_OrthologSet”).

Identification of orthologous transcripts for each taxon was carried out using Orthograph (v.0.5.4) (Petersen et al., 2017). We ran Orthograph with the following settings: max-blast-searches = 50, blast-max-hits = 50, extend-orf = 1, substitute-u-with = X, and leaving other settings to defaults. We further considered the best reciprocal hit (BRH) criterion being fulfilled if the reciprocal BLAST search found in at least one of the four reference taxa the candidate ortholog sequence as best hit (option strict-search turned off). Using these settings, we identified on average 2,370 orthologous genes/groups (OGs) (minimum: 1,534 OGs identified in *Nyctibora* sp., maximum: 2,986 OGs identified in *Prorhinotermes simplex*) (see Table S3.2 for Orthograph results).

Orthograph results were summarized according to each OG with the script summarize\_orthograph\_results.pl provided with the Orthograph package. We removed any terminal stop codon that were not encoded by the corresponding nucleotide sequence (option -t), masked stop symbols (\*) with X on amino-acid and with NNN on the transcript level; terminal stop codons that were not encoded by corresponding nucleotide sequence were not removed (option -s). Additionally, we masked Selenocysteine (symbol U) with “X” and “NNN”, respectively (option -u) since Selenocysteine cannot properly be handled by many software used for downstream analyses. This resulted into a total of 3,244 summarized OGs.

### ***Alignment, protein domain identification, alignment masking, optimizing datasets***

The sequences of retrieved OGs were aligned on amino-acid level (aa) using MAFFT (v.7.245) (Kato & Standley, 2013) with the L-INS-i algorithm. Each aa multiple sequence alignment (MSA) was subsequently checked for the presence of outliers. Identified outliers were refined using a profile alignment approach as described in Misof et al. (2014) but using the -addfragments algorithm implemented in MAFFT. Subsequently, MSAs were checked a second time for outlier sequences, which were then removed from the aa MSAs and nucleotide (nt) OGs as described in Misof et al. (2014). We further removed sequences of the reference species *Ephemera danica*, *Ladona fulva* and *Rhodnius prolixus* from the aa MSAs and nt OGs since we aimed to include only Polyneoptera taxa for the phylogenetic inference. We discarded columns

only containing gaps in the amino-acid MSAs caused by the removal of sequences of the reference genomes. We then generated MSAs of nucleotides corresponding to the amino-acid MSAs with a modified version of the software PAL2NAL (Suyama et al., 2006) (see Misof et al., 2014) using the corresponding amino-acid MSAs as blueprint.

For phylogenetic analyses, we considered regions identified as protein clans, families, single domains or non-annotated regions (so called voids) as evolutionary units in the partitioned analyses. For a rationale see Misof et al. (2014). Using the Pfam database (Punta et al., 2012) release 28.0 (/, including only the Pfam-A database) in conjunction with the software pfam\_scan.pl version 1.5 and HMMER (Eddy, 2011) (), Domain-identification-v1.3 and Domain-parser-v1.4.1-dist, we identified 5,899 Pfam-A domains, and 8,719 void regions using the same strategy as described in Wipfler et al. (unpublished).

Parallel to the protein domain identification, putative ambiguously aligned or randomized MSA sections were identified for each amino-acid MSA with Aliscore (v.1.2) (Kuck et al., 2010; Misof & Misof, 2009) with the default sliding window size, the maximal number of pairwise sequence comparisons (option -r) and a special scoring for gap-rich amino-acid data (option -e). Using custom Perl scripts, the results from the protein domain identification step and the identified randomized MSA sections were merged into a masked supermatrix. Thus, the resulting supermatrix consists of data blocks with regions of putative alignment sections removed. The total alignment length spanned 1,235,884 amino-acid positions. A nucleotide supermatrix that exactly corresponds to the amino-acid supermatrix was created using several custom-made Perl scripts, resulting in a total alignment length of 3,707,652 nucleotide positions (details on the procedure are described in Peters et al. 2017).

In order to optimize these datasets, we removed data blocks with an information content (IC) of zero on amino-acid level as identified by MARE (v.0.1.2-rc) (Misof et al., 2013). Respective data blocks were also removed from the nt dataset. For this full nucleotide dataset we subsequently evaluated whether or not our datasets have evolved under globally stationary, reversible and homogeneous (SRH) conditions with SymTest version 2.0.47 () (Ho & Jermini, 2004a). SymTest uses three matched-pairs tests of homogeneity; details are provided in Misof et al. (2014). We generated heat maps based on *p*-values obtained from the implemented Bowker's matched-pairs test of symmetry (Bowker, 1948) in order to determine those sequence pairs that could be assumed to have evolved under globally SRH conditions. We applied the implemented Bowker Test on the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> codon position separately, on the 1<sup>st</sup> + 2<sup>nd</sup> and keeping all codon positions. Further downstream analyses were performed on the nucleotide dataset (data blocks with IC=0 removed) keeping the 2<sup>nd</sup> codon position only, as this showed a smaller among-lineage heterogeneity compared to the other datasets consisting of either only the 1<sup>st</sup> or only the 3<sup>rd</sup> codon positions or consisting of 1<sup>st</sup> + 2<sup>nd</sup>, or of all codon positions (see Figure S1 A-E). The final full nucleotide data with the 2<sup>nd</sup> codon position comprised 1,205,322 nt sites and 1,546 partitions, i.e. merged data blocks according to the protein domain identified on the corresponding aa dataset and the selection of optimal partitions (see below). To further evaluate the coverage of this full nucleotide data with the 2<sup>nd</sup> codon position only with respect to pairwise



sequence coverage of (missing) data, we used AliStat v.1.6 () (Wong et al., 2017), see also Misof et al. (2014), and generated the respective heat map. In total, 2,145 pairs of sequences were compared and resulted in an overall completeness (C) score for the alignment (Ca) of 0.474262, with a maximum C-score for individual sequences (Cr\_max) of 0.979492 (*Zootermopsis nevadensis*) and a minimum C-score for individual sequences (Cr\_min) of 0.194385 (*Nyctibora* sp.) (see Figure S2).

For the amino-acid dataset, the data coverage was further increased by including only data blocks that contained sequence information for at least one representative of specified taxonomic groups (Table S4) using custom Perl scripts, for a rationale see Dell'Ampio et al. (2014) and Misof et al. (2014). This resulted in a decisive amino-acid dataset comprising 1,315 data blocks and 585,040 amino-acid positions. We again checked whether or not this dataset matched SRH conditions using the Bowker's test implemented in SymTest 2.0.47, (see Figure S1F). We evaluated again the site coverage of this “decisive, optimized” amino-acid dataset with respect to pairwise sequence coverage of unambiguous data with AliStat (v.1.6). The completeness score for the complete alignment (Ca) was remarkably higher (Ca of 0.594592), with a maximum C-score for individual sequences (Cr\_max) of 0.982053 (*Zootermopsis nevadensis*) and a minimum C-score for individual sequences (Cr\_min) of 0.327783 (*Nyctibora* sp.) (see Figure S2B).

## **S1.2 Phylogenetic inference and assessing support**

### ***Dataset partitioning***

In order to select the most appropriate number of partitions (i.e., merged data blocks), we used PartitionFinder 2.0.0 (prerelease 13) (Lanfear et al., 2016) in combination with the provided RaxML version. For the full nucleotide supermatrix with only data blocks with IC=0 removed but not further optimized / reduced, we applied a specific strategy to select the best-fit partitioning schemes and models of molecular evolution for the phylogenetic analyses. Because we used protein domains as an evolutionary unit, we first used the corresponding amino-acid supermatrix (data blocks with IC=0 removed, 66 taxa, 1,205,322 amino-acid positions and 3,916 initial data blocks) to merge data blocks and to select the optimal partitioning scheme in PartitionFinder (Lanfear et al., 2016) (options --rclusterf --rcluster-max 8000 --rcluster-percent 100 -q -p 28 --weights 1,1,0,1 -v --all-states --min-subset-size 100). This partitioning scheme search (merging data blocks) resulted in 1,546 partitions. Please note that the boundaries of the partitions identified on amino-acid level are equivalent to the boundaries we kept for the full nucleotide dataset. To select the best substitution model for each of these partitions on nucleotide level, we applied ModelFinder as implemented in IQ-TREE (v.1.5.0) (Kalyaanamoorthy et al., 2017); options -m TESTNEWONLY -gmedian. The best model for each partition was selected according to the Bayesian information criterion (BIC) (Schwarz, 1978). The full nucleotide alignment with the 2<sup>nd</sup> codon position only and data blocks with IC=0 removed and the corresponding partitioning scheme with the selected substitution models are deposited on Dryad (“S1.2\_Datasets”).

For the “decisive, optimized” amino-acid supermatrix, we again used PartitionFinder 2.0.0 (prerelease 13) to select an optimal partitioning scheme (Lanfear et al., 2014; Lanfear et al., 2016) (options --rclusterf --rcluster-max 4000 --rcluster-percent 100 -q -p 24 --weights 1,1,0,1 -v --all-states --min-subset-size 100; note: the rcluster-max is at least two times the number of initial partitions as recommended by the developers). We restricted the PartitionFinder search to eleven amino-acid substitution models as these are the most selected models for empirical studies on Hexapoda (Misof et al., 2014; Pauli et al., 2018; Peters et al., 2017), namely LG+G, WAG+G, DCMUT+G, JTT+G, BLOSUM62+G, LG+G+F, WAG+G+F, DCMUT+G+F, JTT+G+F, BLOSUM62+G+F, LG4X (Gu et al., 1995; Kosiol & Goldman, 2005; Le & Gascuel, 2008; Müller & Vingron, 2004; Soubrier et al., 2012; Veerassamy et al., 2003; Whelan & Goldman, 2001; Yang, 1994). PartitionFinder revealed 592 partitions in this decisive amino-acid dataset. The decisive amino-acid dataset and the corresponding partitioning scheme with selected substitution models can be found on Dryad (“S1.2\_Datasets”).

### ***Tree search and bootstrapping***

Phylogenetic relationships were inferred under the maximum likelihood (ML) optimality criterion as implemented in IQ-TREE (v.1.4.4) (Chernomor et al., 2016; Nguyen et al., 2015) using the best scoring amino-acid substitution matrix or DNA substitution matrix respectively for each partition and the edge-proportional partition model allowing partitions to have different evolutionary rates (option --ss). We performed 50 independent tree searches with a random start tree, taking the median for each rate category (--gmedian) and with an increased number of unsuccessful iterations before stopping (--numstop 200), and otherwise defaults. The resulting number of unique tree topologies was assessed with Unique Tree (v.1.9), kindly provided by Thomas Wong and available upon request. Maximum likelihood trees inferred from both datasets showed unique tree topologies, but differ only in the position of *Mastotermes* and *Zootermopsis*. The tree inferred from the decisive amino-acid dataset has *Mastotermes* as sister to all other termites and *Zootermopsis* as sister to all other Euisoptera (Figure 1), while tree inferred from the full nucleotide supermatrix has the positions of these taxa switched (Figure S3) (i.e., *Zootermopsis* sister to all other termites and *Mastotermes* sister to Euisoptera - *Zootermopsis*). Statistical node support was estimated via non-parametric bootstrapping of 100 (nucleotide dataset) or 111 (amino-acid dataset) bootstraps replicates as implemented IQ-TREE version (v.1.4.4) with following settings: --gmedian --numstop 200 and otherwise defaults (e.g. parsimony start tree), and mapping them onto the ML tree with the best log-likelihood. We ensured bootstrap convergence (i.e. that a sufficient number bootstrap replicates had been drawn) with *a posteriori* bootstrap criteria (Pattengale et al., 2010) as implemented in RAxML (v.8.2.11) (Stamatakis, 2014) (settings: “autoMRE”, -B 0.01, --bootstop-perms=10000, performing the test 10 times with different random seeds). Bootstrap convergence was fulfilled for all analyses and for both datasets after 50 bootstrap replicates.

### **Rogue taxon analyses**

We tested analyses of both datasets (the full nucleotide dataset with only the second codon position included and the decisive amino-acid dataset) for the presence of rogue taxa using all inferred bootstrap trees with RogueNaRok (v.1.0) (Aberer et al., 2013). We applied four distinct settings: (i) providing the best ML tree, (ii) majority rule consensus (50% threshold), (iii) 75% threshold consensus (the criterion for pruning rogue taxa is to improve the number of edges that have at least 75% bootstrap support), and (iv) strict consensus (100% threshold). Results revealed no rogue taxa in our datasets.

### **S1.3 Identity and phylogenetic position of *Anallacta***

We obtained all samples of *Anallacta methanoides* (Illustration A) used for transcriptome sequencing and morphological verification from one culture stock in Germany (; breeding of Jörg Bernhard). Grandcolas (1996) originally placed *Anallacta* within Blattellinae based on morphological data. Our phylogenetic analyses contradicted this placement and instead robustly placed *Anallacta* as sister to Pseudophyllodromiinae. Since this placement was unexpected we aimed to rule out misidentification, accidental sample swapping or contamination, which would imply an erroneous placement.

Based on various lines of support, we confirmed that our specimen was *Anallacta* (likely, *A. methanoides*). Our supporting evidence based on morphology and molecular analysis is as follows.

Morphological evidence for the identity of a specimen from the same culture stock (deposited in the MNHN-Paris as MNHNEP4277) is as follows:

1. colouration of our specimen matching illustration of *A. methanoides* by Shelford (1908).
2. colouration and gestalt identical to the holotype ()
3. spination of anterior-ventral margin of foreleg femur entirely with large spines ("well armed" as noted by Shelford (1908))
4. hooked phallomere on left (Grandcolas, 1996)
5. styli long and cylindrical (Princis, 1963)
6. subgenital plate slightly asymmetrical, with narrow medial projection, and covered mostly by preceding sternite (Princis, 1963)
7. "parasternite" inserted on left between subgenital plate and preceding segment (Princis, 1963)
8. genitalia similar to *Anallacta undata* as illustrated in Grandcolas (1996).



Illustration A. Adult female of *Anallacta methanoides* from a live culture. The individual is either producing or carrying an ootheca, with the keel oriented dorsally. The photo was kindly provided by Tristan Shanahan, see [invertebratedude.blogspot.com](http://invertebratedude.blogspot.com).

Furthermore, we reassessed the evidence for placement of *Anallacta* within Blattellinae. Grandcolas (1996) only provided an abbreviated character matrix without data specific to *Anallacta*. Our assessment based on morphological characters utilized in Grandcolas (1996) (Table S5) shows that the placement of *Anallacta* within Blattellinae is ambiguous. While the genital symmetry matches that of Blattellinae, the shape of sclerite L3d and R3d are consistent with Pseudophyllodromiinae. The definitions of other relevant characters in the genitalia and wing venation (see Table S5) are not clear enough to make a definitive determination of their states and assignment to either Blattellinae or Pseudophyllodromiinae.

We investigated the molecular support for this identification by comparing the Cytochrome Oxidase I (COI) gene extracted from the transcriptome sample used in our phylogeny and from DNA Sanger sequencing of COI of another sample of the same culture stock. We compared i) the DNA-Barcode sequence of COI extracted from the transcriptome assembly via a BLAST search of the transcriptome with a variety of cockroach COI sequences taken from NCBI and ii) the COI of an ethanol-preserved specimen from the same lab culture as the sample used for transcriptome sequencing. DNA sequencing was done with standard procedures and Sanger sequencing at the Zoological Research Museum A. Koenig (ZFMK), Bonn. The COI barcode regions were identical (see associated data on Dryad: "S1.4\_Anallacta").

Altogether, we exclude any possibility of contamination and confirm that our sample in our best ML trees was indeed *Anallacta methanoides* (see also Discussion in the main text.)

## **S1.4 Topology tests: Approximately Unbiased (AU) tests and Four-cluster Likelihood Mapping (FcLM)**

We utilized two additional statistical approaches to evaluate support for alternative relationships: the Approximately Unbiased (AU) test (Shimodaira, 2002) and Four-cluster Likelihood Mapping (FcLM) (Strimmer & Haeseler, 1997). For both approaches we only used the decisive amino-acid dataset.

For the AU test, alternative tree topologies were constructed using Mesquite (v.3.1) (Maddison & Maddison, 2017). We removed branch lengths from the best ML tree and rooted the topology with Mantodea as outgroup. We then manipulated the tree formulating 13 alternative topologies (described below for each tested topology). The AU test was conducted as implemented in IQ-TREE (v.1.5.5.) testing 14 topologies (including our best ML tree and the 13 alternative topologies). We used the best ML tree found in our previous phylogenetic analysis (amino-acid dataset) as a starting tree, with the same partitioning and modeling schemes. We performed the test with 100,000 pseudoreplicates. Alternative topologies tested (including our best ML tree) are given in the associated tree-file on Dryad (“Blattodea\_AUtest\_trees.tre”).

In contrast to the AU test, FcLM only addresses single splits in a tree. Therefore, this approach enables identification of hidden signal for single relationships that may not be seen in ML trees or cannot be identified by AU tests. In cases where four monophyletic groups could not be defined with respect to the relationship of interest on the best ML tree (one group being the outgroup taxa - a prerequisite to perform FcLM) we only applied the AU Test. With FcLM, we therefore only tested the positions of i) *Lamproblatta* and ii) *Corydioidea* (see below). For each test, we defined four groups and included only partitions for which at least one representative species of the addressed groups was present. Taxa that did not address a particular hypothesis were discarded from the alignment (see Table S6 for included species, group definitions are described below). We additionally checked for confounding signal due to among-lineage heterogeneity, non-random substitution processes and/or distribution of missing data using the FcLM approach with permuted datasets with phylogenetic signal destroyed, for a rationale see Sann et al. (2018) and Misof et al. (2014). FcLM analyses were performed using IQ-TREE version 1.6.beta4. Confounding signal due to not fulfilling stationary, reversible and homogeneous (SRH) conditions (Ho & Jermiin, 2004b; Jermiin et al., 2004) and/or non-randomly distributed missing data might affect FcLM results of the original data and phylogenetic tree inference. To check for potential confounding signal, we permuted both original FcLM datasets in three ways: i) destroying phylogenetic signal but keeping the among-lineage heterogeneity and non-randomly distributed missing data, ii) destroying phylogenetic signal, making the dataset homogeneous among lineages but keeping non-randomly distributed missing data and iii) as ii) but randomly distribute missing data. As substitution model, we used LG for each partition. For a more detailed description on the procedure and settings, see e.g. Simon et al. (2018) and Misof et al. (2014). Group definitions for the two FcLM tests are given in Table S6. Respective species included in each group, and number of drawn quartets are



provided in Table S6. Proportions of quartets that mapped into respective areas in a 2D simplex graph are provided in Figure S4.

Below we describe the rationale for alternative hypotheses being tested. We present results of all alternative phylogenetic relationships of specific taxa tested with the AU test and/or FcLM approaches whenever possible. For each AU test we name the alternative hypotheses as “AUTree #”s, which correspond to alternative hypotheses numbered in Table S7 and file folder “S1.3\_AU\_Tests” on Dryad. See Table S6, Figure S4 and Table S7 for further details.

### 1) Position of *Lamproblatta* (FcLM and AU test)

The position of *Lamproblatta* (as a representative of Lamproblattidae) is of particular interest because of a unique behavioral synapomorphy shared by *Cryptocercus* and *Lamproblatta* (McKittrick, 1964), and *Lamproblatta*’s unique genital morphology. However, phylogenetic studies have been conflicting: Djernæs et al. (2015) support the relationship of *Lamproblatta* as sister to the remaining Blattoidea, or sister to *Tryonicus* and *Cryptocercus* + Isoptera. Legendre et al. (2015) support the relationship of *Lamproblatta* as sister to Cryptocercidae and termites. The latter was confirmed by our ML analysis of the decisive amino-acid dataset supporting *Lamproblatta* as sister to *Cryptocercus* + Isoptera with maximal support.

Applying the AU test, two alternative topologies mentioned in Djernæs et al. (2015) were significantly rejected ( $p=0.00$ ): i) *Lamproblatta* as sister to Blattoidea and ii) *Lamproblatta* as sister to *Tryonicus*, this clade being sister to Blattidae (see AUTree #2 and #3; Table S7 and S1 file folder “S1.3\_AU\_Tests” on Dryad).

FcLM results showed 78.6% of all quartets were unambiguous for *Lamproblatta* + Tutricablattae (T1) as inferred in our analyses and as suggested by Legendre et al. (2015). One fifth of all quartet’s (21.1%) supported *Lamproblatta* + Blattoidae (T2), earlier inferred by Djernæs et al. (2015). There was nearly no support (0.3%) for *Lamproblatta* as sister to remaining Blattoidea (T3), (also inferred by Djernæs et al. 2015). The majority of quartets supporting *Lamproblatta* + Tutricablattae could not be explained by confounding signal (Figure S4). Therefore, we consider the placement of *Lamproblatta* as sister to Tutricablattae as robust and not biased.

### 2) Position of *Tryonicus* (AU test)

*Tryonicus* (as a representative of Tryonicidae) is a unique lineage of Blattoidea and has been proposed as a close relative of Kittrickia or Tutricablattae. Djernæs et al. (2015) support the relationship of *Tryonicus* as sister to *Cryptocercus* + Isoptera (Tutricablattae) but this was highly dependent on modeling and partitioning strategy (Djernæs et al., 2015). The best trees presented by Legendre et al. (2015) and Bourguignon et al. (2018) both place *Tryonicus* in clades sister to Blattidae (or Blattidae + other Tryonicidae).

We tested the position of *Tryonicus* by comparing three topological scenarios: Blattoidae (*Tryonicus* + Blattidae) (AUTree #1, our best ML tree from the decisive aa dataset), *Tryonicus* + Tutricablattae (AUTree #4) (Djernæs et al., 2015), *Tryonicus* + *Lamproblatta* with this clade being sister to Tutricablattae (AUTree #5) (Djernæs et al., 2015; Wang et al., 2017), as well as

*Lamproblatta* sister to Blattidae and *Tryonicus* sister to Tutricablattae (AUTree #6). All alternative topologies (AUTree #4-6) were significantly rejected ( $p=0.00$ ). Therefore, we consider the position of *Tryonicus* as sister to Blattidae as robust under the AU test.

### 3) Position of *Mastotermes* (AU test)

The position of *Mastotermes* and *Zootermopsis* was incongruent between the best tree inferred from the decisive amino-acid and the full nucleotide dataset. The tree reconstructed from the decisive amino-acid dataset and that inferred from our nucleotide dataset differ with respect to which lineage is sister to the remaining termites (*Mastotermes* in the former and *Zootermopsis* in the latter). Other studies have provided little evidence for any relationship other than *Mastotermes* as sister to all other termites (e.g. Bourguignon et al., 2015; Djernæs et al., 2015; Klass & Meier, 2006; Legendre et al., 2015; but see Wang et al. 2017). Note that it was possible to test the position of *Mastotermes* with FcLM but due to the low number of possible quartets (only four) we considered this test as meaningless and only utilized the AU test to assess the alternative topologies.

Specifically, we tested: i) *Mastotermes* as sister to (*Zootermopsis* + all remaining termites) (as in our best ML tree derived from the amino-acid dataset; AUTree #1); ii) *Zootermopsis* + (*Mastotermes* + remaining termites) (AUTree #7) and iii) and (*Mastotermes* + *Zootermopsis*) as sister to remaining termites (AUTree #8) (Wang et al., 2017). While topology (iii) was rejected: (*Mastotermes* + *Zootermopsis*) as sister to remaining termites (AUTree #8), topology (ii): *Zootermopsis* + (*Mastotermes* + remaining termites) (AUTree #7) could not be rejected ( $p=0.2942$ ). This implies incongruent signal in the amino-acid dataset, which is already displayed by low statistical support. Therefore, we consider the position of *Mastotermes* and *Zootermopsis* relative to all other termites as not strongly supported by our dataset. Thus our transcriptome data are not sufficient to make unambiguous conclusions about the earliest splits in Isoptera. One way to identify the conflicting signal could be to include orthologous data of considerably more termite species and perform analyses with the FcLM approach. See main text (Results and discussion: Phylogenetic relationships) for further discussion.

### 4) Position of Corydioidea (FcLM)

Two hypotheses have been repeatedly proposed in molecular phylogenetic studies: i) Blaberoidea + (Corydioidea + Blattoidea) (Djernæs et al., 2015; Legendre et al., 2015), confirmed by both our ML trees with Corydioidea being sister to Blattoidea with maximal support. ii) Corydioidea + (Blattoidea + Blaberoidea) has been suggested by Inward et al, (2007) and Wang et al. (2017). Morphological studies of Corydioidea have largely been misleading on their phylogenetic position (Grandcolas, 1996; Grandcolas, 1999; Klass & Meier, 2006), possibly due to extreme morphological differentiation causing problems with homology assignment.

Here we only applied the FcLM approach since it has advantage of discerning the source of possible incongruence, which is not possible with the AU test. Species included in respective groups are provided in Table S6, possible topologies and results are provided in Figure S4.

Around 4/5 of all quartets supported the relationship we inferred in both ML trees: Corydioidea + Blattoidea (T2: 82.4%). There was nearly no support for Corydioidea + Blaberoidea (T1: 0.8%), and small support (16.5%) for Corydioidea as sister to remaining Blattodea (seen in Wang et al. 2017). Corydioidea + Blattoidea could not be explained by confounding signal (Figure S4 F-H). Therefore, we consider the position of Corydioidea as sister to Blattoidea (and thus Blaberoidea as sister to all other Blattodea) as robust under the FcLM test and strongly supported by our data.

### 5) Position of *Ectobius* (AU test)

Ectobiinae is suggested as sister to all remaining Blaberoidea in Wang et al. (2017), sister to Pseudophyllodromiinae in Inward et al. (2007) and Legendre et al. (2015), and sister to Blaberidae + Pseudophyllodromiinae in Djernæs et al. (2012) and Djernæs et al. (2015).

We tested the following two hypotheses (*Ectobius* as a representative of Ectobiinae): i) *Ectobius* as sister to all other Blaberoidea (AUTree #1), ii) *Ectobius* as sister to Nyctiborinae + Blattellinae (AUTree #12; to our knowledge, this has not been proposed by any study, but we consider it as a possible evolutionary scenario) and iii) *Ectobius* as sister to (Pseudophyllodromiinae + *Anallacta*) (AUTree #13) (Inward et al., 2007; Legendre et al., 2015). Both topologies (ii) and (iii) were significantly rejected ( $p=0.00$ ). Thus, *Ectobius* as sister to the remaining Blaberoidea is robustly supported by our data under the AU test.

### 6) Position of *Anallacta* (AU test)

*Anallacta* has only once been included in a molecular phylogenetic study (Bourguignon et al., 2018) who suggested this taxon as sister to Ectobiinae. Analyzing morphological data, it was placed within Blattellinae by Princis (1969). This morphology-based classification was supported by Grandcolas (1996). However, he did not report the data specific for *Anallacta* and the reasoning is unclear, so it cannot be scrutinized. Morphologically, our specimens of *Anallacta methanoides* have genital symmetry common to most Blattellinae (with the hook on the left) but l3d is not ring shaped, which is consistent with Pseudophyllodromiinae (see section S1.3).

We inferred *Anallacta* as sister to Pseudophyllodromiinae in both ML trees, with maximal support, thus not within Blattellinae. With the AU test we compared: *Anallacta* as sister to Pseudophyllodromiinae (AUTree #1) and *Anallacta* as sister to Blattellinae (AUTree #9). The latter was significantly rejected ( $p=0.00$ ). Thus, our recovered relationship of *Anallacta* as sister to Pseudophyllodromiinae is robust and supported by our data under the AU test.

### 7) Position of Pseudophyllodromiinae (AU test)

Pseudophyllodromiinae was proposed as sister to Blattellinae (Wang et al., 2017), sister to Blaberidae (Djernæs et al., 2012; Djernæs et al., 2015) or to Ectobiinae (Inward et al., 2007; Legendre et al., 2015). We tested: (i) Pseudophyllodromiinae (including *Anallacta*) as sister to (Blattellinae + Nyctiborinae) + Blaberidae (AUTree #1), (ii) Pseudophyllodromiinae sister to Blaberidae (AUTree #14) (Djernæs et al., 2012; Djernæs et al., 2015) and (iii)



Pseudophyllodromiinae as sister to all other Blaberoidea (AUTree #12; discussed above). Topologies (ii) and (iii) were significantly rejected ( $p=0.00$ ). Thus, our recovered position of Pseudophyllodromiinae (including *Anallacta*) as sister to all Blaberoidea except *Ectobius* is robustly supported by our data under the AU test.

## 8) Position of Oxyhaloinae (AU test)

Blaberidae might be the most problematic group in terms of phylogenetic understanding (Evangelista et al., 2017; Legendre et al., 2017) as multiple studies, morphological and molecular, recover widely differing topologies of Blaberidae (Grandcolas, 1997; Grandcolas, 1998; Legendre et al., 2017; Legendre et al., 2015; Maekawa et al., 1999). The only apparently well-established relationships are (Blaberinae + Zetoborinae) and (Panesthiinae + Geoscaphinae). Even suggestions made by Evangelista et al. (2017) based on a comprehensive review are likely incorrect considering the result of this study. We tested the position of Oxyhaloinae in two different topological scenarios.

We tested: (i) Oxyhaloinae as sister to *Diploptera* (AUTree #1; Bourguignon et al. (2018)), (ii) Oxyhaloinae as sister to Blaberinae + Zetoborinae (with *Diploptera* as sister to the remaining Blaberidae) (# tree 10) and Oxyhaloinae as sister to (Blaberinae + Zetoborinae) with (Panchlorinae + Gyninae) as sister to the remaining Blaberidae (AUTree #11). Both (ii) and (iii) were significantly rejected ( $p=0.00$ ). Thus, our recovered position of Oxyhaloinae as sister to *Diploptera* is considered robust under the AU test given our data.

## S1.5 Divergence time estimation

### *Fossil calibrations and maximum bounds*

We selected nine fossils (Table S8; Figure S5) to calibrate our divergence-time analysis. All the calibrations except one (see below), including the root age, were set to hard maximum bound at 412 million years ago (MYA) using uniform priors (Figure S6). We chose uniform priors because we had little information from the fossil record to suggest the shape or parameterization of other distributions. We selected the oldest age of Rhynie Chert (Mark et al., 2013) as the maximum root age because it is a diverse fossil deposit of many well-preserved plants and animals, but lacks winged-insects, and predate all known winged-insect fossils (Trewin, 2008). However, younger ages have been considered for this deposit (Schachat, 2018). Furthermore, predating the origin of tree-form plants (e.g. tree ferns) precludes the possibility that primitive gliding behaviors observed in non-winged hexapods (Dudley & Yanoviak, 2011; Yanoviak et al., 2009) might have evolved. If this behavior is a preadaptation to the evolution of wings in insects as proposed by Yanoviak et al. (2009), it further supports this as an upper age limit for Polyneoptera and all winged insects (Pterygota). The only node given a different maximum age was calibrated by *Archeorhinotermes rossi* (representing the ancestor of Neoisoptera), which we set to have a soft maximum bound at the oldest limit of the Carnian stage (237 MYA). The abundance of described termite fossil taxa (Grimaldi & Engel 2005) since ~130 MYA shows that identifying termites in extinct faunas is straightforward (when they

occur). Therefore, we assume absence in fossil deposits as evidence that termites had not evolved and were not present yet in the Carnian stage. Additionally, this soft maximum provides a ~100-million-year gap before the earliest verified termite fossil and a gap of at least 30 million years prior to the oldest estimate for the origin of termites (see Figure 2). Minimum soft bounds were selected based on the fossil calibrations (see Supplementary material S2).

### *Estimating divergence times*

We used two datasets for divergence date inference: i) the unreduced decisive amino-acid alignment as used for tree inference and ii) a reduced version of this dataset only containing sites with unambiguous data for at least 95% of the 66 taxa (i.e. “reduced decisive amino-acid dataset”). To reduce computational effort, we chose an unpartitioned dating analysis. We ran divergence time analyses on both versions of the unpartitioned dataset using MCMCTree implemented in the software package PAML v.4.9 (Yang & Rannala, 2006). To tailor our modeling scheme to an unpartitioned analysis with substitution matrices implemented in PAML, we estimated the best scoring model for both unpartitioned datasets in IQ-TREE (v. 1.5.0). We restricted the search to the following models as these are available in PAML, options: `-m TESTONLY -mset Dayhoff,JTT,WAG,mtREV,mtMAM -gmedian`. JTT was determined as best scoring model for both datasets. Thus, we set the model JTT (aaRatefile = jones.dat) + G with 5 rate categories, empirically estimated base frequencies (model = 2) and allowed rates to be inferred from individual sites (RateAncestor = 1). We conducted Hessian matrix calculations according to the above specifications with CODEML as implemented in PAML using empirical +F base frequencies estimated from the respective dataset. Model parameters were specified as follows: chronograms for both datasets were estimated under the correlated independent rates clock model as done by Peters et al. (2017). MCMC chains ran for 1,000,000 generations (sfreq = 10) while discarding a burn-in of 100,000 generations. The software package TRACER v1.6 (Rambaut et al., 2018) was used to check for sufficient effective sample size (ESS > 200) for all parameters. For each of the datasets we ran the analysis in four independent replicates to further ensure that parameter space had been searched thoroughly. For each dataset, posterior mean time estimates, as well as lower and upper confidence intervals (CI), from all four independent runs were plotted against each other to check for MCMC chain convergence. All runs converged for both datasets (see Figure S7 & S8). From the four replicates of each dataset, we choose posterior means and CI of one randomly selected run, since all four replicates delivered effectively identical results. The inferred dates and 95% confidence intervals (CI) from all dated trees can be found on Dryad (“S1.5\_Dating”).

Since effective priors in divergence time analyses can strongly deviate from the specified priors (Warnock et al., 2015), we checked that our effective priors were not conflicting with the fossil calibrations. Therefore, we ran our analyses as specified above but without molecular data (i.e., prior-only analysis as described in the PAML documentation and MCMCTree Tutorial; dos Reis et al., 2017; Nascimento et al., 2017). Our effective priors did overlap with specified priors in all cases (Figure S6). For all calibrations, the 95% CI of the recovered posteriors fell within

limits of the specified prior and the effective prior. However, the mean of the effective prior distribution of the clade calibrated by the fossil “*Gyna*” *obesa* was exceptionally disjoint from the minimum age calibration. Hence, we chose a slightly older age justification for this fossil (60 MA; see Supplementary material S2.3 for details of the disagreement) to account for a potential underestimate for the minimum age calibration. We also tested the alternative, younger age (57.7 MA). The results of this test are given in Table S9 and discussed briefly below.

Since there were ambiguities in the ages of a few fossils used for calibration (Supplementary material S2) we checked whether or not these discrepancies had any effect on the inferred posterior mean ages and CIs. Therefore, we ran the divergence time analyses again using alternate calibration ages (“alternate minimum calibration ages”; see Table S9 for original and alternative ages for all three nodes), but restricted to one run and only for the reduced dataset. The results did not significantly deviate from the original analysis of the reduced decisive amino-acid dataset (Table S9). Thus, the discrepancy in fossil age estimates was too small to have a significant effect on our inferred dates.

We deem the final results of the dating analysis to be robust to missing data patterns, and uncertainties in ages of the calibrating fossils. Our inferred ages are generally younger than estimates from previous studies (main text Figure 2; discussed in main text section “The timing of Blattodea’s origins”). Although young, these age estimates still agree with the fossil record, and close or bridge the large gap between molecular estimates of divergence and the last known fossil remains (main text Figure 2). The width of such gaps is exacerbated by the inability to describe known fossils lacking preserved diagnostic characters. In one extreme example, the node calibrated by *Archeorhinotermes rossi*, two-thirds of the confidence interval (including the mean) of the divergence time estimate are younger than the minimum soft-bound calibration for that node (main text Figure 1&2). This could indicate a shift in substitution rates sometime in the early history of Isoptera (see Legendre & Condamine, 2018) or be due to a lack of data on the wing morphology of stem-Cryptocercidae and stem-Lamproblattidae preventing an accurate placement of *Valditermes brenanae*, which is a calibrating fossil. Although such scenario has never before been considered, it is possible that stem-Kittrickia possessed wings with a humeral suture (see Supplementary material S2.1; both Cryptocercidae and Lamproblattidae entirely lack wings). If this bold proposition was true, *Valditermes brenanae* might be placed as stem-Tutricablattae as opposed to stem-Isoptera. Interestingly, an analysis discussed below (section S3.4) provides evidence that stem-Tutricablattae may have dropped their wings in the manner of extant termites.

## **S1.6 Inferring the presence of *Blattabacterium* within transcriptome data**

### ***Current understanding of Blattabacterium in Blattodea***

*Blattabacterium* are bacteroids that are exclusively obligate endocellular mutualists with many cockroach groups. They synthesize amino-acids and recycle nitrogenous wastes for their hosts (Patino-Navarrete et al., 2013; Sabree et al., 2009; Tokuda et al., 2013). This enables their hosts to have a broad physiological repertoire because the symbionts assist in storing nitrogenous

wastes (which are toxic and normally a waste product) in fat body cells that can be metabolized in times of nutrient stress (Clark & Kambhampati, 2003; Mullins & Cochran, 1976). Stored nitrogenous wastes also serve as a resource for both maternal and paternal investment in their progeny (Mullins et al., 1992; Schal & Bell, 1982). The biology of *Blattabacterium* and coevolution with cockroaches has been studied extensively (e.g. Clark & Kambhampati, 2003; Patino-Navarrete et al., 2013; Sabree et al., 2009; Tokuda et al., 2013). Although *Blattabacterium* does not occur in all cockroach species, the ones that they do occur in strongly rely on them (Guthrie & Tindall, 1968). *Blattabacterium* is known to be absent from *Nocticola* and all termites with the exception of *Mastotermes* (Clark & Kambhampati, 2003; Lo et al., 2003; Mullins, 2015). Yet, only a limited number of species have been surveyed in prior studies (Clark & Kambhampati, 2003; Clark et al., 2001; Kinjo et al., 2018; Lo et al., 2003; Milburn, 1966; Patino-Navarrete et al., 2013; Sabree et al., 2009; Tokuda et al., 2013). We assessed the presence of *Blattabacterium* in the transcriptome data of 44 Blattodea species, two Mantodea, and 17 other Polyneoptera outgroups included in this study.

### ***BLAST survey for Blattabacterium***

We compiled a series of reference sequences from the UniProt database (The\_Uniprot\_Consortium, 2015). The reference dataset consisted of 50 genes, of which 20 are known from *Blattabacterium*. To differentiate between host, *Blattabacterium*, and non-target endosymbiont transcripts we included ~770 total protein sequences from a wide variety of metazoan and non-metazoan organisms (see DRYAD data “S1.6\_Blattabacterium” for full list). We performed a BLAST search of each reference sequence against each of the transcriptomes, which we treated as databases for the tBLASTn function in BLAST+ (Camacho et al., 2009). From the results we removed all hits with E-values > 0.1 resulting in 165,865 remaining hits. Among the multiple hits for a given protein within a transcriptome, we chose the sequence with the highest alignment score to be part of the final dataset. We manually checked the output and ensured that a single fragment was not identified as more than one protein type. We then extracted all hits attributed to *Blattabacterium* and used BLAST for each one against the entire NCBI nucleotide collection. Any sequences whose top BLAST result was for *Blattabacterium* was counted as a positive result.

Table S10 shows the presence and absence of *Blattabacterium* in transcriptome data of species included in this study. The associated spreadsheet (“S1.6\_Blattabacterium”) shows all detailed results per organism.

### ***Blattabacterium presence in transcriptomes***

The results show evidence for the presence of *Blattabacterium* in all non-termite cockroaches except *Diploptera* sp., *Tivia* sp., *Nocticola* sp. and *Lamproblatta albipalpus*. As expected, no termites were found to have *Blattabacterium* except for *Mastotermes darwiniensis*. The lack of the bacteroids within the other non-termite cockroaches (*Lamproblatta albipalpus*, *Diploptera* sp. and *Tivia* sp.) is surprising, while there has been reported a lack of

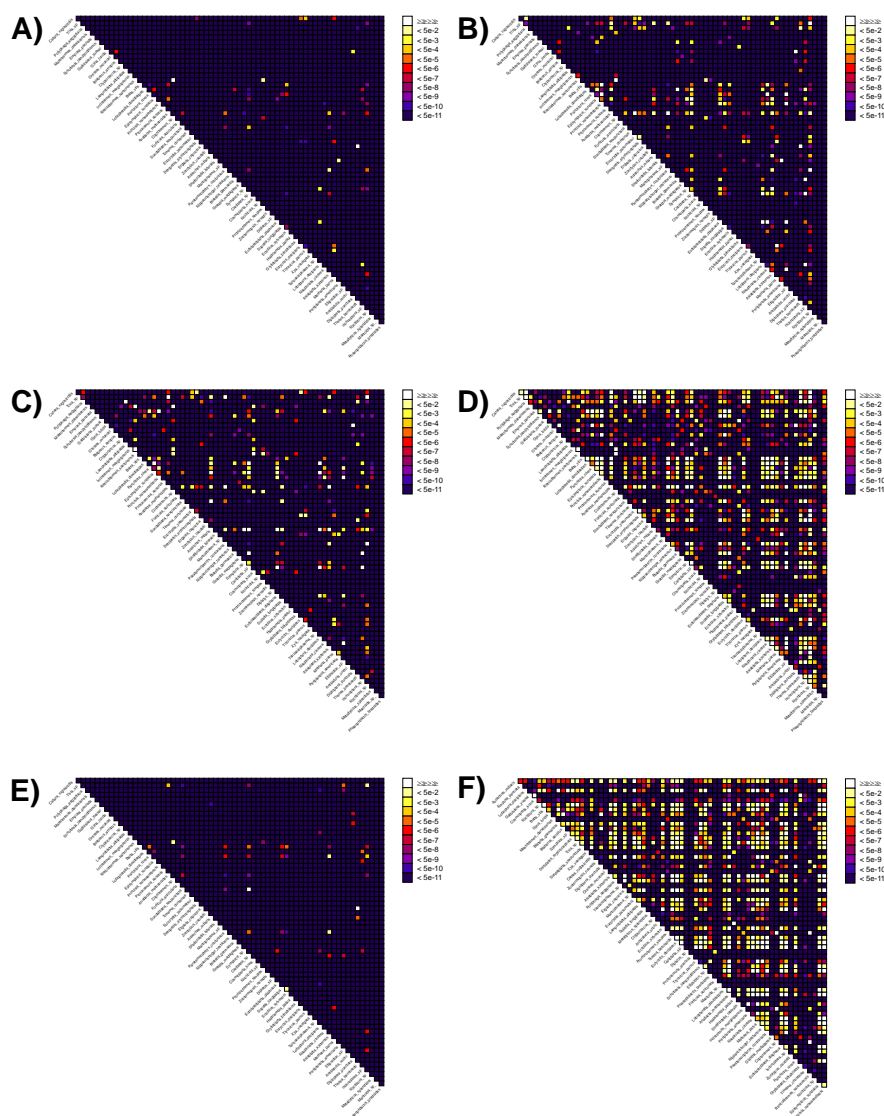
707 *Blattabacterium* already for the genus *Nocticola* (Lo et al. 2003) and is considered as  
708 independent loss. We also corroborate the absence of *Blattabacterium* in Mantodea and other  
709 Polyneoptera.

710       While the nature of our data allows inference of the presence of endosymbionts, inferring  
711 absence is more difficult. For instance, we cannot exclude the possibility that the absence of  
712 BLAST hits could be due to insufficient sequencing, low gene expression or assembly errors.  
713 Indeed, we did not find *Blattabacterium* in the *Diploptera* sp. transcriptome but this sample had  
714 very low starting genomic material (see S1.1 Molecular dataset generation). Of course, further  
715 analysis is needed to verify this. Therefore, BLAST should not be considered as a standard of  
716 evidence by which we confidently identify symbionts in organisms. It is rather an initial  
717 screening, which should be followed up with microscopy or other cytological methods, which  
718 was not feasible in our study and would go beyond our scope. Thus we consider the occurrence  
719 of the symbionts as preliminary suggestions and should be investigated in future studies by more  
720 sophisticated molecular methods as well as other methods, e.g. microscopy or other cytological  
721 methods.  
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## Figures

### Figure S1

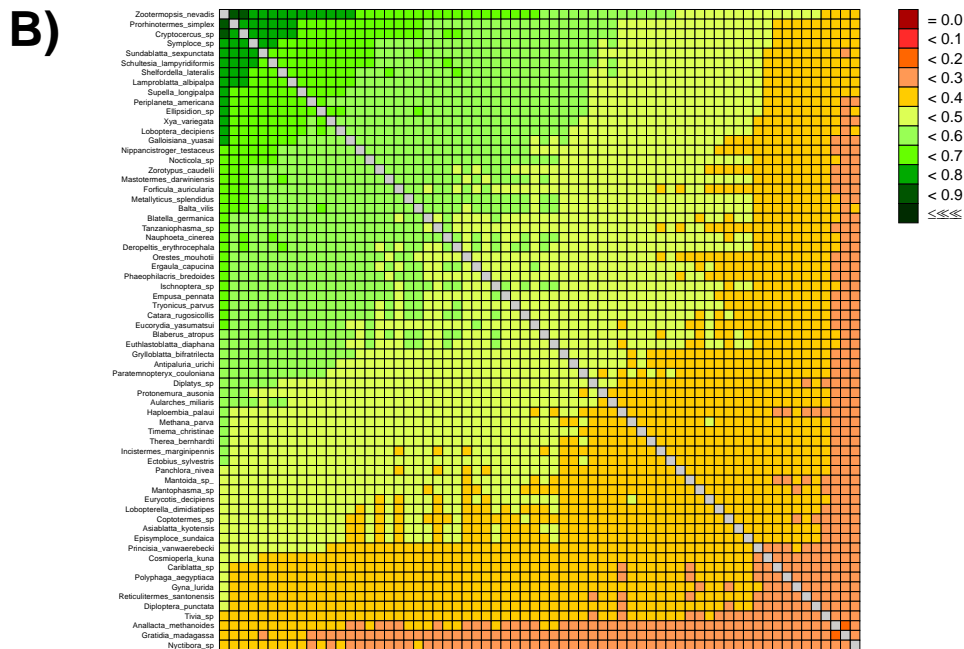
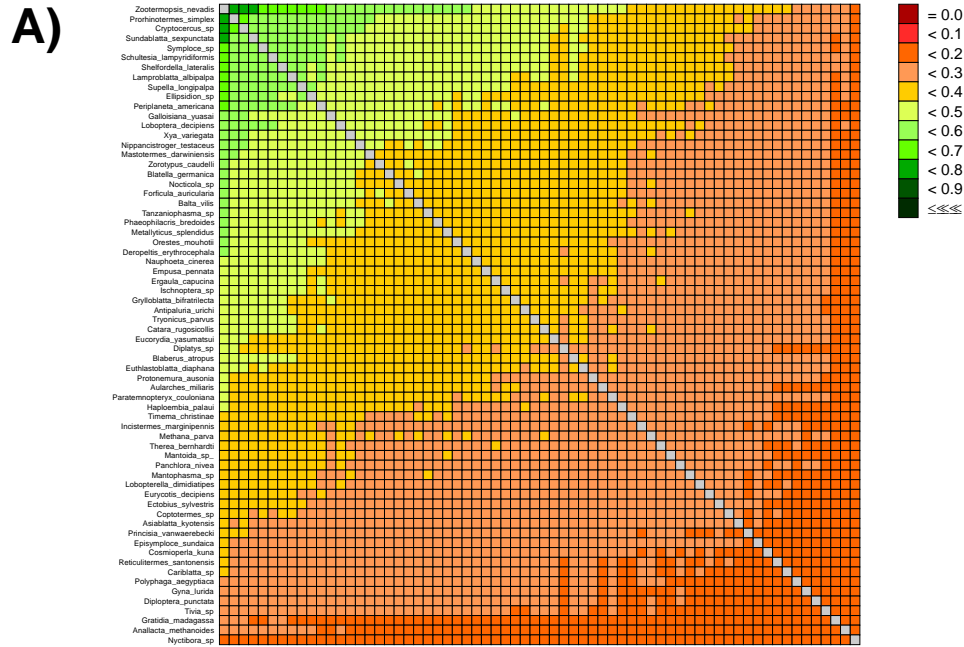
Heat maps show pairwise Bowker's tests visualizing among-lineage heterogeneity as implemented in SymTest 2.0.47. P-values  $> 0.05$  coloured in white indicate sequence pairs that fully match SRH conditions. SymTest was run for the full dataset on nucleotide level after removal of uninformative partitions (A-E) and for the decisive dataset on the amino-acid level (F). Heat map of the nucleotide dataset including A) all codon positions, B) 1st and 2nd codon position, C) 1st codon position only, D) 2nd codon position only, E) 3rd codon position only. F) Heat map of the decisive amino-acid dataset. The nucleotide dataset only including the 2nd codon position and the amino-acid dataset show less model violation compared to other datasets and were thus used for further downstream analyses.



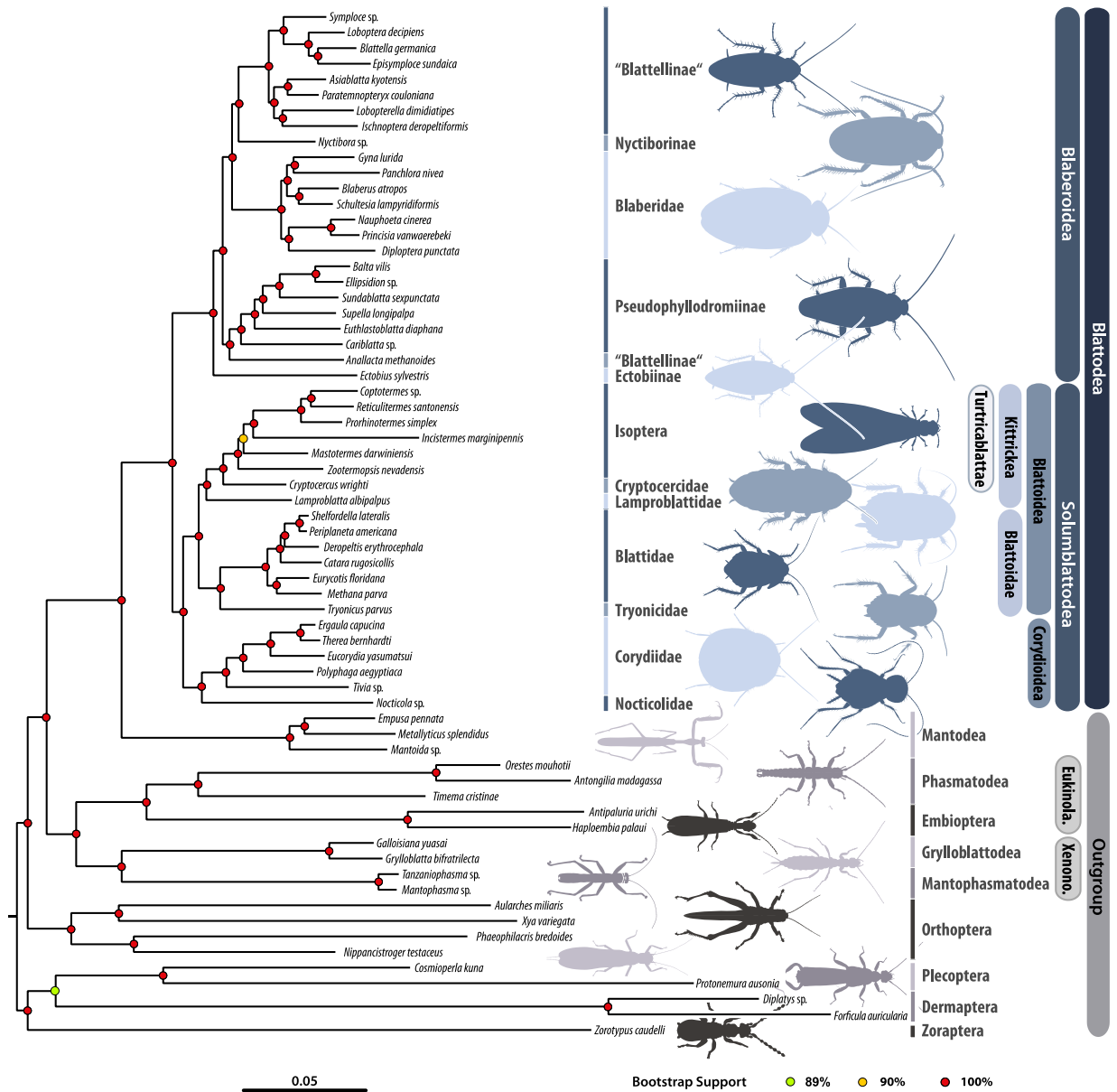


**Figure S2**

**Heat maps show species-pairwise site coverage. A)** Full nucleotide dataset including only 2nd codon positions, and **B)** decisive amino-acid dataset as inferred with AliStat. Low shared site coverage coloured in shades of red; high shared site coverage in shades of green. Pairs of sequences, and completeness scores are provided in the text.



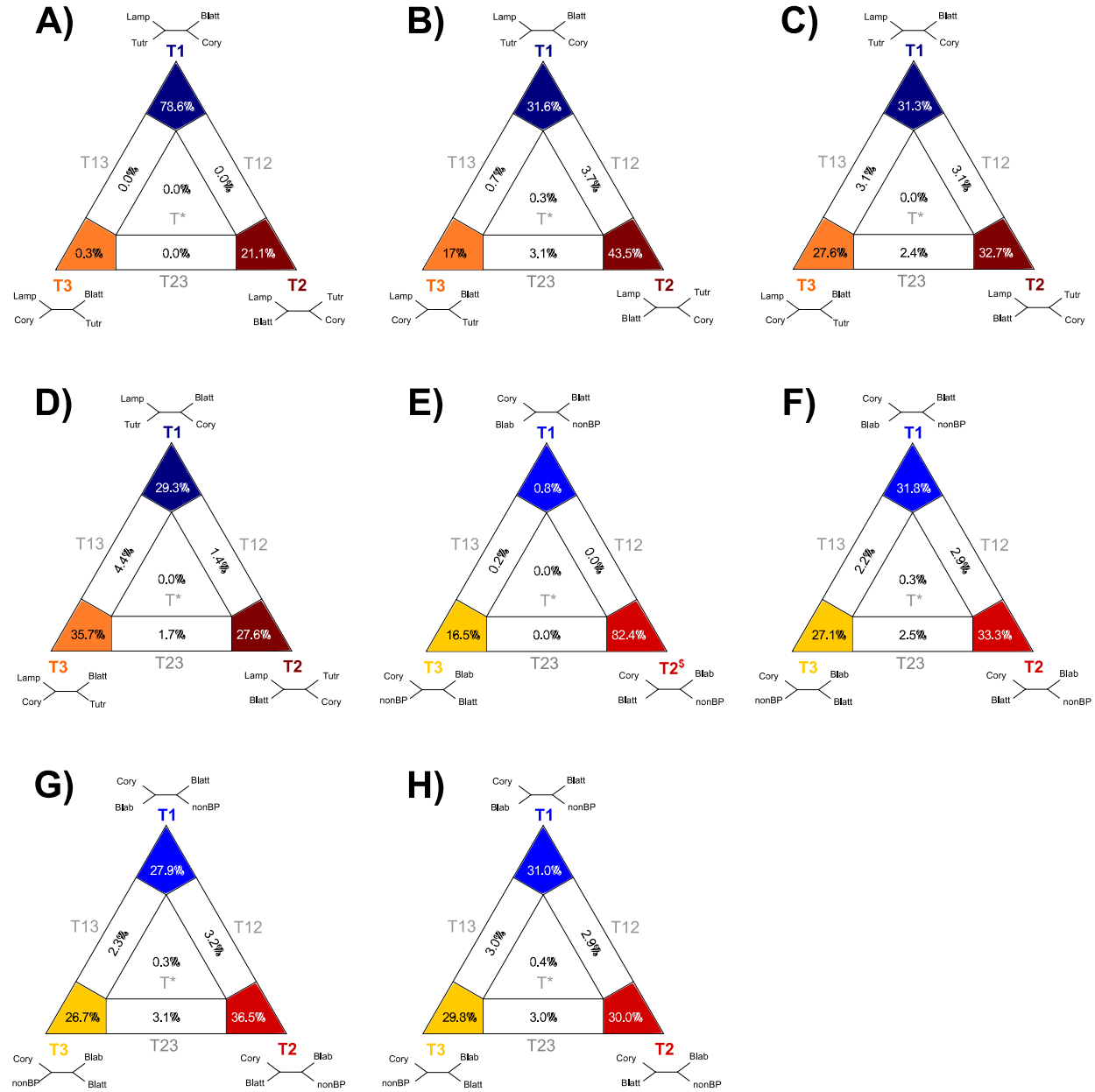
**Figure S3**  
**Best ML tree (phylogram) inferred from the full nucleotide dataset with 2nd positions only with bootstrap support mapped onto the best tree.** The relationships in the tree are identical to those in Figure 1 (inferred from the decisive amino-acid dataset) except for the position of *Mastotermes* and *Zootermopsis* relative to other termites and the position of *Zorotypus*.





## Figure S4

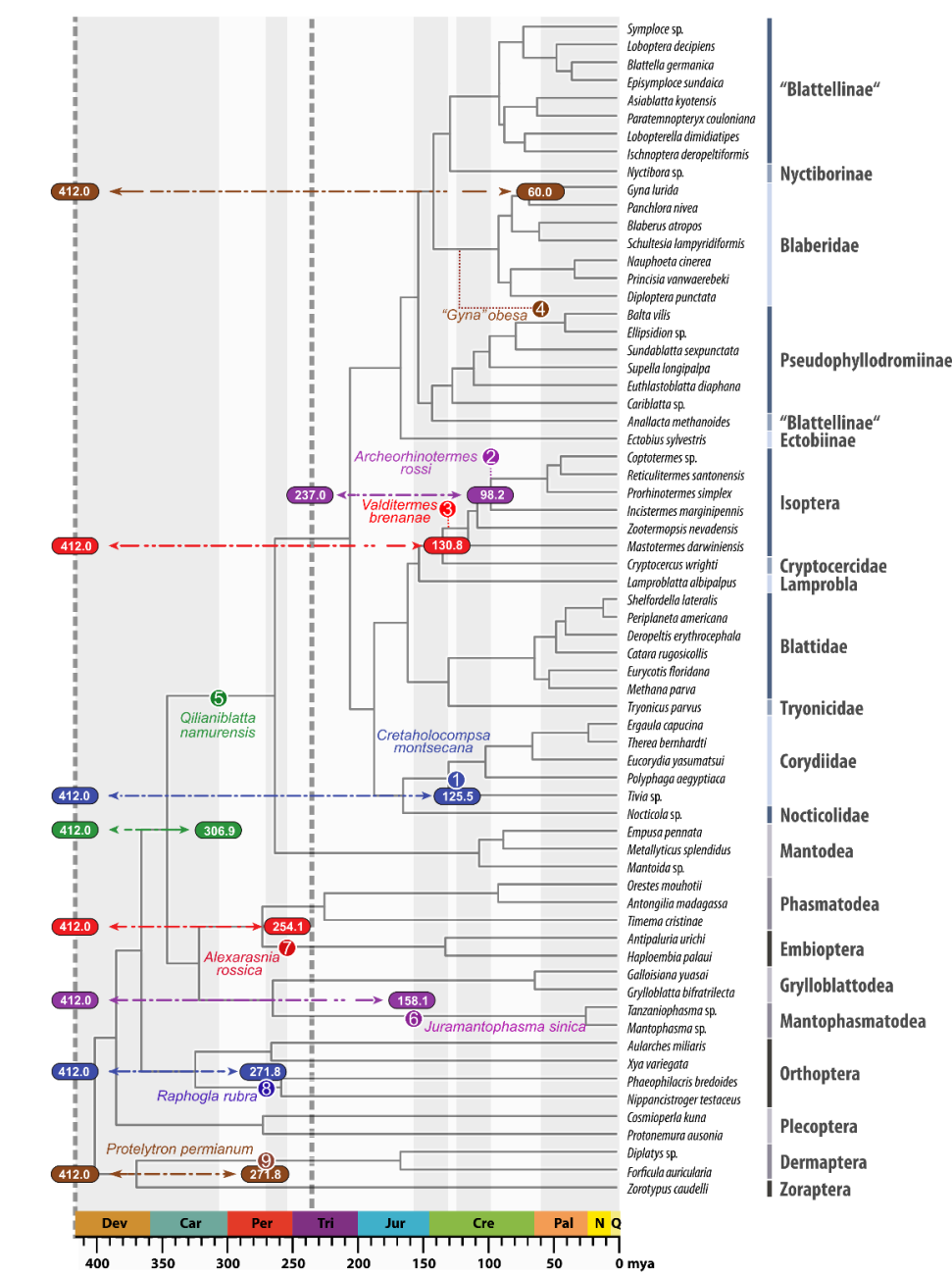
**FcLM results of original and permuted data.** Given are proportions of quartets (in %) that map into respective Voronoi-cells (2D simplex graph). T1 (area 1): unambiguous support for G1,G2 – G3,G4 marked in dark blue/blue. T2 (area 2): unambiguous support for G1,G3 – G2,G4 marked in dark red/red. T3 (area 3): unambiguous support for G1,G4 – G2,G3 marked in orange/yellow. Marked in grey: T12 (area 4), T13 (area 6) and T23 (area 5) provide ambiguous support and quartets mapped into T\* (area 7) are not resolved (star-like). **A-D)** Testing the sister relationship between *Lamproblatta* and Tutricablatta based on the decisive amino-acid alignment (585,040 amino-acid sites, 592 partitions, number of quartets: 294, see Table S6). T1 (indicated by a \$) was supported in our best ML tree. Lamp: Lamproblattidae (group 1); Turt: Tutricablatta (group 2); Blatt: Blattoidea (group 3), Cory: Corydioidea used as outgroup taxa **A)** non-permuted, original data, **B)** permutation I, **C)** permutation II, **D)** permutation III. **E-H)** Testing the sister relationship between Corydioidea and Blattoidea based on the decisive amino-acid alignment (585,040 amino-acid sites, 592 partitions, number of quartets: 45,360, see Table S6). T2 (indicated by a \$) was supported in our best ML tree. Cory: Corydioidea (group 1); Blab: Blaberoidea (group 2); Blatt: Blattoidea (group 3), nonBP: non-blattodean Polyneoptera used as outgroup taxa. **E)** non-permuted, original data, **F)** permutation I, **G)** permutation II, **H)** permutation III.



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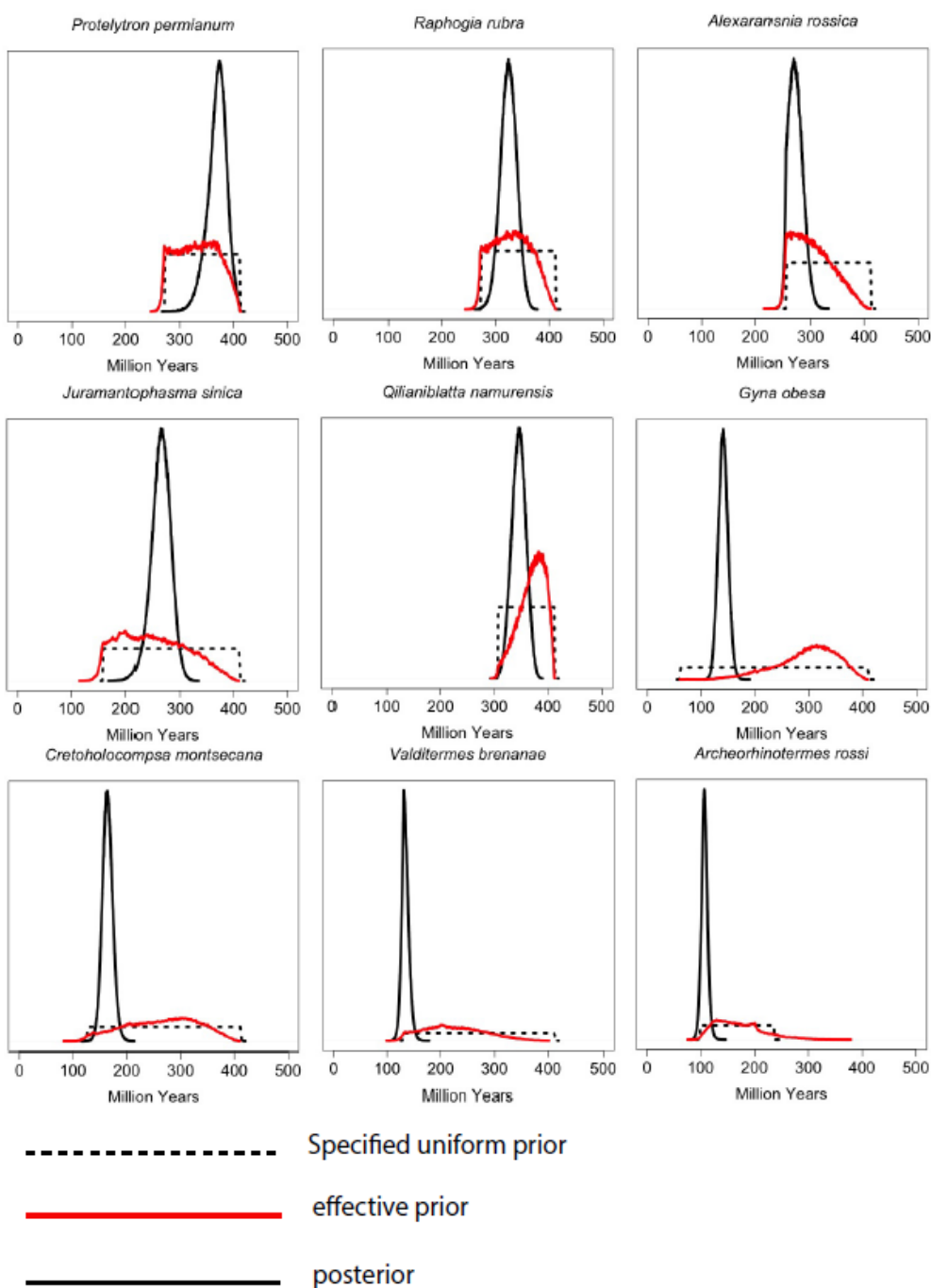
# Figure S5

**Fossil calibrations used for estimating divergence dates of Blattodea.** Numbered circles represent fossils used for calibration and their approximate placement on the phylogenetic tree. Ranges (coloured boxes connected by dashed arrows) include minimum and maximum ages for fossils with the corresponding colour. Dashed black lines correspond to the two maximum age boundaries utilized. Note that the calibration ages used here are implemented in the main analysis and are not the ages referred to as “alternate minimum calibration ages” in peripheral analyses. Further details are given in Table S8.



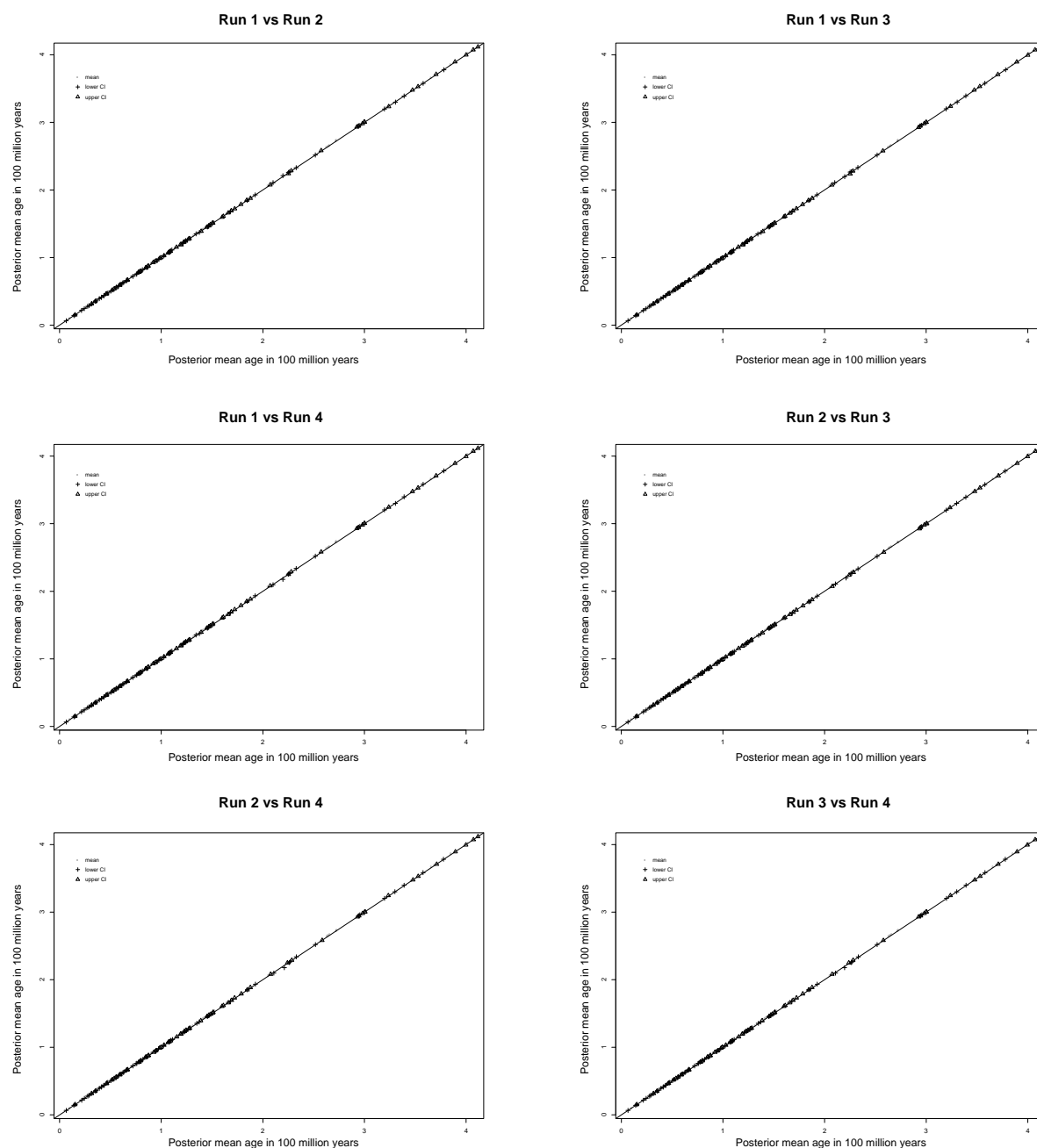
# Figure S6

**Distributions of ages for the nine calibrated nodes of the reduced dataset used for divergence date estimates.** Names above the plots indicate the fossil used for calibration of the respective node. Dashed lines represent the bounds of uniform prior distribution. Red lines show the effective prior distribution of ages; solid black lines show the posterior distribution of ages (reduced decisive amino-acid dataset).



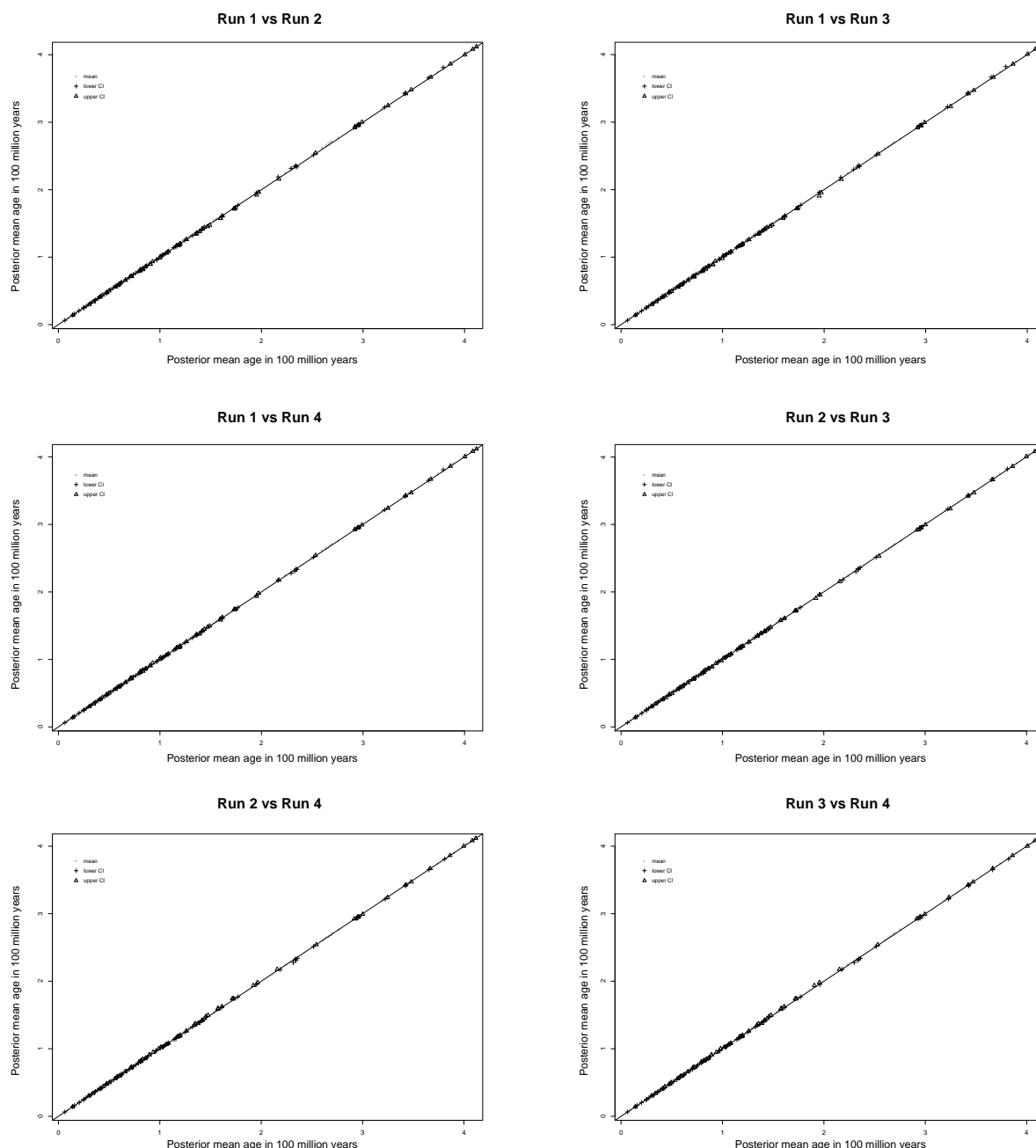
# Figure S7

**Pairwise comparison of posterior mean node age estimates and upper and lower confidence intervals (CI) of four independent runs of the reduced decisive amino-acid dataset (66 taxa, 71,126 aa sites; coverage: at least 95% of included species, see methods section). Runs were performed with the independent-rates clock model and identical settings expect for the seed. Black dots: posterior mean ages; +: lower 95% equal-tail CI; triangles: 95% upper equal-tail CI.**



# Figure S8

**Pairwise comparison of posterior mean node age estimates and upper and lower confidence intervals (CI) of four independent runs of the unreduced decisive amino-acid dataset (66 taxa, 580,040 aa sites). Runs were performed with the independent-rates clock model and identical settings expect for the seed. Black dots: posterior mean ages; +: lower 95% equal-tail CI; triangles: 95% upper equal-tail CI.**



## References

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## S2 Fossil Calibrations

### S2.1 Fossils Selected as Clade Minimum Age Calibrations

Fossil and other calibrations heavily inform prior date distributions, and are the parameters that dating analyses are the most sensitive to (Inoue et al., 2010; Zheng & Wiens, 2015). Hence, they are of utmost importance for the dating analyses. However, the fossil record has not always been effectively scrutinized, and as a result many fossil calibration points have proven invalid. We therefore carefully vetted Blattodea and outgroup fossils based on the five criteria provided by Parham et al. (2012):

**CR1:** single/multiple operational taxonomic units with museum numbers;

**CR2:** apomorphy-based or phylogenetic analysis supporting an unambiguous placement;

**CR3:** agreement of morphology and molecular data in that placement;

**CR4:** detailed locality and stratigraphy data provided;

**CR5:** radioisotopic age or numeric age references given and agreeing with CR4.

Details relevant to the age of each fossil and their stratigraphic context is given in S2.3. We classify character states supporting phylogenetic placement of taxa as ultimate, contextual, and then class 1 or class 2. A character state in the ultimate level is one that assigns a taxon to a specific node (i.e. a synapomorphy for that node). In contrast, a character state in the contextual level is plesiomorphic to that node. A class 1 character is one that occurs only once and a class 2 character is one that is homoplastic.

#### *Selected fossil calibrations*

**Calibrating node:** stem-Corydiidae s.s. / crown-(Nocticola + Corydiidae s.s.)

**Fossil item:** *Cretaholocompsa montsecana* Martínez-Delclòs, 1993

**Original description:** Martínez-Delclòs, X. (1993) Blátidos (Insecta, Blattodea) del Cretácico Inferior de España. Familias Mesoblattinidae, Blattulidae y Poliphagidae. *Boletín Geológico y Minero*, **104**, 52–74.

**Further descriptive accounts:** The position of the species is discussed in Evangelista et al. (2017) based on the original data, including discussion on the age of the fossil.

**Locality:** Montsec (125.5 MYA).

**CR1:** LC-1704-IEI (Fundació Pública Institut d'Estudis Ilerdencs, Lleida, Spain).

**CR2:** See below.

**CR3:** A clade comprising *Nocticola* and Corydiidae is supported by both molecular (Djernæs et al., 2015 ; Legendre et al., 2015; Wang et al., 2017) and morphological (Roth, 1988) data.

**CR4:** Yes (original description and further descriptive account).

**CR5:** Yes (see Section S2.3).

### Phylogenetic justification & discussion:

The placement of *Cretaholocompsa montsecana* as stem-Corydiidae s.s. (hence crown-(Nocticola + Corydiidae s.s.)) is based on the following character states:

#### Ultimate level:

Class 1: (1) in forewing, occurrence of a well-delimited lighter single colour spot, medially located.

Class 2: (2) in forewing, occurrence of a differently sclerotized band parallel to the posterior wing margin; (3) in forewing, venation not substantiated by prominent tubular, sclerotized structures; (4) in forewing, Radius reaching anterior wing margin basally (Radius being branched).

#### Contextual level:

Class 1 [stem-(Tiviinae + (Polyphaginae + Corydiinae))]: (5) in forewing, basally or medially sharply angulate CuP.

Class 2: none found / considered.

Concurring with Evangelista et al. (2017), we consider the placement of this species well-founded. The species displays a mosaic of character states when compared to extant genera, namely *Euthyrrhapha* and *Holocompsa*, both closely related to *Tivia* (itself included in our analysis; Legendre et al., 2015). To our knowledge, the character state (1) occurs in most *Euthyrrhapha* species and some species of *Holocompsa* [based on photos in Beccaloni (2014) and pers. obs.]. The sharply angulate CuP, regarded by Evangelista et al. (2017) as indicative of affinities with *Euthyrrhapha* and *Holocompsa*, actually also occurs in *Polyphaga* (Béthoux et al., 2009; and pers. obs.). Therefore, it is herein relegated to the contextual level. In conjunction with character state (1) it ascertains the placement of the fossil.

Further character states support the affinities of *Cretaholocompsa montsecana* with Tiviinae, Holocompsinae and Euthyrrhaphinae. The character state (2) occurs only in *Euthyrrhapha* [based on photos in Beccaloni (2014) and pers. obs. Olivier Béthoux]. It must be acknowledged, however, that it also occurs in the fossil family Ponopterixidae ('adsutural line' in Nel et al. 2014; Lee, 2016), whose affinities are not evident. The character state (3) occurs in both *Euthyrrhapha* and *Holocompsa*. In the former, the forewings are strongly sclerotized; as a consequence, the venation can only be (partly) observed using transmitted light (pers. obs.). In *Holocompsa*, the forewing distal part is comparatively weakly sclerotized, yet venation is equally very difficult to observe. Based on the original description (depicting a very short M and complete absence of CuA branches) we assume that character state (3) occurs in *Cretaholocompsa montsecana*. To our knowledge, the character state (4) is unique to *Holocompsa* (Rehn, 1951; its occurrence in *Euthyrrhapha* cannot be completely ruled out, given the occurrence of character state (3)).

### Calibrating node: stem-Neoisoptera / crown-(Kalotermitidae + Neoisoptera)

**Preliminary remarks:** We scrutinized several putative crown-Isoptera. We initially relied on the phylogenetic analysis by Engel et al. (2007), based on morphology, and the review



by Ware et al. (2010), combining molecular and morphological data. Note that in fig. 1 in the latter several combinations of genus & species names are erroneous. Comparing these two analyses (both of which used the same morphological matrix for the phylogenetic reconstruction of fossil taxa), we saw a congruent lack of resolution in the ‘Euisoptera assemblage’ (Euisoptera being the sister-group to Mastotermitidae, among crown-Isoptera). Given this lack of phylogenetic resolution among the basal nodes of non-Neoisopteran Isoptera, we did not include fossil calibrations in the vicinity of the most recent common ancestor of Euisoptera.

**Fossil item: *Archeorhinotermes rossi* Krishna & Grimaldi, 2013**

**Original description:** Krishna, K. & Grimaldi, D.A. (2003) The first Cretaceous Rhinotermitidae (Isoptera): a new species, genus, and subfamily in Burmese amber. *American Museum Novitates*, **3390**, 1–10.

**Further descriptive accounts:** none.

**Locality:** Myanmar amber (98.2 MYA).

**CR1:** In. 20160 (Natural History Museum, London, UK).

**CR2:** See below.

**CR3:** The Kalotermitidae-Neoisoptera sister-group relationship is well supported by both molecular (Bourguignon et al., 2015; Cameron et al., 2012; Inward et al., 2007; Thompson et al., 2000; Ware et al., 2010) and morphological (Engel et al., 2009) data.

**CR4:** Yes (original description).

**CR5:** Yes (see Section S2.3).

**Phylogenetic justifications & discussion:**

The placement of *Archeorhinotermes rossi* as stem-Neoisoptera (hence crown-(Kalotermitidae + Neoisoptera)) is based on the following character states:

Ultimate level:

Class 1: (1) frontal gland developed into distinct fontanelle; (2) forewing costalized.

Class 2: none found / considered.

Contextual level:

Class 1 [stem-Isoptera / crown-(*Cryptocercus* + Isoptera)]: (3) in forewing, occurrence of a humeral suture.

Class 2: none found / considered.

The occurrence of a fontanelle (1) is discussed in the original description and the character was subjected to cladistics analysis by Engel et al. (2007). Accordingly, it provides unambiguous support to the clade (*Archeorhinotermes rossi* + Neoisoptera), Neoisoptera including the Rhinotermitidae (represented in our analysis) and several other families. The same applies to character state (2) (Engel et al., 2007). The sister-group to Neoisoptera being the Kalotermitidae, the species is suitable to calibrate the node Kalotermitidae + Neoisoptera.

**Calibrating node: stem-Isoptera / crown-(*Cryptocercus* + Isoptera)**

**Preliminary remarks:** We scrutinized putative stem-Isoptera only (to our knowledge there is no known fossil Cryptocercidae).

**Fossil item:** *Valditermes brenanae* Jarzembowski, 1981

**Original description:** Jarzembowski, E.A. (1981) An early Cretaceous termite from southern England (Isoptera: Hodotermitidae). *Systematic Entomology*, **6**, 91–96.

**Further descriptive accounts:** none.

**Locality:** Clockhouse Brickworks pit (130.3 MYA).

**CR1:** Holotype, In. 64588 (Natural History Museum, London, UK); paratypes, In. 64589-93 (Natural History Museum, London, UK).

**CR2:** Yes (see below).

**CR3:** The sister-group relationship between *Cryptocercus* and the termites is extremely well supported by both molecular (Djernæs et al., 2015; Ware et al., 2008) and morphological (Klass & Meier, 2006) data.

**CR4:** Yes (see original description).

**CR5:** Yes (see Section S2.3).

#### **Phylogenetic justification & discussion:**

The placement of *Valditermes brenanae* as stem-Isoptera (hence crown-(*Cryptocercus* + Isoptera)) is based on the following character states:

##### Ultimate level [stem-Blattodea / crown-Dictyoptera]:

Class 1: (1) in forewing, occurrence of a humeral suture.

Class 2: none found / considered.

##### Contextual level:

Class 1: none found / considered.

Class 2: none found / considered.

The supporting character state has long been recognized as unique to Isoptera (Belayeva, 2002; Grimaldi & Engel, 2005; Hennig, 1981; among recent accounts). Engel et al. (2009) carried out a phylogenetic analysis including this species. They recovered it as stem-Mastotermitidae (i.e., as crown-Isoptera). However, we noticed issues with the support to such placement. Regarding the state ‘occurrence of cross-veins connecting longitudinal veins’ (their character 54, state 1), it is coded absent (state 1) in the species while cross-veins are mentioned and figured in the original description (Jarzembowski, 1981, fig. 6). The next character state change to provide support (ambiguous) and for which the species is documented regards the shape of the humeral margin of the forewing scale (character 64), documented as flat (state 0). However, the condition for this character is not documented for *Cratomastotermes wolfschwennigeri*, their recovered sister-group to the remaining Isoptera, in the close vicinity of *Valditermes brenanae*. Therefore the polarity of the state is not evident. In summary, we consider that the placement of *Valditermes brenanae* as crown-Isoptera is not firmly established, and therefore conservatively consider it as a stem-Isoptera.

Note that this fossil was selected for calibration by Bourguignon et al. (2018) as crown-Euisoptera *sensu* Engel et al. (2009). According to Engel et al. (2009), this fossil is a stem-Mastotermitidae. However, as mentioned above, the topology obtained by Engel et al. (2009) in this area of their proposal of phylogenetic relationships is poorly constrained. As used by Bourguignon et al. (2018), the fossil fails to fulfill **CR2**.

**Calibrating node: stem-Blaberidae / crown-(Blaberidae + (Blattellinae + Nyctiborinae))**

**Fossil item: “Gyna” obesa Piton, 1940**

**Original description:** Piton, L.E., 1940. Paléontologie du gisement éocène de Menat (Puy-de-Dôme) (flore et faune). *Mémoires de la Société d'Histoire Naturelle d'Auvergne*, **1**, 1–303.

**Further descriptive accounts:** Evangelista et al. (2017).

**Locality:** Menat (60.0 MYA).

**CR1:** Holotype, MNHN.F.R06689 (Museum National d'Histoire Naturelle, Paris, France).

**CR2:** Yes (see below).

**CR3:** A clade comprising Blaberidae + Blattellinae + Nyctiborinae has been supported by both molecular (current study) and morphological (Klass & Meier, 2006) data.

**CR4:** Yes (see Evangelista et al., 2017 and references therein).

**CR5:** Yes (see Section S2.3).

#### **Phylogenetic justification & discussion:**

The placement of “Gyna” *obesa* as stem-Blaberidae (hence crown-(Blaberidae + (Blattellinae + Nyctiborinae))) is based on the following character states:

##### Ultimate level:

Class 1: (1) asymmetrically concave margin of subgenital plate.

Class 2: (2) cerci stout.

##### Contextual level:

Class 1: none found / considered.

Class 2: (3) in forewing, anterior and posterior margins parallel for a long distance.

The original treatment of this taxon in Piton (1940) gave an overly specific systematic assignment based on the supposed occurrence of the character state ‘occurrence of a medial lobe along the posterior margin of the pronotum’. From certain angles the posterior edge of the pronotum appears to take two different paths, one tapered and another with a long medial extension (pers. obs.). The latter could indeed indicate a systematic placement to Epilamprinae or Gyninae (family Blaberidae), the latter being the hypothesis favoured by Piton (1940). However, Evangelista et al. (2017) provided detailed evidence showing that the occurrence of the character state is not evident in the fossil specimen.

Yet, other character states present in “Gyna” *obesa* still strongly justify a placement in Blaberidae, in particular the subgenital plate shape [Evangelista et al., 2017; above,

character state (1)], unique to the family (among Blattodea). Blaberidae is strongly considered monophyletic in nearly all molecule-based (Djernæs et al., 2012; Inward et al., 2007; Legendre et al., 2017; Legendre et al., 2015; Pellens et al., 2007; Ware et al., 2008) and morphology-based (Grandcolas, 1996) phylogenetic studies. Two other character states (2, and 3 above) further support this placement.

#### **Calibrating node: stem-Dictyoptera / crown-(Dictyoptera + (Eukinolabia + Xenonomia))**

**Preliminary remarks:** The systematic affinities of the fossil taxon ‘Paoliida’ are herein considered unresolved given the contradictory contributions by Prokop et al. (2012), assuming them to represent stem-Pteryogta, and by Prokop et al. (2014), assuming them to represent stem-Dictyoptera. Moreover, the corresponding fossil species are contemporaneous with the favoured one below. These insects therefore are not further considered.

The identification of Carboniferous and Permian ‘Strephocladidae’ as stem-Mantodea (Béthoux et al., 2010; Béthoux & Wieland, 2009) has been discussed and/or challenged by multiple authors (Gorochoy, 2013; Guan et al., 2016; Hörnig et al., 2013; Kukalová-Peck & Beutel, 2012), resulting in contradicting outputs. The corresponding fossil species therefore were not considered (and see ‘Section S2.2’, case of ‘*Homocladus grandis*’). We consider that the debate equally applies to the ‘Anthracoptilidae’, including the ‘Strephocladidae’ according to Guan et al. (2016).

There is no known putative stem-representative of Xenonomia + Eukinolabia. All considered occurrences represent putative stem-Dictyoptera.

#### **Fossil item: *Qilianiblatta namurensis* Zhang, Schneider & Hong, 2013**

**Original description:** Zhang, Z., Schneider, J.W. & Hong, Y. (2013) The most ancient roach (Blattodea): a new genus and species from the earliest Late Carboniferous (Namurian) of China, with a discussion of the phylomorphogeny of early blattids. *Journal of Systematic Palaeontology*, **11**, 27–40.

**Further descriptive accounts:** A specimen from the same locality as the holotype and forewings and hind wings was described by Guo et al. (2013). Further isolated wings from the same locality were described by Wei et al. (2013).

**Locality:** Xiaheyan (306.9 MYA).

**CR1:** holotype, GMCB 04GNX1001 (Geological Museum of China, Beijing, China); further specimens, CNU-NX1-301 to -304, -336, -337 (Capital Normal University, Beijing, China).

**CR2:** Yes (see below).

**CR3:** There are no morphological analyses that support the node Dictyoptera + (Xenonomia + Eukinolabia) but it is well supported by transcriptomic analysis (Misof et al., 2014; and current study). We thus encourage future workers to review current phylogenetic hypothesis when implementing this fossil in their calibration schemes.

**CR4:** Yes (original description and further descriptive accounts).

**CR5:** Yes (see Section S2.3).

### Phylogenetic justification & discussion:

The placement of *Qilianiblatia namurensis* as stem-Dictyoptera [hence crown-(Dictyoptera + (Eukinolabia + Xenonomia))] is based on the following character states:

#### Ultimate level [stem-Blattodea / crown-Dictyoptera]:

Class 1: (1) in forewing, pectinate fusion of RA onto RP (i.e. R –seemingly– undivided, anteriorly pectinate); (2) in forewing, CuP bent posteriorly.

Class 2: none.

#### Contextual level:

Class 1: none found / considered.

Class 2: none found / considered.

The seemingly undivided R displayed by cockroaches' forewing was demonstrated to be the outcome of a pectinate fusion of RA onto RP by Guo et al. (2013) thanks to material of *Qilianiblatia namurensis*. This trait has been traditionally regarded as diagnostic of Blattodea (Ragge, 1955; Vršanský et al., 2002; represented in figures but not mentioned in Hennig, 1981). As for Mantodea, Béthoux and Wieland (2009) and Brannoch et al. (2017) assumed that RA and RP are distinct in forewings of Mantodea; as a consequence, the character state (1) would be unique to Blattodea. However, the competing hypothesis, viz. that Mantodea also possess the character state (1) [as assumed by Smart (1956) for *Chaeteessa*], cannot be confidently ruled out. Indeed, ongoing research suggests that it must be considered the most plausible interpretation (O. Béthoux and collaborators, in prep.).

It must be noted that *Qilianiblatia namurensis* displays some polymorphism on this character state. However, several other species which are only slightly younger [for example, from the Commeny locality (298.8 MYA)] consistently display the character state [Béthoux et al., 2011; Schneider, 1977, 1978, 1983]; and see Jarzembowski and Schneider (2007) on the sub-contemporaneous occurrence of *Sooblatta villeti* (Pruvost, 1912)]. Moreover, some of these species display the character state 'ScP reaching the anterior wing margin basally', a state highlighted by Hennig (1981: pp. 204–205) as indicative of stem-Blattodea [see also Haas and Kukalová-Peck (2001); the other character state this author considered, viz. 'AA veins reaching the claval furrow', also occurs in Mantodea]. Note that the pronotum is documented in several of them and is expanded laterally (see 'Section S2.2', case '*Miroblatta costalis*', for the relevance of this character state).

It must also be noted here that a pectinate fusion of RP onto RA occurs in forewings of Hemerobiidae (Carpenter, 1940) and of the orthopteran *Exogryllacris ornata* (Anostomatidae; see Béthoux, 2012b). This 'RP onto RA' fusion can be distinguished from that of 'RA onto RP' based on the successive origins of *posterior* branches (representing RP branches; instead of the successive origin of *anterior* branches, representing RA branches).

To our knowledge, the character state is present in all extant Dictyoptera (inclusive of Isoptera; some Blattodea display distal posterior branches possibly representing RP *partim*; and see above regarding Mantodea), a clade which is possibly the best supported in insect phylogeny.

The species is considered by Legendre et al. (2015) as a stem-Dictyoptera owing to the occurrence of a deeply concave CuP in forewing. Indeed, the character state ‘in forewing, CuP bent posteriorly’ has often been associated with the occurrence of a ‘claval furrow’ (e.g. in Grimaldi & Engel, 2005). Such structure has also been considered a putative diagnostic feature of Dictyoptera (Prokop et al., 2014). However a markedly concave CuP (or, a CuP associated with a marked concave fold) occurs in Plecoptera (O. Béthoux, pers. obs.) and stem-Embioptera (Shcherbakov, 2015); despite sclerotization, a process blurring vein elevation, CuP remains concave in its basal half in the stem-Dermaptera *Protelytron permianum* (see reflective transformation imaging, i.e. RTI, data associated with (Béthoux et al., 2016); stem-Paraneoptera also exhibit a markedly concave CuP (Nel et al., 2012); and the posterior branch of CuP is strongly concave in Palaeozoic stem-Orthoptera (O. Béthoux, pers. obs.). The association of CuP (or its posterior branch) with a deep furrow is therefore a trait most likely common to all Neoptera.

We believe the trajectory of CuP must be considered a distinct character from its concavity. In all the above-mentioned cases CuP is straight, hence this state can be considered plesiomorphic within Neoptera, and a bent CuP derived (this polarization being in accordance with the obtained molecular-based topology). Although it has sometimes been considered diagnostic of Blattodea only, this character state is herein regarded as indicative of affinities with the whole Dictyoptera [a proposal in which we concur with Grimaldi and Engel (2005) and Legendre et al. (2015)]. Indeed, the stem-Mantodea *Santanmantis axelrodi* Grimaldi, 2003 (see original description –CuP indicated as ‘CuA<sub>2</sub>’) and *Cretophotina tristriata* Gratshev & Zherikhin, 1993 [see original description and Zherikhin (2002), Grimaldi (2003) –CuP indicated as ‘CuA<sub>2</sub>’], and, to some extent, *Metallyticus* spp. (see Béthoux & Wieland, 2009; Brannoch et al., 2017; Wieland, 2008), display a bent CuP. The occurrence of this character state therefore indicates a Dictyoptera (stem- or crown-).

#### **Calibrating node: stem-Mantophasmatodea / crown-Xenonomia**

**Fossil item:** *Juramantophasma sinica* Huang, Nel, Zompro & Waller, 2008

**Original description:** Huang, D.-y., Nel, A., Zompro, O. & Waller, A. (2008) Mantophasmatodea now in the Jurassic. *Naturwissenschaften*, **95**, 947–952.

**Further descriptive accounts:** none.

**Locality:** Daohugou (158.1 MYA).

**CR1:** NIGP 142171 (Nanjing Institute of Geology and Palaeontology, Nanjing, China).

**CR2:** Yes (see below).

**CR3:** The Mantophasmatodea-Grylloblattodea sister-group relationship (i.e. Xenonomia) is well supported by both molecular (Misof et al., 2014; and current study) and morphological (Wipfler et al., 2015) data.

**CR4:** Yes (original description).

**CR5:** Yes (see Section S2.3).



### Phylogenetic justifications & discussion:

The systematic placement of *Juramantophasma sinica* as stem-Mantophasmatodea (hence crown-Xenonomia) is based on the following character states:

#### Ultimate

Class 1: none found / considered.

Class 2: (1) enlarged and fan-like pretarsal arolia than can be lifted above the ground; (2) head orthognathous.

#### Contextual

Class 1: none found / considered.

Class 2: (3) wings absent (but see below regarding the level at which this state is relevant).

The character state (1) is well documented in *Juramantophasma sinica*. In the original description it is formulated into several character states, including ‘enlarged and fan-like pretarsal arolia with a clearly visible row of dorsal setae’ and ‘last tarsomere making a right angle with the others, keeping it up in the air’. We believe these states form a single one (because they always co-occur and putatively compose a single functional unit), as labelled above. The state is also present in other Polyneopteran groups such as some Phasmatodea (including *Timema*, very generally regarded as sister-group to the remaining crown-Phasmatodea; Beutel & Gorb, 2008; Bradler, 2009; Kristensen, 1975) and the extinct order Alienoptera (Bai et al., 2016; regarded as Dictyoptera). Given that it is absent in Grylloblattodea, the state is therefore considered relevant at the ultimate level but relegated as Class 2.

The holotype (and only known specimen) of *Juramantophasma sinica* displays an orthognathous head (2; this is more evident when merging published photographs of both slabs preserving the specimen). Head orthognathy is also present in the sister-group of Xenonomia + Eukinolabia, namely Dictyoptera. However, given the obtained topology, it is more parsimonious to assume a convergent acquisition in Mantophasmatodea. Therefore we consider that the character state applies at the ultimate level but belongs to Class 2.

The lack of wings (3) is a prominent state. There is no doubt the holotype is an adult, owing to the occurrence of eggs in the abdomen and of developed genitalia. Extant Mantophasmatodea and Grylloblattodea both lack wings, and therefore it has been proposed as diagnostic character state of Xenonomia (Grimaldi & Engel, 2005; Wipfler et al., 2015). However, fossil species regarded by some as stem-Grylloblattodea possess wings (Rasnitsyn, 1976 and multiple more recent accounts on related fossils), and therefore the character state could be considered relevant at the contextual level (as indicated above). It must be noted that the absence of wings is common among Phasmatodea, including *Timema*. On the other hand, well-ascertained stem-Phasmatodea, such as *Renphasma sinica* (Nel & Delfosse, 2011) (see original description and Wang et al., 2014) are winged. Therefore, the state is considered as Class 2.

The third tarsomere with a sclerotized elongated dorsal process was listed by Huang et al. (2008) as demonstrative of the mantophasmatodean affinities of *Juramantophasma sinica*.

Indeed, such a structure has been described for a large variety of extant Mantophasmatodea (Buder & Klass, 2013) where it occurs as a ‘tiny’ convex process. Contrasting, in *Juramantophasma sinica* it is ‘spine-like’ and ‘slightly curved’ (original description) and very long. As a consequence, strict homology of the two structures is not evident. Another character state Huang et al. (2008) considered is ‘female gonoplags (valves 3) short and claw-shaped’. However, in the actual description they state ‘they probably correspond to the gonoplags IX ‘gl9’ sensu Klass et al. (2003; Fig.1d)’. Since the identification and homology of the corresponding structure cannot be ascertained, we consider this an insufficient argument. A further character state Huang et al. (2008) considered is ‘egg with a circular ridge’ and ‘egg large, elongate, and a chorion with a pattern of small spots and a central gibbosity’. However no details about the eggs are provided in the description other than their number and their arrangement. Moreover the polarity of the states is not evident, as well as the intended meaning of the character state itself. Several other character states considered by Huang et al. (2008); e.g. lack of ocelli, morphology of antenna, respective proportions of meso- and metanotum, ovipositor length) were not confirmed or are common among Polyneopteran groups.

In summary the placement of *Juramantophasma sinica* as stem-Mantophasmatodea is only based on a set of Class 2 character states. However, provided that the placement of the species at any other node would imply further homoplasy, and despite the relative weakness of the supporting character states, the species is selected as stem-Mantophasmatodea.

#### **Calibrating node: stem-Embioptera / crown-Eukinolabia**

**Fossil item: *Alexarasnia rossica* Gorochoy, 2011**

**Original description:** Gorochoy, A.V. (2011) A new, enigmatic family for new genus and species of Polyneoptera from the Upper Permian of Russia. *Zookeys*, **130**, 131–136.

**Further descriptive accounts:** Shcherbakov (2015) [see also Aristov (2017) on a congeneric, slightly younger species].

**Locality:** Isady (254.1 MYA).

**CR1:** PIN 3840/63 (Palaeontological Institute, Moscow, Russia).

**CR2:** See below.

**CR3:** The Phasmatodea-Embioptera sister-group relationship (i.e. Eukinolabia) is well supported by both molecular (Misof et al., 2014; among others; and current study) and morphological (Bradler, 2009; Friedmann et al., 2012) data.

**CR4:** Yes (original description and further descriptive account).

**CR5:** Yes (see Section S2.3).

#### **Phylogenetic justification & discussion:**

The placement of *Alexarasnia rossica* as stem-Embioptera (hence crown-Eukinolabia) is based on the following character states:

Ultimate level:

Class 1: (1) in both wing pairs, RA (at least in middle part) margined along both edges by membranous, hyaline lines ('radial border lines'); (2) occurrence of a blood sinus along RA.

Class 2: none found / considered.

Contextual level:

Class 1: (3) in both wing pairs, in the area between ScP and AA1, occurrence of intervenal hyaline & concave lines.

Class 2: none found / considered.

The species was originally considered a Polyneopteran of uncertain affinities (Gorochov, 2011). A thorough account on stem-Embioptera was provided by Shcherbakov (2015) who identified *Alexarasnia rossica* as the most ancient representative of total-Embioptera, on the basis of the character states (1), (2) and (3) (herein slightly reformulated), previously considered unique to Embioptera (Ross, 2000). Herein we consider the occurrence of the 'radial border lines' (1) as a character state distinct from that of a blood sinus (2), itself substantiated by a lumina located in the middle of RA along its course (a distinction considered by D. Shcherbakov's, pers. com. to O. Béthoux, 2017).

The level at which the character state (3) is relevant is not evident, in particular in the context of a Phasmatodea + Embioptera sister-group relationships. Indeed such hyaline lines occur more or less continuous in the distal part of forewings of *Heteropteryx dilatata*, one of the few extant Phasmatodea with long forewings (see Shang et al., 2011, fig. 4A; and O. Béthoux, pers. obs.; and, to a lesser extent, in *Prisopus* sp., O. Béthoux pers. obs.). Note that Shcherbakov (2015) considered, in his discussion, the weakening of cross-veins crossed by these hyaline lines. The occurrence of this condition is not evident in *Alexarasnia rossica* (which, in that respect, resembles *Heteropteryx dilatata*). The character might therefore be relevant at the level of stem-Eukinolabia (and is therefore conservatively relegated at the contextual level above).

For the record, the forewings of some Gripopterygidae (Plecoptera) also display some degree of concavity in intervenal areas in connection with cross-veins weakened in their middle (Béthoux, 2005; Y. Cui and O. Béthoux, pers. obs.), but only in the distal half of the forewing. This is also the case in both wing pairs in *Mantoida* (Mantodea; O. Béthoux, pers. obs.). However none of the corresponding species display continuous hyaline lines.

Finally, Shcherbakov (2015) considered the tendency of veins and/or intercalary hyaline lines to display an alteration of their course when approaching the posterior wing margin. As a consequence veins and/or hyaline lines are parallel to the posterior wing margin for some distance. However this deflection only concerns hyaline lines in crown-Embioptera, and only veins in *Alexarasnia rossica*. The strict homology of these two conditions is therefore not straightforward

The species can be readily excluded from crown-Embioptera owing to the lack of the RP + M fusion, among other character states (Shcherbakov, 2015). For the record, Shcherbakov (2015) also discussed the case of *Soyana* spp. as a putative, slightly more ancient, stem-Embioptera, but evidence was admittedly less conclusive.

**Calibrating node: stem-Ensifera / crown-Orthoptera**

**Preliminary remarks:** Béthoux and Nel (2002) regarded ‘elcanids’ as sister-group related with Caelifera. If so, these are crown-Orthoptera. This proposal was challenged by A. Gorochov (pers. com.), who assumed that the recovered relationships was an artefact due to convergence towards elongate forewings, which is an acceptable criticism. The corresponding species therefore were not considered. Stem-Caelifera were also considered but they represent calibration points younger than the fossil species selected below.

**Fossil item: *Raphogla rubra* Béthoux, Nel, Lapeyrie, Gand & Galtier, 2002**

**Original description:** Béthoux, O., Nel, A., Lapeyrie, J., Gand, G. & Galtier, J. (2002) *Raphogla rubra* gen. n., sp. n., the oldest representative of the clade of modern Ensifera (Orthoptera: Tettigoniidea & Gryllidea) (Lodève Permian basin, France). *European Journal of Entomology*, **99**, 111–116.

**Further descriptive accounts:** A new photograph of the holotype was published as fig. 1C in Wolfe et al. (2016).

**Locality:** Lodève (271.8).

**CR1:** Ld LAP 415 (Musée Fleury, Lodève, France).

**CR2:** Yes (see below).

**CR3:** The Ensifera + Caelifera sister-group relationship (i.e. Orthoptera) is well supported by both molecular ((Misof et al., 2014); and current study) and morphological ((Kristensen, 1981); among many others) data.

**CR4:** Yes (see original description).

**CR5:** Yes (see Section S2.3).

**Phylogenetic justification & discussion:**

The placement of *Raphogla rubra* as stem-Ensifera (hence crown-Orthoptera) is based on the following character states:

Ultimate level:

Class 1: (1) in forewing, branching pattern of CuA + CuPα as follows: first branch (CuPα2) posteriorly directed, second branch (CuA) anteriorly directed, following branches (CuPα1) variable; (2) in forewing, branches of ScP with convex intercalary veins between them.

Class 2: none found / considered.

Contextual level:

Class 1: none found / considered.

Class 2: none found / considered.

The character state (1) was first recognized in the original description of *Raphogla rubra*, but under a different scheme of wing venation homologies from the one favoured herein. The first anterior branch of the CuA + CuPα ‘system’ was interpreted as CuA (alone) by Béthoux (2012a). Stem-Orthoptera also possessing a branched CuPa (such as *Oedischia williamsoni*) have

a CuA + CuPaa overall posteriorly pectinate [which is the assumed plesiomorphic condition, and is interpreted by Béthoux (2012a) as a CuA remaining fused with CuPaa until it reaches the posterior wing margin]. The character state therefore undoubtedly is derived.

Among extant species, the character state (1) occurs in Hagloidea and Tettigonioidea (Béthoux, 2012a; Chivers et al., 2017), but is absent in Grylloidea, as a consequence of a translocation of CuA onto M, and is further altered in Gryllotalpoidea (Béthoux, 2012a). Yet it is present in putative stem-Grylloidea [or stem-(Gryllotalpoidea + Grylloidea); (Béthoux, 2012a; Sharov, 1968, 1971)]. Notably, it is absent in Stenopelmatoidea (Béthoux, 2012b; and see below).

The character state (2) is equally relevant. It was mentioned in the original description of *Raphogla rubra* and its distribution was also formally tested in a cladistic analysis by Béthoux and Nel (2002). It proved diagnostic of a subset of stem-Ensifera, best known from abundant Triassic material (Béthoux & Nel, 2002; Sharov, 1968, 1971), including species for which the occurrence of an ensiferan-type stridulatory file has been ascertained (Béthoux, 2012a). Note that Garrouste et al. (2016) erroneously asserted that the occurrence of this state is rare (and see the *Permotettigonia gallica* case in the ‘Section S2.2’).

According to Béthoux and Nel (2002) and Béthoux (2012b), *Raphogla rubra* is more closely related to Grylloidea, Hagloidea, and Tettigonioidea than to the Stenopelmatoidea, owing to the lack of the character states (1) and (2) in the latter. However, Song et al. (2015) proposed the following topology: (Gryllotalpoidea + Grylloidea) + ((Stenopelmatoidea + Hagloidea) + Tettigonioidea) (and see Zhou et al., 2017). This would imply that the selected character states are homoplastic, and that *Raphogla rubra* could equally be a stem-Ensifera. In summary the position of the species either as stem-Ensifera or crown-Ensifera is contentious due to inconsistencies between morphology and the molecular-based topologies. Note that the issue was not considered in recent surveys on fossil calibration points for insect phylogeny (Wang et al., 2016; Wolfe et al., 2016). The species is then best considered a stem-Ensifera, a level at which morphology and molecules are congruent (assuming reversal in Stenopelmatoidea).

#### **Calibrating node: Stem-Dermaptera / crown-(Zoraptera + Dermaptera)**

**Preliminary remarks:** The earliest putative stem-Zoraptera are very recent (Engel & Grimaldi, 2002) if compared to the favoured case. Therefore, they were not considered.

#### **Fossil item: *Protelytron permianum* Tillyard, 1913**

**Original description:** Tillyard, R.J. (1931) Kansas Permian insects. Part 13. The new order Protelytroptera, with a discussion of its relationships. *American Journal of Science* (5), **21**, 232–266.

**Further descriptive accounts:** The species holotype was revised by Béthoux et al. (2016). Previous descriptive accounts are listed by these authors.

**Locality:** Elmo (271.8 MYA).

**CR1:** holotype, YPM IP 001019 (Yale Peabody Museum, New Haven, USA; additional specimens not considered in (Béthoux et al., 2016).

**CR2:** Yes (see below).

**CR3:** There is no morphological analysis that supports the Zoraptera + Dermaptera sister-group relationship, but it is well supported by transcriptomic analysis (Misof et al., 2014). We thus encourage future workers to review current phylogenetic hypothesis when implementing this fossil in their calibration schemes.

**CR4:** Yes (original description and further descriptive accounts).

**CR5:** Yes (see Section S2.3).

### **Phylogenetic justification & discussion:**

The placement of *Protelytron permianum* as stem-Dermaptera (hence crown-(Zoraptera + Dermaptera) is based on the following character states:

#### Ultimate level:

Class 1: (1) in hind wing, occurrence of vein broadenings forming an arc (i.e., occurrence of a ring fold).

Class 2: (2) forewing sclerotized.

#### Contextual level:

Class 1: none found / considered.

Class 2: none found / considered.

The identification of this species as a stem-Dermaptera can hardly be disputed: the occurrence of character state (1) is well ascertained and it is unique to Dermaptera. Given the obtained topology, the character state (2) can be considered relevant at the ultimate level. The lack of intercalary veins between the main veins of the hind wing vannus indicates that it is not a crown-Dermaptera. Other, related species composing the stem-group of Dermaptera ('Protelytroptera') have been documented from sub-contemporaneous localities (Carpenter, 1992; Kukalová, 1965; Shcherbakov, 2002), but they are not as well-documented as *Protelytron permianum* is.

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## S2.2 Fossils Excluded as Clade Minimum Age Calibrations

During our survey of fossil material potentially useful for temporal calibration we considered a number of species which eventually proved unsuitable. Two main causes prompted us to discard them. Firstly, a given species might not fulfill all the criteria listed by Parham et al. (2012). We found that a poorly supported systematic placement (**CR2**) was the most common cause for exclusion. Secondly, a species which systematic placement was well-ascertained yet might be younger than another equally suited for calibrating the same node. Also, a species could be contemporaneous with, or younger than, a fossil suited for calibrating a node that the obtained rooted topology necessitates as being younger. The case of *Osnogerarus trecwithiensis* exemplifies this situation. This 306.9 Ma-old stem-Orthoptera could have been used to calibrate the split between Orthoptera and Dictyoptera + sister-group. However, the stem-Dictyoptera *Qiliniblatia namurensis*, which is also 306.9 Ma-old, indicates that the Orthoptera / Dictyoptera + sister-group split must have occurred earlier. Hence *Osnogerarus trecwithiensis* does not provide useful temporal data.

As discussed prior, several previous contributions already attempted to time-calibrate the phylogenetic tree of Blattodea. We scrutinized the corresponding fossils. Those found to be unsuitable are listed below. In select cases, some fossils irrelevant to our analysis due to incompatible sampling of extant species are nevertheless briefly discussed.

Age indicated for localities are minimum ages and are, in most cases, not discussed in detail (we often relied on Wolfe et al. (2016), or on the original description; and see Section S2.3). Species are listed according to their current species name, alphabetically.

### *Fossils excluded as calibration points*

#### *Arvernineura insignis* Piton, 1940

**Preliminary remarks:** As indicated in their tab. 1, the fossil was used as calibration point by Legendre et al. (2015) as stem-Chaeteessidae (hence crown-Mantodea).

**Original description:** Piton, L.E. (1940) Paléontologie du gisement éocène de Menat (Puy-de-Dôme) (flore et faune). *Mémoires de la Société d'Histoire Naturelle d'Auvergne*, **1**, 1–303.

**Further descriptive accounts:** The holotype and two new specimens were (re-)described by Nel and Roy (1996).

**Locality:** Menat (ca. 60 MYA).

**Discussion:** Legendre et al. (2015) justified the placement of as stem-Chaeteessidae based on the character states (1) shape of the forewing pseudo-vein, and (2) most posterior branch of CuA simple. None of these states are demonstrative of affinities with stem-Chaeteessidae, the fossil possibly being a stem-Mantodea (Cui et al., 2018). In details the character state (1) (i) is not a character state *per se*, because the shape was not specified, and (ii) if considered long, it is then a putative plesiomorphy, as it occurs in *Cretophotina tristriata* (see Grimaldi, 2003, fig. 5b, c), regarded as a stem-Mantodea (Grimaldi, 2003). The character state (2) is, for the same reasons, a putative plesiomorphy, but is also absent in the holotype of

1897 *Arvernineura insignis*. In summary, as used by Legendre et al. (2015), this fossil fails to fulfill  
 1898 **CR2** for the intended node.

1899 Note that the species could have then been used as stem-Mantodea. However, because it is  
 1900 more or less contemporaneous with several fossils used as calibration within Blattodea (i.e.  
 1901 fossils that calibrate splits expected to have occurred later than the Mantodea + Blattodea split),  
 1902 it follows that *Arvernineura insignis* is not useful for calibration, given the obtained topology.  
 1903 The same comment applies to all known putative Mesozoic stem-Mantodea (herein, see the case  
 1904 of ‘*Baissomantis maculata*’).

1905

1906 ***Baissatermes lapideus* Engel, Grimaldi & Krishna, 2007**

1907 **Preliminary remarks:** As indicated in their tab. 2, the fossil was used as calibration  
 1908 point by Wang et al. (2017) as member of the crown-group (*Cryptocercus* + Isoptera),  
 1909 presumably as stem-Isoptera.

1910 **Original description:** Engel, M.S., Grimaldi, D. & Krishna, K. (2007) Primitive termites  
 1911 from the Early Cretaceous of Asia (Isoptera). *Stuttgarter Beiträge zur Naturkunde (B)*, **371**, 1–  
 1912 32.

1913 **Further descriptive accounts:** A photograph of the holotype was first published as fig.  
 1914 380 in Belayeva (2002).

1915 **Locality:** Baissa (ca. 70 MYA).

1916 **Discussion:** The age of the corresponding locality was re-assessed and proved to be  
 1917 much younger than previously estimated (see Wolfe et al., 2016, and references therein). As a  
 1918 consequence, *Valditermes brenanae*, instead of *Baissatermes lapideus*, is the earliest stem-  
 1919 Isoptera (as well as *Archeorhinotermes rossi*, suited for calibration of a more recent split; see  
 1920 ‘Section S2.1’).

1921

1922 ***Baissomantis maculata* Gratshev & Zherikhin, 1993**

1923 **Preliminary remarks:** As indicated in their tab. 2, the fossil was used as calibration  
 1924 point by Wang et al. (2017). Based on the position of other fossils selected by these authors,  
 1925 suggesting that the ‘Calibration group’ indicates crown-membership, we assume that ‘mantids’  
 1926 accounts for ‘crown-Mantodea’.

1927 **Original description:** Gratshev, V.G. & Zherikhin, V.V. (1993) New fossil mantids  
 1928 (Insect, Mantida). *Paleontological Journal*, **27**, 148–165.

1929 **Further descriptive accounts:** Grimaldi (2003) provided new drawings (under the name  
 1930 ‘*Baissomantis maculatus*’) of the specimens figured by Gratshev and Zherikhin (1993) and  
 1931 reported new observations.

1932 **Locality:** Baissa (ca. 70 MYA).

1933 **Discussion:** According to Grimaldi (2003) the species lacks the stigma (*sensu* Brannoch  
 1934 et al., 2017), one of the few traits allowing isolated wings of Mantodea to be securely identified.  
 1935 The species was therefore regarded by Grimaldi (2003) as a stem-Mantodea. If used as crown-  
 1936 Mantodea by Wang et al. (2017), this fossil fails to fulfill **CR2** for the intended node. Moreover,

the age of the locality the material was recovered from was reconsidered (see Wolfe et al., 2016 and references therein) as much younger than previously assumed (ca. 70 MYA instead of 140 MYA).

Note that even if considered 140 Ma-old, if used as stem-Mantodea (as Wang et al., 2017) might have done), the species is more or less contemporaneous with several fossils used as calibration within Blattodea (i.e. fossils that calibrate splits expected to have occurred later than the Mantodea + Blattodea split). It follows that *Baissomantis maculata* is not useful for calibration, given the obtained topology.

#### ***Balatronis libanensis* Sendi & Azar, 2017**

**Preliminary remarks:** As indicated in their fig. 1 and tab. 1, the fossil was used as calibration point by Bourguignon et al. (2018) as member of crown-group (Blattidae + Tryonicidae), presumably as stem-Blattidae.

**Original description:** Sendi, H. & Azar, D. (2017) New aposematic and presumably repellent bark cockroach from Lebanese amber. *Cretaceous Research*, **72**, 13–17.

**Further descriptive accounts:** None.

**Locality:** Lebanese amber (age uncertain, ca. 130 MYA).

**Discussion:** The fossil genus *Balatronis* was first described based on *Balatronis cretacea*, from Burmese Amber (Šmídová & Lei, 2017), and was placed in the family Blattidae based on its pronotal colouration, supposedly similar to that of extant species of *Neostylopyga*. A more ancient putative representative of this genus, namely *Balatronis libanensis*, was used as calibration point by Bourguignon et al. (2018). However, the pronotum is missing in the known material of this species. Instead Sendi and Azar (2017) relied on some states relating to wing venation which are (i) not diagnostic of the genus, (ii) the occurrence of which is not clearly demonstrated in *Balatronis libanensis*, and/or (iii) the occurrence of which is not clearly demonstrated in *Balatronis cretacea*. Instead of a stem-Blattidae, *Balatronis libanensis* is likely a member of a stem-Blattodea or stem-Dictyoptera clade due to the presence of a central ocellus (not seen in any extant cockroaches).

Given the above, and because the age of Lebanese amber is poorly constrained, we refrained from using this species as calibration point in our analysis. In summary, as used by Bourguignon et al. (2018), the fossil fails to fulfill **CR2** for the intended node, and is not an ideal case regarding **CR4** and **CR5**.

Note that the above reasoning leads one to wonder about the validity of the other species of *Balatronis*. It is also problematic as a fossil used for calibration. The pronotal colouration, while indeed comparable to the recent species of *Neostylopyga*, is also similar to a variety of unrelated extant cockroach species (e.g., *Epilampra azteca*, *Allacta* spp., *Euthlastoblatta* spp.). None of the other character states displayed by the known material indicate affinities with Blattidae, and the fact that the body size is so small makes this placement even more unlikely. In short, there is no known member of the genus *Balatronis* that can be used as a calibration for a dated analysis of the cockroach phylogeny.

1977 ***Cariblattoides labandeirai* Vršanský, Vidlička, Čiampor Jr. & Marsh, 2012**

1978 **Preliminary remarks:** This fossil specimen was suggested to calibrate the node  
 1979 *Cariblattea* + *Cariblattoides* by Evangelista et al. (2017). Given our taxon sampling, this would  
 1980 correspond to a placement as stem-*Cariblattea* (hence crown-Pseudophyllodromiinae) and  
 1981 calibrate the node of *Cariblattea* + remaining Pseudophyllodromiinae.

1982 **Original description:** Vršanský, P., Vidlička, L., Čiampor, F. Jr & Marsh, F. (2012)  
 1983 Derived, still living cockroach genus *Cariblattoides* (Blattida: Blattellidae) from the Eocene  
 1984 sediments of Green River in Colorado, USA. *Insect Science*, **19**, 143–152.

1985 **Further descriptive accounts:** None.

1986 **Locality:** Green River (48.1 MYA).

1987 **Discussion:** Evangelista et al. (2017) referred to the original description for the  
 1988 phylogenetic justification of the fossil. However, the provided evidence is inconclusive when  
 1989 subjected to detailed examination. Character states supporting the phylogenetic placement  
 1990 (namely, cup-like palpi, and hindwing radius simple) are not restricted to the genus  
 1991 *Cariblattoides*, or to the Pseudophyllodromiinae. The colour pattern of the pronotum may be  
 1992 distinctive. However, no systematic treatment of this character has been performed and similar  
 1993 pronotal patterns (at least superficially) occur in geographically disjoint Blattodea of various  
 1994 taxonomic affiliations (Rentz, 2012). In summary, as proposed by Evangelista et al. (2017) this  
 1995 fossil fails to fulfill **CR2** for the intended node.

1996

1997 ***Cratokalotermeis santanensis* Bechly, 2007**

1998 **Preliminary remarks:** As indicated in their fig. 1 and tab. 1, the fossil was used as  
 1999 calibration point by Bourguignon et al. (2018) as member of the crown-group (Kalotermitidae +  
 2000 Neoisoptera *sensu* Engel et al. (2009), presumably as stem-Kalotermitidae. Note that Legendre  
 2001 et al. (2015) also used this fossil and assumed the same placement.

2002 **Original description:** Bechly, G. (2007) Isoptera: termites. *The Crato fossil beds of*  
 2003 *Brazil* (ed. by D.M. Martill, G. Bechly and R.F. Loveridge), pp. 249–262. Cambridge University  
 2004 Press, Cambridge, UK.

2005 **Further descriptive accounts:** Grimaldi et al. (2008).

2006 **Locality:** Crato (112.6 MYA).

2007 **Discussion:** The rationale underlying the placement of this fossil according to  
 2008 Bourguignon et al. (2018) and Legendre et al. (2015) is unclear. Although Bechly (2007)  
 2009 considered the species a Kalotermitidae, Grimaldi et al. (2008) (referred to by Bourguignon et  
 2010 al., 2018), who carried out a re-description of the species, suggested putative relationships with  
 2011 Kalotermitidae. However, Grimaldi *et al.* (2008) also posit that the evidence is indecisive and  
 2012 that it could equally be stem to a larger group. Furthermore, Engel et al. (2009), in their broad  
 2013 scale phylogenetic analysis, retrieved this fossil as a remote stem-relative of Kalotermitidae +  
 2014 Neoisoptera *sensu* (Engel et al., 2009). In summary, as used by Bourguignon et al. (2018) and  
 2015 Legendre et al. (2015), the fossil fails to fulfill **CR2** for the intended node.

2016



***Cratomastotermes wolfschwenningeri* Bechly, 2007**

**Preliminary remarks:** As indicated in their tab. 2, the fossil was used as calibration point by Wang et al. (2017) as member of crown-Isoptera, presumably as a stem-Mastotermitidae.

**Original description:** Bechly, G. (2007) Isoptera: termites. *The Crato fossil beds of Brazil* (ed. by D.M. Martill, G. Bechly and R.F. Loveridge), pp. 249–262. Cambridge University Press, Cambridge, UK.

**Further descriptive accounts:** Grimaldi et al. (2008).

**Locality:** Crato (112.6 MYA).

**Discussion:** For this particular fossil Wang et al. (2017) provided no reference regarding its systematic placement. In this case our ‘Preliminary remarks’ preceding the case of *Archeorhinotermes rossi* (see ‘Section S2.1’) apply: the position of the fossil is not firmly established. As used by Wang et al. (2017), this fossil fails to fulfill **CR2** for the intended node.

***Coptotermes sucineus* Emerson, 1971**

**Preliminary remarks:** As indicated in their fig. 1 and tab. 1, the fossil was used as calibration point by Bourguignon et al. (2018) as member of the crown-group (*Coptotermes* + *Heterotermes*), presumably as member of the genus *Coptotermes*.

**Original description:** Emerson, A.E. (1971) Tertiary fossil species of the Rhinotermitidae (Isoptera), phylogeny of genera, and reciprocal phylogeny of associated Flagellata (Protozoa) and the Staphylinidae (Coleoptera). *Bulletin of the American Museum of Natural History*, **146**, 243–304.

**Further descriptive accounts:** None.

**Locality:** Chiapas amber (age uncertain, Early Miocene).

**Discussion:** Emerson (1971) placed this species in the genus *Coptotermes*, which he regarded as defined by a large number of character states (p. 265). However, their polarity was not formally tested. *Coptotermes priscus*, possibly contemporaneous (Dominican amber; age uncertain, Early Miocene), was retrieved as sister-group to an extant species of *Coptotermes* by Engel et al. (2009), essentially based on a character state of the soldier, a caste unknown for *Coptotermes sucineus*. The character state ‘wing membrane setae present, microsetulose’, described by Emerson (1971) was recovered as a homoplastic support to the assignment of the fossil to the genus *Coptotermes* by Engel et al. (2009).

Given the above, and because the age of Chiapas amber is poorly constrained, we refrained from using this species as calibration point in our analysis. In summary, as used by Bourguignon et al. (2018), the fossil fails to fulfill our strict understanding for **CR2** (see rationale in Section 2.1) for the intended node, and is not an ideal case regarding **CR4** and **CR5**.

***Diploptera* spp.**

**Preliminary remarks:** As indicated in their fig. 1 and tab. 1, unspecified species assigned to the genus *Diploptera* was/were used by as calibration point by Bourguignon et al.



(2018) as member of the crown-group *Diploptera* + Oxyhaloinae, presumably as member of the genus *Diploptera*.

**Original description:** Vršanský, P., Šmídová, L., Valáška, D., Barna, P., Vidlička, L., Takáč, P., Pavlik, L., Kúdelová, T., Karim, T.S., Zelagin, D. & Smith, D. (2016) Origin of origami cockroach reveals long-lasting (11 MYA) phenotype instability following viviparity. *Science of Nature*, **103**, 78.

**Further descriptive accounts:** None.

**Locality:** Green River (ca. 48.1 MYA).

**Discussion:** As discussed in Evangelista et al. (2017), there is no definitive evidence for the placement of the corresponding species. Diagnostic features listed by Vrsansky et al. (2016) are not unique to *Diploptera*; indeed, they can be found in a variety of other Blaberoidea, and possibly Corydiidae *sensu stricto*. Regarding the age of the corresponding locality, Bourguignon et al. (2018) selected the lower boundary for the Eocene, viz. 56.0 Ma. However, the insect-bearing strata are younger, with an upper boundary (i.e. minimum age) at 48.1 MYA (Evangelista et al., 2017). In summary, as used by Bourguignon et al. (2018) the fossil fails to fulfill **CR2**, **CR4** and **CR5** for the intended node.

#### ***Gulou carpenteri* Béthoux, Cui, Kondratieff, Stark & Ren, 2011**

**Preliminary remarks:** As indicated in their tab. 1, the fossil was used as calibration point by Legendre et al. (2015) as stem-Plecoptera.

**Original description:** Béthoux, O., Cui, Y., Kondratieff, B., Stark, B. & Ren, D. (2011) At last, a Pennsylvanian stem-stonefly (Plecoptera) discovered. *BMC Evolutionary Biology*, **11**, 248.

**Further descriptive accounts:** None.

**Locality:** Xiaheyan (306.9 MYA).

**Discussion:** The placement of *Gulou carpenteri* as stem-Plecoptera by Béthoux et al. (2011a); essentially followed by (Legendre et al., 2015) was based on the character states (1) in forewing, broad M/MP-CuA and CuA-CuP areas, (2) in both wing pairs, occurrence of a strong cross-vein connecting M and CuA just distal of the origin of the latter ('arculus'), and (3) in both wing pairs, ScP reaching RA.

The state (1) is generally present in extant Plecoptera but with exceptions. For example, the M/MP-CuA and CuA-CuP areas are not distinctly broader than the R/RP-M area in Austroperlidae (Béthoux, 2005a; Tillyard, 1923); the CuA-CuP area is not distinctly broader than the R/RP-M area in Eustheniidae (Béthoux, 2005a; Tillyard, 1923); the M/MP-CuA area is not distinctly broad in Gripopterygidae, Pteronarcyidae, and Taeniopterygidae (Béthoux, 2005a; among many other contributions); etc. Note that Legendre et al. (2015) considered the state with some reformulation 'presence of a broad MP/CuA and CuA/CuP areas in forewings, with a series of parallel simple crossveins' as relevant. We believe the type of cross-venation should be considered a distinct character. As a matter of fact, a series of parallel simple cross-veins in the areas between MP and CuP occur in many insect groups.

The state (2) is unique among Polyneoptera (an arculus is present in hind wings of Blattodea and Mantodea, but not in forewings), but likely occurs in Paraneoptera. This would lead us to reconsider the homologies proposed for the latter group by Nel et al. (2012), which is largely out of the scope of the current account. Moreover, under the M<sub>5</sub> insect wing venation paradigm, the arculus is the free part of M<sub>5</sub>, and this condition exhibited by Plecoptera and *Gulou carpenteri* is then to be regarded as a plesiomorphy within Pterygota.

The character state (3) is not unique to Plecoptera. It is also documented in fossils contemporaneous to *Gulou carpenteri* and regarded as stem-Orthoptera by some (Béthoux, 2005b, 2008b; Du et al., 2017; among others), in Psocodea (Carpenter, 1992), and several lineages of some Neuroptera such as Polystoechotidae, Osmylidae, and Myrmeleontidae (among others; (New, 1983; Tillyard, 1916; Winterton & Makarkin, 2010); among many others).

Note that Aristov (2014) places *Gulou carpenteri* in the order Cnemidolestodea, within a super-order ‘Perlidae’, itself including Plecoptera/Perlida, Dermaptera/Forficulida, Embioptera/Embiida and Grylloblattodea/Grylloblattida. However, the rationale for this placement is not obvious. Note that the clade ‘Perlidae’ is not recovered by our analysis.

In summary, the identification of *Gulou carpenteri* as a stem-Plecoptera, although likely, is not based on an autapomorphic character state (see selection rational in Section S2.1). Finally, because *Gulou carpenteri* is contemporaneous to *Qilianiblatia namurensis*, and because the latter calibrates a split expected to have occurred later than the Plecoptera + sister-group split, it follows that *Gulou carpenteri* is not useful for calibration, given the obtained topology (this applies to the case of *Palaeotaeniopteryx elegans*, see herein).

### ***Homocladus grandis* Carpenter, 1966**

**Preliminary remarks:** As indicated in their tab. 1, the fossil was used as calibration point by Bourguignon et al. (2018), in some of their analyses, as member of crown-Dictyoptera, presumably as stem-Mantodea.

**Original description:** Carpenter, F.M. (1966) The Lower Permian insects of Kansas. Part 11: The orders Protorthoptera and Orthoptera. *Psyche*, **73**, 46–88.

**Further descriptive accounts:** New photographs and drawings of material of *Homocladus grandis* were reproduced in Béthoux et al. (2010). Specimens of other species from the same fossil family (viz. the Strephocladidae, itself considered a junior synonym of Anthracoptilidae by some) were documented in Béthoux and Wieland (2009), Guan et al. (2016) and Kukalová-Peck and Beutel (2012), among recent contributions.

**Locality:** Elmo (271.8 MYA).

**Discussion:** As discussed by Bourguignon et al. (2018), the systematic placement of this species, and of other Strephocladidae (including representatives more ancient than *Homocladus grandis*), has been debated. Based on particular conjectures of homologies for the forewing venation of Mantodea Béthoux and Wieland (2009) suggested that Strephocladidae were stem-Mantodea. This proposal was challenged by many (Gorochov, 2013; Guan et al., 2016; Kukalová-Peck & Beutel, 2012; Legendre et al., 2015). Indeed, Béthoux and Wieland (2009)’s

interpretation is herein discarded: members of this family possess, in the forewing, distinct stems of RA and RP, and therefore lack one of the distinctive apomorphy of crown-Dictyoptera (namely, a pectinate fusion of RA onto RP; see ‘Section S2.1’, case of *Qilianiblatta namurensis*). Strephocladidae (or, Anthracoptilidae) could be stem-Dictyoptera (Legendre et al., 2015, and references therein). In summary, as used by Bourguignon et al. (2018), the fossil fails to fulfill **CR2** for the intended node.

#### ***Ischnoptera gedanensis* (Germar & Berendt, 1856)**

**Preliminary remarks:** As indicated in their fig. 1 and tab. 1, the fossil was used as calibration point by Bourguignon et al. (2018) as member of the crown-group (*Ischnoptera* + *Carbruneria* + *Beybienkoa*), presumably as member of the genus *Ischnoptera*.

**Original description:** Germar, E.F. & Berendt, G.C. (1856) Die im Berstein befindlichen Hemipteren und Orthopteren der Vorwelt. *Die im Berstein befindlichen organischen Reste der Vorwelt, Zweiter Band* (ed. by G.C. Berendt), pp. 1–40, pl. 4. Gerbardschen Officin, Berlin, Germany.

**Further descriptive accounts:** Berendt (1836) first described material of this species, including a figure (pl. 16, fig. 6). Shelford (1910) provided a re-description based on additional material.

**Locality:** Baltic amber (age uncertain, Eocene).

**Discussion:** Roth (2002) proposed the character states (1) front leg spination type B<sub>2</sub> or B<sub>3</sub> and (2) sulci in pronotum as diagnostic of *Ischnoptera*. However, both states can be regarded as diagnostic only in the context of Neotropical Blattellinae, as both are homoplastic in Blattodea. Given that extant species of *Ischnoptera* are restricted to the New World, and that the fossil is from the Old World Baltic region, the assignment appears dubious. Moreover, the available descriptive data is insufficient to assess the occurrence of the diagnostic states in the fossil species.

Given the above, and because the age of Baltic amber is poorly constrained, we refrained from using this species as calibration point in our analysis. In summary, as used by Bourguignon et al. (2018), the fossil fails to fulfill **CR2** for the intended node, and is not an ideal case regarding **CR4** and **CR5**.

#### ***Mastotermes nepropadyom* Vršanský & Aristov, 2014**

**Preliminary remarks:** As indicated in their tab. 1, the fossil was used as calibration point by Legendre et al. (2015) as member of stem-Mastotermitidae.

**Original description:** Vršanský, P. & Aristov, D.S. (2014) Termites (Isoptera) from the Jurassic/Cretaceous boundary: Evidence for the longevity of their earliest genera. *European Journal of Entomology*, **111**, 137–141.

**Further descriptive accounts:** None.

**Locality:** Chernovskie Kopi (ca. 70 MYA).

**Discussion:** Legendre et al. (2015) relied on the character state ‘hindwing with *Mastotermes*-like anal field’ to assigned this species to the extant family *Mastotermes*. However, this state is a plesiomorphy within crown-Dictyoptera. In other words, the species could be a stem-Isoptera, a stem-Mastotermitidae or a stem-Euisoptera. The most conservative option is to consider it a stem-Isoptera.

The age of the locality the material was recovered from was reconsidered as much younger than previously assumed (ca. 70 MYA instead of 140 Ma; see (Wolfe et al., 2016) and references therein). As a consequence, *Mastotermes nepropadyom* is not suited as stem-Isoptera, given that the stem-Isoptera *Valditermes brenanae* is more ancient (see ‘Section S2.1’). In summary, as used by Legendre et al. (2015), the fossil fails to fulfill **CR2** and **CR5** for the intended node.

### ***Mastotermes sarthensis* Schlüter, 1989**

**Preliminary remarks:** As indicated in their tab. 2, an undetermined species of *Mastotermes* described by Schlüter (1978) was used as calibration point by Wang et al. (2017) as member of crown-Isoptera, presumably as stem-Euisoptera *sensu* Engel et al. (2009). The corresponding specimen was assigned to a species on its own, namely *Mastotermes sarthensis*, by Schlüter (1989).

**Original description:** Schlüter, T. (1989) Neue Daten über harzkonservierte Arthropoden aus dem Cenomanium NW-Frankreichs. *Documenta Naturae*, **56**, 59–70.

**Further descriptive accounts:** The holotype and only know specimen has been described by Schlüter prior to his 1989 account (see references therein), including Schlüter (1978), referred to by Wang et al. (2017).

**Locality:** Bezonnais (age uncertain, ca. 94 MYA)

**Discussion:** The rationale adopted by Wang et al. (2017) to use this fossil to calibrate the group ‘termites excluding *Mastotermes*’ is not evident given that it has been consistently assigned to *Mastotermes* (see original description, references therein, and Engel et al., 2007a; Nel & Paicheler, 1993) or regarded as *incertae sedis* (Wappler & Engel, 2006). The available data suggests that the species possessed an expanded plicatum in the hind wing, which is a plesiomorphy within Dictyoptera. In other words, the species could be a stem-Isoptera, a stem-Mastotermitidae or a stem-Euisoptera. The most conservative option is to consider it a stem-Isoptera. Given that it is more recent than the stem-Isoptera *Valditermes brenanae* (see ‘Section S2.1’), it follows that *Mastotermes sarthensis* is not useful as calibration point. In summary, as used by Wang et al. (2017), the fossil fails to fulfill **CR2** for the intended node.

### ***Miroblattites costalis* (Laurentiaux-Vieira & Laurentiaux, 1987)**

**Preliminary remarks:** Owing to its age, the species was putatively useful as stem-Dictyoptera.

**Original description:** Laurentiaux-Vieira, F. & Laurentiaux, D. (1987) Un remarquable Archimylacride du Westphalien inférieur belge. Ancienneté du dimorphisme sexuel des Blattes. *Annales de la Société Géologique du Nord*, **106**, 37–47.

**Further descriptive accounts:** Photographs of both sides of the specimen were first reproduced on pl. 29 in Laurentiaux (1958). A new drawing and photograph of the holotype was published as fig. 5 in Béthoux et al. (2011b); species erroneously referred to as ‘*Miroblatta costalis*’; a photograph of the holotype was published as fig. 4A in Prokop et al. (2014a).

**Locality:** Charbonnages de Rieu-du-Coeur (ca. 314 MYA).

**Discussion:** The putative placement of *Miroblatta costalis* as stem-Dictyoptera is based on the character state ‘pronotum expanded anteriorly and laterally’. This state was listed by Rasnitsyn (2002) and Grimaldi (2005) as synapomorphic of (total-)Dictyoptera (termed ‘Blattidea’ in the former). This option then assumes a loss in Mantodea. In the absence of stem-Mantodea possessing a large pronotum, this proposal can be considered speculative, yet not unrealistic, given that Isoptera had to have experienced such a loss, as well as some Mesozoic stem- or crown-Blattodea (Grimaldi, 2005). The occurrence of the character state could be conservatively considered as indicative of a stem-Dictyoptera.

However at least some of the ‘Protorthoptera-Protoblattodea-Paraplecoptera’, regarded by some as relatives of Grylloblattodea, possess a laterally expanded pronotum. This is at least the case of *Euryptilon blattoides* (Martynov, 1940) (Euryptilonidae; see original description and Sharov (1962, 1991), and of some Epideigmatidae (Béthoux, 2007b) and Geinitziidae (Cui et al., 2012; Huang & Nel, 2008). According to Storozhenko (2002) the absence of ‘pronotal paranota’ (a formulation also including lateral lobes provided with vein-like reticulations) is diagnostic of a taxon within the ‘Protorthoptera-Protoblattodea-Paraplecoptera’, implying that a laterally expanded pronotum occurs widely in the group. These data suggest that the character state cannot be considered a definitive indication of Dictyopteran affinities.

In summary the species was not considered because it fails to fulfill **CR2**.

### ***Morphna paleo* Vršanský, Vidlička, Barna, Bugdaeva & Markevich, 2013**

**Preliminary remarks** As indicated in their tab. 1, the fossil was used as calibration point by Legendre et al. (2015) as member of stem-“Asian” Epilamprinae.

**Original description:** Vršanský, P., Vidlička, L., Barna, P., Bugdaeva, E. & Markevich, V. (2013) Paleocene origin of the cockroach families Blaberidae and Corydiidae: evidence from Amur River region of Russia. *Zootaxa*, **3635**, 117–126.

**Further descriptive accounts:** None.

**Locality:** Archara-Boguchan (61.6 MYA).

**Discussion:** Evangelista et al. (2017) discussed the weaknesses of the morphological character justification for this fossil’s phylogenetic placement. Regardless, we herein consider this fossil as of equal age to “*Gyna*” *obesa*. Then, given our taxon sampling, at best we could only consider *Morphna paleo* as stem-Blaberidae (or a crown group Blaberidae whose



relationship to the Blaberidae we include is unknown). Given this, as a calibration point, this fossil would be redundant with “*Gyna*” *obesa*.

### ***Mylacris anthracophila* Scudder, 1868**

**Preliminary remarks:** As indicated in their fig. 1 and tab. 1, the fossil was used as calibration point by Bourguignon et al. (2018), in some of their analyses, as member of the crown-group (Dictyoptera + Phasmatodea + Grylloblattodea + Mantophasmatodea), presumably as stem-Dictyoptera.

**Original description:** Scudder, S.H. (1868) Description of fossil insects found on Mazon Creek, and near Morris, Grundy co., Ill. *Geological Survey of Illinois. Volume III. Geology and Palaeontology. Palaeontology* (ed. by M.F. B. and A.H. Worthen), pp. 566-572. Schmidt, L. W., New York.

**Further descriptive accounts:** A drawing of one of the syntypes of the species (the forewing) was reproduced as fig. 1 in Durden (1969).

**Locality:** Mazon Creek (306.9 MYA).

**Discussion:** Neither apomorphy nor phylogenetic analysis was referred to by Bourguignon et al. (2018) in support of the systematic assignment of the species. These authors possibly followed Tong et al. (2015) who selected ‘late Carboniferous roachoid fossils (from ~315 MYA)’ for temporal calibration. These authors referred to Labandeira (1994) and Garwood and Sutton (2010). In the former, which is a compendium of fossil insect families (and therefore relies on earlier, systematics-orientated accounts) the Mylacridae, among other ‘Carboniferous roachoid families’ are considered members of the order Blattodea. This is not the option followed by Tong et al. (2015) nor Bourguignon et al. (2018), who regard *Mylacris anthracophila* as a stem-Dictyoptera. Garwood and Sutton (2010), who focused on a re-description of the Late Carboniferous *Aphthoroblattina eggintoni*, did not reveal any character state allowing to place the species either as a stem-Blattodea or a stem-Dictyoptera. Our own analysis, however, suggests that Bourguignon et al. (2018)’s use of this fossil is indeed appropriate: according to Durden (1969) *Mylacris anthracophila* possessed a pectinate fusion of RA onto RP, herein regarded as indicative of a stem-Dictyoptera indeed (see ‘Section S2.1’).

Bourguignon et al. (2018) as well as Tong et al. (2015) applied an age of 315 MYA for this fossil. However, the minimum age for the corresponding locality, namely Mazon Creek, is 306.9 MYA (Wolfe et al., 2016). The contemporaneous *Qilianiblatia namurensis*, selected herein (see ‘Section S2.1’), is then equally suitable.

### ***Nanotermes isaacae* Engel & Grimaldi in Engel, Grimaldi, Nascimbene & Singh, 2011**

**Preliminary remarks:** As indicated in their fig. 1 and tab. 1, an unspecified species of *Nanotermes* was used as calibration point by Bourguignon et al. (2018) as member of the crown-group (Termitidae + *Coptotermes* + *Heterotermes* + *Reticulitermes*), presumably as stem-Termitidae. To our knowledge, the only known species of this genus is *Nanotermes isaacae*.



**Original description:** Engel, M.S., Grimaldi, D., Nascimbene, P.C. & Singh, H. (2011) The termites of Early Eocene Cambay amber, with the earliest record of the Termitidae (Isoptera). *Zookeys*, **148**, 105–123.

**Further descriptive accounts:** None.

**Locality:** Cambay amber (age uncertain, Early Eocene).

**Discussion:** The original description does not provide supporting evidence for the placement in Termitidae. Unambiguous character states retrieved by Engel et al. (2009) as supporting the monophyly of Termitidae and Termitidae *nec Macrotermes* are either not applicable or not documented in *Nanotermes isaacae*. Engel et al. (2011) discuss how the comparatively poor preservation of the available material preclude a positive assignment at the sub-familial level.

Given the above, and because the age of Cambay amber is poorly constrained, we refrained from using this species as calibration point in our analysis. In summary, as used by Bourguignon et al. (2018), the fossil fails to fulfill **CR2** for the intended node, and is not an ideal case regarding **CR4** and **CR5**.

Note that our sample of extant species would make this fossil suitable to date the node we calibrated using *Archeorhinotermes rossi*, which is older than *Nanotermes isaacae* by ca. 50 MYA (see ‘Section S2.1’).

### ***Oedischia williamsoni* Brongniart, 1885**

**Preliminary remarks:** The species was putatively useful as stem-Orthoptera.

**Original description:** Brongniart, C. (1885) Les insectes fossiles des terrains primaires. Coup d'oeil rapide sur la faune entomologique des terrains paléozoïques. *Bulletin de la Société des Amis des Sciences naturelles de Rouen*, **1885**, 50-68.

**Further descriptive accounts:** The holotype was further documented in Brongniart (1893). A new photograph of the holotype was published as fig. 94 in Carpenter (1992), complemented by a new drawing (fig. 95.4a), largely similar to that published as text-fig. 15 in Carpenter (1966).

**Locality:** Commentry (298.8 MYA).

**Discussion:** The placement of *Oedischia williamsoni* as a putative stem-Orthoptera is based on the following character states:

#### Ultimate level:

Class 1: (1) in forewing, large area between the anterior wing margin and ScA (‘precostal area’).

Class 2: (2) CuPa forked (into CuPa $\alpha$  and CuPa $\beta$ ) just basal of the fusion of its anterior branch (CuPa $\alpha$ ) with CuA; (3) base of hind femur broad.

#### Contextual level:

Class 1: none further considered.

Class 2: none further considered.

The holotype of *O. williamsoni* is remarkable because it represents the earliest occurrence of jumping hind leg, diagnostic of (but not unique to) Orthoptera (or, Saltatoria), in association with wing venation character states unique to total-Orthoptera (see section on *Osnogerarus trecwithiensis*). The identification of this species as a stem-Orthoptera is hardly disputable. Indeed, it has been widely accepted [(Brongniart, 1885; Carpenter, 1992; Gorochoy & Rasnitsyn, 2002; Grimaldi, 2005; Hennig, 1981; Kukalová-Peck, 1991; Sharov, 1968; Zeuner, 1939); among others; see Kluge (2016) for alternative wording of character state (3)] and, to our knowledge, never been challenged.

The current location of the material of this species is unknown (i.e. **CR1** is not fulfilled). However, there is no doubt that the holotype existed. In addition to XIX<sup>th</sup> century accounts, Carpenter (1966) reported that he made direct observations during his visits at the Muséum National d'Histoire Naturelle (Paris) during the decade preceding his publication. The Museum of Comparative Zoology (Harvard) houses the original negative of the photograph he took of the holotype (OB, pers. obs., 2002); and the Muséum National d'Histoire Naturelle houses the original photographic glass plate of the photograph reproduced in Brongniart (1885, 1893), a scan of which will be made available on request (O. Béthoux). The available data leaves no doubts on the occurrence of the relevant character states.

However, the species was not selected because, as stem-Orthoptera, it represents a younger occurrence compared to *Osnogerarus trecwithiensis* (which, itself, was not selected for similar reasons; see the corresponding case, herein).

#### ***Osnogerarus trecwithiensis* Kukalová-Peck & Brauckmann, 1992**

**Preliminary remarks:** The species was putatively useful as stem-Orthoptera. The identification of a number of Carboniferous species as stem-Orthoptera, such as *Miamia* spp. and the Cnemidolestodea, is debated. While one school (e.g., Béthoux, 2005b; Béthoux & Nel, 2002, 2005; Prokop et al., 2014b) identifies them as stem-Orthoptera, another (e.g., Aristov, 2012; Rasnitsyn, 2002) considers them as stem representatives of various Polyneopteran lineages, or stem-Polyneoptera. The grounds for discrepancies essentially lay in the favoured insect wing venation groundplan, a topic addressed elsewhere (Béthoux, 2008a) but which can still be considered unsettled. Yet, both schools concur on the identification of a subset of these species as genuine stem-Orthoptera, including the one discussed below.

**Original description:** Kukalová-Peck, J. & Brauckmann, C. (1992) Most Paleozoic Protorthoptera are ancestral hemipteroids: major wing braces as clues to a new phylogeny of Neoptera (Insecta). *Canadian Journal of Zoology*, **70**, 2452–2473.

**Further descriptive accounts:** A photograph of a portion of the forewing of a cast of the holotype was reproduced as fig. 12 in Béthoux and Nel (2002); a drawing of the same cast, done by O. Béthoux, was reproduced as fig. 3 by Brauckmann and Herd (2006).

**Locality:** Piesberg quarry (306.9 MYA).

**Discussion:** The placement of *Osnogerarus trecwithiensis* as a putative stem-Orthoptera is based on the following character states:

Ultimate level:

Class 1: (1) in forewing, large area between the anterior wing margin and ScA ('precostal area').

Class 2: (2) CuPa forked (into CuPa $\alpha$  and CuPa $\beta$ ) just basal of the fusion of its anterior branch (CuPa $\alpha$ ) with CuA.

Contextual level:

Class 1: none found / considered.

Class 2: none found / considered.

The species was assigned to the Geraridae, a family which has been the focus of much debate. As a preliminary remark, the Paraneopteran affinities defended by Kukalová-Peck and Brauckmann (1992) can be readily excluded: they were proved to rely on artefacts of preparation (Béthoux & Briggs, 2008). Other accounts generally regarded the corresponding species as stem-Orthoptera. according to Sharov (1968), who restricted the Protorthoptera to this single family (he termed Sthenaropodidae), the corresponding species are stem-Orthoptera (inclusive of the 'Titanopterids', he regarded as deriving from stem-Caelifera); Gorochov (2001) regarded the Geraridae as putatively sister-group related with 'Titanopterids', the common clade representing stem-Orthoptera; on 'Titanopterids', see Béthoux (2007a). Note that Carpenter (1992) placed the family within his Protorthoptera, equivalent to 'stem-Polyneoptera & stems to some of the main Polyneopteran lineages'.

The character state (1) has been generally recognized as indicative of affinities with Orthoptera (Gorochov, 2001; Gorochov & Rasnitsyn, 2002; Sharov, 1968, 1971). Among Geraridae, it is present with certainty at least in the selected species and in the slightly younger *Gerarus fischeri* (Brongniart, 1885) (see Béthoux & Nel, 2003; locality Commentry, 298.8 MYA). Note that the character state is regarded by Hennig (1981) as a plesiomorphy, a position contradicted by the tree topology obtained herein. It is therefore listed above under Class 1. Among extant species, it unambiguously occurs only in Caelifera, Stenopelmatoidea and Tettigonioidea (Béthoux, 2012a; Béthoux et al., 2012; Ragge, 1955).

The character state (2) corresponds to a venation pattern that has been interpreted differently under the same insect wing venation groundplan, but also under different insect wing venation groundplans. Under the serial insect wing venation groundplan, the Zeuner-Sharov-Gorochov interpretation diagnoses the occurrence of a fork of CuA (into CuA<sub>1</sub> and CuA<sub>2</sub>) before the fusion of the anterior branch (CuA<sub>1</sub>) with MP with, regarded as both unique and derived. Under the same groundplan, the Béthoux and Nel (2002)'s interpretation (of the same structure) diagnoses the occurrence of a fusion of CuA (emerging from M + CuA) with the anterior branch of CuPa. The fact that CuPa is branched before the fusion (of its anterior branch) with CuA is considered both unique and derived.

Under the M<sub>5</sub> wing venation groundplan, Rasnitsyn (2002) placed the Geraridae within 'Eoblattida', an assemblage regarded as including both putative stem-Polyneoptera and stem-lineages of Polyneopteran main groups (therefore, to some extent, similar to Carpenter's Protorthoptera). Based on fig. 360 in this contribution (second item) it can be derived that this

author diagnoses a fusion of  $M_5$  with  $CuA_1$ . In the same book, Rasnitsyn in Gorochov and Rasnitsyn (2002) diagnose the same pattern for Orthoptera (fig. 432). According to the  $M_5$  wing venation groundplan,  $CuA$  (emerging from  $Cu$ ; concave) fuses with  $M_5$  (convex), the resulting vein being convex. A fork of  $CuA$  before the fusion with  $M_5$  is then to be regarded as derived and diagnostic of a stem- or total-Orthoptera (our analysis, and A. P. Rasnitsyn pers. com. to O. Béthoux, 2017; see also Aristov, 2014, pp. 40–41).

To our knowledge, under this wing venation groundplan, the only resemblance to this pattern can be found in Archimylacridae [considered putative stem-Dictyoptera by some (see (Béthoux, 2008b; Laurentiaux-Vieira & Laurentiaux, 1980); among others] in which an oblique structure bridges branches belonging to  $M$  on one hand and  $CuA$ , after it forked, on the other (the oblique structure then being  $M_5$  under the eponym groundplan). According to A. P. Rasnitsyn (pers. com. to O. Béthoux, 2017), this organization was acquired convergently in stem-Blattodea/Dictyoptera ( $M_5$  being then lost in crown-Blattodea/Dictyoptera and stem-groups closer to the crown than Archimylacridae).

In summary, the Geraridae can be confidently identified as stem-Orthoptera based on character state (2) under all interpretations, but the state is relegated into Class 2 because it is homoplastic under one of the proposed insect wing venation groundplans. Among extant species the corresponding structure is preserved only in Hagloidea, Tettigonioidea, and some Stenopelmatoidea (Béthoux, 2012a, b; Béthoux et al., 2012; Chivers et al., 2017; among recent accounts). It was altered in Caelifera, Grylloidea and Gryllotalpoidea (visible in putative stem-groups of these lineages; Béthoux, 2012a; Sharov, 1968, 1971). The character is present in related species from several contemporaneous localities, including Mazon Creek and Xiaheyan (Béthoux et al., 2012; Gu et al., 2017).

However, because *Osnogerarus trecwithiensis* is contemporaneous to *Qilianiblatia namurensis*, and because the latter calibrates a split expected to have occurred later than the Orthoptera + sister-group split, it follows that *Osnogerarus trecwithiensis* is not useful for calibration, given the obtained topology.

### ***Palaeotaeniopteryx elegans* Sharov, 1961**

**Preliminary remarks:** The species was putatively useful as stem-Plecoptera.

**Original description:** Sharov, A.G. (1961) Otryad Plecoptera in Paleozojskoe nasekomye Kuznetskovo bassejna. *Trudy Paleontologicheskogo instituta, Akademiya Nauk SSSR*, **85**, 225–234.

**Further descriptive accounts:** None.

**Locality:** Kuznetsk (268.3 MYA).

**Discussion:** The placement of *Palaeotaeniopteryx elegans* as stem-Plecoptera is based on the following character states:

Ultimate level:

Class 1: (1) in both wing pairs, occurrence of a single cross-vein in the areas between RA and RP, and between RP and M/MA; (2) in hind wing, occurrence of a single cross-vein in the area between M/MP and CuA (in addition to the arculus).

Class 2: (3) in hind wing, RP fused with MA; (4) in both wing pairs, occurrence of a strong cross-vein connecting M and CuA just distal of the origin of the latter ('arculus'); (5) in both wing pairs, ScP reaching RA.

Contextual level:

Class 1: none found / considered.

Class 2: none found / considered.

The species was originally placed in the family Taeniopterygidae, indicating that it would be a crown-Plecoptera. Sharov (1961) provided a brief description of the family, including the character states 'RP branched distally, with 2-4 branches', 'CuA with 1-4 branches', and 'CuP straight'. None of these character states are unique to the family.

The species was further placed in the Palaeonemouridae by Sinitshenkova (1987). According to Sinitshenkova (2002) this family is composed of stem-Euholognatha (i.e. crown-Plecoptera). However, the character supporting the corresponding taxon (therein termed 'Nemourina') refers to antennae, which are documented in a specimen putatively assigned to the species but which lacks wings, and therefore cannot be unambiguously related to the holotype of *Palaeotaeniopteryx elegans* (composed of a forewing). Among character state regarded as synapomorphic of the Plecoptera by Sinitshenkova (2002), the only putatively relevant one is 'fore wing with 2 rows cross-veins aligned CuA'; however, the meaning of this formulation is obscure to us.

The family Palaeonemouridae is relegated as stem-Plecoptera by Grimaldi (2005), echoing the general caution expressed by Zwick (2000) regarding the placement of fossil species (but who nevertheless admits the occurrence of Permian stem-Plecoptera). Note that Wolfe et al. (2016) relied on a personal communication to propose that *Palaeotaeniopteryx elegans* could be a crown-Plecoptera. Substantiation for such statement is yet to be published (and see below).

The placement of *Palaeotaeniopteryx elegans* as stem-Plecoptera can be firmly established based on the occurrence of the character state (1). Note that its occurrence in this species' hind wing is based on a hind wing imprint found isolated from the holotype (a forewing) from the same locality. That the two specimens are conspecific (similar size, distal branching of RP) is likely but cannot be positively assessed based on the available data (at worst they represent closely related species). Yet, the character state remains relevant if converted into 'in forewing, occurrence of a single cross-vein [...]' or 'in hind wing, occurrence of a single cross-vein [...]'. Within stoneflies there are exceptions in which the distal part of the corresponding areas is filled with cross-veins. This is the case in Pteronarcyidae and Peltoperlidae (both Arctoperlaria), and generally in Antarctoperlaria. Given that the character state is undoubtedly derived, its occurrence either indicates that (i) Antarctoperlaria retain a plesiomorphic condition, hence that *Palaeotaeniopteryx elegans* is at least a stem-Arctoperlaria (hence a crown-Plecoptera), or that (ii) Antarctoperlaria display a secondary acquisition of cross-venation in the



distal part of the corresponding areas (as is likely the case of the arctoperlarian Pteronarcyidae and Peltoperlidae), implying that *Palaeotaeniopteryx elegans* is a stem-Plecoptera. In the absence of suitable review on the wing venation of Antarctoperlaria, which would allow a proper appreciation of the character state distribution in the group, we conservatively considered *Palaeotaeniopteryx elegans* a stem-Plecoptera. The same discussion applies to character state (2). Given the relevance of character states (1) and (2), further supporting character states listed above do not need to be evaluated in detail.

Considered a stem-Plecoptera, *Palaeotaeniopteryx elegans* is younger than *Qilianiblatta namurensis*. Because the latter calibrates a split expected to have occurred later than the Plecoptera + sister-group split, it follows that *Palaeotaeniopteryx elegans* is not useful for calibration, given the obtained topology.

### ***Periplaneta houlberti* Piton, 1940**

**Preliminary remarks:** As indicated in their fig. 1 and tab. 1, the fossil was used as calibration point by Bourguignon et al. (2018) as member of the crown-group Blattinae (including an Archiblattinae), presumably as stem-(*Blatta* + *Neostylopyga*) + (*Protagonista* + *Shelfordella* + *Periplaneta* spp.).

**Original description:** Piton, L.E. (1940) Paléontologie du gisement éocène de Menat (Puy-de-Dôme) (flore et faune). *Mémoires de la Société d'Histoire Naturelle d'Auvergne*, **1**, 1–303.

**Further descriptive accounts:** None (but see <https://science.mnhn.fr/institution/mnhn/collection/f/item/r07034>).

**Locality:** Menat (ca. 60.0 MYA).

**Discussion:** Piton (1940) described this fossil and placed it in the Blattinae based on the character states (1) narrow and lanceolate elytra, (2) large size, and (3) secondary branching of RA+RP branches. Based on our observation of the specimen (and see further descriptive accounts), the actual occurrence of the character state (1) is not substantiated: the posterior and apical wing margins are not preserved/exposed, and a half of the wing is probably missing, including the whole AA area. As for character state (2), it is obviously not decisive at such taxonomic level. As for character state (3), it is not unique to Blattinae (it can be found in many Blaberidae and Nyctiborinae, among others). The assignment to the genus *Periplaneta* proposed by Piton (1940) was not based on any explicit character state ('it seems to us that it is towards the genus *Periplaneta* (*sensu lato*) that our fossil should be accommodated'; transl. O. Béthoux, 2018). Moreover, Piton (1940) refers to South American representatives of *Periplaneta*, a genus nowadays regarded as strictly afro-tropical and indo-malayan. It is then possible that Piton (1940) had *Pelmatosilpha* species in mind, which are the only macropterous representatives of Blattinae occurring in South America. Were this proposal substantiated with morphological evidence (which it is not), the fossil would then be a proper calibration point for Polyzosteriinae (as opposed to Blattinae). In summary, as used by Bourguignon et al. (2018), the fossil fails to fulfill **CR2** for the intended node.



***Permotettigonia gallica* Nel & Garrouste in Garrouste, Hugel, Jacquelin, Rostan, Steyer, Desutter-Grandcolas & Nel 2016**

**Preliminary remarks:** The species was putatively useful as stem-Tettigonioidea (in our species sample, as sister-group to *Nippancistroger*, assuming a Stenopelmatoidea-Tettigonioidea sister-group relationship).

**Original description:** Garrouste, R., Hugel, S., Jacquelin, L., Rostan, P., Steyer, J.-S., Desutter-Grandcolas, L. & Nel, A. (2016) Insect mimicry of plants dates back to the Permian. *Nature Communications*, **7**, 13735.

**Further descriptive accounts:** None.

**Locality:** Roua Valley (ca. 270 MYA).

**Remarks:** Garrouste et al. (2016) interpreted *Permotettigonia gallica* as a Permian stem-Tettigonioidea. Therefore the species is a potentially critical calibration point, given the relative uncertainties on the position of *Raphogla rubra* (see the case in the ‘Section S2.1’). Indeed, according to Song et al. (2015), Tettigonioidea are more closely related to Stenopelmatoidea than to Grylloidea + Gryllotalpoidea.

The assignment of *Permotettigonia gallica* was based on the character states (1) complete absence of the vein [sic] MA, M and CuA, with no secondary branches, and (2) occurrence of an archaediectyon. The meaning of the state (1) is obscure to us, as the authors labelled a vein ‘M’ on their reconstruction of the forewing of the species (fig. 1b), and ‘M’ and ‘CuA’ veins on a forewing of an extant Tettigonioidea they illustrated (suppl. fig. 2). The lack of ‘secondary branches’ is equally obscure. Understood as ‘lack of secondary intercalary veins’, it is at best a plesiomorphy. As for character state (2), based on our personal observations (O. Béthoux, 2017), it does not occur; cross-venation is instead of scalariform type (i.e. without reticulation), a state known in Late Palaeozoic and Triassic stem-Ensifera (Gorochoy, 1986; Marchal-Papier et al., 2000; Sharov, 1968, 1971). It follows that the species occupies a phylogenetic position similar to that of *Raphogla rubra*, which is older. *Permotettigonia gallica* was therefore not further considered.

***Piniblattella vitimica* (Vishniakova, 1964)**

**Preliminary remarks:** As indicated in their tab. 1, the fossil was used as calibration point by Legendre et al. (2015) as stem-Blaberoidea (hence crown-Blattodea).

**Original description:** Vishniakova, V.N. (1964) Osobennosti jilkovaniya perednikh krylev novogo nozdneyurskovo tarakana. *Paleontologicheskii Zhurnal*, **1964**, 82–87.

**Further descriptive accounts:** The species and closely related ones were documented by Vršanský (1997).

**Locality:** Baissa (ca. 70 MYA).

**Discussion:** While *Piniblattella* species are generally considered to be Blaberoidea (Legendre et al., 2015), and possibly Blattellinae (Vršanský, 1997), we consider a generic revision necessary to clarify some character states conflicting with such placements. Vršanský (1997) notes that the subgenital plate in some individuals of *Piniblattella vitimica* is valvate, a

character state well-known to be lost in the ancestral Blaberoidea (Hörnig et al., 2018). The presence of tergal glands on abdominal segments VII and VIII is also indicated by Vršanský (1997). While we cannot corroborate whether the supposed glands are internal or external, we note that tergal glands are present in all cockroaches but not always associated with external tergal modification (Roth, 1969, 2003). In other words, we considered structures described by Vršanský (1997) as insufficiently demonstrative. Moreover, while sclerotized and externally visible tergal glands posterior to abdominal segment I are most common in Blaberoidea, they are also present in Blattoidea (Roth, 1969). In summary, as used by Legendre et al. (2015), the fossil fails to fulfill **CR2** for the intended node.

#### ***Praelocustopsis mirabilis* Sharov, 1968**

**Preliminary remarks:** The species was putatively useful as stem-Caelifera.

**Original description:** Sharov, A.G. (1968) *Filogeniya orthopteroidnykh nasekomykh. Trudy Paleontologicheskogo instituta, Akademiya Nauk SSSR*, **118**, 1–216.

**Further descriptive accounts:** None.

**Locality:** Bugarikta (ca. 250 MYA)

**Remarks:** *Praelocustopsis mirabilis* was regarded by Sharov (1968) as a stem-Caelifera comparatively closer to crown-Caelifera than the slightly younger ‘Locustaviidae’ (itself regarded as composed of the most ‘remote’ stem-Caelifera). The character state ‘in forewing, basal displacement (or translocation onto CuPa) of the free portion of CuA’ [interpreted by Sharov (1968) as ‘MP converted into a cross-vein’] as diagnostic of the ‘Locustaviidae’ (i.e. total-Caelifera; and see Béthoux & Ross, 2005). However, our examination of the material (O. Béthoux, pers. obs., 2002, 2017) let us believe that a distinct CuA occurs in this species [Sharov (1968) indeed represented a cross-vein stronger than others where the portion of CuA occurs]. Another relevant character state could have been ‘in hind wing, distal emergence of M from a common stem with RP’, as figured by Sharov (1968: fig. 34D). However, our examination of the material (O. Béthoux, pers. obs., 2017) revealed that M is actually parallel to RP since the split of M + CuA (into M and CuA). There is no other unambiguous character state supporting the placement of the species as stem-Caelifera, although the general habitus of the species definitely suggests close affinities with this group.

In any case, *Praelocustopsis mirabilis* would have been relevant to date the same split as *Raphogla rubra* (given the placement herein adopted for this species), but the latter is older (see ‘Section S2.1’). The same comment applies to other known putative stem-Caelifera.

#### ***Prochaeradodis enigmaticus* Piton, 1940**

**Preliminary remarks:** As indicated in their tab. 1, the fossil was used as calibration point by Legendre et al. (2015) as stem-Choeradodinae (hence crown-Mantodea). As indicated in their tab. 2, a similar placement was assumed by Wang et al. (2017).

**Original description:** Piton, L.E. (1940) Paléontologie du gisement éocène de Menat (Puy-de-Dôme) (flore et faune). *Mémoires de la Société d'Histoire Naturelle d'Auvergne*, **1**, 1–303.

**Further descriptive accounts:** Nel and Roy (1996) provided a first revision of the known material, followed by Cui et al. (2018).

**Locality:** Menat (ca. 60.0 MYA).

**Discussion:** Cui et al. (2018) demonstrated that the species is a member of Blattodea (as opposed to a Mantodea) of uncertain familial affinities. Therefore, as used by Legendre et al. (2015) and Wang et al. (2017), the fossil fails to fulfill **CR2** for the intended node.

***Prochresmoda grauvogeli* Nel, Marchal-Papier, Béthoux & Gall, 2004.**

**Preliminary remarks:** The species was putatively useful as stem-Phasmatodea.

**Original description:** Nel, A., Marchal-Papier, F., Béthoux, O. & Gall, J.-C. (2004) A 'stick insect-like' from the Triassic of The Vosges (France) (Insecta: 'Phasmatodea'). *Annales de la Société Entomologique de France*, **40**, 31–36.

**Further descriptive accounts:** None.

**Locality:** Grès à Voltzia (240.5 My).

**Discussion:** The systematic affinities of the species were considered uncertain in the original description. Moreover the affinities of the Chresmodidae, the family it is assigned to, remain unclear, despite recent discoveries (Delclòs et al., 2008; Zhang et al., 2017; Zhang et al., 2009). In summary the species was not considered because it fails to fulfill **CR2** for the intended node.

***Reticulitermes antiquus* (Germar, 1813)**

**Preliminary remarks:** As indicated in their fig. 1 and tab. 1, the fossil was used as calibration point by Bourguignon et al. (2018) as member of the crown-group (*Reticulitermes* + *Coptotermes* + *Heterotermes*), presumably as member of the genus *Reticulitermes*.

**Original description:** Germar, C.G. (1813) Insekten in Bernstein eingeschlossen, beschrieben aus dem akademischen Mineralien-Cabinet zu Halle. *Magazin der Entomologie*, **1**, 11–18.

**Further descriptive accounts:** See Emerson (1971) and Engel et al. (2007b) and references therein. A photograph of a specimen assigned to this species was reproduced as fig. 382 in Belayeva (2002).

**Locality:** Baltic amber (age uncertain, Eocene).

**Discussion:** The phylogenetic position of the species was tested by Engel et al. (2009), who retrieved a relationship (*Coptotermes* + (*Heterotermes* + *Reticulitermes*), as opposed to Bourguignon et al. (2018)'s (*Reticulitermes* + (*Heterotermes* + *Coptotermes*)). Moreover, Engel et al. (2009) retrieved *Reticulitermes antiquus* as sister-group to a clade including extant species assigned to both *Reticulitermes* and *Heterotermes*, indicating that the generic placement is dubious (assuming that genera should be monophyletic). In the absence of a re-evaluation of the

character states supporting the placement of the fossil species, there is disagreement between morphology and molecular data.

Given the above, and because the age of Baltic amber is poorly constrained, we refrained from using this species as calibration point in our analysis. In summary, as used by Bourguignon et al. (2018) the fossil fails to fulfill **CR2** and **CR3** for the intended node, and is not an ideal case regarding **CR4** and **CR5**.

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## 2924 S2.3 Locality Ages

2925 Here we provide the ages for each locality relevant to our chosen fossil calibrations.  
 2926 While we may have discussed other localities not mentioned here, particularly in regards to  
 2927 fossils we have excluded from the analysis, here we only focus on those localities relevant to our  
 2928 fossil calibrations.

### 2929 *Localities*

2930 Xiaheyan (306.9 MYA)

2931 **Geographic location:** Xiaheyan, Ningxia, China.

2932 **Geological settings:** Tupo Formation.

2933 **Stratigraphic data:** See below.

2934 **Age justification:** Ongoing studies are revealing that the age of the insect-yielding strata are  
 2935 younger than previously assumed. While the lowest (most ancient) strata are late Bashkirian  
 2936 (minimum age 315.0 Ma; Cohen et al., 2013), the upper (youngest) ones are Moscovian  
 2937 (minimum age 306.9 Ma; Cohen et al., 2013). The conservative 306.9 Ma age is to be used for  
 2938 Xiaheyan species whose precise origin was not documented.

2939

2940 Lodève (271.8 MYA)

2941 **Geographic location:** Lodève, Hérault, France.

2942 **Geological settings:** Mérifrons Member, Salagou Formation.

2943 **Stratigraphic data:** Artinskian to Kungurian, Early Permian.

2944 **Age justification:** See Wolfe et al. (2016); and references therein.

2945

2946 Elmo (271.8 MYA)

2947 **Geographic location:** Elmo, KS, USA.

2948 **Geological settings:** Carlton Member, Wellington Formation.

2949 **Stratigraphic data:** Leonardian regional stage, Early Permian.

2950 **Age justification:** See Wolfe et al. (2016); and references therein.

2951

2952 Isady (254.1 MYA)

2953 **Geographic location:** Isady, Vologda Region, Russian Federation.

2954 **Geological settings:** Kichuga Member, Poldarsa Formation.

2955 **Stratigraphic data:** Capitanian, Upper Permian.

2956 **Age justification:** The insect-bearing locality Isady belongs to the Kichuga Member of the  
 2957 Pondarsa Formation, itself dated as late Severodvinian based on multiple biostratigraphic  
 2958 markers (Golubev in Aristov et al., 2013; Arefiev et al. 2015). Recent accounts have considered  
 2959 the Severodvinian to correlate closely with the Capitanian (Biakov et al., 2017a; Biakov et al.,  
 2960 2017b; Davydov et al., 2018; Henderson et al., 2012; Lucas & Shen, 2018). Datings of the  
 2961 Capitanian-Wuchiapingian boundary converge towards 258–260 Ma (minimum age of 258.6 Ma  
 2962 according to Lucas & Shen, 2018). However, the Severodvinian has also been regarded as



- 2963 ending within the Wuchiapingian (e.g. Arefiev et al., 2015), a stage which upper boundary is  
 2964 254.1 Ma-old (Cohen et al., 2013).
- 2965
- 2966 Daohugou (158.1 MYA)
- 2967 **Geographic location:** Nincheng County, Inner Mongolia, China.
- 2968 **Geological settings:** Tiaojishan Formation.
- 2969 **Stratigraphic data:** Oxfordian, Upper Jurassic.
- 2970 **Age justification:** See Wolfe et al. (2016); and references therein.
- 2971
- 2972 Clockhouse Brickworks pit (130.3 MYA)
- 2973 **Geographic location:** Surrey, United-Kingdom.
- 2974 **Geological settings:** Lower Weald Clay.
- 2975 **Stratigraphic data:** Hauterivian/Barremian boundary, Lower Cretaceous.
- 2976 **Age justification:** See Wolfe et al. (2016); and references therein.
- 2977
- 2978 Montsec (125.5 MYA)
- 2979 **Geographic location:** Montsec, Spain.
- 2980 **Geological settings:** "La Pedrera Rúbies" and "La Cabrua" Formations.
- 2981 **Stratigraphic data:** Late Barremian or Early Aptian.
- 2982 **Age justification:** We followed Evangelista et al. (2017); see references therein). Note that a  
 2983 slightly older age (viz. 125.71 MYA) was proposed by Wolfe et al. (2016).
- 2984
- 2985 Myanmar amber (98.2 MYA)
- 2986 **Geographic location:** Kachin, Myanmar.
- 2987 **Geological settings:** Hukawng Basin.
- 2988 **Stratigraphic data:** Cenomanian, Upper Cretaceous.
- 2989 **Age justification:** See Wolfe et al. (2016); and references therein.
- 2990
- 2991 Menat (60.0 MYA)
- 2992 **Geographic location:** Puy-de-Dôme, France.
- 2993 **Geological settings:** Menat maar (?).
- 2994 **Stratigraphic data:** Thanetian, Paleocene.
- 2995 **Age justification:** The age of the Menat locality has been long discussed (see (Mayr et al., in  
 2996 press), and references therein; among many others), but the conflicting outcomes coupled with,  
 2997 occasionally, improper reports on previous accounts, prompted us to carry out an exhaustive  
 2998 review. Vincent et al. (1977), based on K/Ar ratios analyses of basaltic layers and of a  
 2999 hornblende occurring at the Menat fossiliferous outcrop, obtained ages ranging from  $51 \pm 2$  to  
 3000  $64 \pm 2$  Ma (average: 56.6 MYA). They also reported a reversed polarity Chron.
- 3001       Kedves in Kedves and Russell (1982; see also Kedves, 1967) studied the palynological  
 3002 content of the Menat fossiliferous layers and concluded that they are of Thanetian age (i.e. 56.0

to 59.2 Ma; Cohen et al., 2013), owing to the occurrence of decisive biostratigraphic markers. In turn, these markers were recovered in marine sediments of the Kroisbach Member, as part of a palynological assemblage similar to that of Menat (Draxler, 2007). Calcarerous nannoplankton contained in the same sediments indicated the *Heliolithus riedelii* zone (i.e. zone NP8; Stradner in Gohrbandt, 1963). The only Chron of reversed polarity overlapping this zone is Chron C25r (Vandenberghe et al., 2012). This gives a maximum age of 58.8 Ma (upper boundary of NP8) and a minimum age of 57.7 Ma (lower boundary of C25r) for the Menat locality, fitting within the age range obtained by Vincent et al. (1977; especially that obtained from the hornblende,  $58 \pm 2$  MYA).

Wappler et al. (2009, suppl. data; and see Wedmann et al., 2009) suggested an age in the range of 60–61 Ma (Selandian) based on a personal communication from P. Gingerich to T. Wappler (2008), which indicated that *Plesiadapis insignis*, a fossil mammal recovered from Menat, is mostly similar to the North American species *Plesiadapis praecursor* and *Plesiadapis anceps*, themselves of Selandian age. Gingerich (1976) indeed reported that teeth length of the Menat species makes it overall similar to the two above-mentioned North American ones. However, he also stated on multiple occasions that the Menat species is difficult to compare with its supposed American counterparts. One possible reason underlying such caution is that the second lower premolar (P<sub>2</sub>) of the Menat species is much larger than it is in most other species of the genus. Also, and maybe more importantly, the former is known from more or less complete compressed skeletons ('road kill' fossilisation), this making a detailed study of the teeth and cranial morphology difficult, while the latter are known from isolated, 3-dimensionally preserved elements, including mandibles bearing teeth. We doubt the proposed Selandian age as currently justified, but it remains plausible. In summary, we hold that the minimum age with strongest justification is 57.7 Ma, but leave room for new evidence to support the older age of 60 Ma.

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## **S3 Evolution of morphology, behavior and life history**

### **S3.1 Current knowledge about phenotypic evolution in Blattodea**

The study of phenotypes is a key issue in understanding evolutionary relationships among Blattodea. In precladistic times there were systematic studies of cockroach wings (Rehn, 1951), tergal glands (Roth, 1969), proventriculus and genitalia (McKittrick, 1964). More formal analyses were put forth in cladistics times, utilizing mostly genital morphology (Deitz et al., 2003; Grandcolas, 1996; Klass & Meier, 2006; Klass, 2001). In the era of phylogenetic systematics similar character systems have been used in conjunction with molecular data (Djernæs et al., 2012; Djernæs et al., 2015; Ware et al., 2008).

Although one can discuss the evolution of phenotypic characters in the context of the above cited studies, it is perhaps more useful to discuss phenotypic evolution with a phylogenetic topology derived from independent data (to avoid circular reasoning). Yet, few studies have used phylogenetic topologies inferred from non-morphological data to investigate the evolution of phenotypic features. Maekawa et al. (2003) inferred evolutionary patterns of soil and wood burrowing among Panesthiinae cockroaches using their molecular phylogeny. Legendre et al. (2014) discerned evolution of social behaviors among species of Blaberidae. Bourguignon, et al. (2018) inferred patterns of biogeographical evolution among all cockroaches and termites. Finally, most Dictyopteran systematic studies have, usually without formal analyses, inferred the evolution of eusociality, sub-sociality, and parental care through phylogenetic hypotheses inferred from independent data.

### **S3.2 Ancestral state reconstruction of phenotypic traits**

Morphological data was collected from previous publications (Djernæs et al., 2012; Djernæs et al., 2015; Klass & Meier, 2006; McKittrick, 1964). Table S11 provides a list of the included characters including their literature sources. Body size data was either taken from taxonomic publications, or measured directly using specimens in the collection of Museum national d'Histoire naturelle (Paris) or specimens identified by Dominic Evangelista. Body length was measured as the length along the mid-line from the anterior most point to the end of the abdomen (wings not considered). Body width was measured as the greatest body width (wings not considered). Information on sociality and parental care was taken from published literature (Bell et al., 2007; Gilbert & Manica, 2015; Wong et al., 2013). Additional characters were considered (e.g. presence of *Blattabacterium*) but were excluded because of inappropriate sampling or incomplete information.

Species were assigned categorical character states for our 19 selected morphological, behavioral and parental care characters (Table S11 and Supplementary File “Blattodea\_ancStates\_datamatrix.nex” provided on Dryad). We inferred the most parsimonious ancestral states of all characters along our best ML tree topology with the software Mesquite v. 3.3 (trace all characters / Parsimony Ancestral State) (Maddison & Maddison, 2017). Note that Mesquite does not allow ancestral state reconstruction under the Dollo parsimony (Maddison &

Maddison, 2008) optimality criterion, which penalizes the loss and subsequent regain of a character. Thus, certain reconstructions appear to show the loss of a character state in one lineage and its subsequent re-evolution in a descendant lineage.

In addition to the parsimony ancestral state reconstruction of all characters, we also used a Bayesian ancestral state reconstruction with stochastic character mapping for all characters related to wing morphology (characters 15-18, see below). There are many lineages, particularly in Blattoidea, that are missing wings in the extant species, but whose ancestors may or may not have been missing wings. Stochastic character mapping allows us to infer the state of ancestors when the state of extant taxa is unknown. We specified this by providing a prior probability of 0.5 for both states when wings were absent (in characters 16-18). The exact prior probabilities (“morpho\_data\_selected\_PriorProb\_version.csv”), input tree (“95datedTree.run1.tre”), and R script (“PhytoolsCode.R”) utilized can be found in the Supplementary File folder “Stochastic character analysis” found on DRYAD. Specifically, we used the software package Phytools (Revell, 2012) to stochastically map characters onto the phylogeny using specified prior probabilities and an equal rates matrix with the function make.simmap (Bollback, 2006). We did this for 100 trials and visualized the summary of all results mapped onto the tree.

### S3.3 Detailed description of selected characters

**Character 1: Body size (anterior margin of head - posterior margin of last segment, without cerci or other appendages) in mm: (0) smaller than 6.5; (1) 6.5-15; (2) 15-30; (3) above 30.** Information about body size of specimens was measured preferably on adult females of the collection of the Muséum national d'Histoire naturelle (Paris, France). In some cases, we relied on published species descriptions. Body size can be an indicator of other biological traits such as: reproductive strategy (Werner, 2011), population size (Blackburn, 1993), or locomotion and dispersal (Gutierrez, 1997; Ness, 2004).

**Character 2: Ratio between body length and maximum width: (0) smaller than 2; (1) 2-2.5; (2) 2.6-3.5; (3) 3.5-5; (4) above 5.** Information about this ratio was measured preferably on females of the collection of the Muséum national d'Histoire naturelle (Paris, France). In some cases, we relied on species descriptions or images.

**Character 3: Postovipositional maternal care: (0) absent; (1) present.** This form of care is found in Blaberids, *Blattella*, *Supella* and *Cryptocercus* (Bell et al., 2007; Gilbert & Manica, 2015; Wong et al., 2013). Termites also show this behaviour during the early stages of colony foundation (Nalepa, 1984; Nalepa et al., 2001; Park et al., 2002; Seelinger & Seelinger, 1983; Watson et al., 1985).

**Character 4: Long-lasting biparental care: (0) absent; (1) present.** Although biparental care is known in various roaches, long-term biparental care sensu Klass et al. (2008) is only found in *Cryptocercus* and the termites (Klass et al., 2008).

**Character 5: Eusociality: (0) absent, (1) present.** Among Blattodea, only termites exhibit eusociality (Bell et al., 2007).



**Character 6: Mode of reproduction: (0) Oviparity; (1) ovoviviparous or false viviparous; (2) viviparity.** We define oviparity as development of young in an ootheca that is not incubated within the mother until birth. We define ovoviviparity as development of young inside an ootheca that is incubated within the mother until birth. We define viviparity as development of young inside the mother, without the presence of an ootheca, until birth. Ovoviviparity, a.k.a. false viviparity, are found in Blaberidae with the remaining Dictyoptera being oviparous. Viviparity is found only in *Diploptera punctata* (Djernæs et al., 2012; Klass & Meier, 2006; McKittrick, 1964).

**Character 7: Ootheca: (0) absent; (1) present.** An ootheca is present in all Dictyoptera with the exception of Euisoptera (i.e. termites excluding *Mastotermes*) (Nalepa & Lenz, 2000) and viviparous taxa (Bell et al., 2007).

**Character 8: Ootheca: (0) deposited; (1) carried around.** Most oviparous cockroaches deposit their ootheca (either by attaching it to substrate or just dropping it) but some Blattellinae carry it around for a significant amount of time. All ovoviviparous cockroach species carry their ootheca, by definition (Djernæs et al., 2012; Klass & Meier, 2006; McKittrick, 1964).

**Character 9: Ootheca deposition: (0) laid directly into dug hole; (1) laid first, hole prepared, then ootheca moved into hole; (2) laid and ignored.** Corydioidea are known to drop their ootheca without putting it into a hole whereas the oviparous Blaberoidea and Blattodea usually dig a hole and then drop the ootheca into it. In *Lamproblatta* and *Cryptocercus*, mothers lay the ootheca first, then dig the hole, and finally place the ootheca into the hole (Cleveland, 1934; McKittrick, 1964).

**Character 10: Advanced rotation of ootheca in vestibulum: (0) absent; (1) present.** The rotation refers to a turn of the ootheca such that its keel is lateral rather than dorsal. This character is coded according to Klass & Meier (2006).

**Character 11: Position of the ootheca during formation: (0) outside the body; (1) inside the body.** Mantodea form their ootheca externally, on substrate. In contrast, Blattodea form it internally, although it is sometimes glued to substrate after its internal formation.

**Character 12: Orientation of phallomere asymmetry: (0) normal: left complex on left side, right phallomere on right side; (1) reversed: left complex on right side, right phallomere on left side.** Character 107 of Klass & Meier (2006). Following Klass (1997): the left phallomere complex is defined as the complex bearing the hooked sclerite (hla) ancestrally in Mantodea and Blattodea. In living taxa, this state is seen in most Mantodea, and most lineages of Blattodea (all Solumblattodea and many Blaberoidea). Additional information on this character is provided in Klass (1997).

**Character 13: Symmetry of external genitalia: (0) strongly asymmetrical; (1) (almost) bilaterally symmetrical (including virtual absence).** External genitalia refers to the genital phallomeres (not paraprocts or subgenital plate, or supraanal plate). This character is coded according to Klass & Meier (2006).

**Character 14: Nest (built galleries): (0) absent; (1) present.** Subsocial and social taxa in Blattodea build nests by boring through wood or soil. This is ubiquitous among termites and

*Cryptocercus* (Deitz et al., 2003). It is also present in some Blaberidae (Legendre et al., 2014; Maekawa et al., 2003), but we do not include these taxa here.

**Character 15: Wings: (0) absent; (1) present.** The coding is based on specimens of the collection of the Muséum national d'Histoire naturelle (Paris, France). In some cases, we relied on species descriptions.

**Character 16: Wings: (0) cannot be dropped; (1) can be dropped.** Termites drop their wings along a predefined breaking line (Myles, 1988).

**Character 17: Plicatum of hindwing folding: (0) folding simple; (1) folded fan-like.** Corydioidea have a simple, non-fanwise fold in their wing. All non-termite cockroaches with large wings have a plicatum that folds along the radiant-shaped anal veins, like an accordion (Rehn, 1951). Euisoptera lack a folded plicatum entirely.

**Character 18: Tegminization of forewings: (0) absent; (1) present.** All studied Blattodea, with the exception of termites and *Nocticola*, have sclerotized front wings. They are considered to be a protection against damage in small and narrow spaces. Termites dealate before entering a life in the ground and *Nocticola* lives in caves.

**Character 19: Number of ocelli: (0) zero; (1) two; (2) three.** All studied extant Blattodea have two ocelli, although in some they are severely reduced. The only exception is *Cryptocercus* that lacks them completely. Extant Mantodea have three ocelli (Brannoch et al., 2017).

### S3.4 Results and discussion

Table S12 provides the results of the parsimony mapping for the major clades. Full results of parsimony ancestral state reconstructions can be found in Supplementary File “Blattodea\_ancStates\_datamatrix.nex” deposited on Dryad. Here we will focus on overall trends in the results as well as interpretations not discussed in the main text.

Overall, our results of the parsimony ancestral state reconstructions are mostly consistent with expected patterns or they remain ambiguous because of missing data. Ovovivipary and vivipary are limited to Blaberidae and did not arise elsewhere. Similarly, extended care of ootheca is also shared among all Blaberidae, even though it seems to have evolved independently at least once, but missing character information may be affecting this inference. Nearly all Corydioidea are thought to lay ootheca and subsequently ignore it. Our reconstruction for the ancestor of Corydioidea and Corydiidae s.s., however, is ambiguous because of missing data in *Nocticola* and *Tivia*.

Results for the parsimony ancestral state reconstructions of characters 16-18 were ambiguous for many nodes in Blattoidea because a few major line-ages (Lamproblattidae, Cryptocercidae, Tryonicidae) lack wings. The Bayesian analysis, utilizing stochastic character mapping, brings some insight into the evolution of these characters. This analysis finds that the ancestor of Kittrickia, Tutricablattae and Blattoidea most likely had wings (either brachypterous or macropterous) whereas this was ambiguous in the parsimony reconstruction. Given that, it is meaningful to discuss the inferred state of characters 16-18 for these nodes. The results of all the Bayesian analyses can be seen in the tree visualizations on the data uploaded to DRYAD.

The parsimony and Bayesian analyses agree that the ancestor of Blattoidea did not drop its wings (as seen in termites; although see Evangelista, 2017 for a behavioral observation regarding Eurycotis that might affect these re-sults). The parsimony reconstruction was ambiguous for the ancestors of Kittrickia and Tutricablattae. The Bayesian reconstruction gives the ancestor of Kittrickia has ~0.25 posterior probability (pp) for wing dropping and the ancestor of Tutricablattae has a >0.50 pp of dropping its wings. This finding is noteworthy because it indicates it is plausible for wing-dropping to be plesiomorphic to Isoptera.

We coded hindwing folding (character 17) as a categorical state in the parsimony analysis but as a binary state (presence or absence of fan-wise folding) in the Bayesian analysis with prior probability of 0.5 for wing-less taxa. The results of the Bayesian analysis show that the hindwing of the ancestors for Solumblattodea, Blattoidea, Kittrickia and Tutricablattae were likely not folded fan-wise and the ancestor of Blattodea is ambiguous.

The parsimony reconstruction inferred character 18 as an ambiguous state for Kittrickia and Tutricablattae. However, the Bayesian analysis shows that it is likely the ancestor of Kittrickia had tegminized forewings (>0.75 pp) but tegminization was unclear for the ancestor of Tutricablattae with marginally more support (>0.50 pp) for a membranous forewing in this species.

There were some cases where missing data for extant taxa was limiting our inference for ancestral nodes. Having a more complete character matrix would improve our reconstructions in this regard. Increased taxon sampling would also improve our inference of some traits. This is particularly true for our inference of body size evolution, which we find changes very often among the subgroups of Blattodea.

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## S4 Taxonomic terminology

### S4.1 Criteria for naming clades

In Table S13 and below, we clarify the meaning of taxonomic names used in the main text and formally propose new ones for previously unnamed clades. The names utilized here follow Beccaloni and Eggleton (2013), which is the naming scheme utilized for the cockroach species file online database (Beccaloni, 2018). We treated names for clades based on the following criteria: implied monophyly (e.g. Nocticolinae, instead of Nocticolidae, which makes Corydiidae paraphyletic), clarity (e.g. while Corydiidae, Corydiidae *s.s.* or Corydiidae *s.l.* can be ambiguous, Corydioidea should easily be understood to contain the Corydiidae and Nocticolidae), ease of use (e.g. Blattoidea instead of “Blattidae + Tryonicidae + Lamproblattidae + Cryptocercidae + Termitoidae”), or historical usage (e.g. we treat Isoptera as an unranked name for termites, since it is still in such wide usage despite being an invalid ordinal rank). Also, we voted to use the name of genera in our dataset for clades we sampled poorly (e.g. we refer to *Lamproblatta* instead of Lamproblattidae, even though this one genus could be a representative of the whole family, which contains only 10 species). Finally, we propose new names for important clades recovered with strong support. In some cases, we attempt to make these names consistent with traditional Linnaean taxonomic procedure despite the fact that any rank above species does not represent any natural classification but rather an artificial, man-made system. We provide detailed justification and character support for each newly defined clade. See Table S13 for a list of all preferred names and their synonymy.

### S4.2 Systematic entomology for the newly proposed terms

**Solumblattodea** Evangelista and Wipfler *nom. nov.*

**Systematic scope.** The taxon includes Corydioidea Saussure, 1864 and Blattoidea Latreille, 1810.

**Diagnosis.** Adult female subgenital plate valvate (divided), as opposed to simple and undivided. Male genital sclerites not separated and left phallomere (side with hook) complex, as opposed to separated by soft membranes and simplified left (side with hook) phallomeres.

**Remarks.** We found strong statistical support for this clade (bootstrap analysis and Four-cluster Likelihood Mapping; Figure S4). Also, the character “female subgenital plate valvate” is highly conserved and does not appear in any other extant taxa (i.e., in the sister group, Blaberoidea). However, the character is likely plesiomorphic as it is present in Mantodea (Hörnig et al., 2018).

**Etymology.** Derived from latin meaning “ground cockroaches”. This refers to the affinity of many species for sand (Corydiinae), caves (Nocticolinae), or soil (many termites). Additionally, many major lineages are flightless (Cryptocercidae, Lamproblattidae, Tryonicidae, Duchailuinae, *Eurycotis*) or poor fliers (e.g., Corydiinae, Blattinae) limiting these taxa to the ground-dwelling or arboreal lifestyles.

**Kittrickea** Wipfler and Evangelista *nom. nov.*

**Systematic scope.** This taxon includes Lamproblattidae McKittrick, 1964, Cryptocercidae Handlirsch, 1925, and Isoptera Brullé, 1832 (or synonymously called Termitoidae, Latreille, 1802).

**Diagnosis.** A-B-C ootheca laying sequence (A = lay ootheca; B = dig protective hole; C = deposit ootheca in hole), as opposed to B-A-C. Absence of muscle 14, as opposed to presence of muscle 14.

**Remarks.** McKittrick (1965) discussed the relationship between *Cryptocercus* and Isoptera, and stated “*L. albipalpus* is phylogenetically the closest known cockroach to *Cryptocercus*” citing her then recent work McKittrick (1964) as supporting evidence. McKittrick (1964) did not actually show this in a formal phylogenetic analysis but did in fact discuss morphological and behavioral similarity among the two clades. Particularly, she discusses similarities in the proventriculus, female genitalia, and ootheca protection sequence.

The formalized morphological analysis of Klass and Meier (2006) did not recover this clade but did give one male genital character (absence of muscle 14) common to *Lamproblatta*, *Anaplecta* and *Cryptocercus*. We do not include *Anaplecta*, or any Anaplectidae in this clade as of yet, but further systematic studies could prove its presence in this clade. Prior studies have suggested this as a possibility (Djernæs et al., 2015; Evangelista et al., 2018).

**Etymology.** This taxon is named after Frances Ann McKittrick, who was the first to give evidence for this phylogenetic hypothesis.

**Tutricablattae** Wipfler and Evangelista *nom. nov.*

**Systematic scope.** The taxon includes Cryptocercidae Handlirsch, 1925, and Isoptera Brullé, 1832 (or Termitoidae Latreille, 1802).

**Diagnosis.** Living in colonies with social structure. Having biparental care at least in some colonial stages. Wood feeding. Forming galleries or nests in wood. Hindgut fauna of oxymonadid and hypermastigid flagellates. Performs anal trophallaxis. Antenna moniliform as opposed to filiform. In male genitalia muscle 15 absent. In proventriculus primary pulvilli greatly expanded (partially overlapping neighboring sclerites).

**Remarks.** Behavioral, life history, and physiological support for this relationship is given in Klass et al. (2008) and morphological support for the relationship is given in Klass & Meier (2006). Molecular phylogenetic studies support this relationship unequivocally (Djernæs et al., 2012; Djernæs et al., 2015; Inward et al., 2007; Legendre et al., 2015; Ware et al., 2008).

**Etymology.** The name Tutricablattae derives from the latin “tutrices” meaning female guardians and “blatta” meaning cockroaches.

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## Appendix: Supplementary files and descriptions provided via the Digital Repository DRYAD

Files can be found here:

### **Supplementary Archive 1.**

This archive contains all files relevant to molecular analyses (see Supplementary text S1: Datasets and molecular analyses).

[Supplementary\_Archive\_1.zip: 54 MB]

The archive contains the following subdirectories:

#### **S1.1\_OrthologSet**

Files can be used as input for Orthograph, The subdirectory includes a table with all orthologous sequence groups (OG) as used in this study from the OrthoDB8 database. We provide the table with annotation and without, latter serves as input Orthograph (\*\_orthograph.txt), and the four official gene sets, on amino-acid (\*.protein.fas) and nucleotide level (\*.CDS.fas), cleaned and with corresponding headers (FASTA format) for the reciprocal BLAST search (= ready to use for HaMStRad). Shortcuts: EDAN: *E. danica*, LFUL: *L. fulva*, RPRO: *R. prolixus*, ZNEV: *Z. nevadensis*, see Supplementary Table S3 and Methods section.

#### **S1.2\_Datasets**

This subdirectory includes two supermatrices (PHYLIP format) and respective partition files (NEXUS format) including selected models (\*partitions.nex, assigned models to each partition are listed at the bottom). The partitioning based on protein domains annotation.

- decisive supermatrix (superalignment) on amino-acid level + corresponding partition file including selected models (“decisive amino-acid dataset”, see Figure 1)
- supermatrix on nucleotide level including only 2<sup>nd</sup> codon positions + corresponding partition file including selected models (“full nucleotide dataset”, see Supplementary Figure S3)

#### **S1.3\_AU\_Tests**

This subdirectory includes supplementary files and results of all AU-test analyses in plain text format.

- Blattodea\_AUtest\_command.txt: command as used in IQTree
- Blattodea\_AUtest\_trees.tre: all trees tested in Newick format (best ML tree inferred plus 13 alternative topologies, see comments and Supplementary Table S7)
- Blattodea\_AUtest.log; Blattodea\_Autest.iqtree: IQTree output files
- Blattodea\_AUtest\_information\_on\_tested\_trees.txt: additional information corresponding to the log file and AUtest result

#### **S1.4\_Anallacta**

This subdirectory includes the alignment (nucleotide level, FASTA format) of cytochrome oxidase I (COI) sequences of i) a cultured specimen of *Anallacta* sp. (SANGER sequences) and ii) extracted from transcriptome used in this study, see also Supplementary text S1).

### S1.5\_Dating

This subdirectory includes files and subdirectories related to divergence time analyses (plain text files).

- *Blattodea\_calibrations.tre*: tree with minimum and maximum prior age calibrations (NEWICK format as required for MCMCTree), see Figure S5 and Supplementary material S2.
- *Blattodea\_mcmctree.cti*: MCMCTree control file (example filenames) for the main analyses after generation of the Hessian matrix. Note that for all runs parameters were kept identical.
- *Blattodea\_supermatrix\_aa\_reduced\_95.fas*: reduced amino-acid dataset. See Supplementary texts S1. For resulting divergence dates see Figure 1.
- *Blattodea\_priorOnly\_noData\_1run.tre*: tree inferred from the analysis utilizing priors only used to check if priors were conflicting and to map effective and specified priors in relation to each other. For further details see Supplementary text S1 and Figure S6.
- *Blattodea\_FigTree\_full\_aa\_run3.tre*: Chronogram inferred from unreduced amino-acid dataset (replicate run 3, chosen arbitrarily among 4 replicates, for details see Supplementary text S1).
- *Blattodea\_FigTree\_95\_aa\_run1.tre*: Chronogram inferred from the reduced amino-acid dataset (replicate run 3, chosen arbitrarily among 4 replicates, for details see Supplementary text S1).
- *Blattodea\_FigTree\_95\_aa\_run1\_altCalibrations.tre*: Chronogram inferred from the reduced amino-acid dataset with alternative prior calibrations (for details see Supplementary text S1 and Table S9).
- Subdirectory “dated\_trees\_unreduced”: Chronograms (NEWICK format) inferred from the unreduced amino-acid dataset, 4 replicates; the chronogram from run 3 was arbitrarily chosen as the representative for dates reported in our stud (also see Supplementary text S1 and Table S9).  
Files: *FigTree\_full\_run1.tre*; *FigTree\_full\_run2.tre*; *FigTree\_full\_run3.tre*; *FigTree\_full\_run4.tre*
- Subdirectory “dated\_trees\_reduced”: Chronograms (NEWICK format) inferred from the reduced amino-acid dataset, 4 replicates; the chronogram from run 1 was arbitrarily chosen as the representative for dates reported in our study (also see Supplementary text S1 and Table S9).  
Files: *FigTree\_95\_run1.tre*; *FigTee\_95\_run2.tre*; *FigTree\_95\_run3.tre*; *FigTree\_95\_run4.tre*

### S1.6\_Blattabacterium

This subdirectory includes a spreadsheet with the number of transcripts identified as *Blattabacterium* for each taxon (sheet 1) and the sequence name and organismal identity of each candidate transcript (sheet 2), details are provided in Supplementary text S1.

## Supplementary Archive 2.

This archive refers to analyses as described in Supplementary text S3: Evolution, Morphology & Behavior.

[Supplementary\_Archive\_2.zip: 93.73 KB]

### Ancestral state reconstruction

The file “*Blattodea\_ancStates\_datamatrix.nex*” includes the input character matrix (NEXUS format) and output (Figure 3) of the ancestral state reconstructions for 19 morphological characters listed in the Supplementary text S3. Details on character states are provided in S3.3, see Table S11 for a more readable summary of the input matrix and Table S12 for the ancestral states of selected nodes only. This matrix was used as input for parsimony analysis with Mesquite.

File: *Blattodea\_ancStates\_datamatrix.nex*.

### Subdirectory “Stochastic character analysis”



This subdirectory includes supplementary files and results of the stochastic character mapping Bayesian ancestral state inference with Phytools.

- PhytoolsCode.R - R-script used to analyze the input data.
- 95datedTree.run1.tre - a copy of the divergence dated tree obtained with the reduced dataset.
- morpho\_data\_selected\_PriorProb\_version.csv - Input data matrix with prior probabilities for the two states of each character analyzed.
- **Character 15** - subdirectory with results of the analyses for character 15 (wing presence and absence).
  - **Info.txt** - Output from the Phytools (make.simmap) analysis, summary output , description of the color code used in the corresponding figure, and notes about the results.
  - **Wing presence and absence.png** – result of the Bayesian ancestral state reconstruction of wing presence and absence in Blattodea. Red coloration indicates posterior probability of wing presence; black coloration indicates posterior probability of wing absence.
- **Character 16** - subdirectory with results of the analyses for character 16 (wings dropped or retained).
  - Info.txt - Output from the Phytools (make.simmap) analysis, summary output, description of the color code used in the corresponding figure, and notes about the results.
  - Wing dropping.png – results of the Bayesian ancestral state reconstruction of wing dropping and retention in Blattodea. Red coloration indicates posterior probability of wing dropping; black coloration indicates posterior probability of wing retention.
- **Character 17** - subdirectory with results of the analyses for character 17 (hindwing folding simple or fanlike).
  - Info.txt - Output from the Phytools (make.simmap) analysis, summary output from a summary of that analysis, a description of the color code used in the corresponding figure, and notes about the results.
  - Hindwing folding.png - A Bayesian ancestral state reconstruction of hindwing folding in Blattodea. Red coloration indicates posterior probability of a non-fan-like fold; black coloration indicates posterior probability of fan-like fold.
- **Character 18** - subdirectory with results of the analyses for character 18 (forewing tegminization).
  - Info.txt - Output from the Phytools (make.simmap) analysis, summary output, a description of the color code used in the corresponding figure, and a notes about the results.
  - Tegminization.png - A Bayesian ancestral state reconstruction of hindwing folding in Blattodea. Red coloration indicates posterior probability of a tegminized forewings; black coloration indicates posterior probability of membranous forewings.