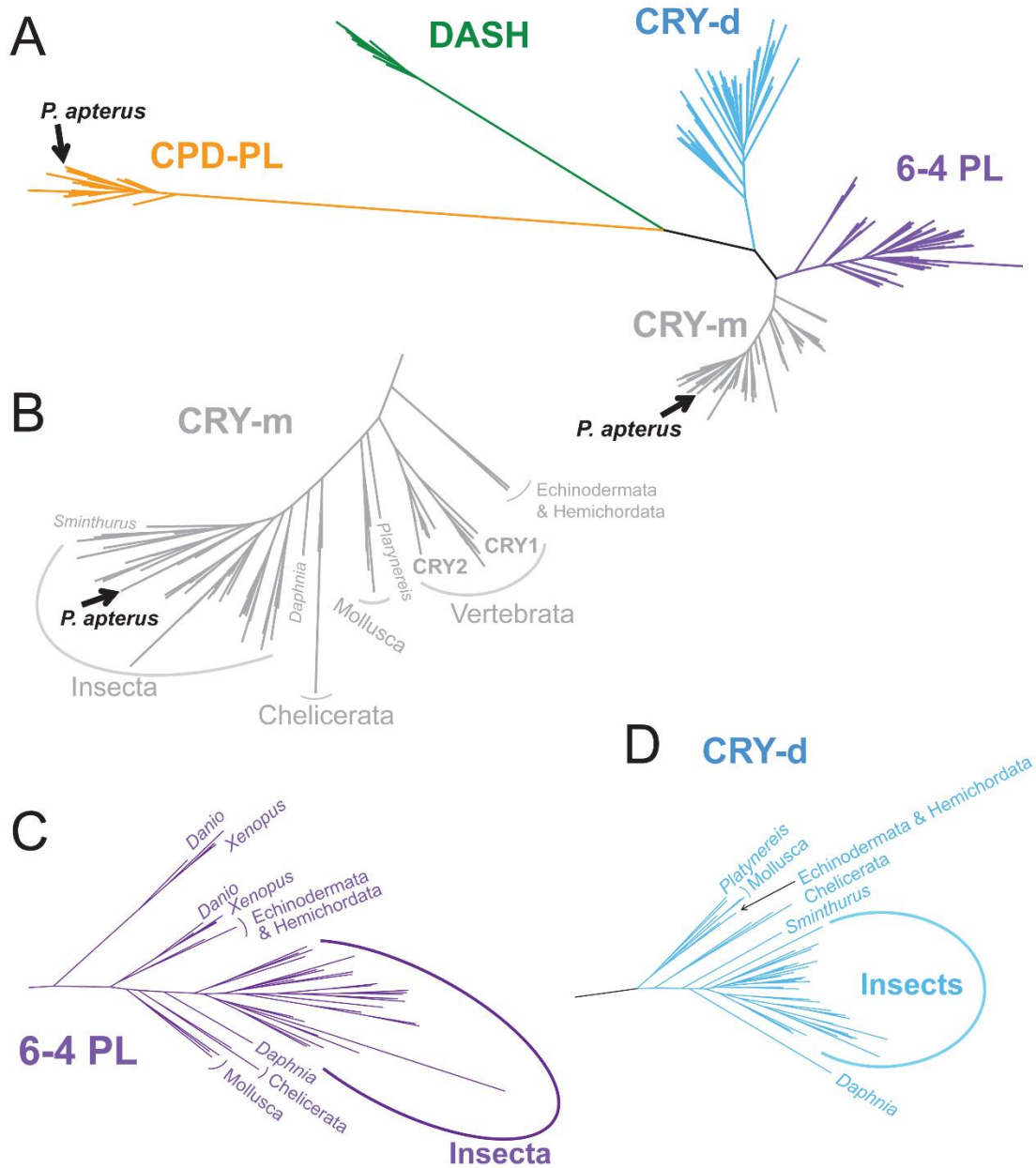
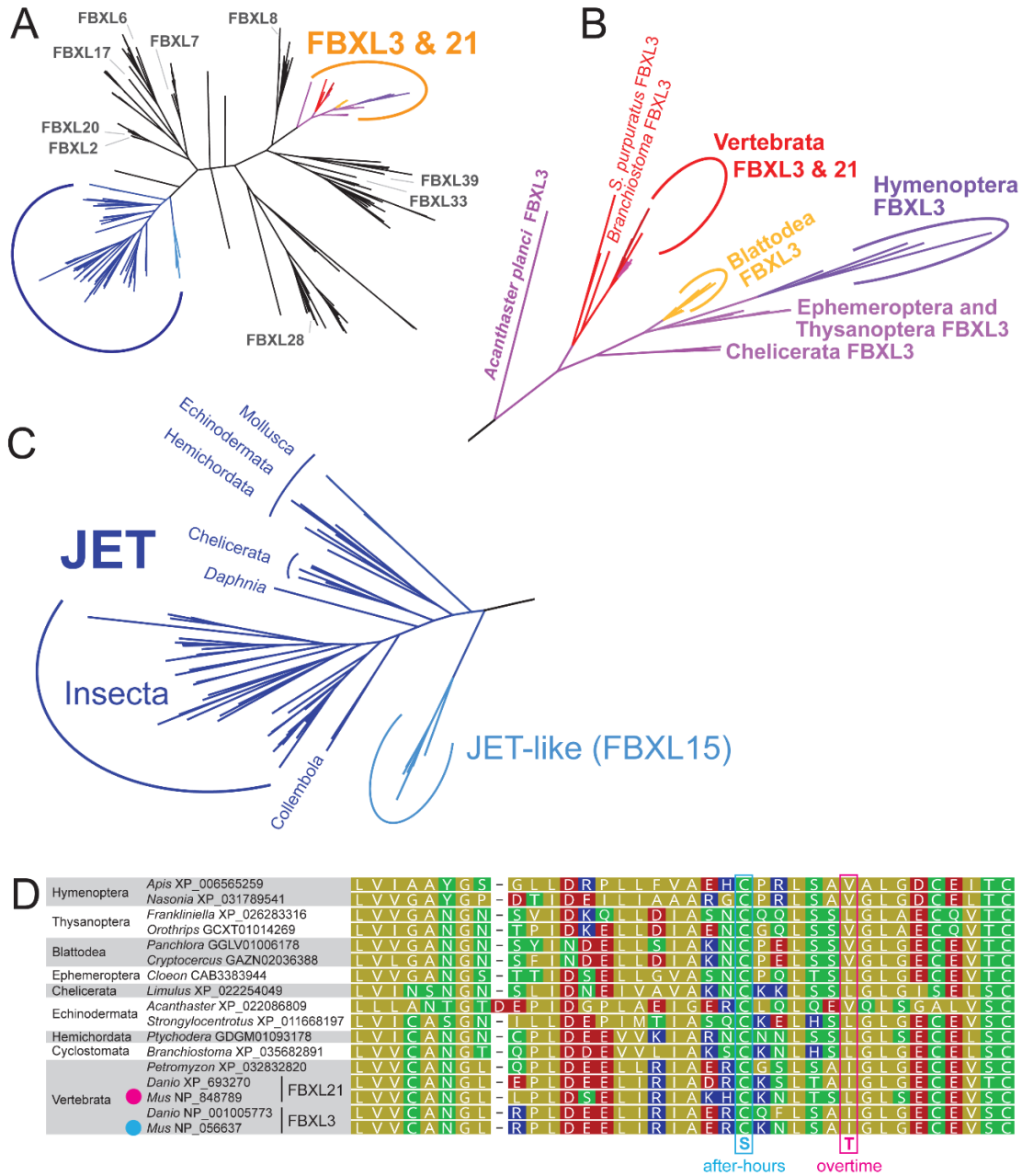


Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
 SUPPLEMENTARY MATERIAL ONLINE



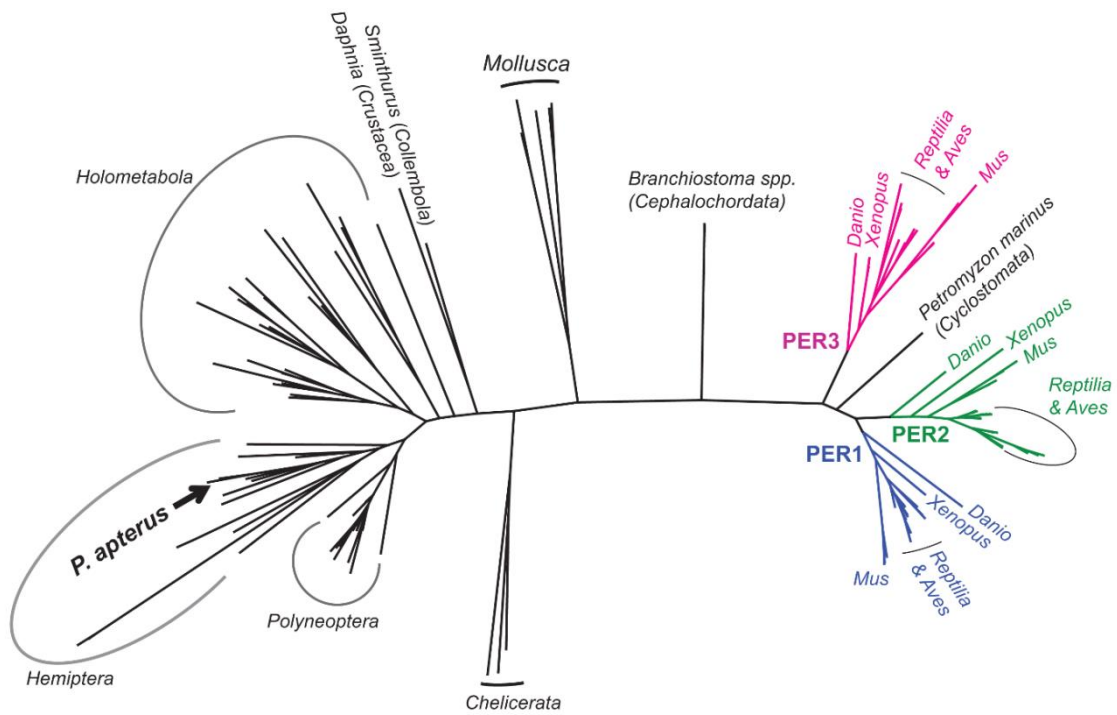
**Supplementary Figure 1. Phylogeny of Metazoan CRYPTOCHROMES.** (A) Unrooted tree of 210 Metazoan protein sequences (RAxML, GAMMA-based Likelihood: log -118860.635040) illustrates that CRYs cluster to five distinct groups: CYCLOBUTANE PYRIMIDINE DIMER PHOTOLYASE (CPD-PL, orange), DASH (*Drosophila*, *Arabidopsis*, *Synechocystis*, *Human*)-type cryptochrome (green), mammalian and *Drosophila*-type of cryptochrome (CRY-m, grey; CRY-d, blue), and 6-4 DNA photolyase (6-4 PL, deep purple). (B-D) Depict details of particular tree regions with representative taxa. Positions of *P. apterus* CRYs are highlighted.

SUPPLEMENTARY MATERIAL ONLINE



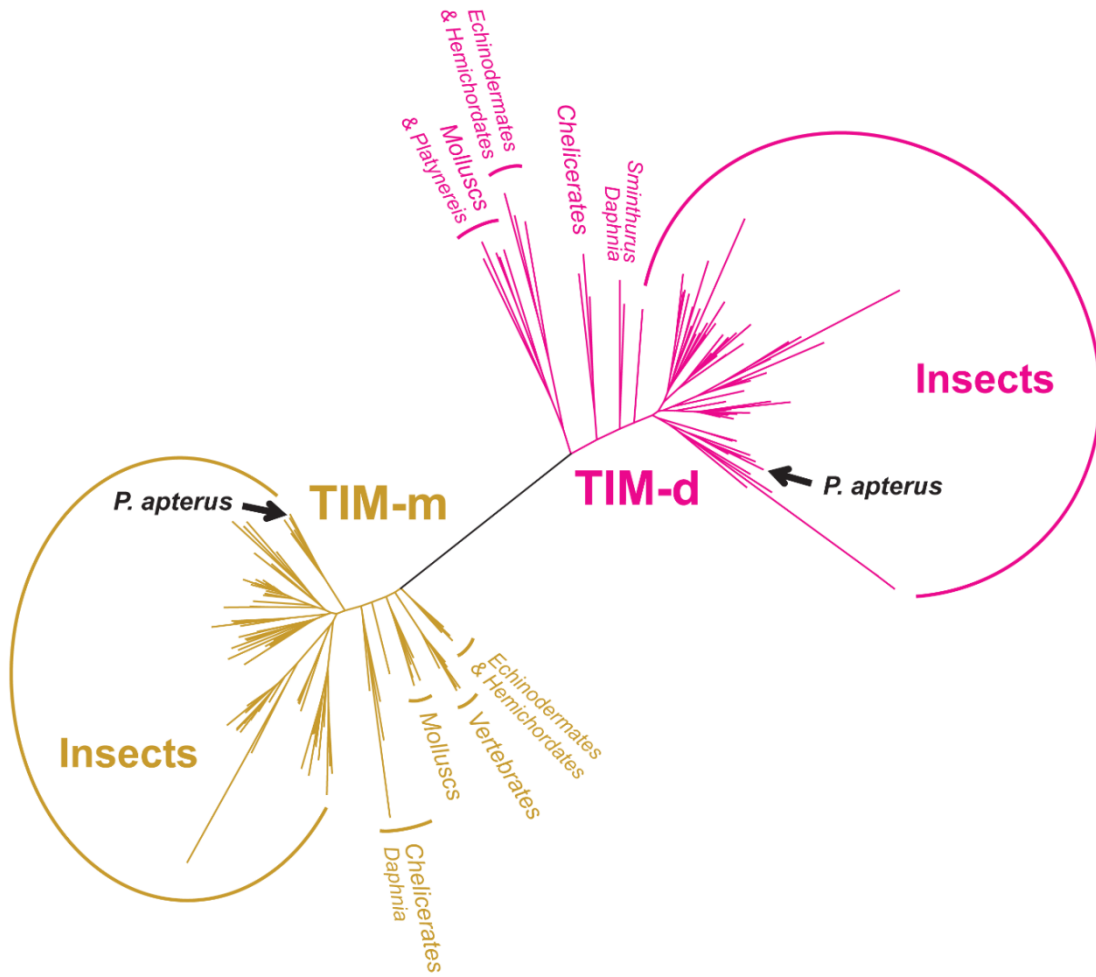
**Supplementary Figure 2. Phylogeny of Metazoan JETLAG and FBXL3/21 proteins.** (A) The unrooted tree containing representative metazoan FBXL proteins (mouse proteins are by grey names) with a detailed dataset of JETLAG (JET) and FBXL21/3 sequences (242 proteins in total, RAxML, GAMMA-based Likelihood: -108138.373258). (B) Detail of the tree depicting phylogeny of FBXL3 / FBXL21. (C) Phylogeny of JETLAG homologs and its related JET-like (FBXL15). (D) Alignment of FBXL3 and FBXL21, two vertebrate paralogs represented by the zebrafish (*Danio rerio*) and mouse (*Mus musculus*), compared to the ancestral sequence from *Petromyzon* and to FBXL3 representing major metazoan groups in which the protein was identified. The blue and red rectangles highlight the positions that are substituted in *after-hours* in the mouse FBXL3 (31), highlighted by the light blue full circle, and *overtime* mutant in the mouse FBXL21 (13), highlighted by magenta full circle, respectively.

Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
SUPPLEMENTARY MATERIAL ONLINE



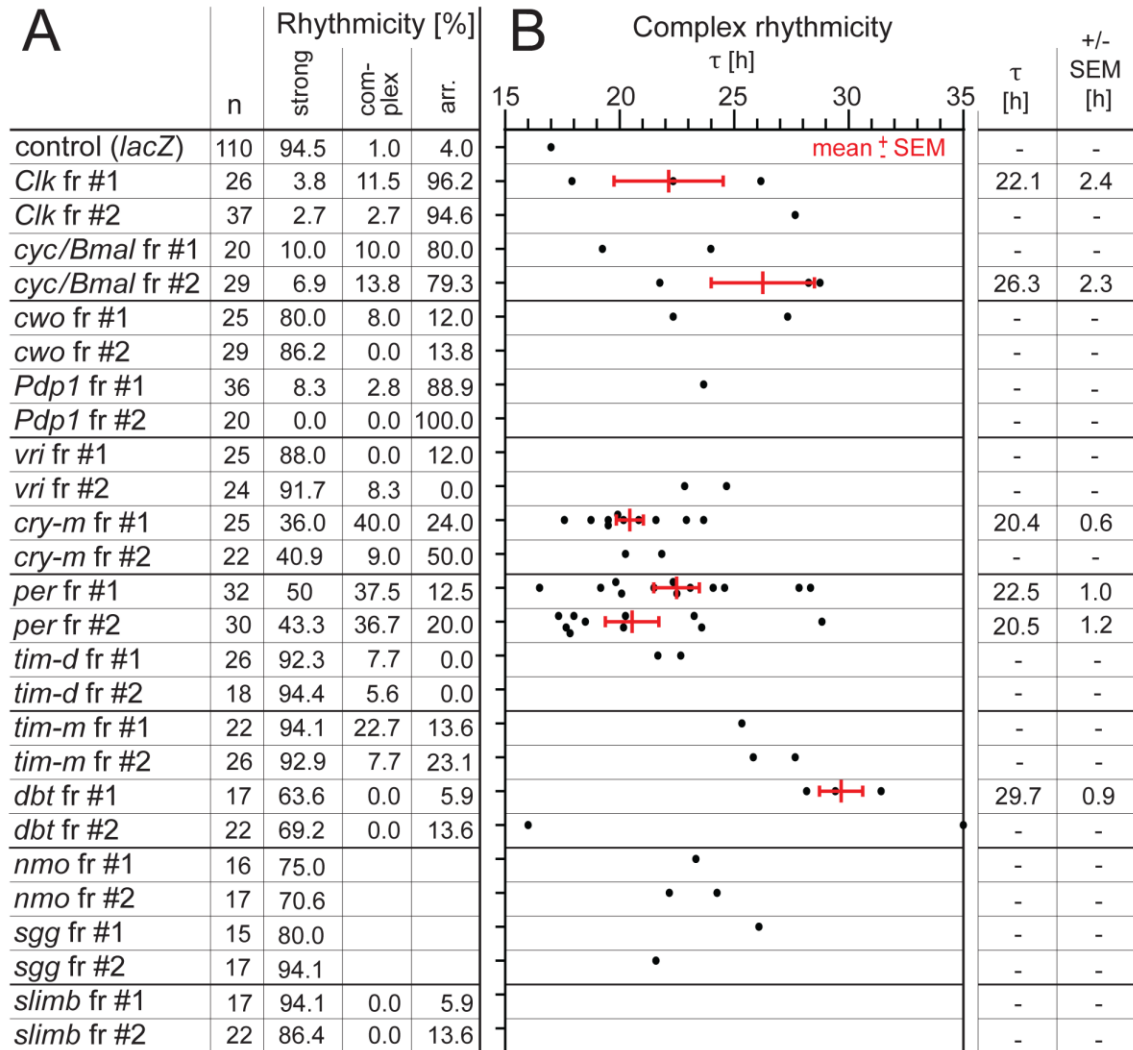
**Supplementary Figure 3. Phylogeny of Metazoan PERIOD proteins (PER).** The unrooted tree containing representative Metazoan PER proteins (133 proteins in total, RAxML, GAMMA-based Likelihood: -185735.690583).

Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
SUPPLEMENTARY MATERIAL ONLINE



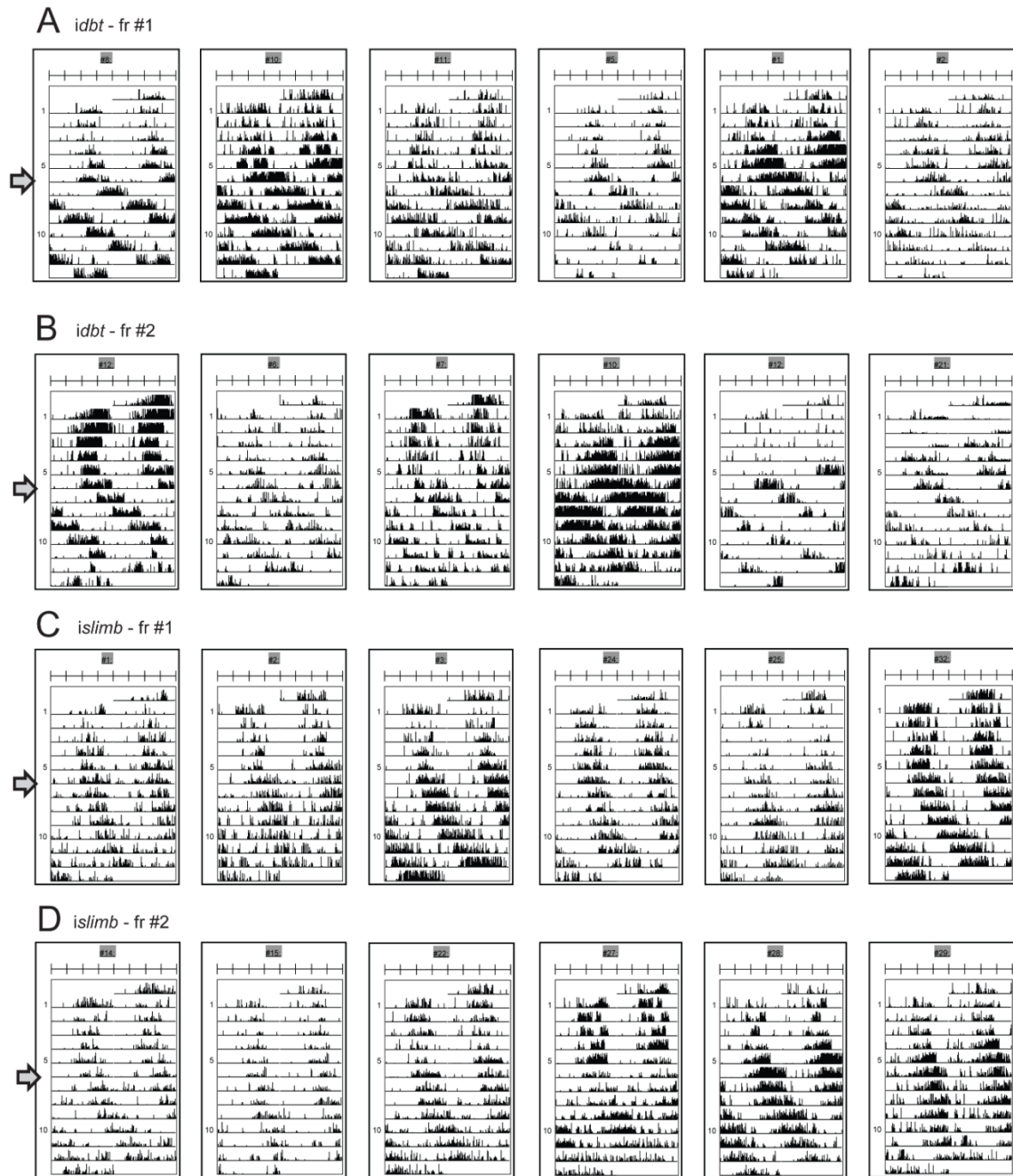
**Supplementary Figure 4. Phylogeny of TIMELESS proteins indicates clear and unambiguous separation of TIM-d and TIM-m clusters.** The tree was constructed from the protein alignment of 167 metazoan sequences using RAxML (GAMMA-based Likelihood: -245239.706545). The mammalian-type of TIM (TIM-m, in gold) was found in all representatives of Metazoan species (see table S1 for detail list), whereas the *Drosophila*-type TIM (TIM-d, in red) is present in the majority of insect species, Collembola (*Sminthurus*), Crustacea (*Daphnia*), Chelicerata, Mollusca, Hemichordata and Echinodermata. Positions of *P. apterus* TIM-m and TIM-d are highlighted by black arrows.

Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
 SUPPLEMENTARY MATERIAL ONLINE



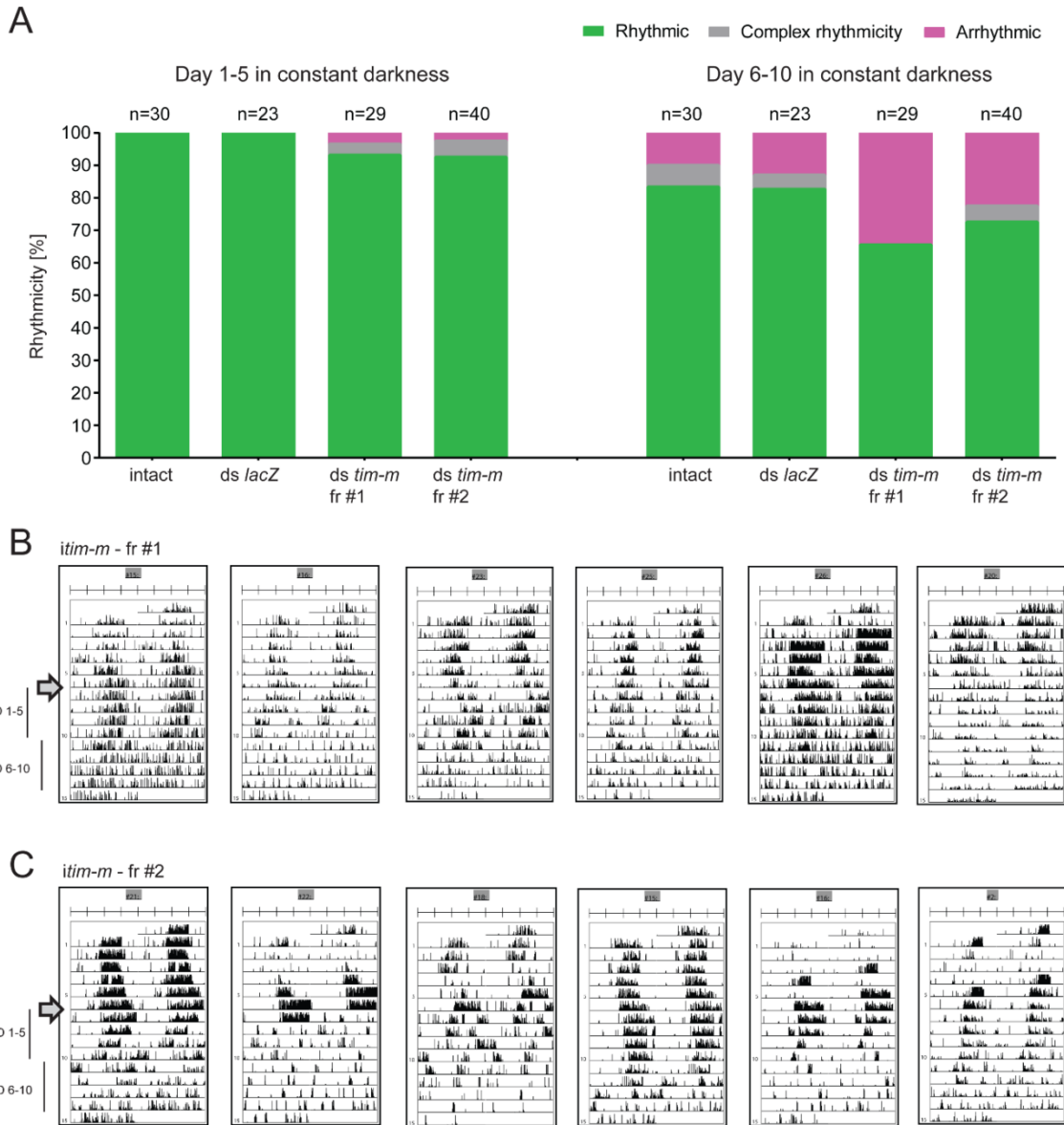
**Supplementary Figure 5. Free-running period of RNAi animals with complex rhythmicity.** (A) Summary of the gene silencing describing its impact on the behavioral rhythmicity shown as % of males demonstrating strong rhythmicity, complex rhythmicity, and arrhythmicity. fr #1 and fr #2 = non-overlapping dsRNA fragment 1 and 2, respectively. (B) Individual free-running period ( $\tau$ ) values are shown as a dot for each male with complex rhythmicity; red bars represent means  $\pm$  SEMs (calculated if >10 % individuals demonstrated rhythmicity). Columns depict the mean free-running period ( $\tau$ ), standard error of mean ( $\pm$  SEM).

## SUPPLEMENTARY MATERIAL ONLINE



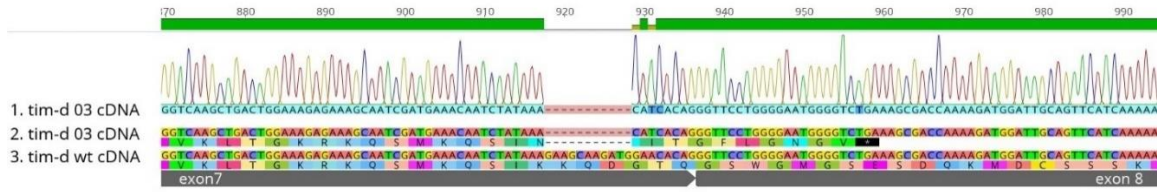
**Supplementary Figure 6.** *P. apterus* activity after *dbt* (*idbt*) and *slimb* (*islimb*) knock-down shown as double-plotted actograms. The activity of adult males was recorded at 25 °C and light-dark cycles for five days. Then, males were released to constant-dark conditions (indicated by grey arrow). Typical examples of: (A) *idbt* fr #1, (B) *idbt* fr #2, (C) *islimb* fr #1, (D) *islimb* fr #2 knock-down are shown. fr #1 and fr #2 refer to non-overlapping dsRNA fragment 1 and 2, respectively.

Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
 SUPPLEMENTARY MATERIAL ONLINE



**Supplementary Figure 7. *Timeless-m* silencing influences rhythmicity in *P. apterus*.** (A) Summary of the rhythmicity during first 5 days of constant darkness (left) and second half of constant darkness (all recorded at 25 °C). (B-C) Examples of double-plotted actograms of *tim-m* silenced males (*itim-m*). The activity of adult males was recorded at 25 °C and light-dark cycles for five days. Then, males were released to constant-dark conditions (indicated by grey arrow).

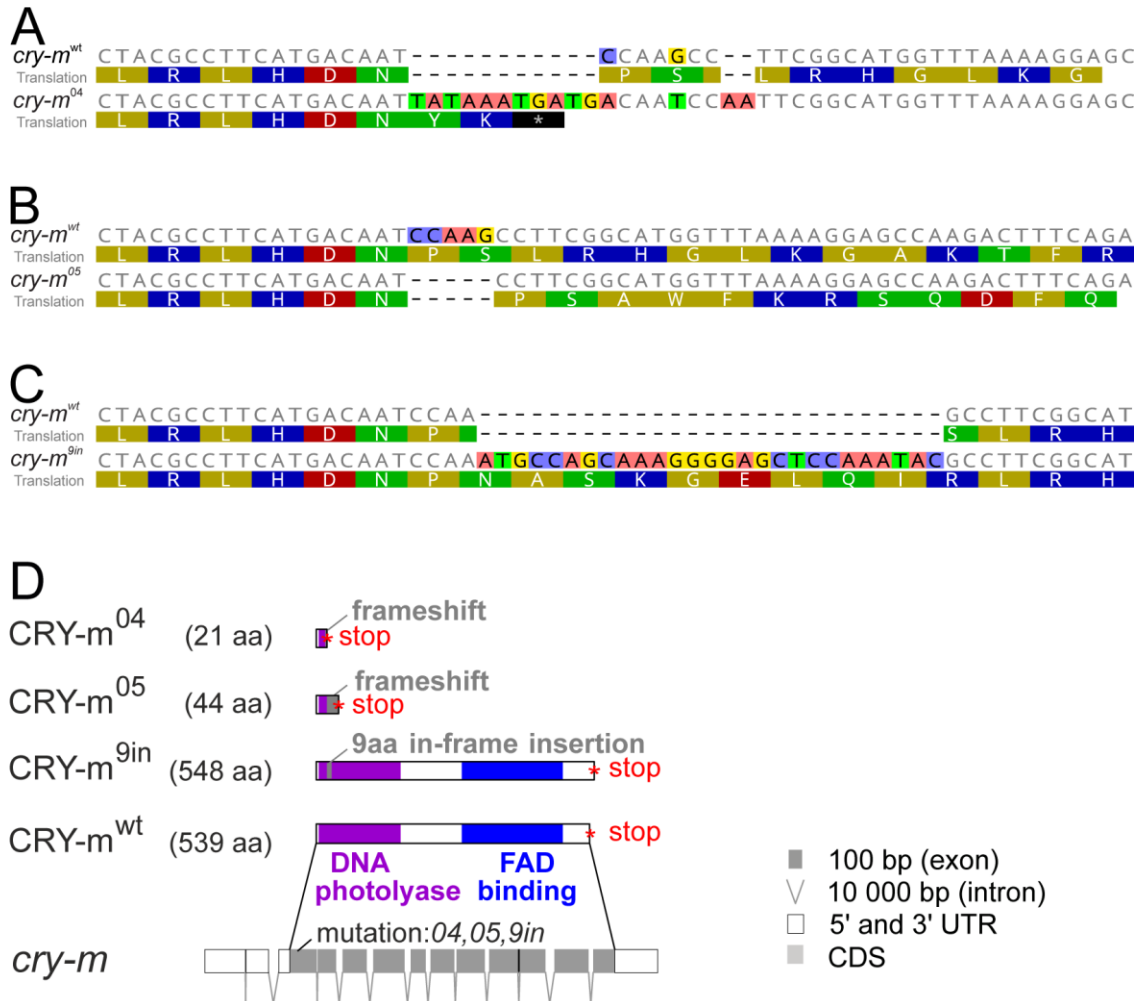
Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
 SUPPLEMENTARY MATERIAL ONLINE



**Supplementary Figure 8. Deletion in *tim-d*<sup>03</sup> does not influence splicing of exon 7 and 8.** To clarify if the 11bp deletion and two in-frame substitutions might influence *tim-d* splicing, total RNA was isolated from the brains of *tim-d*<sup>03</sup> homozygous mutant animals, mRNA reverse transcribed, and the corresponding region of *tim-d* transcript PCR amplified. The chromatogram obtained from direct sequencing of *tim-d*<sup>03</sup> PCR product is shown in the first line of the alignment. The third line corresponds to the cDNA of the wild-type control with exon position highlighted underneath. Protein sequences are shown below *tim-d*<sup>03</sup> and *tim-d*<sup>wt</sup>, respectively. The black box corresponds to the stop codon (coded by TGA).

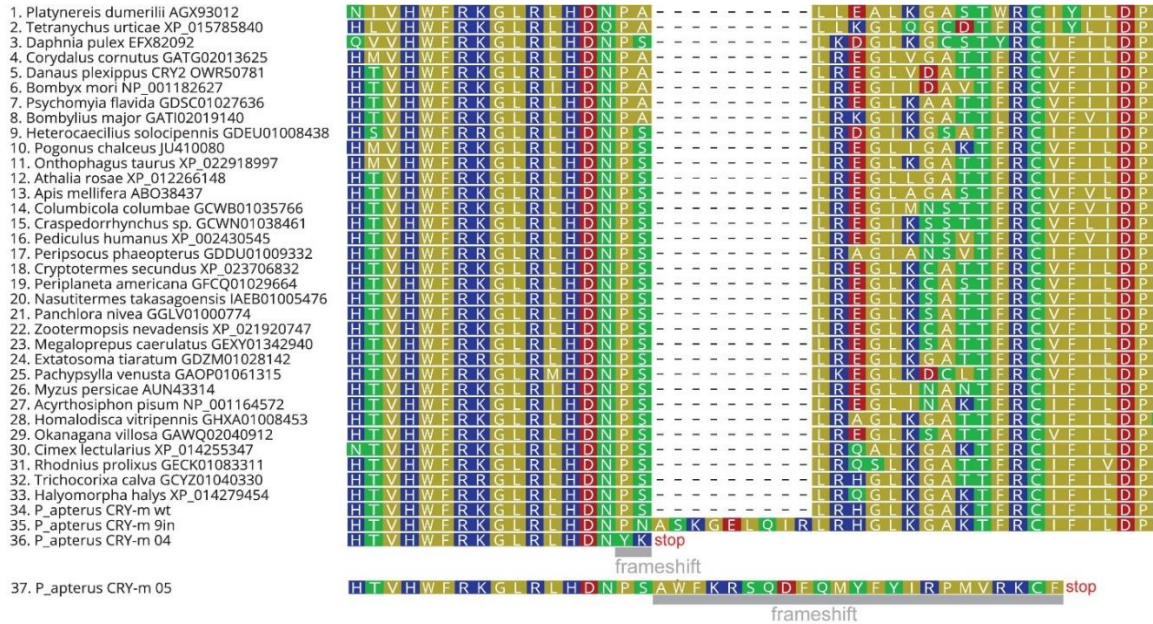


Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
 SUPPLEMENTARY MATERIAL ONLINE



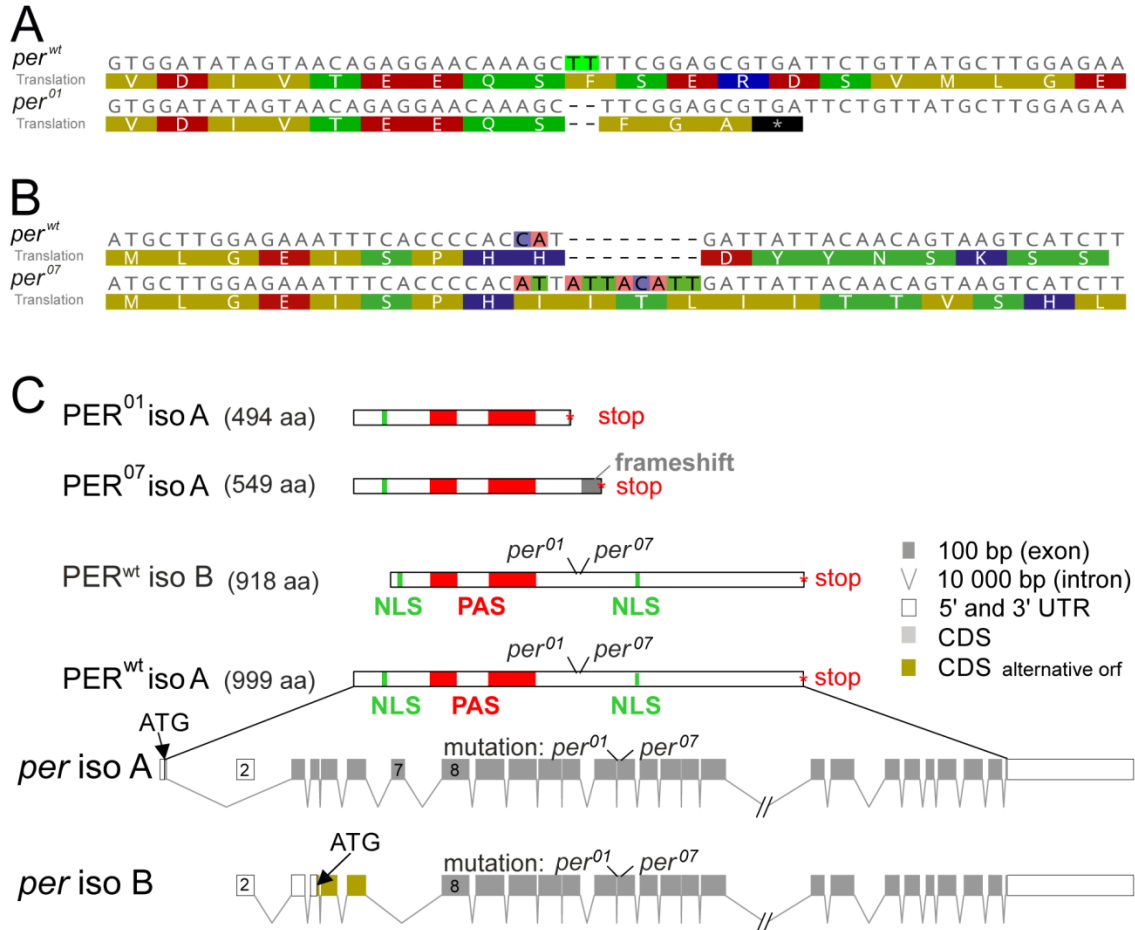
**Supplementary Figure 9. Overview of *P. apterus* cryptochrome-*m* engineered mutants.** (A) DNA insertion of a sequence in *cry-m<sup>04</sup>* mutant contains an in-frame stop codon. (B) Two-base pair deletion in *cry-m<sup>05</sup>* mutant results in a frameshift. The stop codon is downstream of the presented region. (C) 27-base pair in-frame insertion results in additional 9 amino acids in CRY-m<sup>9in</sup>. (D) *cryptochrome-m* gene model (bottom) with highlighted exons and introns (note different scale for exons and introns). Corresponding wild-type protein is shown above the gene model with the highlighted position of mutations, protein domains, and the C-terminus (stop). Predicted CRY-m<sup>9in</sup> mutant protein contains nine-amino acid insertion (see Supplementary Data Fig. 10 for protein alignment of this region with CRY proteins from different insect species). In contrast to *cry-m<sup>9in</sup>*, two other mutants, *cry-m<sup>04</sup>* and *cry-m<sup>05</sup>*, are *bona fide* loss-of-function mutants because the indels result in frameshifts leading to premature stop codons. Predicted protein sequences are shown at the top of the panel.

Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
 SUPPLEMENTARY MATERIAL ONLINE



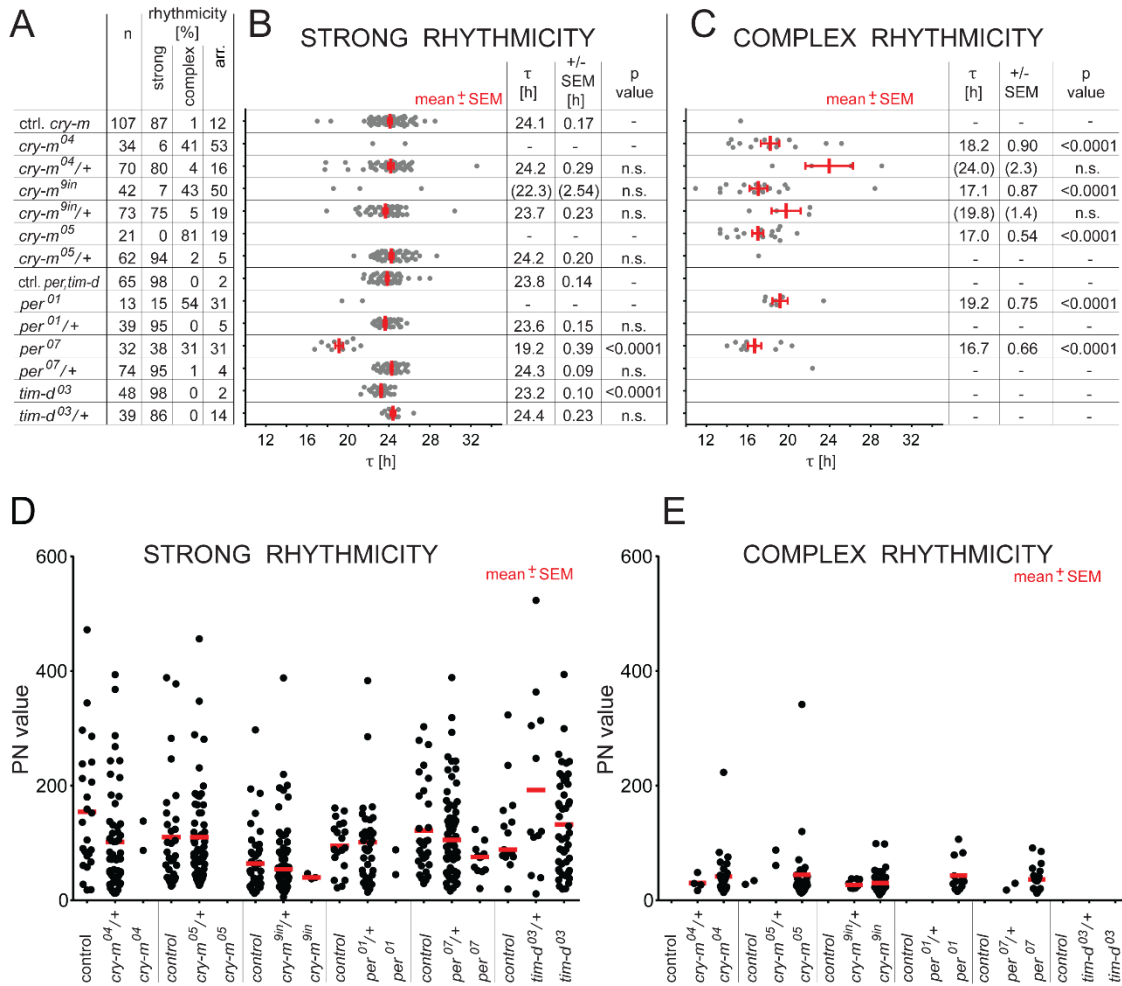
**Supplementary Figure 10. Cryptochrome-m engineered mutants target evolutionarily conserved region in CRY-m.** Representative CRY-m proteins were aligned with protein sequences of three *P. apterus* mutants. Two of them, CRY-m<sup>04</sup> and CRY-m<sup>05</sup> (No. 36 and No. 37, respectively), are terminated prematurely (indicated as a stop). The third mutant, CRY-m<sup>9in</sup> (No. 35), contains an in-frame insertion of nine amino acids into the evolutionarily conserved CRY-m region.

Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
 SUPPLEMENTARY MATERIAL ONLINE



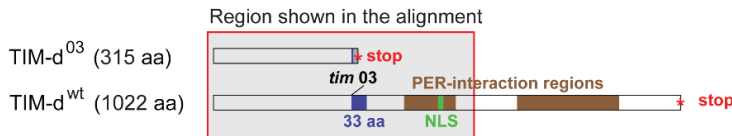
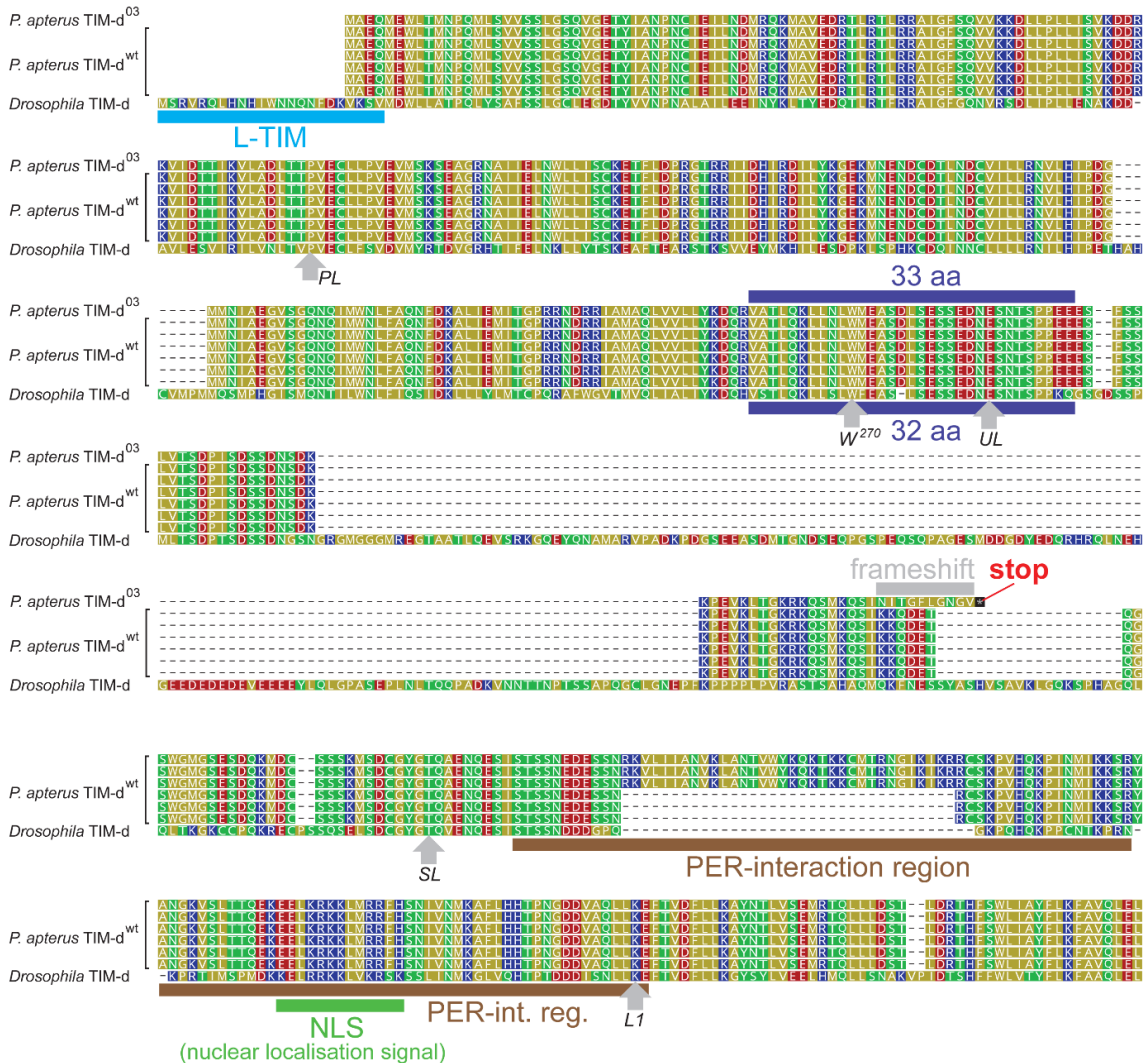
**Supplementary Figure 11. Overview of *P. apterus period* engineered mutants.** (A) A two-base pair DNA deletion in *per*<sup>01</sup> mutant results in a frameshift and stop codon (depicted as an asterisk in black background). (B) Eight-base pair deletion in *per*<sup>07</sup> mutant results in a frameshift. The stop codon is downstream of the presented region. (C) Two transcript isoforms differing by alternative transcription starts (ATG) and alternative retention of exon 7 are presented in *period* gene models (bottom) with highlighted exons and introns (note different scale for exons and introns). The open reading frames are identical for isoforms A and B from exon 8. Therefore, despite the different N terminal regions, mutations *per*<sup>01</sup> and *per*<sup>07</sup> identically shorten the resulting mutant proteins. Corresponding wild-type protein is shown above the gene model with the highlighted position of mutations, protein domains, and the C-terminus (stop). Predicted protein sequences are shown at the top of the panel.

Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
 SUPPLEMENTARY MATERIAL ONLINE



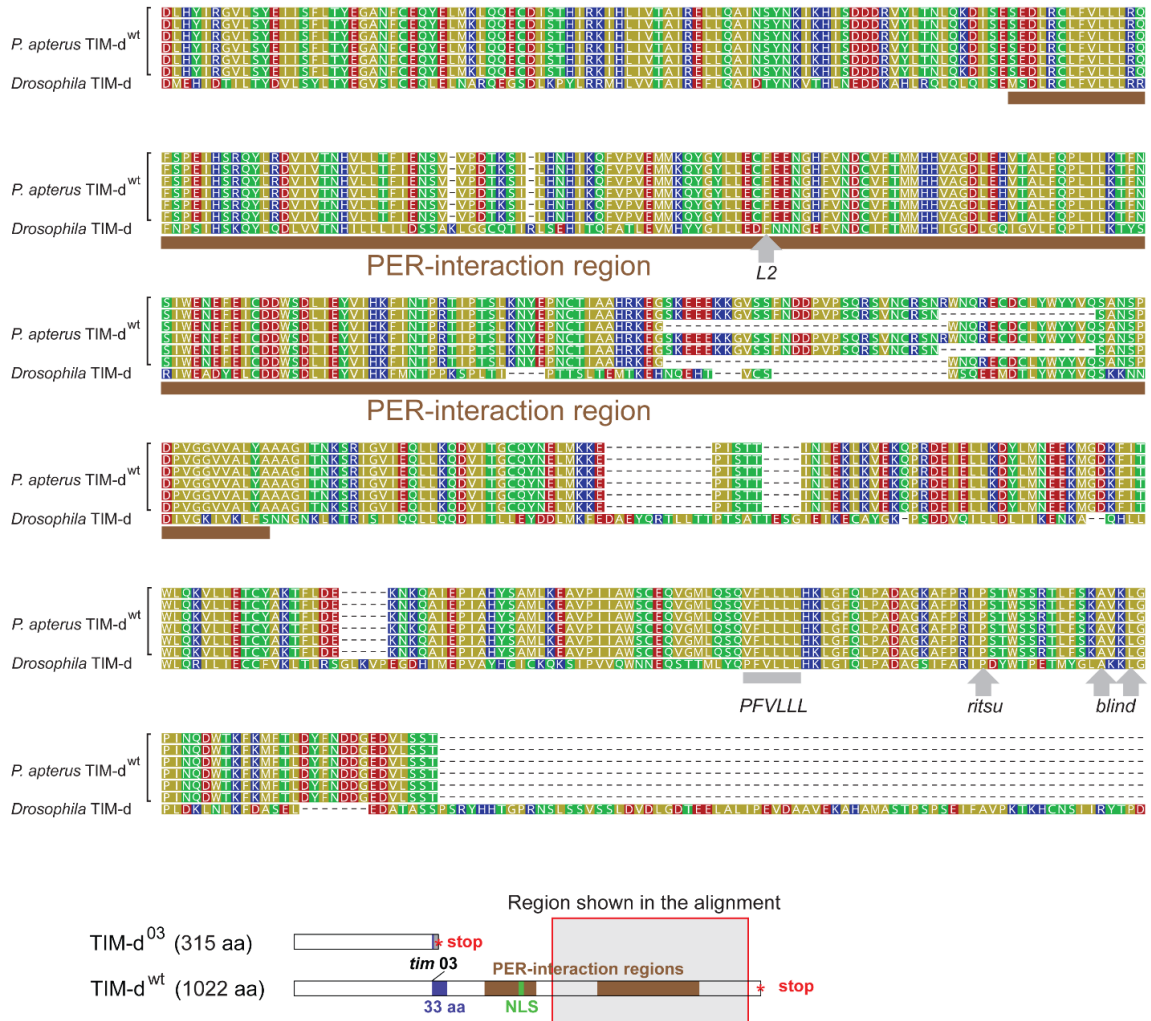
**Supplementary Figure 12. A detailed summary of the free-running period ( $\tau$ ) in the wild-type and genetic mutants.** (A) Summary indicating number and phenotypes of measured mutant and heterozygous animals compared to their corresponding wild-type control siblings (labelled as 'ctrl. *cry-m*' and 'ctrl. *per*, *tim-d*', respectively). The statistical difference from the controls is shown as a p-value. (B) Individual  $\tau$  values are plotted as a dot for each male, red bars depict means  $\pm$  SEMs (values shown in parenthesis if <10 % of individuals were rhythmic). (C) Individual  $\tau$  values plotted for males that showed either multiple periodic components or the  $\tau$  changed during the recording. (D) The strength of the rhythmicity in the 'strong rhythmicity' group is shown as the PN value and red bars depict mean. (E) The strength of the rhythmicity in the 'complex rhythmicity' group is shown as the PN value. Wild-type control siblings are plotted for each genotype separately in panels A and E).

SUPPLEMENTARY MATERIAL ONLINE



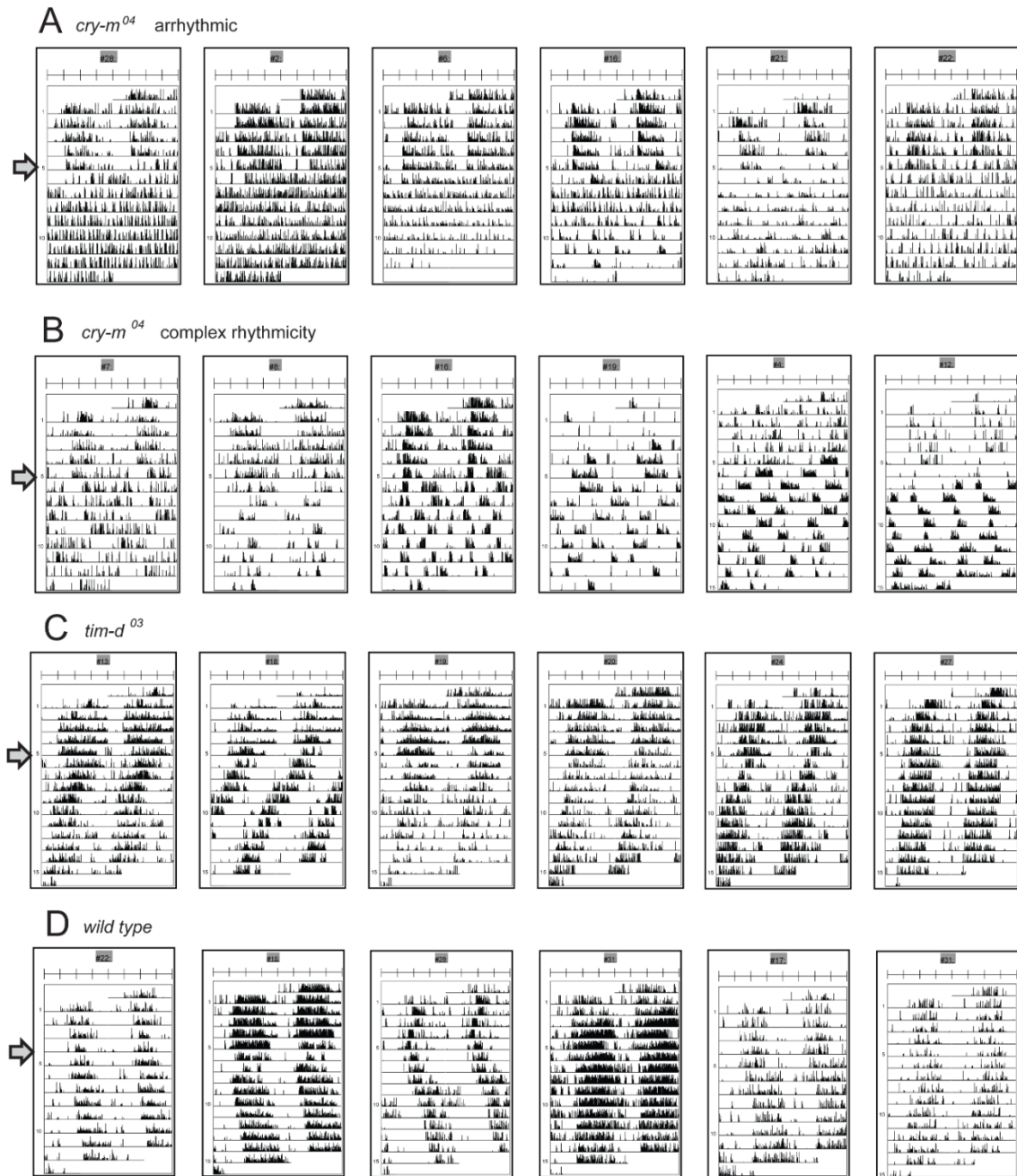
**Supplementary Figure 13. TIM-d domains and protein alignment, part A.** *P. apterus* TIM-d<sup>03</sup> mutant protein sequence and six *P. apterus* wt TIM-d isoforms were aligned to *Drosophila* TIM-d (NP\_001334730.1). Dashes (-) indicate gaps in the alignment, amino acids are color-coded according to their biochemical properties, and asterisks correspond to positions of a stop codons, which in mutant results in a premature termination of the TIM-d<sup>03</sup> protein. Major functional domains were highlighted under *Drosophila* TIM-d together with important residues (indicated by grey arrows), whose mutations in *Drosophila* produce altered  $\tau$  (33-36). L-TIM indicates N-terminal protein extension resulting from alternative translation start in *Is-tim* allele (37, 38). All major functional domains and key residues of TIM-d seem to be conserved in *P. apterus*.

Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
 SUPPLEMENTARY MATERIAL ONLINE



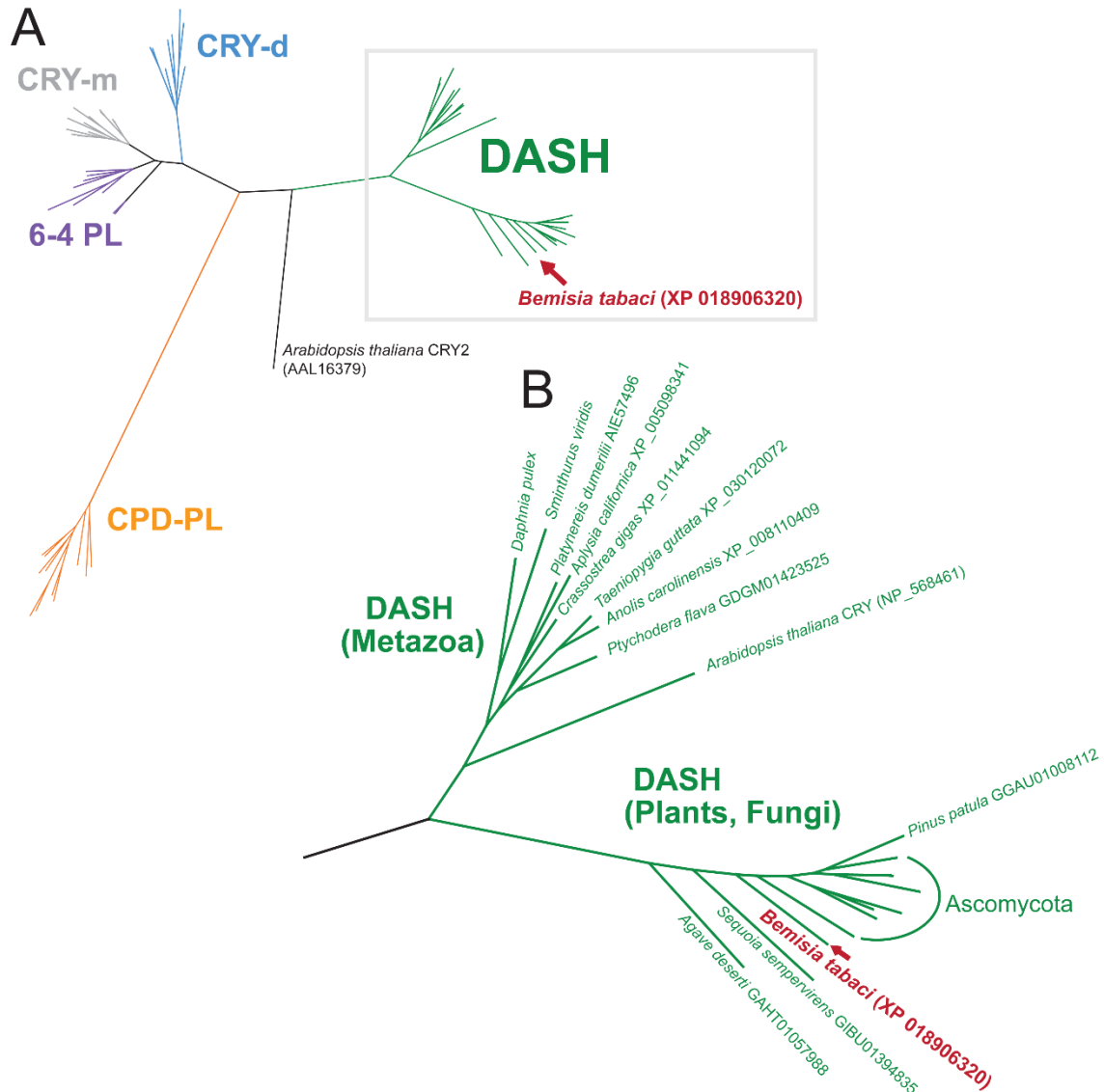
**Supplementary Figure 14. TIM-d domains and protein alignment, part B.** The second part of the protein alignment depicting six *P. apterus* wt TIM-d isoforms aligned to *Drosophila* TIM-d (NP\_001334730.1). Dashes (-) indicate gaps in the alignment, and amino acids are color-coded according to their biochemical properties. Major functional domains were highlighted under *Drosophila* TIM-d together with important residues (indicated by grey arrows), whose mutations in *Drosophila* produce altered  $\tau$  (33, 36, 39, 40). All major functional domains and key residues of TIM-d seem to be conserved in *P. apterus*.

## SUPPLEMENTARY MATERIAL ONLINE



**Supplementary Figure 15. Examples of *P. apterus* locomotor activity shown as double-plotted actogram.** The activity of adult males was recorded at 25 °C and light-dark cycles for five days. Then, males were released to constant-dark conditions (indicated by grey arrow). (A) Typical examples of arrhythmic *cry-m<sup>04</sup>* homozygotes, (B) *cry-m<sup>04</sup>* homozygotes with multiple periodic components, or the  $\tau$  changed during the recording, (C) *tim-d<sup>03</sup>* homozygotes, and (D) wild-type males are shown.

Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
 SUPPLEMENTARY MATERIAL ONLINE



**Supplementary Figure 16. Available *Bemisia tabaci* DASH-CRY sequence is highly suspicious.** (A) Unrooted tree containing representative Metazoan CRYS, two *Arabidopsis thaliana* CRY proteins, *Bemisia tabaci* XP\_018906320, and CRY proteins from plants and Ascomycota (Fungi) (66 protein sequences in total, RAxML, GAMMA-based Likelihood: -49344.467111). *B. tabaci* branches within DASH group, however, (B) Detailed inspection points to its clear relatedness to plant and fungal CRYS. Although horizontal gene transfer from plant or fungi to *Bemisia* cannot be excluded, the cross-contamination seems to be the most parsimonious explanation for the origin of the sequence.



## Supplementary Materials and Methods

### Animal model

Linden bugs, *Pyrrhocoris apterus*, were reared at  $25 \pm 0.5$  °C under diapause-preventing long photoperiod of 18 h light and 6 h dark with access to linden seeds (*Tilia cordata*) and water *ad libitum*. The majority of experiments (RNA interference, CRISPR/Cas9 gene-editing of *per* and *tim-d* genes) were performed on Roana strain, whereas Oldrichovec strain was used for *cry-m* gene editing. See (1) for details of the strain's origin.

### Circadian clock gene discovery in *Pyrrhocoris apterus*

Circadian clock gene homologs were identified in *P. apterus* in-house Illumina-based brain transcriptomes using the BLAST algorithm in the Geneious Prime 21.0.3 program (Biomatters, New Zealand, <https://www.geneious.com/>). As queries, protein sequences of circadian clock genes from *Drosophila melanogaster* and mouse were used. The full-length sequences were confirmed, and alternatively-spliced isoforms were identified in full-cDNA sequences obtained by Oxford Nanopore Technology (Oxford Nanopore, Oxford, UK) from male and female brains. These curated transcriptomic data were further compared to the in-house *P. apterus* genome draft to define exons and introns. Sequences used in this study were uploaded to GenBank (see Table S3 for accession numbers). The entire *P. apterus* genome and transcriptome will be published elsewhere. Putative functional domains in PER, TIM-d, and CRY-m were predicted on the homology to already established domains in *D. melanogaster* and mouse orthologs.

Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
SUPPLEMENTARY MATERIAL ONLINE

RNA interference (RNAi)

RNAi was performed as in (2-5). Briefly, two non-overlapping fragments located within the open reading frame of each gene were amplified using PCR (see Table S4 for the exact position of each fragment), cloned into pGEM-T Easy (Promega), and inserts were verified by Sanger sequencing. Templates for dsRNA *in-vitro* synthesis were prepared from pGEM-T Easy clones by PCR using M13 forward and pGEM-RNAi reverse 5'-TAATACGACTCACTATAGGGGACACTATAGAATACT-3' primer replacing SP6 to T7 promoter. Double-stranded RNA was synthesized using MEGAscript T7 transcription kit (Ambion/ThermoFisher) following the manufacturer's protocol. As a negative control, 178 bp long *beta-galactosidase (lacZ)* dsRNA was used. Adult males received 2 µl of dsRNA at a concentration of 4 mg/ml in Ringer's solution.

CRISPR/Cas9 gene editing

Details of *P. apterus* gene editing are described in (6), here is just a brief overview: 0-12 h after egg laying embryos were injected with gRNA/Cas9 mix. Early experiments (*cry-m* editing) were performed with Cas9 mRNA, whereas the later experiments (*per* and *tim-d* editing) relied on CAS9 protein (CP01 from PNA Bio). After 8-9 days, hatched larvae were transferred to a Petri dish supplied with water and linden seeds and allowed to grow until adulthood (G0 adults). Adults were mated to wild-type and, after first batches of eggs were laid, their gonads were dissected. PCR heteroduplex mobility assay was used to assess levels of mosaicism in gonads, and only the offspring from animals with the highest level of mosaicism were kept. In the next generation, individuals with successfully modified genes were identified from antennal-squish PCR.

Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
SUPPLEMENTARY MATERIAL ONLINE

Seven to nine generations of backcrosses to wt strain (~one year of backcrossing) were used to outcross possible off-target modifications. To further dissect the impact of the engineered mutation from an off-target effect and the bottleneck effect of these backcrosses, the following protocol was applied: heterozygotes were crossed together, and their adult male progeny (which consisted of the mixture of wt, heterozygotes, and homozygotes) were used to perform locomotor activity run. After the run ended and analyses of the behavior were performed, individuals were genotyped by PCR.

Locomotor activity recording and analysis

In all experiments, adult males were used for locomotor activity analysis. Bugs were individually housed in the test tubes (2.5 cm diameter, 15 cm in length) supplemented *ad libitum* with dry linden seeds and water and placed in the Locomotor Activity Monitors (LAM 25, TriKinetics Inc., Waltham, MA, USA). All activity measurements were performed in the Cooled Incubator MIR-154 (Sanyo/Panasonic, Japan) equipped with a built-in electronic timer, where bugs were synchronized for 5 days in LD conditions (18 h light, 6 h darkness) at 25 °C, followed by at least 10 days in constant darkness (DD) at 25 °C. The locomotor activity of *P. apterus* was recorded automatically in 5-min bins.

Males injected with dsRNA and their controls (intact males and males injected with *lacZ* dsRNA) were immediately placed in the Locomotor Activity Monitors. To evaluate the impact of CRISPR/Cas9-induced mutations, 2-5 day after adult ecdysis male siblings (wt, heterozygotes, and homozygotes; see the 'CRISPR/Cas9 gene editing' section for details) were run in parallel.

Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
SUPPLEMENTARY MATERIAL ONLINE

To determine rhythmicity and  $\tau$  in the constant darkness, the Lomb-Scargle periodogram in ActogramJ plugin of ImageJ (7) was employed to analyze activity from 10 consecutive days in DD. In the case of *tim-m* knockdowns, we also analyzed days 1-5 and days 6-10 separately. All actograms were further inspected by an independent investigator, who was not aware of the genotype. To describe *P. apterus* behavior in DD, three categories were defined following the description in (1): 1) strong rhythm, if the PN value of the periodogram (significance level of the periodogram) crossed significance threshold and actogram passed visual inspection test; 2) complex rhythm, if multiple periodic components were identified, or if the  $\tau$  changed during the recording; 3) arrhythmic, if the PN value did not reach significance threshold or actogram did not pass visual inspection test.

The statistical analysis of the differences in  $\tau$  was performed using Kruskal–Wallis test with Dunn’s post-hoc test using Prism 7 (GraphPad Software, La Jolla, CA, USA). RNAi males were compared to corresponding controls, intact and *ds/lacZ*, whereas homozygous mutants were compared to their heterozygous and wt siblings (see the CRISPR/Cas9 gene-editing section for explanation).

#### Phylogenetic analyses

A systematic search for circadian clock genes in Metazoa was performed in GenBank (NCBI) protein and genomic databases, and in transcriptome shotgun assemblies (TSA) using BLAST-P and tBLASTn algorithms, respectively, with taxon limits for searches in particular lineages at the level of orders, suborders, infraorders, and species. In some cases, the genome or whole-genome shotgun contigs (wgs) were explored. As queries, protein sequences of circadian clock genes from *D. melanogaster*,

Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
SUPPLEMENTARY MATERIAL ONLINE

mouse, and *P. apterus* were used. To identify precisely the type of CRY (-m, -d, 6-4 photolyase, CPD photolyase, or DASH) (8-11), TIM (-d or -m) (12), and PER (PER1, PER2, or PER3), protein sequences were aligned using MAFFT algorithm (13) in Geneious Prime 21.0.3 (Biomatters, New Zealand), unambiguously aligned regions were trimmed and phylogenetic analysis was performed using RAxML version 8.2.11 (14) in Geneious Prime 21.0.3 (Biomatters, New Zealand). Final figure illustration additions, such as the description of taxa, were performed in Illustrator CS5 (Adobe).

The Metazoan phylogeny shown in Fig. 2 is a consensus of recent molecular phylogenomic studies; namely those focused on insects (15), chelicerates, and arthropods (16), mollusks (17), vertebrates (18), protostomian/deuterostomian split (19). Within the richest group, insects, phylogenomic studies focused on Polyneoptera (20), Blattodea (21), hemipteroid assembly (22), Hymenoptera (23), Coleoptera (24), and Lepidoptera (25), were used as a reference for the corresponding part of the tree depicting the metazoan phylogeny.

## **Extended description of the Circadian Clock Gene Phylogeny**

### Phylogeny and evolution of circadian clock genes in Metazoa

The remarkable recent progress in Metazoan phylogenomics, the amount of TSA data, and a growing number of sequenced genomes allowed us to explore the circadian clock genes systematically across all major Metazoan groups. Here, we focus on lineage-specific gene losses which are well supported from multiple species, whole-genome assemblies, and deep sequencing of the entire transcriptomes. These losses are also highlighted in Fig. 2 of the study and summarized in Table S2.

Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
SUPPLEMENTARY MATERIAL ONLINE

PERIOD

PER protein-coding sequences were identified in all Metazoan species with the exception of one basal deuterostomian lineage consisting of Echinodermata and Hemichordata. The phylum Echinodermata, with starfish, sea urchins, and sea cucumbers, contains several species with well-sequenced genomes, including the purple sea urchin *Strongylocentrotus purpuratus* (26) and the crown-of-thorns starfish *Acanthaster planci* (27). The genome of hemichordates is also available (28). Given the reasonable quality of these genomes, and the solid amount of TSA data, the absence of *per* in those two phyla is the most parsimonious explanation. Gene multiplication was observed in all vertebrates leading to three *period* gene paralogs, albeit reduced to only two of them in some lineages.

TIMELESS

Two TIM paralogs, TIM-d (the *Drosophila*-type) and TIM-m (the mammalian-type) exist in Metazoa (12). The phylogenetic analysis undoubtedly separated them into two clusters (Fig. S3). TIM-m is present in all analyzed organisms consistently with its essential role in development (29, 30). TIM-d is present in both Deuterostomia and Protostomia, however, three apparent gene losses were identified in: (i) Chordata, a lineage containing the lancelet *Branchiostoma* (Cephalochordata), the lamprey *Petromyzon* (Cyclostomata), and vertebrates; (ii) termites, with the exception of one basal termite species *Porotermes*; and (iii) Hymenoptera, an order containing wasps, bees, ants, and bumblebees. In all three cases, the gene loss is supported by multiple well-assembled genomes.

Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
SUPPLEMENTARY MATERIAL ONLINE

JETLAG (JET)

JET, a protein responsible for degradation of TIM-d and CRY-d is reliably identifiable in Protostomia, where it was lost several times in (i) Cimicomorpha and Pentatomorpha, two large heteropteran groups, (ii) in aphids, (iii) Psocodea, a group containing lice, (iv) Hymenoptera, (v) Neuropterida, and (vi) crown Coleoptera, including the red flour beetle *Tribolium castaneum*. In Deuterostomia, we identified FBXL15 as proteins similar in sequence to JET and branching at the base of the protostomian JET cluster, thus, we refer to them as JET-like.

FBXL 3/21 proteins

FBXL3 and FBXL21 are two closely related CRY-m-interacting vertebrate paralogs (13, 31, 32) that result from genome duplication, which took place in the ancestor of all known vertebrates. Thus, the original protein-coding gene is described as *FBXL3/21*. *FBXL3/21* was lost in: (i) mollusks and annelids, (ii) hemipteroid assembly (Heteroptera, Auchenorrhyncha, Sternorrhyncha), that is a large group containing true bugs, cicadas, planthoppers, aphids, psyllids, etc., (iii) Psocodea, (iv) and Holometabola except for Hymenoptera, where the sequence is well conserved (Fig. S2 panels B and D).

CRYPTOCHROMES

Metazoan CRYPTOCHROMES and PHOTOLYASES can be organized into five groups: CRY-d known as the *Drosophila*-type, CRY-m known from the mammalian clock, 6-4 PHOTOLYASE (6-4 PL), CPD PHOTOLYASE (CPD-PL), which is also known in *Drosophila* as PHOTOREPAIR, and DASH (*Drosophila*, *Arabidopsis*, *Synechocystis*, *Human*)-type CRY. Although the sequences can reliably be assigned to a specific group

Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
SUPPLEMENTARY MATERIAL ONLINE

reflecting its origin, the phylogenetic relationship does not guarantee the protein still possesses specific biochemical properties.

CRY-d was lost: (i) in all chordates (but is present in basal deuterostomian lineage, Echinodermata and Hemichordata), (ii) in the subset of Blattodea containing all termites and three roach families: Cryptocercidae, Lamproblattidae, and Blattidae. (iii) In Cimicomorpha and Pentatomorpha, two large heteropteran groups, (iv) parasitic lice, (v) Hymenoptera, and (vi) crown Coleoptera (beetles).

CRY-m was only lost in Cyclorrhapha, a crown group of Diptera.

6-4 PL forms a sister group to CRY-m and was lost in: (i) mammals, (ii) Cephalopoda (squids, octopuses), (iii) all Blattodea (cockroaches and termites), (iv) all Heteroptera, (v) parasitic lice, (vi) Hymenoptera, and (vii) crown Coleoptera. 6-4 PL was independently duplicated in the lancelet *Branchiostoma* and vertebrates, where it was reduced to one copy in birds and lost in mammals.

CPD PL was lost in: (i) placental mammals, (ii) termites and sister group Cryptocercidae, (iii) parasitic lice, (iv) and crown Coleoptera.

DASH was lost in: (i) mammals, (ii) cephalopods, (iii) chelicerates, (iv) and all insects. Although we were able to find a DASH-like sequence in *Bemisia*. However, its detailed phylogenetic reconstruction indicates the sequence is branching among DASH of plants and fungi, thus we probably witness sample contamination, which is in a plant-sucking insect fairly conceivable option. However, we cannot rule out a horizontal gene transfer at this point, albeit it is an unlikely explanation.



Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
SUPPLEMENTARY MATERIAL ONLINE

*P. apterus timeless* gene structure and its alternatively spliced isoforms

In *P. apterus tim-d*, the deletion was engineered within the 7<sup>th</sup> exon, resulting in a frameshift followed by a premature stop codon. The only alternative splicing of *tim-d* was detected in downstream exons 9, 17, and 18 (Fig. 4A and Figs. S13 and S14), with the engineered deletion itself not affecting the splicing of exons 7 and 8 (Fig. S8). Therefore, *tim-d*<sup>o3</sup> encodes only the initial one-third of the TIM-d protein lacking the evolutionarily conserved PER-interaction regions, nuclear localization signal, and several key amino acid residues downstream (Fig. 4A, Figs. S13 and S14). These features, essential for its proper function in *Drosophila*, are remarkably conserved also in *P. apterus* TIM-d.

Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
 SUPPLEMENTARY MATERIAL ONLINE

**Supplementary Table 1.** Circadian clock gene homologs identified in Metazoa (graphically depicted in Fig. 2)

Species/group	gene, cDNA, or protein	acc #	note
<b><i>Drosophila melanogaster</i></b> Metazoa; Arthropoda; Insecta; Holometabola; Diptera; Brachycera; Cyclorrhapha; Drosophilidae	<i>cryptochrome-d</i>	4JZY_A	
	<i>cryptochrome-m</i>	--	Absent in the genome
	<i>6-4 photolyase</i>	NP_001260632	
	<i>CPD photolyase</i>	NP_724613	
	<i>DASH</i>	--	
	<i>timeless-d</i>	AAC46920	
	<i>timeless-m</i>	NP_524341	
	<i>period</i>	AAA28752	
	<i>jetlag</i>	ABF57911	
	<i>FBXL3/21</i>	--	Absent in the genome
<b><i>Musca domestica</i></b> Metazoa; Arthropoda; Insecta; Holometabola; Diptera; Brachycera; Cyclorrhapha, Muscidae	<i>cryptochrome-d</i>	XP_019890502	
	<i>cryptochrome-m</i>	--	
	<i>6-4 photolyase</i>	XP_005182773	
	<i>CPD photolyase</i>	GARN01046938	
	<i>DASH</i>	--	
	<i>timeless-d</i>	AFP61060	
	<i>timeless-m</i>	XP_005185938	
	<i>period</i>	AAD39163	
	<i>jetlag</i>	XP_005176477	
	<i>FBXL3/21</i>	--	Absent in the genome
<b><i>Ceratitis capitata</i></b> Metazoa; Arthropoda; Insecta; Holometabola; Diptera; Brachycera; Cyclorrhapha, Tephritidae	<i>cryptochrome-d</i>	XP_004529289	
	<i>cryptochrome-m</i>	--	
	<i>6-4 photolyase</i>	XP_004531295	
	<i>CPD photolyase</i>	XP_004535731	
	<i>DASH</i>	--	
	<i>timeless-d</i>	JAB93858	
	<i>timeless-m</i>	XP_023158675; XP_023158519	partial sequence
	<i>period</i>	ABB20914	
	<i>jetlag</i>	CAD6999318	
	<i>FBXL3/21</i>	--	Not found in the TSA
<b><i>Heteropsilopus ingenuus</i></b> Metazoa; Arthropoda; Insecta; Holometabola; Diptera; Brachycera;	<i>cryptochrome-d</i>	GCGO01027349	
	<i>cryptochrome-m</i>	GCGO01039126	partial sequence
	<i>6-4 photolyase</i>	GCGO01026479	partial sequence
	<i>CPD photolyase</i>	GCGO01028698	partial sequence
	<i>DASH</i>	--	
	<i>timeless-d</i>	GCGO01029282	

Kotwica-Rolinska et al. Evolutionary transitions in animal clock

SUPPLEMENTARY MATERIAL ONLINE

Eremoneura; Dolichopodidae	<i>timeless-m</i>	GCGO01016606	partial sequence
	<i>period</i>	GCGO01031901	partial sequence
	<i>jetlag</i>	GCGO01024636	
	<i>FBXL3/21</i>	--	Not found in the TSA
<b><i>Machimus arthriticus</i></b>  Metazoa; Arthropoda; Insecta; Holometabola; Diptera; Brachycera; Asiloidea; Asilidae	<i>cryptochrome-d</i>	GFFZ01008305	
	<i>cryptochrome-m</i>	GFFZ01012401	
	<i>6-4 photolyase</i>	--	
	<i>CPD photolyase</i>	GFFZ01009761	
	<i>DASH</i>	--	
	<i>timeless-d</i>	GFFZ01001760	
	<i>timeless-m</i>	GFFZ01000199; GFZQ01002839; GFZQ01020989	3 partial fragments
	<i>period</i>	GFFZ01021722	
	<i>jetlag</i>	GFZQ01008008	
<b><i>Bombylius major</i></b> Metazoa; Arthropoda; Insecta; Holometabola; Diptera; Brachycera; Asiloidea; Bombyliidae	<i>cryptochrome-d</i>	--	Not found in the TSA
	<i>cryptochrome-m</i>	GATI02019140	
	<i>6-4 photolyase</i>	GATI02019132	
	<i>CPD photolyase</i>	GATI02014885; GATI02016082	2 partial fragments
	<i>DASH</i>	--	
	<i>timeless-d</i>	GATI02019706; GATI02016907; GATI02009616; GATI02009555	4 partial fragments
	<i>timeless-m</i>	GATI02002591; GATI02005167; GATI02012230; GATI02014497	4 partial fragments
	<i>period</i>	GATI02019485	
	<i>jetlag</i>	--	Not found in the TSA
<b><i>Culex quinquefasciatus</i></b>  Metazoa; Arthropoda; Insecta; Holometabola; Diptera; Nematocera; Culicidae	<i>cryptochrome-d</i>	XP_001851403	
	<i>cryptochrome-m</i>	AXG24360	
	<i>6-4 photolyase</i>	XP_001867895	
	<i>CPD photolyase</i>	XP_001845657	
	<i>DASH</i>	--	
	<i>timeless-d</i>	XP_001848611	
	<i>timeless-m</i>	XP_001842224	
	<i>period</i>	AKE07650	
	<i>jetlag</i>	CPIJ015883-RA	
<b><i>Anopheles gambiae</i></b>  Metazoa; Arthropoda; Insecta; Holometabola;	<i>FBXL3/21</i>	--	Not found in the TSA
	<i>cryptochrome-d</i>	XP_321104	
	<i>cryptochrome-m</i>	ABB29887	
	<i>6-4 photolyase</i>	XP_314748	
	<i>CPD photolyase</i>	XP_313925	
	<i>DASH</i>	--	

Kotwica-Rolinska et al. Evolutionary transitions in animal clock

SUPPLEMENTARY MATERIAL ONLINE

Diptera; Nematocera; Culicidae	<i>timeless-d</i>	XP_001689006	
	<i>timeless-m</i>	XP_001689129	
	<i>period</i>	GIBN01001963	
	<i>jetlag</i>	XP_310005	
	<i>FBXL3/21</i>	--	Not found in the TSA
<b><i>Bombyx mori</i></b>  Metazoa; Arthropoda; Insecta; Holometabola; Lepidoptera; Ditrysia; Bombycidae	<i>cryptochrome-d</i>	NP_001182628	
	<i>cryptochrome-m</i>	NP_001182627	
	<i>6-4 photolyase</i>	GFCY01030226	
	<i>CPD photolyase</i>	XP_004930027	
	<i>DASH</i>	--	
	<i>timeless-d</i>	NP_001037622	
	<i>timeless-m</i>	XP_004925203	
	<i>period</i>	XP_021205994	
	<i>jetlag</i>	XP_012543943	
	<i>FBXL3/21</i>	--	Not found in the TSA or genome
<b><i>Danaus plexippus</i></b>  Metazoa; Arthropoda; Insecta; Holometabola; Lepidoptera; Ditrysia; Nymphalidae	<i>cryptochrome-d</i>	OWR41136	
	<i>cryptochrome-m</i>	OWR50781	
	<i>6-4 photolyase</i>	ABO38436	
	<i>CPD photolyase</i>	XP_032529216	
	<i>DASH</i>	--	
	<i>timeless-d</i>	XP_032510676	
	<i>timeless-m</i>	XP_032512746	
	<i>period</i>	XP_032527423	
	<i>jetlag</i>	XP_032519306	
	<i>FBXL3/21</i>	--	Not found in the TSA or genome
<b><i>Palaephatus luteolus</i></b>  Metazoa; Arthropoda; Insecta; Holometabola; Lepidoptera; Palaephatoidea; Palaephatidae	<i>cryptochrome-d</i>	GEON01018930	
	<i>cryptochrome-m</i>	GENI01028497	
	<i>6-4 photolyase</i>	GENI01143689	
	<i>CPD photolyase</i>	GEON01022982	
	<i>DASH</i>	--	
	<i>timeless-d</i>	GENI01053289	
	<i>timeless-m</i>	GEON01026825	
	<i>period</i>	GEON01019583	
	<i>jetlag</i>	GEON01009399	
	<i>FBXL3/21</i>	--	Not found in the TSA
<b><i>Psychomyia flavida</i></b> Metazoa; Arthropoda; Insecta; Holometabola; Trichoptera; Psychomyiidae	<i>cryptochrome-d</i>	GDSC01031720	
	<i>cryptochrome-m</i>	GDSC01027636	
	<i>6-4 photolyase</i>	GDSC01002868	
	<i>CPD photolyase</i>	GDSC01034993	
	<i>DASH</i>	--	
	<i>timeless-d</i>	GDSC01005764	
	<i>timeless-m</i>	GDSC01034894	
	<i>period</i>	GDSC01030560	

Kotwica-Rolinska et al. Evolutionary transitions in animal clock

SUPPLEMENTARY MATERIAL ONLINE

	<i>jetlag</i>	--	Not found in the TSA
	<i>FBXL3/21</i>	--	Not found in the TSA
<b><i>Corydalus cornutus</i></b> Metazoa; Arthropoda; Insecta; Holometabola; Megaloptera; Corydalidae	<i>cryptochrome-d</i>	GATG02017634	
	<i>cryptochrome-m</i>	GATG02013625	
	<i>6-4 photolyase</i>	GATG02016608	partial sequence
	<i>CPD photolyase</i>	GATG02012842	
	<i>DASH</i>	--	
	<i>timeless-d</i>	GATG02015528; GATG02017314	2 partial fragments
	<i>timeless-m</i>	GATG02005567; GATG02010398; GATG02005981	3 partial fragments
	<i>period</i>	GATG02014479	
	<i>jetlag</i>	--	Not found in the TSA
	<i>FBXL3/21</i>	--	Not found in the TSA
<b><i>Conwentzia psociformis</i></b> Metazoa; Arthropoda; Insecta; Holometabola; Neuroptera; Coniopterygidae	<i>cryptochrome-d</i>	GAYH02027555	
	<i>cryptochrome-m</i>	GAYH02030033	
	<i>6-4 photolyase</i>	GAYH02021873	
	<i>CPD photolyase</i>	GAYH02012585	
	<i>DASH</i>	--	
	<i>timeless-d</i>	GATG02015528; GATG02017314	2 partial fragments
	<i>timeless-m</i>	GAYH02012647; GAYH02012108	2 partial fragments
	<i>period</i>	GAYH02013843	
	<i>jetlag</i>	--	Not found in the TSA
	<i>FBXL3/21</i>	--	Not found in the TSA
<b><i>Xanthostigma xanthostigma</i></b> Metazoa; Arthropoda; Insecta; Holometabola; Raphidioptera; Raphidiidae	<i>cryptochrome-d</i>	--	Not found in the TSA
	<i>cryptochrome-m</i>	GAUI02044449	
	<i>6-4 photolyase</i>	GAUI02043242	
	<i>CPD photolyase</i>	GAUI02012114; GAUI02012911	2 partial fragments
	<i>DASH</i>	--	
	<i>timeless-d</i>	GAUI02033942	partial fragment
	<i>timeless-m</i>	GAUI02050009	
	<i>period</i>	GAUI02046259	
	<i>jetlag</i>	--	Not found in TSA
	<i>FBXL3/21</i>	--	Not found in TSA
<b><i>Tribolium castaneum</i></b> Metazoa; Arthropoda; Insecta; Holometabola; Coleoptera; Polyphaga; Tenebrionidae	<i>cryptochrome-d</i>	--	Not found in the genome, transcriptome, or TSA
	<i>cryptochrome-m</i>	EFA04537	
	<i>6-4 photolyase</i>	--	Not found in the genome,

## Kotwica-Rolinska et al. Evolutionary transitions in animal clock

## SUPPLEMENTARY MATERIAL ONLINE

			transcriptome, or TSA
	<i>CPD photolyase</i>	--	Not found in the genome, transcriptome, or TSA
	<i>DASH</i>	--	
	<i>timeless-d</i>	XP_008192983	
	<i>timeless-m</i>	XP_008201051	
	<i>period</i>	XP_015835617	
	<i>jetlag</i>	--	Not found in the genome, transcriptome, or TSA
	<i>FBXL3/21</i>	--	Not found in the genome, transcriptome, or TSA
<b><i>Onthophagus taurus</i></b>	<i>cryptochrome-d</i>	--	
	<i>cryptochrome-m</i>	XP_022918997	
Metazoa; Arthropoda; Insecta; Holometabola; Coleoptera; Polyphaga; Scarabaeidae	<i>6-4 photolyase</i>	--	
	<i>CPD photolyase</i>	--	
	<i>DASH</i>	--	
	<i>timeless-d</i>	XP_022903307	
	<i>timeless-m</i>	XP_022919507	
	<i>period</i>	XP_022899891	
	<i>jetlag</i>	--	Not found in TSA
	<i>FBXL3/21</i>	--	Not found in TSA
<b><i>Agrilus planipennis</i></b>	<i>cryptochrome-d</i>	XP_025837056	
	<i>cryptochrome-m</i>	XP_018331212	
Metazoa; Arthropoda; Insecta; Holometabola; Coleoptera; Polyphaga; Buprestidae	<i>6-4 photolyase</i>	XP_018321940	
	<i>CPD photolyase</i>	XP_018319593	
	<i>DASH</i>	--	
	<i>timeless-d</i>	GDOC01018042	
	<i>timeless-m</i>	XP_018327682	
	<i>period</i>	XP_025836066	
	<i>jetlag</i>	XP_018321770	
	<i>FBXL3/21</i>	--	Not found in the TSA
<b><i>Pogonus chalceus</i></b>	<i>cryptochrome-d</i>	JU432971	
	<i>cryptochrome-m</i>	JU410080	
Metazoa; Arthropoda; Insecta; Holometabola; Coleoptera; Adephaga; Carabidae	<i>6-4 photolyase</i>	JU430199	
	<i>CPD photolyase</i>	JU423416	
	<i>DASH</i>	--	
	<i>timeless-d</i>	JU426733; JU418895	2 partial fragments
	<i>timeless-m</i>	JU429343	
	<i>period</i>	JU419193	
	<i>jetlag</i>	JU405152	

## Kotwica-Rolinska et al. Evolutionary transitions in animal clock

## SUPPLEMENTARY MATERIAL ONLINE

	<i>FBXL3/21</i>	--	Not found in the TSA
<b><i>Halipilus fluviatilis</i></b>  Metazoa; Arthropoda; Insecta; Holometabola; Coleoptera; Adephaga; Halipilidae	<i>cryptochrome-d</i>	GDMW01041568	
	<i>cryptochrome-m</i>	GDMW01034020	
	<i>6-4 photolyase</i>	GDMW01027856	
	<i>CPD photolyase</i>	GDMW01027923	
	<i>DASH</i>	--	
	<i>timeless-d</i>	GDMW01027768; GDMW01036386	2 partial fragments
	<i>timeless-m</i>	GDMW01027750	
	<i>period</i>	GDMW01032314; GDMW01022608	2 partial fragments
	<i>jetlag</i>	GDMW01045097	
	<i>FBXL3/21</i>	--	Not found in TSA
<b><i>Apis mellifera</i></b>  Metazoa; Arthropoda; Insecta; Holometabola; Hymenoptera; Apocrita; Aculeata; Apoidea; Apidae	<i>cryptochrome-d</i>	--	Not found in the genome, transcriptome, or TSA
	<i>cryptochrome-m</i>	ABO38437	
	<i>6-4 photolyase</i>	--	Not found in the genome, transcriptome, or TSA
	<i>CPD photolyase</i>	XP_006564509	
	<i>DASH</i>	--	Not found in the genome, transcriptome, or TSA
	<i>timeless-d</i>	--	Not found in the genome, transcriptome, or TSA
	<i>timeless-m</i>	XP_006565496	
	<i>period</i>	ARB43935	
	<i>jetlag</i>	--	Not found in the genome, transcriptome, or TSA
	<i>FBXL3/21</i>	XP_006565259	
<b><i>Nasonia vitripennis</i></b>  Metazoa; Arthropoda; Insecta; Holometabola; Hymenoptera; Apocrita; Parasitoidea; Chalcidoidea; Pteromalidae	<i>cryptochrome-d</i>	--	Not found in the genome, transcriptome, or TSA
	<i>cryptochrome-m</i>	XP_001606405	
	<i>6-4 photolyase</i>	--	Not found in the genome, transcriptome, or TSA
	<i>CPD photolyase</i>	XP_016844206	

## Kotwica-Rolinska et al. Evolutionary transitions in animal clock

## SUPPLEMENTARY MATERIAL ONLINE

	<i>DASH</i>	--	Not found in the genome, transcriptome, or TSA
	<i>timeless-d</i>	--	Not found in the genome, transcriptome, or TSA
	<i>timeless-m</i>	XP_031783081	
	<i>period</i>	XP_016844400	
	<i>jetlag</i>	--	Not found in the genome, transcriptome, or TSA
	<i>FBXL3/21</i>	XP_008205714	
<b><i>Orussus abietinus</i></b> Metazoa; Arthropoda; Insecta; Holometabola; Hymenoptera; Orussoidea; Orussidae	<i>cryptochrome-d</i>	--	
	<i>cryptochrome-m</i>	XP_012284947	
	<i>6-4 photolyase</i>	--	
	<i>CPD photolyase</i>	XP_012272812	
	<i>DASH</i>	--	
	<i>timeless-d</i>	--	
	<i>timeless-m</i>	XP_012279584	
	<i>period</i>	XP_023290616	
	<i>jetlag</i>	--	Not found in the TSA
	<i>FBXL3/21</i>	XP_012281024	
<b><i>Athalia rosae</i></b> Metazoa; Arthropoda; Insecta; Holometabola; Hymenoptera; Tenthredinoidea; Tenthredinidae	<i>cryptochrome-d</i>	--	
	<i>cryptochrome-m</i>	XP_012266148	
	<i>6-4 photolyase</i>	--	
	<i>CPD photolyase</i>	XP_012254557	
	<i>DASH</i>	--	
	<i>timeless-d</i>	--	
	<i>timeless-m</i>	XP_020709520	
	<i>period</i>	XP_012265524	
	<i>jetlag</i>	--	Not found in the TSA
	<i>FBXL3/21</i>	XP_012263772	
<b><i>Cerobasis guestfalica</i></b> Metazoa; Arthropoda; Insecta; Paraneoptera; Psocodea; Psocoptera, Trogiiidae	<i>cryptochrome-d</i>	GDEA01029438	
	<i>cryptochrome-m</i>	GDEA01042853	
	<i>6-4 photolyase</i>	GDEA01039570	
	<i>CPD photolyase</i>	GDEA01047900	
	<i>DASH</i>	--	
	<i>timeless-d</i>	GDEA01045279	
	<i>timeless-m</i>	GCWJ01017645; GDEA01020989, GDEA01028135	3 partial fragments
	<i>period</i>	GDEA01042435	
	<i>jetlag</i>	--	Not found in the TSA
	<i>FBXL3/21</i>	--	Not found in the TSA



Kotwica-Rolinska et al. Evolutionary transitions in animal clock

SUPPLEMENTARY MATERIAL ONLINE

<b><i>Heterocaecilius solocipennis</i></b>  Metazoa; Arthropoda; Insecta; Paraneoptera; Psocodea; Psocoptera, Pseudocaeciliidae	<i>cryptochrome-d</i>	GDEU01005874, GDEU01000308	2 partial fragments
	<i>cryptochrome-m</i>	GDEU01008438	
	<i>6-4 photolyase</i>	GDEU01006910	
	<i>CPD photolyase</i>	GDEU01029020	
	<i>DASH</i>	--	
	<i>timeless-d</i>	GDEU01008518	
	<i>timeless-m</i>	GDEU01008713	
	<i>period</i>	GDEU01008592	
	<i>jetlag</i>	--	Not found in the TSA
<i>FBXL3/21</i>	--	Not found in the TSA	
<b><i>Peripsocus phaeopterus</i></b>  Metazoa; Arthropoda; Insecta; Paraneoptera; Psocodea; Psocoptera, Peripsocidae	<i>cryptochrome-d</i>	GDDU01009455	
	<i>cryptochrome-m</i>	GDDU01009332	
	<i>6-4 photolyase</i>	GDDU01010248	
	<i>CPD photolyase</i>	GDDU01022857	
	<i>DASH</i>	--	
	<i>timeless-d</i>	GDDU01010920; GDDU01007930; GDDU01005991	3 partial fragments
	<i>timeless-m</i>	GDDU01022229; GDDU01008138	2 partial fragments
	<i>period</i>	GDDU01011596	
	<i>jetlag</i>	--	Not found in the TSA
<i>FBXL3/21</i>	--	Not found in the TSA	
<b><i>Lachesilla contraforcepeta</i></b>  Metazoa; Arthropoda; Insecta; Paraneoptera; Psocodea; Psocoptera, Lachesillidae	<i>cryptochrome-d</i>	GCWJ01017645	
	<i>cryptochrome-m</i>	GCWJ01022319	
	<i>6-4 photolyase</i>	GCWJ01026396	
	<i>CPD photolyase</i>	GCWJ01019774	
	<i>DASH</i>	--	
	<i>timeless-d</i>	GCWJ01021271	
	<i>timeless-m</i>	GCWJ01024943	
	<i>period</i>	GCWJ01026302	
	<i>jetlag</i>	--	Not found in the TSA
<i>FBXL3/21</i>	--	Not found in the TSA	
<b><i>Craspedorrhynchus sp.</i></b>  Metazoa; Arthropoda; Insecta; Paraneoptera; Psocodea; Phthiraptera; Philopteridae	<i>cryptochrome-d</i>	--	
	<i>cryptochrome-m</i>	GCWN01038461	
	<i>6-4 photolyase</i>	--	
	<i>CPD photolyase</i>	--	
	<i>DASH</i>	--	
	<i>timeless-d</i>	GCWN01015667	
	<i>timeless-m</i>	GCWN01030588	
	<i>period</i>	GCWN01026715	

## Kotwica-Rolinska et al. Evolutionary transitions in animal clock

## SUPPLEMENTARY MATERIAL ONLINE

	<i>jetlag</i>	--	Not found in the TSA
	<i>FBXL3/21</i>	--	Not found in the TSA
<b><i>Columbicola columbae</i></b> Metazoa; Arthropoda; Insecta; Paraneoptera; Psocodea; Phthiraptera; Phlopteridae	<i>cryptochrome-d</i>	--	
	<i>cryptochrome-m</i>	GCWB01035766	
	<i>6-4 photolyase</i>	--	
	<i>CPD photolyase</i>	--	
	<i>DASH</i>	--	
	<i>timeless-d</i>	GCWB01033489	
	<i>timeless-m</i>	GCWB01038026	
	<i>period</i>	GCWB01036402	
	<i>jetlag</i>	--	Not found in the TSA
	<i>FBXL3/21</i>	--	Not found in the TSA
<b><i>Pediculus humanus</i></b> Metazoa; Arthropoda; Insecta; Paraneoptera; Psocodea; Phthiraptera; Pediculidae	<i>cryptochrome-d</i>	--	
	<i>cryptochrome-m</i>	XP_002430545	
	<i>6-4 photolyase</i>	--	
	<i>CPD photolyase</i>	--	
	<i>DASH</i>	--	
	<i>timeless-d</i>	EEB19683	
	<i>timeless-m</i>	EEB13198	
	<i>period</i>	XP_002426301	
	<i>jetlag</i>	--	Not found in the genome, transcriptome, or TSA
	<i>FBXL3/21</i>	--	Not found in the genome, transcriptome, or TSA
<b><i>Frankliniella occidentalis</i></b> Metazoa; Arthropoda; Insecta; Paraneoptera; Thysanoptera; Thripidae	<i>cryptochrome-d</i>	GCYR01014055	
	<i>cryptochrome-m</i>	GAXD01023628	
	<i>6-4 photolyase</i>	GCYR01020055	
	<i>CPD photolyase</i>	KAE8748624	
	<i>DASH</i>	--	
	<i>timeless-d</i>	XP_026280944	
	<i>timeless-m</i>	XP_026294102	
	<i>period</i>	XP_026278055	
	<i>jetlag</i>	XP_026275107	
	<i>FBXL3/21</i>	XP_026283316	
<b><i>Orothrips kelloggi</i></b> Metazoa; Arthropoda; Insecta; Paraneoptera; Thysanoptera; Aelothripidae	<i>cryptochrome-d</i>	GCXT01021188	
	<i>cryptochrome-m</i>	GCXT01026378	
	<i>6-4 photolyase</i>	GCXT01023161	
	<i>CPD photolyase</i>	GCXT01015659	
	<i>DASH</i>	--	
	<i>timeless-d</i>	GCXT01027714	

## Kotwica-Rolinska et al. Evolutionary transitions in animal clock

## SUPPLEMENTARY MATERIAL ONLINE

	<i>timeless-m</i>	GCXT01022059		
	<i>period</i>	GCXT01018680		
	<i>jetlag</i>	GCXT01014931		
	<i>FBXL3/21</i>	GCXT01014269	Partial sequence	
<b><i>Halyomorpha halys</i></b> Metazoa; Arthropoda; Insecta; Paraneoptera; Hemiptera; Heteroptera; Pentatomomorpha; Pentatomidae	<i>cryptochrome-d</i>	--	Not found in the genome, transcriptome, or TSA	
	<i>cryptochrome-m</i>	XP_014279454		
	<i>6-4 photolyase</i>	--	Not found in the genome, transcriptome, or TSA	
	<i>CPD photolyase</i>	XP_014272968		
	<i>DASH</i>	--	Not found in the genome, transcriptome, or TSA	
	<i>timeless-d</i>	XP_014278196		
	<i>timeless-m</i>	XP_024217293		
	<i>period</i>	XP_014285208		
	<i>jetlag</i>	--	Not found in the genome, transcriptome, or TSA	
	<i>FBXL3/21</i>	--	Not found in the genome, transcriptome, or TSA	
	<b><i>Cimex lectularius</i></b> Metazoa; Arthropoda; Insecta; Paraneoptera; Hemiptera; Heteroptera; Cimicomorpha; Cimicidae	<i>cryptochrome-d</i>	--	Not found in the genome, transcriptome, or TSA
		<i>cryptochrome-m</i>	XP_014255347	
<i>6-4 photolyase</i>		--	Not found in the genome, transcriptome, or TSA	
<i>CPD photolyase</i>		XP_014256100		
<i>DASH</i>		--	Not found in the genome, transcriptome, or TSA	
<i>timeless-d</i>		XP_014260027		
<i>timeless-m</i>		XP_014246467		
<i>period</i>		XP_014250731		
<i>jetlag</i>		--	Not found in the genome, transcriptome, or TSA	

## Kotwica-Rolinska et al. Evolutionary transitions in animal clock

## SUPPLEMENTARY MATERIAL ONLINE

	<i>FBXL3/21</i>	--	Not found in the genome, transcriptome, or TSA
<b><i>Rhodnius prolixus</i></b> Metazoa; Arthropoda; Insecta; Paraneoptera; Hemiptera; Heteroptera; Cimicomorpha; Reduviidae	<i>cryptochrome-d</i>	--	Not found in the genome, transcriptome, or TSA
	<i>cryptochrome-m</i>	GECK01083311	
	<i>6-4 photolyase</i>	--	Not found in the genome, transcriptome, or TSA
	<i>CPD photolyase</i>	GECK01013387	
	<i>DASH</i>	--	Not found in the genome, transcriptome, or TSA
	<i>timeless-d</i>	GECK01071020	
	<i>timeless-m</i>	GECK01068203	
	<i>period</i>	GECK01038303	
	<i>jetlag</i>	--	Not found in the genome, transcriptome, or TSA
	<i>FBXL3/21</i>	--	Not found in the genome, transcriptome, or TSA
<b><i>Trichocorixa calva</i></b> Metazoa; Arthropoda; Insecta; Paraneoptera; Hemiptera; Heteroptera; Nepomorpha; Corixidae	<i>cryptochrome-d</i>	GCRYZ01037595	
	<i>cryptochrome-m</i>	GCRYZ01040330	
	<i>6-4 photolyase</i>	--	Not found in the TSA
	<i>CPD photolyase</i>	GCRYZ01037238	
	<i>DASH</i>	--	Not found in the TSA
	<i>timeless-d</i>	GCRYZ01029016	
	<i>timeless-m</i>	GCRYZ01027556	
	<i>period</i>	GCRYZ01025514	
	<i>jetlag</i>	GCRYZ01034783	
	<i>FBXL3/21</i>	--	Not found in the TSA
<b><i>Rhagovelia antilleana</i></b> Metazoa; Arthropoda; Insecta; Paraneoptera; Hemiptera; Heteroptera; Gerromorpha; Veliidae	<i>cryptochrome-d</i>	GFOS01101543	
	<i>cryptochrome-m</i>	GFOS01070464	
	<i>6-4 photolyase</i>	--	Not found in the genome, transcriptome, or TSA
	<i>CPD photolyase</i>	GFOS01110824	

## Kotwica-Rolinska et al. Evolutionary transitions in animal clock

## SUPPLEMENTARY MATERIAL ONLINE

	<i>DASH</i>	--	Not found in the genome, transcriptome, or TSA
	<i>timeless-d</i>	GFOS01032396	
	<i>timeless-m</i>	GFOS01100367	
	<i>period</i>	GFOS01044706	
	<i>jetlag</i>	GFOS01057713	
	<i>FBXL3/21</i>	--	Not found in the genome, transcriptome, or TSA
<b><i>Homalodisca vitripennis</i></b> Metazoa; Arthropoda; Insecta; Paraneoptera; Hemiptera; Auchenorrhyncha; Cicadomorpha; Cicadellidae	<i>cryptochrome-d</i>	GHXA01007521	
	<i>cryptochrome-m</i>	GHXA01008453	
	<i>6-4 photolyase</i>	GHXA01015203	
	<i>CPD photolyase</i>	GHXA01011277	
	<i>DASH</i>	--	Not found in the genome, transcriptome, or TSA
	<i>timeless-d</i>	GHXA01018537	
	<i>timeless-m</i>	GHXA01016937	
	<i>period</i>	GHXA01003322	
	<i>jetlag</i>	GICT01181535	
	<i>FBXL3/21</i>	--	Not found in the genome, transcriptome, or TSA
<b><i>Bemisia tabaci</i></b> Metazoa; Arthropoda; Insecta; Paraneoptera; Hemiptera; Sternorrhyncha; Aleyrodoidea; Aleyrodidae	<i>cryptochrome-d</i>	GEZK01146017	
	<i>cryptochrome-m</i>	GAUC01050840	
	<i>6-4 photolyase</i>	GEZK01161051	
	<i>CPD photolyase</i>	XP_018905815	
	<i>DASH</i>	XP_018906320	Plant/fungal contamination?
	<i>timeless-d</i>	XP_018904703	
	<i>timeless-m</i>	XP_018907854	
	<i>period</i>	18910230	
	<i>jetlag</i>	XP_018903799	
	<i>FBXL3/21</i>	--	Not found in the genome, transcriptome, or TSA
<b><i>Pachypsylla venusta</i></b> Metazoa; Arthropoda; Insecta; Paraneoptera; Hemiptera; Sternorrhyncha; Psylloidea; Aphalaridae	<i>cryptochrome-d</i>	GAOP01084163	
	<i>cryptochrome-m</i>	GAOP01061315	
	<i>6-4 photolyase</i>	GAOP01114724	
	<i>CPD photolyase</i>	GAOP01112348	
	<i>DASH</i>	--	Not found in the TSA
	<i>timeless-d</i>	GAOP01060320	
	<i>timeless-m</i>	GAOP01095293	

## Kotwica-Rolinska et al. Evolutionary transitions in animal clock

## SUPPLEMENTARY MATERIAL ONLINE

	<i>period</i>	GAOP01098646; GAOP01098647	2 partial fragments
	<i>jetlag</i>	GAOP01062272	
	<i>FBXL3/21</i>	--	Not found in TSA
<b><i>Acyrtosiphon pisum</i></b>  Metazoa; Arthropoda; Insecta; Paraneoptera; Hemiptera; Sternorrhyncha; Aphidomorpha; Aphididae	<i>cryptochrome-d</i>	NP_001164532	
	<i>cryptochrome-m</i>	NP_001164572 ACYPI087167-PA	ACYPI087167-PA was retrieved from <a href="http://bf2i200.insa-lyon.fr:2555/">http://bf2i200.insa-lyon.fr:2555/</a> ; protein seq. seems to be only partial
	<i>6-4 photolyase</i>	XP_001946012	
	<i>CPD photolyase</i>	XP_001949151	
	<i>DASH</i>	--	Not found in the genome, transcriptome, or TSA
	<i>timeless-d</i>	ARM65417	
	<i>timeless-m</i>	XP_008186746	
	<i>period</i>	NP_001164576	
	<i>jetlag</i>	--	Not found in the genome, transcriptome, or TSA
	<i>FBXL3/21</i>	--	Not found in the genome, transcriptome, or TSA
	<b><i>Myzus persicae</i></b>  Metazoa; Arthropoda; Insecta; Paraneoptera; Hemiptera; Sternorrhyncha; Aphidomorpha; Aphididae	<i>cryptochrome-d</i>	XP_022178570
<i>cryptochrome-m</i>		AUN43314	
<i>6-4 photolyase</i>		XP_022169149	
<i>CPD photolyase</i>		XP_022172380	
<i>DASH</i>		--	
<i>timeless-d</i>		XP_022161049	
<i>timeless-m</i>		XP_022179877	
<i>period</i>		XP_022163849	
<i>jetlag</i>		--	Not found in the TSA
<i>FBXL3/21</i>		--	Not found in the TSA
<b><i>Gryllus bimaculatus</i></b>  Metazoa; Arthropoda; Insecta; Polyneoptera; Orthoptera; Ensifera; Gryllidae	<i>cryptochrome-d</i>	BAX56238	
	<i>cryptochrome-m</i>	BAX56241	
	<i>6-4 photolyase</i>	GFMG01298695	
	<i>CPD photolyase</i>	GFMG01363798	
	<i>DASH</i>	--	Not found in the TSA
	<i>timeless-d</i>	BBD17785	
	<i>timeless-m</i>	BAJ16356	
	<i>period</i>	BAG48878	
	<i>jetlag</i>	GFMG02019192	
	<i>FBXL3/21</i>	--	Not found in the TSA

## Kotwica-Rolinska et al. Evolutionary transitions in animal clock

## SUPPLEMENTARY MATERIAL ONLINE

<b><i>Extatosoma tiaratum</i></b>  Metazoa; Arthropoda; Insecta; Polyneoptera; Phasmatodea; Phasmatidae	<i>cryptochrome-d</i>	GAWG01038287	
	<i>cryptochrome-m</i>	GDZM01028142	
	<i>6-4 photolyase</i>	GAWG01077025	
	<i>CPD photolyase</i>	GAWG01042139	
	<i>DASH</i>	--	Not found in the TSA
	<i>timeless-d</i>	GAWG01055520	
	<i>timeless-m</i>	GAWG01082569	
	<i>period</i>	GAWG01065288	
	<i>jetlag</i>	GAWG01085039	
	<i>FBXL3/21</i>	--	Not found in the TSA
<b><i>Blattella germanica</i></b>  Metazoa; Arthropoda; Insecta; Polyneoptera; Blattodea; Blaberoidea; Ectobiidae	<i>cryptochrome-d</i>	PSN30513	
	<i>cryptochrome-m</i>	PSN44595	
	<i>6-4 photolyase</i>	--	Not found in the genome, transcriptome, or TSA
	<i>CPD photolyase</i>	PSN38921	
	<i>DASH</i>	--	Not found in the genome, transcriptome, or TSA
	<i>timeless-d</i>	PSN50864	
	<i>timeless-m</i>	GDCR01079487	
	<i>period</i>	PSN42098	
	<i>jetlag</i>	PSN54911	
	<i>FBXL3/21</i>	PSN30125	
<b><i>Panchlora nivea</i></b>  Metazoa; Arthropoda; Insecta; Polyneoptera; Blattodea; Blaberoidea; Blaberidae	<i>cryptochrome-d</i>	GGLV01004172	
	<i>cryptochrome-m</i>	GGLV01000774	
	<i>6-4 photolyase</i>	--	Not found in the TSA
	<i>CPD photolyase</i>	GGLV01037008	
	<i>DASH</i>	--	Not found in the TSA
	<i>timeless-d</i>	GGLV01036753	
	<i>timeless-m</i>	GGLV01058516	
	<i>period</i>	GGLV01007810	
	<i>jetlag</i>	GGLV01043282	
	<i>FBXL3/21</i>	GGLV01006178	
<b><i>Sundablatta sexpunctata</i></b>  Metazoa; Arthropoda; Insecta; Polyneoptera; Blattodea; Blaberoidea; Ectobiidae	<i>cryptochrome-d</i>	GDCJ01022923; GDCJ01060550	2 partial fragments
	<i>cryptochrome-m</i>	GDCJ01050009	
	<i>6-4 photolyase</i>	--	Not found in the TSA
	<i>CPD photolyase</i>	GDCJ01035462	
	<i>DASH</i>	--	Not found in the TSA
	<i>timeless-d</i>	GDCJ01051298; GDCJ01039928	2 partial fragments

## Kotwica-Rolinska et al. Evolutionary transitions in animal clock

## SUPPLEMENTARY MATERIAL ONLINE

	<i>timeless-m</i>	GDCJ01050612	
	<i>period</i>	GDCJ01035574; GDCJ01032306; GDCJ01030317; GDCJ01027825	4 partial fragments
	<i>jetlag</i>	GDCJ01049871	
	<i>FBXL3/21</i>	GDCJ01040350	
<b><i>Periplaneta americana</i></b> Metazoa; Arthropoda; Insecta; Polyneoptera; Blattodea; Blattoidea; Blattidae	<i>cryptochrome-d</i>	--	Not found in the TSA
	<i>cryptochrome-m</i>	GFCQ01029664	
	<i>6-4 photolyase</i>	--	Not found in the TSA
	<i>CPD photolyase</i>	GBJC01001632; GBJC01038845; GFCQ01030805	3 partial fragments
	<i>DASH</i>	--	Not found in the TSA
	<i>timeless-d</i>	AAM77468; GFCQ01024702	2 partial fragments
	<i>timeless-m</i>	GFCQ01021782	
	<i>period</i>	AAA64677	
	<i>jetlag</i>	GFCQ01021894	
	<i>FBXL3/21</i>	GBJC01001306	
<b><i>Lamproblatta albipalpus</i></b> Metazoa; Arthropoda; Insecta; Polyneoptera; Blattodea; Blattoidea; Lamproblattidae	<i>cryptochrome-d</i>	--	Not found in the TSA
	<i>cryptochrome-m</i>	GCPS01043497; GCPS01059498	2 partial fragments
	<i>6-4 photolyase</i>	--	Not found in the TSA
	<i>CPD photolyase</i>	GCPS01044676	
	<i>DASH</i>	--	Not found in the TSA
	<i>timeless-d</i>	GCPS01048998; GCPS01038665	2 partial fragments
	<i>timeless-m</i>	GCPS01012790; GCPS01037465	2 partial fragments
	<i>period</i>	GCPS01045300	
	<i>jetlag</i>	GCPS01042505	
	<i>FBXL3/21</i>	GCPS01046280	
<b><i>Cryptocercus wrighti</i></b> Metazoa; Arthropoda; Insecta; Polyneoptera; Blattodea; Blattoidea; Cryptocercidae	<i>cryptochrome-d</i>	--	
	<i>cryptochrome-m</i>	GAZN02037693	
	<i>6-4 photolyase</i>	--	
	<i>CPD photolyase</i>	--	Not found in the TSA
	<i>DASH</i>	--	
	<i>timeless-d</i>	GAZN02048784	
	<i>timeless-m</i>	GAZN02032331; GAZN02036043; GAZN02043003	3 partial fragments
	<i>period</i>	GAZN02046497	
	<i>jetlag</i>	GAZN02047778	
<i>FBXL3/21</i>	GAZN02036388		
<b><i>Porotermes quadricollis</i></b>	<i>cryptochrome-d</i>	--	Not found in the TSA
	<i>cryptochrome-m</i>	GIAG01049439	



Kotwica-Rolinska et al. Evolutionary transitions in animal clock

SUPPLEMENTARY MATERIAL ONLINE

Metazoa; Arthropoda; Insecta; Polyneoptera; Blattodea; Blattoidea; Termitoidae; Termopsidae	<i>6-4 photolyase</i>	--	Not found in the TSA
	<i>CPD photolyase</i>	--	Not found in the TSA
	<i>DASH</i>	--	Not found in the TSA
	<i>timeless-d</i>	GIAG01134881	partial fragment
	<i>timeless-m</i>	GIAG01078513; GIAG01138183; GIAG01068524	3 partial fragments
	<i>period</i>	GIAG01071532, GIAG01071533	2 fragments
	<i>jetlag</i>	GIAG01123673	partial sequence
	<i>FBXL3/21</i>	GIAG01051392 GIAG01098752 GIAG01132428	3 partial fragments
<b><i>Zootermopsis nevadensis</i></b>  Metazoa; Arthropoda; Insecta; Polyneoptera; Blattodea; Blattoidea; Termitoidae; Termopsidae	<i>cryptochrome-d</i>	--	Absent in the genome
	<i>cryptochrome-m</i>	XP_021920747	
	<i>6-4 photolyase</i>	--	Absent in the genome
	<i>CPD photolyase</i>	--	Absent in the genome
	<i>DASH</i>	--	Absent in the genome
	<i>timeless-d</i>	--	Absent in the genome
	<i>timeless-m</i>	XP_021923761	
	<i>period</i>	XP_021930792	
	<i>jetlag</i>	XP_021920889	
<i>FBXL3/21</i>	XP_021926215 2		
<b><i>Cryptotermes secundus</i></b> Metazoa; Arthropoda; Insecta; Polyneoptera; Blattodea; Blattoidea; Termitoidae; Kalotermitidae	<i>cryptochrome-d</i>	--	Absent in the genome
	<i>cryptochrome-m</i>	XP_023706832	
	<i>6-4 photolyase</i>	--	Absent in the genome
	<i>CPD photolyase</i>	--	Absent in the genome
	<i>DASH</i>	--	Absent in the genome
	<i>timeless-d</i>	--	Absent in the genome
	<i>timeless-m</i>	XP_0219237610	
	<i>period</i>	XP_021930792	
	<i>jetlag</i>	XP_023715918	
<i>FBXL3/21</i>	XP_023723291		
<b><i>Nasutitermes takasagoensis</i></b> Metazoa; Arthropoda; Insecta; Polyneoptera;	<i>cryptochrome-d</i>	--	Not found in the TSA
	<i>cryptochrome-m</i>	IAEB01005476	
	<i>6-4 photolyase</i>	--	Not found in the TSA

Kotwica-Rolinska et al. Evolutionary transitions in animal clock

SUPPLEMENTARY MATERIAL ONLINE

Blattodea; Blattoidea; Termitoidea; Termitidae	<i>CPD photolyase</i>	--	Not found in the TSA
	<i>DASH</i>	--	Not found in the TSA
	<i>timeless-d</i>	--	Not found in the TSA
	<i>timeless-m</i>	IAEB01013049	
	<i>period</i>	IAEB01013495	
	<i>jetlag</i>	IAEB01011397	
	<i>FBXL3/21</i>	IAEB01020786	
<b><i>Megaloprepus caerulatus</i></b>  Metazoa; Arthropoda; Insecta; Palaeoptera; Odonata; Pseudostigmatidae	<i>cryptochrome-d</i>	GEXY01066891	
	<i>cryptochrome-m</i>	GEXY01342940	
	<i>6-4 photolyase</i>	GEXY01149320	
	<i>CPD photolyase</i>	GEXY01187320	
	<i>DASH</i>	--	Not found in the TSA
	<i>timeless-d</i>	GEXY01409322	
	<i>timeless-m</i>	GEXY01429671	
	<i>period</i>	GEXY01189581	
	<i>jetlag</i>	--	Not found in the TSA
	<i>FBXL3/21</i>	--	Not found in the TSA
<b><i>Ephemera danica</i></b>  Metazoa; Arthropoda; Insecta; Palaeoptera; Ephemeroptera; Scaphodonta; Ephemeridae; Ephemera	<i>cryptochrome-d</i>	KAF4522540	
	<i>cryptochrome-m</i>	KAF4523712	
	<i>6-4 photolyase</i>	KAF4518708	
	<i>CPD photolyase</i>	KAF4519522	
	<i>DASH</i>	--	Not found in the TSA
	<i>timeless-d</i>	KAF4523325 KAF4523326 KAF4529908	3 partial fragments
	<i>timeless-m</i>	KAF4529908 KAF4531667	2 partial fragments
	<i>period</i>	KAF4520745 KAF4527595 KAF4528952 KAF4528976	4 partial fragments
	<i>jetlag</i>	KAF4516930	
	<i>FBXL3/21</i>	KAF4517907	
<b><i>Sminthurus viridis</i></b>  Metazoa; Arthropoda; Collembola; Symphyleona; Sminthuridae	<i>cryptochrome-d</i>	GATZ02010310	
	<i>cryptochrome-m</i>	GATZ02009173	
	<i>6-4 photolyase</i>	--	Not found in the genome, transcriptome, or TSA
	<i>CPD photolyase</i>	GATZ02022208	
	<i>DASH</i>	GATZ02022546	
	<i>timeless-d</i>	GATZ02010498	
	<i>timeless-m</i>	GATZ02017020	

## Kotwica-Rolinska et al. Evolutionary transitions in animal clock

## SUPPLEMENTARY MATERIAL ONLINE

	<i>period</i>	GATZ02006475	
	<i>jetlag</i>	GATZ02008436	
	<i>FBXL3/21</i>	--	Not found in the TSA
<b><i>Daphnia pulex</i></b> Metazoa; Arthropoda; Crustacea; Diplostraca; Daphniidae	<i>cryptochrome-d</i>	EFX85418	
	<i>cryptochrome-m</i>	EFX82092	
	<i>6-4 photolyase</i>	EFX85418	
	<i>CPD photolyase</i>	EFX71237	
	<i>DASH</i>	EFX86680	
	<i>timeless-d</i>	EFX80642	
	<i>timeless-m</i>	EFX80319	
	<i>period</i>	EFX76293	
	<i>jetlag</i>	EFX83363	
		<i>FBXL3/21</i>	--
<b><i>Limulus polyphemus</i></b> Metazoa; Arthropoda; Chelicerata; Merostomata; Xiphosura; Limulidae	<i>cryptochrome-d</i>	XP_022257009	
	<i>cryptochrome-m</i>	ANO53972	
	<i>6-4 photolyase</i>	XP_022247971	
	<i>CPD photolyase</i>	XP_013787452	
	<i>DASH</i>	--	Not found in the genome, transcriptome, or TSA
	<i>timeless-d</i>	XP_022254152	
	<i>timeless-m</i>	XP_022243537	
	<i>period</i>	ANO53971	
	<i>jetlag</i>	XP_013780499	
		<i>FBXL3/21</i>	XP_022254049
<b><i>Araneus ventricosus</i></b> Metazoa; Arthropoda; Chelicerata; Arachnida; Araneae; Araneidae	<i>cryptochrome-d</i>	GBN19159	
	<i>cryptochrome-m</i>	GBM49213	
	<i>6-4 photolyase</i>	GBM67888	
	<i>CPD photolyase</i>	GBN22197	
	<i>DASH</i>	--	Not found in the TSA
	<i>timeless-d</i>	GBM21781	
	<i>timeless-m</i>	GBN56120	
	<i>period</i>	GBM95439	
	<i>jetlag</i>	GBN49390	
		<i>FBXL3/21</i>	--
<b><i>Tetranychus urticae</i></b> Metazoa; Arthropoda; Chelicerata; Arachnida; Acari; Trombidiformes; Tetranychidae	<i>cryptochrome-d</i>	XP_025017376	
	<i>cryptochrome-m</i>	XP_015785840	
	<i>6-4 photolyase</i>	--	Not found in the TSA, transcriptome, or genome
	<i>CPD photolyase</i>	XP_015787507	

Kotwica-Rolinska et al. Evolutionary transitions in animal clock

SUPPLEMENTARY MATERIAL ONLINE

	<i>DASH</i>	--	Not found in the TSA, transcriptome, or genome
	<i>timeless-d</i>	XP_025017966	
	<i>timeless-m</i>	XP_015789018	
	<i>period</i>	XP_015786774	
	<i>jetlag</i>	XP_015786213	
	<i>FBXL3/21</i>	--	Not found in the TSA, transcriptome, or genome
<b><i>Platynereis dumerilii</i></b>  Metazoa; Annelida; Polychaeta; Phyllodocida; Nereididae	<i>cryptochrome-d</i>	AEJ87227	
	<i>cryptochrome-m</i>	AGX93012	
	<i>6-4 photolyase</i>	AGX93015	
	<i>CPD photolyase</i>	AIE57497	
	<i>DASH</i>	AIE57496	
	<i>timeless-d</i>	HALR01337992	
	<i>timeless-m</i>	HAMO01053063	
	<i>period</i>	HALR01343176	
	<i>jetlag</i>	HAMO01014748	
	<i>FBXL3/21</i>	--	Not found in the TSA, transcriptome, or genome
<b><i>Crassostrea gigas</i></b>  Metazoa; Mollusca; Bivalvia; Ostreida; Ostreidae	<i>cryptochrome-d</i>	ANJ02841	
	<i>cryptochrome-m</i>	AQM57602	
	<i>6-4 photolyase</i>	XP_011414697	
	<i>CPD photolyase</i>	XP_011422754	
	<i>DASH</i>	XP_011441094	
	<i>timeless-d</i>	AQM57605	
	<i>timeless-m</i>	XP_011441580	
	<i>period</i>	XP_011434453	
	<i>jetlag</i>	XP_011444755	
		<i>FBXL3/21</i>	--
<b><i>Aplysia californica</i></b>  Metazoa; Mollusca; Gastropoda; Aplysiida; Aplysiidae	<i>cryptochrome-d</i>	XP_005089742	
	<i>cryptochrome-m</i>	XP_012941094	
	<i>6-4 photolyase</i>	XP_012944453	
	<i>CPD photolyase</i>	XP_012935158	
	<i>DASH</i>	XP_005098341	
	<i>timeless-d</i>	XP_012943985	
	<i>timeless-m</i>	XP_012938690	
	<i>period</i>	XP_012944985	
	<i>jetlag</i>	XP_005101190	
	<i>FBXL3/21</i>	--	Not found in the TSA
<b><i>Octopus vulgaris</i></b>	<i>cryptochrome-d</i>	XP_029634730	
	<i>cryptochrome-m</i>	XP_029646224	

Kotwica-Rolinska et al. Evolutionary transitions in animal clock

SUPPLEMENTARY MATERIAL ONLINE

Metazoa; Mollusca; Cephalopoda; Octopoda; Octopodidae	<i>6-4 photolyase</i>	--	not in TSA of any Cephalopoda (taxid:6605)
	<i>CPD photolyase</i>	XP_029648244	
	<i>DASH</i>	--	not in TSA of any Cephalopoda (taxid:6605)
	<i>timeless-d</i>	XP_029642111	
	<i>timeless-m</i>	XP_029654177	
	<i>period</i>	XP_029633045	
	<i>jetlag</i>	JR436235	
	<i>FBXL3/21</i>	--	Not found in the TSA
<b><i>Sepia esculenta</i></b>  Metazoa; Mollusca; Cephalopoda; Sepiida; Sepiidae	<i>cryptochrome-d</i>	GGQU01009236	
	<i>cryptochrome-m</i>	GGQU01028147	
	<i>6-4 photolyase</i>	--	not in TSA of any Cephalopoda (taxid:6605)
	<i>CPD photolyase</i>	GGQU01005854	
	<i>DASH</i>	--	not in TSA of any Cephalopoda (taxid:6605)
	<i>timeless-d</i>	GGQU01018501	
	<i>timeless-m</i>	GGQU01119589	
	<i>period</i>	GGQU01013326	
	<i>jetlag</i>	GGQU01008949	
<i>FBXL3/21</i>	--	Not found in the TSA	
<b><i>Strongylocentrotus purpuratus</i></b> Metazoa; Echinodermata; Echinoidea; Camarodonta; Strongylocentrotidae	<i>cryptochrome-d</i>	XP_030843606	
	<i>cryptochrome-m</i>	XP_785873	
	<i>6-4 photolyase</i>	XP_030853363	
	<i>CPD photolyase</i>	XP_030838243	
	<i>DASH</i>	XP_030855350	
	<i>timeless-d</i>	XP_011666280	
	<i>timeless-m</i>	XP_784350	
	<i>period</i>	--	Absent in the TSA, proteins, and genome
	<i>jetlag-related</i>	XP_030835612	
<i>FBXL3/21</i>	XP_011668197		
<b><i>Acanthaster planci</i></b>  Metazoa; Echinodermata; Asteroidea; Valvatida; Acanthasteridae	<i>cryptochrome-d</i>	XP_022112080	
	<i>cryptochrome-m</i>	XP_022093922	
	<i>6-4 photolyase</i>	XP_022085333	
	<i>CPD photolyase</i>	XP_022095977	
	<i>DASH</i>	XP_022106989	
	<i>timeless-d</i>	XP_022095151	
	<i>timeless-m</i>	XP_022107896	
<i>period</i>	--	Absent in the TSA, proteins, and genome	

## SUPPLEMENTARY MATERIAL ONLINE

	<i>jetlag-related</i>	XP_022086279	
	<i>FBXL3/21</i>	XP_022086809	
<b><i>Ptychodera flava</i></b> Metazoa; Hemichordata; Enteropneusta; Ptychoderidae	<i>cryptochrome-d</i>	GDGM01320022	
	<i>cryptochrome-m</i>	GDGM01087240	
	<i>6-4 photolyase</i>	GDGM01051165	
	<i>CPD photolyase</i>	GDGM01188624	
	<i>DASH</i>	GDGM01423525	
	<i>timeless-d</i>	GDGM01251144	
	<i>timeless-m</i>	GDGM01011345GDGM01415766	2 partial fragments
	<i>period</i>	--	Absent in the TSA, proteins, and genome
		<i>jetlag-related</i>	GDGM01503414
	<i>FBXL3/21</i>	GDGM01093178	
<b><i>Branchiostoma floridae</i></b> Metazoa; Chordata; Leptocardii; Branchiostomidae	<i>cryptochrome-d</i>	--	Absent in the genome
	<i>cryptochrome-m</i>	XP_035662695	
	<i>6-4 photolyase</i>	XP_035662689 XP_035688923	2 paralogs
	<i>CPD photolyase</i>	XP_035694008	
	<i>DASH</i>	--	
	<i>timeless-d</i>	--	Absent in the genome
	<i>timeless-m</i>	XP_035657364	
	<i>period</i>	XP_035696876	
	<i>jetlag</i>	XP_035692716	
	<i>FBXL3/21</i>	XP_035682891	
<b><i>Petromyzon</i></b>	<i>cryptochrome-d</i>	--	Absent in the genome
	<i>cryptochrome-m</i>	XP_032805096	
	<i>6-4 photolyase</i>	--	
	<i>CPD photolyase</i>	XP_032801148	
	<i>DASH</i>	--	
	<i>timeless-d</i>	--	Absent in the genome
	<i>timeless-m</i>	XP_032813202	
	<i>period</i>	XP_032817908	
	<i>jetlag-related</i>	XP_032805591	
	<i>FBXL3/21</i>	XP_032832820	
<b><i>Danio rerio</i></b> Metazoa; Chordata; Vertebrata; Actinopteri; Teleostei; Cypriniformes; Danionidae	<i>cryptochrome-d</i>	--	Absent in the genome, transcriptome, and TSA
	<i>cryptochrome-m</i>	BAA96850; BAA96848; BAA96847; BAA96846;	4 paralogs
	<i>6-4 photolyase</i>	AAI64413; XP_005155462	2 paralogs
	<i>CPD photolyase</i>	XP_005168471	
	<i>DASH</i>	NP_991249	

## Kotwica-Rolinska et al. Evolutionary transitions in animal clock

## SUPPLEMENTARY MATERIAL ONLINE

	<i>timeless-d</i>	--	Absent in the genome, transcriptome and TSA
	<i>timeless-m</i>	NP_001265529	
	<i>period1</i>	XP_005172684	
	<i>period2</i>	AAI63549	
	<i>period3</i>	AAI62472	
	<i>jetlag-related</i>	NP_998107	
	<i>FBXL3/21</i>	NP_001005773; XP_693270	2 paralogs
<b><i>Xenopus laevis</i></b>	<i>cryptochrome-d</i>	--	Absent in the genome, transcriptome, and TSA
Metazoa; Chordata; Vertebrata; Tetrapoda; Amphibia; Anura; Pipidae	<i>cryptochrome-m</i>	AAK94667; AAK94665	2 paralogs
	<i>6-4 photolyase</i>	NP_001088990; NP_001081421	2 paralogs
	<i>CPD photolyase</i>	NP_001089127	
	<i>DASH</i>	NP_001084438	
	<i>timeless-d</i>	--	Absent in the genome, transcriptome, and TSA
	<i>timeless-m</i>	XP_018105525	
	<i>period1</i>	XP_018106090	
	<i>period2</i>	NP_001081098	
	<i>period3</i>	XP_018081638; XP_018083480	
	<i>jetlag-related</i>	NP_001079747	
	<i>FBXL3/21</i>	XP_018105395; XP_018107511	
<b><i>Anolis carolinensis</i></b>	<i>cryptochrome-d</i>	--	Absent in the genome
Metazoa; Chordata; Vertebrata; Tetrapoda; Sauria; Lepidosauria; Squamata; Dactyloidea	<i>cryptochrome-m</i>	XP_003220970; XP_003214689	2 paralogs
	<i>6-4 photolyase</i>	XP_008108122; XP_003225762	2 paralogs
	<i>CPD photolyase</i>	XP_003227011	
	<i>DASH</i>	XP_008110409	
	<i>timeless-d</i>	--	Absent in the genome
	<i>timeless-m</i>	XP_016846705	
	<i>period1</i>	XP_008117781	
	<i>period2</i>	XP_008104509	
	<i>period3</i>	--	
	<i>jetlag-related</i>	XP_003223176	
	<i>FBXL3/21</i>	XP_008113732; XP_003218703	
<b><i>Taeniopygia guttata</i></b>	<i>cryptochrome-d</i>	--	Absent in the genome
Metazoa; Chordata; Vertebrata; Tetrapoda; Sauria; Archelosauria;	<i>cryptochrome-m</i>	XP_030130159; XP_030118992	2 paralogs
	<i>6-4 photolyase</i>	XP_012426408	
	<i>CPD photolyase</i>	XP_030136454	
	<i>DASH</i>	XP_030120072	

## Kotwica-Rolinska et al. Evolutionary transitions in animal clock

## SUPPLEMENTARY MATERIAL ONLINE

Dinosauria; Aves; Passeriformes; Estrildidae	<i>timeless-d</i>	--	
	<i>timeless-m</i>	XP_030115154	
	<i>period1</i>	--	
	<i>period2</i>	XP_030136609	
	<i>period3</i>	XP_012425833	
	<i>jetlag-related</i>	XP_030131830	
	<i>FBXL3/21</i>	XP_030139641; XP_002199560	
<b><i>Vombatus ursinus</i></b>  Metazoa; Chordata; Vertebrata; Tetrapoda; Mammalia; Diprotodontia; Vombatidae	<i>cryptochrome-d</i>	--	Absent in the genome
	<i>cryptochrome-m</i>	XP_027693622; XP_027728959	2 paralogs
	<i>6-4 photolyase</i>	--	Absent in the genome
	<i>CPD photolyase</i>	XP_027701119	
	<i>DASH</i>	--	Absent in the genome
	<i>timeless-d</i>	--	Absent in the genome
	<i>timeless-m</i>	XP_027710880	
	<i>period1</i>	XP_027725776	
	<i>period2</i>	XP_027731061	
	<i>period3</i>	XP_027699731	
	<i>jetlag-related</i>	XP_027707763	
<i>FBXL3/21</i>	XP_027700340		
<b><i>Mus musculus</i></b>  Metazoa; Chordata; Vertebrata; Tetrapoda; Mammalia; Rodentia; Murinae	<i>cryptochrome-d</i>	--	Absent in the genome
	<i>cryptochrome-m</i>	4K0R_A; NP_034093	2 paralogs
	<i>6-4 photolyase</i>	--	Absent in the genome
	<i>CPD photolyase</i>	--	Absent in the genome
	<i>DASH</i>	--	Absent in the genome
	<i>timeless-d</i>	--	Absent in the genome
	<i>timeless-m</i>	NP_001157553	
	<i>period1</i>	NP_001152839	
	<i>period2</i>	NP_035196	
	<i>period3</i>	XP_011248505	
	<i>jetlag-related</i>	NP_001365702	
<i>FBXL3/21</i>	NP_056637; NP_848789		
<b><i>Homo sapiens</i></b>  Metazoa; Chordata; Vertebrata; Tetrapoda; Mammalia; Primates; Hominidae	<i>cryptochrome-d</i>	--	Absent in the genome
	<i>cryptochrome-m</i>	BAG64048; NP_004066	2 paralogs
	<i>6-4 photolyase</i>	--	Absent in the genome
	<i>CPD photolyase</i>	--	Absent in the genome
	<i>DASH</i>	--	Absent in the genome



Kotwica-Rolinska et al. Evolutionary transitions in animal clock

SUPPLEMENTARY MATERIAL ONLINE

	<i>timeless-d</i>	--	Absent in the genome
	<i>timeless-m</i>	NP_003911	
	<i>period1</i>	NP_002607	
	<i>period2</i>	NP_073728	
	<i>period3</i>	NP_001276790	
	<i>jetlag-related</i>	XP_005270206	
	<i>FBXL3/21</i>	FXL21_HUMAN; NP_036290	

## Kotwica-Rolinska et al. Evolutionary transitions in animal clock

## SUPPLEMENTARY MATERIAL ONLINE

**Supplementary Table 2.** Summary on genes absence in entire metazoan lineages

Metazoan lineage	Absent gene	note
<b>Higher Diptera</b>	<i>cryptochrome-m</i>	Absent in all Cyclorrhapha (taxid:480117)
<b>Crown Coleoptera</b>	<i>jetlag</i>	absent in TSAs and genomes of Scarabaeoidea (taxid:75546), Tenebrionoidea (taxid:71527), Chrysomeloidea (71528), Curculionoidea (71529)
	<i>cryptochrome-d</i>	absent in TSAs and genomes of Scarabaeoidea (taxid:75546), Tenebrionoidea (taxid:71527), Chrysomeloidea (71528), Curculionoidea (71529)
	<i>6-4 photolyase</i>	absent in TSAs and genomes of Scarabaeoidea (taxid:75546), Tenebrionoidea (taxid:71527), Chrysomeloidea (71528), Curculionoidea (71529)
	<i>CPD photolyase</i>	absent in TSAs and genomes of Scarabaeoidea (taxid:75546), Tenebrionoidea (taxid:71527), Chrysomeloidea (71528), Curculionoidea (71529)
<b>Hymenoptera</b>	<i>timeless-d</i>	Absent in all Hymenoptera (taxid:7399)
	<i>jetlag</i>	Absent in all Hymenoptera (taxid:7399)
	<i>cryptochrome-d</i>	Absent in all Hymenoptera (taxid:7399)
	<i>6-4 photolyase</i>	Absent in all Hymenoptera (taxid:7399)
<b>Aphids</b>	<i>jetlag</i>	Absent in all Aphids (taxid:33380)
<b>Heteroptea</b>	<i>6-4 photolyase</i>	Absent in all Heteroptera (taxid:33345)
	<i>cryptochrome-d</i>	absent in TSAs, proteins, and genomes Cimicomorpha (taxid:33354) and Pentatomomorpha (taxid:33357)
<b>Hemipteroid assembly (Heteroptera, Auchenorrhyncha, Sternorrhyncha, Sternorrhyncha,</b>	<i>FBXL3/21</i>	Absent in all Heteroptera (taxid:33345) Absent in all Auchenorrhyncha (taxid:1955247) Absent in all Sternorrhyncha (taxid:33373)
<b>Blattodea (including termites)</b>	<i>6-4 photolyase</i>	absent in TSAs, proteins, and genomes of all available Blattodea (taxid:85823) & Termites (taxid:7499)
	<i>cryptochrome-d</i>	absent in TSAs, proteins, and genomes of all available Termites (taxid:7499), Cryptocercidae (taxid:36982), Lamproblattidae (taxid:1080998), Blattidae (taxid:6974)
	<i>CPD photolyase</i>	absent in TSAs, proteins, and genomes of all available Termites (taxid:7499) and Cryptocercidae (taxid:36982)
	<i>timeless-d</i>	With the exception of <i>Porotermes quadricollis</i> , absent in TSAs, proteins, and genomes of all available Termites (taxid:7499)
<b>Insecta</b>	<i>DASH</i>	Absent in TSAs, proteins, and genomes of all available insects, except for <i>Bemisia</i> (which seems to be plant contamination)
<b>Chelicerata</b>	<i>DASH</i>	Absent in TSAs, proteins, and genomes of all available Chelicerata (taxid:6843)

## SUPPLEMENTARY MATERIAL ONLINE

<b>Mollusca</b>	<i>6-4 photolyase</i>	Absent in TSAs, proteins, and genomes of all available Cephalopoda (taxid:6605)
	<i>DASH</i>	Absent in TSAs, proteins, and genomes of all available Cephalopoda (taxid:6605)
<b>Echinodermata</b>	<i>period</i>	Absent in TSAs, proteins, and genomes of all available Echinodermata (taxid:7586)
<b>Hemichordata</b>	<i>period</i>	Absent in TSAs, proteins, and genomes of all available Hemichordata (taxid:10219)
<b>Vertebrata</b>	<i>timeless-d</i>	Absent in TSAs, proteins, and genomes of all available Vertebrata (taxid:7742)
	<i>cryptochrome-d</i>	Absent in TSAs, proteins, and genomes of all available Vertebrata (taxid:7742)
	<i>6-4 photolyase</i>	Absent in TSAs, proteins, and genomes of all available Mammalia (taxid:40674)
	<i>DASH</i>	Absent in TSAs, proteins, and genomes of all available Mammalia (taxid:40674)
	<i>CPD photolyase</i>	Absent in TSAs, proteins, and genomes of all available Placentalia (taxid:9347)

## Kotwica-Rolinska et al. Evolutionary transitions in animal clock

## SUPPLEMENTARY MATERIAL ONLINE

**Supplementary Table 3.** Circadian clock gene homologs and closely related genes identified in the Linden bug, *Pyrrhocoris apterus*

<b>gene</b>	<b>acc#</b>	<b>note</b>
<i>period</i>	MW662133	gene model
<i>timeless-d</i>	MW662134	gene model
<i>cryptochrome-m</i>	MW662132	gene model
<i>Clock</i>	MW662127	cDNA sequence
<i>cycle</i>	MW662128	cDNA sequence
<i>clockwork orange</i>	MW662123	cDNA sequence
<i>Pdp1</i>	MW662129	cDNA sequence
<i>vriille</i>	MW662124	cDNA sequence
<i>double time</i>	MW662125	cDNA sequence
<i>shaggy</i>	MZ399198	cDNA sequence
<i>nemo</i>	MZ399197	cDNA sequence
<i>slimb</i>	MW662126	cDNA sequence
<i>CPD photolyase</i>	MW662130	cDNA sequence
<i>timeless-m</i>	MW662131	cDNA sequence

Kotwica-Rolinska et al. Evolutionary transitions in animal clock  
**SUPPLEMENTARY MATERIAL ONLINE**

**Supplementary Table 4.** Primers used to clone cDNA templates used for dsRNA synthesis

<b>gene</b>		<b>Forward (5'→3')</b>	<b>Reverse (5'→3')</b>	<b>product</b>
<i>period</i>	fr #1	ACACAGTTTTACGGGGACCAC	GTTATTGGAGCCGGGGATTGT	605 bp
	fr #2	CAAAGGATATGTGGGCTGGTAG	GTGCTGGCAAATGACAAACTC	623 bp
<i>tim-d</i>	fr #1	ATGGCAGAACAGATGGAATGGT	TCGGATATGGGGTCTGATGTTAC	839 bp
	fr #2	CACCGAACGGAGACGATGTA	TTTTAAGCGAAGTAGGTATTGTGC	885 bp
<i>tim-m</i>	fr #1	GGGAAATTTGGCCAGAAAACGA	TTCTGTTCTTCGGTCCAGGATG	805 bp
	fr #2	AATTATTTGACTTGATATTAAGGCTTA TAGT	GCCTGGAGCCAAGGTATTGT	600 bp
<i>cry-m</i>	fr #1	GCTGCAACAAAAGAACCCAAAT	TAGAGGCTGGAGGAGCAAATA	697 bp
	fr #2	TGGTTCGCAAATGCTTCTAATG	GACTTGTCCTGTAGAGAAAGAG	752 bp
<i>Clock</i>	fr #1	GAAGGGGCAACAGTGGATA	GATGCTGAAGGGAATGTCTC	300 bp
	fr #2	ATTTTAGGCCCTTCTGCTGACT	CGAGACTATGCCTTGATGTGAAT	307 bp
<i>cycle</i>	fr #1	ATGGCTATGGACGTTGTACAGGT	GGAGGATGATAGGCCCTGATTG	956 bp
	fr #2	ATCAACGTACGCCACTAC	CATGTCTCGGCCGATATT	453 bp
<i>cwo</i>	fr #1	CCAACCTCGAACCCATCTAA	AGCCCAGGATCCAAACATATC	399 bp
	fr #2	CACGGCCATCAAATACGAAAG	GTAACCTCCGAGTCAACAAGA	403 bp
<i>Pdp1</i>	fr #1	AAAAAGATGAAGCAGAGC	CAGCAGGTGAAGGTGGTG	318 bp
	fr #2	AACATTATCATTTCCTCATC	GACAGCCTTTCCTTATGTG	315 bp
<i>vrille</i>	fr #1	CATCTCCTCCAATAGTCCCTTAC	CTGCTACCTTCGCCATCCCAAG	385 bp
	fr #2	TGACCTGGGATCAATACCTAGA	TTGGAACCTGAATTGGCTGGA	473 bp
<i>dbt</i>	fr #1	CTCAGAGTCGGAAATAAATA	TTCTAAATGAGTGTGAGA	556 bp
	fr #2	TAGGAATTGAACAAAGCAGAAGAG	CTAGGGGGTAAGGCGAGTG	502 bp
<i>sgg</i>	fr #1	CTGAGGGTAACAAGCAAACCT	CGATGGCAGATACCGAGAG	502 bp
	fr #2	AGCCGCAAAATCTTCTCCTC	GATGGGTCTCTAATTCGTCAAAA	493 bp
<i>nmo</i>	fr #1	GTAGGGGAGTGAGAACAGCCAGTATG	GCGAGCCAGCCGAAGTCAC	542 bp
	fr #2	CGCCGTGGACGTCTGGAGTGTAG	TTGAACGCAGCCGATTGTGGATTT AT	561 bp
<i>slimb</i>	fr #1	CACAGCTTAGCGGAATGACA	TCCTGGCTTTGGTTTGAATA	458 bp
	fr #2	ATTGGAGGCTAGGAAGATTTA	TCGCCGGAAGCAGATAC	481 bp

## Supplementary References

1. Pivarciova L, *et al.* (2016) Unexpected Geographic Variability of the Free Running Period in the Linden Bug *Pyrrhocoris apterus*. *Journal of biological rhythms*.
2. Bajgar A, Jindra M, & Dolezel D (2013) Autonomous regulation of the insect gut by circadian genes acting downstream of juvenile hormone signaling. *Proceedings of the National Academy of Sciences of the United States of America* 110(11):4416-4421.
3. Kotwica-Rolinska J, Pivarciova L, Vaneckova H, & Dolezel D (2017) The role of circadian clock genes in the photoperiodic timer of the linden bug *Pyrrhocoris apterus* during the nymphal stage. *Physiological Entomology* 42(3):266-273.
4. Smykal V, *et al.* (2014) Juvenile hormone signaling during reproduction and development of the linden bug, *Pyrrhocoris apterus*. *Insect biochemistry and molecular biology* 45:69-76.
5. Smykal V, *et al.* (2020) Complex Evolution of Insect Insulin Receptors and Homologous Decoy Receptors, and Functional Significance of Their Multiplicity. *Molecular biology and evolution* 37(6):1775-1789.
6. Kotwica-Rolinska J, *et al.* (2019) CRISPR/Cas9 Genome Editing Introduction and Optimization in the Non-model Insect *Pyrrhocoris apterus*. *Frontiers in physiology* 10:891.
7. Schmid B, Helfrich-Forster C, & Yoshii T (2011) A new ImageJ plug-in "ActogramJ" for chronobiological analyses. *Journal of biological rhythms* 26(5):464-467.
8. Yuan Q, Metterville D, Briscoe AD, & Reppert SM (2007) Insect cryptochromes: Gene duplication and loss define diverse ways to construct insect circadian clocks. *Molecular biology and evolution* 24(4):948-955.
9. Zhu HS, *et al.* (2005) The two CRYs of the butterfly. *Curr Biol* 15(23):R953-R954.
10. Daiyasu H, *et al.* (2004) Identification of cryptochrome DASH from vertebrates. *Genes to cells : devoted to molecular & cellular mechanisms* 9(5):479-495.
11. Mei Q & Dvornyk V (2015) Evolutionary History of the Photolyase/Cryptochrome Superfamily in Eukaryotes. *PloS one* 10(9):e0135940.
12. Benna C, *et al.* (2000) A second timeless gene in *Drosophila* shares greater sequence similarity with mammalian *tim*. *Curr Biol* 10(14):R512-513.
13. Siepka SM, *et al.* (2007) Circadian mutant overtime reveals F-box protein FBXL3 regulation of cryptochrome and period gene expression. *Cell* 129(5):1011-1023.
14. Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30(9):1312-1313.
15. Misof B, *et al.* (2014) Phylogenomics resolves the timing and pattern of insect evolution. *Science* 346(6210):763-767.
16. Thomas GWC, *et al.* (2020) Gene content evolution in the arthropods. *Genome biology* 21(1):15.

SUPPLEMENTARY MATERIAL ONLINE

17. Tanner AR, *et al.* (2017) Molecular clocks indicate turnover and diversification of modern coleoid cephalopods during the Mesozoic Marine Revolution. *Proceedings. Biological sciences* 284(1850).
18. Irisarri I, *et al.* (2017) Phylotranscriptomic consolidation of the jawed vertebrate timetree. *Nature ecology & evolution* 1(9):1370-1378.
19. Dohrmann M & Worheide G (2017) Dating early animal evolution using phylogenomic data. *Scientific reports* 7(1):3599.
20. Wipfler B, *et al.* (2019) Evolutionary history of Polyneoptera and its implications for our understanding of early winged insects. *Proceedings of the National Academy of Sciences of the United States of America*.
21. Evangelista DA, *et al.* (2019) An integrative phylogenomic approach illuminates the evolutionary history of cockroaches and termites (Blattodea). *Proceedings. Biological sciences* 286(1895):20182076.
22. Johnson KP, *et al.* (2018) Phylogenomics and the evolution of hemipteroid insects. *Proceedings of the National Academy of Sciences of the United States of America* 115(50):12775-12780.
23. Branstetter MG, *et al.* (2017) Phylogenomic Insights into the Evolution of Stinging Wasps and the Origins of Ants and Bees. *Curr Biol* 27(7):1019-1025.
24. McKenna DD, *et al.* (2019) The evolution and genomic basis of beetle diversity. *Proceedings of the National Academy of Sciences of the United States of America* 116(49):24729-24737.
25. Kawahara AY, *et al.* (2019) Phylogenomics reveals the evolutionary timing and pattern of butterflies and moths. *Proceedings of the National Academy of Sciences of the United States of America* 116(45):22657-22663.
26. Sea Urchin Genome Sequencing C, *et al.* (2006) The genome of the sea urchin *Strongylocentrotus purpuratus*. *Science* 314(5801):941-952.
27. Hall MR, *et al.* (2017) The crown-of-thorns starfish genome as a guide for biocontrol of this coral reef pest. *Nature* 544(7649):231-234.
28. Simakov O, *et al.* (2015) Hemichordate genomes and deuterostome origins. *Nature* 527(7579):459-465.
29. Benna C, *et al.* (2010) *Drosophila timeless2* is required for chromosome stability and circadian photoreception. *Curr Biol* 20(4):346-352.
30. Gotter AL, *et al.* (2000) A time-less function for mouse Timeless. *Nat Neurosci* 3(8):755-756.
31. Godinho SI, *et al.* (2007) The after-hours mutant reveals a role for Fbx13 in determining mammalian circadian period. *Science* 316(5826):897-900.
32. Hirano A, *et al.* (2013) FBXL21 Regulates Oscillation of the Circadian Clock through Ubiquitination and Stabilization of Cryptochromes. *Cell* 152(5):1106-1118.
33. Rothenfluh A, Abodeely M, Price JL, & Young MW (2000) Isolation and analysis of six timeless alleles that cause short- or long-period circadian rhythms in *Drosophila*. *Genetics* 156(2):665-675.
34. Saez L & Young MW (1996) Regulation of nuclear entry of the *Drosophila* clock proteins period and timeless. *Neuron* 17(5):911-920.

SUPPLEMENTARY MATERIAL ONLINE

35. Rothenfluh A, Young MW, & Saez L (2000) A TIMELESS-independent function for PERIOD proteins in the Drosophila clock. *Neuron* 26(2):505-514.
36. Singh S, *et al.* (2019) New Drosophila Circadian Clock Mutants Affecting Temperature Compensation Induced by Targeted Mutagenesis of Timeless. *Frontiers in physiology* 10:1442.
37. Rosato E, *et al.* (1997) Conceptual translation of timeless reveals alternative initiating methionines in Drosophila. *Nucleic Acids Res* 25(3):455-458.
38. Tauber E, *et al.* (2007) Natural selection favors a newly derived timeless allele in Drosophila melanogaster. *Science* 316(5833):1895-1898.
39. Matsumoto A, Tomioka K, Chiba Y, & Tanimura T (1999) timrit Lengthens circadian period in a temperature-dependent manner through suppression of PERIOD protein cycling and nuclear localization. *Mol Cell Biol* 19(6):4343-4354.
40. Wulbeck C, Szabo G, Shafer OT, Helfrich-Forster C, & Stanewsky R (2005) The novel Drosophila tim(blind) mutation affects behavioral rhythms but not periodic eclosion. *Genetics* 169(2):751-766.