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SHORT COMMUNICATION

Identification of the molecular components of a putative *Jasus edwardsii* (Crustacea; Decapoda; Achelata) circadian signaling system

Andrew E. Christie[1]

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Abstract

Like all organisms, members of the crustacean order Decapoda must coordinate their physiology and behavior to accommodate recurring patterns of environmental change. Genetically encoded biological clocks are responsible, at least in part, for the proper timing of these organism-environment patternings. While biological clocks cycling on a wide range of timescales have been identified, the circadian signaling system, which serves to coordinate physiological/behavioral events to the solar day, is perhaps the best known and most thoroughly investigated. While many circadian patterns of physiology/behavior have been documented in decapods, few data exist concerning the identity of circadian genes/proteins in members of this taxon. In fact, large collections of circadian genes/proteins have been described from just a handful of decapod species. Here, a publicly accessible transcriptome, produced from tissues that included the nervous system (brain and eyestalk ganglia), was used to identify the molecular components of a circadian signaling system for rock lobster, *Jasus edwardsii*, a member of the decapod infraorder Achelata. Complete sets of core clock (those involved in the establishment of the molecular feedback loop that allows for ~ 24-h cyclical timing), clock-associated (those involved in modulation of core clock output), and clock input pathway (those that allow for synchronization of the core clock to the solar day) genes/proteins are reported. This is the first description of a putative circadian signaling system from any member of the infraorder Achelata, and as such, expands the decapod taxa for which complete complements of putative circadian genes/proteins have been identified.

Keywords Bioinformatics · Transcriptome shotgun assembly (TSA) · Circadian signaling

Introduction

To survive, all organisms must coordinate their physiology and behavior to recurring patterns of environmental change, e.g., tidal cycles, day versus night, changing seasons, etc. This organism-environment coordination is achieved through the actions of innate, genetically encoded time keeping systems, commonly referred to as biological clocks, which operate on multiple timescales to coordinate physiological and behavioral processes to a wide array of environmental rhythms (e.g., Golombek et al. 2014). While a number of biological clocks have been described, the best known and most thoroughly investigated is undoubtedly the circadian signaling system, which is responsible for controlling physiological and behavioral events that are synchronized to the solar day and recur on an approximately 24-h cycle (e.g., Allada and Chung 2010; Hardin 2011; Mendoza-Viveros et al. 2017; Ozkaya and Rosato 2012; Yoshii et al. 2015).

In decapod crustaceans, many physiological and behavioral processes have been shown to exhibit ~ 24-h cyclical timing and are known or have been hypothesized to be regulated, at least in part, by circadian signaling systems (e.g., Strauss and Dircksen 2010). These physiological/behavioral events include, but are not limited to, larval metamorphosis (e.g., Fitzgibbon and Battaglene 2012; Matsuda et al. 2003), locomotion (e.g., Ziegler et al. 2010), and feeding and its associated specific dynamic action (e.g., Radford et al. 2004). Interestingly, and somewhat surprisingly given the extensive documentation of circadian rhythms in decapods, little is known about the identity of the genes and proteins

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✉ Andrew E. Christie
crabman@pbrc.hawaii.edu

[1] Békésy Laboratory of Neurobiology, Pacific Biosciences Research Center School of Ocean and Earth Science and Technology, University of Hawaii at Manoa, 1993 East-West Road, Honolulu, HI 96822, USA

Table 1 Putative *Jasus edwardsii* circadian signaling system transcripts/proteins identified via *in silico* transcriptome mining

Circadian signaling system protein		Transcript/protein identifications					
Clock component	Family	Transcript			Deduced protein		
		Accession no.	Trinity no.	Length*	Name	Length†	Type
Core clock protein	CLK	GGHM01081537	TRINITY_DN43397_c0_g1_i1	1908	Jased-CLK	557	N
	CRY2	GGHM01093483	TRINITY_DN45252_c0_g1_i1	2432	Jased-CRY2	549	F
	CYC	GGHM01054737	TRINITY_DN35788_c0_g1_i1	2432	Jased-CYC	661	F
	PER	GGHM01119147	TRINITY_DN47951_c0_g2_i6	6153	Jased-PER-v1	1272	F
		GGHM01119137	TRINITY_DN47951_c0_g2_i1	6115	Jased-PER-v1	1272	F
		GGHM01119145	TRINITY_DN47951_c0_g2_i5	6123	Jased-PER-v2	1262	F
		GGHM01119144	TRINITY_DN47951_c0_g2_i4	5207	Jased-PER-v3	1038	C
		GGHM01119139	TRINITY_DN47951_c0_g2_i3	4770	Jased-PER-v4	892	C
	TIM	GGHM01114036	TRINITY_DN47593_c5_g1_i1	1027	Jased-TIM-v1-NT	266	N
		GGHM01114041	TRINITY_DN47593_c5_g1_i6	1260	Jased-TIM-v1-NT	266	N
		GGHM01114039	TRINITY_DN47593_c5_g1_i4	1349	Jased-TIM-v1-NT	266	N
		GGHM01114042	TRINITY_DN47593_c5_g1_i7	947	Jased-TIM-v2-NT	289	N
		GGHM01114040	TRINITY_DN47593_c5_g1_i5	764	Jased-TIM-v3-NT	228	N
		GGHM01114038	TRINITY_DN47593_c5_g1_i3	1086	Jased-TIM-v3-NT	228	N
		GGHM01114037	TRINITY_DN47593_c5_g1_i2	997	Jased-TIM-v4-NT	288	N
		GGHM01132999	TRINITY_DN48984_c0_g1_i5	4677	Jased-TIM-CT	1269	C
		GGHM01132997	TRINITY_DN48984_c0_g1_i3	4706	Jased-TIM-CT	1269	C
Clock-associated protein	CKIIα	GGHM01030525	TRINITY_DN24195_c0_g1_i1	2245	Jased-CKIIα	348	F
	CKIIβ	GGHM01085212	TRINITY_DN43957_c1_g1_i2	951	Jased-CKIIβ-v1	220	F
		GGHM01085211	TRINITY_DN43957_c1_g1_i1	1375	Jased-CKIIβ-v2	230	F
	CWO	GGHM01087814	TRINITY_DN44402_c0_g1_i1	4013	Jased-CWO-v1	739	F
		GGHM01087815	TRINITY_DN44402_c0_g1_i2	3965	Jased-CWO-v2	723	F
	DBT	GGHM01062150	TRINITY_DN38250_c0_g1_i5	2697	Jased-DBT-v1	393	F
		GGHM01062148	TRINITY_DN38250_c0_g1_i3	2592	Jased-DBT-v1	393	F
		GGHM01062153	TRINITY_DN38250_c0_g1_i8	1869	Jased-DBT-v2	345	F
		GGHM01062149	TRINITY_DN38250_c0_g1_i4	1974	Jased-DBT-v2	345	F
		GGHM01062147	TRINITY_DN38250_c0_g1_i2	1914	Jased-DBT-v2	345	F
		GGHM01062146	TRINITY_DN38250_c0_g1_i1	1809	Jased-DBT-v2	345	F
		GGHM01062152	TRINITY_DN38250_c0_g1_i7	2298	Jased-DBT-v3	412	F
		GGHM01062151	TRINITY_DN38250_c0_g1_i6	2193	Jased-DBT-v3	412	F
	JET	GGHM01099316	TRINITY_DN46054_c3_g1_i4	1748	Jased-JET	284	F
		GGHM01099314	TRINITY_DN46054_c3_g1_i2	1825	Jased-JET	284	F
		GGHM01099313	TRINITY_DN46054_c3_g1_i1	1843	Jased-JET	284	F
	PDP1	GGHM01070131	TRINITY_DN40690_c0_g1_i3	1764	Jased-PDP1-v1	312	F
		GGHM01070130	TRINITY_DN40690_c0_g1_i2	1788	Jased-PDP1-v2	320	F
		GGHM01070135	TRINITY_DN40690_c0_g1_i6	1832	Jased-PDP1-v3	226	F
		GGHM01070137	TRINITY_DN40690_c0_g1_i8	2308	Jased-PDP1-v4	432	F
		GGHM01070139	TRINITY_DN40690_c0_g1_i10	1856	Jased-PDP1-v5	234	F
		GGHM01070133	TRINITY_DN40690_c0_g1_i4	2332	Jased-PDP1-v6	440	F
		GGHM01070138	TRINITY_DN40690_c0_g1_i9	1587	Jased-PDP1-v7	82	F
		GGHM01070136	TRINITY_DN40690_c0_g1_i7	2005	Jased-PDP1-v8	77	F
		GGHM01070129	TRINITY_DN40690_c0_g1_i1	1412	Jased-PDP1-v9	213	C
		GGHM01070134	TRINITY_DN40690_c0_g1_i5	1739	Jased-PDP1-v10	322	C
		GGHM01070140	TRINITY_DN40690_c0_g1_i11	1436	Jased-PDP1-v11	221	C
	PP1	GGHM01013421	TRINITY_DN11624_c0_g1_i1	653	Jased-PP1-I-NT	213	N
		GGHM01064652	TRINITY_DN39038_c0_g1_i1	4129	Jased-PP1-I-CT	169	C
		GGHM01099655	TRINITY_DN46037_c0_g1_i1	2910	Jased-PP1-II	329	F

Table 1 (continued)

Circadian signaling system protein		Transcript/protein identifications					
Clock component	Family	Transcript			Deduced protein		
		Accession no.	Trinity no.	Length*	Name	Length†	Type
		GGHM01053218	TRINITY_DN35289_c0_g1_i1	1942	Jased-PP1-III	313	F
	MTS	GGHM01091013	TRINITY_DN44869_c0_g1_i1	1310	Jased-MTS-I-v1	309	F
		GGHM01091015	TRINITY_DN44869_c0_g1_i3	984	Jased-MTS-I-v2	115	F
		GGHM01053241	TRINITY_DN35222_c0_g1_i2	2385	Jased-MTS-II-v1	319	F
		GGHM01053240	TRINITY_DN35222_c0_g1_i1	2358	Jased-MTS-II-v2	333	F
	TWS	GGHM01064655	TRINITY_DN39048_c0_g1_i2	2914	Jased-TWS	446	F
	WDB	GGHM01135811	TRINITY_DN49148_c0_g1_i2	5867	Jased-WDB-v1	459	F
		GGHM01135810	TRINITY_DN49148_c0_g1_i1	5848	Jased-WDB-v1	459	F
		GGHM01135812	TRINITY_DN49148_c0_g1_i3	5962	Jased-WDB-v2	431	F
	SGG	GGHM01081871	TRINITY_DN43405_c0_g1_i2	5852	Jased-SGG-v1	410	F
		GGHM01081872	TRINITY_DN43405_c0_g1_i3	5864	Jased-SGG-v2	414	F
		GGHM01081870	TRINITY_DN43405_c0_g1_i1	5912	Jased-SGG-v3	430	F
	SLIMB	GGHM01050857	TRINITY_DN34369_c0_g1_i1	3235	Jased-SLIMB	556	F
	VRI	GGHM01044837	TRINITY_DN31714_c0_g1_i1	1759	Jased-VRI	585	I
Clock input pathway protein	CRY1	GGHM01084229	TRINITY_DN43841_c0_g1_i1	1762	Jased-CRY1	528	F

*Length in nucleotides; †Length in amino acids

Protein family abbreviations: CLK, clock; CRY2, cryptochrome 2; CYC, cycle; PER, period; TIM, timeless; CKIIα, casein kinase IIα; CKIIβ, casein kinase IIβ—CWO, clockwork orange; DBT, doubletime; JET, jetlag; PDP1, PAR-domain protein 1; PP1, protein phosphatase 1; MTS, protein phosphatase 2A catalytic subunit microtubule star; TWS, protein phosphatase 2A regulatory subunit twins; WDB, protein phosphatase 2A regulatory subunit widerborst; SGG, shaggy; SLIMB, supernumerary limbs; VRI, vrille; CRY1, cryptochrome 1

Protein type abbreviations: F, full-length; N, amino-terminal partial; C, carboxyl-terminal partial; I, internal fragment

Homarus americanus (Homam) circadian signaling system proteins identified and described in Christie et al. (2018a, 2018b) were used as the query proteins for all tblastn searches: CLK, Homam-CLK; CRY2, Homam-CRY2; CYC, Homam-CYC; PER, Homam-PER-v1; TIM, Homam-TIM-v1; CKIIα, Homam-CKIIα-v1; CKIIβ, Homam-CKIIβ—CWO, Homam-CWO-v1; DBT, Homam-DBT-v1; JET, Homam-JET-v1; PDP1, Homam-PDP1-v1; PP1, Homam-PP1-I-v1, Homam-PP1-II-v1 and Homam-PP1-III; MTS, Homam-MTS-I and Homam-MTS-II-v1; TWS, Homam-TWS-v1; WDB, Homam-WDB; SGG, Homam-SGG; SLIMB, Homam-SLIMB-v1; VRI, Homam-VRI; CRY1, Homam-CRY1-v1

responsible for the establishment of circadian pacemakers in members of this crustacean order. In fact, just a handful of studies currently exist describing seemingly complete sets of the genes/proteins needed for the establishment circadian signaling systems in decapods, all from members of the infraorder Astacidea (e.g., Christie et al. 2018a, b; Sbragaglia et al. 2015). In the study presented here, a spiny lobster, *Jasus edwardsii*, transcriptome (**BioProject No. PRJNA386609**; Souza et al. 2018), was used to elucidate the first complete set of circadian signaling system genes/proteins for a member of the decapod infraorder Achelata. The sources of RNA used to generate the *J. edwardsii* transcriptome included both the supraesophageal ganglion (brain) and the eyestalk ganglia (Souza et al. 2018), two nervous system regions shown previously to be loci of clock gene expression in other decapod species (e.g., Christie et al. 2018a; Sbragaglia et al. 2015).

Materials and methods

Transcriptome mining

Methods modified from those employed previously for circadian gene/protein discovery in other crustacean species (Christie et al. 2013, 2018a, b, c; Nesbit and Christie 2014; Roncalli et al. 2017; Tilden et al. 2011) were used to elucidate the molecular components of the *J. edwardsii* clock system. In brief, the database of the online program tblastn (http://blast.ncbi.nlm.nih.gov/Blast.cgi; National Center for Biotechnology Information [NCBI], Bethesda, MD, USA) was set to transcriptome shotgun assembly (TSA) and restricted to data from **BioProject PRJNA386609** (Souza et al. 2018). The query sequences used for all BLAST searches were lobster, *Homarus americanus*, circadian proteins that were described in several recent publications (Christie et al. 2018a, b). The complete list of clock proteins searched for and the specific queries used are provided in Table 1.

A Alignment of *Jasus edwardsii* and *Homarus americanus* cryptochrome 2

```
Jased-CRY2       MTGETYKSRPKNVVHWFRKGLRLHDNPALRAGLKNAQTYRCIFILDPWFAGSSNVGINKW
Homam-CRY2       MTGEASKVKAKHLVHWFRKGLRLHDNPALRAGLKNAHTFRCIFILDPWFAGSSNVGINKW
                 ****: * :.*::***********************:*:*********************

Jased-CRY2       RFLLQSLEDLDCSLRKLNSRLYVIRGQPAHVLPELFREWGTTCLTFEKDPEPFGKVRDAN
Homam-CRY2       RFLLQSLEDLDSSLRKLNSRLFVIRGQPAHVLPELFREWGTTCLTFEKDPEPFGKARDAN
                 ***********.*********:*********************************.****

Jased-CRY2       IIAIAREMGINVIVKTSHTLYKPEKIIDRNGGKPPLTYKTFQNILMNMDLPPLPQSTIGL
Homam-CRY2       IVAIAREMGINVIVKTSHTLYKPEKIIDKNGGKPPLTYKSFQNILMSMDLPLLPQSTITL
                 *:**************************:**********:******.**** ****** *

Jased-CRY2       EDVEDKYTPISDDHDEKYGVPTLEELGFDTESLQQTGWKGGESEALARLEHHLERKAWVA
Homam-CRY2       EDLEDKSTPLSDDHDEKYGVPTLEELGFETETLHQTGWKGGESEALTRLEHHLERKAWVA
                 **:*** **:******************:**:*:************:*************

Jased-CRY2       SFGRPKMTPQSLYPSRTGLSPYLRFGCLSCRRFFVELNDLYRKIKKSPPPLSLHGQMLWR
Homam-CRY2       SFGRPKMTPQSLYPSRTGLSPYLRFGCLSCRRFFAELNDLYRKIRKSPPPLSLHGQLLWR
                 **********************************.*********:***********:***

Jased-CRY2       EFYYTAATNNPKFDHMEGNPICVQIPWDKNPEALAKWANGQTGYPWVDAIMTQLRKEGWI
Homam-CRY2       EFYYTAATNNPKFDHMEGNPICVQIPWDKNPEALAKWANGQTGYPWVDAIMTQLRKEGWI
                 ************************************************************

Jased-CRY2       HNVARHAVACFLTRGDLWVSWEEGMKVFDELLLDADWSVNAGSWMWLSCSSFFQQFFHCY
Homam-CRY2       HNVARHAVACFLTRGDLWVSWEEGMKVFDELLLDADWSVNAGSWMWLSCSSFFQQFFHCY
                 ************************************************************

Jased-CRY2       CPVRYGRKADPNGDYIRTYLPVLKNFPTKYIHEPWTAPESVQRAAKCVVGRDYPMPMVDH
Homam-CRY2       CPVRYGRKADPNGDYIRMYLPVLKNFPTKYIHEPWTAPESVQRAAKCVIGRDYPMPMVDH
                 ***************** ******************************:***********

Jased-CRY2       IKQSQNNIERMKQVYQQLAHYRGKINPAIKSPTVDHYPSPPTEFEKKRSNKTGKNDIQRD
Homam-CRY2       IKQSQNNIERMKQVYQQLAHYRGKINPAIKSPTVDHYPSPPPEFEKKRSSKTNKNDLQYD
                 *****************************************.*******.**.***:* *

Jased-CRY2       SNYRVQVQA
Homam-CRY2       SNYRVQVQA
                 *********
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B Alignment of *Jasus edwardsii* CRYPTOCHROME 2 and CRYPTOCHROME 1

```
Jased-CRY2       MTGETYKSRPKNVVHWFRKGLRLHDNPALRAGLKNAQTYRCIFILDPWFAGSSNVGINKW
Jased-CRY1       MS--------KKSVHWFRHGLRIHDNPALLESIKDAEKFYAIFIFDGETTAKNRTGYNRL
                 *:        *: *****:***:******  .:*:*:.: .***:*   :.....* *:

Jased-CRY2       RFLLQSLEDLDCSLRKLNSRLYVIRGQPAHVLPELFREWGTTCLTFEKDPEPFGKVRDAN
Jased-CRY1       HYLTEALNDLDSYFRELGSQLFVLKGEPVEVLRSLHKEAGINRVTFEQDCEAIWNKCDKA
                 ::* ::*:***. :*:*.*:*:*::*:*..** .*.:* * . :***:* *.: :  *

Jased-CRY2       IIAIAREMGINVIVKTSHTLYKPEKIIDRNGGKPPLTYKTFQNILMNMDLPPLPQSTIGL
Jased-CRY1       VHHICDELGIELVERISHTLWDPFKIIDTNGGQPPLTYDMFLQVAQALGPPPRPC-----
                 :  *. *:**::: : ****:.* **** ***:*****. * ::   :. ** *

Jased-CRY2       EDVEDK----YTPISDDHDEKYG----VPTLEELGFDTESLQQTGWKGGESEALARLEHH
Jased-CRY1       -DYPDKDNIIFGEISDELALKLKLYPHVPTPEELGFTQECGEFPVYVGGEHAALAHLEDR
                  *  **    :  ***:   *      *** *****  *. : . : ***  ***:**.:

Jased-CRY2       L--ERKAWVASFGRPKMTPQSLYPSRTGLSPYLRFGCLSCRRFFVELNDLYRKIKKSPPP
Jased-CRY1       LRVEEDAFEDGYILPNQVNPDLLGPPMSMSAALRFGCLSVRKFYWDIQNVYSKLFSTAPP
                 *  *..*:  .:  *: .  .*  .  .:*. ******* *:*: :::::* *: .:.**

Jased-CRY2       L--SLHGQMLWREFYYTAATNNPKFDHMEGNPICVQIPWDKNPEALAKWANGQTGYPWVD
Jased-CRY1       LSHSLTAQLIWREFFYCMSANNPNYDQMKGNPICIDIPWSTNDEYLSAWGEGLTGYPFID
                 *  ** .*::****:*  ::***::*:*:*****::***..* * *: *.:* ****::*

Jased-CRY2       AIMTQLRKEGWIHNVARHAVACFLTRGDLWVSWEEGMKVFDELLLDADWSVNAGSWMWLS
Jased-CRY1       ACMRQLRKEGWIHHVCRTAVACFLTRGDLWISWESGLKVFYKYLIDADWSVSAGNWMWVS
                 * * *********:*.* ************:***.*:*** : *:******.**.***:*

Jased-CRY2       CSSFFQQF--FHCYCPVRYGRKADPNGDYIRTYLPVLKNFPTKYIHEPWTAPESVQRAAK
Jased-CRY1       SSAFERQLDCSTCICPVNYGRTVEPSGDYIRRYVPEVATLPQEYIFEPWKAPVKVQKRAK
                 .*:* :*:    * ***.***..:*.***** *:* : .:* :**.***.** .**: **

Jased-CRY2       CVVGRDYPMPMVDHIKQSQNNIERMKQVYQQLAHYRGKINPAIKSPTVDHYPSPPTEFEK
Jased-CRY1       CIVGRDYPHRIVIHEEVSKVNRKKMEDVCQKLKKKPPHICPSNTEETKSFLRLPKSCFHN
                 *:******  :* * : *: * ::*::* *:* :   :* *: .. * ..   * : *.:

Jased-CRY2       KRSNKTGKNDIQRDSNYRVQVQA
Jased-CRY1       ---------------------VL
```

◂**Fig. 1** MAFFT alignments of *Jasus edwardsii* cryptochrome 2 (Jased-CRY2) and related proteins. **a** Alignment of Jased-CRY2 and *Homarus americanus* cryptochrome 2 (Homam-CRY2). **b** Alignment of Jased-CRY2 and *J. edwardsii* cryptochrome 1 (Jased-CRY1). In the line immediately below each sequence grouping, "*" indicates identical amino acid residues, while ":" and "." denote amino acids that are similar in structure between sequences. In this figure, DNA photolyase domains and FAD binding domains of DNA photolyase identified by Pfam analyses are highlighted in pink and blue, respectively. Homam-CRY2 (**Accession No. AWC08575**; Christie et al. 2018a) (color figure online)

Confirmation of protein identifications

A workflow used previously to increase confidence in the annotation of a variety of proteins, including those involved in circadian signaling (Christie et al. 2013, 2018a, b, c; Nesbit and Christie 2014; Roncalli et al. 2017; Tilden et al. 2011), was employed to provide support for the protein family annotations ascribed to the sequences deduced from *J. edwardsii* transcripts. Specifically, nucleotide sequences were translated using the Translate tool of ExPASy (http://web.expasy.org/translate/) and assessed for completeness. Proteins listed as full-length exhibit a start methionine and are flanked on their carboxyl (C)-termini by a stop codon. Proteins described here as partial lack a start methionine (referred to as C-terminal partial proteins), a stop codon (referred to as amino [N]-terminal partial proteins), or both of these features (referred to as internal fragment proteins). Next, each *J. edwardsii* protein (except cryptochrome 1 [CRY1] and cryptochrome 2 [CRY2]) was used as the input query in a blastp search of annotated *Drosophila melanogaster* protein dataset present in FlyBase (version FB2018_05; Gramates et al. 2017) to confirm that its top hit is a *D. melanogaster* homolog of the same protein family that it was annotated as being a member of; *Drosophila* has arguably the most thoroughly investigated/vetted circadian system of any organism (e.g., Allada and Chung 2010; Hardin 2011; Mendoza-Viveros et al. 2017; Ozkaya and Rosato 2012; Yoshii et al. 2015), and FlyBase is the most complete and thoroughly characterized single-species protein database extant for any member of the Arthropoda (Gramates et al. 2017). For CRY1 (also referred to *D. melanogaster*-type CRY or dCRY) and CRY2 (also referred to as mammalian-type CRY or mCRY), blastp searches of the annotated monarch butterfly, *Danaus plexippus*, protein dataset in NCBI (taxid:13037) were substituted for the FlyBase search, as *D. melanogaster* lacks CRY2, but *Danaus* possesses well-characterized genes for both CRYs (e.g., Yuan et al. 2007). Next, the top arthropod protein hit for each *Jasus* sequence was identified by conducting a blastp search of the non-redundant arthropod protein dataset curated at NCBI (taxid:6656). Finally, protein structural motifs were analyzed for each *J. edwardsii* protein using the online program Pfam (http://pfam.xfam.org/; El-Gebali et al. 2019) version 32.0, and the identified domains compared to those identified by the program for the protein's top FlyBase and NCBI non-redundant arthropod protein hits. This vetting workflow was conducted on or before December 24, 2018. All protein alignments were done using the online program MAFFT version 7.408 (http://mafft.cbrc.jp/alignment/software/; Katoh and Standley 2013).

Results

To generate a functional circadian signaling system requires members of four distinct classes of genes/proteins, i.e., core clock, clock-associated, clock input pathway, and clock output pathway genes/proteins (e.g., Allada and Chung 2010). The genes/proteins of the core clock are responsible for establishing the basic molecular feedback loop that allows for approximately 24-h cyclical timing, while members of the clock-associated group provide modulatory control over the core clock cascade. Members of the clock input pathway provide a mechanism for synchronizing the core clock to the solar day. Finally, members of the clock output pathway provide a means for transmitting timing information from the cellular locus of the core clock to affect physiology and behavior either directly or indirectly. In the sections that follow, the *J. edwardsii* members of each of first three classes of genes/proteins are discussed in turn. A description of potential *J. edwardsii* clock output pathway members (e.g., peptide paracrines/hormones and their cognate receptors) was the subject of an earlier report (Christie and Yu 2019).

Core clock proteins

The fruit fly, *D. melanogaster*, has arguably the most thoroughly investigated clock system of any organism (Allada and Chung 2010; Hardin 2011; Mendoza-Viveros et al. 2017; Ozkaya and Rosato 2012; Yoshii et al. 2015). In this species, four interacting genes/proteins, clock (CLK), cycle (CYC), period (PER), and timeless (TIM), are responsible for the generation of the molecular cascade of the core clock. Binding of a CLK-CYC protein heterodimer to E-box elements in the promoter regions of the PER and TIM genes initiates a core clock molecular cascade by activating their transcription (and allowing for their translation). PER and TIM proteins subsequently accumulate in the cytoplasm where they dimerize. Following dimerization, the PER-TIM protein heterodimer is translocated to the nucleus where it binds to CLK-CYC, inhibiting its activation of the PER and TIM genes. While arguably the most thoroughly investigated, the *D. melanogaster* circadian system is not necessarily stereotypical of arthropod clocks in that it lacks CRY2, a core clock component in ancestral-type clock systems,

Table 2 Most similar *Drosophila melanogaster* protein* to each predicted *Jasus edwardsii* circadian protein sequence

Jasus protein		Top FlyBase *Drosophila* annotated protein hit			
Clock component	Query protein	Accession no.	Name	BLAST statistics	
				Score	*E*-value
Core clock protein	Jased-CLK	AAF50516	Clock, isoform A	425	9e-119
	Jased-CRY2	ABA62409	Cryptochrome 2*	800	0.0
	Jased-CYC	AAF49107	Cycle	453	4e-127
	Jased-PER-v1	AAF49107	Cycle	89	4e-17
	Jased-PER-v2	AAF49107	Cycle	89	4e-17
	Jased-PER-v3	AAF49107	Cycle	88	6e-17
	Jased-PER-v4	AAF49107	Cycle	76	2e-13
	Jased-TIM-v1	AAF51098	Timeless, isoform B	351	6e-96
	Jased-TIM-v2	API64979	Timeless, isoform T	350	9e-96
	Jased-TIM-v3	API64979	Timeless, isoform T	350	8e-96
	Jased-TIM-v4	AAF51098	Timeless, isoform B	351	5e-96
Clock-associated proteins	Jased-CKIIα	AAN11415	Casein kinase II alpha, isoform A	570	1e-162
	Jased-CKIIβ-v1	AAF48093	Casein kinase II beta subunit, isoform B	407	6e-114
	Jased-CKIIβ-v2	AAF48093	Casein kinase II beta subunit, isoform B	408	3e-114
	Jased-CWO-v1	AAF54527	Clockwork orange, isoform A	127	7e-29
	Jased-CWO-v2	AAF54527	Clockwork orange, isoform A	127	8e-29
	Jased-DBT-v1	AAF57110	Discs overgrown, isoform A	546	2e-155
	Jased-DBT-v2	AAF57110	Discs overgrown, isoform A	547	1e-155
	Jased-DBT-v3	AAF57110	Discs overgrown, isoform A	548	5e-156
	Jased-JET	ABI31287	Jetlag, isoform B	128	1e-29
	Jased-PDP1-v1	AAN12026	PAR-domain protein 1, isoform J	199	4e-51
	Jased-PDP1-v2	AGB94240	PAR-domain protein 1, isoform M	192	7e-49
	Jased-PDP1-v3	AAN12026	PAR-domain protein 1, isoform J	160	1e-39
	Jased-PDP1-v4	AAN12026	PAR-domain protein 1, isoform J	199	7e-51
	Jased-PDP1-v5	AAN12026	PAR-domain protein 1, isoform J	153	2e-37
	Jased-PDP1-v6	AAN12026	PAR-domain protein 1, isoform J	192	1e-48
	Jased-PDP1-v7	AAN12021	PAR-domain protein 1, isoform F	109	1e-24
	Jased-PDP1-v8	AAN12021	PAR-domain protein 1, isoform F	96	5e-21
	Jased-PDP1-v9	AAN12026	PAR-domain protein 1, isoform J	154	1e-37
	Jased-PDP1-v10	AAN12026	PAR-domain protein 1, isoform J	200	3e-51
	Jased-PDP1-v11	AAN12026	PAR-domain protein 1, isoform J	147	1e-35
	Jased-PP1-I	AAF46583	Flapwing, isoform B	607	9e-174
	Jased-PP1-II	AAF56306	Protein phosphatase 1alpha at 96A, isoform A	623	8e-179
	Jased-PP1-III	AAF56306	Protein phosphatase 1alpha at 96A, isoform A	423	1e-118
	Jased-MTS-I-v1	AAF52567	Microtubule star, isoform A	609	2e-174
	Jased-MTS-I-v2	AAF52567	Microtubule star, isoform A	243	3e-65
	Jased-MTS-II-v1	AAF52567	Microtubule star, isoform A	363	2e-100
	Jased-MTS-II-v2	AAF52567	Microtubule star, isoform A	359	3e-99
	Jased-TWS	AAF54499	Twins, isoform B	810	0.0
	Jased-WDB-v1	AAF56720	Widerborst, isoform A	792	0.0
	Jased-WDB-v2	AAF56720	Widerborst, isoform A	759	0.0
	Jased-SGG-v1	AAN09082	Shaggy, isoform A	673	0.0
	Jased-SGG-v2	AAN09082	Shaggy, isoform A	666	0.0
	Jased-SGG-v3	AAN09082	Shaggy, isoform A	663	0.0
	Jased-SLIMB	AGB96182	Supernumerary limbs, isoform B	825	0.0
	Jased-VRI	AAN10539	Vrille, isoform C	173	6e-43
Clock input pathway proteins	Jased-CRY1	AAX58599	Cryptochrome*	580	0.0

BLAST searches conducted on or before December 24, 2018

Abbreviations: CLK, clock; CRY2, cryptochrome 2; CYC, cycle; PER, period; TIM, timeless; CKIIα, casein kinase IIα; CKIIβ, casein kinase IIβ—CWO, clockwork orange; DBT, doubletime; JET, jetlag; PDP1, PAR-domain protein 1; PP1, protein phosphatase 1; MTS, microtubule star; TWS, twins; WDB, widerborst; SGG, shaggy; SLIMB, supernumerary limbs; VRI, vrille; CRY1, cryptochrome 1

Table 2 (continued)

**D. plexippus* proteins were used as the query sequences for the BLAST searches for CRY1 and CRY2, as this species possesses both proteins, while *D. melanogaster* lacks CRY2 (*e.g.*, Yuan et al. 2007). Thus, for reciprocal BLASTs for these two proteins, the extant *D. plexippus* protein sequences curated in NCBI were the targeted dataset

Table 3 Most similar non-redundant arthropod protein to each predicted *Jasus edwardsii* circadian protein sequence

Jasus protein		Top NCBI non-redundant arthropod protein hit				
Clock component	Name	Accession no.	Species	Name	BLAST statistics	
					Score	*E*-value
Core clock protein	Jased-CLK	AWC08577	*Homarus americanus*	Clock	804	0.0
	Jased-CRY2	AWC08575	*Homarus americanus*	Cryptochrome 2	1093	0.0
	Jased-CYC	AWC08576	*Homarus americanus*	Cycle	1241	0.0
	Jased-PER-v1	AWC08578	*Homarus americanus*	Period isoform 1	1903	0.0
	Jased-PER-v2	AWC08578	*Homarus americanus*	Period isoform 1	1889	0.0
	Jased-PER-v3	ALC74274	*Nephrops norvegicus*	Period-like protein	1592	0.0
	Jased-PER-v4	ALC74274	*Nephrops norvegicus*	Period-like protein	1316	0.0
	Jased-TIM-v1	AWC08579	*Homarus americanus*	Timeless isoform 1	1902	0.0
	Jased-TIM-v2	AWC08579	*Homarus americanus*	Timeless isoform 1	1899	0.0
	Jased-TIM-v3	AWC08580	*Homarus americanus*	Timeless isoform 2	1905	0.0
	Jased-TIM-v4	AWC08580	*Homarus americanus*	Timeless isoform 2	1905	0.0
Clock-associated protein	Jased-CKIIα	ARJ31756	*Litopenaeus vannamei*	Casein kinase II subunit alpha	699	0.0
	Jased-CKIIβ-v1	ASW35085	*Procambarus clarkii*	Casein kinase II subunit beta isoform X3	467	6e-168
	Jased-CKIIβ-v2	ASW35085	*Procambarus clarkii*	Casein kinase II subunit beta isoform X3	470	3e-169
	Jased-CWO-v1	AXF35707	*Homarus americanus*	Clockwork orange	855	0.0
	Jased-CWO-v2	AXF35707	*Homarus americanus*	Clockwork orange	850	0.0
	Jased-DBT-v1	ROT67534	*Litopenaeus vannamei*	Casein kinase 1 epsilon	757	0.0
	Jased-DBT-v2	AXF35708	*Homarus americanus*	Doubletime	704	0.0
	Jased-DBT-v3	AXF35708	*Homarus americanus*	Doubletime	696	0.0
	Jased-JET	XP_013780499	*Limulus polyphemus*	F-box/LRR-repeat protein 15-like	172	4e-50
	Jased-PDP1-v1	XP_014614214	*Polistes canadensis*	Thyrotroph embryonic factor isoform X2	225	2e-70
	Jased-PDP1-v2	XP_014614214	*Polistes canadensis*	Thyrotroph embryonic factor isoform X2	218	2e-67
	Jased-PDP1-v3	KFB48620	*Anopheles sinensis*	AGAP006376-PB-like protein	175	2e-52
	Jased-PDP1-v4	XP_014614214	*Polistes canadensis*	Thyrotroph embryonic factor isoform X2	221	3e-67
	Jased-PDP1-v5	KFB48620	*Anopheles sinensis*	AGAP006376-PB-like protein	167	2e-49
	Jased-PDP1-v6	XP_016836686	*Nasonia vitripennis*	Hepatic leukemia factor isoform X4	220	7e-65
	Jased-PDP1-v7	PNF27330	*Cryptotermes secundus*	Hepatic leukemia factor	111	1e-30
	Jased-PDP1-v8	XP_002404320	*Ixodes scapularis*	Thyrotroph embryonic factor	99	4e-26
	Jased-PDP1-v9	XP_002404320	*Ixodes scapularis*	Thyrotroph embryonic factor	162	5e-49
	Jased-PDP1-v10	XP_012218190	*Linepithema humile*	Thyrotroph embryonic factor isoform X13	221	5e-68
	Jased-PDP1-v11	XP_002404320	Ixodes scapularis	Thyrotroph embryonic factor	155	7e-46
	Jased-PP1-I	AXF35713	*Homarus americanus*	Protein phosphatase 1a-B	669	0.0
	Jased-PP1-II	AQZ36563	*Penaeus monodon*	Serine/threonine protein phosphatase 1	684	0.0
	Jased-PP1-III	AXF35715	*Homarus americanus*	Protein phosphatase 1a-D	622	0.0

Table 3 (continued)

Jasus protein		Top NCBI non-redundant arthropod protein hit				
Clock component	Name	Accession no.	Species	Name	BLAST statistics	
					Score	*E*-value
	Jased-MTS-I-v1	AXF35717	*Homarus americanus*	Protein phosphatase 2A microtubule star-B	650	0.0
	Jased-MTS-I-v2	KMQ93778	*Lasius niger*	Serine–threonine protein phosphatase 2a catalytic subunit alpha isoform-like protein	266	2e-86
	Jased-MTS-II-v1	AXF35719	*Homarus americanus*	Protein phosphatase 2A microtubule star-D	559	0.0
	Jased-MTS-II-v2	AXF35719	*Homarus americanus*	Protein phosphatase 2A microtubule star-D	527	0.0
	Jased-TWS	AXF35703	*Homarus americanus*	Protein phosphatase 2A twins	926	0.0
	Jased-WDB-v1	AXF35720	*Homarus americanus*	Protein phosphatase 2A widerborst-A	941	0.0
	Jased-WDB-v2	AXF35720	*Homarus americanus*	Protein phosphatase 2A widerborst-A	883	0.0
	Jased-SGG-v1	ASW35107	*Procambarus clarkii*	Glycogen synthase kinase 3 beta	847	0.0
	Jased-SGG-v2	ASW35107	*Procambarus clarkii*	Glycogen synthase kinase 3 beta	840	0.0
	Jased-SGG-v3	ASW35107	*Procambarus clarkii*	Glycogen synthase kinase 3 beta	837	0.0
	Jased-SLIMB	ROT73729	*Litopenaeus vannamei*	F-box/WD repeat-containing protein 1A	1013	0.0
	Jased-VRI	ROT80122	*Litopenaeus vannamei*	Nuclear factor interleukin-3-regulated protein-like	379	2e-124
Clock input pathway protein	Jased-CRY1	ROT73690	*Litopenaeus vannamei*	Cryptochrome precursor	829	0.0

BLAST searches conducted on or before December 24, 2018

Abbreviations: CLK, clock; CRY2, cryptochrome 2; CYC, cycle; PER, period; TIM, timeless; CKIIα, casein kinase IIα; CKIIβ, casein kinase IIβ—CWO, clockwork orange; DBT, doubletime; JET, jetlag; PDP1, PAR-domain protein 1; PP1, protein phosphatase 1; MTS, microtubule star; TWS, twins; WDB, widerborst; SGG, shaggy; SLIMB, supernumerary limbs; VRI, vrille; CRY1, cryptochrome 1

e.g., the monarch butterfly, *D. plexippus,* and clock system, which serves as inhibitor of CLK-CYC-mediated transcription (e.g., Yuan et al. 2007).

Using known *D. melanogaster* or *D. plexippus* proteins as query sequences, all components of an "ancestral-type" core clock were recently predicted from *H. americanus* neural transcriptomes (Christie et al. 2018a, b), including both brain- and eyestalk ganglia-specific assemblies (Christie et al. 2018a). Here, the deduced *Homarus* proteins were used to identify transcripts encoding putative *J. edwardsii* CLK, CRY2, CYC, PER, and TIM isoforms (Table 1 and Supplemental Fig. 1). Translation of the identified *Jasus* transcripts revealed one isoform of CLK, one isoform of CRY2 (Fig. 1), one isoform of CYC, four isoforms of PER, and four isoforms of TIM. All of the deduced *J. edwardsii* proteins appear to be full-length sequences, with the exceptions of Jased-CLK, which is an N-terminal partial protein, and Jased-PER-v3 and v4, both of which are C-terminal partial proteins. Based on nucleotide and amino acid sequence variation, as well as on the unique Trinity numbers assigned to the transcripts encoding the proteins, it appears that alternative splicing of single genes is responsible for PER and TIM protein diversity in *J. edwardsii*. Blastp searches of the annotated *D. melanogaster* proteins in FlyBase (the annotated *D. plexippus* protein dataset used for Jased-CRY2) and the non-redundant arthropod proteins curated in NCBI largely support the provisional annotations of the deduced *Jasus* proteins as members of the core clock families to which they have been ascribed (Tables 2 and 3). In fact, the only exceptions were the top FlyBase hits for the four Jased-PER variants, where CYC rather than PER was returned as the top *D. melanogaster* hit (Table 2), a conundrum also found for some, but not all, of the previously reported *Homarus* PER variants and attributed to the presence/absence of 16 amino acid insertion/deletion, with the sequence DGKSSTHPAFSLCGGS (see Christie et al. 2018a for details); all four *Jasus* PERs possess a nearly identical sequence, i.e., DGKSISHPAFSMCDGS. The results of structural/functional domain analysis conducted on each of the *Jasus* core clock sequences using the online program Pfam (and comparison with those identified by the program for the top

Table 4 Structural domains/regions identified by Pfam in deduced *Jasus edwardsii* circadian signaling system proteins

Jasus protein		Identified domains/regions (amino acid coordinates)
Clock component	Name	
Core clock protein	Jased-CLK	Helix-loop-helix DNA-binding (28–78); PAS fold (102–203); PAS (290–398)
	Jased-CRY2	DNA photolyase (13–177); FAD binding domain of DNA photolyase (293–493)
	Jased-CYC	Helix-loop-helix DNA-binding (56–107); PAS fold (150–196); PAS (329–437)
	Jased-PER-v1	PAS (364–472); Period protein 2/3C-terminal region (1042–1237)
	Jased-PER-v2	PAS (364–472); Period protein 2/3C-terminal region (1043–1227)
	Jased-PER-v3	PAS (148–256); Period protein 2/3C-terminal region (806–1003)
	Jased-PER-v4	PAS (12–120); Period protein 2/3C-terminal region (671–857)
	Jased-TIM-v1	Timeless protein (62–359)
	Jased-TIM-v2	Timeless protein (81–382)
	Jased-TIM-v3	Timeless protein (26–321)
	Jased-TIM-v4	Timeless protein (86–381)
Clock-associated protein	Jased-CKIIα	Protein kinase (37–322)
	Jased-CKIIβ-v1	Casein kinase II regulatory subunit (8–190)
	Jased-CKIIβ-v1	Casein kinase II regulatory subunit (18–200)
	Jased-CWO-v1	Helix-loop-helix DNA-binding (122–176); Hairy orange (201–242)
	Jased-CWO-v2	Helix-loop-helix DNA-binding (122–176); Hairy orange (201–241)
	Jased-DBT-v1	Protein kinase (9–280)
	Jased-DBT-v2	Protein kinase (9–280)
	Jased-DBT-v3	Protein kinase (9–280)
	Jased-JET	F-box (8–44); Leucine rich repeat (203–227)
	Jased-PDP1-v1	Basic region leucine zipper (245–310)
	Jased-PDP1-v2	Basic region leucine zipper (253–318)
	Jased-PDP1-v3	Basic region leucine zipper (159–225)
	Jased-PDP1-v4	Basic region leucine zipper (365–429)
	Jased-PDP1-v5	Basic region leucine zipper (167–226)
	Jased-PDP1-v6	Basic region leucine zipper (373–437)
	Jased-PDP1-v7	Basic region leucine zipper (15–81)
	Jased-PDP1-v8	Basic region leucine zipper (10–76)
	Jased-PDP1-v9	Basic region leucine zipper (146–201)
	Jased-PDP1-v10	Basic region leucine zipper (255–320)
	Jased-PDP1-v11	Basic region leucine zipper (154–209)
	Jased-PP1-I	Serine–threonine protein phosphatase N-terminal (8–55); Calcineurin-like phosphoesterase (56–251)
	Jased-PP1-II	Serine–threonine protein phosphatase N-terminal (9–56); Calcineurin-like phosphoesterase (57–252)
	Jased-PP1-III	Serine–threonine protein phosphatase N-terminal (16–62); Calcineurin-like phosphoesterase (63–258)
	Jased-MTS-I-v1	Calcineurin-like phosphoesterase (50–245)
	Jased-MTS-I-v2	No domains/regions identified
	Jased-MTS-II-v1	Calcineurin-like phosphoesterase (58–256)
	Jased-MTS-II-v2	Calcineurin-like phosphoesterase (58–256)
	Jased-TWS	WD40 repeat (11–56)
	Jased-WDG-v1	B56 family protein phosphatase 2A regulatory B subunit (44–449)
	Jased-WDG-v2	B56 family protein phosphatase 2A regulatory B subunit (44–431)
	Jased-SGG-v1	Protein kinase (54–338)
	Jased-SGG-v2	Protein kinase (58–342)
	Jased-SGG-v3	Protein kinase (54–358)
	Jased-SLIMB	D domain of beta-TrCP (89–127); F-box-like (134–181); WD40 repeat (241–278, 282–318, 365–401, 405–441, 445–481, 495–531)
	Jased-VRI	Basic region leucine zipper (293–346)
Clock input pathway protein	Jased-CRY1	DNA photolyase (5–170); FAD binding domain of DNA photolyase (291–493)

CLK, clock; CRY2, cryptochrome 2; CYC, cycle; PER, period; TIM, timeless; CKIIα, casein kinase IIα; CKIIβ, casein kinase IIβ—CWO, clockwork orange; DBT, doubletime; JET, jetlag; PDP1, PAR-domain protein 1; PP1, protein phosphatase 1; MTS, microtubule star; TWS, twins; WDB, widerborst; SGG, shaggy; SLIMB, supernumerary limbs; VRI, vrille; CRY1, cryptochrome 1

A Alignment of *Jasus edwardsii* doubletime variants

```
Jased-DBT-v1     MELRVGNKYRLGRKIGSGSFGDIYLGTNISTGEEVAIKLECIKTKHPQLHIESKFYKMMS
Jased-DBT-v2     MELRVGNKYRLGRKIGSGSFGDIYLGTNISTGEEVAIKLECIKTKHPQLHIESKFYKMMS
Jased-DBT-v3     MELRVGNKYRLGRKIGSGSFGDIYLGTNISTGEEVAIKLECIKTKHPQLHIESKFYKMMS
                 ************************************************************

Jased-DBT-v1     GGVGIPGIKWCGSEGDYNVMVMELLGPSLEDLFNFCSRKFSLKTVLLLADQLISRIEFIH
Jased-DBT-v2     GGVGIPGIKWCGSEGDYNVMVMELLGPSLEDLFNFCSRKFSLKTVLLLADQLISRIEFIH
Jased-DBT-v3     GGVGIPGIKWCGSEGDYNVMVMELLGPSLEDLFNFCSRKFSLKTVLLLADQLISRIEFIH
                 ************************************************************

Jased-DBT-v1     SKNFIHRDIKPDNFLMGLGKKGNLVYIIDFGLAKKYRDSRTHQHIVYRENKNLTGTARYA
Jased-DBT-v2     SKNFIHRDIKPDNFLMGLGKKGNLVYIIDFGLAKKYRDSRTHQHIVYRENKNLTGTARYA
Jased-DBT-v3     SKNFIHRDIKPDNFLMGLGKKGNLVYIIDFGLAKKYRDSRTHQHIVYRENKNLTGTARYA
                 ************************************************************

Jased-DBT-v1     SVNTHLGIEQSRRDDLESLGYVLMYFNRGSLPWQGLKAATKRQKYERISEKKMQTPIEEL
Jased-DBT-v2     SVNTHLGIEQSRRDDLESLGYVLMYFNRGSLPWQGLKAATKRQKYERISEKKMQTPIEEL
Jased-DBT-v3     SVNTHLGIEQSRRDDLESLGYVLMYFNRGSLPWQGLKAATKRQKYERISEKKMQTPIEEL
                 ************************************************************

Jased-DBT-v1     CKGFPNEFATYLNICRSLPFEEKPDYSHLRQLFRQLFHRQGFTYDYVFDWNMLKFGGSRN
Jased-DBT-v2     CKGFPNEFATYLNICRSLPFEEKPDYSHLRQLFRQLFHRQGFTYDYVFDWNMLKFGGSRN
Jased-DBT-v3     CKGFPNEFATYLNICRSLPFEEKPDYSHLRQLFRQLFHRQGFTYDYVFDWNMLKFGGSRN
                 ************************************************************

Jased-DBT-v1     QENEIERRDRQSHKPQATGATSRIRHDTTVGGVLPSPTAGELQAPPPPPAGSALPLPGHH
Jased-DBT-v2     QENEIERRDRQSHKPQATGATSRIRHDTTVGGVLPSPTAGK-------------------
Jased-DBT-v3     QENEIERRDRQSHKPQATGATSRIRHDTTVGGVLPSPTAGIGRMRSGSESEQRVSMRVHR
                 ****************************************

Jased-DBT-v1     TPSRDL---EPVRPVLLKTSWVPRSRSVPNRTGCLE----------------
Jased-DBT-v2     -------------PLVI-----------------------------------
Jased-DBT-v3     SGNSNAQTPELSRPTDRCVETWANSVSPPVSGGVIRRGSAGRDGTFRYPAKK
```

B Alignment of *Jasus edwardsii* shaggy variants

```
Jased-SGG-v1     MSGRPRTTSFAEGNKGPPSVSFPGMKISS----KDGNKITTVIATPGQGSDRPQEVSYMD
Jased-SGG-v2     MSGRPRTTSFAEGNKGPPSVSFPGMKISNTLFGKDGNKITTVIATPGQGSDRPQEVSYMD
Jased-SGG-v3     MSGRPRTTSFAEGNKGPPSVSFPGMKISS----KDGNKITTVIATPGQGSDRPQEVSYMD
                 ****************************.    ***************************

Jased-SGG-v1     TKVIGNGSFGVVFQAKLCETGELVAIKKVLQDKRFKNRELQIMRRLEHCNIVKLMYFFYS
Jased-SGG-v2     TKVIGNGSFGVVFQAKLCETGELVAIKKVLQDKRFKNRELQIMRRLEHCNIVKLMYFFYS
Jased-SGG-v3     TKVIGNGSFGVVFQAKLCETGELVAIKKVLQDKRFKNRELQIMRRLEHCNIVKLMYFFYS
                 ************************************************************

Jased-SGG-v1     SGDKKEEVFLNLVLEFIPETVYKVARHHSKQKQTIPISYIKLYMYQLFRSLAYIHSLGVC
Jased-SGG-v2     SGDKKEEVFLNLVLEFIPETVYKVARHHSKQKQTIPISYIKLYMYQLFRSLAYIHSLGVC
Jased-SGG-v3     SGDKKEEVFLNLVLEFIPETVYKVARHHSKQKQTIPISYIKLYMYQLFRSLAYIHSLGVC
                 ************************************************************

Jased-SGG-v1     HRDIKPQNLLLDPETGVLKLCDFGSAKHLVRGEPNVSYICSRYYRAPELIFGATDYTTNI
Jased-SGG-v2     HRDIKPQNLLLDPETGVLKLCDFGSAKHLVRGEPNVSYICSRYYRAPELIFGATDYTTNI
Jased-SGG-v3     HRDIKPQNLLLDPETGVLKLCDFGSAKHLVRGEPNVSYICSRYYRAPELIFGATDYTTNI
                 ************************************************************

Jased-SGG-v1     DVWSAGCVLAELLLGQPIFPGDSGVDQLVEIIKVLGTPTREQIREMNPNYTEFKFPQIKS
Jased-SGG-v2     DVWSAGCVLAELLLGQPIFPGDSGVDQLVEIIKVLGTPTREQIREMNPNYTEFKFPQIKS
Jased-SGG-v3     DVWSAGCVLAELLLGQPIFPGDSGVDQLVEIIKVLGTPTREQIREMNPNYTEFKFPQIKS
                 ************************************************************

Jased-SGG-v1     HPWQK--------------------VFRQRTPEDAINLVSRLLEYTPSARITPLQACAHK
Jased-SGG-v2     HPWQK--------------------VFRQRTPEDAINLVSRLLEYTPSARITPLQACAHK
Jased-SGG-v3     HPWQKALLSRMADSSNNDKKHQKLRVFRQRTPEDAINLVSRLLEYTPSARITPLQACAHK
                 *****                    ***********************************

Jased-SGG-v1     FFDELRDPNTRLPNNRELPPLYNFTEQEIKIQPELNSKLIPPHYRGEVANSSAGGGSSAV
Jased-SGG-v2     FFDELRDPNTRLPNNRELPPLYNFTEQEIKIQPELNSKLIPPHYRGEVANSSAGGGSSAV
Jased-SGG-v3     FFDELRDPNTRLPNNRELPPLYNFTEQEIKIQPELNSKLIPPHYRGEVANSSAGGGSSAV
                 ************************************************************

Jased-SGG-v1     DSSEGAAAAAINDN
Jased-SGG-v2     DSSEGAAAAAINDN
Jased-SGG-v3     DSSEGAAAAAINDN
                 **************
```

◂**Fig. 2** MAFFT alignments of *Jasus edwardsii* doubletime (Jased-DBT) and shaggy (Jased-SGG) variants. **a** Alignment of Jased-DBT-v1, v2, and v3. **b** Alignment of Jased-SGG-v1, v2, and v3. In the line immediately below each sequence grouping, "*" indicates identical amino acid residues, while ":" and "." denote amino acids that are similar in structure between sequences. In this figure, protein kinase domains identified by Pfam analyses are highlighted in teal (color figure online)

FlyBase and NCBI non-redundant arthropod protein hits) also support the provisional annotations ascribed to them (Table 4).

Clock-associated proteins

In *D. melanogaster*, a large number of genes/proteins have been shown to modulate core clock output (Allada and Chung 2010; Hardin 2011; Mendoza-Viveros et al. 2017; Ozkaya and Rosato 2012; Yoshii et al. 2015). These so-called clock-associated genes/proteins include those that control the state of phosphorylation and/or degradation of core clock proteins, i.e., the kinases casein kinase II (CKII; which consists of an α and a β subunit), doubletime (DBT), and shaggy (SGG), the phosphatases protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A, which consists of one catalytic subunit, microtubule star [MTS], and either of two regulatory subunits, twins [TWS] or widerborst [WDB]), the ligase supernumerary limbs (SLIMB), and the F-box protein jetlag (JET), as well as others that are hypothesized to play roles in modulating the phase, amplitude, and/or rhythmic output of the core clock, i.e., the transcription factors clockwork orange (CWO), PAR-domain protein 1 (PDP1), and vrille (VRI).

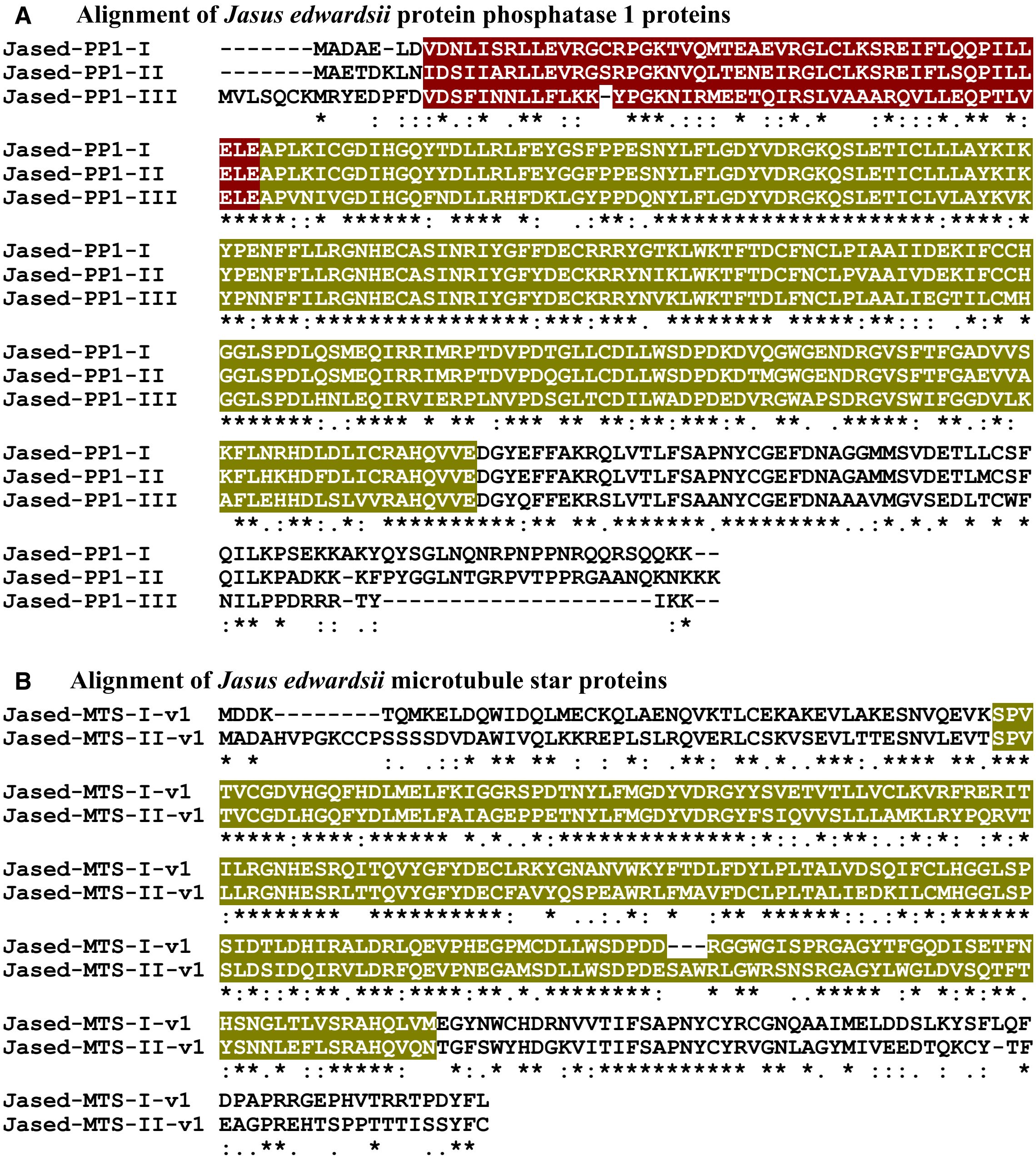
Here, recently predicted *H. americanus* isoforms of the above-mentioned clock-associated proteins (Christie et al. 2018a) were used to identify *J. edwardsii* transcripts encoding putative homologs of each family (Table 1 and Supplemental Fig. 1). Translation of the identified transcripts revealed one CKII α-subunit, two CKII β-subunits, two CWOs, three DBTs, one JET, 11 PDP1s, three PP1s, four PP2A MTSs, one PP2A TWS, two PP2A WDBs, three SGGs, one SLIMB, and one VRI. All of the deduced *Jasus* putative clock-associated proteins are full-length sequences except for Jased-PDP1-v9-11, each of which is a C-terminal partial protein, and Jased-VRI, which is an internal protein fragment (Table 1). The protein diversity seen in all *J. edwardsii* clock-associated protein families except for PP1 and MTS appears to arise from alternative splicing (e.g., the DBT and SGG isoforms shown in Fig. 2). Protein diversity for PP1 appears to be due to gene duplication, while that seen for MTS appears to be the result of both gene duplication and alternative splicing (Fig. 3). As for the putative *J. edwardsii* core clock proteins, searches of FlyBase returned an isoform of the expected protein family as the top *D. melanogaster* blastp hit for each of the putative *Jasus* clock-associated proteins listed above (Table 2). Similarly, the top hits from the blastp searches of the non-redundant arthropod proteins curated in NCBI also support the protein family attributions ascribed to each of the putative *Jasus* clock-associated proteins identified here (Table 3). Lastly, Pfam structural domain analyses support each of *J. edwardsii* clock-associated proteins as being members of clock-associated protein families to which they were annotated (Table 4).

Clock input pathway proteins

Clock input pathway genes/protein are responsible for synchronizing the core clock to the solar day. In *D. melanogaster*, and most other arthropods, CRY1, an ultraviolet/blue light photoreceptor, has been shown to serve this functional role (e.g., Allada and Chung 2010; Hardin 2011; Mendoza-Viveros et al. 2017; Ozkaya and Rosato 2012; Yoshii et al. 2015). Using the recently predicted sequence of a *H. americanus* CRY1 as the input query (Christie et al. 2018a), a single *J. edwardsii* transcript was identified as encoding a putative CRY1 homolog (Table 1, Fig. 2b and Supplemental Fig. 1). As for the *Jasus* core clock and clock-associated proteins, reciprocal BLAST and Pfam analyses strongly support the annotation of this *J. edwardsii* protein as a member of the CRY1 family (Tables 2, 3 and 4).

Discussion

Via in silico transcriptome mining, the genes/proteins putatively involved in the establishment of a *J. edwardsii* circadian signaling system were identified. These genes/proteins, in combination with those for peptide precursors and receptors reported previously (Christie and Yu 2019), represent the first description of a complete circadian system for any member of the Achelata, and one of just three currently extant for the Decapoda generally, the other two being from the American lobster, *H. americanus* (Christie et al. 2017, 2018a, b), and the Norway lobster, *Nephrops norvegicus* (Sbragaglia et al. 2015), both members of the infraorder Astacidea. Like the other crustaceans thus far studied (Christie et al. 2013, 2018a, b, c; Nesbit and Christie 2014; O'Grady et al. 2016; Roncalli et al. 2017; Sbragaglia et al. 2015; Tilden et al. 2011), the *J. edwardsii* circadian system appears to be of ancestral-type, in that both CRY2 and TIM are present, likely serving as transcriptional repressors in the core clock feedback loop, as is CRY1, which likely serves to provide solar entrainment to the core clock. Given that the description of the *J. edwardsii* circadian system reported here is the only one currently extant for any member of the Achelata, it represents a unique resource for



A Alignment of *Jasus edwardsii* protein phosphatase 1 proteins

```
Jased-PP1-I      -------MADAE-LDVDNLISRLLEVRGCRPGKTVQMTEAEVRGLCLKSREIFLQQPILL
Jased-PP1-II     -------MAETDKLNIDSIIARLLEVRGSRPGKNVQLTENEIRGLCLKSREIFLSQPILL
Jased-PP1-III    MVLSQCKMRYEDPFDVDSFINNLLFLKK-YPGKNIRMEETQIRSLVAAARQVLLEQPTLV
                        *   : :::*.:* .** ::   ***.::: * ::*.*   :*:::*.** *:

Jased-PP1-I      ELEAPLKICGDIHGQYTDLLRLFEYGSFPPESNYLFLGDYVDRGKQSLETICLLLAYKIK
Jased-PP1-II     ELEAPLKICGDIHGQYYDLLRLFEYGGFPPESNYLFLGDYVDRGKQSLETICLLLAYKIK
Jased-PP1-III    ELEAPVNIVGDIHGQFNDLLRHFDKLGYPPDQNYLFLGDYVDRGKQSLETICLVLAYKVK
                 *****::* ******: **** *:  .:**:.*********************:****:*

Jased-PP1-I      YPENFFLLRGNHECASINRIYGFFDECRRRYGTKLWKTFTDCFNCLPIAAIIDEKIFCCH
Jased-PP1-II     YPENFFLLRGNHECASINRIYGFYDECKRRYNIKLWKTFTDCFNCLPVAAIVDEKIFCCH
Jased-PP1-III    YPNNFFILRGNHECASINRIYGFYDECKRRYNVKLWKTFTDLFNCLPLAALIEGTILCMH
                 **:***:****************:***:***. ******** *****:**::: .*:* *

Jased-PP1-I      GGLSPDLQSMEQIRRIMRPTDVPDTGLLCDLLWSDPDKDVQGWGENDRGVSFTFGADVVS
Jased-PP1-II     GGLSPDLQSMEQIRRIMRPTDVPDQGLLCDLLWSDPDKDTMGWGENDRGVSFTFGAEVVA
Jased-PP1-III    GGLSPDLHNLEQIRVIERPLNVPDSGLTCDILWADPDEDVRGWAPSDRGVSWIFGGDVLK
                 *******:.:**** * ** :*** ** **:**:***:*. **. .*****: **.:*:

Jased-PP1-I      KFLNRHDLDLICRAHQVVEDGYEFFAKRQLVTLFSAPNYCGEFDNAGGMMSVDETLLCSF
Jased-PP1-II     KFLHKHDFDLICRAHQVVEDGYEFFAKRQLVTLFSAPNYCGEFDNAGAMMSVDETLMCSF
Jased-PP1-III    AFLEHHDLSLVVRAHQVVEDGYQFFEKRSLVTLFSAANYCGEFDNAAAVMGVSEDLTCWF
                  **.:**:.*: **********:** **.*******.*********..:*.*.* * * *

Jased-PP1-I      QILKPSEKKAKYQYSGLNQNRPNPPNRQQRSQQKK--
Jased-PP1-II     QILKPADKK-KFPYGGLNTGRPVTPPRGAANQKNKKK
Jased-PP1-III    NILPPDRRR-TY--------------------IKK--
                 :** *  :: .:                     :*
```

B Alignment of *Jasus edwardsii* microtubule star proteins

```
Jased-MTS-I-v1  MDDK--------TQMKELDQWIDQLMECKQLAENQVKTLCEKAKEVLAKESNVQEVKSPV
Jased-MTS-II-v1 MADAHVPGKCCPSSSSDVDAWIVQLKKREPLSLRQVERLCSKVSEVLTTESNVLEVTSPV
                * *         :. .::* ** ** : : *: .**: **.*..***:.**** **.***

Jased-MTS-I-v1  TVCGDVHGQFHDLMELFKIGGRSPDTNYLFMGDYVDRGYYSVETVTLLVCLKVRFRERIT
Jased-MTS-II-v1 TVCGDLHGQFYDLMELFAIAGEPPETNYLFMGDYVDRGYFSIQVVSLLLAMKLRYPQRVT
                *****:****:****** *.*..*:**************:*::.*:**:.:*:*: :*:*

Jased-MTS-I-v1  ILRGNHESRQITQVYGFYDECLRKYGNANVWKYFTDLFDYLPLTALVDSQIFCLHGGLSP
Jased-MTS-II-v1 LLRGNHESRLTTQVYGFYDECFAVYQSPEAWRLFMAVFDCLPLTALIEDKILCMHGGLSP
                :********  **********:  * ..:.*: *  :** ******::.:*:*:******

Jased-MTS-I-v1  SIDTLDHIRALDRLQEVPHEGPMCDLLWSDPDD---RGGWGISPRGAGYTFGQDISETFN
Jased-MTS-II-v1 SLDSIDQIRVLDRFQEVPNEGAMSDLLWSDPDESAWRLGWRSNSRGAGYLWGLDVSQTFT
                *:*::*:**.***:****:**.*.********:   * **  ..***** :* *:*:**.

Jased-MTS-I-v1  HSNGLTLVSRAHQLVMEGYNWCHDRNVVTIFSAPNYCYRCGNQAAIMELDDSLKYSFLQF
Jased-MTS-II-v1 YSNNLEFLSRAHQVQNTGFSWYHDGKVITIFSAPNYCYRVGNLAGYMIVEEDTQKCY-TF
                :**.* ::*****:   *:.* ** :*:*********** ** *. * :::. : .:  *

Jased-MTS-I-v1  DPAPRRGEPHVTRRTPDYFL
Jased-MTS-II-v1 EAGPREHTSPPTTTISSYFC
                :..**.  .  *   ..**
```

Fig. 3 MAFFT alignments of *Jasus edwardsii* protein phosphatase 1 (Jased-PP1) and protein phosphatase 2A catalytic subunit microtubule star (Jased-MTS) proteins. **a** Alignment of Jased-PP1-I, II, and III. **b** Alignment of Jased-MTS-I-v1 and Jased-MTS-II-v1. In the line immediately below each sequence grouping, "*" indicates identical amino acid residues, while ":" and "." denote amino acids that are similar in structure between sequences. In this figure, serine–threonine protein phosphatase N-terminal and calcineurin-like phosphoesterase domains identified by Pfam analyses are highlighted in dark red and dark yellow, respectively (color figure online)

initiating investigations of photoperiodic control in members of this decapod infraorder.

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Compliance with ethical standards

Conflict of interest None.

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