

Supplementary Information

Rhythms during the polar night: Evidence of clock-gene oscillations in the Arctic scallop *Chlamys islandica*

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Methods S1

Identification of candidate light perception and clock genes in *C. islandica*

For gene identification, total RNA was extracted from the gills and mantle edge tissues of scallops (the individual samples used for gene identification were not those used for the gene expression analysis) using TRIzol (Ambion, AM9738) according to the manufacturer's instructions. The total RNA quantity and quality were assessed by spectrophotometry (OD260, OD280), and 5 µg of total RNA was individually submitted to reverse transcription using oligo dT17 and Moloney murine leukaemia virus (M-MLV) reverse transcriptase (Promega, Madison, WI, USA). Specific primers were designed from conserved regions and are available in the electronic supplemental material, table S1. *C. islandica* candidate genes were amplified by using specific primers. PCR products were separated on agarose gel and purified using a Wizard® SV gel and PCR clean up system (Promega, Madison, USA). Purified products were ligated into the pGEM-T vector (Promega, Madison, USA) and used to transform DH5α bacteria (Invitrogen, USA). Recombinants were cultured in Luria-Bertani broth medium containing 100 µg·ml⁻¹ ampicillin, and plasmids containing inserts were extracted and sequenced from both ends using T7 and SP6 universal primers. Putative *C. islandica* light perception and clock genes and also *Cief1* and *Cigapdh* (used as reference genes for qPCR) cDNA partial sequences were sent to GATC Biotech (GATC Biotech SARL, Marseille, France) for sequencing. Forward and reverse sequences were assembled using BioEdit 7.0 software. The cDNA sequence and deduced amino acid sequence of candidates were analysed and compared using the BLAST algorithm (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Choice of the tissues sampled

The three tissues sampled for gene expression analysis were selected as follows. Due to the lack of a well-identified master clock in bivalve tissue, we chose three major tissues according to their functions and locations where autonomous peripheral clocks could be present. The mantle edge, where the eyes of the scallop are located, was chosen due to its involvement in light transduction. The muscle was chosen because of its action as an adductor muscle responsible for valve movement (VOA). Finally, the gills were selected because this organ is the main organ involved in respiration and nutrition processes. The activity of the gills is directly dependent on the valve apertures and mantle edge, and could be directly connected to environmental parameters such as phytoplankton (chlorophyll a concentration), temperature or light irradiance.

Expression of candidate light perception and clock genes in *C. islandica*

At each sampling time, tissues (mantle edge, gills, muscle) from individuals were quickly excised on site and under red light when sampling was done during the night and the polar night. Then, samples were kept at 4°C overnight in RNAlater and stored at -80°C until processing for gene expression assays. Total RNA was extracted using TRIzol (Ambion, AM9738) according to the manufacturer's instructions, and quantity and quality were assessed by spectrophotometry (OD260, OD280) and reverse transcribed using Moloney murine leukaemia virus reverse transcriptase (M-MLV; Promega). Specific primers for the measurement of mRNA expression levels of *Cicry1*, *Cicry2*, *Ciclock*, *Cibmal*, *Ciper*, *Ciror*, *Cirhodopsin*, *Cimelanopsin*, *Cief1* and *Cigapdh* were designed based on sequenced fragments (electronic supplemental material, table S1).

Real-time qPCR was performed on individual samples using GoTaq qPCR SYBR green chemistry (Promega, Madison, USA) on a LightCycler 480 System. Elongation factor 1, *Cief1*, and glyceraldehyde 3-phosphate dehydrogenase, *Cigapdh*, were used as housekeeping genes

following analysis with geNorm, BestKeeper and Normfinder [3]. PCR efficiency (E) was assessed for each primer pair by determining the slope of standard curves obtained from serial dilution analysis of cDNA from different experimental samples. Reactions were initiated with the activation of the DNA polymerase at 95°C for 10 min followed by amplification of the target cDNA (40 cycles: denaturation at 95°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 30 s). Reaction specificity was controlled using a melting curve step from 95°C to 60°C (decrease of temperature of 0.5°C every 10 s). Both candidate genes and references were run in technical duplicates for each sample (n = 5 biological replicates). A third technical replicate was performed if the Ct values of the technical replicates were not homogenous. The relative mRNA expression level was calculated by the comparative Ct method [4].

References

1. Perrigault M, Tran D. 2017 Identification of the molecular clockwork of the oyster *Crassostrea gigas*. *PloS One* **12**, e0169790. (doi:10.1371/journal.pone.0169790)
2. Wang S *et al.* 2017 Scallop genome provides insights into evolution of bilaterian karyotype and development. *Nat. Ecol. Evol.* **1**, 120. (doi:10.1038/s41559-017-0120)
3. Wang Q, Ishikawa T, Michiue T, Zhu B-L, Guan D-W, Maeda H. 2012 Stability of endogenous reference genes in postmortem human brains for normalization of quantitative real-time PCR data: comprehensive evaluation using geNorm, NormFinder, and BestKeeper. *Int. J. Legal Med.* **126**, 943–952. (doi:10.1007/s00414-012-0774-7)
4. Livak KJ, Schmittgen TD. 2001 Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402–408. (doi:10.1006/meth.2001.1262)

Sampling time	Date	Time	Night	Astronomical Twilight	Nautical Twilight	Solar noon and altitude	Moon meridian time and angle	Moon illumination	Sea level (m)
	27/01/2017								
1		15h00			15h11				1.287
2		17h00		17h26					0.797
3		19h00				11h25 (-7,3°)	10h55 (-7°)	0,40%	0.402
4		21h00							0.489
5		23h00							0.941
6	28/01/2017	1h00							1.347
7		3h00							1.263
8		5h00		5h20					0.755
9		7h00			7h34	11h25 (-7°)	11h46 (-4,7°)	0,30%	0.32
10		9h00							0.373
11		11h00							0.89

Figure S1A. Details of sampling frequency and environmental parameters during polar night period (PN). All times are presented in UTC (Coordinated Universal Time).

Sampling time	Date	Time	Day	Civil Twilight	Nautical Twilight	Solar noon and altitude	Moon meridian time and angle	Moon illumination	Sea level (m)
1	22/09/2017	10h00	17h23	19h16		11h04 (11,3°)	12h54 (4,1°)	5,90%	0.508
2		12h00							1.169
3		14h00							1.447
4		16h00							1.1
5		18h00	0.451						
6		20h00	0.172						
7		22h00	0.541						
8	23/09/2017	0h00	4h50	2h55		11h04 (10,9°)	13h40 (0,5°)	11,70%	1.205
9		2h00							1.564
10		4h00							1.3
11		6h00							0.636
12		8h00							0.248
13		10h00							0,491

Figure S1B. Details of sampling frequency and environmental parameters during equinox period (Eq). All times are presented in UTC (Coordinated Universal Time) .

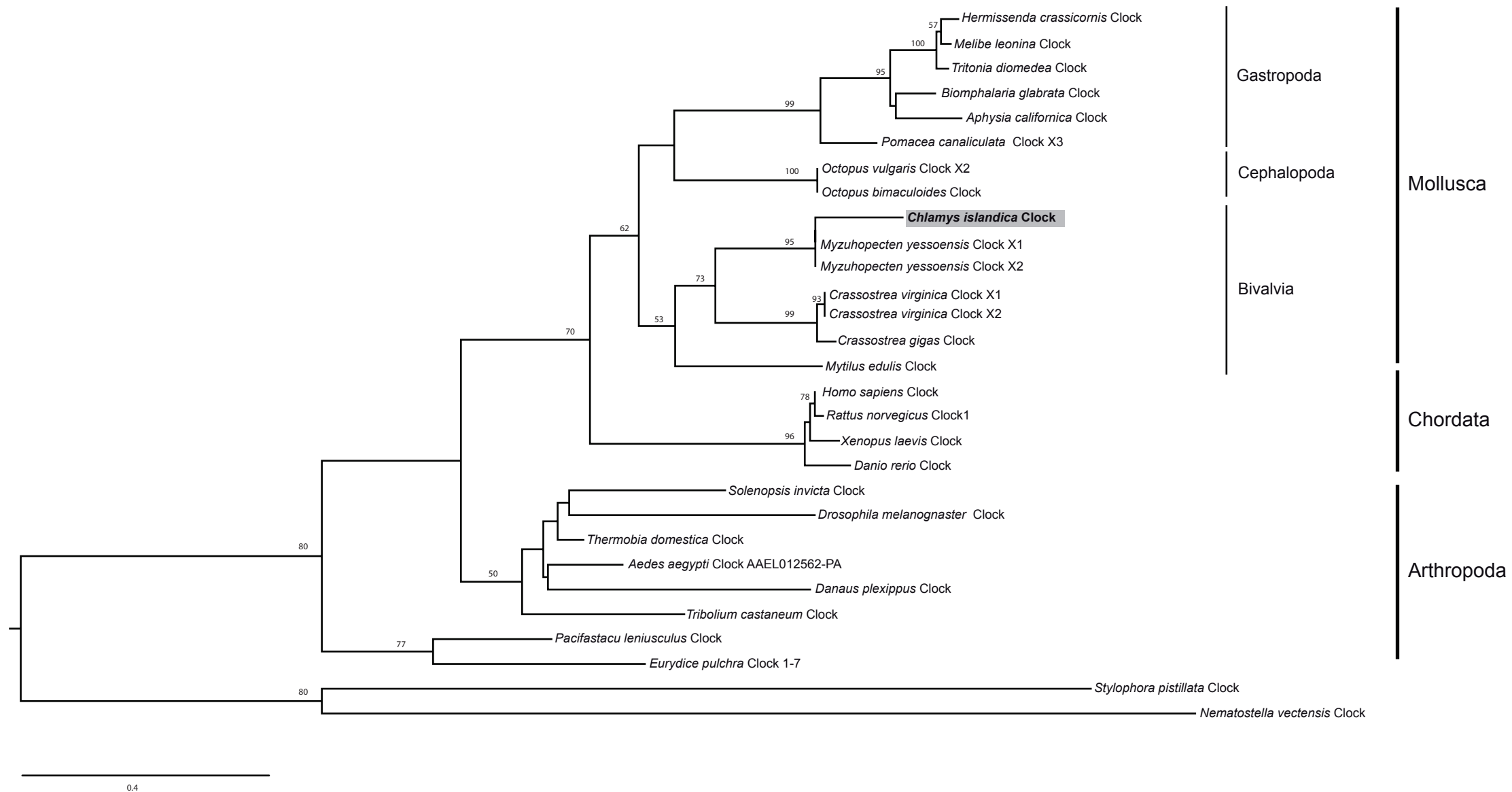


figure S2 A. Phylogenetic tree indicating the evolutionary relationships between CLOCK orthologs reconstructed by maximum likelihood. Numbers are bootstraps proportion, reflecting clade support (value below 50 are not indicated). Phylum and class (only for Mollusca) origin of sequences are given on the right.

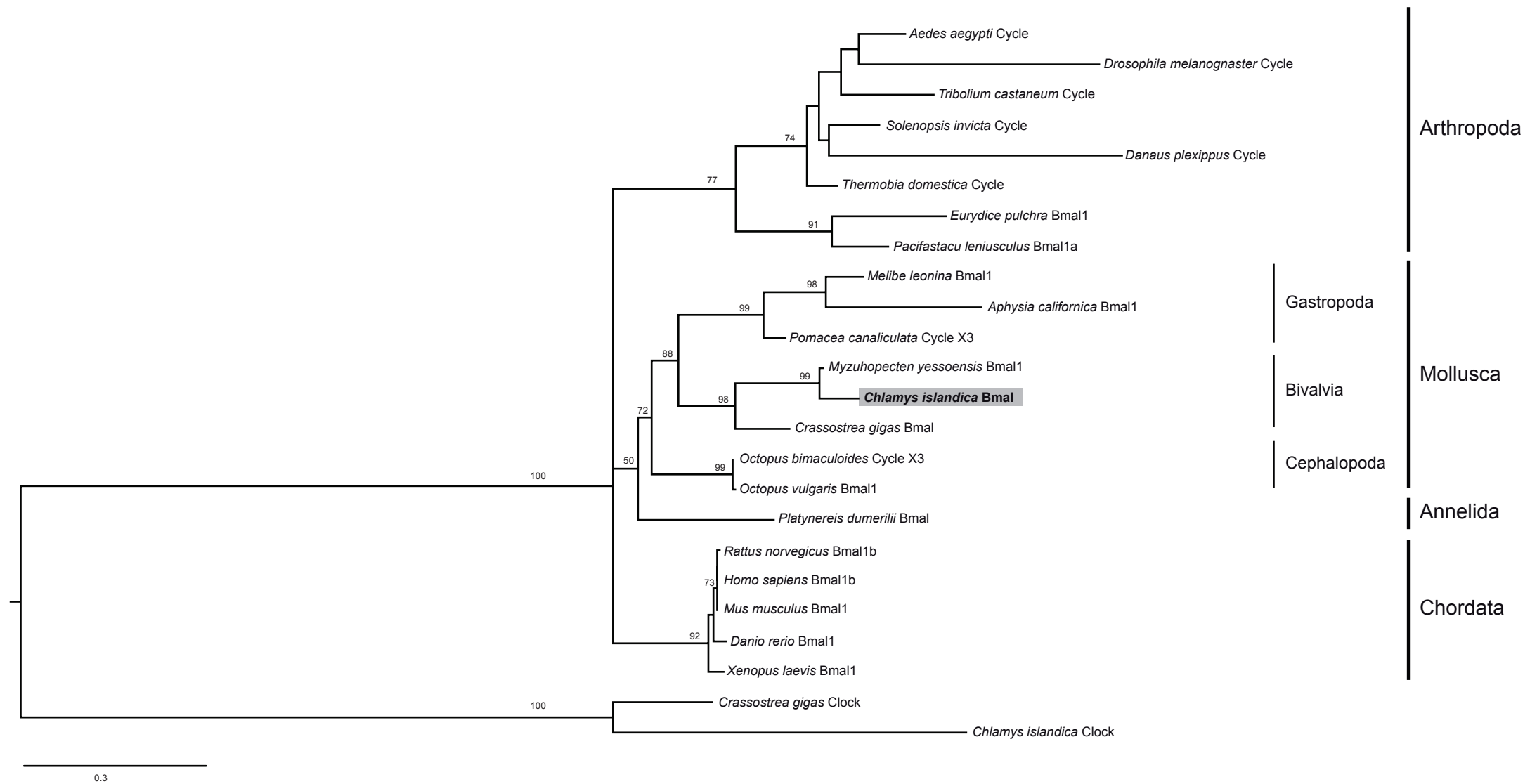


figure S2 B. Phylogenetic tree indicating the evolutionary relationships between BMAL1/CYCLE orthologs reconstructed by maximum likelihood. Numbers are bootstraps proportion, reflecting clade support (value below 50 are not indicated). Phylum and class (only for Mollusca) origin of sequences are given on the right.

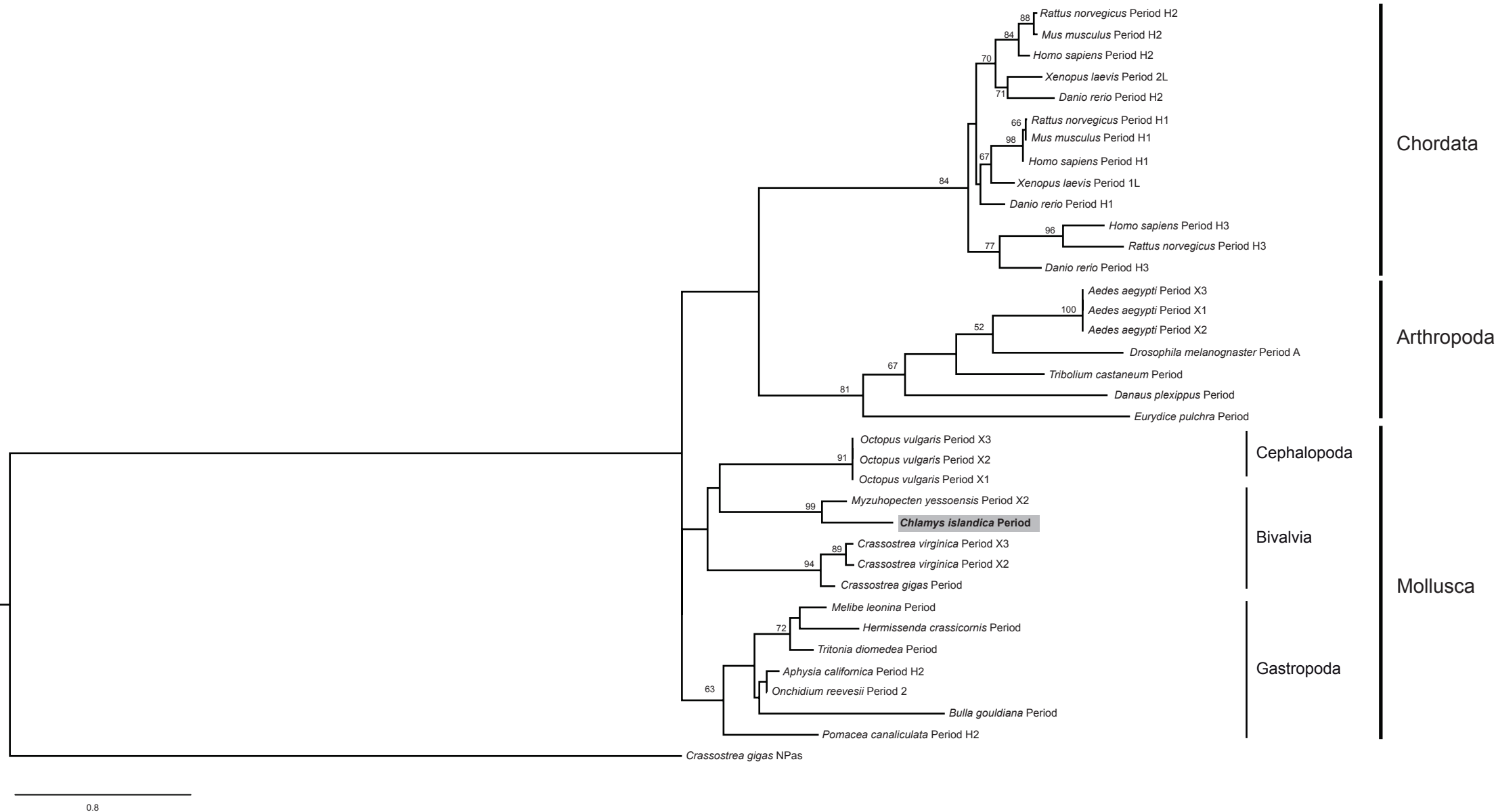


figure S2 C. Phylogenetic tree indicating the evolutionary relationships between PERIOD orthologs reconstructed by maximum likelihood. Numbers are bootstraps proportion, reflecting clade support (value below 50 are not indicated). Phylum and class (only for Mollusca) origin of sequences are given on the right.

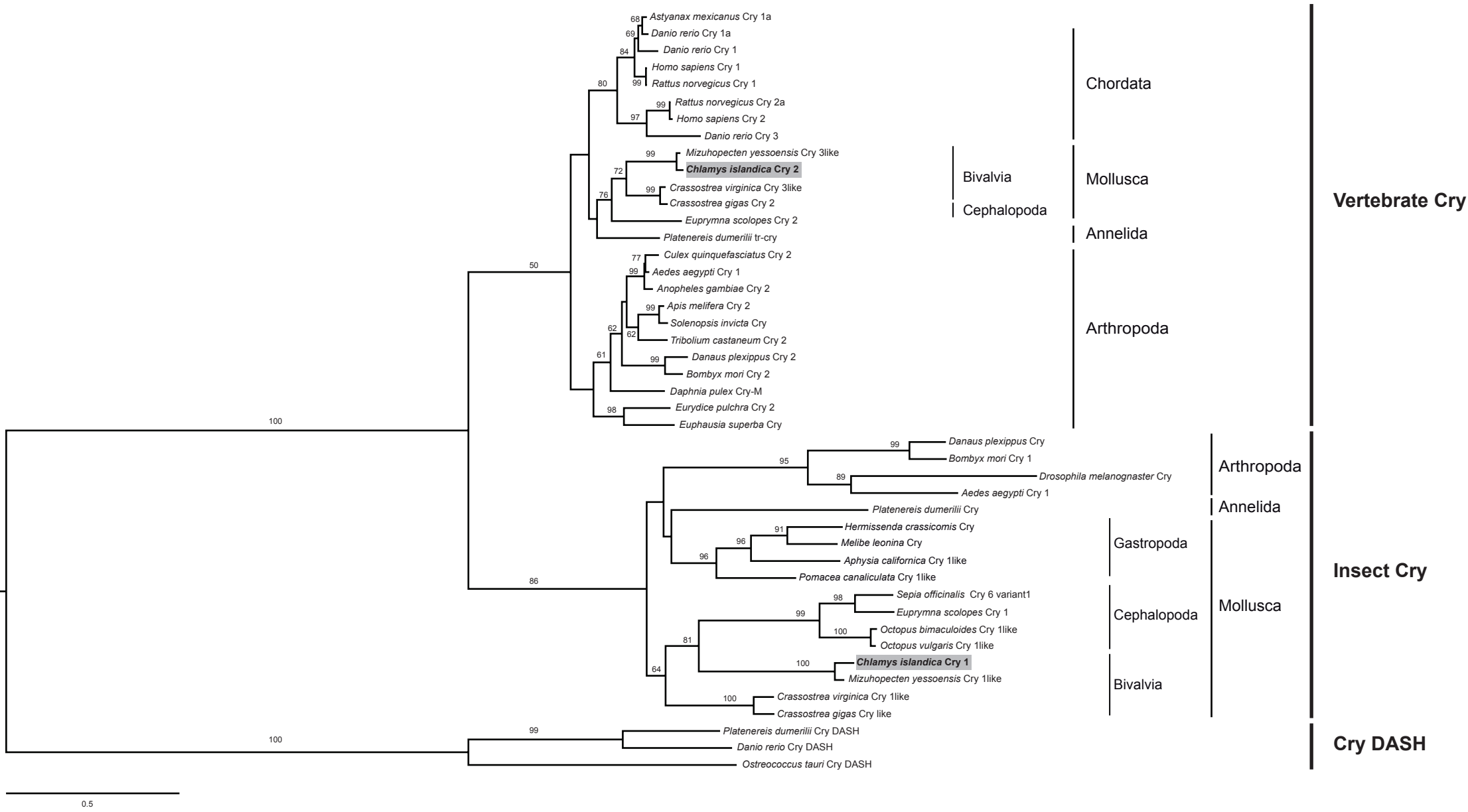


figure S2 D. Phylogenetic tree indicating the evolutionary relationships between CRY orthologs reconstructed by maximum likelihood. Numbers are bootstraps proportion, reflecting clade support (value below 50 are not indicated). Phylum and class (only for Mollusca) origin of sequences are given on the right.

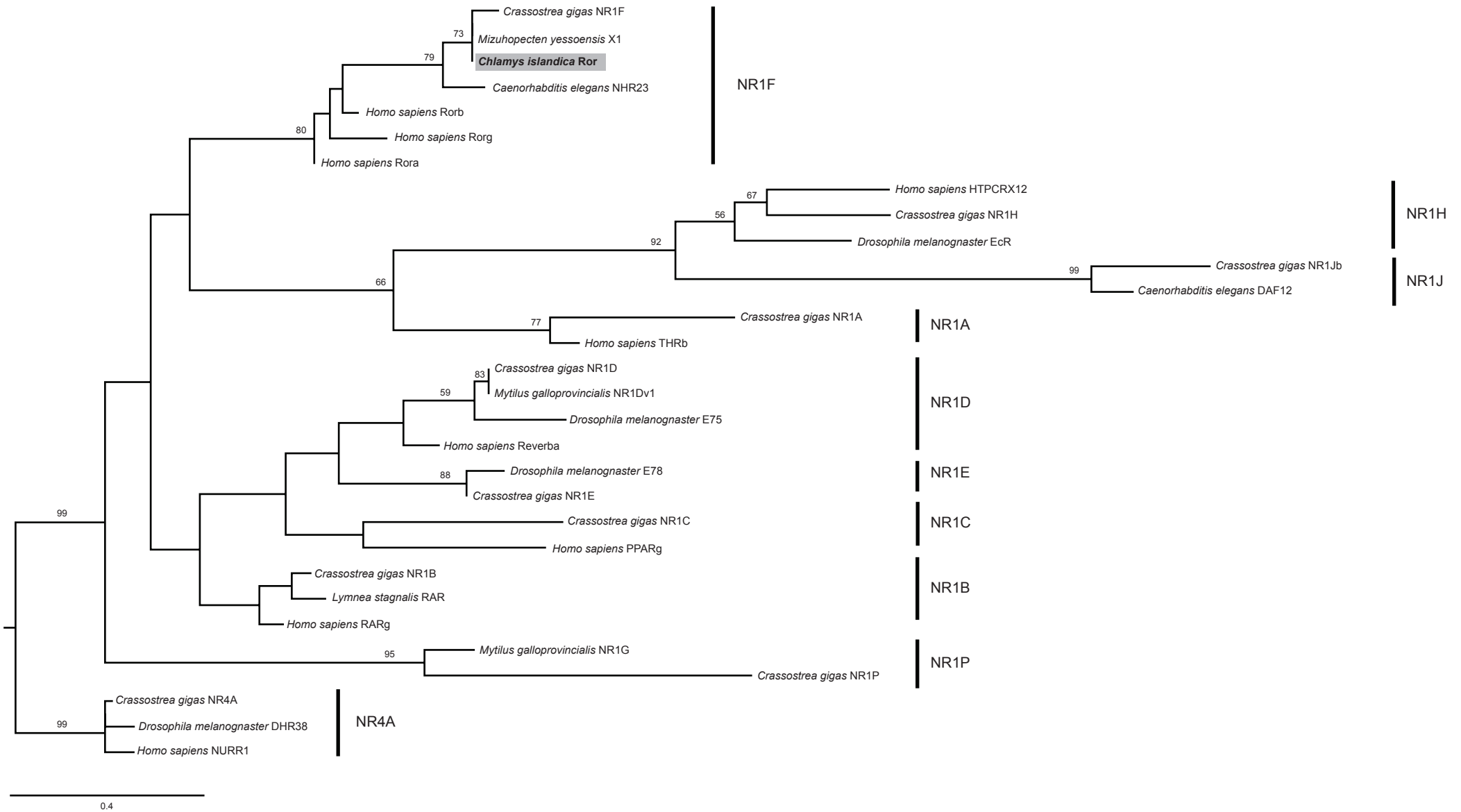


figure S2 E. Phylogenetic relationship of nuclear receptors in *Chlamys islandica*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Homo sapiens* and mollusk species. The phylogenetic tree was conducted by maximum likelihood. Numbers are bootstraps proportion, reflecting clade support (value below 50 are not indicated). NR subfamilies are given on the right.

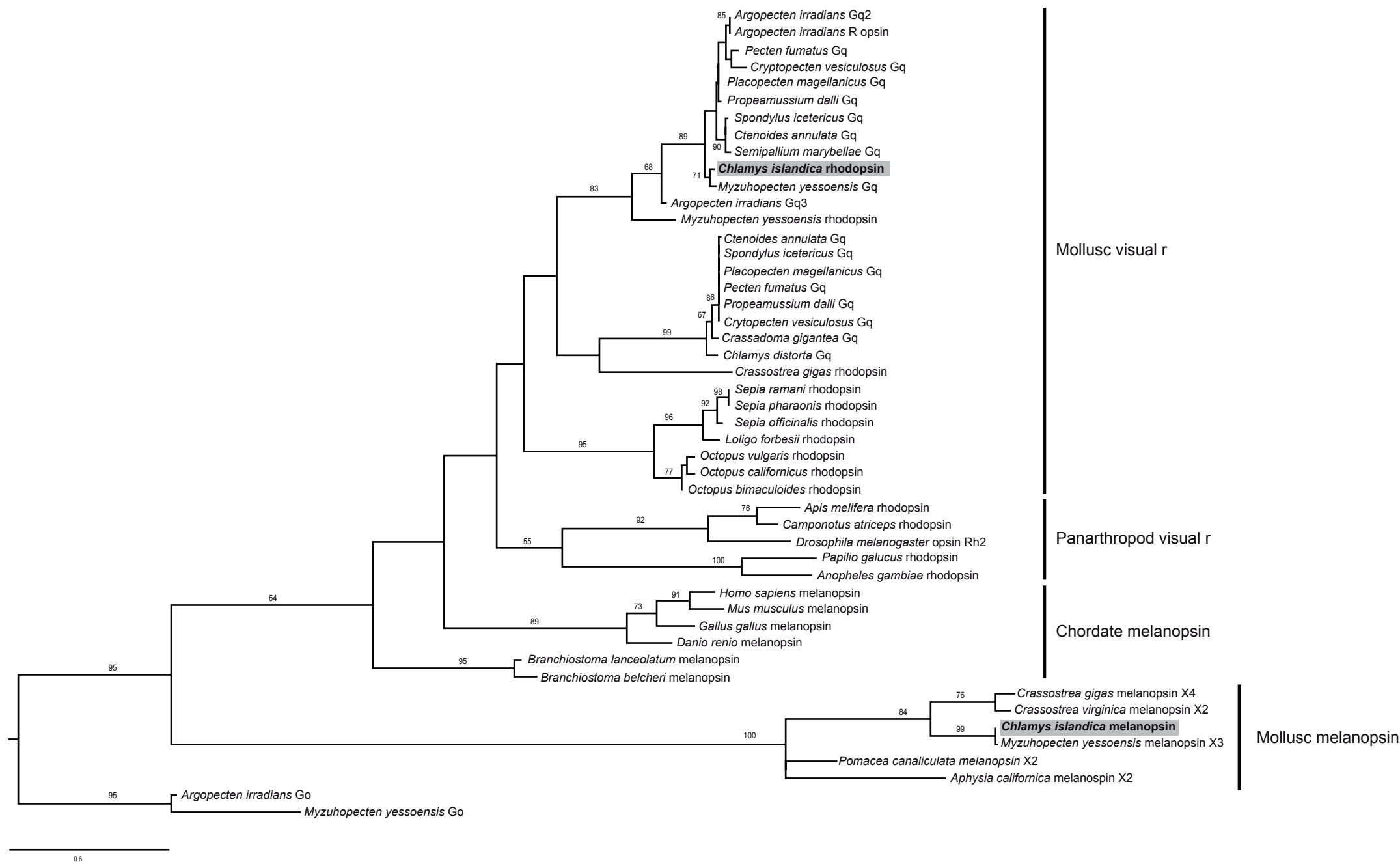


figure S2 F. Phylogenetic tree indicating the evolutionary relationships between opsin subfamily orthologs reconstructed by maximum likelihood. Numbers are bootstraps proportion, reflecting clade support (value below 50 are not indicated).

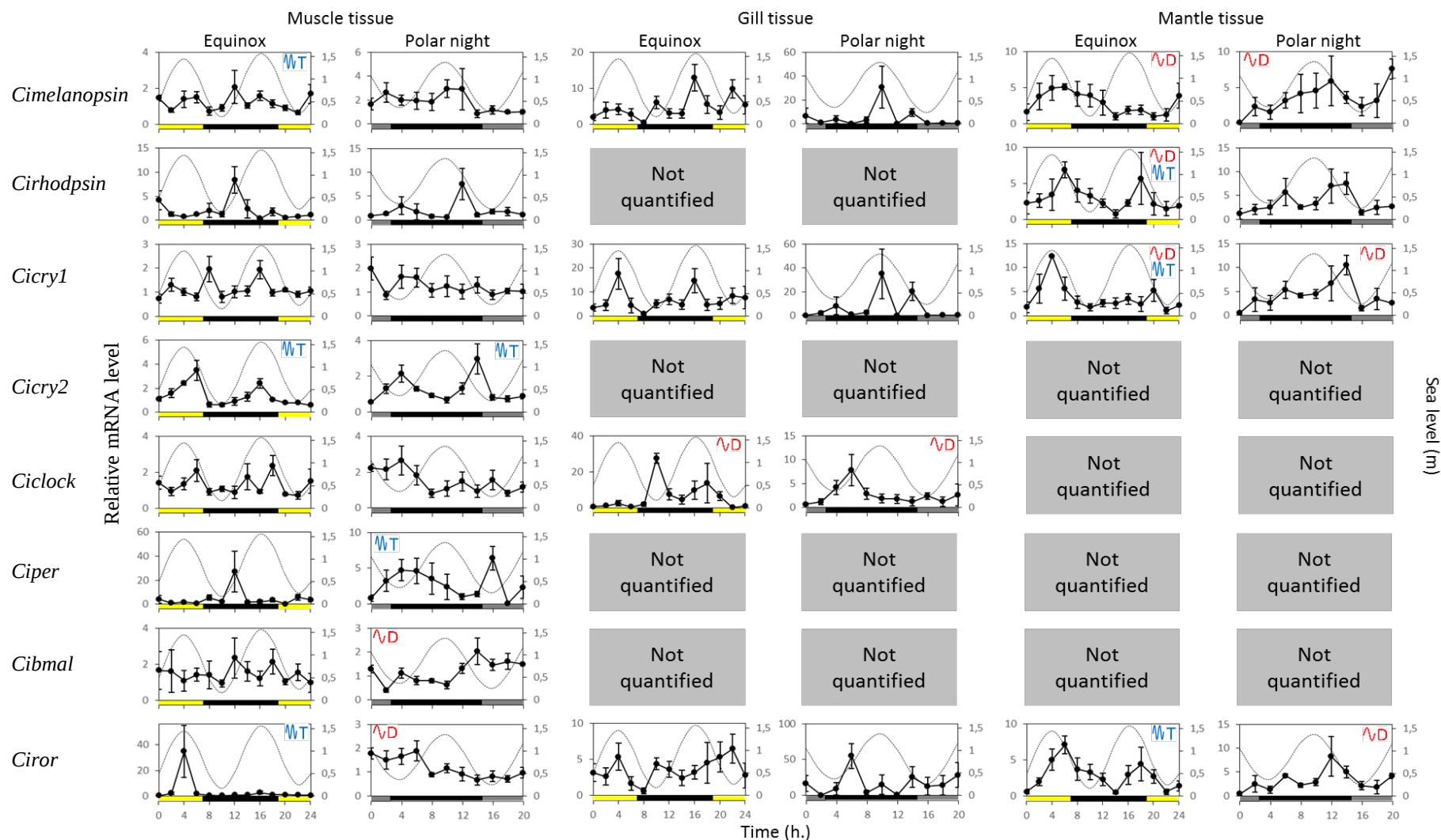
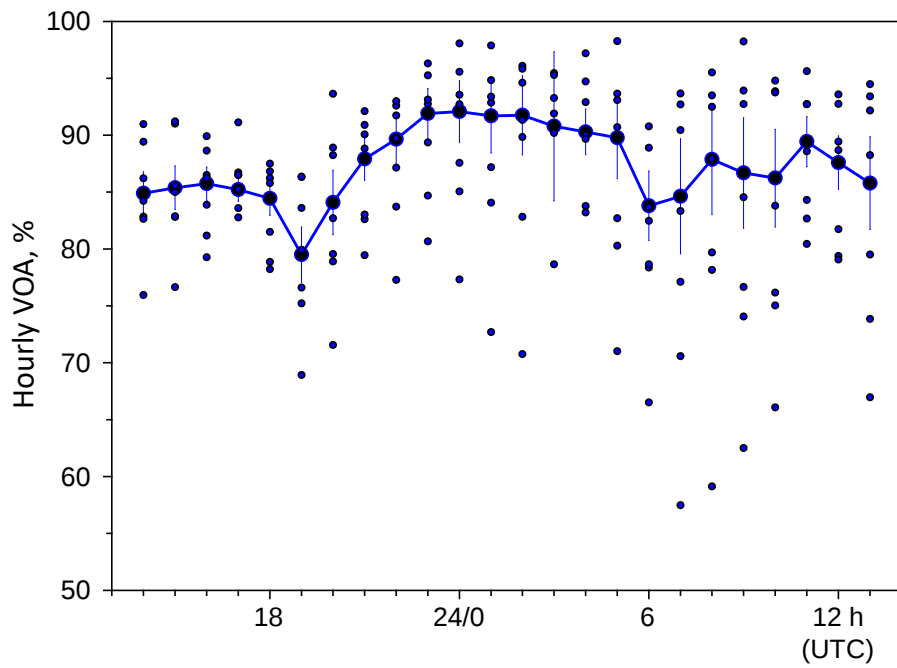
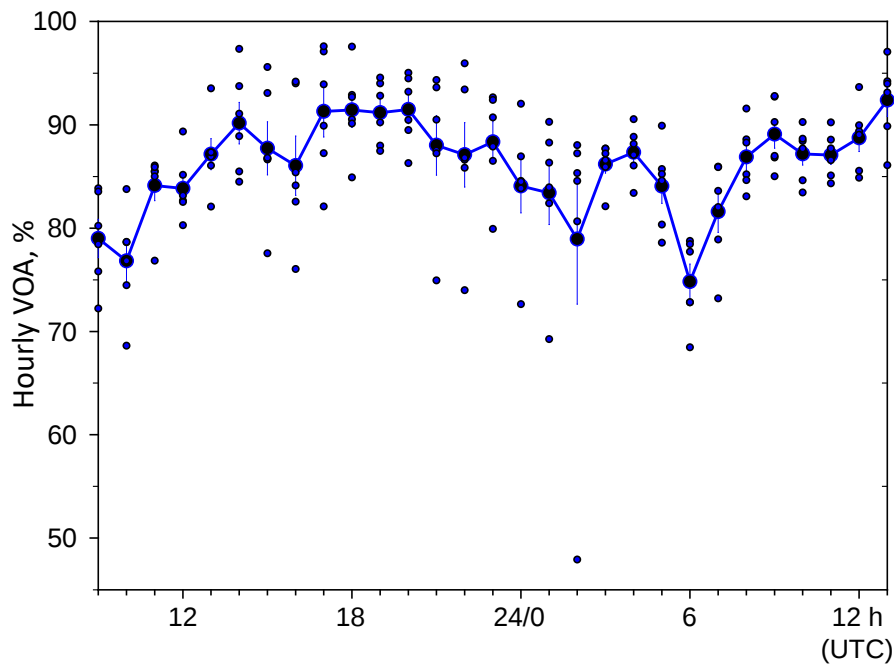


Figure S3. Cyclic expression patterns of *C. islandica* light perception and core clock genes. Relative transcription levels (mean $\pm$ SEM, $n = 5$) of relative mRNA level in muscle, gill and mantle tissues of *C. islandica* during equinox and polar night. Dotted lines refer to tide cycles. Yellow and dark areas indicated photophase and scotophase during Eq; dark and grey areas referred to the night and nautical twilight periods respectively during PN. The significant rhythmicity of gene was analyzed by RAIN algorithm. T : significant periodicity in the tidal range (12 ± 2 h), D significant daily periodicity (24 ± 4 h). Exact p -values of significant periodicities were presented in table S3.



Chlamys 1	Chlamys 2	Chlamys 3	Chlamys 4	Chlamys 5	Chlamys 6	Chlamys 7	Hours of the day	Mean (n=7)	Standard Error
86.205	89.407	84.221	82.827	82.641	90.962	75.944375	14	84.8967292	1.878468255
91.006	85.574	84.994	82.798	82.864	91.198	76.635375	15	85.3785625	1.916061908
88.636	83.882	86.49	81.167	86.237	89.907	79.263625	16	85.7359375	1.462619405
86.717	86.488	83.581	91.114	85.233	86.502	82.764125	17	85.2141875	1.024058368
86.815	87.495	86.225	78.222	85.786	81.491	78.85025	18	84.4437083	1.488792404
86.348	86.319	76.594	79.931	83.592	68.914	75.20975	19	79.496125	2.435915862
88.907	93.627	82.696	78.902	88.234	71.551	79.547375	20	84.0937292	2.821006163
90.89	92.107	88.817	79.449	90.057	82.601	83.01125	21	87.913875	1.861440923
92.588	89.722	87.135	77.278	91.73	92.979	83.7005	22	89.6424167	2.164684461
92.746	96.299	89.348	80.655	93.116	95.25	84.6865	23	91.9075833	2.181343973
92.704	95.555	87.563	77.309	93.54	98.062	85.053875	24	92.0796458	2.704296399
92.85	94.83	87.181	72.687	97.881	93.371	84.05675	1	91.6949583	3.239064831
91.321	96.086	89.829	70.749	94.598	95.833	82.82275	2	91.7482917	3.467974043
91.896	93.26	90.185	47.563	95.449	95.273	78.645375	3	90.7847292	6.543316057
89.973	94.717	83.758	89.658	92.897	97.191	83.201125	4	90.2895208	1.996306613
90.691	93.636	80.273	71.012	93.084	98.261	82.693	5	89.773	3.588485948
82.459	83.673	78.364	66.5	88.889	90.76	78.644375	6	83.7982292	3.04242325
83.325	90.441	70.562	57.478	92.699	93.661	77.1	7	84.6313333	5.044380229
87.926	92.479	79.682	59.122	93.492	95.5	78.15175	8	87.8717917	4.832995186
84.538	92.722	74.059	62.499	93.904	98.222	76.65525	9	86.683375	4.849183022
83.795	94.784	75.025	66.067	93.853	93.748	76.163125	10	86.2280208	4.279031512
88.579	92.719	84.296	80.435	92.715	95.623	82.662	11	89.4323333	2.194350853
89.427	92.738	79.38	79.071	88.681	93.57	81.72675	12	87.587125	2.344053589
92.163	93.397	66.952	73.836	88.24	94.475	79.500375	13	85.7878958	4.07309373

Figure S4 A. Mean values ($\pm$ SE, blue line) and individual plots ($n = 7$ *C. islandica*) of hourly scallop VOA (valve opening amplitude, %) during polar night (from 14 h 27/01/2017 to 13 h 28/01/2017, UTC). (B) Table of individual hourly data of VOA and mean value and standard errors.



Chlamys 1	Chlamys 2	Chlamys 3	Chlamys 4	Chlamys 5	Chlamys 6	Hours of the day	Mean (n=6)	Standard error
75.806	72.213	83.855	78.426	83.542	80.208	9	79.00833333	1.846204371
76.837	68.608	74.467	78.625	83.764	78.6631667	10	76.82736111	2.064363835
85.516	86.047	76.845	85.449	85.899	84.993	11	84.12483333	1.463794622
80.27	85.147	82.648	82.521	89.341	83.1515	12	83.84641667	1.269495441
82.073	87.083	86.919	87.323	93.514	86.0516667	13	87.16061111	1.500776072
85.476	84.489	91.06	93.73	97.343	88.891	14	90.16483333	2.007159741
86.719	77.561	86.638	93.067	95.582	86.7986667	15	87.72761111	2.560129987
82.557	84.13	76.035	94.001	94.176	85.377	16	86.046	2.862928477
93.903	87.246	82.101	97.084	97.593	89.906	17	91.3055	2.468681737
90.516	92.878	84.907	92.677	97.553	90.1175	18	91.44141667	1.695578969
94.002	87.983	87.467	92.808	94.565	90.2236667	19	91.17477778	1.251584074
95.032	86.287	89.481	94.476	93.198	90.4533333	20	91.48788889	1.373088551
93.615	74.933	90.495	87.505	94.321	87.2271667	21	88.01602778	2.882681246
95.938	73.995	85.836	86.614	93.416	86.8031667	22	87.10036111	3.118039588
92.654	86.497	79.919	90.702	92.408	87.8901667	23	88.34502778	1.960020003
92.031	86.932	72.638	83.849	84.561	84.5051667		84.08602778	2.599595736
90.271	88.267	69.245	83.938	86.335	82.4051667	1	83.41019444	3.061345531
88.031	87.232	47.911	84.557	85.317	80.6458333	2	78.94897222	6.296370882
87.697	87.689	82.111	87.215	86.564	85.9198333	3	86.19930556	0.864470213
90.535	88.154	83.396	88.836	86.053	87.079	4	87.34216667	1.006449781
89.909	85.705	80.339	84.586	78.593	85.2238333	5	84.05930556	1.656119331
78.759	72.809	78.441	72.822	68.441	77.7156667	6	74.83127778	1.690554646
85.948	82.034	85.891	78.898	73.201	83.5943333	7	81.59438889	1.994386477
88.579	85.204	91.558	84.643	83.072	88.2723333	8	86.88805556	1.278312648
92.718	86.831	92.768	85.011	86.978	90.254	9	89.09333333	1.344702735
90.255	84.627	88.432	83.445	87.692	88.6425	10	87.18225	1.062892012
87.718	86.562	90.225	85.085	84.325	88.547	11	87.077	0.899786604
84.866	85.533	93.637	89.374	89.03	89.9551667	12	88.73252778	1.306849711
86.082	89.857	97.057	94.2	93.964	93.1181667	13	92.37969444	1.57343581

Figure S4 B. Mean values ($\pm$ SE, blue line) and individual plots ($n = 6$ *C. islandica*) of hourly scallop VOA (valve opening amplitude, %) during equinox (from 09 h 22/09/2017 to 13 h 23/09/2017, UTC). (B) Table of individual hourly data of VOA and mean value and standard errors.

Table S1. Primer sequences.

	Gene names	Forward primer	Reverse primer	Amplicon size (bp)
PCR identification				
	<i>Cirhodopsin</i>	AGGAAGCGGATAAACAGGAG	AAGCTTGGATCTCCAACTG	428
	<i>Cimelanopsin</i>	CTCTCGTATGTTCCGGTGA	CTTCTTCAGCAGACGGAGTT	491
	<i>Cicry1</i>	CTTCGTATCCATGACAACC	TCCGGTACAAACTGATT	720
	<i>Cicry2</i>	GAAGGCAGCCAAATGTGTG	AGCACTTGAGTACACAGCAAA	1352
	<i>Ciclock</i>	TGGTTTCTACGACCAACAAGAA	CCAGATGGAAGGTTGGTGAA	516
	<i>Cibmal</i>	CCACAGCCTTAGCAGACAG	CTCTATGGGTCGGACGTTAATC	690
	<i>CiPer</i>	ACAGAATGGTCGAGGTTTG	TTATCACTGCTAGGGTC	882
	<i>Ciror</i>	CTTGTAAGGTCTGTGGTGAC	GCGATACATTCTGTAG	217
	<i>Cigapdh</i>	ATCAACGATCCCTTCATT	ACACGGAAAGCCATACCAGT	518
	<i>Cief1</i>	GATGCTCCTGGACACAGA	GTTTGACGCATGTCACG	1035
qPCR				
	<i>Cirhodopsin</i>	CCGCTCCAAGCTATGAGAATTA	CGAAGAGTGGCAACAAGGA	100
	<i>Cimelanopsin</i>	CCCTGTGATAACGACGAAATCT	TCTCACCGAACATACGAGAGTA	110
	<i>Cicry1</i>	GTCCTGTCCCTGTTCTTAAAC	CTAGTTCTTTGACGCTGGGTAG	93
	<i>Cicry2</i>	GAGGAGGGCATGAAGGTATTT	CTGAAAGAAGGAGCTACAGGATAG	99
	<i>Ciclock</i>	CCTAGGCCACTTACCAAATGAT	GGTTCTTCTCTTTCTCCATCTCC	137
	<i>Cibmal</i>	CAATGTGAGCTCTGGGATAGG	CACTCCCGGCACATTTAGT	109
	<i>CiPer</i>	ACTAACGCAGGAAGTGCTATG	CATCTGACTGTTGCTCCTTAG	112
	<i>Ciror</i>	TGGTCCGGTGAACCTACCA	AGTACTGACAGCGGTTCCCT	122
	<i>Cigapdh</i>	GGACTGCTGGTGCTGATTAT	TTGGCACCTCCGGTAATATG	88
	<i>Cief1</i>	ATGGCTCAGGTTATTGTCCTAAA	GCAAGCAATGTGAGCAGTATG	112

Partial sequences of glyceraldehyde-3-phosphate dehydrogenase (*Cigapdh*) and elongation factor 1-alpha (*Cief1*) were submitted to GenBank under the accession numbers MN025527 and MN025528 respectively.

Table S2. Details and accession numbers of sequences used in phylogenetic analyses.

Protein	Phylum	Class	Species	Accession Number
Bmal	Annelida		<i>Platynereis dumerilii</i>	AGX93014.1
	Arthropoda		<i>Aedes aegypti</i>	AEX32872.1
			<i>Danaus plexippus</i>	EHJ64590.1
			<i>Drosophila melanogaster</i>	AAF49107.1
			<i>Eurydice pulchra</i>	AGV28715.1
			<i>Pacifastacu leniuscutus</i>	AFV39705.1
			<i>Solenopsis invicta</i>	AGD94518.1
			<i>Thermobia domestica</i>	BAJ16354.1
			<i>Tribolium castanuem</i>	NP_001107795.1
	Chordata		<i>Danio rerio</i>	AAF64395.1
			<i>Homo sapiens</i>	BAA19935.1
			<i>Mus musculus</i>	Q9WTL8.2
			<i>Rattus norvegicus</i>	BAA33450.1
			<i>Xenopus laevis</i>	AAW80970.1
	Mollusca	Bivalvia	<i>Chlamys islandica</i>	AYE92104
			<i>Crassostrea gigas</i>	AQM57601.1
			<i>Myzohopecten yessoensis</i>	OWF54991.1
		Cephalopoda	<i>Octopus bimaculoides</i>	XP_014775904.1
			<i>Octopus vulgaris</i>	XP_029639557.1
		Gastropoda	<i>Aphysia californica</i>	XP_012941991.1
			<i>Melibe leonina</i>	AWY10924.1
			<i>Pomacea canaliculata</i>	XP_025086096.1
Clock	Arthropoda		<i>Aedes aegypti</i>	XP_001662706.1
			<i>Danaus plexippus</i>	AAR13011.1
			<i>Drosophila melanogaster</i>	O61735.3
			<i>Eurydice pulchra</i>	AGV28721.1
			<i>Pacifastacu leniuscutus</i>	AFV39704.1
			<i>Solenopsis invicta</i>	AGD94516.1
			<i>Thermobia domestica</i>	BAJ16353.1
			<i>Tribolium castanuem</i>	NP_001106937.1
	Chordata		<i>Danio rerio</i>	NP_571032.1
			<i>Homo sapiens</i>	AAB83969.1

		<i>Rattus norvegicus</i>	NP_068628.1
		<i>Xenopus laevis</i>	AAF34772.1
Cnidaria		<i>Nematostella vectensis</i>	XP_001639742.1
		<i>Stylophora pistillata</i>	PFX31666.1
Mollusca	Bivalvia	<i>Chlamys islandica</i>	MN025525
		<i>Crassostrea gigas</i>	AQM57601.1
		<i>Crassostrea virginica</i>	XP_022324520.1; XP_022324521.1
		<i>Mytilus edulis</i>	AJI43744.1
		<i>Myzohopecten yessoensis</i>	XP_021367044.1; XP_021367049.1
	Cephalopoda	<i>Octopus bimaculoides</i>	XP_014780975.1
		<i>Octopus vulgaris</i>	XP_029648807.1
	Gastropoda	<i>Aphysia californica</i>	XP_012945840.1
		<i>Biomphalaria glabrata</i>	XP_013079457.1
		<i>Hermisenda crassicornis</i>	AWY10518.1
		<i>Melibe leonina</i>	AWY10519.1
		<i>Pomacea canaliculata</i>	XP_025087431.1
		<i>Tritonia diomedea</i>	AWY10650.1
Cry	Annelida	<i>Platynereis dumerilii</i>	AEJ87227.1; AGX93012.1
		<i>Platynereis dumerilii</i> Cry-DASH	AIE57496.1
	Arthropoda	<i>Aedes aegypti</i>	XP_001648498.2; XP_001655778.2
		<i>Anopheles gambiae</i>	ABB29887.1
		<i>Apis mellifera</i>	NP_001077099.1
		<i>Bombyx mori</i>	NP_001182628.1; NP_001182627.1
		<i>Culex quinquefasciatus</i>	XP_001869456.1
		<i>Danaus plexippus</i>	AAX58599.1; ABA62409.1
		<i>Daphnia pulex</i>	EFX82092.1
		<i>Drosophila melanogaster</i>	NP_732407.1
		<i>Euphausia superba</i>	CAQ86665.1
		<i>Eurydice pulchra</i>	AGV28717.1

		<i>Solenopsis invicta</i>	AGD94517.1
		<i>Tribolium castaneum</i>	NP_001076794.1
Chlorophyta		<i>Ostreococcus tauri</i> Cry-DASH	AAU14279.1
Chordata		<i>Astyanax mexicanus</i>	AHA91700.1
		<i>Danio rerio</i>	BAA96846.1; NP_571865.4; BAA96850.1
		<i>Danio rerio</i> Cry-DASH	NP_991249.1
		<i>Homo sapiens</i>	NP_004066.1; AAH41814.1
		<i>Rattus norvegicus</i>	NP_942045.2; EDL79575.1
Mollusca	Bivalvia	<i>Chlamys islandica</i>	AYE92098; AYE92099
		<i>Crassostrea gigas</i>	AQM57607.1; AQM57602.1
		<i>Crassostrea virginica</i>	XP_022291740.1; XP_022292411.1
		<i>Myzohpecten yessoensis</i>	XP_021380007.1; XP_021355346.1
	Cephalopoda	<i>Euprymna scolopes</i>	AGJ94014.1; AGJ94015.1
		<i>Octopus bimaculoides</i>	XP_014774572.1
		<i>Octopus vulgaris</i>	XP_029634730.1
		<i>Sepia officinalis</i>	QHA94772.1
	Gastropoda	<i>Aphysia californica</i>	XP_005089742.1
		<i>Hermisenda crassicornis</i>	AWY10935.1
		<i>Melibe leonina</i>	AWY11207.1
		<i>Pomacea canaliculata</i>	XP_025095473.1
Melanospin	Chordata	<i>Branchiostoma belcheri</i>	Q4R1I4.1
		<i>Branchiostoma lanceolatum</i>	AWU78807.1
		<i>Danio rerio</i>	ADN39430.1
		<i>Gallus gallus</i>	NP_00103818.1
		<i>Homo sapiens</i>	NP_150598.1
		<i>Mus musculus</i>	Q9QXZ9.1
Mollusca	Bivalvia	<i>Chlamys islandica</i>	AYE92102

Rhodopsin	Gastropoda		<i>Crassostrea gigas</i>	XP_011425688.1	
			<i>Crassostrea virginica</i>	XP_022303048.1	
			<i>Myzohoepecten yessoensis</i>	XP_021356368.1	
			<i>Aphysia californica</i>	XP_005090194.1	
			<i>Pomacea canaliculata</i>	XP_025094777.1	
	Arthropoda		<i>Anopheles gambiae</i>	Q5TR54	
			<i>Apis mellifera</i>	Q17053.1	
			<i>Camponotus atriceps</i>	Q17292.1	
			<i>Drosophila melanogaster</i>	P08099.1	
			<i>Papilio glaucus</i>	Q9UAMB	
	Mollusca	Bivalvia		<i>Argopecten irradians</i>	ALO02515.1; ALO02516.1; AUR34014.1
				<i>Argopecten irradians</i> Go	AUR34012.1
				<i>Chlamys islandica</i>	AYE92101
				<i>Chlamys discorta</i>	AGN95795.1
				<i>Ctenoides annulata</i>	AGN95785.1; AGN95794.1
				<i>Crassadoma gigantea</i>	AGN95797.1
				<i>Crassostrea gigas</i>	XP_011447068.1
				<i>Cryptopecten vesiculosus</i>	AGN95792.1; AGN95803.1
				<i>Myzohoepecten yessoensis</i>	AGN95793.1; OWF40989.1
				<i>Myzohoepecten yessoensis</i> Go	O15974.1
				<i>Pecten fumatus</i>	AGN95786.1; AGN95796.1
				<i>Placopecten magellanicus</i>	AGN95788.1; AGN95799.1
				<i>Propeamussium dalli</i>	AGN95790.1; AGN95801.1
				<i>Semipallium marybellae</i>	AGN95789.1
				<i>Spondylus icetericus</i>	AGN95791.1; AGN95802.1
		Cephalopoda		<i>Loligo forbesii</i>	AAS67115.1
				<i>Octopus bimaculoides</i>	AAS67103.1
				<i>Octopus californicus</i>	ADX42273.1

		<i>Octopus vulgaris</i>	ADZ05934.1		
		<i>Sepia officinalis</i>	ADT78509.1		
		<i>Sepia pharaonis</i>	AAT47923.1		
		<i>Sepia ramani</i>	ADR77766.1		
Period	Arthropoda	<i>Aedes aegypti</i>	XP_021710526.1; XP_021710527.1; XP_021710528.1		
		<i>Danaus plexippus</i>	AAO48719.1		
		<i>Drosophila melanogaster</i>	AAF45804.1		
		<i>Eurydice pulchra</i>	AGV28714.1		
		<i>Tribolium castanuem</i>	NP_001106933.1		
	Chordata	<i>Danio rerio</i>	NP_997604.2; NP_878277.1; NP_571659.1		
		<i>Homo sapiens</i>	O15534.2; O15055.2; P56645.4		
		<i>Mus musculus</i>	O35973.2; O54943.3		
		<i>Rattus norvegicus</i>	Q8CHI5.2; Q9Z301.1; Q8CJE2.1		
		<i>Xenopus laevis</i>	NP_001079172.2; NP_001081098.1		
	Mollusca	Bivalvia	<i>Chlamys islandica</i>	AYE92100	
			<i>Crassostrea gigas</i>	AQM57604.1	
			<i>Crassostrea gigas</i> NPas	EKC18855.1	
			<i>Crassostrea virginica</i>	XP_022345656.1; XP_022345657.1	
			<i>Myzohopecten yessoensis</i>	XP_021375509.1	
		Cephalopoda	<i>Octopus vulgaris</i>	XP_029633044.1; XP_029633045.1; XP_029633046.1	
			Gastropoda	<i>Aphysia californica</i>	XP_005111020.1
				<i>Bulla gouldiana</i>	AAK97374.1
				<i>Hermisenda crassicornis</i>	AWY10929.1
				<i>Melibe leonina</i>	AWY10930.1
<i>Onchidium reevesii</i>	QDK59986.1				

		<i>Pomacea canaliculata</i>	XP_025083583.1
		<i>Tritonia diomedea</i>	AWY10931.1
Ror	Arthropoda	<i>Drosophila melanogaster</i>	P17672; P45447; P34021;
		<i>D. melanogaster</i> DHR38	P49869.3
	Chordata	<i>Homo sapiens</i>	P35398.2; Q92753.3; P51449.2; P10828; P13631; P37231; Q14995; Q96R11
		<i>H. sapiens</i> NURR1	P22736.1
	Mollusca	Bivalvia	<i>Chlamys islandica</i> MN025526
		<i>Crassostrea gigas</i>	EKC30897; EKC30866; EKC18691; KJ188106; EKC33126; EKC18621; EKC19773; EKC27734; EKC35897
		<i>C. gigas</i> NR4A	EKC18844.1
		<i>Mytilus galloprovincialis</i>	ABU89807; ABU89802
		<i>Myzohpecten yessoensis</i>	XP_021341211.1
	Nematoda	Gastropoda	<i>Limnea stagnalis</i> <i>Caenorhabditis elegans</i> ADF43963 P41828; AAD34462

Table S3. Phylogenetic reconstructions details for each dataset.

Phylogeny	Alignment final length	Number of sequences	Best model used	Outgroup
Bmal (<i>Cibmal</i>)	184	24	LG+G+F	Clock: <i>C. islandica</i> ; <i>C. gigas</i>
Clock (<i>Ciclock</i>)	138	29	LG+G	Clock: Cnidaria
Cry 1&2 (<i>Cicry1/Cicry2</i>)	420	45	LG+G+I	Cry Dash: <i>D. rerio</i> ; <i>P. dumemerilii</i> ; <i>O. tauri</i>
Rhodopsin (<i>Cirhodopsin</i>)/ Melanopsin (<i>Cimelanopsin</i>)	102	48	LG+G+F+I	Go opsin: Bivalvia
Period (<i>Ciper</i>)	161	36	LG+G+F	NPas: <i>C. gigas</i>
Ror (<i>Ciror</i>)	64	30	LG+G+I	NR4A: <i>C. gigas</i> ; <i>H. sapiens</i> ; <i>D. melanogaster</i>

F = Equilibrium frequencies; G = Gamma shape parameter; I = Proportion of invariable sites;

Table S4. Significance of rhythmic expression analyses (RAIN algorithm) of light perception and clock genes in different tissues of *C. islandica* in the field (Ny-Alesund, 78° 56' N, 11°56' E, Kongsfjorden, Spitsbergen Island, Svalbard). Tidal periodicity range is defined by significant period of 12 ± 2 h. Daily periodicity range is defined by significant period of 24 ± 4 h. (-) means non-significant periodicity ($p > 0.05$). Grey areas denote non quantified candidates.

Gene name	Periodicity range	Muscle		Mantle edge		Gills	
		Polar night	Equinox	Polar night	Equinox	Polar night	Equinox
<i>CiCry1</i>	Tidal	-	-	$p = 0.0323$	$p = 0.0037$	-	-
	Daily	-	-	$p = 0.0196$	$p = 0.07$	-	-
<i>CiCry2</i>	Tidal	$p = 0.0042$	$p = 0.0014$				
	Daily	-	-				
<i>CiClock</i>	Tidal	-	-			-	-
	Daily	-	-			$p = 0.0278$	$p = 0.0078$
<i>Ciper</i>	Tidal	$p = 0.0212$	-				
	Daily	-	-				
<i>Cibmal</i>	Tidal	-	-				
	Daily	$p = 0.0117$	-				
<i>Ciror</i>	Tidal	-	$p = 0.0032$	-	$p = 0.0026$	-	-
	Daily	$p = 0.0272$	-	$p = 0.0345$	$p = 0.0377$	-	-
<i>Cimelanopsin</i>	Tidal	-	$p = 0.0317$	-	-	-	-
	Daily	-	-	$p = 0.0346$	$p = 0.0017$	-	-
<i>Cirhodopsin</i>	Tidal	-	-	-	$p = 0.0114$		
	Daily	-	-	-	$p = 0.038$		