Supplemental Materials and Methods

Specimen collection

We identified most species by the patterns of male courtship signals, targeting each particular display type with a single net and later measuring length and height of individual animals using a dissecting microscope and ocular micrometer. Several of the species, especially from Panama, are undescribed and we refer to those here by field codes, consisting of two or three capital letters. We classify species into genera based on length:height ratio, which is a reliable genus-level characteristic (1, 2). For emission spectra specimen preservation, methods varied by locale. In Roatan and Puerto Rico, we dried ostracods in direct sunlight. In Panama and Belize, we used a drying oven and transported animals in Eppendorf tubes with silica beads as a dessicant.

Luciferase discovery and amplification.

We designed luciferase-specific primers (Table S7) to amplify from cDNA and obtain sequences that do not include signal peptides (18 or 19 amino acids from the n-terminus, as inferred with SignalP) because we later used yeast-specific signal peptides during protein expression. we used an initial denaturation of 95°C for 2 min. For 30x cycles, we performed a 95°C denaturation step for 1 min., followed by an annealing phase at varying temperatures per species (*K. hastingsi*: 45.5°C, *P. morini*: 48°C, *M. sp.* SVU: 41.1°C , *M. sp.* SVD: 43.7°C, *M chicoi*: 41.4°C, *V. tsujii*: 45.5°C) for 1:45 min., and then by an extension step at 73°C for 1 min. For *V. tsujii* primers designed from the published transcriptome, we used thermal profile: 40 cycles of 94°C for 35s, 55°C for 30s, 72°C for 1min) and amplified the native signal peptide.

Luciferase Expression In Vitro.

We expressed three luciferases in mammalian HEK293 cells. To construct a *V. tsujii* luciferase (VtL) expression vector, we first amplified VtL using primers with engineered restriction sites to clone into a pCR4-TOPO vector. We next excised VtL-pCR4 with XhoI and EcoRI (Promega), and subcloned into a modified pCMV3B mammalian expression vector with a C-terminal mCherry reporter (mCherry-C). The luciferase genes of *P. morini* and *M. sp. SVU* were synthesized and cloned into the mCherry-C vector by Genscript (Piscataway, New Jersey, USA) with flanking restriction enzyme sites. We planned to use mCherry to quantify the concentration of expressed luciferase, but we found high autofluorescence of cell media and/or other secreted proteins to preclude this use. We first transformed cloned constructs into competent *E. coli* cells using the One Shot Chemical Transformation Kit (Invitrogen), and cultured for 24 hours in standard lysogeny broth (LB) with 0.1% kanamycin at 37°C. We verified construct transformation using the engineered restriction enzyme sites in digests and compared them to their expected product size. We extracted these plasmids using the FastPlasmid Mini kit (Qaigen) and assessed concentrations with the Qubit high standard DNA kit (Qubit). For transfection, we cultured mammalian HEK293 cells in Dulbecco's modified

Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin (P/S) at 37°C with 5% CO_2. We then plated 5 x 10^4 cells in each well of a 24-well plate one to three days before transfection. Cell medium was changed to DMEM without serum and antibiotics before transfection. We transfected cells with 0.5 μg of vector using Lipofectamine 2000 (Invitrogen), performed according to the manufacturer's instructions. After 4 hours of incubation, we replaced the transfection medium with DMEM+FBS+P/S and allowed the cells to recover for 24 hours. We collected cells via trypsin digestion and reseeded them into 10mL of DMEM+FBS+P/S+1% G418 to select against untransfected cells in 90cm cell plates. We cultured the transfected cells for 3 to 5 days before harvesting and using in light catalysis assays.

For expression in *Pichia* yeast, we cloned sequences into the pPICZ-αC vector at the XhoI and NotI sites following standard procedures (Invitrogen Easy Select *Pichia* kit). First, we analyzed predicted c-luciferases for the presence of a signal peptide at the n-terminal end using SignalP v4.1 (3). We then designed primers for cloning and protein expression to amplify the entire c-luciferase sequence without the native signal peptide, beginning usually 51-54 bp inside the 5' end from the predicted start codon. 3' end primers excluded the native stop codon so that a fusion construct could be generated. Fusion constructs were made via the EasySelection Pichia expression kit (Invitrogen) using the pPICZ-αC vector according to the manufacturer's instructions. Briefly, we used the 5' XhoI site in order to generate fusion c-luciferases with an alpha secretion signal from yeast. We reconstructed the Kecx2 cleavage site with one Glycine Alanine repeat region via PCR. On the 3' end, we used NotI; this would result in the addition of extra amino acids in our expressed proteins on the c-terminal end before inclusion of the fusion c-myc epitope and histidine tags. We transformed newly-made, linearized constructs into *Pichia* using electroporation with a BioRad Micro-Pulser using the Sc2 program (1.5 V). After electroporation, *Pichia* were allowed to recover in selective media for an hour before plating. We initially selected for recombinant *Pichia* colonies using two concentrations of zeocin (100 and 500 mg/mL). After three days of growth, individual colonies were replica-plated at high zeocin concentrations (1,000 and 2,000 mg/mL) to try and screen for high copy-number integrants for our gene of interest. After one day, we selected single colonies that grew best at high zeocin concentrations to induce protein expression according to the manufacturer's guidelines. To stabilize the pH of the media for extended expression, colonies were grown in 25mL buffered media with glycerol in baffled flasks until the OD_{600} reached 2.0 - 8.0. For our colonies, this occurred after 72 hrs due to suboptimal shaking conditions. We then calculated the amount of original growth we would need for an OD_{600} of 1.0 in 30mL expression media, spun down the appropriate volume of the original colonies at 3,000 g for 5min., removed the glycerol media, and resuspended the pellet in 30mL of buffered media with methanol in a 125 mL baffled flask. Flasks were shaken in a table-top incubator at 29.5C at 300 rpm for 3 days, with media supplemented with 100% methanol every 24hrs to maintain a 0.5% volume of methanol in culture.

Emission Spectra

In earlier trials of emission spectra data collection, we introduced specimens into a test tube placed inside Spectralon-coated 150 mm diameter integrating sphere (Labsphere). But because we report relative rather than absolute levels of light at different wavelengths, we abandoned the integrating sphere in later trials for a rectangular quartz cuvette to increase sensitivity of our analyses.

To correct for variation in emission, background noise, and quality during data collection, we first summed all collection time points for one sample, then subtracted each background value for each wavelength, and corrected using measurements from the black body radiator before standardizing each spectrum, setting the maximum value to 1.0 as the wavelength with the most photons. Some specimens did not yield strong light emission, probably due to variation in drying. We filtered low quality data using signal to noise ratio. Specifically, we sorted emission values at each wavelength from lowest to highest and averaged the lowest 1000 data points to estimate a baseline emission value (E_{min}). We then found the maximum emission value (E_{max}). From this, we calculated a signal to noise value as E_{min}/E_{max}, removing trials where $E_{min}/E_{max} < 0.02$.

Notes on alignment and site numbering

Throughout the main text, as we mentioned, sites are referred to based on their homologous position within the reference reporter luciferase, CnL (the orthologous sequence from the luminous Japanese ostracod *Cypridina noctiluca*). All result files from HyPhy however, report sites based on alignment number, and Figure S3 is plotted with respect to those sites. We provide a conversion key (Table S8) in this SI for ease of interpretation, and our Github code has two functions to easily convert back and forth between the numbering systems (aligned2cyp & cyp2aligned).

Supplemental Figures and Tables

Supplemental Figure S1. Multiple sequence alignment for putative and functional c-luciferase sequences

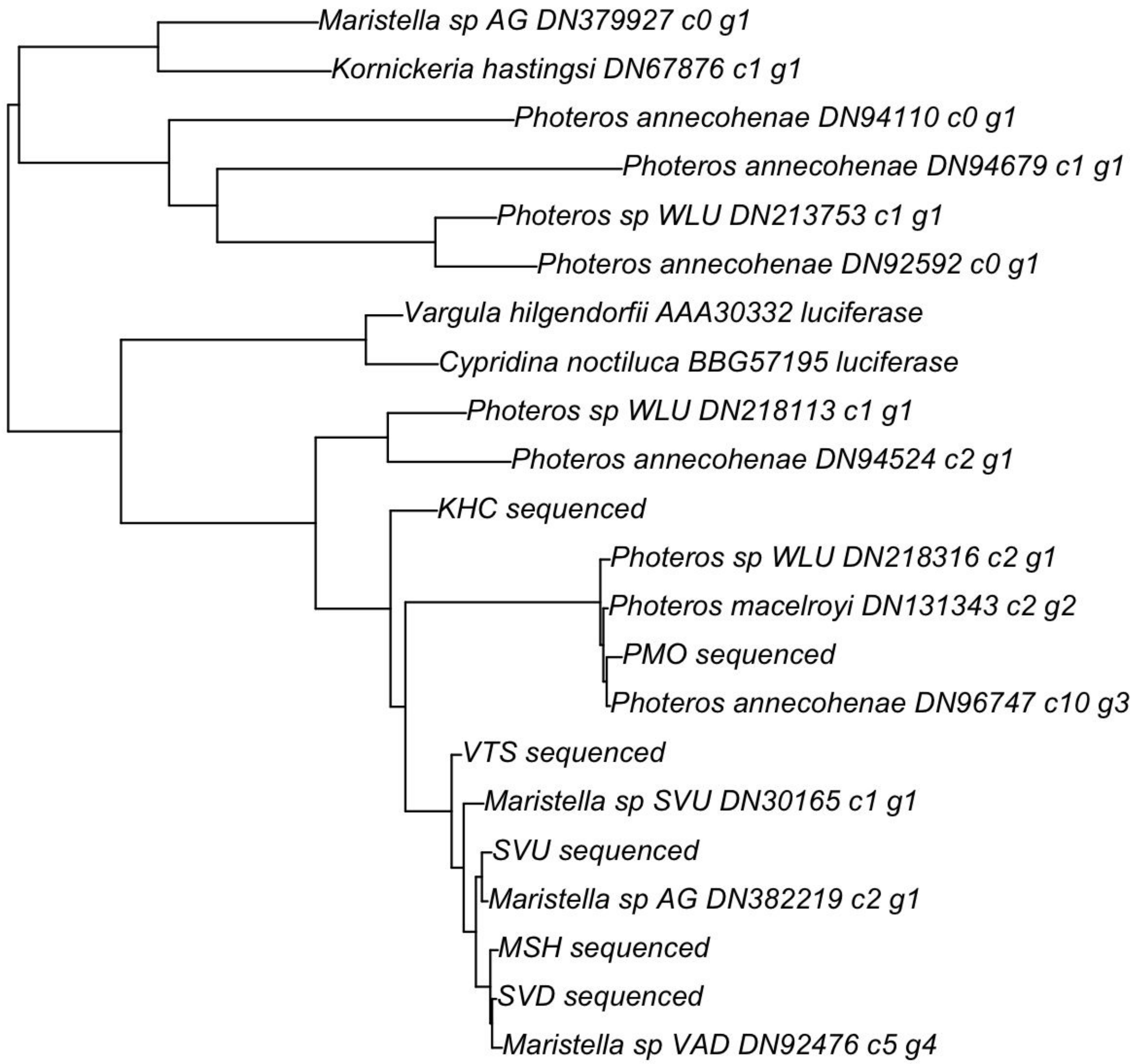
```
Vargula_hilgendorfii      ----------------------MKIIILSVILAYCVTDNCQDACPVEAEP  28
Cypridina_noctiluca_      ----------------------MKTLILAVALVYCVTVNCQE-CPYVADP  27
Kornickeria_hastings      -------------------------------------KDCFESSFHSLE-  12
Vargula_tsujii_seque      -------------------------------------QDCYESTWASND-  12
Maristella_chicoi_se      -------------------------------------EDCYEFTWASND-  12
Maristella_sp_SVD_se      ----------------------------------------YEFTWASND-  9
Maristella_sp_SVU_se      -------------------------------------QDCYEFTWASND-  12
Maristella_sp_AG_DN3      MGLKTHSAFVISREIRSGIMWLQSLL-LLAGICFCAGQDCYEFTWASND-  48
Maristella_sp_VAD_DN      -------------------MWLQSLL-LLAGICFCAGEDCYEFTWASND-  29
Photeros_sp_WLU_DN21      -------------------MWLQNLLILLVGIYFSSGEECAQTSPTYLD-  30
Photeros_macelroyi_D      -------------------MWLQNLLILLVGIYFSSGEECAQTSLTSLD-  30
Photeros_morini_sequ      -------------------------------------QECAQTSLTSLD-  12
Photeros_annecohenae      -------------------MWLQNLLILLVGIYFSSGEECAQTSLTSLD-  30

Vargula_hilgendorfii      PSSTPTVPTSCEAKEGECIDTRCATCKRDILSDGLCENKPGKT--CCRMC  76
Cypridina_noctiluca_      PN---TVPTSCEAKEGECIDSSCSTCTRDILSDGLCENKPGKT--CCRMC  72
Kornickeria_hastings      ------FPSSCEAQNGICIDSECKDCNEVMFGDGLCENAGGASPKCCRDC  56
Vargula_tsujii_seque      ------YPSSCEALNGRCVDSACGSCDEVLFGDGLCENAGGASPKCCRDC  56
Maristella_chicoi_se      ------YPSSCEAINGRCVDSACGSCDQVLFGEGLCENAGGASPKCCRDC  56
Maristella_sp_SVD_se      ------YPSSCEAINGRCVDSACGSCDQVLFGDGLCENAGGASPKCCRDC  53
Maristella_sp_SVU_se      ------FPSSCEAINGRCVDSACGSCDEVLFGDGLCENAGGASPKCCRDC  56
Maristella_sp_AG_DN3      ------FPSSCEAINGRCVDSACGSCDEVLFGDGLCENAGGASPKCCRDC  92
Maristella_sp_VAD_DN      ------YPSSCEAINGRCVDSACGSCDQVLFGDGLCENAGGASPKCCRDC  73
Photeros_sp_WLU_DN21      -----SVPSSCEAQRGVCKDSSCSGCDKALYGDDMCENSGVANAKCCRDC  75
Photeros_macelroyi_D      -----SVPSSCEAQRGVCKDSSCSGCDKALYGDDMCENSGIDSAKCCRDC  75
Photeros_morini_sequ      -----SAPSSCEAQKGVCKDPSCSGCDKALYGDDMCENSGVANAKCCRDC  57
Photeros_annecohenae      -----SVPSSCEAQKGVCKDSSCSGCDKALYGDDMCENSGVANAKCCRDC  75

Vargula_hilgendorfii      QYVIECRVEAAGYFRTFYGKRFNFQEPGKYVLARGTKGGDWSVTLTMENL  126
Cypridina_noctiluca_      QYVIECRVEAAGWFRTFYGKRFQFQEPGTYVLGQGTKGGDWKVSITLENL  122
Kornickeria_hastings      PEIVRCRASAAGFFTTFYGKKFNLQEPGTYLLSEDCVGGLWSLYVTLVNI  106
Vargula_tsujii_seque      PEIVRCRVSAAGYFQTFYGQRFNLQVPGTYLLSEDCVGGLWSLYVNLANI  106
Maristella_chicoi_se      PEIVRCRASAAGFFHTFYGQRFNLQVPGTYLLSEDCVGGLWSLYVNLANV  106
Maristella_sp_SVD_se      PEIVRCRASAAGFFHTFYGQRFNLQVPGTYLLSEDCVGGLWSLYVNLANV  103
Maristella_sp_SVU_se      PEIVRCRASAAGFFHTFYGQRFNLQVPGTYLLSEDCVGGLWSLYVNLANI  106
Maristella_sp_AG_DN3      PEIVRCRASAAGFFHTFYGQRFNLQVPGTYLLSEDCVGGLWSLYVNLANI  142
Maristella_sp_VAD_DN      PEIVRCRASAAGFFHTFYGQRFNLQVPGTYLLSEDCVGGLWSLYVNLANV  123
Photeros_sp_WLU_DN21      PEVVRCRAAGAGYFTTFYGKRFNFQVPGKYLLSEDCVGGLWSLYVNLAPI  125
Photeros_macelroyi_D      PEVVRCRAAGGGYFTTFYGKRFNFQVPGKYLLSEDCVGGLWSLYVNLAPI  125
Photeros_morini_sequ      PAVVKCRAAGAGYFTTFYGKRFNFQVPGKYLLSEDCVGGLWSLYVNLAPI  107
Photeros_annecohenae      PAVVKCRAAAAGYFTTFYGKRFNFQVPGKYLLSEDCVGGLWSLYVNLAPI  125

Vargula_hilgendorfii      DGQKGAVLTKTTLEVAGDV-IDITQATADPITVNGGADPVIANPFTIGEV  175
Cypridina_noctiluca_      DGTKGAVLTKTRLEVAGDI-IDIAQATENPITVNGGADPIIANPYTIGEV  171
Kornickeria_hastings      AGEKGAVLGSVKM-IVGEVTVDIVQKGG-PVTVNGGSVAIDSNPFSIGDV  154
Vargula_tsujii_seque      EGEKGAVLDSVKM-VVGDVTVDIKQKVG-AVTVNGGSVEIDSNPFSIGDV  154
Maristella_chicoi_se      EREKGAVLDSVKM-VVGDVTLDIKQKVG-SITVNGGTVDIDSNPFSIGDV  154
Maristella_sp_SVD_se      EGEKGAVLDSVKM-VVGDVTLDIKQKVA-SITVNGGTVDIDSNPFSIGDV  151
Maristella_sp_SVU_se      EGEKGAVLDSVKM-VVGDVTLDIKQKVG-SITVNGGSVEIDSNPFSIGDV  154
Maristella_sp_AG_DN3      EGEKGAVLDSVKM-VVGDVTLDIKQKVG-SITVNGGSVAIDSNPFSIGDV  190
Maristella_sp_VAD_DN      EGEKGAVLDSVKM-VVGDVTLDIKQRLG-YITVNGGAVAIDSNPFSIGDV  171
Photeros_sp_WLU_DN21      EGQKGAALESVNM-IVGDVTVDITQKGG-PIVVNKGDVPIDSNPFSIGDV  173
Photeros_macelroyi_D      EGQKGAALESVNM-IVGDVTVDITQKGG-PIVVNKGDVPIDSNPFSIGDV  173
Photeros_morini_sequ      EGQKGAALESVNM-IFGDVTIDITQKGG-PIVVNKGDVQIDSNPLSIGDV  155
Photeros_annecohenae      EGQKGAALESVNM-IVGDVTVDITQKGG-PILVNKGDVPIDSNPFSIGDV  173

Vargula_hilgendorfii      TIAVVEIPGFNITVIEFFKLIVIDILGGRSVRIAPDTANKGLISGICGNL  225
Cypridina_noctiluca_      TIAVVELPGYNITVIEFFKLIVIDILGGRSVRIAPDTANKGMISGLCGDL  221
Kornickeria_hastings      TIAIVHTPNFDVAVIEFLKLVTFDIIHGRAFRLAPDFLYRDRTCGLCG-V  203
Vargula_tsujii_seque      TIAIVHTPNFDVSVIEFLKLVTFDILQGRAFRLAPDFLYADRTCGLCG-V  203
Maristella_chicoi_se      TIAIVHTPYFDVSVIEFLKLVTFNILQGRAFRLAPDFLYADRTCGLCG-V  203
Maristella_sp_SVD_se      TIAIVHTPFFDVSVIEFLKLVTFDILQGRAFRLAPDFLYADRTCGLCG-L  200
Maristella_sp_SVU_se      TIAVVYTPYFSVSVIEFLKLVTFDILQGRAFRLAPDFLYADRTCGLCG-L  203
Maristella_sp_AG_DN3      TIAVVYTPYFSVSVIEFLKLVTFDILQGRAFRLAPDFLYADRMCGLCG-L  239
Maristella_sp_VAD_DN      TIAIVHTPYFDVSVIEFLKLVTFDILQGRAFRLAPDFLYADRTCGLCG-L  220
Photeros_sp_WLU_DN21      TIAVVTPPFSHVSVIEFRRIVTFDIISGRAFRLAPDFYYRDRTCGLCG-L  222
Photeros_macelroyi_D      TIAIVTPPFSKVSVIEFLRIVTFDIISGRAFRLAPDFYYRDRTCGLCG-L  222
Photeros_morini_sequ      TIAIVTPPFSKVSVIEFLRIVTFDIISGRAFRLAPDFYYRDRTCGLCG-L  204
Photeros_annecohenae      TIAIVTPPFSKVSVIEFLRIVTFDIISGRAFRLAPDFYYRDRTCGLCG-L  222

Vargula_hilgendorfii      EMNDADDFTTDADQLAIQPNINKEFDGCPFYGNPSDIEYCKGLMEPYRAV  275
Cypridina_noctiluca_      KMMEDTDFSSDPEQLAIQPKINQEFDGCPLYGNPEDITYCKGLLEPYKDS  271
Kornickeria_hastings      MSNEPTDFIDNPDQLAVQDKINKDIDGCPLSGNPSDVEYCKNKMQPYKVA  253
Vargula_tsujii_seque      MSDEPTDFIDNPDQLAIQDQMNQDIDGCPLSGNPSDVEYCVNKMQPYKDG  253
Maristella_chicoi_se      MSDEPSDFIDNPDQLAIQDLMNQDVEGCPLSGNPSDIEYCVNKMQPYKDG  253
Maristella_sp_SVD_se      MSDEPSDFIDNPDQLAIQDLMNQDVEGCPLSGNPSDIEYCVNKMQPYKDG  250
Maristella_sp_SVU_se      MSDEPSDFIDDPDQLAIQDIMNQDVEGCPLSGNPSDAEYCLNKMQPYKDG  253
Maristella_sp_AG_DN3      MSDVPSDFIDNPDQLAIQDIMNQDVEGCPLSGNPSDVEYCLNKMQPYKDG  289
Maristella_sp_VAD_DN      MSDEPSDFIDNPDQLAIQDLMNQDVEGCPLSGNPSDIEYCVNKMQPYKDG  270
Photeros_sp_WLU_DN21      MTTEASDFVNNPDQLAIKDQIDQDVEGCPLSGNPSDVEYCTKYLKPHQDN  272
Photeros_macelroyi_D      MTTEASDFVNCPDQLAIKDQIDQDVEGCPLSGNPSDVEYCTKYLKPHQDN  272
Photeros_morini_sequ      MTTEASDFVNCPDQLSIKDQIDQDVEGCPLSGNPSDVEYCTKYLKPHQDN  254
Photeros_annecohenae      MTTEASDFVNCPDQLAIKDQIDHDVEGCPLSGNPSDVEYCTKYLKPHQDN  272
```

Supplemental 5

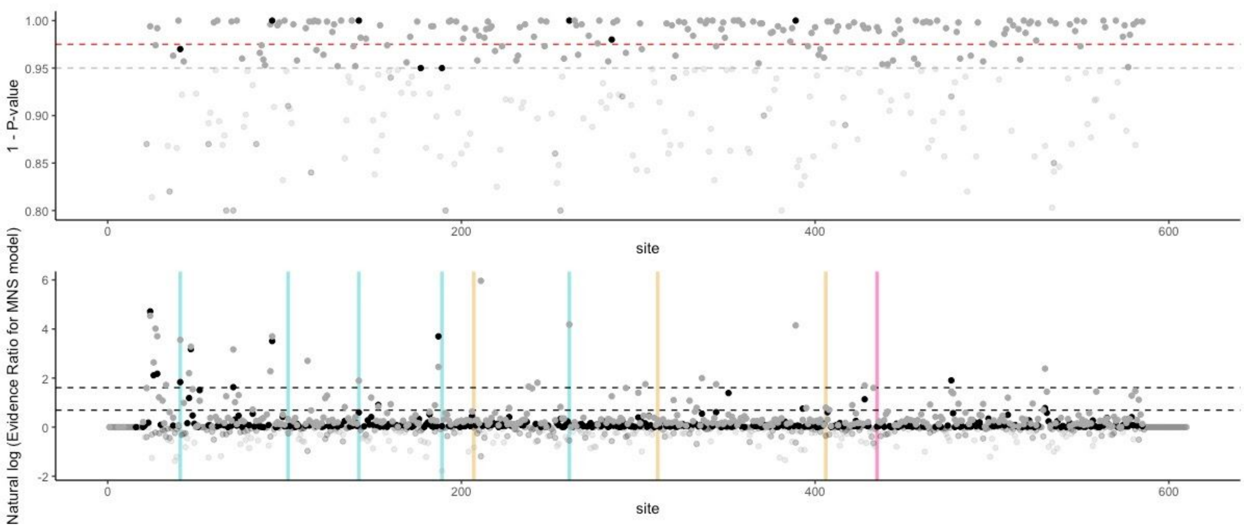
```
Vargula_hilgendorfii     CRN--NINFYYYTLSCAFAYCMGGEERAKHVLFDYVETCAAPETRGTCVL   323
Cypridina_noctiluca_     CRN--NINFYYYTLSCAFARCMGGDERASHVVLDYRETCAAPETRGTCVL   319
Kornickeria_hastings     CINYNDVNFATYLYSCALAYCMGGEDRVEDVIFDYVEACVEPITRATCVM   303
Vargula_tsujii_seque     CVNKNDVHFGTYLYACALAYCMGGDDRVEDVIFEYAEACVEPIGRATCVM   303
Maristella_chicoi_se     CINKNDVHFGTYLYACALAYCMGGDDRVEDVIFEYAEACVEPIGRATCVM   303
Maristella_sp_SVD_se     CINKNDVHFGTYLYACALAYCMGGDDRVEDVIFEYAEACVEPIGRATCVM   300
Maristella_sp_SVU_se     CINKNDVHFGTYLYACALAYCMGGDDRVEDVIFEYAEACVEPIGRATCVM   303
Maristella_sp_AG_DN3     CINKNDVHFGTYLYACALAYCMGGDDRVEDVIFEYAEACVEPIGRATCVM   339
Maristella_sp_VAD_DN     CINKNDVHFGTYLYACALAYCMGGDDRVQDVIFEYAEACVEPIGRATCVM   320
Photeros_sp_WLU_DN21     CNNGDAIHFATYVYACALAYCMGGDDRAEDVAMDYQEACVDPIGRGTCVM   322
Photeros_macelroyi_D     CKNGDAIHFATYVYACALAYCMGGDDRAEDVAMDYQEACVDPIGRGTCVM   322
Photeros_morini_sequ     CKNGDAIHFATYVYACALAYCMGGDDRAEDVAMDYQEACVDPIGRGTCVM   304
Photeros_annecohenae     CKNGDAIHFATYVYACALAYCMGGDDRAEDVAMDYQEACVDPIGRGTCVM   322

Vargula_hilgendorfii     SGHTFYDTFDKARYQFQGPCKEILMAADCYWNTWDVKVSHRDVESYTEVE   373
Cypridina_noctiluca_     SGHTFYDTFDKARYQFQGPCKEILMAADCYWNTWDVKVSHRNVDSYTEVE   369
Kornickeria_hastings     NGHTYYDTFDKTSYQFQAPCK-VLFAKDCAGDEWEVTITHKAAGTYTEVE   352
Vargula_tsujii_seque     NGHTYYDTFDKTSYQFQAPCK-VLFAKDCTDDDWEVTITHKAVGEYTEVE   352
Maristella_chicoi_se     NGHTYYDTFDKTSYQFQAPCK-VLFAKDCTDDDWEVTITHKAAGEYTEVE   352
Maristella_sp_SVD_se     NGHTYYDTFDKTSYQFQAPCK-VLFAKDCTDDDWEVTIVHKAAGEYTEVE   349
Maristella_sp_SVU_se     NGHTYYDTFDKSSYQFQAPCK-VLFAKDCTDDEWEVTITHKEAGEYTEVE   352
Maristella_sp_AG_DN3     NGHTYYDTFDKTSYQFQAPCK-VLFAKDCTDDEWEVTITHKAAGEYTEVE   388
Maristella_sp_VAD_DN     NGHTYYDTFDKTSYQFQAPCK-VLFAKDCTDDEWEVTIVHKAAGEYTEVE   369
Photeros_sp_WLU_DN21     SGHTFYDTFDKTSFQYQAPCK-VQFSKDCIGDDWEVSITYKPKADYTVVD   371
Photeros_macelroyi_D     SGHTFYDTFDKTSFQYQTPCK-VQFSKDCIGDDWEVSITYKPKADYTVVD   371
Photeros_morini_sequ     SGHTFYDTFDKTSFQYQTPCR-VQFSKDCVGDDWEVSITYKPKADYTVVD   353
Photeros_annecohenae     SGHTFYDTFDKTSFQYQTPCK-VQFSKDCIGDDWEVSITYKPKADYTVVD   371

Vargula_hilgendorfii     KVTIRKQSTVVDLIVDGKQVKVGGVDVSIPYSSENTSIYWQDGDILTTAI   423
Cypridina_noctiluca_     KVRIRKQSTVVELIVDGKQILVGGEAVSIPYSSQNTSIYWQDGDILTTAI   419
Kornickeria_hastings     KVTVRYFQTLIDLISEGKQVLVNGTEVSVPYNKGDTSIYMYD-NLITTAV   401
Vargula_tsujii_seque     KVTVRYFQTLIDLISETKKVFVNGTEVSVPYNYGDTSIYMYD-NLITTAV   401
Maristella_chicoi_se     KVTVRYFQTLIDLIAESKKVFVNGTEVSVPYNYGDTSIYMYD-NLITTAV   401
Maristella_sp_SVD_se     KVTVRYFQTLIDLVAENKKVFVNRTEVSVPYNYGDTSIYMYD-NLITTAV   398
Maristella_sp_SVU_se     KVTVRYFQTLIDLVAENKKVFVNGTEVSVPYNYGDTSIYMYD-NLVTTAV   401
Maristella_sp_AG_DN3     KVTVRYFQTLIDLIAENKKVFVNGTEVSAPYNYGDTSIYMYD-NLITTAV   437
Maristella_sp_VAD_DN     KVTVRYFQTLIDLVAESKKVFVNGTEVSVPYNYGDTSIYMYD-NLVTTAV   418
Photeros_sp_WLU_DN21     KVTVRYFATLIDLIPEGRQVLVNGSAVSVPFNYADTSVYMYE-NLITTAV   420
Photeros_macelroyi_D     KVTVRYFATLIDLIPEGRQVLVNGSAVSVPFNYADTSIYMYE-NLITTAV   420
Photeros_morini_sequ     KVTVRYFATLIDLIPEGRQVLVNGSAVSVPFNYADTSIYMYE-NLITTAV   402
Photeros_annecohenae     KVTVRYFATLIDLIPEGRQVLVNGSAVSVPFNYADTSIYMYE-NLITTAV   420

Vargula_hilgendorfii     LPEALVVKFNFKQLLVVHIRDPFD-GKTCGICGNYNQDSTDDFFDAEGA-   471
Cypridina_noctiluca_     LPEALVVKFNFKQLLVVHIRDPFD-GKTCGICGNYNQDFSDDSFDAEGA-   467
Kornickeria_hastings     LPGAVVVKYNFEQMLALHIRDPYE-RRSCGLCGIWDLDKSNDGPDNQYVD   450
Vargula_tsujii_seque     LPGAVVVKYNFEQMLALHIRDPYE-ADSCGLCGIWDLDKSNEGPDTQYVD   450
Maristella_chicoi_se     LPGAVVVKYNFAQMLALHIRDPYE-ADSCGLCGIWDLDKSNDGPDTQYVD   450
Maristella_sp_SVD_se     LPGAVVVKYNFAQMLALHIRDPYE-ADSCGLCGIWDLDKSNDGPDTQYVD   447
Maristella_sp_SVU_se     LPGAVVVKYNFEQMLALHIRDPYE-ADSCGLCGIWDLDKSNDGPDAQHAD   450
Maristella_sp_AG_DN3     LPGAVVVKYNFAQMLALHIRDPYE-ADSCGLCGIWDLDKSNDGPDTQYAD   486
Maristella_sp_VAD_DN     LPGAVVVKYNFAQMLALHIRDPYE-ADSCGLCGIWDLDKSNDGPDTQYVD   467
Photeros_sp_WLU_DN21     LPGAVVVKYNFDQMLALHIRDPYEHGRSCGLCGLWDLDNTNDEPQRQYSE   470
Photeros_macelroyi_D     LPGAVVVKYNFDQMLALHIRDPYEHGRSCGLCGLWDLDNTNDEPQRQYSE   470
Photeros_morini_sequ     LPGAVVVKYNFDQMLALHIRDPYENGRACGLCGLWDLDNTNDEPQRQYSE   452
Photeros_annecohenae     LPGAVVVKYNFDQMLALHIRDPYEHGRSCGLCGLWDLDNTNDEPQRQYSE   470

Vargula_hilgendorfii     CALTPNPPGCTEEQKPEAERLCNNLF--DSSIDEKCNVCYKPDRIARCMY   519
Cypridina_noctiluca_     CDLTPNPPGCTEEQRPEAERLCNSLFVGQSDLDQKCNVCYKPDRVERCMY   517
Kornickeria_hastings     CEPTPNPATCTADQEAEARELCQNMF--PASLDDECDICYKSDRVERCMY   498
Vargula_tsujii_seque     CEPTPNPPTCTADKEAEARELCQNMF--PASLDDQCNICYKADRVERCMY   498
Maristella_chicoi_se     CEPTPNPPTCTADKEAEARELCQNMF--PASIDDECDICYKADRVERCMY   498
Maristella_sp_SVD_se     CEPTPNPPTCTADKEAEARELCQNMF--PASIDDECDICYKADRVERCMY   495
Maristella_sp_SVU_se     CEPTPNPPTCTADKEAEARELCQNMF--PASIDDKCNICYKADRVERCMY   498
Maristella_sp_AG_DN3     CEPTPNPPTCTADKEAEARELCQNMF--PASIDDKCNICYKADRVERCMY   534
Maristella_sp_VAD_DN     CEPTPNPPTCTADKEAEARELCQNMF--PASIDDECDICYKADRVERCMY   515
Photeros_sp_WLU_DN21     CDPTPNPPHCSAEKEAEARDLCANMF--PANLDDVCNICYNADRMKRCMY   518
Photeros_macelroyi_D     CDPTPNPPHCSAEKEAEARDLCANMF--PANLDDVCNICYNADRMKRCMY   518
Photeros_morini_sequ     CDPTPSPPHCSAEKEAEARDLCANMF--PANLDDVCNICYNADRMKRCMY   500
Photeros_annecohenae     CDPTPNPPHCSAEKEAEARDLCANMF--PANLDDVCNICYNADRMKRCMY   518

Vargula_hilgendorfii     EYCLRGQQGFCDHAWEFKKECYIKHGDTLEVPPECQ-   555
Cypridina_noctiluca_     EYCLRGQQGFCDHAWEFKKECYIKHGDTLEVPDECK-   553
Kornickeria_hastings     EYCLGGMDYFCKHAGTVIDECFVRHGDDLQLPPQCTK   535
Vargula_tsujii_seque     EYCLGGLEGFCQHAGTVIDECFVRHGDDLQYPPQCK-   534
Maristella_chicoi_se     EYCLGGLEGFRQHAGTVIDECFVRHGDDLQYPPQCK-   534
Maristella_sp_SVD_se     EYCLGGLEGFCQHAGTVIDECFVRHGDDLQYPPQCK-   531
Maristella_sp_SVU_se     EYCLGGLEAFCQHAGTVIDECFVRHGDDLQYPPQCK-   534
Maristella_sp_AG_DN3     EYCLGGLEAFCQHAGTVIDECFVRHGDDLQYPPQCK-   570
Maristella_sp_VAD_DN     EYCLGGLEGFCQHAGTVIDECFVRHGDDLQYPPQCK-   551
Photeros_sp_WLU_DN21     EYCLNGKEGICQHATSILDECFVRHGDDYKVPAICQ-   554
Photeros_macelroyi_D     EYCLNGMEGICQHANSILDECFVRHGDDYKVPAICQ-   554
Photeros_morini_sequ     EYCLNGMEGICQHANSILDECFVRHGDDYKVPAICQ-   536
Photeros_annecohenae     EYCLNGMEGICQHANSILDECFVRHGDDYKVPAICQ-   554
```

Supplemental 6

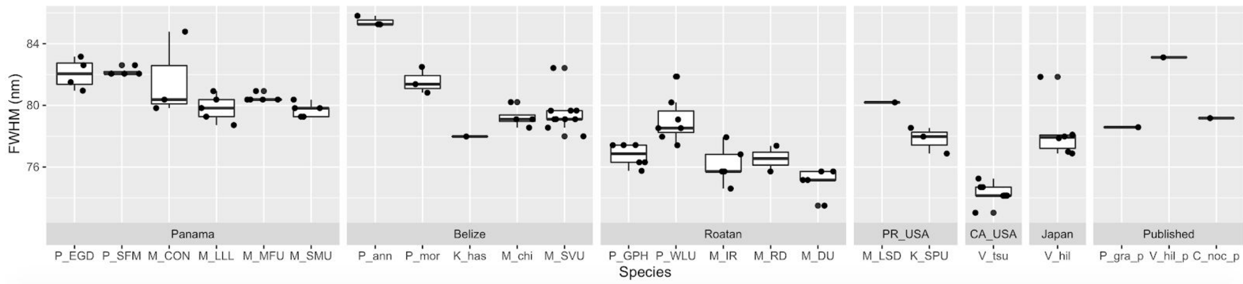
Supplemental Figure S2 - Maximum likelihood phylogeny of sequences most similar to published c-luciferases.



Supplemental Figure S3**.** Plots of significance tests (top panel) and evidence ratios (bottom panel) for each codon site. Top panel: 1 - p-values from HyPhy MEME tests (black) or FEL (gray). Dashed lines are significance level at p ≤ 0.05 (grey) or q ≤ 0.025 (corrected for multiple tests). Sites above the dotted lines have signatures of diversifying or purifying selection. Bottom panel: Natural log of the evidence ratio from HyPhy Multihit tests comparing the support for a model with a dinucleotide mutation over a single mutation (gray), or the support for a model with a trinucleotide mutation over a single hit (black). Opaque data have an evidence ratio greater than 1. Dashed horizontal lines are evidence ratio levels supporting a model with a multinucleotide substitution over a model with a single nucleotide substitution by 2x (lower line) or 5x (upper line) greater chance of support. Note that evidence ratio support is continuous between alternative models with no distinct cut-off; lines are added for aid in interpretation. Horizontal colored bars correspond to sites with functional correlates from Table 1. Bar colors represent dn:ds values consistent with diversifying selection (blue), purifying selection (red), or neutral (yellow).



Supplemental Figure S4. Full Width of emission spectrum at Half of the Maximum value (FWHM) in nanometers (nm) from new emission spectra from 20 species and previously published spectra from 3 species.



Supplemental Figure S5. We find no relationship between the color of light emission and light decay constants (Pearson's correlation test, t = , df = 16, p = 0.4). Scatter plot of the average decay (x-axis) and peak emission spectra (y-axis) measured for different species of luminescent ostracod (N = 17 species). Data are colored by genus, and shape by country of origin. Although *Photeros* greatly differ in their peak emission spectra than other genera, there is no strong pattern for differences in light decay constant.

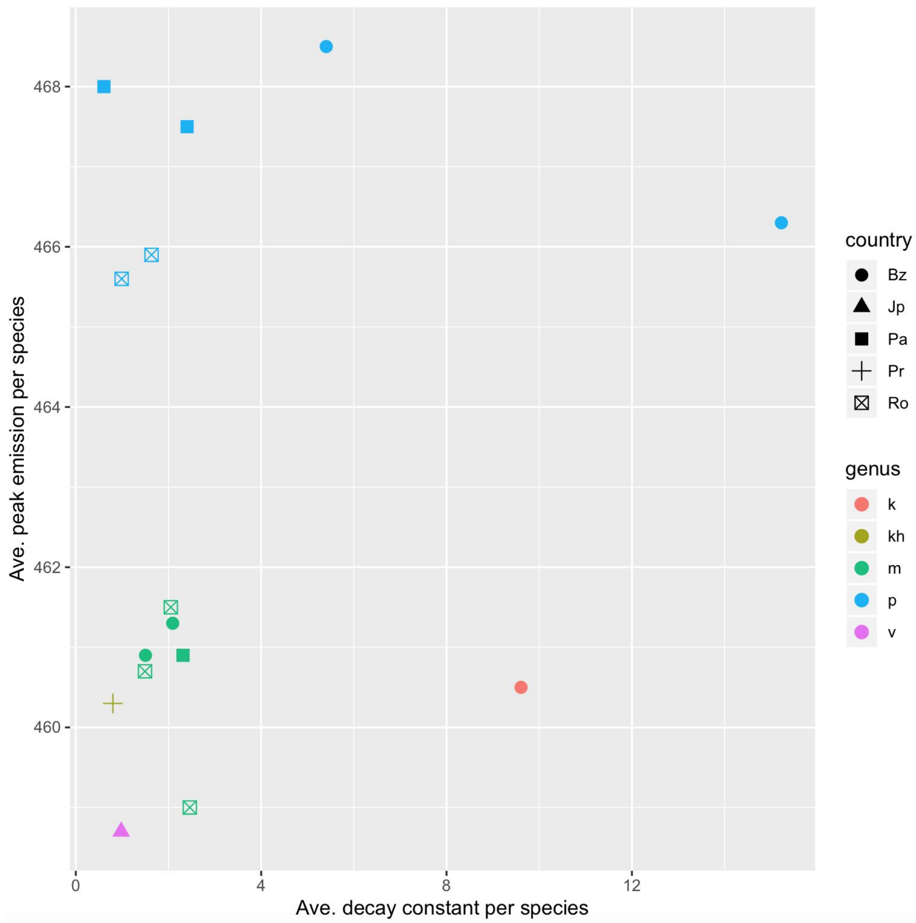


Table S1 - Previously published emission spectra for Cypridinidae

Species	λ max(nm)	FWHM (nm)	Method	Light Units	Citation
Vargula hilgendorfii	459		Absolute max	Relative Quanta	(4)
Vargula hilgendorfii	465	84	Savitzky-Golay with 2°polynomial and 25 channel smoothing, slit = 1, .1 mm	Energy	(5)
Vargula tsujii	466	87	Savitzky-Golay with 2°polynomial and 25 channel smoothing, slit=1 mm	Energy	(5)
Cypridina noctiluca	465		Not described	Not described	(6)
Cypridina noctiluca	454		Absolute Max following FFT/LPT (>0.05) smoothing in OriginPro. N=2	Not described	(7)
Photeros shulmanae	473	80	Cited (Widder et al. 1983)		(8)
Photeros graminicola	473	80	Cited (Widder et al. 1983)		(8)

Table S2 - Collection localities, size measurements, and accession numbers for vouchers for specimens used in this study

Inferred genus	Species or field code	Locality	Latitude	Longitude	Carapace length	Carapace height	Carapace ratio	BioProject	Luciferase Genbank Accession	SRA Accession	RNA Isolation	Library Preparation	Sequencing Instrument
Photeros	EGD	Bocas del Toro, Panama	9.331385	-82.25434	1.625	1.044	1.56		N/A	N/A			
Photeros	SFM	Bocas del Toro, Panama	9.331326	-82.25327	1.703	1.077	1.58		N/A	N/A			
Photeros	annecohenae	Southwater Caye, Belize	16.8116	-88.08243	1.623	1.02	1.59	PRJNA589015		SRR1086080	Qiagen Rneasy Fibrous Tissue Mini Kit	Illumina TruSeq v3	Illumina HiSeq 2500
Photeros	morini	Southwater Caye, Belize	16.8116	-88.08243	2.056	1.282	1.6	PRJNA589015		SRR1086077	Trizol	NEB Ultra RNA Library Prep Kit for	Illumina HiSeq 1500

												Illumina	
Photeros	morini	Southwater Caye, Belize	16.8116	-88.08243	2.056	1.282	1.6	PRJNA589015		SRR10860876	Qiagen Rneasy	Illumina TruSeq v2	Illumina HiSeq 1500
Photeros	GPH	Roatan, Honduras	16.40272	-86.409	1.683	1.038	1.62		N/A	N/A			
Photeros	WLU	Roatan, Honduras	16.35806	-86.43291	1.978	1.222	1.62	PRJNA589015		SRR10811635	Trizol	NEB Next Ultra IIRNA Library Prep Kit for Illumina	NextSeq 500
Photeros	mcelroyi	Discovery Bay, Jamaica						PRJNA589015		SRR10811638	Qiagen RNA	Illumina TruSeq v3	Illumina HiSeq 1500
Photeros	mcelroyi	Discovery Bay, Jamaica						PRJNA589015		SRR10811637	Qiagen RNA	Illumina TruSeq v3	Illumina HiSeq 1500
Photeros	mcelroyi	Discovery Bay, Jamaica						PRJNA589015		SRR10811645	Trizol	NEB Next Ultra RNA Library Prep	Illumina HiSeq 1500

												Kit for Illumina	
Maristella	MFU	Bocas del Toro, Panama	9.331326	-82.253267	1.657	1.002	1.65		N/A	N/A			
Maristella	IR	Roatan, Honduras	16.358806	-86.43291	2.282	1.386	1.65		N/A	N/A			
Maristella	SVU (MWU)	Southwater Caye, Belize	16.8116	-88.08243	2.172	1.305	1.66	PRJNA589015		SRR10811644	Trizol	NEB Next Ultra RNA Library Prep Kit for Illumina	Illumina HiSeq 1500
Maristella	SVU (MWU)	Southwater Caye, Belize	16.8116	-88.08243	2.172	1.305	1.66	PRJNA589015		SRR10811643	Qiagen RNA	Illumina TruSeq v2	Illumina HiSeq 1000
Maristella	SVD	Southwater Caye, Belize	16.8116	-88.08243				PRJNA589015		SRR10860875	Trizol	NEB Ultra RNA Library Prep Kit for Illumina	Illumina HiSeq 1500

Maristella	SVD	Southwater Caye, Belize	16.8116	-88.08243				PRJNA589015		SRR10860874	Qiagen Rneasy	Illumina TruSeq v2	Illumina HiSeq 1500
Maristella	DU	Roatan, Honduras	16.35806	-86.43291	1.631	0.981	1.66		N/A	N/A			
Maristella	RD	Roatan, Honduras	16.358061	-86.432906	1.637	0.98	1.67		N/A	N/A			
Maristella	LLL	Bocas del Toro, Panama	9.331707	-82.255633	1.775	1.057	1.68		N/A	N/A			
Maristella	SMU	Bocas del Toro, Panama	9.331707	-82.255633	2.162	1.289	1.68		N/A	N/A			
Maristella	chicoi	Southwater Caye, Belize	16.8116	-88.08243	1.624	0.963	1.69	PRJNA589015	N/A	SRR10811640	Trizol	NEB Next Ultra RNA Library Prep Kit for Illumina	Illumina HiSeq 1500
Maristella	chicoi	Southwater Caye, Belize	16.8116	-88.08243	1.624	0.963	1.69	PRJNA589015	N/A	SRR10811639	Qiagen RNA	Illumina TruSeq v2	Illumina HiSeq 1500

Maristella	VAD	Discovery Bay, Jamaica						PRJNA589015		SRR10811642	Qiagen RNA	Illumina TruSeq v3	Illumina HiSeq 1500
Maristella	VAD	Discovery Bay, Jamaica						PRJNA589015		SRR10811641	Trizol	NEB Next Ultra RNA Library Prep Kit for Illumin	Illumina MiSeq
Maristella	AG	Roatan, Honduras	16.358061	-86.432906				PRJNA589015		SRR10811636	Trizol	NEB Next Ultra IIRNA Library Prep Kit for Illumina	NextSeq 500
C-group	CONT	Bocas del Toro, Panama	9.3317077	-82.2556333	1.846	1.066	1.73		N/A	N/A			
"Vargula"	tsujii	Catalina Island, CA, USA	33.445139	-118.4845	1.581	0.913	1.73	PRJNA287212		SRR1269674			
Kornickeria	SPU	Isla Magueye	17.96	-67.05	1.383	0.78	1.77		N/A	N/A			

		s, PR, USA	10 89	215 6									
Kornickeria	hastingsi carriebowae	Southwater Caye, Belize	16.8116	-88.08243	1.799	1.014	1.77	PRJNA589015		SRR10860879	Qiagen Rneasy	Unknown; amplified by Novogene Co (Davis CA)	Illumina HiSeq 1500
Kornickeria	hastingsi carriebowae	Southwater Caye, Belize	16.8116	-88.08243	1.799	1.014	1.77	PRJNA589015		SRR10860878	Qiagen Rneasy	Illumina TruSeq v2	Illumina HiSeq 1500
Maristella	LSD	Isla Magueyes, PR, USA	17.961089	-67.052156					N/A				
"Vargula"	hilgendorfii	Carolina Biological Purchase	N/A		N/A				AAA30332				
Cypridina	noctiluca	NCBI							BBG57195				

Supplementary Table S3 - Comparison between phylogenetically uncorrected (OLS) or corrected (BM) models. Models were compared only within datasets (shading) by lowest AIC or AICc score.

Model	AIC or AICc	AIC / AICc	Dataset
OLS	AICc	261.1865	Old mutagenesis, new color, & tx-ome combined
BM	AICc	355.176	Old mutagenesis, new color, & tx-ome combined
OLS	AIC	-3.98076	New color & tx-ome only
BM	AIC	3.636894	New color & tx-ome only
OLS	AICc	-285.7653	Previously published decay & new tx-ome only
BM	AICc	-244.4218	Previously published decay & new tx-ome only

Table S4 - Statistical results for luciferase expression *in vitro* from key comparisons

Culture	Comparison	Test	Value	Std. Error	DF	T-value	P-value	Correted P-value (if needed)
Mammalian Cells	HEK & P_mor	T-test	na	na	8.39	-4.76	0.00	0.00
Mammalian Cells	HEK & M_SVU	T-test	na	na	11.92	-14.91	0.00	0.00
Mammalian Cells	HEK & V_tsu	T-test	na	na	11.25	-15.92	0.00	0.00
Yeast Cells	Before & After substrate (C_noc)	T-test	na	na	8.75	5.79	0.00	0.00
Yeast Cells	Before & After substrate (K_has)	T-test	na	na	4.68	3.18	0.03	0.11
Yeast Cells	Before & After substrate (M_SVU)	T-test	na	na	11.50	2.62	0.02	0.09
Yeast Cells	Before & After substrate (V_tsu)	T-test	na	na	4.60	4.88	0.01	0.02
	Model effects							
Yeast Cells	Pichia after substrate addition	Linear Mixed Effect Model	2.13	0.85	53.00	2.50	0.02	
Yeast Cells	C_noc	Linear Mixed Effect Model	3.17	0.98	19.00	3.23	0.00	
Yeast Cells	K_has	Linear Mixed Effect	0.88	1.04	19.00	0.85	0.41	

		Model						
Yeast Cells	M_SVU	Linear Mixed Effect Model	0.57	0.95	19.00	0.60	0.55	
Yeast Cells	V_tsu	Linear Mixed Effect Model	0.49	1.04	19.00	0.47	0.65	
Yeast Cells	Before substrate addition	Linear Mixed Effect Model	-0.09	0.28	40.00	-0.31	0.75	
Yeast Cells	C_noc * Before substrate addition	Linear Mixed Effect Model	-2.83	0.36	40.00	-7.87	0.00	
Yeast Cells	K_has * Before substrate addition	Linear Mixed Effect Model	-2.00	0.39	40.00	-5.09	0.00	
Yeast Cells	M_SVU * Before substrate addition	Linear Mixed Effect Model	-0.70	0.32	40.00	-2.19	0.03	
Yeast Cells	V_tsu * Before substrate addition	Linear Mixed Effect Model	-1.26	0.39	40.00	-3.21	0.00	

Table S5 - Means of parameters of emission spectra

Species abbreviation	N	Lmax_Mean	Lmax_SD	FWHM_Mean	FWHM_SD
P_EGD	4	468	0.71	82.05	1
P_SFM	4	467.5	0.71	82.19	0.28
M_CON	3	461.5	0.32	81.66	2.7
M_LLL	5	461	0.6	79.82	0.87
M_MFU	5	461.6	0.46	80.48	0.25
P_ann	3	468.5	1.8	85.44	0.32
P_mor	3	466.3	0.63	81.56	0.85
M_SMU	5	460.9	0.49	79.71	0.46
K_has	1	460.5	NA	77.98	NA
M_chi	4	461.3	0.55	79.24	0.7
M_SVU	10	460.9	0.85	79.43	1.2
P_GPH	6	465.6	0.78	76.77	0.74
P_WLU	7	465.9	1.5	79.08	1.5
M_LSD	1	459.4	NA	80.19	NA
M_IR	5	460.7	1.5	76.16	1.3
M_RD	2	461.5	2.7	76.54	1.2
M_DU	5	459	1.1	75.05	0.91
V_tsu	8	460.6	1.2	74.28	0.65
K_SPU	3	460.3	1.8	77.79	0.85
V_hil	6	458.7	2	78.28	1.8
P_gra_p	1	471.1	NA	78.58	NA
V_hil_p	1	460.5	NA	83.11	NA
C_noc_p	1	454.3	NA	79.17	NA

Table S6 - All parameters measured from individual emission spectra in this study, including those removed for further analysis due to low signal:noise

Abbre-viation	locality	genus	species	replicate	sex	preservation	source	sgMax	sgfwhm	error
P_EGD	Panama	Photeros	Photeros_EGD	EGD1	male	dried	ucsb	467.73	81.50	0.00
P_EGD	Panama	Photeros	Photeros_EGD	EGD3	male	dried	ucsb	467.18	82.60	0.00
P_EGD	Panama	Photeros	Photeros_EGD	EGD4	male	dried	ucsb	468.83	80.95	0.00
P_EGD	Panama	Photeros	Photeros_EGD	EGD5	male	dried	ucsb	468.28	83.16	0.00
P_SFM	Panama	Photeros	Photeros_SFM	SFM1	male	dried	ucsb	469.38	75.99	0.05
P_SFM	Panama	Photeros	Photeros_SFM	SFM2	male	dried	ucsb	467.18	82.05	0.00
P_SFM	Panama	Photeros	Photeros_SFM	SFM3	male	dried	ucsb	466.63	82.05	0.00
P_SFM	Panama	Photeros	Photeros_SFM	SFM4	male	dried	ucsb	467.73	82.60	0.00
P_SFM	Panama	Photeros	Photeros_SFM	SFM5	male	dried	ucsb	468.28	82.05	0.00
M_CON	Panama	Contragula	contragula	cont1	male	dried	ucsb	461.13	79.82	0.00
M_CON	Panama	Contragula	contragula	cont2	male	dried	ucsb	461.68	80.37	0.01
M_CON	Panama	Contragula	contragula	cont3	male	dried	ucsb	461.68	84.78	0.00
M_LLL	Panama	Maristella	Maristella_LLL	LLL1	male	dried	ucsb	461.13	79.82	0.00
M_LLL	Panama	Maristella	Maristella_LLL	LLL2	male	dried	ucsb	461.68	80.37	0.00
M_LLL	Panama	Maristella	Maristella_LLL	LLL3	male	dried	ucsb	461.13	78.72	0.00

M_LLL	Panama	Maristella	Maristella_LLL	LLL4	male	dried	ucsb	460.03	79.27	0.00
M_LLL	Panama	Maristella	Maristella_LLL	LLL5	male	dried	ucsb	461.13	80.92	0.00
M_MFU	Panama	Maristella	Maristella_MFU	MFU1	male	dried	ucsb	461.13	80.37	0.00
M_MFU	Panama	Maristella	Maristella_MFU	MFU2	male	dried	ucsb	461.68	80.93	0.00
M_MFU	Panama	Maristella	Maristella_MFU	MFU3	male	dried	ucsb	461.13	80.37	0.00
M_MFU	Panama	Maristella	Maristella_MFU	MFU4	male	dried	ucsb	461.68	80.38	0.00
M_MFU	Panama	Maristella	Maristella_MFU	MFU5	male	dried	ucsb	462.23	80.38	0.00
P_ann	Belize	Photeros	Photeros_annecohenae	Pann1	unknown	live	ucsb	466.51	85.25	0.00
P_ann	Belize	Photeros	Photeros_annecohenae	Pann2	unknown	live	ucsb	469.81	85.81	0.01
P_ann	Belize	Photeros	Photeros_annecohenae	Pann3	unknown	live	ucsb	469.26	85.25	0.00
P_mor	Belize	Photeros	Photeros_morini	Pmor1	male	live	ucsb	464.32	79.72	0.05
P_mor	Belize	Photeros	Photeros_morini	Pmor2	male	live	ucsb	467.06	81.37	0.00
P_mor	Belize	Photeros	Photeros_morini	Pmor3	male	live	ucsb	467.61	83.58	0.04
P_mor	Belize	Photeros	Photeros_morini	Pmor4	male	live	ucsb	465.96	82.49	0.00
P_mor	Belize	Photeros	Photeros_morini	Pmor5	male	live	ucsb	465.96	80.82	0.00
M_SMU	Panama	Maristella	Maristella_SMU	SMU1	male	dried	ucsb	461.13	79.82	0.00

M_SMU	Panama	Maristella	Maristella_SMU	SMU2	male	dried	ucsb	460.58	79.27	0.00
M_SMU	Panama	Maristella	Maristella_SMU	SMU3	male	dried	ucsb	460.58	79.82	0.00
M_SMU	Panama	Maristella	Maristella_SMU	SMU4	male	dried	ucsb	461.68	80.37	0.00
M_SMU	Panama	Maristella	Maristella_SMU	SMU5	male	dried	ucsb	460.58	79.27	0.00
K_has	Belize	Kornickeria	Kornickeria_hastingsi	Khas1	male	dried	ucsb	464.32	80.74	0.11
K_has	Belize	Kornickeria	Kornickeria_hastingsi	Khas2	male	live	ucsb	471.46	75.78	0.09
K_has	Belize	Kornickeria	Kornickeria_hastingsi	Khas3	male	live	ucsb	460.48	77.98	0.00
M_chi	Belize	Maristella	Maristella_chicoi	MSH1	male	live	ucsb	461.57	79.10	0.00
M_chi	Belize	Maristella	Maristella_chicoi	MSH2	male	live	ucsb	461.57	80.21	0.00
M_chi	Belize	Maristella	Maristella_chicoi	MSH3	male	live	ucsb	458.83	79.10	0.02
M_chi	Belize	Maristella	Maristella_chicoi	MSH4	male	live	ucsb	460.48	79.10	0.00
M_chi	Belize	Maristella	Maristella_chicoi	MSH5	male	live	ucsb	461.57	78.55	0.00
M_SVU	Belize	Maristella	Maristella_SVD	SVD1	male	live	ucsb	461.02	79.66	0.00
M_SVU	Belize	Maristella	Maristella_SVD	SVD2	male	live	ucsb	462.12	82.42	0.00
M_SVU	Belize	Maristella	Maristella_SVD	SVD3	male	live	ucsb	459.93	79.10	0.00
M_SVU	Belize	Maristella	Maristella_SVD	SVD4	male	live	ucsb	461.57	79.65	0.00

M_SVU	Belize	Maristella	Maristella_SVD	SVD5	male	live	ucsb	461.02	78.55	0.00
M_SVU	Belize	Maristella	Maristella_SVU	SVU1	male	live	ucsb	461.02	79.10	0.00
M_SVU	Belize	Maristella	Maristella_SVU	SVU2	male	live	ucsb	462.12	79.66	0.00
M_SVU	Belize	Maristella	Maristella_SVU	SVU3	male	live	ucsb	459.93	77.99	0.00
M_SVU	Belize	Maristella	Maristella_SVU	SVU4	male	live	ucsb	460.48	79.10	0.00
M_SVU	Belize	Maristella	Maristella_SVU	SVU5	male	live	ucsb	459.93	79.10	0.00
P_GPH	Roatan	Photeros	Photeros_GPH	GPH1	male	live	ucsb	466.19	75.75	0.01
P_GPH	Roatan	Photeros	Photeros_GPH	GPH2	male	live	ucsb	466.19	75.75	0.08
P_GPH	Roatan	Photeros	Photeros_GPH	GPH3	male	live	ucsb	465.63	76.30	0.01
P_GPH	Roatan	Photeros	Photeros_GPH	GPH4	male	live	ucsb	467.84	75.21	0.06
P_GPH	Roatan	Photeros	Photeros_GPH	GPH5	male	live	ucsb	466.74	77.42	0.00
P_GPH	Roatan	Photeros	Photeros_GPH	GPH6	male	live	ucsb	465.63	77.41	0.00
P_GPH	Roatan	Photeros	Photeros_GPH	GPH7	male	live	ucsb	464.53	76.31	0.00
P_GPH	Roatan	Photeros	Photeros_GPH	GPH8	male	live	ucsb	465.08	77.42	0.01
P_WLU	Roatan	Photeros	Photeros_WLU	WLU1	male	live	ucsb	466.19	79.08	0.00
P_WLU	Roatan	Photeros	Photeros_WLU	WLU2	male	live	ucsb	463.98	77.41	0.00

P_WLU	Roatan	Photeros	Photeros_WLU	WLU3	male	live	ucsb	466.19	78.52	0.00
P_WLU	Roatan	Photeros	Photeros_WLU	WLU4	male	live	ucsb	464.53	78.52	0.00
P_WLU	Roatan	Photeros	Photeros_WLU	WLU5	male	live	ucsb	466.74	80.19	0.00
P_WLU	Roatan	Photeros	Photeros_WLU	WLU6	male	live	ucsb	465.63	77.97	0.00
P_WLU	Roatan	Photeros	Photeros_WLU	WLU7	male	dried	ucsb	466.74	78.53	0.03
P_WLU	Roatan	Photeros	Photeros_WLU	WLU8	male	dried	ucsb	468.40	81.87	0.01
M_LSD	PR_USA	Maristella	Maristella_LSD	LSD1	male	live	ucsb	459.38	80.19	0.02
M_LSD	PR_USA	Maristella	Maristella_LSD	LSD2	male	live	ucsb	459.93	79.08	0.03
M_IR	Roatan	Maristella	Maristella_IR	IR1	male	dead	ucsb	459.56	75.71	0.00
M_IR	Roatan	Maristella	Maristella_IR	IR2	male	dead	ucsb	459.56	76.82	0.00
M_IR	Roatan	Maristella	Maristella_IR	IR3	male	live	ucsb	466.74	83.46	0.13
M_IR	Roatan	Maristella	Maristella_IR	IR4	male	dead	ucsb	459.56	74.60	0.01
M_IR	Roatan	Maristella	Maristella_IR	IR5	male	dead	ucsb	462.32	77.93	0.01
M_IR	Roatan	Maristella	Maristella_IR	IR6	male	dead	ucsb	462.32	75.71	0.01
M_RD	Roatan	Maristella	Maristella_RD	RD1	male	dead	ucsb	463.42	75.71	0.02
M_RD	Roatan	Maristella	Maristella_RD	RD2	male	dead	ucsb	459.56	77.38	0.01

M_RD	Roatan	Maristella	Maristella_RD	RD3	male	dead	ucsb	466.74	77.92	0.10
M_RD	Roatan	Maristella	Maristella_RD	RD4	male	dead	ucsb	465.08	78.48	0.03
M_RD	Roatan	Maristella	Maristella_RD	RD5	male	dead	ucsb	465.63	75.16	0.04
M_DU	Roatan	Maristella	Maristella_DU	DU1	male	live	ucsb	460.11	75.16	0.00
M_DU	Roatan	Maristella	Maristella_DU	DU2	male	live	ucsb	459.56	75.16	0.00
M_DU	Roatan	Maristella	Maristella_DU	DU3	male	live	ucsb	457.35	75.71	0.00
M_DU	Roatan	Maristella	Maristella_DU	DU4	male	live	ucsb	458.45	73.49	0.01
M_DU	Roatan	Maristella	Maristella_DU	DU5	male	live	ucsb	459.56	75.71	0.00
V_tsu	CA_USA	Fred	Vargula_tsujii	Vtsu1	female	live	ucsb	462.12	74.14	0.01
V_tsu	CA_USA	Fred	Vargula_tsujii	Vtsu2	female	live	ucsb	461.02	74.70	0.00
V_tsu	CA_USA	Fred	Vargula_tsujii	Vtsu3	female	live	ucsb	462.12	74.14	0.01
V_tsu	CA_USA	Fred	Vargula_tsujii	Vtsu4	male	live	ucsb	458.83	74.14	0.00
V_tsu	CA_USA	Fred	Vargula_tsujii	Vtsu5	male	live	ucsb	459.93	74.14	0.00
V_tsu	CA_USA	Fred	Vargula_tsujii	Vtsu6	male	live	ucsb	459.93	73.03	0.01
V_tsu	CA_USA	Fred	Vargula_tsujii	Vtsu7	male	live	ucsb	459.93	75.25	0.00
V_tsu	CA_USA	Fred	Vargula_tsujii	Vtsu8	male	live	ucsb	461.02	74.70	0.01

K_WCU	PR_USA	Kornickeria	Kornickeria_WCU	WCU1	male	live	ucsb	462.12	81.78	0.14
K_WCU	PR_USA	Kornickeria	Kornickeria_WCU	WCU2	male	live	ucsb	458.83	79.07	0.03
K_WCU	PR_USA	Kornickeria	Kornickeria_WCU	WCU3	male	live	ucsb	463.22	82.36	0.08
K_WCU	PR_USA	Kornickeria	Kornickeria_WCU	WCU5	male	live	ucsb	450.06	74.09	0.08
K_SPU	PR_USA	Kornickeria	Kornickeria_SPU	SPU1	male	live	ucsb	457.19	76.89	0.02
K_SPU	PR_USA	Kornickeria	Kornickeria_SPU	SPU2	male	live	ucsb	458.28	76.87	0.00
K_SPU	PR_USA	Kornickeria	Kornickeria_SPU	SPU3	male	live	ucsb	461.57	78.54	0.01
K_SPU	PR_USA	Kornickeria	Kornickeria_SPU	SPU4	male	live	ucsb	454.99	73.56	0.07
K_SPU	PR_USA	Kornickeria	Kornickeria_SPU	SPU5	male	live	ucsb	460.48	81.85	0.08
K_SPU	PR_USA	Kornickeria	Kornickeria_SPU	SPU6	male	live	ucsb	461.02	77.97	0.01
K_SPU	PR_USA	Kornickeria	Kornickeria_SPU	SPU7	male	live	ucsb	451.16	71.31	0.13
V_hil	Japan	Vargula	Vargula_hilgendorfii	Vhil0123171	unknown	dried	ucsb	460.48	76.88	0.01
V_hil	Japan	Vargula	Vargula_hilgendorfii	Vhil0123172	unknown	dried	ucsb	459.38	77.98	0.00
V_hil	Japan	Vargula	Vargula_hilgendorfii	Vhil0123173	unknown	dried	ucsb	459.38	74.68	0.04
V_hil	Japan	Vargula	Vargula_hilgendorfii	Vhil0907161	unknown	dried	ucsb	459.66	80.87	0.03
V_hil	Japan	Vargula	Vargula_hilgendorfii	Vhil0907162	unknown	dried	ucsb	460.21	77.54	0.06

V_hil	Japan	Vargula	Vargula_hilgendorfii	Vhil0907163	unknown	dried	ucsb	460.21	80.87	0.03
V_hil	Japan	Vargula	Vargula_hilgendorfii	Vhil0907164	unknown	dried	ucsb	456.33	78.10	0.02
V_hil	Japan	Vargula	Vargula_hilgendorfii	Vhil0907165	unknown	dried	ucsb	456.33	76.99	0.01
V_hil	Japan	Vargula	Vargula_hilgendorfii	Vhil0907166	unknown	dried	ucsb	460.76	79.76	0.04
V_hil	Japan	Vargula	Vargula_hilgendorfii	Vhil100520161	unknown	dried	ucsb	458.28	81.85	0.01
V_hil	Japan	Vargula	Vargula_hilgendorfii	Vhil0920161	unknown	dried	ucsb	465.19	73.11	0.02
V_hil	Japan	Vargula	Vargula_hilgendorfii	Vhil0920162	unknown	dried	ucsb	465.19	72.01	0.02
V_hil	Japan	Vargula	Vargula_hilgendorfii	Vhil0920163	unknown	dried	ucsb	461.32	72.01	0.03
V_hil	Japan	Vargula	Vargula_hilgendorfii	Vhil_Japan	unknown	live	Japan	461.14	77.87	0.00
P_gra_p	Published	Photeros	Photeros_gramminicola	Pgra_huvard	unknown	unknown	published	471.14	78.58	0.32
V_hil_p	Published	Vargula	Vargula_hilgendorfii	Vhil_tsuji	unknown	unknown	published	460.54	83.11	0.33
C_noc_p	Published	Cypridina	Cypridina_noctiluca	Cnoc_ohmiya	unknown	unknown	published	454.27	79.17	0.06

Table S7 - Luciferase-specific primers to amplify from cDNA

Primer name	Target species	Sequence	Purpose
KHC Forward	*Kornickeria hastingsi carriebowae*	GGACTCGAGAAGAGAGAGGCTAAAGATTGTTTTGAATCATCTTTCC	Amplify from cDNA
KHC Reverse	*Kornickeria hastingsi carriebowae*	AGCGGCCGCTTTGGTGCATTGAGGTGG	Amplify from cDNA
PMO Forward	*Photeros morini*	TAACTCGAGAAGAGAGAGGCTCAAGAATGCGCTCAGACA	Amplify from cDNA
PMO Reverse	*Photeros morini*	AGCGGCCGCCTGGCATATGGCTGGTAC	Amplify from cDNA
SVU Forward	SVU (undescribed)	GGGCTCGAGAAGAGAGAGGCTCAAGATTGTTATGAATTCACA	Amplify from cDNA
SVU Reverse	SVU (undescribed)	AGCGGCCGCTTTACACTGAGGGGGATA	Amplify from cDNA
SVD Forward	SVD (undescribed)	GTGCTCGAGAAGAGAGAGGCTGAAGATTGTTATGAATTCACATG	Amplify from cDNA
SVD Reverse	SVD (undescribed)	AGCGGCCGCTTTACACTGAGGTGGATAC	Amplify from cDNA
MSH Forward	*Maristella chicoi*	CGGCTCGAGAAGAGAGAGGCTGAAGATTGTTATGAATTCACAT	Amplify from cDNA
MSH Reverse	*Maristella chicoi*	AGCGGCCGCTTTACACTGAGGGGGATAC	Amplify from cDNA
VTS Forward	*Vargula tsujii*	CCGCTCGAGAAGAGAGAGGCTCAAGATTGTTATGAATCAACATG	Amplify from cDNA
VTS Reverse	*Vargula tsujii*	AGCGGCCGCTTTACACTGAGGAGGATACT	Amplify from cDNA
454-Forward-Vt	*Vargula tsujii*	CTCGAGATCAGTCGAGAAACGAAACGTGATA	Amplify from cDNA
454-Reverse-	*Vargula tsujii*	GAATTCCTTTACACTGAGGG	Amplify from cDNA

Vt		GGATACTG	
AOX Forward	n/a	GACTGGTTCCAATTGACAAGC	Sequence from clones
AOX Reverse	n/a	GCAAATGGCATTCTGACATCC	Sequence from clones

S8 - Translation between the alignment site number (column 1) and the corresponding site number in the reference sequence, the c-luciferase of *C. noctiluca* (column 2).

Alignment site number	Cypridina site number
1	0
2	0
3	0
4	0
5	0
6	0
7	0
8	0
9	0
10	0
11	0
12	0
13	0
14	0
15	0
16	0
17	0
18	0
19	0
20	0
21	0
22	0
23	1
24	2
25	3
26	4
27	5
28	6
29	7
30	8
31	9

32	10
33	11
34	12
35	13
36	14
37	15
38	16
39	17
40	18
41	19
42	20
43	20
44	21
45	22
46	23
47	24
48	25
49	26
50	27
51	28
52	29
53	29
54	29
55	29
56	30
57	31
58	32
59	33
60	34
61	35
62	36
63	37
64	38
65	39

66	40
67	41
68	42
69	43
70	44
71	45
72	46
73	47
74	48
75	49
76	50
77	51
78	52
79	53
80	54
81	55
82	56
83	57
84	58
85	59
86	60
87	61
88	62
89	63
90	64
91	65
92	66
93	67
94	67
95	67
96	68
97	69
98	70
99	71

100	72
101	73
102	74
103	75
104	76
105	77
106	78
107	79
108	80
109	81
110	82
111	83
112	84
113	85
114	86
115	87
116	88
117	89
118	90
119	91
120	92
121	93
122	94
123	95
124	96
125	97
126	98
127	99
128	100
129	101
130	102
131	103
132	104
133	105

134	106
135	107
136	108
137	109
138	110
139	111
140	112
141	113
142	114
143	115
144	116
145	117
146	118
147	119
148	120
149	121
150	122
151	123
152	124
153	125
154	126
155	127
156	128
157	129
158	130
159	131
160	132
161	133
162	134
163	135
164	136
165	137
166	138
167	139

168	140
169	141
170	141
171	142
172	143
173	144
174	145
175	146
176	147
177	148
178	149
179	150
180	151
181	152
182	153
183	154
184	155
185	156
186	157
187	158
188	159
189	160
190	161
191	162
192	163
193	164
194	165
195	166
196	167
197	168
198	169
199	170
200	171
201	172

202	173
203	174
204	175
205	176
206	177
207	178
208	179
209	180
210	181
211	182
212	183
213	184
214	185
215	186
216	187
217	188
218	189
219	190
220	191
221	192
222	193
223	194
224	195
225	196
226	197
227	198
228	199
229	200
230	201
231	202
232	203
233	204
234	205
235	206

236	207
237	208
238	209
239	210
240	211
241	212
242	213
243	214
244	215
245	216
246	217
247	218
248	219
249	220
250	221
251	222
252	223
253	224
254	225
255	226
256	227
257	228
258	229
259	230
260	231
261	232
262	233
263	234
264	235
265	236
266	237
267	238
268	239
269	240

270	241
271	242
272	243
273	244
274	245
275	246
276	247
277	248
278	249
279	250
280	251
281	252
282	253
283	254
284	255
285	256
286	257
287	258
288	259
289	260
290	261
291	262
292	263
293	264
294	265
295	266
296	267
297	268
298	269
299	270
300	271
301	272
302	273
303	274

304	274
305	274
306	275
307	276
308	277
309	278
310	279
311	280
312	281
313	282
314	283
315	284
316	285
317	286
318	287
319	288
320	289
321	290
322	291
323	292
324	293
325	294
326	295
327	296
328	297
329	298
330	299
331	300
332	301
333	302
334	303
335	304
336	305
337	306

338	307
339	308
340	309
341	310
342	311
343	312
344	313
345	314
346	315
347	316
348	317
349	318
350	319
351	320
352	321
353	322
354	323
355	324
356	325
357	326
358	327
359	328
360	329
361	330
362	331
363	332
364	333
365	334
366	335
367	336
368	337
369	338
370	339
371	340

372	341
373	342
374	343
375	344
376	345
377	346
378	347
379	348
380	349
381	350
382	351
383	352
384	353
385	354
386	355
387	356
388	357
389	358
390	359
391	360
392	361
393	362
394	363
395	364
396	365
397	366
398	367
399	368
400	369
401	370
402	371
403	372
404	373
405	374

406	375
407	376
408	377
409	378
410	379
411	380
412	381
413	382
414	383
415	384
416	385
417	386
418	387
419	388
420	389
421	390
422	391
423	392
424	393
425	394
426	395
427	396
428	397
429	398
430	399
431	400
432	401
433	402
434	403
435	404
436	405
437	406
438	407
439	408

440	409
441	410
442	411
443	412
444	413
445	414
446	415
447	416
448	417
449	418
450	419
451	420
452	421
453	422
454	423
455	424
456	425
457	426
458	427
459	428
460	429
461	430
462	431
463	432
464	433
465	434
466	435
467	436
468	437
469	438
470	439
471	440
472	441
473	442

474	443
475	443
476	444
477	445
478	446
479	447
480	448
481	449
482	450
483	451
484	452
485	453
486	454
487	455
488	456
489	457
490	458
491	459
492	460
493	461
494	462
495	463
496	464
497	465
498	466
499	467
500	467
501	468
502	469
503	470
504	471
505	472
506	473
507	474

508	475
509	476
510	477
511	478
512	479
513	480
514	481
515	482
516	483
517	484
518	485
519	486
520	487
521	488
522	489
523	490
524	491
525	492
526	493
527	494
528	495
529	496
530	497
531	498
532	499
533	500
534	501
535	502
536	503
537	504
538	505
539	506
540	507
541	508

542	509
543	510
544	511
545	512
546	513
547	514
548	515
549	516
550	517
551	518
552	519
553	520
554	521
555	522
556	523
557	524
558	525
559	526
560	527
561	528
562	529
563	530
564	531
565	532
566	533
567	534
568	535
569	536
570	537
571	538
572	539
573	540
574	541
575	542

576	543
577	544
578	545
579	546
580	547
581	548
582	549
583	550
584	551
585	552
586	553
587	553
588	553
589	553
590	553
591	553
592	553
593	553
594	553
595	553
596	553
597	553
598	553
599	553
600	553
601	553
602	553
603	553
604	553
605	553
606	553
607	553
608	553
609	553

610	553

References Cited in Supplement

1. N. M. Hensley, *et al.*, Phenotypic evolution shaped by current enzyme function in the bioluminescent courtship signals of sea fireflies. *Proceedings of the Royal Society B* **286**, 20182621 (2019).

2. N. J. Reda, *et al.*, Maristella, a new bioluminescent ostracod genus in the Myodocopida (Cypridinidae). *Zool. J. Linn. Soc.* (2019) https:/doi.org/10.1093/zoolinnean/zlz082 (November 8, 2019).

3. J. D. Bendtsen, H. Nielsen, G. von Heijne, S. Brunak, Improved prediction of signal peptides: SignalP 3.0. *J. Mol. Biol.* **340**, 783–795 (2004).

4. F. I. Tsuji, B. G. Nafpaktitis, T. Goto, M. J. Cormier, Spectral characteristics of the bioluminescence induced in the marine fish, Porichthys notatus, byCypridina (ostracod) luciferin. *Molecular and cellular* (1975).

5. E. A. Widder, M. I. Latz, J. F. Case, MARINE BIOLUMINESCENCE SPECTRA MEASURED WITH AN OPTICAL MULTICHANNEL DETECTION SYSTEM. *Biol. Bull.* **165**, 791–810 (1983).

6. Y. Nakajima, K. Kobayashi, K. Yamagishi, T. Enomoto, Y. Ohmiya, cDNA cloning and characterization of a secreted luciferase from the luminous Japanese ostracod, Cypridina noctiluca. *Biosci. Biotechnol. Biochem.* **68**, 565–570 (2004).

7. K. Kawasaki, Y. Morita, S. Ohgiya, Y. Ohmiya, Y. Ohyama, Mutant luciferase. *US Patent* (2012) (August 29, 2016).

8. A. L. Huvard, Analysis of visual pigment absorbance and luminescence emission spectra in marine ostracodes (Crustacea: Ostracoda). *Comp. Biochem. Physiol. A Physiol.* **104**, 333–338 (1993).