

Evolution of Bioluminescence in Marine Planktonic Copepods

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Объект исследования:

Copepods are the most numerous taxa of zooplankton fauna in the ocean worldwide.

Within copepods, the luminescent behavior of *Metridinidae* and most *Heterorhabdidae*, *Lucicutiidae*, and *Augaptilidae* are well known. Except for the *Augaptiloidea* superfamily, a single species in *Megacalanoidea* (*Megacalanus princeps*) was reported as luminous with very limited evidence. It is also unclear whether species in other superfamilies, such as *Centropagoidea*, *Clausocalanoidea*, and *Eucalanoidea*, are luminous.

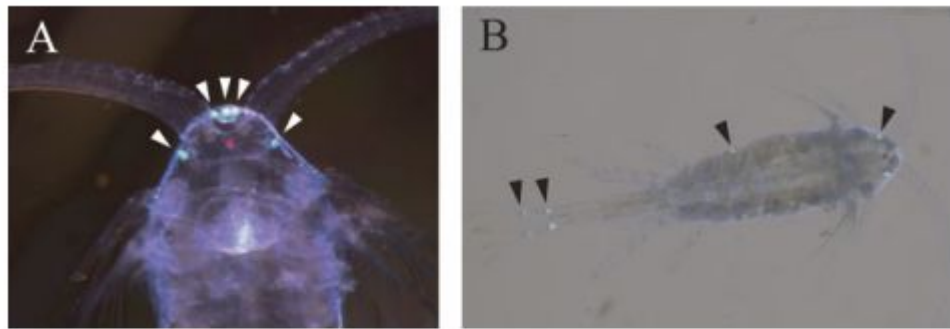
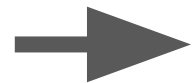


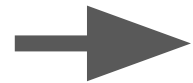
FIG. 1. (A) Fluorescent microscopic image of bioluminescent Copepoda, *Metridia pacifica*, illuminated with ultraviolet. Arrow-heads indicate the position of putative luminous glands on head. (B) Bright-field and fluorescent image of *M. pacifica*. Putative luminous glands were on the legs and abdomen adjacent to the head.

Цель исследования (и путь):

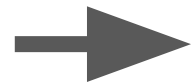
In this study, we **isolated and characterized novel luciferase genes** from five species of calanoid copepods and **conducted the quantitative analyses of copepod bioluminescence** (using zooplankton homogenates and recombinant luciferases).



By quantitative measurements, we could **reveal the presence or absence of luminescence of unverified species** with confirmation of verified ones.



Furthermore, we **compared the luminous intensity** of homogenates among calanoid superfamilies and those of luciferases among genera in Augaptiloidea to **infer the defensive function and molecular evolution of copepod bioluminescence**.



Материал	Используемый метод
Выделенный белок из гомогенизированной пробы	Люминометр Спектрофлуориметр
6Гистидин-меченый по С-концу белок люциферазы из белковых проб (ген люциферазы на вектор, вектор в бактерию, бактерию на культивацию, а потом на выделение белка)	Выделение РНК реверсия + ПЦР Трансформация Культивирование Иммуноблоттинг (хемилюминесценция)
18S rRNA гены для всех видов	Выделение ДНК+ПЦР Сиквенс для Филогенетики
участки генов люцифераз по консервативным кускам люцифераз других таксонов	в филоген анализ: the deduced amino acid sequences of copepod luciferases were trimmed manually to domains 1 and 2 with internal sequences because of their divergence at their N- and C-terminals. Resulting core regions consisting of 135–142 amino acid

Результаты: 18S

In this study, we have confirmed the phylogenetic relationships among luminous and nonluminous calanoid copepods using approximately 1738 nt of 18S ribosomal DNA sequences using ML (fig. 3) and NJ analyses (supplementary fig. S1, Supplementary Material online). The 18S alignment contained 35 species of 22 genera of copepods plus four malacostracan (*Gonodactylus viridis*, *Odontodactylus havanensis*, *Squilla rugosa*, and *Euphausia superba*) and two ostracod (*V. hilgendorffii* and *Conchoecia* sp.) species as outgroup taxa.

SUPPORT PREVIOUS MORPHOLOGY AND SEQUENCE-BASED PHYLOGENY

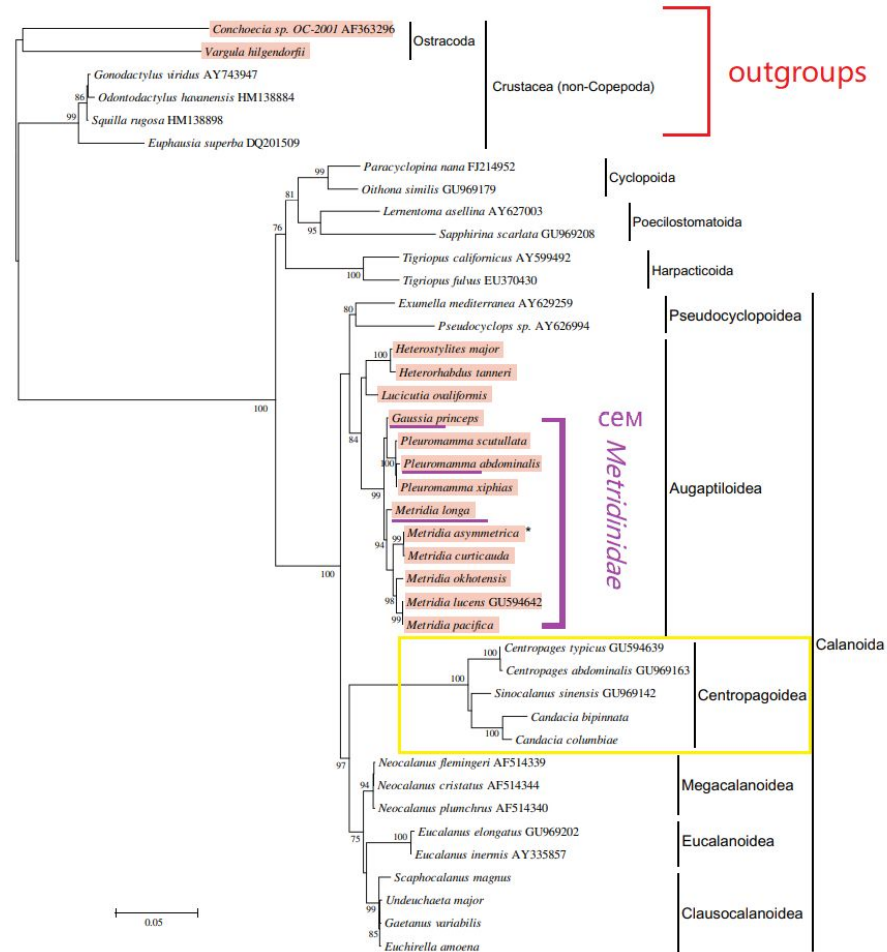


Fig. 3. ML analysis of the 18S rRNA nucleotide sequences from calanoid copepods and marine bioluminescent organisms. Species with bioluminescence assay in this study or reported elsewhere are shown in red boxes. Accession numbers are indicated with names of species whose 18S rRNA sequences are available from public databases. Bootstrap values are shown on only highly or moderately supported nodes (>74%) as a percentage of 1,000 replicates. Scale bar indicates genetic distance. *Metridia asymmetrica* (*) was not included in the luminescence assay of zooplankton lysate (Fig. 2) but is capable of bioluminescence (Takenaka Y, unpublished data).

The phylogeny of 13 calanoid luciferases suggests a different evolution of the Metridinidae and Heterorhabdidae families' luciferases. In the clade containing Metridinidae luciferases (clade 1), two proteins in a single species are likely to have evolved independently.

The similarity of primary structures among MpLuc1, MoLuc1, and PaLuc1 (type-I) luciferases were greater than those of their counterparts (MpLuc2, MoLuc2, and PaLuc2) (type-II) luciferases.

The mRNA coding for MoLuc1 or MoLuc2 would be transcribed from two different loci or alleles since studies of genomic sequences revealed the presence of different lengths and positions of the introns in MoLuc genes.

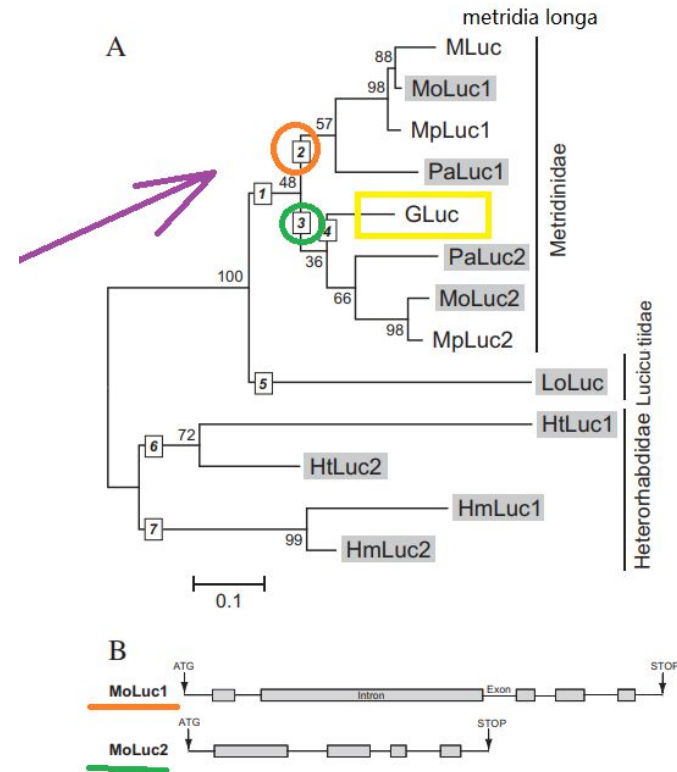


FIG. 5. (A) ML phylogeny of copepod luciferases based on an amino acid sequence alignment of domains 1 and 2 with a linker sequence. The tree is midpoint rooted, and numbers on the nodes indicate % bootstrap values from 1,000 replicates. Luciferases isolated and analyzed in this study are shown in shaded boxes. Scale bar indicates evolutionary distance and is in the units of the number of amino acid substitutions per site. (B) Gene structures of MoLuc1 and MoLuc2. Introns and exons are shown as open boxes and lines, respectively.

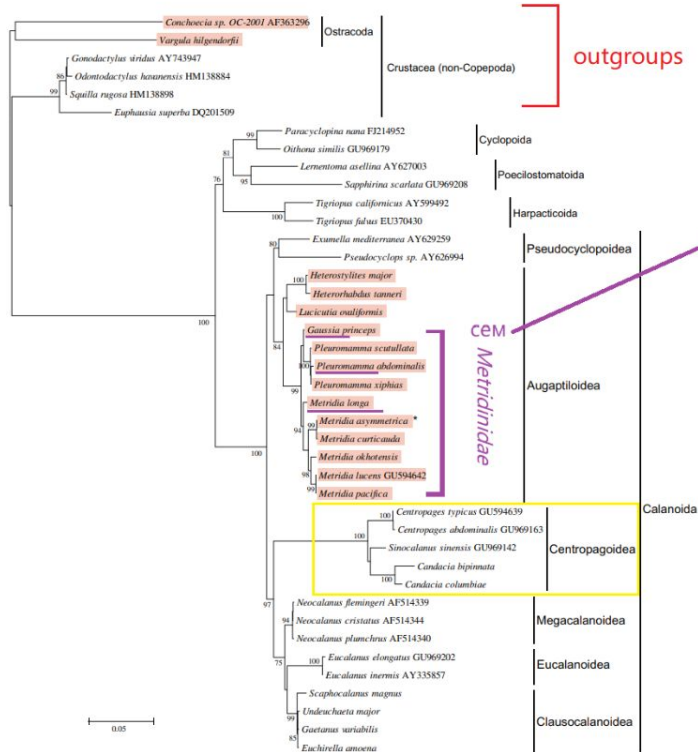


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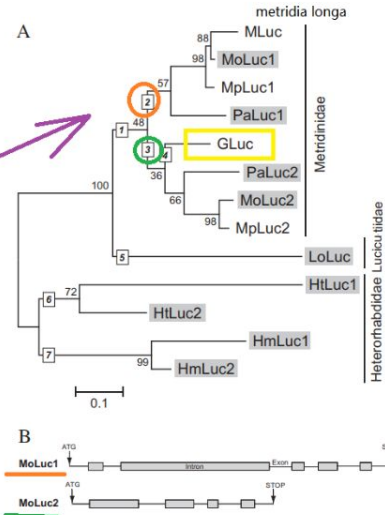


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