Bioluminescence of the polychaete *Tharyx* sp. (Annelida: Cirratulidae) in deep-seawater from Toyama Bay, Japan

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**Abstract:** Commercially pumped deep-seawater is an easily accessible source of deep-sea animals. In this study, we report the bioluminescence of a cirratulid polychaete, *Tharyx* sp., obtained from pipeline seawater pumped up from a depth of 384 m in Toyama Bay. Two living worms collected from the nylon mesh attached to the pump outlet produced greenish light when disturbed. They were identified as *Tharyx* sp. (Cirratulidae) by morphological examination and DNA sequencing. Bioluminescence of cirratulid worms is not widely reported and, to our knowledge, this is the first report of bioluminescence in the genus *Tharyx*.

**Key words:** Bioluminescence, deep-seawater, Cirratulidae, *Tharyx*

In Nyuzen City, Toyama Prefecture, Japan, deep-seawater 3 km offshore in Toyama Bay is pumped up from a depth of 384 m, at a speed of 100 m³ h⁻¹. This water is used for diverse applications such as cultivating oysters, air conditioning of factories, and in various fishery and food industries. Organic matter (primarily zooplankton) in the water is removed through a nylon filter (mesh size: 200 μm) attached to the pipeline outlet. We have collected living deep-sea zooplankton retained by this filter for our bioluminescence studies. For example, we have identified luciferin of the ostracod *Discoconchoecia pseudodiscophora* as coelenterazine (Oba et al. 2004) and demonstrated coelenterazine biosynthesis of the copepod *Metridia pacifica* (Oba et al. 2009). This deep-seawater residue contains various creatures besides crustaceans, including chaetognaths, hydrozoans, echinoderms, and polychaetes; thus, we expected to find other bioluminescent organisms in the residue.

In terms of bioluminescence, polychaetes are one of the most diverse but least studied animal groups (Verdes & Gruber 2017). Bioluminescence in the family Cirratulidae has only been described several times during twentieth century (Table 1). Intriguingly, some of these observations suggest the involvement of light production in the process of reproduction (Lund 1911; Gibbs 1971); however, this has not been studied in detail.

In this study, we surveyed the luminosity of deep-sea animals from Toyama Bay and report bioluminescence of the cirratulid *Tharyx* sp. in Japan for the first time. Deep-sea animals were collected at the deep-seawater pumping facility in Toyama Bay. The residue in the filter (mesh size, 200 μm) was collected for 24 h (11:00 on May 13 to 11:00 on May 14, 2019). A sample of this residue (wet weight, approximately 100 g) was transported to our lab and sorted under stereoscopic microscopes. During transport and sorting, the sample was kept in deep-seawater on ice. For luminosity tests, each specimen was on a separate dish and was poked using tweezers; photographs of the luminescence were then immediately taken under dark conditions using a digital camera (Nikon D5500, F 3.5, ISO 25,600, exposure 30 sec). The specimens that emitted light were preserved in 99.5% ethanol. Species identification was performed either genetically or morphologically. For both specimens (Specimens 01 and 02), the partial DNA sequence of mitochondrial cytochrome c oxidase subunit I (DNA barcoding region) was tried to be determined by PCR and sequencing, based on the method described by Folmer et al. (1994), but PCR was not amplified in Specimen 01. The sequence obtained from Specimen 02 was deposited in GenBank/EMBL/DDBJ (accession number, LC536956). A neighbor joining phylogenetic tree with other Cirratulidae sequences from GenBank and BOLD was constructed using MEGA 7.0.

Our sample comprised a large number of living zooplankton (mainly copepods and chaetognaths) and several types of benthic animals such as ophiuroids and polychaetes (including opheliids and sphæroderoids). A significant number of luminous organisms identified were either *M. pacifica* or *D.
Only two luminous specimens were polychaetes, and these were considered to be the same species or morphologically close relatives. Both specimens had a conical head without antennae and a brownish body 3 mm in length. The bioluminescence of the specimens was greenish (Figure 1). Specimen 01 (Figure 1A) was morphologically examined and identified as Tharyx sp. by the following characteristics: simple capillary chaetae, acicular spines not arranged in partial or complete cinctures, acicular spines with short nobs, and chaetal fascicles laterally placed on the body wall and close together.

The morphology of Specimen 01 was as follows: body

Table 1. Bioluminescence in cirratulid worms.

| No. | Taxa                                      | Notes of luminescence                                      | References                          |
|-----|-------------------------------------------|-----------------------------------------------------------|-------------------------------------|
| 1   | *Cirrhineres phosphorea*                  | —                                                         | Verrill 1882                         |
|     | (= *Cirratulus fragilis* sensu Petersen 1999) | (currently, *Caulleriella fragilis*)                        | (Petersen 1999)                     |
| 2   | *Heterocirrus bioculata*                  | Yellow green, stimulated by fresh water                    | Bonhomme 1944                       |
|     | (currently, *Caulleriella bioculata*)     |                                                           |                                     |
| 3   | *Heterocirrus saxicola*                   | Green, on algae                                           | Molisch 1904                        |
|     | (currently, *Dodecaceria saxicola*)       |                                                           |                                     |
| 4   | *Cirratulidae* sp.                       | Greenish yellow, swarming in harbor                        | Lund 1911                           |
| 5   | *Cirratulus*                              | —                                                         | Harvey 1952                         |
|     | *Macrochaeta*                             |                                                           |                                     |
| 6   | *Caulleriella caput-esocis*               | Greenish                                                 | Gibbs 1971                          |
|     | (currently, *Chaetozone capotesocis*)     |                                                           |                                     |
| 7   | *Tharyx multibranchis*                    | “has epidermal cells opening between the chaetae of each segment” | Temple 1974                         |
|     | (= *Heterocirrus multibranchis* (currently, *Aphelochaeta multibranchis*)) |                                                           | (Herring 1978b)                     |
| 8   | *Tharyx* (= *Heterocirrus* in part)*      | —                                                         | Herring 1978a                       |
|     | *Dodecaceria* (= *Heterocirrus* in part)* |                                                           |                                     |
|     | *Cirratulus* (= *Heterocirrus* in part)*  |                                                           |                                     |
|     | (still inadequate, yet often suggestive)  |                                                           |                                     |
| 9   | *Dodecaceria* (= *Heterocirrus*)          | —                                                         | Herring 1987                        |
|     | *Tharyx* (= *Heterocirrus*)               |                                                           |                                     |
|     | *Cirratulus* (uncertain)                  |                                                           |                                     |
| 10  | *Caulleriella parva*                      | Bluish, pinched or irritated with fresh water             | Petersen 1999                       |
| 11  | *Tharyx* sp.                              | Greenish, pinched, in deep-sea                            | present study                       |

1Petersen (1999) cited Verrill (1882) as the literature describing the luminescence.
2The genus *Macrochaeta* is currently accepted as a member of Acrocirridae.
3Herring (1978b) cited an unpublished reference by Temple (1974).

Fig. 1. Bioluminescence of *Tharyx* sp. specimens collected from deep-seawater. (A) Specimen 01, (B) Specimen 02. Left panels (bright field) correspond to the middle panels (dark field, after stimulation). Right panels are the magnified views of the left panels. Scale bar: 2 mm.
Bioluminescence of the polychaete *Tharyx* sp.

incomplete (posterior part absent), 19 chaetigers, 2 mm in length, 300 µm in width, yellowish in life. Prostomium conical (right side broken; Figure 2A), eyespots absent, nuchal organs not seen. Peristomium elongate with a pair of dorsal tentacles. Pairs of branchiae on dorsolateral side of body, first pair of branchiae on achaetous segment located posterior to peristomium (Figure 2A). Parapodia biramous, with 4–5 capillary notochaetae and 2–3 capillary neurochaetae/2–3 neurospines. Neurospines with knobbed tips (Figure 2B). The spines do not form cinctures within the obtained segments.

The exact species could not be determined because the posterior end of the specimen was lost. Specimen 02 (Figure 1B) was subjected to DNA analysis and the sequence was compared to the DNA barcoding database in GenBank. The blastn result showed that sequences for *Tharyx* sp. were the best match to our specimen sequence (96%–97% identities). The second-best match was the genus *Chaetozone* (90%). The phylogenetic tree showed that Specimen 02 was grouped with *Tharyx* sp. and *Chaetozone* sp. (Figure 3). Herring (1978a; 1987) listed the genus *Tharyx* as a group containing bioluminescent species with the notes of “*=Heterocirrus in part” or “*=Heterocirrus.” However, currently all species of *Heterocirrus* are combined with other genera (*Acrocirrus*, *Aphelochaeta*, *Chaetozone*, *Caullerella*, and *Dodecaceria*). Thus, the status of bioluminescence in presently accepted *Tharyx* has been ambiguous. Consequently, we believe that the present study confirms bioluminescence in the currently accepted genus *Tharyx* for the first time.

The ecological role of bioluminescence in some cirratulids is thought to be related to reproduction (Verdes & Gruber

![Fig. 2. *Tharyx* sp. Specimen 01: (A) anterior end, (B) neurospines. Arrowheads indicate dorsal tentacles. Arrows indicate branchiae.](image)

![Fig. 3. Phylogenetic tree of Cirratulidae based on partial COI sequences. The bootstrap values (500 replications) over 95% are shown on the nodes.](image)
Table 2. List of taxa used for molecular analysis.

| Registered taxon name                     | GenBank ACCN (BOLD ID) |
|-------------------------------------------|------------------------|
| Cirratulidae                              |                        |
| *Apherocochaeta filiformis*               | (HZPLY461-13)          |
| *Aphelochaeta glandaria*                  | (BBPS219-19)           |
| *Aphelochaeta montlaris*                  | (BBPS222-19)           |
| *Aphelochaeta petersenae*                 | (CMBIA146-11)          |
| *Aphelochaeta philipsi*                   | (CMBIA148-11)          |
| *Caulieriella pacifica*                   |                        |
| *Chaeotozone* sp.                         |                        |
| *Chaeotozone hartmanae*                   |                        |
| *Cirriformia capixabensis*                | KM192164               |
| *Cirriformia chicoi*                      | KM192164               |
| *Cirratulus cirratus*                     | GU672480               |
| *Cirratulus spectabilis*                  | MF121473               |
| *Clenodrilus serratus*                    | KP794932               |
| *Dodecaceria ater*                        | KP794933               |
| *Dodecaceria concharum*                   | DQ209262               |
| *Dodecaceria fowkesi*                     | (HZPLY476-13)          |
| *Dodecaceria sestentaculata*              |                        |
| *Kirkegaardia sp.*                        | (CMBIA229-11)          |
| *Monticellina silbina* (=*Kirkegaardia silbina*) | (CMBIA1229-11)        |
| *Monticellina* sp. (=*Kirkegaardia sp.*) | MK971188               |
| *Protocirrineris chrysoderma*             | KP096407               |
| *Rarricirrus jennae*                      | MF417425               |
| *Tharyx sp.*                              | KT307703               |
| *Tharyx multifilis* (=*Apherocochaeta multifilis*) | (HZPLY476-13)        |
| *Tharyx* sp.                              |                        |
| *Tomartinae*                              |                        |
| *Timarete* sp.                            |                        |
| *Timarete* sp.                            |                        |
| *Tharyx* sp.                              |                        |
| *Tharyx* sp.                              |                        |
| *Tharyx* sp.                              |                        |
| *Tharyx* sp.                              |                        |
| *Tharyx* sp.                              |                        |
| *Tomartinae*                              |                        |
| *Timarete* sp.                            |                        |
| *Timarete* sp.                            |                        |
| *Tharyx* sp.                              |                        |
| *Tharyx* sp.                              |                        |
| *Acroscirridae*                           |                        |
| *Acroscirrus validus*                     |                        |
| *Acroscirrus* sp.                         |                        |
| *Flabelligera* sp.                        |                        |
| *Flabelligerina* sp.                      |                        |
| *Flabelligerina* sp.                      |                        |
| *Flabelligerina* sp.                      |                        |
| *Flabelligerina* sp.                      |                        |
| *Flaeta* sp.                              |                        |
| *Flaeta* sp.                              |                        |
| *Flaeta* sp.                              |                        |
| *Flaeta* sp.                              |                        |
| *Poeobius* meseres                         |                        |

2017), as in fireflies. Lund (1911) described the mating behavior of shallow water cirratulids as similar to that of syllid worms (genus *Odontosyllis*), while Bonhomme (1944) found seasonal maturation of photocytes in the epidermis of *Caulieriella bioculata*. In *Tharyx*, epitokous swarms have been reported for several species (Petersen 1999); thus, green light may have a mate-attracting function. This *Tharyx* worm is benthic and it is more practical to keep the specimens alive in laboratory aquariums than planktonic luminous polychaetes such as *Tomopteris* and *Swima*. We propose *Tharyx* sp. from deep-seawater as a model organism useful for studying the non-blue bioluminescence system of polychaetes and its evolution, which are still poorly understood.

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