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New record of bioluminescence in *Odontosyllis* cf. *australiensis* (Annelida: Syllidae: Eusyllinae) in Japan

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Abstract

Bioluminescence is widespread in the marine environment. The bioluminescence of some species of the fireworm *Odontosyllis* (Annelida: Syllidae: Eusyllinae) has been well studied, although the presence or absence of bioluminescence in most species of this genus is yet to be revealed. The bioluminescent worms were observed after sunset around the new moon day in July and October 2020 and in July to October 2021 in Nagasaki Prefecture, Japan. Molecular phylogenetic analysis based on two mitochondrial and one nuclear gene sequence showed that the worms were closely related to *Odontosyllis australiensis*, but the partial 16S rRNA gene sequences differed by 2% between those of the Japanese and Australian material. Because only epitokes, i.e. morphologically modified sexually mature worms, were collected, further studies on morphological characters of atokes would be required in the future. We therefore tentatively refer to them as *Odontosyllis* cf. *australiensis*. Molecular phylogenetic analysis also showed that known bioluminescent *Odontosyllis* species belong to various lineages.

Introduction

Bioluminescence, the emission of visible light by a natural chemical reaction, is found in a wide variety of organisms of different phyla, from bacteria to eukaryotes (Haddock *et al.*, 2010). According to a recent estimate by Lau & Oakley (2021), bioluminescence has evolved at least 94 times in the phylogenetic tree of life. Bioluminescence is considered to play an important role in biological interactions such as protection from predators (e.g. Latz, 1995; Deheyn *et al.*, 2000; Jones & Nishiguchi, 2004; von der Heyden *et al.*, 2010) and mate attraction (e.g. Morin & Bermingham, 1980; Lewis & Cratsley, 2007; Rivers & Morin, 2008, 2009; Morin & Cohen, 2010). A well-known example of bioluminescence is the light emitted by fireflies on land. However, bioluminescent organisms are more common in the ocean than in terrestrial environments (Widder, 2001). Stable, dark without light, and optically clear environmental conditions, as well as the variety of biological interactions in the ocean may favour the evolution of luminescence (Haddock *et al.*, 2010). Marine organisms are therefore important subjects in the study of bioluminescence.

Annelida includes over 20,000 described species and is one of the major taxa in the marine environment (Capa & Hutchings, 2021). Bioluminescence occurs in various lineages in Annelida, such as scale worms (Polynoidae), spaghetti worms (Terebellidae) and earthworms (Megascolecidae) (reviewed by Verdes & Gruber, 2017). The family Syllidae includes bioluminescent species and is one of the most specious families of Annelida with more than 1100 described species (Martin *et al.*, 2021). Many syllid species are benthic worms with a pelagic stage for reproduction, exhibiting altered morphology of the parapodia and chaetae (bristles), and bioluminescence (Daly, 1975; Tsuji & Hill, 1983; Deheyn & Latz, 2009). A few syllid genera, such as *Eusyllis* (Zörner & Fischer, 2007), *Nudisyllis* (as *Pionosyllis* in Bassot, 1979), and *Odontosyllis*, contain luminescent species.

The bioluminescence of *Odontosyllis* spp., known as 'fireworms' (not the Amphinomidae here), is the best documented in this family. At least nine described species, namely *Odontosyllis ctenostoma* Claparède, 1868 (see Verdes *et al.*, 2018), *Odontosyllis guillermoi* Fukuda & Nogueira, 2006 (see Verdes *et al.*, 2018), *Odontosyllis hyalina* Grube, 1878 (Van Lummel, 1932), *Odontosyllis enopla* Verrill, 1900 (Galloway & Welch, 1911; Huntsman, 1948; Markert *et al.*, 1961; Shimomura *et al.*, 1963; Haneda, 1971; Wilkens & Wolken, 1981; Wolken & Florida, 1984; Fischer & Fischer, 1995; Brugler *et al.*, 2018; Prentiss, 2020), *Odontosyllis luminosa* San Martín, 1990 (San Martín, 1990; Gaston & Hall, 2000), *Odontosyllis octodentata* Treadwell, 1917 (Erdman, 1965), *Odontosyllis phosphorea* (Potts, 1913; Fraser, 1915; Berkeley, 1935; Tsuji & Hill, 1983; Deheyn & Latz, 2009), *Odontosyllis polycera* (Schmarda, 1861) (Daly, 1975), and *Odontosyllis undecimdonta* Imajima & Hartman, 1964 (Horii, 1982; Inoue *et al.*, 1990), and several unidentified species of *Odontosyllis* (Haneda, 1971; McCloskey *et al.*, 2017; Ramesh *et al.*, 2017; Verdes *et al.*, 2018) have been reported as bioluminescent. Interestingly, the swarming of luminescent *Odontosyllis* spp. reportedly starts from about an hour after sunset on days close to the full moon for reproduction (Markert *et al.*, 1961; Gaston & Hall, 2000). In *O. enopla*, females swim in circles with a

strong and continuous glow and release their gametes (Galloway & Welch, 1911). Subsequently, males swim directly towards the luminous circle with sharper and intermittent flashes and locate a glowing female (Galloway & Welch, 1911). Recently, the luciferases of *O. undecimdonta* (Schultz *et al.*, 2018) and *O. enopla* (Brugler *et al.*, 2018) were sequenced and those of syllids were uniquely obtained among all known luminescent taxa (Schultz *et al.*, 2018). The biological traits of several luminescent *Odontosyllis* spp. have become apparent as mentioned above, but the presence or absence of bioluminescence in *Odontosyllis* is still not sufficiently understood to discuss the evolutionary gain or loss of bioluminescence, considering the relatively high species diversity of this genus with over 50 described species.

To date, bioluminescence of a single syllid species, *Odontosyllis undecimdonta*, has been reported from Japan (Horii, 1982), and this species has become the subject of subsequent studies on chemistry and genetics (Inoue *et al.*, 1990, 1991, 1993; Tanino *et al.*, 1994, 1996; Kakoi *et al.*, 1995; Schultz *et al.*, 2018; Kotlobay *et al.*, 2019). In this study, we found a bioluminescent *Odontosyllis* cf. *australiensis* from Japan for the first time and determined the phylogenetic placement of this species by reconstructing the molecular phylogenetic tree of Eusyllinae based on two mitochondrial (COI and 16S rRNA) and one nuclear (18S rRNA) gene sequences.

Materials and methods

Visual observations and net sampling were conducted in 2020 and 2021 (Table 1) at a pier in Nagasaki, Japan. Protected by breakwaters, the sea off the pier is generally calm and underwater video camera observations showed that the seabed around the pier is mainly covered with rocks. Luminant specimens swimming near the sea surface were collected with a spoon net at about 21:00 (JST) on 17–19 and 21 July 2020. Additional observations were conducted at the same location ~1.5 h after sunset on days near the new moon in October 2020 and April through October 2021. Sunset times and ages of the moon in Nagasaki city (32°45′N 129°52′E) during observation days were obtained from the National Astronomical Observatory of Japan homepage (https://eco.mtk.nao.ac.jp/koyomi/dni/index.html.en). Seawater temperatures on observation days were represented by the sea surface temperature measured and published weekly by the Nagasaki Prefectural Institute of Fisheries at an aquaculture cage located several hundred metres from the pier (https://www.pref.nagasaki.jp/bunrui/shigoto-sangyo/suisangho/suisan-shiken-suishi-teichi-water-temperature/). Benthic samples, including the surface of seaweeds, stones, the shells of dead and living oysters in the intertidal to subtidal (~8 m depth) zone, were examined on 13 April 2021 to detect the benthic mode of the swimming specimens. The specimens were fixed and preserved in 70 or 99% ethanol. The morphological characters of the specimens were examined under a stereomicroscope and photographed (Figure 1).

Protocols of preparation of template DNA and PCR were basically conducted by following the methods described in Kobayashi *et al.* (2021). Portions of the body wall of the specimens were cut out and treated with a mixture of 10 µl of proteinase K solution (Nacalai Tesque, Kyoto, Japan) and 100 µl of 10% solution of Chelex 100 Resin (Bio-Rad, Hercules, CA) at 56°C for 20 min and then 100°C for >20 min to use the supernatant fluid as template DNA.

The partial mitochondrial 16S rRNA and COI gene sequences, and the nuclear 18S rRNA gene sequences of three specimens were determined. The PCR mixtures were as follows: (Mixture 1) 8.75 µl of sterilized water, 0.07 µl of TaKaRa Ex *Taq* Hot Start Version (TaKaRa Bio, Kusatsu, Japan), 1.25 µl of 10 × Ex *Taq* Buffer, 1.0 µl of 2.5 µM dNTP mixture, 0.15 µl of 20 µM forward and reverse primers (Table 2), and 1.0 µl of template DNA; or (2) 4.50 µl of sterilized water, 6.25 µl of KOD One PCR Master Mix (TOYOBO), 0.375 µl of 10 µM forward and reverse primers (Table 2), and 1.0 µl of template DNA. PCR amplifications were performed as follows: (Mixture 1) initial denaturation at 94°C for 120 s; followed by 35 cycles comprising denaturation at 94°C for 30 s, annealing at 50°C for 20 s, and extension at 72°C for 20 s; and then final extension at 72°C for 300 s (16S and COI) or (Mixture 2) initial denaturation at 98°C for 10 s; followed by 30 cycles comprising denaturation at 98°C for 10 s, annealing at 60°C for 5 s, and extension at 68°C for 2 s (18S). The PCR product was purified using ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA). Sequencing was outsourced to Eurofins Genomics (Tokyo, Japan). The obtained nucleotide sequences were deposited in the DNA Data Bank of Japan (DDBJ) with DDBJ/EMBL/GenBank accession numbers LC641713–LC641716, LC654403–LC654404.

Phylogenetic analysis based on the concatenated dataset (18S + 16S + COI) was conducted using 33 ingroup and four outgroup taxa to reveal the phylogenetic position of our specimen (Table 3). Taxa were selected based on a previous study of phylogenetic relationships within Syllidae (Aguado *et al.*, 2012). All sequences except our specimens were obtained from GenBank. Alignment was performed using MAFFT v7.294b (Katoh *et al.*, 2017). The following substitution models were selected based on the corrected Akaike information criterion (AICC) using PartitionFinder2 (Lanfear *et al.*, 2017): TRN + I + G for 18S rRNA, GTR + I + G for 16S rRNA, and TRN + I + G, TVM + I and HKY + G for the 1st 2nd and 3rd codon of COI, respectively. Molecular phylogenetic analyses using the concatenated dataset were conducted with Bayesian inference and maximum likelihood (ML) methods. Bayesian analysis was performed using MrBayes v3.2.6. (Ronquist & Huelsenbeck, 2003) with the setting 'branch lengths unlinked'. Two parallel runs were made for 5,000,000 generations (with a sampling frequency of 1000), using the default value of four Markov chains. The initial 25% of samples were discarded and the following 75% were accepted to ensure that the four chains reached stationary distributions based on the average standard deviation of split frequencies (Ronquist & Huelsenbeck, 2003). A ML phylogenetic analysis was conducted with IQ-TREE v1.6.12 (Nguyen *et al.*, 2014) using 1000 ultrafast bootstrap replicates. The resultant tree was edited using FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

The glowing creatures swimming near the surface of the sea around the pier were first spotted around 21:00 (JST) on 17 July 2020. They turned out to be worms about 2–4 cm long by visual estimate after being collected with a spoon net (Figure 1A). They were found within 10 m of the pier at a density of several individuals per m^2 and were swimming spirally or straight ahead at a speed of about 5 cm s^{-1} (Supplementary data 1). They glowed green and released luminescent particles as they swam, leaving glowing trails behind them for several seconds. Glowing fogs were also observed at about 30 cm depth from the surface. The glowing worms were observed on 5 consecutive nights until 21 July, the new moon day, around 21:00, but not on 22 July between 20:30 and 21:30. The sea surface temperature measured at a nearby station on 22 July was 27.0°C and the sunset times during 17–21 July were about 19:30. The bioluminescence of the worm was sighted again on 18 and 19 October 2020, 6–11 July 2021, and 4, 5 and 7 August 2021, 4 and 7 September 2021, and 3 October 2021 about 1.2–1.5 h after sunset. Moon ages on these days were 25–29 or 0–2, and sea surface temperatures measured nearby were 20.2–29.3°C. On these days, we typically observed several to several tens of glowing worms only

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Table 1. Metadata of visual observations

Date	Age of the moon	Time of observation (JST)	Time of sunset (JST)	SST (°C)	Luminant worms
17–21 July 2020	26–29, 0	20:30–21:30	19:27–19:29	24.6 (15 July)	Found (21:00)
22 July 2020	1	20:30–21:30	19:26	27.0 (22 July)	Not found
18–19 October 2020	1–2	19:10–19:40	17:43–17:44	20.2 (21 October)	Found (19:20)
9 April 2021	27	20:00–20:20	18:46	16.8 (7 April)	Not found
9–11, 13 May 2021	27–29, 1	20:30–21:00	19:08–19:12	19.5 (13 May)	Not found
28 May 2021	16	20:45–21:10	19:21	20.7 (26 May)	Not found
7–11, 14 June 2021	26–29, 1, 4	20:45–21:20	19:27–19:30	23.5 (9 June)	Not found
6–11 July 2021	26–29, 0–1	20:50–21:30	19:31–19:32	26.5 (7 July)	Found (21:00)
12–13 July 2021	2–3	20:55–21:20	19:30–19:31	28.2 (14 July)	Not found
4, 5, 7 August 2021	25, 26, 28	20:25–21:35	19:14–19:17	29.3 (4 August)	Found (20:25)
10 August 2021	2	20:25–20:50	19:11	27.8 (11 August)	Not found
4, 7 September 2021	27–29	19:30–20:30	18:40–18:42	27.6 (8 September)	Found (20:00)
3 October 2021	26	19:20–19:50	18:03	24.8 (6 October)	Found (19:25)

Fig. 1. *Odontosyllis* cf. *australiensis* collected from Japan. (a) Swimming bioluminescent specimen. A movie is available in Supplementary data 1. (b) Eggs released from a specimen during observation under a microscope. (c, d) Living specimen. (e, f) Ethanol fixed specimen. (e) Dorsal view of the anterior body. (f) Ventral view of chaetigers on the middle part of the body. An arrow indicates elongated notochaetae.

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Table 2. Nucleotide sequences of primers used in this study

Locus	Primer	Sequence (5′-3′)	Direction[a]	Usage[b]	Reference
16S rRNA	16SarL	CGCCTGTTTATCAAAAACAT	F	P/S	Palumbi (1996)
	16SbrH	CCGGTCTGAACTCAGATCACGT	R	P/S	Palumbi (1996)
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	F	P/S	Folmer *et al.* (1994)
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	R	P/S	Folmer *et al.* (1994)
18S rRNA	18A1	CCTACCTGGTTGATCCTGCCAG	F	P	Steiner & Dreyer (2003)
	NS2	GGCTGCTGGCACCAGACTTGC	R	S	White *et al.* (1990)
	NS5	AACTTAAAGGAATTGACGGAAG	F	S	White *et al.* (1990)
	189r	TCGGAATTAACCAGACAAATC	R	S	Nakamura *et al.* (2007)
	1800r	ATGATCCTTCCGCAGGTTCACC	R	P	Steiner & Dreyer (2003)

[a]Forward (F) or reverse (R); [b]PCR (P) or sequencing (S).

Table 3. Species used in the phylogenetic analysis based on the concatenated dataset with GenBank accession numbers

Taxon	18S	16S	COI
Ingroup			
Eusyllis blomstrandi	EF123887	EF123788	EF123749.
Eusyllis kupfferi	JF903595	JF903697	JF903758
Eusyllis lamelligera	–	JF913952	JF913975
Nudisyllis pulligera	AF474286	–	EF123754
Odontosyllis australiensis (Australia)	JF903615 and KP974807	JF913955	–
***Odontosyllis* cf. *australiensis* (Japan)**	**LC654404**	**LC641716**	**LC641714**
Odontosyllis ctenostoma	JF903616 and JF903617	JF913956	–
Odontosyllis detecta	JF903618	JF913957	–
Odontosyllis freycinetensis	JF903619	JF903755	JF903772
Odontosyllis fulgurans	EF123882	EF123792	EF123755
Odontosyllis gibba	AF474282	–	–
Odontosyllis gibba	EF123850	EF123793	EF123756
Odontosyllis globulocirrata	JF903620	JF903702	JF903773
Odontosyllis maculata	JF903621	JF903703	–
Odontosyllis phosphorea	–	JF903754	–
Odontosyllis polycera	JF913967	–	–
Odontosyllis sp.	JF903572	–	–
Synmerosyllis lamelligera	EF123864	EF123796	EF123759
Synmerosyllis yolandae	JF913973	JF903747	–
Synmerosyllis sp.	JF903573	JF903681	JF903759
Outgroup			
Autolytus rubrolineatus	AF474307	AF474261	–
Epigamia macrophthalma	AF474293	GQ856207	GQ856184
Proceraea pseudopicta	AF474318	AF474272	–
Virchowia clavata	AF474314	AF474268	GQ856171

during 10–30 min from the first worm sighting. However, on 4 August 2021, a total of more than 100 of them were observed continuously for more than an hour from the first sighting. The specimens captured alive on 10 and 11 July released thousands of white particles that appeared to be eggs during observation under a microscope (Figure 1B). On 9 April, 9–14 May 2021 and 7–14 June, all of which were near the new moon day and when sea surface temperatures were 16.8–23.5°C, no glowing worms were observed around 1.5 h after sunset. The results of the observations are summarized in Table 1.

The bodies of the fixed specimens (N = 2) were about 17–19 mm long and 1.3–1.4 mm wide (Figure 1C & D). The pharynx possessed at least four teeth. Occipital flaps of the specimens covered the posterior part of the prostomium (Figure 1E). The notochaetae were capillary and the ventral compound chaetae in midbody are bidentate. Long notochaetae were present on the

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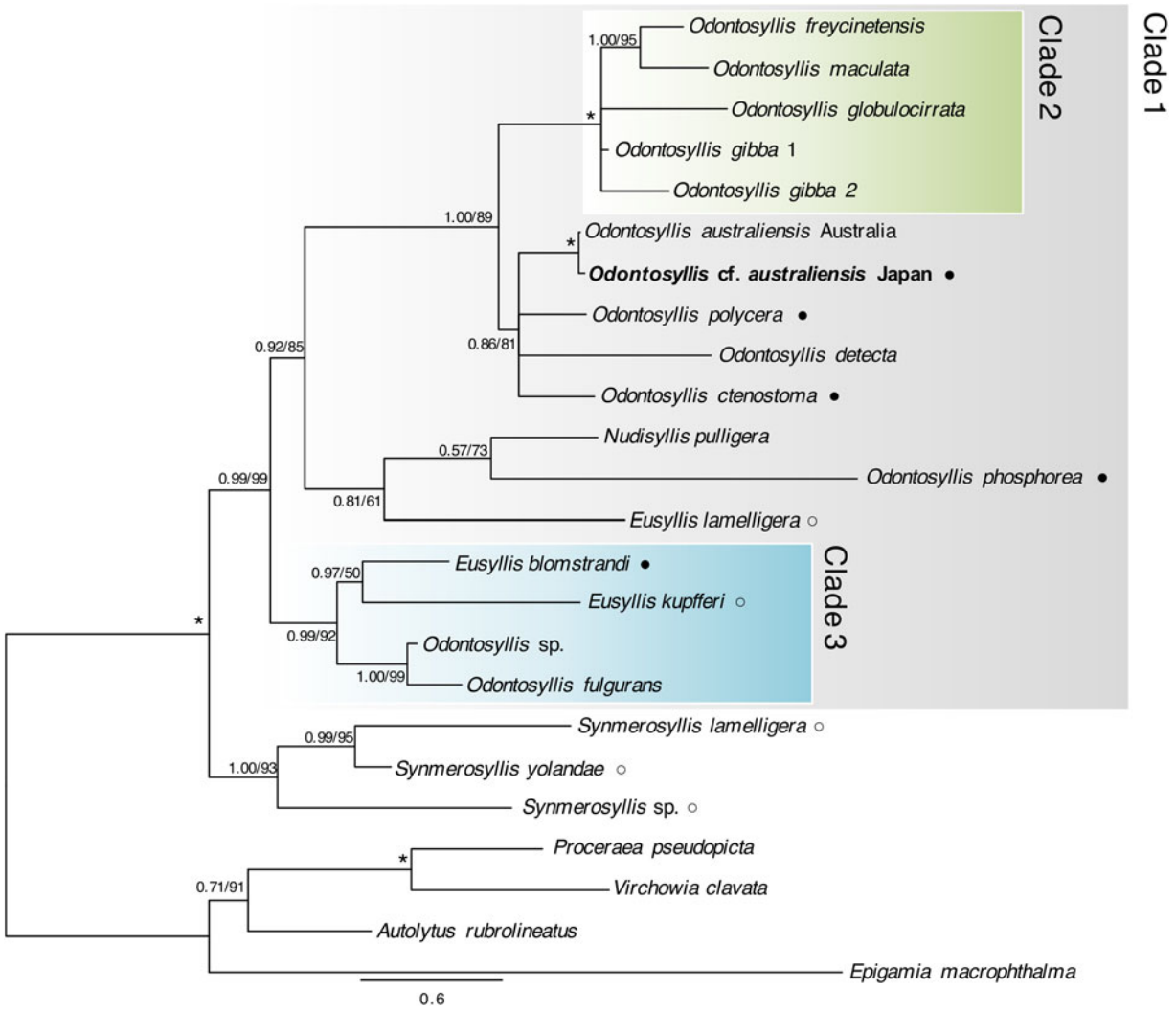

Fig. 2. Bayesian phylogeny of *Odontosyllis* and closely related syllids based on the combined dataset, which comprises nuclear 18S rRNA (1916 characters), mitochondrial 16S rRNA (546 characters) and mitochondrial COI (659 characters) gene sequences. The numbers above the branches indicate posterior probability (PP), followed by the percentage of maximum likelihood bootstrap (BS) values. Asterisks indicate full support (PP = 1, BS = 100). Closed and open circles represent bioluminescent species or not, respectively (after Verdes *et al*., 2018, except for *Odontosyllis* cf. *australiensis* Japan). The status of bioluminescence is known for species without circles. An operational taxonomic unit with newly obtained DNA sequences is shown in bold.

chaetiger 34 or 35 and subsequent 19 chaetigers (Figure 1F). Dorsal cirri were long and smooth, and ventral cirri were ovoid shape. Morphologically matching specimens to the swimming worms were not found in the sediment samples at the site where the swimming worms were collected.

The concatenated dataset for molecular phylogenetic analysis comprised 3165 characters of COI (659 characters), 16S rRNA (556 characters) and 18S rRNA (1950 characters) gene sequences. The results of phylogenetic analysis showed that the ingroup was monophyletic with full support, i.e. posterior probability (PP) = 1.00 and maximum likelihood bootstrap value (BS) = 100%. The monophyly of several lineages was recovered (Figure 2): species of *Synmerosyllis* (PP = 1.00, BS = 93%); *Odontosyllis*, *Eusyllis* and *Nudisyllis* (PP = 0.99, BS = 99%; Clade 1); *Odontosyllis*, except for *O. phosphorea*, *Odontosyllis fulgurans* (Audouin & Milne Edwards, 1833) and *Odontosyllis* sp. (PP = 1.00, BS = 89%); *Odontosyllis freycinetensis* Augener, 1913, *Odontosyllis maculata* Uschakov in Annenkova, 1939, *Odontosyllis globulocirrata* Hartmann-Schröder, 1981, and *Odontosyllis gibba* Claparède, 1863 (PP = 1.00, BS = 100%; Clade 2); *O. fulgurans*, *Odontosyllis* sp., *Eusyllis blomstrandi* Malmgren, 1867, and *Eusyllis kupfferi* Langerhans, 1879 (PP = 0.99, BS = 92%; Clade 3). Our specimen was clustered with Australian *Odontosyllis australiensis* Hartmann-Schröder, 1979 with full support. Although the overlapping regions of the 18S rRNA gene sequences of our specimen and *Odontosyllis australiensis* were identical (1147 bp), their 16S rRNA sequences differed by 2% (10 of 477 bp). At least five taxa, including *Odontosyllis* cf. *australiensis*, were bioluminescent in the current analysis (reviewed by Verdes *et al*., 2018). They belonged to various lineages in Clade 1 except for Clade 2 (Figure 2).

Discussion

Our specimens are morphologically distinct from the other species of *Odontosyllis* known from Japan, including another bioluminescent syllid species *Odontosyllis undecimdonta* (Imajima, 1996). The morphological characters of our specimens were consistent with the description of *Odontosyllis australiensis* in San Martín & Hutchings (2006), except for the larger size of our specimens (17–19 mm) than the known maximum size (6.6 mm) (Hartmann-Schröder, 1981; see San Martín & Hutchings, 2006). However, the genetic divergence between Japanese and Australian specimens of *O. australiensis* was relatively large when compared with the intra-specific differences between other coastal invertebrates of Japan and Australia, and the differences between syllid species. The variation in the 16S rRNA gene sequences of *O.*

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australiensis from Japan (LC641716) and Australia (JF913955) was 2%. The 16S rRNA gene sequences of the ring top cowrie *Monetaria annulus* (Linnaeus, 1758) collected from Japan (LC469295) and Australia (KT753889) were identical. Contrarily, the 16S rRNA gene sequences of the blue swimming crab *Portunus pelagicus* (Linnaeus, 1758) from Japan (LC469668) and Australia (FJ152161 and FM208750) differed (5 of 169 bp; 3%). The genetic difference between our specimens and *O. australiensis* from Australia is much greater than that of *M. annulus* but is within the intra-specific variation of *P. pelagicus*. Considering the genetic differences among syllid species, the inter-specific difference in the 16S of *Amblyosyllis* is 4–28% with two exceptions (1.5 and 2.9%), while the intra-specific difference was less than 1.9% (Aguado *et al.*, 2019). The *Syllis gracilis* species complex shows higher variation in the genetic divergence of 16S between species; the inter-specific divergence is 3.9–59.2%, while the intra-specific variation in some lineages is also high (≤26%) (Álvarez-Campos *et al.*, 2017). Therefore, it is difficult to judge whether our specimens are *O. australiensis* or not based on molecular data. Considering the above morphological and molecular information, we tentatively refer to our specimens as *Odontosyllis* cf. *australiensis*. Scrutinizing morphological characters of the atoke (sexually immature) specimens of Japanese *Odontosyllis* cf. *australiensis* would be needed in the future after a comprehensive revision of the diagnostic characters and phylogenetic relationships of the genus.

Our findings on bioluminescence in *Odontosyllis* cf. *australiensis* also represent the first observation of bioluminescence in relevant species of *O. australiensis*. Swarming of Japanese *Odontosyllis* cf. *australiensis* may exhibit lunar periodicity, as has been reported for other *Odontosyllis* species (Potts, 1913; Huntsman, 1948; Markert *et al.*, 1961; Gaston & Hall, 2000). For example, the swarming for reproduction of *O. enopla* and *O. luminosa* peaks about one hour after sunset on several days after full moon (Markert *et al.*, 1961; Gaston & Hall, 2000). On the other hand, *Odontosyllis* cf. *australiensis* was found to be luminous on days within 5 days from the new moon day, 1.2–1.5 h after sunset in July and October 2020, and July through October 2021. It is therefore possible that swarming of *Odontosyllis* cf. *australiensis* occurs during certain moon ages and from certain times after sunset, similar to other species of *Odontosyllis* but with different timing parameters. While swarming of *O. enopla* and *O. luminosa*, which inhabit tropical waters, can be observed during most months of the year, we found no glowing worms during the months of April to June 2021, indicating that occurrence of swarming of *Odontosyllis* cf. *australiensis* is a seasonal event, perhaps because temperatures above a certain threshold are required for maturation. Further systematic collection of positive and negative observational data throughout the entire year is essential to further test the hypotheses.

The article posted on bioRxiv includes a phylogenetic analysis with more species than our analysis (Verdes *et al.*, 2018). Overall, the topology was similar between the tree shown in Verdes *et al.* (2018) and this study. However, *Synmerosyllis* was clustered with *Eusyllis lamelligera* 1–3 in Verdes *et al.* (2018), whereas *Synmerosyllis* was sister to the clade comprising the rest of eusylline species (Clade 1) in our analysis (Figure 2). The paraphyletic status of *Eusyllis* and *Odontosyllis* was shown by previous studies on the syllid phylogeny (Aguado *et al.*, 2007, 2012). Verdes *et al.* (2018) suggested that bioluminescence in Eusyllinae was obtained once at the common ancestors of the lineages of Eusyllinae and *Odontosyllis* based on ancestral state reconstruction analysis. It should be noted that the information on the presence or absence of bioluminescence of most lineages remains unknown in the analysis. Parsimoniously, it is legitimate that the common ancestors of Eusyllinae and *Odontosyllis* were luminous, i.e. bioluminescence was obtained once and lost independently at least in *Eusyllis lamelligera* and in *Eusyllis kupfferi*, because the loss of complex traits is more plausible than their multiple acquisitions (Kobayashi *et al.*, 2018; Audino *et al.*, 2020). Further discussion of the ancestral character states and loss/gain of bioluminescence in syllids requires considerable efforts to confirm the presence/absence of bioluminescence for each species, in addition to the obtaining of a more robust phylogenetic framework for the Eusyllinae in the future.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0025315421000850

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