Reply to referee #2 on bg-2020-64

Interactive comment on "Reviews and syntheses: Bacterial bioluminescence – ecology and impact in the biological carbon pump" by Lisa Tanet et al.

Anonymous Referee #2

General comments:
This is a fascinating subject for a review and I read it with much interest. It is extremely thorough, and in some places even a bit too detailed and requires a step back for the non-expert (see specific comments below). It is well organized and generally well written, although requires a thorough editing for grammar (some examples below).
The one figure and Table are well done, but in a review of this detail and length a few more figures to help illustrate some of the concepts would be helpful. One example that comes to mind is a diagram showing the mechanisms of expulsion.
The discussion on impacts on the biological C pump need to be qualified more. Luminescent bacteria are not always a catalyst for sequestration. If bioluminescence leads to disaggregation and "slowing down the sinking rate of particles and consequently increasing their degradation and the remineralization rate" and this happens in the mixed layer, that will decrease carbon export and sequestration.
Answer: We thank referee#2 for his favorable comments. We will have the manuscript proofread by a language specialist. We agree that a review such as ours would benefit from a little more illustration. However, we chose not to add the illustration suggested by the referee because the mechanisms of expulsion are little known and, as far as we know, differ from one organism to another. Indeed, there are numerous types of light organs, with a large diversity of both structure and location. Only a few of them have been described in detail. The most studied is that of the squid but, in accordance with the comments of referee #1, we have chosen to avoid systematically focusing our interest on this organism so as not to make its functioning a generality. However, in order to integrate additional information on the localisation of the ejections of bioluminescent bacteria, either directly into the surrounding seawater or indirectly through the gut, we will complete Table 1 (see at the end of this document). The Table caption will be changed as followed:

Table 1: List of luminous bacterial species found in light organ symbiosis in fishes and squids. The diagrammatic fish, from Nealson and Hastings (1979), was used to indicate, in blue, the approximate locations of the light organ of the different families of symbiotically-luminous fishes. E: indicates an external expulsion of the bioluminescent bacteria, directly into the seawater. I: indicates an internal expulsion of the bioluminescent bacteria, in the digestive tract. (E) or (I) indicate a putative localisation of the expulsion.

Moreover, we propose the addition of another illustration, that will explain in more detail the importance of bioluminescence in the accessibility of organic matter for marine organisms, in section 4.4.

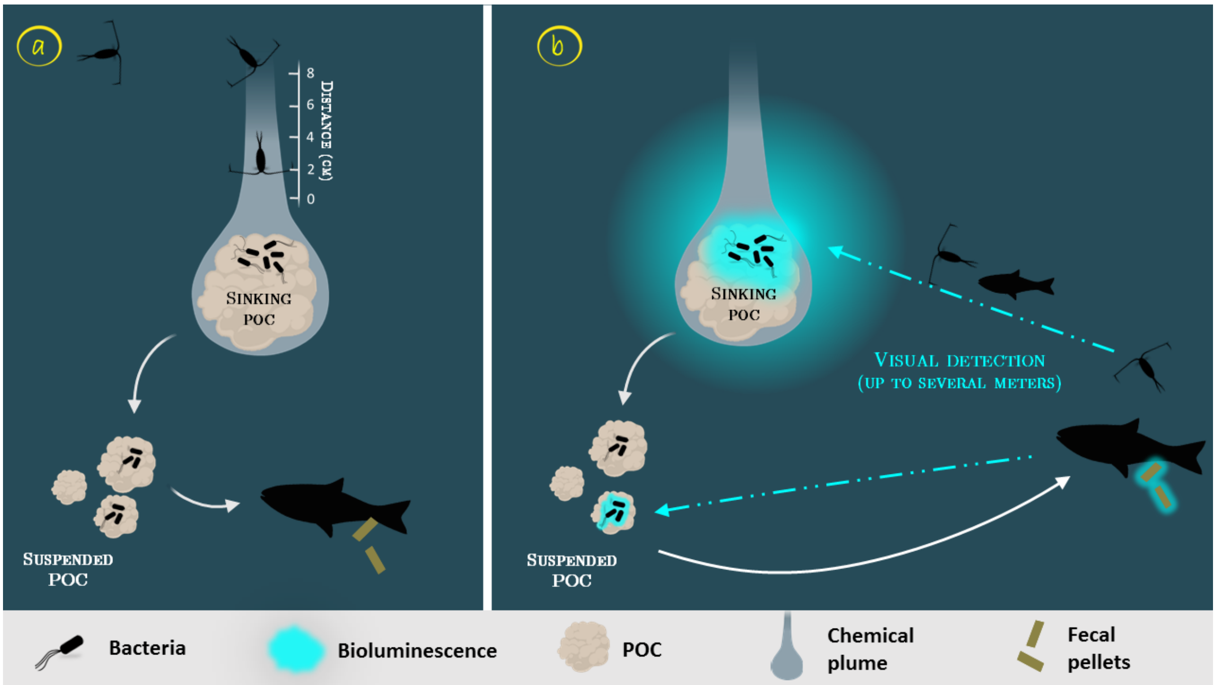


Figure 2: Zoom on the carbon fluxes at the level of a gravitational sinking particle (inspired by Azam and Long 2001). The sinking POC is moving downward followed by the chemical plume (Kiørboe 2011). The plain white arrows represent the carbon flow. Panel (a) represents the classical view of a non-bioluminescent particle. The length of the plume is identified by the scale on the side (Kiørboe and Jackson 2001). Panel (b) represents the case of a glowing particle in the bioluminescence shunt hypothesis. Bioluminescent bacteria are represented aggregated onto the particle. Their light emission is shown as a bluish cloud around it. Blue dotted arrows represent the visual detection and the movement toward the particle of the consumer organisms. Increasing the visual detection allows a better detection by upper trophic levels, potentially leading to the fragmentation of sinking POC into suspended POC due to sloppy feeding. The consumption of the bioluminescent POC by fish can lead to the emission of bioluminescent fecal pellets (repackaging), which can also be produced with non-bioluminescent POC if the fish gut is already charged with bioluminescent bacteria.

Specific comments:

Paper uses 'bacteria' throughout. Are Archaea bioluminescent too? (This should be mentioned somewhere).

Answer: No archaea has been characterized as bioluminescent. The sentence "To our knowledge, no archaea has been characterized as bioluminescent" will be added in the introduction section.

p. 2, L 34 beneficies (should this be benefits?)

Answer: Done

p. 3, L 68 spelling- evidence

Answer: Done

p. 3, L 77 pyrosomes are not fishes (they are pelagic tunicates)
Answer: We will modify the paragraph to clarify. In this paragraph, we will discuss the symbioses with luminous bacteria in general and not only with fishes.

p. 3, L 87 Anglerfishes- would be more clear if you give the rule first then the exception (isn't it that nearly all the esca in Angler fishes are symbiotic luminous bacteria and not intrinsic light organs?
Answer: This part will be removed subsequently to the referee #1 comments in order to lighten the text.

p. 4, L 91 spelling- internal
Answer: Done

Section 2.2; p. 4, L 101-118 This section gives examples, but does not actually explain how symbiont selection or colonization occurs. What is 'microbial recognition and molecular dialog' and how does it work? How colonization occurs is not described at all.
Answer: This review is already very thorough as both referees commented. We would rather not add more information regarding subjects that are not directly related to the BCP since many authors have already extremely well reviewed information on symbiont selection or colonization and the more described are the squid's ones. These publications are indicated in the text. As suggested by referee #1, we don't want to talk systematically about the squid so that it doesn't become the general case. Moreover, the text will slightly be modified at some points in order to clarify what is known only for the squid symbiosis and what is valid for all symbioses. These changes are indicated in the reply to referee#1.

p. 6, L 174- spelling- reduces
Answer: Done

p. 6, L 176- The bacterial ...
Answer: Done

p. 7, L 193- More detail needed here. How does the expulsion actually take place? How do the bacteria get from the tubules into the digestive tract (are all light organs directly connected to the digestive tract, and through what)? Or from tubules into the surrounding water, for that matter- do all tubules have an opening on the animal surface- seawater interface, or only some ? For example, I have always wondered in an Anglerfish esca, how are the bacteria expulsed? A figure would be helpful to illustrate.
Answer: As mentioned above, there is an important diversity in the structure and location of the light organs, and actually, with the squid exception, many points of the other symbioses (symbiont selection, population regulation, frequency of the symbiont expulsion...) remain unclear. That's why it is not possible to have a simple description of the process. Since this is not the topic of our review and as explained in the 2.2 answer, we chose not to add a figure. However, this comment prompted us to add, in the Table 1, an information related to the expulsion pathway of the luminous bacteria (directly connected to the environment if the light organ has pores or ducts opening into the surrounding sea, or indirectly if the light organ has ducts connected to the gut). We think it is an interesting piece of information and thank the referee#2 for that.

p. 7, L 193- "Most hosts with internal light organs…"
Answer: Done.

p.8, L237- "in an herbivorous fish compared to a carnivore." p.8, L240- prey

Answer: Done

p. 9, L273- what is meant by 'A rare item'? Do you mean that one rare piece of information we do have is that luminescent bacteria are known to help in chitin digestion, or that in rare cases luminescent bacteria are known to help in chitin digestion.
Answer: The former suggestion is the right one. However, this section will be deleted to reduce the length of the manuscript according to referee #1 comments.

p.11 , L329-330 'prior eaten' is awkward
Answer: This part will be removed since this idea is already discussed all along the paragraph and the turn of phrase was not ideal.

p.12 , L353 'and is always associated with luminous bacteria'
Answer: Done

p.13, L387- replace the word 'unbelievable'
Answer: 'unbelievable' will be replaced by "huge".
The sentence will be as follows: "As indicated previously, the release of bioluminescent bacteria from light organs and fecal pellets could represent a huge quantity of bioluminescent bacteria in the water column."

p.13, L394 - 'amphipods were attracted'
Answer: Done

p.13, L398- do you mean 'the attraction of luminous bacteria to zooplankton'?
Answer: No, we mean the contrary. Since the sentence was confusing, the two last sentences of the paragraph will be modified as follows : "To our knowledge, the only one known is from Zarubin et al. (2012), who demonstrated that zooplankton is attracted to luminous particles and feeds on the luminous bacteria-rich organic matter. Because of the ingestion of the luminous bacteria, the zooplankton itself starts to glow. Then, they experimentally measured 8-times-higher ingestion rate of glowing (due to ingestion of bioluminescent bacteria) zooplankton by fishes, compared to non-luminous zooplankton."

p.13, L404- replace 'excreted' with 'egested'
Answer: Done

p.13, L414- replace 'excreted' with 'egested'
Answer: Done

p. 14, L424-429. As mentioned in general comments, need to be careful here- it is not always a catalyst for sequestration: if bioluminescence leads to disaggregation and slowing down the sinking rate of particles and consequently increasing their degradation and the remineralization rate, and this happens in the mixed layer, that will decrease carbon flux and sequestration.
Answer: We agree with the comment. Bioluminescence can impact the BCP in both ways and we clearly indicate these two hypotheses several times through the text. We realize that the term catalyst can be misinterpreted. We will modify the specific paragraph to clarify as follows :

"Considering this bioluminescence shunt hypothesis, all the processes described above show that bioluminescence affects the biological gravitational carbon pump (Boyd et al., 2019), by either increasing the carbon sequestration into the deep ocean, or by slowing down the sinking rate of particles and consequently increasing their degradation and the remineralization rate. Bioluminescence and especially luminous bacteria may therefore influence the export and sequestration of biogenic carbon in the deep oceans (either positively or negatively). A better quantification of these processes and impacts in the biological carbon pump are a requirement in future studies."

p. 14, L438- relies
Answer: Done

p. 14, L448- replace 'pulled' with 'combines'
Answer: Done

p. 15, L467- 'role of bioluminescence bacteria..."
Answer: In this subpart, we not only propose to investigate bioluminescent bacteria but more generally to quantify bioluminescence globally (as indicated for exemple in "1) the assessment of the global importance of bioluminescence in the oceans"). This justifies the use of a more general title.

p. 15, L473- 'pursuit' of investigations
Answer: Done

p. 15, L475-476- be specific- vertical migration of what ? (diel vertical migration zooplankton and fish?)
Answer:We will define the vertical migration more precisely as suggested.

p.16, L486-487; suggest make this more broad/ global statement than just European initiatives (mention of ARGO is good, and Bioargo should be mentioned too).
Answer: We agree with the comment. We will modify the text as follows :
'For temporal scales, in the last decades, the multiplication of long-term observatories such as Ocean Network Canada (ONC), the Ocean Observatories Initiative (OOI), the station ALOHA, the European Multidisciplinary Seafloor and water column Observatory (EMSO-ERIC), or the Biogeochemical Argo International Program have increased global-ocean observations at long time scales (more than 10 years) and high sampling frequency.'

p. 17, L518- The 'pursuit' of investigations
Answer: The section title will be changed according to the referee #1.

p. 17, L528- what about use of acrylamide gels in sediment traps, which preserve the integrity of the particle, and presumably the attached bacteria? Fecal pellets should be mentioned in this section
Answer: Acrylamide gel is efficient for the conservation of the pellets. It might be worth trying for cell conservation but will certainly alter the bioluminescence. For that reason, we decided not to add this methodology into the subsection.

p. 17, section 5.2.3- I found this section unfocused (too much of 'catch all'), and it also does not discuss vertical migration, which is mentioned in the section heading. Fecal pellets should be mentioned in this section

Answer: We will follow reviewer #2's comment and will remove this section. Two sentences will be moved into the next subsection 5.2.4, since we believe that this information, based on already existing literature, is of major importance for future investigations.

"As an example, Vibrio are important contributors to particulate organic carbon fluxes that have been observed at abyssal depths in the Pacific Ocean (Preston et al., 2019, Boeuf et al., 2019).

A better characterization at species or functional level should highlight the luminous potential related to the presence of such organisms, even at low abundance."

The description of the effects of vertical migration of zooplankton and fish on luminous bacteria dispersal will be added in part 4.4 (Figure 1, step 4), we will include the following details:

"Additionally, the consumption of organic material colonized by bioluminescent bacteria increases their dispersal rate provided by migrating zooplankton, and even more so by actively swimming fish, following the conveyor-belt hypothesis (Grossart et al., 2010) (Figure 1, step 4). After being ingested, bacteria (including luminous ones), attached to the particles consumed by zooplankton and fish, stay in their digestive tract. At night, these organisms migrate in the upper part of the water column and release feces in niches and at depth that, eventually, would not have been otherwise colonized by luminous bacteria. This dispersion, due to the expelling of luminous feces, is several orders of magnitude greater than that of water-borne free bacteria."

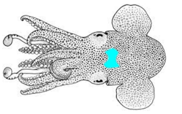
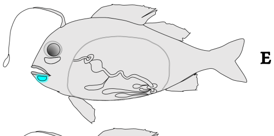
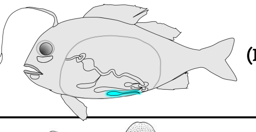
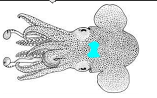
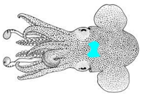
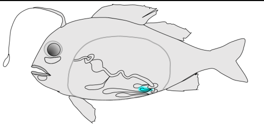
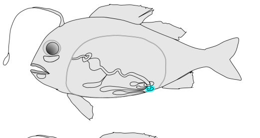
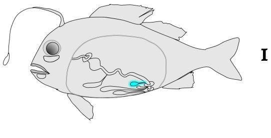
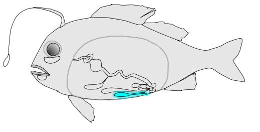
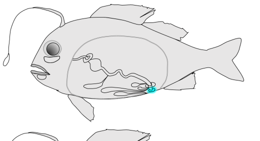
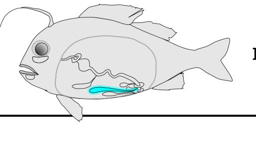
p. 18, section 5.2.4 L554- the word 'lock' needs to be replaced whole section- I thought bioluminescence in zooplankton was used mainly to startle or confuse a predator. Also, bacteria in fecal pellets should be mentioned in this section.

Answer: We will remove the word 'lock' and use "One current challenge". In this subsection we mainly described future actions to quantify the attraction rate of particles (including fecal pellets), glowing due to bioluminescent bacteria, by higher trophic levels. As the reviewer says, it is commonly admitted that bioluminescence from bacteria attracts, while flashes of light in most zooplankton deters. Here we describe the attraction of bacteria on zooplankton. We will add the sentence as follows to avoid misunderstanding and take into account the comment of the reviewer:

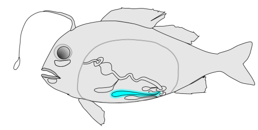
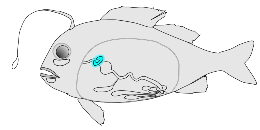
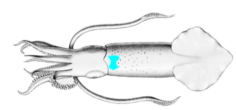
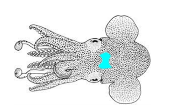
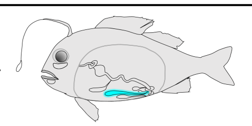
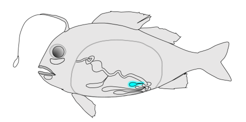
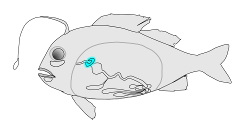
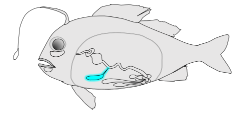
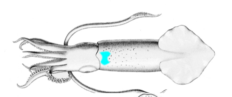
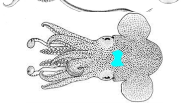
"Few studies related the preferential consumption of luminous bacteria by zooplankton (copepods in Nishida et al., 2002) or fish (Zarubin et al., 2012). It is well-known that marine snow is intensively colonized by bacteria (about 10^9 bacteria per millilitre) (Azam & Long, 2001). Amongst them, luminous bacteria attract zooplankton by emitting light continuously (while flashes of light emitted by zooplankton deter, as mentioned earlier)."

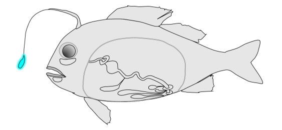
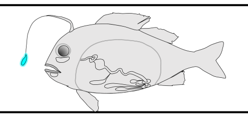
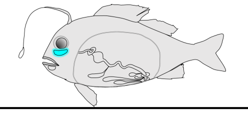
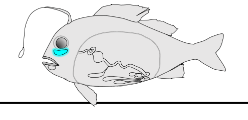
Figure 1- not clear to me why the arrow in 4 denotes slow sinking (why are particles released from vertical migrators slower than those repackaged or from sloppy feeding?)
Answer: We agree with the remark and the arrow will be corrected from dotted arrow to solid arrow.

Table 1.- Caption should specify 'in fishes and squids' (as there are also luminescent bacteria in zooplankton, which are not shown here). "List of luminous bacterial species found in light organ symbiosis in fishes and squids"
Answer: We will add 'fishes and squids' to Table 1 caption as suggested.

Species	Host Collection	Hosts	Light Organ Location
Aliivibrio fischeri (Vibrio fischeri)	***Euprymna* spp.** Western Pacific (Fidopiastis et al., 1998) ***Sepiola* spp.** Mediterranean Sea, European Atlantic coast, Japan, Philippines (Fidopiastis et al., 1998) ***Moconcentris japonica*** Japan (Dunlap et al., 2007)	SEPIOLIDAE ***Euprymna* spp.** *E. morsei* *E. berryi* *E. scolopes* *E. tasmanica* ***Sepiola* spp.** *S. affinis* *S. atlantica* *S. intermedia* *S. ligulata* *S. robusta*	 E
	Cleidopus gloriamaris East coast of Australia (Fitzgerald, 1977)	MONOCENTRIDAE ***Monocentris* spp.** *M. japonica* ***Cleidopus* spp.** *C. gloriamaris*	 E
	***Caelorinchus* spp.** Taiwan (C. *formosanus*) Japan (C. *multispinulosus*) (Dunlap et al., 2007)	MACROURIDAE ***Caelorinchus* spp.** *C. formosanus* *C. multispinulosus*	 (I)
Aliivibrio thorii	***Sepiola affinis*** Mediterranean Sea (Fidopiastis et al., 1998 ; Ast et al., 2007)	SEPIOLIDAE ***Sepiola* spp.** *S. affinis*	 E
Aliivibrio wodanis *	***Sepiola* spp.** Mediterranean Sea (Fidopiastis et al., 1998 ; Ast et al., 2007)	SEPIOLIDAE ***Sepiola* spp.** *S. affinis* *S. robusta*	 E
Photobacterium kishitanii	***Opisthoproctus* spp.** Atlantic Ocean (*O. grimaldii*) Atlantic Ocean and Indian Ocean (*O. soleatus*) (Haygood et al., 1992; Dunlap et al., 2007)	OPISTHOPROCTIDAE ***Opisthoproctus* spp.** *O. grimaldii* *O. soleatus*	 (I)
	***Chlorophthalmus* spp.** Japan (Dunlap et al., 2007)	CHLOROPHTHALMIDAE ***Chlorophthalmus* spp.** *C. acutifrons* *C. albatrossis* *C. nigromarginatus*	 (I)
	***Caelorinchus* spp.** Taiwan (*C. kishinouyei*) Japan (Other species) (Dunlap et al., 2007)	MORIDAE ***Physiculus* spp.** *P. japonicus*	 I
	Malacocephalus laevis Indian Ocean (Dunlap et al., 2007) ***Ventrifossa* spp.** Japan (*V. garmani* and *V. longibardata*) Taiwan (*V. rhidodorsalis*) (Dunlap et al., 2007) ***Physiculus japonicus*** Japan (Dunlap et al., 2007) ***Aulotrachichthys prosthemius*** Japan (Ast and Dunlap, 2004)	MACROURIDAE ***Caelorinchus* spp.** *C. anatirostris* *C. denticulatus* *C. fasciatus* *C. hubbsi* *C. japonicus* *C. kamoharai* *C. kishinouyei* ***Malacocephalus* spp.** *M. laevis* ***Ventrifossa spp.*** *V. garmani* *V. longibarbata* *V. rhipidodorsalis*	 (I)
	Acropoma hanedai Taiwan (Kaeding et al., 2007; Dunlap et al., 2007)	TRACHICHTHYIDAE ***Aulotrachichthys* spp.** *A. prosthemius*	 I
		ACROPOMATIDAE ***Acropoma spp.*** *A. hanedai*	 I

* firstly identified as *Vibrio logei* by Fidopiastis et al., 1998

Species	Host Collection	Hosts	Light Organ Location
Photobacterium leiognathi	***Acropoma japonicum*** Taiwan (Kaeding et al., 2007)	**ACROPOMATIDAE** ***Acropoma spp.*** *A.japonicum*	 I
	Gazza **spp.** Philippines (Dunlap et al., 2004, 2007) ***Leiognathus*** **spp.** Taiwan (*L. equulus*) Okinawa (*L. fasciatus*) Philippines (*L. jonesi, L. philippinus*) Japan (*L. nuchalis*) Gulf of Siam (*L. splendens*) (Dunlap et al., 2004, 2007) ***Equulites*** **spp.** Japan (*E. elongatus, E. rivulatus*) Philippines (*E. leucistus*) (Dunlap et al., 2004, 2007) ***Photopectoralis*** **spp.** Japan (*P. bindus*) Philippines (*P. panayensis*) (Kaeding et al., 2007) ***Photolateralis*** **spp.** Philippines *(P. stercorarius)* (Dunlap et al., 2007) ***Secutor*** **spp.** Philippines (Dunlap et al., 2007)	**LEIOGNATHIDAE** ***Gazza*** **spp.** *G. achlamys* *G. minuta* ***Leiognathus*** **spp.** *L. equulus* *L. fasciatus* *L. jonesi* *L. nuchalis* *L. philippinus* *L. splendens* ***Equulites*** **spp.** *E. elongatus* *E. leucistus* *E. rivulatus* ***Photopectoralis*** **spp.** *P. bindus* *P. panayensis* ***Photolateralis*** **spp.** *P. stercorarius* ***Secutor*** **spp.** *S. insidiator* *S. megalolepis*	 I
	Uroteuthis noctilus Sydney, Australia (Guerrero-Ferreira et al., 2013)	**LOLIGINIDAE** ***Uroteuthis*** **spp.** *U. noctiluca*	 E
	Rondeletiola minor Mediterranean Sea, France (Guerrero-Ferreira et al., 2013) ***Sepiolina nipponensis*** Japan (Nishiguchi and Nair, 2003)	**SEPIOLIDAE** ***Rondeletiola*** **spp.** *R. minor* ***Sepiolina*** **spp.** *S. nipponensis*	 E
Photobacterium mandapamensis	***Acropoma japonicum*** Taiwan (Kaeding et al., 2007)	**ACROPOMATIDAE** ***Acropoma spp.*** *A. japonicum*	 I
	Gadella jordani Taiwan (Kaeding et al., 2007)	**MORIDAE** ***Gadella spp.*** *G. jordani*	 I
	Photopectoralis **spp.** Japan (*P. bindus*) Philippines (*P. panayensis*) (Kaeding et al., 2007)	**LEIOGNATHIDAE** ***Photopectoralis spp.*** *P. bindus* *P. panayensis*	 I
	Siphamia versicolor Japan (Kaeding et al., 2007)	**APOGONIDAE** ***Siphamia*** **spp.** *S. versicolor*	 I
Vibrio harveyi	***Uroteuthis chinensis*** Thailand (Guerrero-Ferreira et al., 2013)	**LOLIGINIDAE** ***Uroteuthis*** **spp.** *U. chinensis*	 E
	Euprymna hyllbergi Thailand (Guerrero-Ferreira et al., 2013)	**SEPIOLIDAE** ***Euprymna*** **spp.** *E. hyllebergi*	 E

Species	Host Collection	Hosts	Light Organ Location
Candidatus Enterovibrio escacola	***Ceratias* spp.** NE Atlantic (C. sp) Gulf of Mexico (*C. uranoscopus*) ***Lynophryne maderensis*** NE Atlantic ***Melanocetus johnsoni*** Gulf of Mexico and NE Atlantic ***Melanocestus murrayi*** Gulf of Mexico ***Chaenophryne* spp.** NE Atlantic ***Oneirodes* sp.** Gulf of Mexico (Baker et al., 2019)	**CERATIIDAE** ***Ceratias* spp.** *C. uranoscopus* *C.* sp **LINOPHRYNIDAE** ***Linophryne* spp.** *L. maderensis* **MELANOCETIDAE** ***Melanocetus* spp.** *M. johnsoni* *M. murrayi* **ONEIRODIDAE** ***Chaenophryne* spp.** *C. longiceps* *C.* sp ***Oneirodes* spp.** *O.* sp	 **E**
Candidatus Enterovibrio luxaltus	***Cryptopsaras couesii*** Gulf of Mexico and NE Atlantic (Baker et al., 2019)	**CERATIIDAE** ***Cryptopsaras* spp.** *C. couesii*	 **E**
Candidatus Photodesmus blepharus	***Photoblepharon* spp.** Pacific Ocean *(P. palpebratus)* Western Indian Ocean (*P. steinitzi)* (Hendry and Dunlap, 2014)	**ANOMALOPIDAE** ***Photoblepharon* spp.** *P. palpebratus* *P. steinitzi*	 **E**
Candidatus Photodesmus katoptron	***Anomalops* spp.** Philippines (Hendry and Dunlap, 2011)	**ANOMALOPIDAE** ***Anomalops* spp.** *A. katoptron*	 **E**