The manuscript "Microbial community analysis in the gills of coastal shellfish and molecular identification of the potentially dominant epsilonproteobacterium on the gills of *Haliotis gigantea"* presents a relevant and interesting study, describing the gill bacterial composition of 4 species of gastropds (*Haliotis gigantea, H. discus, H. diversicolor and Turbo cornutus*) and 2 species of molluscs species (*Meretrix lusoria* and *Cyclina sinensis*) using high throughput sequencing, cloning-sequencing and microscopic methods. Besides, the authors revealed the evidence of a specific taxon of uncultivated *Epsilonproteobacteria* closely affiliated to *Helicobacteraceae* family, using specific primers designed for this purpose, in the gill of the abalone *Haliotis gigantea*
The manuscript flow well and it well written
However, there is several main points, that should be added or clarified before the paper is recommended for publication

Major comments:
The manuscript will really gain in clarity if the main aims of this study were clearly described in the introduction: why studying these specific species, why comparing molluscs and gastropods Which scientific gaps this study ambitions to explore. That will really help the readers to understand the study and its rationale.
Also, the authors should highlight why they did a specific focus on the uncultured *Epsilonproteobacteria* detection in different tissues of *Haliotis gigantea.* Is this specific taxon also present in the others abalone gills (*Haliotis discus* and *H. diversicolor*)? Did the authors checked that using the specific primers that they designed for the uncultivated *Epsilonproteobacteria* detected in *Haliotis gigantea*?

The manuscript: discussion and conclusion mostly, will gain in relevance if the authors dig deeper in the analysis of the gill microbiome, meaning not only the classes but maybe until the family or genus level when possible. For instance, the histogram shows only the classes with a relative abundance >10% and in the text, only few genera are highlighted as *Vibrio, Arcobacter*. Then, for example, I wonder what are their relative abundance within the *Gammaproteobacteria* and *Epsilonproteobacteria* respectively.

The manuscript will improve a lot if the authors can highlight both the core microbiome and variable microbiome, up to the genus level, in the gills of *Haliotis gigantea* itself and in *H. diversicolor* and compare and point out some similarity or dissimilarity among the core microbiote of these 2 species belonging to the same genus.
See for example: Neu, Alexander T., Eric E. Allen, and Kaustuv Roy. "Diversity and composition of intertidal gastropod microbiomes across a major marine biogeographic boundary." *Environmental microbiology reports* 11.3 (2019): 434-447.
Reich, Inga, et al. "16S rRNA sequencing reveals likely beneficial core microbes within faecal samples of the EU protected slug Geomalacus maculosus." *Scientific reports* 8.1 (2018): 1-9.
Hernandez-Agreda, Alejandra, Ruth D. Gates, and Tracy D. Ainsworth. "Defining the core microbiome in corals' microbial soup." *Trends in Microbiology* 25.2 (2017): 125-140.

The material and method need some clarifications:
Does sample Hgig1 corresponds to the pool of 5 specimen collected at the farm? Not clear
"Other abalone specimens (*H. gigantea, H. discus and H. diversicolor*) and *Turbo cornutus* were obtained from a fish-market in Mie, Japan, from April 2017 to May 2019": Any idea of where they come from? Where had they been collected?
"Gill tissues from *H. discus* (n=3), *H. diversicolor* (n=5), and *T. cornutus* (n=3) specimens were pooled into three tubes, one for each species (sample code: Hdis1, Hdiv1, and Tcor1)": Can you explain why you have decided to pool the gill of each specie together?

H. discus specimens corresponding to samples Hdis2-4: I Can't reckon where come from the sample 1 for H Discus? Line 100 H. discus n=3; line 103 sample name Hdis2-4 so no Hdis1 in the manuscript BUT in graph yes there is a sample Hdis 1 and in NCBI as well. Here some explanations are missing.
"Seawater and stone samples were collected from Minami-ise" Some indications are missing as the sampling point coordinates, when have they been collected, Which year? How have they have been collected Are these samples been collected close to the Farming Fishery Center, from the Farming Fishery Center?
Also, line 369: "sediments smelt …"why did you investigated the microbial diversity in the stones and not in the sediment and to look for potential symbionts inside?

Obtaining the 16S rRNA gene sequences of the uncultured epsilonproteobacterium by cloning:

"The phylogenetic tree of representative members of Epsilonproteobacteria inferred from 16S rRNA gene sequences was estimated by the Maximum Likelihood method using MEGA 7.0 (Kumar et al. 2016)." That's not clear, I get that the authors wanted to obtain the almost full length of 16S rRNA gene in order to get a fair idea of the affiliation and taxonomy of this uncultured epsilonproteobacterium taxon but here I missed a link because the authors used universal primers that target all the *Bacteria* and not only the epsilonproteobacteria. Also, why only the epsilonproteobacteria are in the phylogenetic tree? How did the authors checked that send only Epsilonproteobactria clones to be sequenced?

Nested PCR analysis and sequencing of the uncultured epsilonproteobacterium:

"The nested PCR assay targeted 356 bp of the uncultured epsilonproteobacterial 16S rRNA gene using bacterial universal primers 27F and 1492R, as well as the uncultured epsilonproteobacterium-specific primers, Eps222F (5'-CGCTAAGAGATTGGACTATAT-3') and Eps578R (5'-GACTTAATAGGACACCTACATACC-3') (designed in this study)." Here the sentence is not clear, does that mean that the authors have used the same PCR products as for the cloning-sequencing? About the Eps222F/ Eps578R primers, more precisions are needed: are these primers been design for the NGS data, from the almost full length 16S rRNA gene? Specificity of the primers?
"Sequencing and phylogenetic analysis were performed as previously described in the subsection "Obtaining the 16S rRNA gene sequences of the uncultured epsilonproteobacterium using cloning method" above" then, why only one sequence of 1436 pb has been deposited in GenBank (LC511979.1). There is no accession number for the nested PCR data.
"Four out of sixteen cloned sequences were closely related to the sequence of the uncultured epsilonproteobacterium obtained from the 16S rRNA gene amplicon sequencing undertaken in this study. The longest sequence length was 1436 bp (LC511979)." How many clones have been sequenced? How did the authors checked they send only epsilonproteobacteria clones to be sequenced? Why only 16 sequences: it's very few especially if the nested PCR have been done from the DNA from the gills of the 5 organisms? If the sequences were closely related, what is the percentage of similarity? Why only 1 accession number? Why only 1 sequence in the phylogenetic tree? How many clones did the authors managed to obtain per Hgig sample?

Minor comments:

Abstract:

-"Recently, it has been reported that chemosynthetic bacteria in the gills of some shallow-water bivalves have the ability to fix nitrogen carbon, and synthesize amino acids for their hosts." This sentence is not useful as the study is not dealing with the metabolic activities of the symbionts and neither on which compounds the symbionts can supply to their hosts.
- "Microbiome analysis suggested that the gills of *H. gigantea*, *M. lusoria* and *C. sinensis* each have unique bacterial community structures that differ from those in the surrounding environment"; add detected between differ and those in the surrounding… In addition, some results about the microbial communities inhabiting the gills of *Haliotis* discus, *H. diversicolor and Turbo cornutus* would be appreciated.
-Few words about the core and variable microbiome within *Haliotis gigantea and H. diversicolor* will improve a lot the abstract.

Introduction:
The introduction described well the state of the art of the gills' symbiont topic with relevant and right amount of references but in my opinion, focuses too much on deep sea hydrothermal or cold seep systems, even if the comparison between shallow and deep sea on lines 82-86 is well appropriated.
From line 67 to 71, on the Solemyidae description, it could be good to add few references about the symbiotic sulfur-oxidizing symbionts and to link that with the Thyasiridae description:
Dmytrenko, Oleg, et al. "The genome of the intracellular bacterium of the coastal bivalve, Solemya velum: a blueprint for thriving in and out of symbiosis." *BMC genomics* 15.1 (2014): 924 ; see also other papers
Also, the authors described the Solemyidae and the Thyasiridae and their symbionts' roles but then, somehow it felt like the description of the species used in this study is lacking. Indeed, several information about their life's styles, previous evidences of symbiont in the gill of specimens of the same family etc… will

Results:
"Greengenes database": Which version

"These sequences were observed in all abalone specimens, but were not dominant, except in Hgig5 (68.4%).", any idea why Hgig 5 is so different from the others

"PCoA": Utilization of which software, R? Need to be described in the Materials & Methods

Discrepancy between the text and the phylogenetic tree: with the tree lines: 276-279 "BLAST analysis of the uncultured epsilonproteobacterial sequence revealed that the sequence could be assigned to various described Epsilonproteobacteria species, such as *Arcobacter canalis* strain F138-33 (87.43% identity), *Arcobacter marinus* strain CL-S1 (87.19% identity), *Sulfurovum lithotrophicum* strain 42BKT (87.01% identity) and *Helicobacter pullorum* strain ATCC51801 (86.78% identity), but these identity scores were low" BUT in the phylogenetic tree (Figure 2), clone Hgig1 is closest to *Sulfurimonas autotrophica* and *Sulfuricurvum kujiense* and bit further from *Arcobacter nitrofigilis.* That differs from the manuscript. Also, why not using *Arcobacter canalis, Arcobacter marinus, Sulfurovum lithotrophicum, Helicobacter pullorum* to do the phylogenetic tree??

Discussion:
"This assumption was also supported by the finding that the gills of Hgig6 gave a clearer band than the gills of Hgig4 in the PCR assay using the same concentration of DNA template." qPCR is needed to infer the number of representatives of this specific taxon in each gill' abalone.

References:
Newton ILG, et al., is followed by Katoh K et all and Kuwahara H, et al is followed by On S et al., : Check this order.

Wickham, 2016 in the manuscript while it's 2009 in the ref Wickham H. 2009. ggplot2: Elegant Graphics for Data Analysis. New York: Springer. DOI: 10.1007/978-0-387-98141-3 in the reference list

Figure 2: species name in italic