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研究課題名(和文) Exploring the unknown biodiversity of apicomplexan parasites (Apicomplexa) in bivalve shellfish (Mollusca) in Japan

研究課題名(英文) Exploring the unknown biodiversity of apicomplexan parasites (Apicomplexa) in bivalve shellfish (Mollusca) in Japan

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研究成果の概要(和文)：私たちは北海道からホタテ貝、カキ、ムール貝を採取し、生物多様性と貝類と寄生虫の関係を研究しました。私たちが研究した寄生虫は、繊毛虫とアピコンプレックス寄生虫です。、ヨーロッパの組織学的セクションでのみ知られている繊毛虫。これまで日本では報告されたことのない寄生虫たち。私たちはそれらの DNA を単離し、分子系統図を作成しました。

研究成果の学術的意義や社会的意義

This research was some of the first molecular work generated from parasites of shellfish (bivalves) in Japan. Here we were able to link traditional histological observations to genetic data. We also made several reports of parasitic species that are newly reported in Japan.

研究成果の概要(英文)：For this project, we collected 40 blue mussels, 40 Pacific Oysters and a total of 180 Hokkaido Scallop, from Hokkaido, Japan. Generally, samples were processed for histology, genomic DNA isolation, and also prepared for laser dissection microscopy. We started with the processing of scallop because we had more samples of those. We were able to amplified 18S rDNA and also conduct phylogenetic analyses in order to assess relationships of these parasites compared to samples collected in previous studies around the world. As a results, we found two ciliates, *Tichodina pectenis* and *Ancistrum* sp. We also were able to isolate and identify an apicomplexan parasite, *Merocystis kathae*. This was the first report of all three of these organisms in Japan. For each of these organisms, we were able to gather histology and amplify 18S rDNA. We are currently processing the blue mussels and pacific oysters.

研究分野：Protistology

キーワード：Alveolates Apicomplexans Ciliates Phylogeny

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様式 C-19、F-19-1、Z-19 (共通)

1. 研究開始当初の背景

Harmful apicomplexans infect invertebrates, and they are also in the ocean.

The vast majority of knowledge and research on apicomplexans is based on parasites that harm humans and livestock such as *Plasmodium* and *Toxoplasma*, two pathogens responsible for malaria and toxoplasmosis, respectively. *Plasmodium* and *Toxoplasma*, together with the blood-parasitizing piroplasmids (e.g., *Babesia*) are often referred to as the “core apicomplexans”, due to their harmful nature and relevance to humans. However, the largest diversity of apicomplexans, by far, infects invertebrates such as worms, insects, and even molluscs (shellfish), particularly in the ocean, where there is a high diversity of invertebrates. These marine apicomplexans have been largely understudied because their significance and impact on human health and the economy is not as obvious.

2. 研究の目的

Core apicomplexans in bivalve shellfish: widespread occurrences and negative implications.

The first descriptions of apicomplexans in bivalve shellfish were reported in 1897 from *Tellina* sp. and *Donax* sp. Since this time others have been described from a number of bivalves around the world, even in Japan. Infection stages (sporozoites/cysts) and feeding stages (trophozoites) infect various tissue layers and organs. Some of these parasites have been blamed for massive (whole stock) die-offs in scallop fisheries in Europe; others have been implicated for damaging the economic value of mussels and oyster aquaculture in New Zealand. In each of these cases, it is hypothesized that the apicomplexans were damaging the tissues and organs of the host bivalves beyond repair, resulting in decreased size of host individuals and reproductive fitness of the population.

More information on apicomplexans in shellfish is needed to assess their diversity and impacts.

While it is true that apicomplexans have been commonly found in shellfish, the fact remains that very little information is really known about these parasites. Basic questions about their diversity are unclear, as molecular data only exists for a few species throughout the whole world; the vast majority of descriptions are based only on histological data; meaning that the true diversity and distribution of these parasites is likely misjudged. Importantly, potential causality issues exist because many of the studies that have implicated apicomplexans as being the causal agent for disease have only examined diseased (dead or dying) stocks and did not examine healthy populations of the same species, or other species of shellfish that may contain the same parasite(s). Therefore, this field of study would benefit from a comprehensive approach that combines a broader sampling scheme, as well as histological and molecular methodologies, taking advantage of what both techniques have to offer.

3. 研究の方法

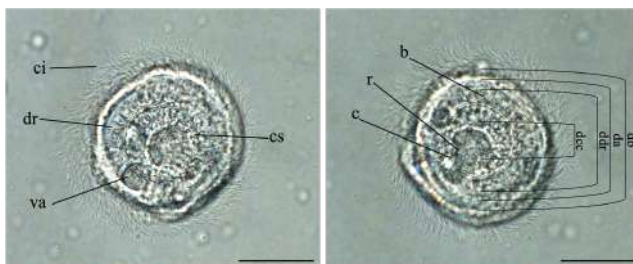
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histology, genomic DNA isolation, and also prepared for laser dissection microscopy. We started with the processing of scallop because we had more samples of those. We were able to amplified 18S rDNA and also conduct phylogenetic analyses in order to assess relationships of these parasites compared to samples collected in previous studies around the world. As a results, we found two ciliates, *Tichodina pectenis* and *Ancistrum* sp. We also were able to isolate and identify an apicomplexan parasite, *Merocystis kathae*. This was the first report of all three of these organisms in Japan. For each of these organisms, we were able to gather histology and amplify 18S rDNA. We were able to amplify 700 bp of the samples with the laser dissection microscope, but only under certain conditions using specific primers and when the PCR product was short. We also made an interesting discovery of a ciliate, MPX, that to date has only been reported in Europe. We are currently processing the blue mussels and pacific oysters.

4. 研究成果

Examining the biodiversity of alveolate in Akkeshi Scallop:

In this study, we found examples of Ciliates within the genus *Tichodina pectenis* and *Ancistrum* sp. We also found examples of *Merocystis kathae*, an apicomplexan parasites. This is the first time that these species have been observe and sequenced from Japanese Scallop.



Tichodina pectenis



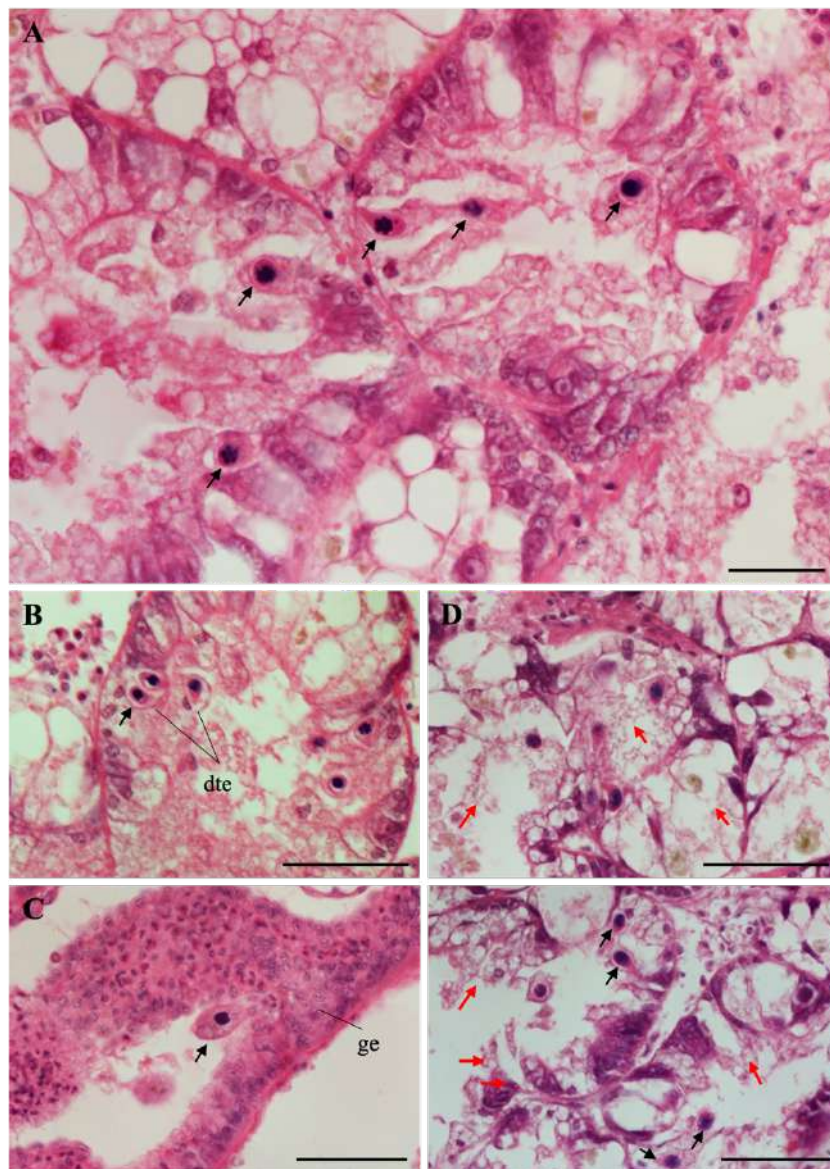
Ancistrum sp.

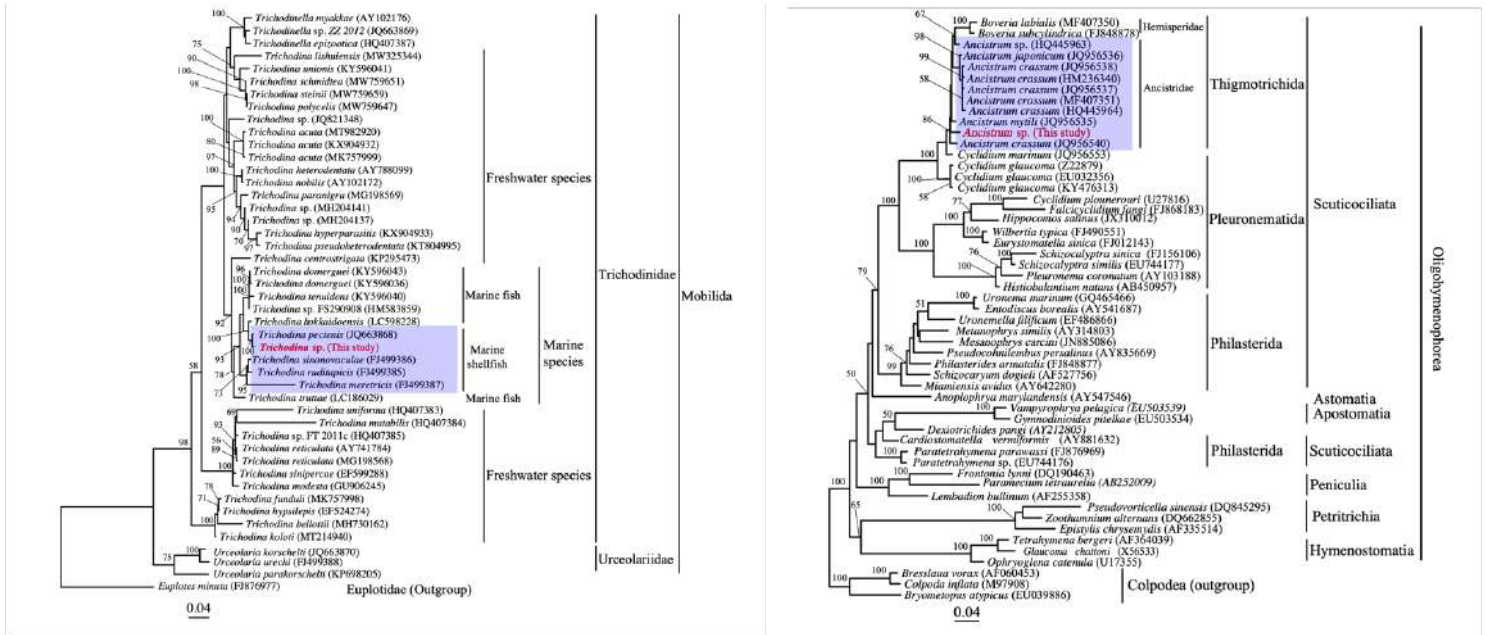
Observation of MPX: first report from Japanese scallop

We also observed and reported, for the first time in Japan, MPX. This ciliate is a pathogen of scallops in Europe. It is unknown how harmful this parasite is. Our future work will be examining this parasite.



Merocystis kathae (Above) and Histotlogical sections of MPX (Below).





Phylogeny of *Tichidina* sp. based on 18S rDNA (Top left); *Ancistrum* sp. 18S rDNA (Top right); and *Merocystis kathae* (Bottom)

Phylogenetic analysis of alveolate pathogens:

In this study, we also collected the first genetic data for several alveolate from scallop in Japan. Below are the phylogenetic trees. It is likely that these are not necessarily genetically distinct from other pathogens found in other regions of the world (e.g., Europe). This result is interesting because it suggests that these species are either 1) not reproductively isolated from each other, or 2) have only recently been introduced to either Japan, or other parts of the world. Future work will examine the distribution and connection between these parasites and other shellfish species.

5. 主な発表論文等

〔雑誌論文〕 計7件（うち査読付論文 7件/うち国際共著 6件/うちオープンアクセス 2件）

1. 著者名 Kevin Wakeman	4. 巻 171
2. 論文標題 Molecular Phylogeny of Marine Gregarines (Apicomplexa) from the Sea of Japan and the Northwest Pacific Including the Description of Three Novel Species of <i>Selenidium</i> and <i>Trollidium akkeshiense</i> n. gen. n. sp.	5. 発行年 2020年
3. 雑誌名 Protist	6. 最初と最後の頁 125710
掲載論文のDOI (デジタルオブジェクト識別子) 10.1016/j.protis.2019.125710	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 -
1. 著者名 Mana Yamamoto, Kevin Wakeman, Takeo Horiguchi	4. 巻 59
2. 論文標題 Molecular phylogeny and ultrastructure of two novel parasitic dinoflagellates, <i>Haplozoon gracile</i> sp. nov. and <i>H. pugnus</i> sp. nov.	5. 発行年 2020年
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掲載論文のDOI (デジタルオブジェクト識別子) 10.1080/00318884.2020.1753427	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する
1. 著者名 Cano et al., (26 authors)	4. 巻 11
2. 論文標題 Cosmopolitan Distribution of Endozoicomonas-Like Organisms and Other Intracellular Microcolonies of Bacteria Causing Infection in Marine Mollusks	5. 発行年 2020年
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1. 著者名 Varsha Mathur, Kevin Wakeman, Patrick Keeling	4. 巻 31
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1. 著者名 Kevin Wakeman, Shimpei Hiruta, Yosuke Kondo, Susumu Ohtsuka	4. 巻 172
2. 論文標題 Evidence for Host Jumping and Diversification of Marine Cephaloidophorid Gregarines (Apicomplexa) Between Two Distantly Related Animals, viz., Crustaceans and Salps	5. 発行年 2021年
3. 雑誌名 Protist	6. 最初と最後の頁 125822
掲載論文のDOI (デジタルオブジェクト識別子) 10.1016/j.protis.2021.125822	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

1. 著者名 Yong Heng Phua, Michael C. Roy, Sarah Lemer, Filip Husnik, Kevin Wakeman	4. 巻 109
2. 論文標題 Diversity and toxicity of Pacific strains of the benthic dinoflagellate <i>Coolia</i> (Dinophyceae), with a look at the <i>Coolia canariensis</i> species complex	5. 発行年 2021年
3. 雑誌名 Harmful Algae	6. 最初と最後の頁 102120
掲載論文のDOI (デジタルオブジェクト識別子) 10.1016/j.hal.2021.102120	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

1. 著者名 Koh Yokouchi, Davis Iritani, Kay Hian Lim, Yong Heng Phua, Takeo Horiguchi, Kevin Wakeman	4. 巻 173
2. 論文標題 Description of an Enigmatic Alveolate, <i>Platyproteum noduliferae</i> n. sp., and Reconstruction of its Flagellar Apparatus	5. 発行年 2022年
3. 雑誌名 Protist	6. 最初と最後の頁 125878
掲載論文のDOI (デジタルオブジェクト識別子) 10.1016/j.protis.2022.125878	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕 計2件（うち招待講演 2件 / うち国際学会 0件）

1. 発表者名 Kevin Wakeman
2. 発表標題 Alveolate parasites of shellfish
3. 学会等名 HARP seminar between Russia and Japan (招待講演)
4. 発表年 2021年～2022年

1. 発表者名 Kevin Wakeman
2. 発表標題 Diversity of alveolate parasites
3. 学会等名 Dinophyte conference (招待講演)
4. 発表年 2022年

〔図書〕 計0件

〔産業財産権〕

〔その他〕

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6. 研究組織

氏名 (ローマ字氏名) (研究者番号)	所属研究機関・部局・職 (機関番号)	備考

7. 科研費を使用して開催した国際研究集会

〔国際研究集会〕 計0件

8. 本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関