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研究課題名（和文）Cellular identity, systematics and biogeography of apicomplexan parasites Genotype-N and Gemmocystis infecting coral reefs

研究課題名（英文）Cellular identity, systematics and biogeography of apicomplexan parasites Genotype-N and Gemmocystis infecting coral reefs

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研究成果の概要（和文）：この作業は、サンゴ礁に関連するアピコンプレックス門のサンプリングに焦点を当てました。サンプルは沖縄から、そして石垣、鹿児島、広島を含む南日本周辺の場所で収集されました。 18S rDNAを使用して、アピコンプレックス門の組織学およびPCRスクリーニングのためにサンプルを処理しました。これらのサンプルに関連するアピコンプレックス門の多様性をよりよく理解するために、サンプルは次世代シーケンシング用にも準備されました。合計80種以上のサンゴがサンプリングされ、シーケンスに送られました。

研究成果の学術的意義や社会的意義
社会的および科学的影響から、これらの結果はサンゴの生物学を理解するために重要であり、生物が寄生虫と持つ相互作用についての見通しを得るための最初のステップのいくつかです。言い換えれば、この作業はサンゴ礁の健康をよりよく管理するのに役立ちます。この研究からの重要な派生物の1つは、微生物叢と、これが特に気候変動時にサンゴの健康にどのように影響するかに関する研究です。健康なサンゴ微生物叢を構成するものを理解することは、サンゴの白化現象の予測因子となり、その影響を制限するのに役立つ可能性があります

研究成果の概要（英文）：This work focused on sampling apicomplexans associated with coral reefs. Samples were collected from Okinawa and in locations around Southern Japan, including Ishigaki, Kagoshima and Hiroshima. Samples were processed for histology and PCR screening of apicomplexans using the 18S rDNA. Samples were also prepared for next generation sequencing, in order to gain a better understanding of the diversity of apicomplexans associated with these samples. In total over 80 species of coral were sampled and sent for sequencing. As a general result, we found that apicomplexans that associate with coral are broadly distributed, geographically and throughout coral reefs. To this end, coral in Japan have similar communities of apicomplexans to those found in other localities such as the Caribbean. While similar, these species are likely different, and appear to be unique to the waters around Japan.

研究分野：Biodiversity

キーワード：Biology　Coral reefs　Parasitology

１．研究開始当初の背景

I. Background

A. Core Apicomplexans are important parasites of vertebrates (including humans)

Apicomplexans are notorious for parasitizing vertebrates, infecting the gut, tissue, and blood of their hosts. The causative agent of malaria, which is still one of the most deadly and infamous human pathogens, is the haemosporidian, *Plasmodium*. Haemosporidians (e.g., *Plasmodium*), coccidians (e.g., *Sarcocystis* and *Toxoplasma*) and piroplasmids (e.g., *Babesia*) compose what is referred to as "core apicomplexans", due to their severe impact on their hosts. Infections, and especially coccidian infections, are far more harmful (even deadly) to hosts with weakened immune systems; an example of this in humans is AIDS patients.

B. Genotype-N: Genetic evidence for a coral-associated core apicomplexan !

Genetic evidence (18S rDNA) for a group of apicomplexans infecting hard and soft coral was initially reported by Toller et al., (2002) (Fig 1). These novel sequences have gained attention due to their close relationship to core apicomplexans like the coccidians, *Sarcocystis* and *Toxoplasma* (Kirk et al., 2013). The cellular identity of these Genotype-N sequences remains unknown. Genotype-N has only been sequenced from coral tissue, suggesting a tight relationship between Genotype-N and coral.

C. *Gemmocystis*: A poorly studied apicomplexan lineage described in coral

In 1986, a novel coccidian-like apicomplexan, *Gemmocystis cylindrus*, was described from the gastric cavities of six genera of Caribbean hard coral (Cnidaria, Anthozoa) (Upton and Peters 1986) (Fig 2). Researchers speculate that the molecular signature of Genotype-N belongs to *Gemmocystis*, although the connection has never been shown. Still, no further work on *Gemmocystis'* geographic distribution, or its diversity across coral lineages has been reported.

２．研究の目的

D. Potential impact of apicomplexans on coral health

No study has assessed the relationship between apicomplexans and coral. Transcriptomic data showed that coral under thermal-stress or which lost their symbiotic algae (bleached) had significantly weakened immune systems, making it weak to pathogens and diseases (Pinzón et al., 2014) (Fig 3). Interestingly, genetic evidence has shown that Genotype-N is still associated with bleached coral (Kirk et al., 2013). Under such conditions, apicomplexans likely severely impact coral fitness, and as bleaching events become more widespread with climate change, it's probable apicomplexans will play an important part in the health and survivability of coral reef ecosystems.

A. Preliminary work on *Gemmocystis* and Genotype-N infecting coral from Okinawa, Japan

From September 2012 – January 2013, I travelled to Okinawa, Japan with the goal of finding early ancestors of core apicomplexans parasitizing marine invertebrates (Wakeman et al., 2014). During this time, I also looked for *Gemmocystis* and (genetic) evidence for Genotype-N in coral. I was able to consistently identify *Gemmocystis* from histological sections of hard coral and soft coral. The 18S rDNA of Genotype-N was also amplified from these same coral. Nonetheless, I lacked the time and resources to conclusively link the genetics of Genotype-N to the morphology of *Gemmocystis*, or to study its lifecycle, diversity, and geographic distribution. Still, it is a very promising preliminary finding that these apicomplexan parasites can be reliably found in a wide range of coral in Okinawa, Japan.

B. Summary of the proposed project goals

- **Provide a cellular identity for Genotype-N,** and to understand if the 18S rDNA from Genotype-N belongs to *Gemmocystis*, or alternatively, represents a different coral-associated apicomplexan lineage.
- Understand the **geographic distribution** of *Gemmocystis*/Genotype-N by sampling the diversity of hard coral (Hexacorallia) and soft coral (Octocorallia) in locations in Japan's Southern Pacific Ocean.

• Use genetics and histology to study the **biodiversity of *Gemmocystis*/Genotype-N**, specifically **host-specificity**, and to assess **parasite-host interactions and relationships** (e.g. pathenogicity).

３．研究の方法

A. Collection and fixation coral from Okinawa, Japan
Acropora digitifera (Hexacorallia, hard coral) and *Sinularia lochmodes* (Octocorallia, soft coral) will be sampled while snorkeling and SCUBA diving
at Odo, Onna, Sesoko, Ginowan, and Teniya on the island of Okinawa, Japan (Fig 4). *A. digitifera* and *S. lochmodes* will be used in this part of the study due to their relative abundance and wide distribution, and because protocols for performing histological embedding and *in situ* hybridization work on these lineages has already been establish (Ainsworth and Hoegh-Guldberg 2009). Coral tissue from both lineages measuring approximately $2cm^2$ will be fixed in 100% ethanol or 4% paraformaldehyde, for molecular work or histological
work, respectively (Fig 5).

B. Molecular work

Genomic DNA will be isolated from ethanol-fixed coral. Genotype-N 18S rDNA will the amplified and sequenced using specific primers (Toller et al., 2002), and PCR products will be cloned into vectors, in order to identify specific genotypes. The relationships between Genotype-N/*Gemmocystis* sequences produced in this study, and those from previous work in the Caribbean and in temperate locations will be compared using phylogenetic analyses. Unique regions of the 18S rDNA will be used to design probes specific to Genotype-N for use in fluorescent *in situ* hybridization (FISH).

C. Histological work

Coral will be initially fixed for 12 hours in paraformaldehyde and decalcified using acidic acid or EGTA (for *in situ* work). Tissue will then be embedded
in paraffin wax. Wax-tissue blocks will be sectioned to a thickness of 4μm using a LeicaRM2135 rotary microtome. Sections will be prepared for florescent *in situ* hybridization (FISH) or stained with hematoxylin and eosin.

D. Determining the biogeographic distribution and diversity of Genotype-N/*Gemmocystis*
Species representing the orders within Hexacorallia (Ceriantharia, Actiniaria, Antipatharia, Corallimorpharia, Scleractinia, and Zoantharia) and Octocorallia (Alcyonacea, Helioporacea, and Pennatulacea) will be collected while SCUBA diving and snorkeling in locations around Japan known to have a diversity of coral including Okinawa, Kagoshima, Hiroshima, Kochi, and Shimoda (Fig. 6).

Okinawa contains a more diverse range of coral lineages, and so sampling around this island will be more extensive. Nonetheless, coral lineages do exist in other parts of Japan, and collection at these locations would be fundamentally important to understanding the distribution of this parasite in Japan. For sampling, pieces of coral ($2cm^2$) will be collected and fixed in ethanol or paraformaldehyde. Once fixed, the samples will be shipped via currier to Hokkaido University for further molecular and histological work in the lab.

E. Molecular phylogenetics and histological techniques

Coral samples will be processed and fixed for DNA extraction and PCR amplification of 18S rDNA of *Gemmocystis*/Genotype-N using specific primers. Samples will also be prepared for hematoxylin and eosin staining following a similar protocol established in FY2017. Collecting molecular and histological data from multiple coral species will allow us to report on host-parasite specificity, and to what degree the parasites interact or influence the host, as well as their pathenogicity.

４．研究成果

This work focused on sampling apicomplexans associated with coral reefs. Samples were collected from Okinawa and in locations around Southern Japan, including Ishigaki, Kagoshima and Hiroshima. Samples were processed for histology and PCR screening of apicomplexans using the 18S rDNA. Samples were also prepared for next generation sequencing, in order to gain a better understanding of the diversity of apicomplexans associated with these samples. In total over 80 species of coral were sampled and sent for sequencing. As a general result, we found that apicomplexans that associate with coral are broadly distributed, geographically and throughout coral reefs. To this end, coral in Japan have similar communities of apicomplexans to those found in other localities such as the Caribbean. While similar, these species are likely different, and appear to be unique to the waters around Japan.

Below are some of the results of the histological work and the genetic sequencing. These photos are from Ocampo et al. 2019 ‘A universal set of primers to study animal associated microeukaryotic communities’.

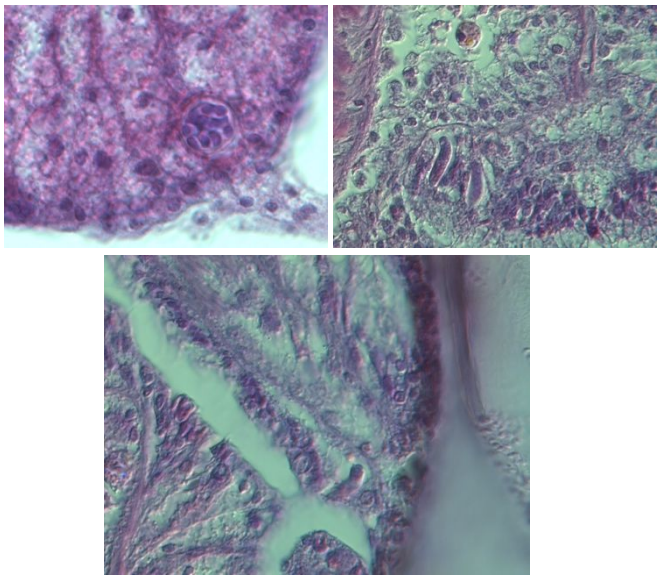

Figure 1. Histology performed on soft coral (octocoralia) showing cysts and zoit stages of apicomplexans.

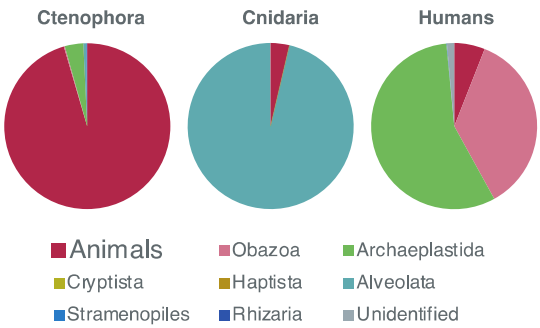

Figure 2. Metagenomic reads comparing the types of sequences attained using a universal set of primers.

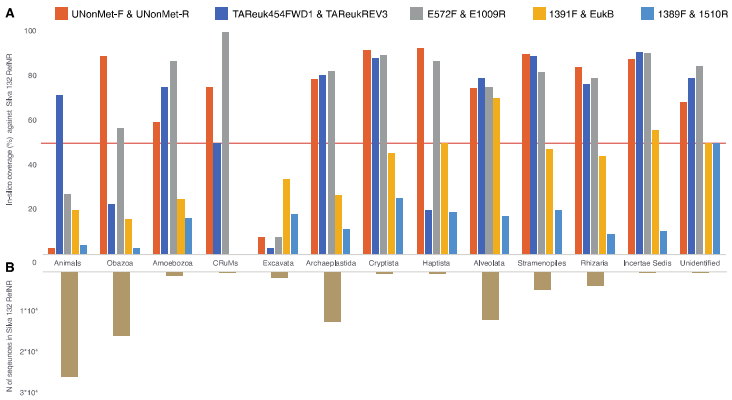

Figure 3. Comparison of general eukaryotic primers on metagenomic data output.

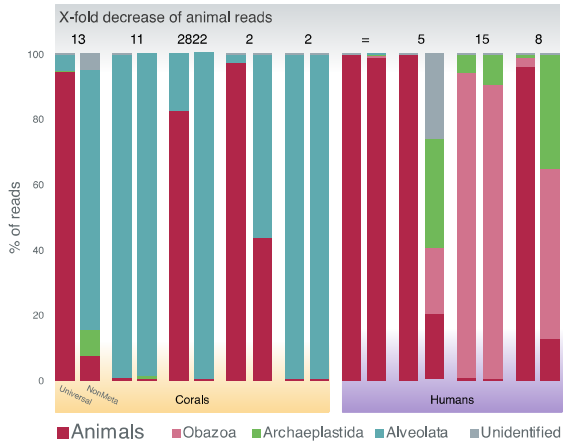

Figure 4. Comparison of primers and the results looking at metazoan read and microeukaryotic reads.

５．主な発表論文等

〔雑誌論文〕　計10件（うち査読付論文　8件／うち国際共著　8件／うちオープンアクセス　1件）

１．著者名 LEC Siaden, KC Wakeman, SC Webb, K Hasegawa, H Kajihara	４．巻 4551
２．論文標題 Morphological and molecular diversity of rissoellids (Mollusca, Gastropoda, Heterobranchia) from the Northwest Pacific island of Hokkaido, Japan.	５．発行年 2019年
３．雑誌名 Zootaxa	６．最初と最後の頁 415-431
掲載論文のDOI（デジタルオブジェクト識別子） 10.11646/zootaxa.4551.4.2.	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 GS Gavelis, M Herranz, KC Wakeman, C Ripken, S Mitarai, GH Gile, ...	４．巻 9
２．論文標題 Dinoflagellate nucleus contains an extensive endomembrane network, the nuclear net	５．発行年 2019年
３．雑誌名 Scientific reports	６．最初と最後の頁 839
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41598-018-37065-w	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 NT Suong, JC Banks, A Fidler, A Jeffs, KC Wakeman, S Webb	４．巻 132
２．論文標題 PCR and histology identify new bivalve hosts of Apicomplexan-X (APX), a common parasite of the New Zealand flat oyster Ostrea chilensis	５．発行年 2019年
３．雑誌名 Diseases of Aquatic Organisms	６．最初と最後の頁 181-189
掲載論文のDOI（デジタルオブジェクト識別子） 10.3354/dao03318	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 A Yamaguchi, KC Wakeman, M Hoppenrath, T Horiguchi, H Kawai	４．巻 57
２．論文標題 Molecular phylogeny of the benthic dinoflagellate Cabra matta (Dinophyceae) from Okinawa, Japan	５．発行年 2018年
３．雑誌名 Phycologia	６．最初と最後の頁 630-640
掲載論文のDOI（デジタルオブジェクト識別子） 10.2216/18-7.1	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	4．巻
KC Wakeman, T Horiguchi	48
2．論文標題	5．発行年
Morphology and molecular phylogeny of the marine gregarine parasite Selenidium oshoroense n. sp. (Gregarina, Apicomplexa) isolated from a Northwest Pacific Hydroides ezoensis Okuda 1934 (Serpulidae, Polychaeta)	2018年
3．雑誌名	6．最初と最後の頁
Marine Biodiversity	1489-1498
掲載論文のDOI（デジタルオブジェクト識別子）	査読の有無
10.1007/s12526-017-0643-1	有
オープンアクセス	国際共著
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	該当する

1．著者名	4．巻
NT Suong, JC Banks, SC Webb, A Jeffs, KC Wakeman, A Fidler	129
2．論文標題	5．発行年
PCR test to specifically detect the apicomplexan ‘X’(APX) parasite found in flat oysters Ostrea chilensis in New Zealand	2018年
3．雑誌名	6．最初と最後の頁
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10.3354/dao03244	有
オープンアクセス	国際共著
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	該当する

1．著者名	4．巻
KC Wakeman, A Yamaguchi, T Horiguchi	169
2．論文標題	5．発行年
Molecular Phylogeny and Morphology of Haplozoon ezoense n. sp.(Dinophyceae): A Parasitic Dinoflagellate with Ultrastructural Evidence of Remnant Non-photosynthetic Plastids	2018年
3．雑誌名	6．最初と最後の頁
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掲載論文のDOI（デジタルオブジェクト識別子）	査読の有無
10.1016/j.protis.2018.04.008	有
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1．著者名	4．巻
KC Wakeman, A Yabuki, K Fujikura, K Tomikawa, T Horiguchi	65
2．論文標題	5．発行年
Molecular Phylogeny and Surface Morphology of Thiriotia hyperdolphinae n. sp. and Cephaloidophora oradareae n. sp. (Gregarinasina, Apicomplexa) Isolated from a Deep Sea Oradarea sp. (Amphipoda) in the West Pacific	2018年
3．雑誌名	6．最初と最後の頁
Journal of Eukaryotic Microbiology	372-381
掲載論文のDOI（デジタルオブジェクト識別子）	査読の有無
10.1111/jeu.12480	有
オープンアクセス	国際共著
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1．著者名 D Iritani, T Horiguchi, KC Wakeman	4．巻 65
2．論文標題 Molecular Phylogenetic Positions and Ultrastructure of Marine Gregarines (Apicomplexa) Cuspisella ishikariensis n. gen., n. sp. and Loxomorpha cf. harmothoe from Western Pacific scaleworms (Polynoidae)	5．発行年 2018年
3．雑誌名 Journal of Eukaryotic Microbiology	6．最初と最後の頁 637-647
掲載論文のDOI（デジタルオブジェクト識別子） 10.1111/jeu.12509	査読の有無 無
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 Wakeman KC, Hoppenrath M, Yamaguchi A, Gavelis GS, Leander BS, Nozaki H.	4．巻 57
2．論文標題 Ultrastructure of the marine benthic dinoflagellatePlagiodinium belizeanum(Dinophyceae) from the southeast Pacific island of Okinawa, Japan	5．発行年 2018年
3．雑誌名 Phycologia	6．最初と最後の頁 209-222
掲載論文のDOI（デジタルオブジェクト識別子） 10.2216/17-43.1	査読の有無 無
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 －

〔学会発表〕　計0件

〔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