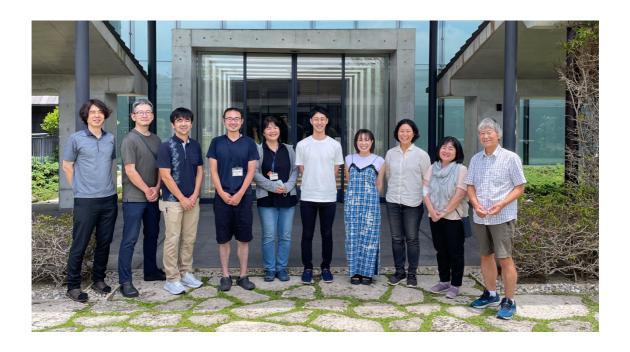
FY2023 Annual Report

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Abstract

The genome contains all the genetic information of a given organism. Decoding the genome provides the molecular basis for understanding every biological phenomenon. Since 2009, the Marine Genomics Unit (MGU) has conducted research in the realm of genome-based biological sciences. By sequencing genomes of target marine organisms (mainly marine invertebrates), we wish to understand genetic and developmental mechanisms underlying evolution and diversification of marine organisms. The major research fields are (a) evolutionary and developmental genomics of marine invertebrates, (b) environmental genomics of coral reefs, and (c) functional genomics of marine organisms including pearl oyster and algae. To date, we have reported draft genomes of a coral in 2011, a pearl oyster in 2012, and coral-symbiotic dinoflagellate (Breviolum) in 2013. We further decoded genomes of hemichordates and a brachiopod in 2015; a brown alga (Okinawa-mozuku) in 2016; Crown-of-Thorns starfish in 2017; a nemertean, phoronid, and two dinoflagellate clades in 2018; jellyfish, dicyemids, acoel flatworm, siphonous macroalga (umi-budo), brown alga (itomozuku) in 2019; and hydra and four strains of "Okinawa mozuku" brown alga in 2020. In 2021 we have reported sequenced genome of 19 coral species (collaboration with Tokyo Univ.), the kuruma shrimp (collaboration with Tokyo U. of Marine Sci. Tech.), and nearly complete genome of the tunicate Ciona (collaboration with Kyoto U.). In this year, we reported haplotype-phased genome of a pearl oyster. In addition, we have advanced genome-based coral research, especially coral-specific eDNA projects, and one research result shall be reported below.



[Photo on June 7, 2023]

1. Staffs and Students

- Professor Noriyuki Satoh
- Staff Scientists
 - o Eiichi Shoguchi (Group Leader)
 - o Keisuke Nakashima
 - o Takeshi Takeuchi
 - o Takeshi Noda
- Technical Staffs
 - o Kanako Hisata
 - o Sakura Kikuchi
 - o Haruhi Narisoko
 - o Mayuki Suwa
- Research Assistants
 - o Aya Koseki (COI-NEXT)
- Research Administrators
 - o Tomomi Teruya

- o Kazuko Toyoda
- Students
 - o Ph.D students (co-superviser)
 - Rio Kashimoto (Supervisor: Prof. Laudet, V.)

2. Collaborations

- 2-1 Genome scientific studies of chordate evolution
 - Type of collaboration: Scientific collaboration
 - Researchers: Prof. Daniel Rokshar, OIST
- 2-2 Genome sequencing of marine invertebrates at haplotype-resolution level
 - Type of collaboration: Scientific collaboration
 - Researchers: Prof. Gene Myers, OIST
- 2-3. Molecular biological study of COTS communications
 - Type of collaboration: Scientific collaboration
 - Researchers: Prof. Scott Cummins, Univ. Sunshine Coast, Australia
- 2-4. Genome scientific study of dinoflagellates
 - Type of collaboration: Scientific collaboration
 - Researchers: Prof. Pengchen Fu, Hainan University
- 2-5. Genome scientific study of coral-dinoflagellate symbiosis
 - Type of collaboration: Scientific collaboration
 - Researchers: Profs. Shigeki Fujiwara & Kaz Kawamura, Kochi University
- 2-6. Genome scientific study of left-right asymmetry of snails

- Type of collaboration: Scientific collaboration
- Researchers: Prof. Takehiro Asami, Shinshu University
- 2-7. Genome scientific study of amphioxus development
 - Type of collaboration: Scientific collaboration
 - Researchers: Dr. Hiroki Takahashi, National Institute for Basic Biology
- 2-8. Genome scientific study of hemichordate development
 - Type of collaboration: Scientific collaboration
 - Researchers: Profs. Kunifumi Tagawa, Asuka Arimoto & Tatsuya Ueki, Hiroshima University

3. Research activities and findings

Of several studies published in 2023, we report here an advance in scleractinian (stony, reef-building) corals, which was published in Proc. Roy. Soc. B, 290: 20230026 (2023).

Coral reefs have the highest biodiversity of all marine ecosystems in tropical and subtropical oceans. However, scleractinian corals, keystone organisms of reef productivity, are facing a crisis due to climate change and anthropo- genic activities. A broad survey of reef-building corals is essential for worldwide reef preservation. To this end, direct observations made by coral-specialist divers might be supported by another robust method. We improved a recently devised environmental DNA (eDNA) metabarcoding method to identify more than 43 scleractinian genera by sampling 21 of sur- face seawater above reefs. Together with direct observations by divers, we assessed the utility of eDNA at 63 locations spanning approximately 250 km near Okinawa Island. Slopes of these islands are populated by diverse coral genera, whereas shallow 'moats' sustain fewer and less varied coral taxa. Major genera recorded by divers included Acropora, Pocillopora, Porites and Montipora, the presence of which was confirmed by eDNA analyses. In addition, eDNA identified more genera than direct observations and documented the presence of previously unrecorded species. This scleractinian coral-specific eDNA method promises to be a powerful tool to survey coral reefs broadly, deeply and robustly.

In this study, together with direct observations by divers (a part of Monitoring Sites 1000 Project supported by the Ministry of the Environment of Japan), we assessed the utility of eDNA at 63 locations spanning ~250 km near Okinawa Island (Fig. 1). Of 63 monitoring sites, 51 were slopes (3-10m in depth), 8 were moats (1-3m in depth), and 4 sites were both slopes and moats.

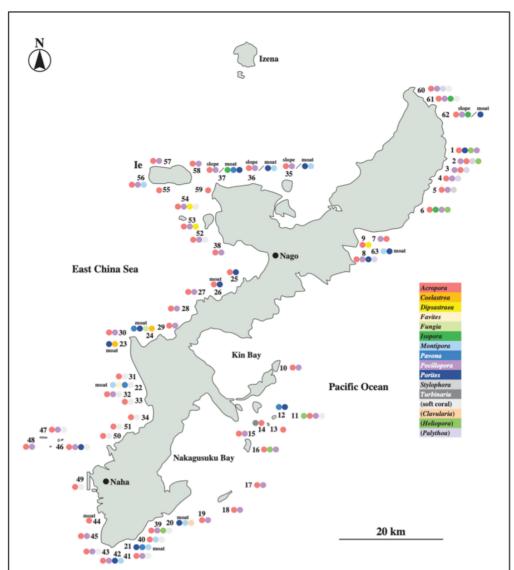


Figure 1. Sixty-three coral reef monitoring locations around Okinawa Island. Collections sites and representative coral genera are shown. Colour codes for genera are shown in the right corner. The order (from left to right) does not always indicate relative dominance of genera. Moats are indicated. Other locations are slopes. At locations #35, 36, 37 and 62, both slopes and moats were examined by diving, whereas only eDNA data were obtained from moats.

Major genera recorded by divers included *Acropora*, *Pocillopora*, *Porites*, and *Montipora* (Fig. 1). Simultaneously, scleractinian coral-specific eDNA barcoding analyses were carried out at 62 locations. We obtained amplicons from all samples at 62 sites, confirming that 2 L of surface seawater are enough for scleractinian-specific eDNA metabarcoding (Fig. 1). Results of the scleractinian coral-specific eDNA barcoding analyses confirmed genera recorded by direct observations by divers. To evaluate this scleractinian coral-specific eDNA method, we compared results of eDNA barcoding with direct observations (Table 1). As a result, 41 of 62 points were well matched (67%), 15 were moderately matched (24%), 4 were partially match (6%), and only 2 points showed no match (3%). In other words, at more than 91% (67%+24%) of monitored locations, eDNA results were confirmed by direct observations.

No	point name	geomorphic classification	direct observat	on		number of genus detected	eDNA method				number of genus detected	match	Overlap coefficien
25	Busenamisaki west	slope	Acropora	Porites		2	Acropora	Porites	Pocillopora		3		1
27		slope	Acropora	Pocillopora		2	Acropora	Pocillopora	Montipora		3		1
28	Onnason Akasaki west	slope	Acropora	Pocillopora		2	Acropora	Montipora	Pocillopora		3		1
29	Maedamisaki west	slope	Acropora	Pocillopora		2	Acropora	Pocillopora	Montipora		3		1
30	Zanpamisaki west	slope	Acropora	Pocillopora		2	Acropora	Pocillopora	Dipsastraea		3		1
31	Toguchi west	slope	Acropora			1	Acropora	Montipora	Pocillopora		3		1
32	Mizugama	slope	Acropora	Pocillopora		2	Acropora	Porites	Dipsastraea		3		0.5
33	Sunabe	slope	Acropora			1	Acropora	Montipora	Plesiastrea		3	, -	1
34	Isa	slope	Acropora			1	Acropora	Pocillopora	Goniastrea		3		1
38	Shiokawa Port south	slope	Acropora	Pocillopora		2	Acropora	Montipora	Porites		3		0.5
49	Oominezaki Oose	slope	Acropora			1	Acropora	Montipora	Pocillopora		3	, -	1
50	Karasuzaki west	slope	Acropora			1	Acropora	Porites	Goniastrea		3	, 0	1
51	Janase	slope	Acropora			1	Acropora	Goniastrea	Dipsastraea		3	, -	1
52	Sesokojima south	slope	Acropora	Pocillopora		2	Acropora	Pocillopora	Montipora		3		1
53	Minnajima east	slope	Acropora	Pocillopora	Dipsastraea	3	Acropora	Pocillopora	Porites	Dipsastraea	4	. 0	1
54	Nakanshi cast	slope	Acropora	Pocillopora	Dipsastraea	3	Acropora	Pocillopora	Porites		3	3 0	0.666666667
55	Iejima Funazubaru south	slope	Acropora			1	Porites	Acropora	Goniastrea		3	3 0	1
56	Iejima West	slope	Acropora	Pocillopora	Montipora	3	Acropora				1	. 0	1
57	Ieijma Waji north	slope	Acropora	Pocillopora		2	Acropora	Montipora	Pocillopora		3	3 0	1
58	Iejima Isharabaru east	slope	Acropora	Pocillopora		2	Acropora	Pocillopora			2	2 0	1
59	Aquarium west	slope	Acropora		•	1	Acropora	Pocillopora	Montipora		3	. 0	1
45	Kvan Port west	slope	Acropora	Pocillopora		2	Acropora	Dipsastraea	Porites	Pocillopora	4	. 0	1
											1		
1	Adagashima north	slope	Acropora	Porites		2	Acropora	Porites	Montipora		3	-	1
2	Adagashima south	slope	Pocillopora	Acropora	Stylophora	3	Plesiastrea	Acropora	Montipora	Pocillopora	4		0.00000000
3	Ishikinazaki southwest	slope	Pocillopora	Acropora		2	Acropora	Montipora	Porites	Pocillopora	4		1
4	Katsusenozaki south	slope	Acropora	Pocillopora		2	Acropora	Pavona	Porites	Pocillopora	4		1
5	Aha south	slope	Acropora	Pocillopora	Stylophora	3	Acropora	Pavona	Porites	Dipsastraea	4		0.333333333
6	Pumped Storage Hydropower Station southeast		Isopora	Pocillopora	Acropora	3	Acropora	Montipora	Pocillopora		3		0.666666667
7	Higashison Miyagi Unse south	slope	Acropora	Pocillopora		2	Acropora	Montipora	Pocillopora		3		1
8	Gesashi Uppama east	slope	Acropora		Porites	3	Acropora	Montipora	Porites	Pocillopora	4		1
9	Gesashi north	slope	Acropora	Dipsastraea		2	Acropora	Porites	Montipora		3		0.5
10	Ikeijima east	slope	Acropora	Pocillopora		2	Montipora	Acropora	Pocillopora		3		1
11	Ukibaru Yokobishi east	slope	Acropora	Pocillopora		2	Acropora	Pavona	Pocillopora		3		1
12	Ukibaru Yokobishi south	slope	Pavona	Porites		2	Montipora	Acropora	Palythoa		3		0
13	Minamiukibaru southeast	slope	Acropora			1	Acropora	Montipora	Porites		3	3 0	1
14	Minamiukibaru south	slope	Turbinaria	Acropora		2	Acropora	Pavona	Montipora		3	3 0	0.5
15	Ginogiiwa northeast	slope	Acropora	Pocillopora		2	Porites	Acropora	Montipora		3	3 0	0.5
16	Tsukenjima Agihama east	slope	Acropora	Pocillopora		2	Montipora	Acropora	Pachyseris	Pocillopora	4	. 0	1
17	Uganiwa south	slope	Acropora	Pocillopora		2	Acropora	Porites			2	2 0	0.5
18	Kudakajima Erabuiwa east	slope	Acropora	Pocillopora		2	Montipora	Acropora	Pocillopora		3	3 0	1
19	Kumakajima south	slope	Acropora	Pocillopora		2	Montipora				1	. X	0
39	Ohjima south	slope	Acropora	Pocillopora		2	Montipora	Acropora	Porites		3	3 0	0.5
40	Mabuni south	slope	Acropora	Montipora		2	Acropora	Montipora	Goniastrea		3		1
41	Ohdo	slope	Acropora	Pocillopora		2	Acropora	Montipora	Porites		3	3 0	0.5
43	Arasaki west	slope	Acropora	Pocillopora		2	Acropora	Pocillopora	Montipora		3		1
46	Chiibishi Kamiyama south	slope	Acropora		Porites	3	Acropora	Pocillopora	Porites		3	. 0	1
47		slope	Pocillopora	Acropora		2	Pocillopora	Acropora	Montipora		3	3 0	1
48	Chiibishi Nagannu west	slope	Acropora	Pocillopora		2	Acropora	Pocillopora	Montipora		3		
48 60		slope	Acropora	Pocillopora		2	Isonora	Acropora	Dipsastraea	Pocillopora	4		1
61	Oku Port north	slope	Acropora Acropora		Isopora	3	Acropora	Porites Porites	Goniastrea	Limopord	3		0.33333333
					ssopora	•					-		************
26	Afuso north	moat	Acropora	Porites		2	Acropora	Porites	Turbinaria		3		1
35	Kourijima Tokeihama	moat	Porites	Montipora		2	Montipora	Acropora	Porites		3		1
36	Nakijinson Nagahama	moat	Porites	Montipora		2	Montipora	Acropora	Porites		3		1
37	Bisczaki east	moat	Isopora	Pavona	Porites	3	Astreopora	Pavona	Porites		3		0.666666667
44	Itoman Port Kurantogai north	moat	Acropora			1	Acropora	Montipora	Pocillopora		3	. 0	1
20	Ohjima south	moat	Porites	Montipora		2	Montipora				1	. 0	1
21	Ohdo east	moat	Porites	Рачопа	Montipora	3	Pavona	Montipora	Acropora	Porites	4	. 0	0.6666666
22	Mizugama	moat	Montipora	Favites	Pavona	3	Montipora	Goniastrea	Porites	Pavona	4		0.33333333
23	Zanpamisaki west	moat	Porites	Coelastrea		2	Montipora	Porites	Dipsastraea	Goniastrea	4	. 0	0.
													0.33333333
24	Maedamisaki west	moat	Pavona	Porites		3	Dipsastraea	Pavona			3		
24 62	Maedamisaki west Kunigamison Akasaki north	moat moat	Pavona Porites	Porites	Fungia	3	Dipsastraea Acropora	Montipora	Porites Pocillopora	Porites	4		43333333

Therefore, we concluded that this scleractinian coral-specific eDNA method promises to be a powerful tool to survey coral reefs broadly, deeply, and robustly.

4. Publications

(a) Developmental and Evolutionary Genomics

- 1. Tominaga, H., Nishitsuji, K., Satoh, N. A single-cell RNA-seq analysis of early larval cell-types of the starfish, *Patiria pectinifera*: Insights into evolution of the chordate body plan. Developmental Biology, 496:52-62 (2023).
- 2. <u>Li, K., Nakashima, K., Hisata, K., Satoh, N.</u> Expression and possible functions of a horizontally transferred glycosyl hydrolase gene, *GH6-1*, in *Ciona* embryogenesis EvoDevo, 14:11 (2023) **PUBMED**
- 3. Arimoto, A., Nishitsuji, K., Hisata, K., Satoh, N., Tagawa, K. Transcriptomic evidence for *Brachyury* expression in the caudal tip region of adult *Ptychodera* flava (Hemichordata). DGD, 65: 370-480 (2023)
- 4. Noda, T., Satoh, N., Gittenberger, E., Asami, T. Left-right reversals recurrently evolved regardless of diaphanous-related formin gene duplication or loss in snails Journal of Molecular Evolution, 91:721-729 (2023)

(b) Environmental Genomics

- Nishitsuji, K., Nagata, F., Narisoko, H., Kanai, M., Hisata, K., Shinzato, C., Satoh, N. An environmental DNA metabarcoding survey reveals generic-level occurrence of scleractinian corals at reef slopes of Okinawa Island. Proc. Royal Soc. B.290:20230026 (2023).
- Hillberg, A., Smith, M., Lausen, B., Suwansa-ard, S., Johnston, R., Mitu, S., MacDonald, L., Zhao, M., Motti, C., Wang, T., Elizur, A., Nakashima, K., <u>Satoh, N.</u>, Cummins, S. Crown-of-thorns starfish spines secrete defense proteins PeerJ 11:e15689 (2023)
- Davies, S.W., Gamache, M.H., Howe-Kerr, L.I., Kriefall, N.G., Baker, A.C., Banaszak, A.T., Bay, L.K., Bellantuono, A.J., Bhattacharya, D., Chan, C.X., Claar, D.C., Coffroth, M.A., Cunning, R., Davy, S.K., del Campo, J., Díaz-Almeyda, E.M., Frommlet, J.C., Fuess, L.E., González-Pech, R.A., Goulet, T.L., Hoadley, K.D., Howells, E.J., Hume, B.C.C., Kemp, D.W., Kenkel, C.D., Kitchen, S.A., LaJeunesse, T.C., Lin, S., McIlroy,

- S.E., McMinds, R., Nitschke, M.R., Oakley, C.A., Peixoto, R.S., Prada, C., Putnam, H.M., Quigley, K., Reich, H.G., Reimer, J.D., Rodriguez-Lanetty, M., Rosales, S.M., Saad, O.S., Sampayo, E.M., Santos, S.R., Shoguchi, E., Smith, E.G., Stat, M., Stephens, T.G., Strader, M.E., Suggett, D.J., Swain, T.D., Tran, C., Traylor-Knowles, N., Voolstra, C.R., Warner, M.E., Weis, V.M., Wright, R.M., Xiang, T., Yamashita, H., Ziegler, M., Correa, A.M.S., Parkinson, J.E. Building consensus around the assessment and interpretation of Symbiodiniaceae diversity. PeerJ 11:e15023
- 8. Kawamura, K., Shioguchi, E., Nishitsuji, K., Sekida, S., Narisoko, H., Zhao, H., Shu, H., Fu, P., Yamashita., H., Fujiwara, S., Satoh, N. In vitro Phagocytosis of Different Dinoflagellate Species by Coral Cells. Zoological Science, 40:444-454 (2023).

(c) Functional Genomics

- 9. Smith, M.K., Rotgans, B.A., Lang, T., Johnston, R., Wang, T., Suwansa-Ard, S., Bose, U., Satoh, N., Egertova, M., Hall, M.R., Bryne, M., Elphick, M.R., Motti, C.A., Cummins, S.F. Structure and proteomic analysis of the crown-of-thorns starfish (*Acanthaster* sp.) radial nerve cord. Sci Rep. 13(1):3349. doi: 10.1038/s41598-023-30425-1 (2023).
- 5. Seminar: no seminar due to COVID problem.