FY2022 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, dicyemid, acoel flatworm, siphonous macroalga (umi-budo), brown alga (itomozuku) in 2019; and hydra and four 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 shrinp (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, especialy coral-specific eDNA projects, and one research result shall be reported below.



[Photo on October 12, 2022]

1. Staffs and Students

- Professor Noriyuki Satoh
- Staff Scientists
 - o Eiichi Shoguchi (Group Leader)
 - o Keisuke Nakashima
 - o Konstantin Khalturin (~Sept. 2022)
 - o Takeshi Takeuchi
 - o Koki Nishitsuji
 - o Takeshi Noda
- Technical Staffs
 - o Kanako Hisata
 - Sakura Kikuchi
 - o Haruhi Narisoko
- Research Assistants
 - o Yoshie Nishitsuji (part time)
 - Mayuki Suwa (part time)

- Research Administrators
 - Shoko Yamakawa
 - o Tomomi Teruya
- 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 acoel development

- Type of collaboration: Scientific collaboration
- Researchers: Profs. Kunifumi Tagawa, Asuka Arimoto & Tatsuya Ueki, Hiroshima University

3. Research activities and findings

An environmental DNA metabarcoding survey reveals generic-level occurrence of scleractinian corals at reef slopes of Okinawa Island

Proc. Roy. Soc. B, 290: 20230026

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 anthropogenic activities. A broad survey of reef-building corals is essential for world-wide 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 by out team to identify more than 43 scleractinian genera by sampling 2 L of surface seawater above reefs (Shinzato et al., 2021, Front. Marine Sci. 8, 758207).

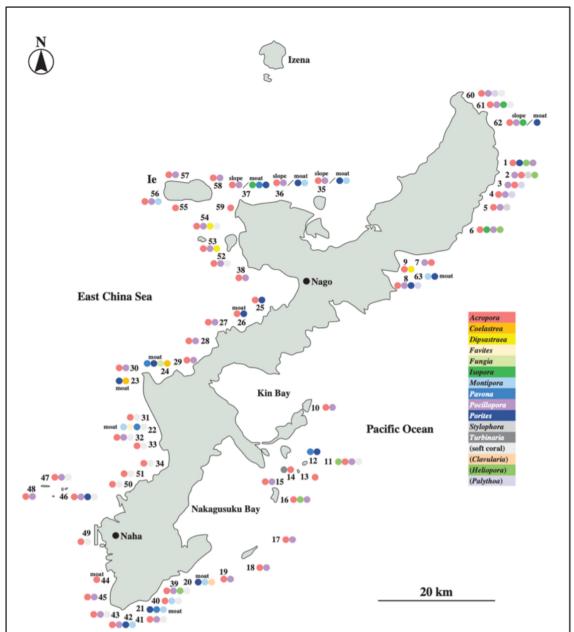


Figure 1. Sixty-three coral reef monitoring locations around Okinawa Island. Collections sites and representative coral genera are shown. Colour codes for genera are shown in the right corner. The order (from left to right) does not always indicate relative dominance of genera. Moats are indicated. Other 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.

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; Fig. 2a), 8 were moats (1-3m in depth; Fig. 2b), and 4 sites were both slopes and moats (#35-37, 62).

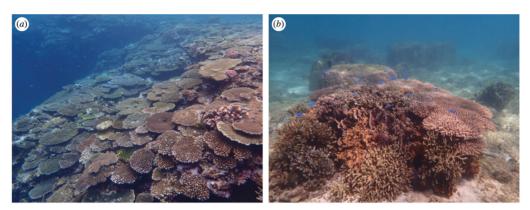


Figure 2. Distribution of various scleractinian corals at a reef slope (#8, Gesashi Uppama East Slope) (a) and a moat (#21, Ohdo East Moat) (b). Dominant genera in (a) are tabular *Acropora* and branching *Pocillopora* and (b) massive and branching *Porites* and tabular *Acropora*.

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. 3). Results of the scleractinian coral-specific eDNA barcoding analyses confirmed genera recorded by direct observations by divers.

In addition, the eDNA method identified more genera than direct observations and documented the presence of previously unrecorded species. For example, eDNA likely provided evidence for genera that were so far unknown from Okinawa Island. For example, two species in the genus *Heliofungia* have been reported, *H. actiniformis* (Quoy and Gaimard 1833) and *H. fralinae* (Nemenzo 1959), the latter being recorded in southern Asia, including Japan. However, this species reportedly reaches its northern limit at Miyako Island, and there have been no reports of its presence at Okinawa or Amami Islands (Fig. 3). Therefore, it was suggest a more northward distribution of *H. fralinae*, reaching at least as far as Okinawa Island.

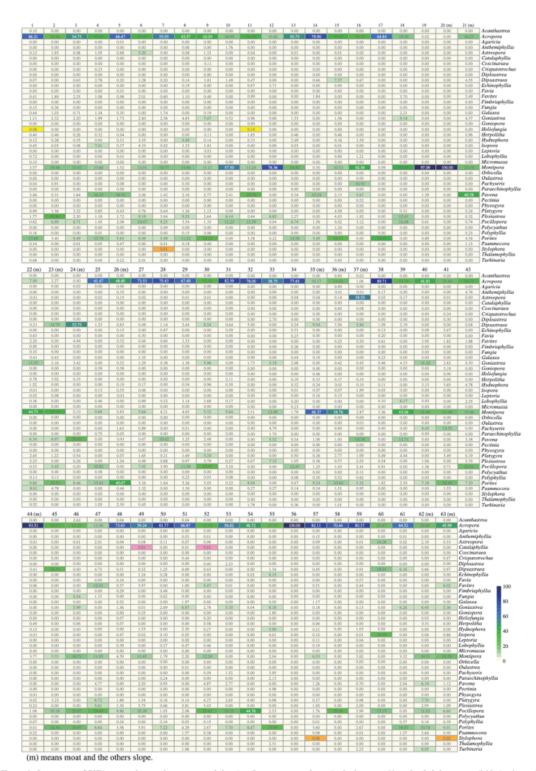


Figure 3. Percentages of ZOTUs mapped to coral genera at each location. Percentages are shown in the heatmap. More detailed data are available in electronic supplementary material, figure S2. Occurrences of Herpolitha, Catalaphyllia and Stylophora are marked with yellow, magenta and brown, respectively.

Finally, 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.

Table 1. Comparison of scleractinian corals called by direct diver obseration and coral-specific eDNA barcoding method

No	point name	geomorphic classification	direct observati	on		number of genus detected	eDNA method				number of genus detected	match	Overlap coefficient
25	Busenamisaki west	slope	Acropora	Porites		2	Acropora	Porites	Pocillopora			3 O	1
27	Onna	slope	Acropora	Pocillopora		2	Acropora	Pocillopora	Montipora			3 0	1
28	Onnason Akasaki west	slope	Acropora	Pocillopora		2	Acropora	Montipora	Pocillopora			3 O	1
29	Maedamisaki west	slope	Acropora	Pocillopora		2	Acropora	Pocillopora	Montipora			3 O	1
		slope	Acropora	Pocillopora		2	Acropora	Pocillopora	Dipsastraea			3 O	1
31	Toguchi west	slope	Acropora			1	Acropora	Montipora	Pocillopora			3 O	1
32	Mizugama	slope	Acropora	Pocillopora		2	Acropora	Porites	Dipsastraea			3 0	0.5
33	Sunabe	slope	Acropora			1	Acropora	Montipora	Plesiastrea			3 O	1
34	Isa	slope	Acropora			1	Acropora	Pocillopora	Goniastrea			3 0	1
38	Shiokawa Port south	slope	Acropora	Pocillopora		2	Acropora	Montipora	Porites			3 0	0.5
49	Oominezaki Oose	slope	Acropora			1	Acropora	Montipora	Pocillopora			3 O	1
50	Karasuzaki west	slope	Acropora			1	Acropora	Porites	Goniastrea			3 O	1
51		slope	Acropora			1	Acropora	Goniastrea	Dipsastraea			3 O	1
52		slope	Acropora	Pocillopora		2	Acropora	Pocillopora	Montipora			3 0	1
		slope	Acropora	Pocillopora	Dipsastraea	3	Acropora	Pocillopora	Porites	Dipsastraea		4 0	1
	-	slope	Acropora	Pocillopora	Dipsastraea	3	Acropora	Pocillopora	Porites	- poussiacu		3 0	0.66666667
55		slope	Acropora	2 осторога	Dipsusiratu	1	Porites	Acropora	Goniastrea			3 0	1
56		slope	Acropora	Pocillopora	Montipora	3		жтороги	Goniusirea			1 0	
					монирога	2	Acropora	Montile	Pocillopora			1 0	
57		slope	Acropora	Pocillopora		_	Acropora	Montipora	Pociliopora			2 0	
58		slope	Acropora	Pocillopora		2	Acropora	Pocillopora				3 0	
59		slope	Acropora			1	Acropora	Pocillopora	Montipora				1
45	Kyan Port west	slope	Acropora	Pocillopora		2	Acropora	Dipsastraea	Porites	Pocillopora		4 0	1
1	Adagashima north	slope	Acropora	Porites	ı	2	Acropora	Porites	Montipora			3 0	1
2	-	slope	Pocillopora	Acropora	Stylophora	3	Plesiastrea	Acropora	Montipora	Pocillopora		4 0	0.66666667
3		slope	Pocillopora	Acropora	-,-,	2	Acropora	Montipora	Porites	Pocillopora		4 0	1
4		slope	Acropora	Pocillopora		2	Acropora	Pavona	Porites	Pocillopora		4 0	1
5		slope	Acropora	Pocillopora	Stylophora	3	Acropora	Pavona	Porites	Dipsastraea		4 Δ	0.33333333
-	Pumped Storage Hydropower Station southeast		Isopora	Pocillopora	Acropora	3	Acropora	Montipora	Pocillopora	Dipsusirueu		3 0	0.666666667
			_		лсгорога	2						3 0	1
		slope	Acropora	Pocillopora	Porites	3	Acropora	Montipora	Pocillopora Porites	Pocillopora		4 0	
9		slope	Acropora	_	Porties		Acropora	Montipora		Росшорога		3 0	0.5
-		slope	Acropora	Dipsastraea		2	Acropora	Porites	Montipora			3 0	1
10		slope	Acropora	Pocillopora		2	Montipora	Acropora	Pocillopora			, ,	1
11		slope	Acropora	Pocillopora		2	Acropora	Pavona	Pocillopora			3 0	1
12		slope	Pavona	Porites	ı	2	Montipora	Acropora	Palythoa			3 X	0
13		slope	Acropora			1	Acropora	Montipora	Porites			3 0	1
14		slope	Turbinaria	Acropora		2	Acropora	Pavona	Montipora			3 0	0.5
15	Ginogiiwa northeast	slope	Acropora	Pocillopora		2	Porites	Acropora	Montipora			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 0	0.5
18	Kudakajima Erabuiwa east	slope	Acropora	Pocillopora		2	Montipora	Acropora	Pocillopora			3 O	1
19	Kumakajima south	slope	Acropora	Pocillopora		2	Montipora					1 X	0
39	Ohjima south	slope	Acropora	Pocillopora		2	Montipora	Acropora	Porites			3 0	0.5
40	Mabuni south	slope	Acropora	Montipora		2	Acropora	Montipora	Goniastrea			3 0	1
41		slope	Acropora	Pocillopora		2	Acropora	Montipora	Porites			3 0	0.5
43		slope	Acropora	Pocillopora		2	Acropora	Pocillopora	Montipora			3 0	1
46		slope	Acropora	Pocillopora	Porites	3	Acropora	Pocillopora	Porites	1		3 0	1
		slope	Pocillopora	Acropora		2	Pocillopora	Acropora	Montipora			3 0	1
48		slope	Acropora	Pocillopora		2	Acropora	Pocillopora	Montipora			3 0	1
60		slope	Acropora	Pocillopora		2	Isopora	Acropora	Dipsastraea	Pocillopora		4 0	1
61		slope	Acropora	Pocillopora	Isopora	3	Acropora	Porites	Goniastrea	2 Semopora		₃ Δ	0.33333333
		stope		_	130pora								**************
26	Afuso north	moat	Acropora	Porites		2	Acropora	Porites	Turbinaria			3 O	1
35	Kourijima Tokeihama	moat	Porites	Montipora		2	Montipora	Acropora	Porites			3 O	1
36	Nakijinson Nagahama	moat	Porites	Montipora		2	Montipora	Acropora	Porites			3 O	1
37	Bisezaki east	moat	Isopora	Pavona	Porites	3	Astreopora	Pavona	Porites			3 0	0.66666667
	Itoman Port Kurantogai north	moat	Acropora			1	Acropora	Montipora	Pocillopora			3 0	1
	-	moat	Porites	Montipora		2	Montipora					1 0	1
		moat	Porites	Payona	Montipora	3	Pavona	Montipora	Acropora	Porites	l	4 0	0.666666667
		moat	Montipora	Favites	Pavona	3	Montipora	Goniastrea	Porites	Payona		4 Δ	0.33333333
	_	moat	Montipora Porites	Coelastrea	1 d vona	2	Montipora Montipora	Porites	Portes Dipsastraea	Goniastrea -		4 0	0.3333333
				CO CIMON CM	E.maia	3				Goniastrea		4 Ο 3 Δ	0.333333333
		moat	Pavona	Porites	Fungia		Dipsastraea	Pavona	Porites	Destruc			0.33333333
		moat	Porites			1	Acropora	Montipora	Pocillopora	Porites			1
63	Gesashi Uppama east	moat	Montipora	Porites		2	Acropora	Montipora	Pocillopora			3 0	0.5

 $[\]mathbb{O}$, strong match; \bigcirc , moderate match; \triangle , partial match; X , no match

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. <u>Khalturin, K.</u>, Shunatova, N., Shchenkov, S., Sasakura, Y., Kawamitsu, M., <u>Satoh, N.</u> Polyzoa is back: The effect of complete gene sets on the placement of Ectoprocta and Entoprocta

Science Advances, 8(26):eabo4400. (2022) **PUBMED**

2. <u>Satoh, N., Hisata, K., Foster, S., Morita, S., Nishitsuji, K., Oulhen, N., Tominaga, H.,</u> Wessel, G.

A single-cell RNA-seq analysis of Brachyury-expressing cell clusters suggests a morphogenesis-associated signal center of oral ectoderm in sea urchin embryos Developmental Biology, 483: 128-142 (2022)

- 3. Humphreys, T., Weiser, K., Arimoto, A., Sasaki, A., Uenishi, G., Fujimoto, B., Kawashima, T., Taparra, K., Molnar, J., Satoh, N., Marikawa, Y., Tagawa, K. Ancestral stem cell reprogramming genes active in hemichordate regeneration Frontiers in Ecology and Evolution, 10: 769433 (2022)
- 4. Kashimoto, R., Tanimoto, M., Miura, S., <u>Satoh, N.</u>, Laudet, V., <u>Khalturin, K.</u>

 Transcriptomes of giant sea anemones from Okinawa as a tool for understanding their phylogeny and symbiotic relationships with anemonefish Zoological Science, 39(4) (2022) **LITUK PUBMED**

(b) Environmental Genomics

- 5. Nishitsuji, K., Nagata, T., 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.

 Proceedings B, 290:20230026 (2023)
- Yasuda, N., Inoue, J., Hall, M.R., Nair, M.R., Adjeroud, M., Miguel D. Fortes, M.D., Nishida, M., Tuivavalagi, N., Ravago-Gotanco, R., Forsman, Z.H., Soliman, T., Koyanagi, R., Hisata, K., Motti, C.A., Satoh, N.
 Two Hidden mtDNA-Clades of Crown-of-Thorns Starfish in the Pacific Ocean Frontiers Marine Science, 9: 831240, doi: 10.3389/fmars.2022.831240 (2022)
- 7. Shimakawa, G., <u>Shoguchi, E.</u>, Burlacot, A., Ifuku, K., Che, Y., Kumazawa, M., Tanaka, K., Nakanishi, S.

Coral symbionts evolved a functional polycistronic flavodiiron gene. Photosynth Res. 151(1):113-124. doi: 10.1007/s11120-021-00867-7.

(2022) PUBMED

- 8. Tsuchiya, K., Zayasu, Y., Nakajima, Y., Arakaki, N., Suzuki, G., Satoh, N., Shinzato, C. Genomic analysis of a reef-building coral, *Acropora digitifera*, reveals complex population structure and a migration network in the Nansei Islands, Japan Molecular Ecology, 31, 5270–5284. (2022)
- Kitchen, S.,A., Jiang, D., Harii, S., <u>Satoh, N.</u>, Weis, V.M., and Shinzato, C.
 Coral larvae suppress the heat stress response during the onset of symbiosis thereby decreasing their odds of survival
 Molecular Ecology, 31:5813-5830. (2022) **PUBMED**

(c) Functional Genomics

11. <u>Takeuchi, T.,</u> Suzuki, Y., Watabe, S., Nagai, K., Tetsuji Masaoka, T., Fujie, M., Kawamitsu, M., <u>Satoh, N.</u>, Myers, E.W.

A high-quality, haplotype-phased genome reconstruction reveals unexpected 3 haplotype diversity in a pearl oyster

DNA Research, 29:dsac035. (2022) [PUBMED]

12. Isomoto, A., <u>Shoguchi, E., Hisata, K., Inoue, J., Inaba, K., Satoh, N., Ogawa, T., Hiroki Shibata, H.</u>

Active Expression of Genes for Protein Modification Enzymes in Habu Venom Glands

toxins, 14, 300. (2022) **FUBMED LINK**

13. Nishitsuji, K., Nishitsuji, Y., Yonashiro, Y., Satoh, N.

Development of DNA markers that distinguish male and female haploid germlings of the brown alga, *Cladosiphon okamuranus*

Phycological Research, 70: 160-166.

14. Shimizu, K., <u>Takeuchi, T.</u>, Negishi, L., Kurumizaka, H., Kuriyama, I., Endo, K., Suzuki, M.

Evolution of Epidermal Growth Factor (EGF)-like and Zona Pellucida Domains Containing Shell Matrix Proteins in Mollusks.

Mol Biol Evol. 39(7):msac148. doi: 10.1093/molbev/msac148. (2022) PUBMED LINK

15. Adi, T.K., Fujie, M., Satoh, N., Ueki, T.

The acidic amino acid-rich C-terminal domain of VanabinX enhances reductase activity, attaining 1.3- to 1.7-fold vanadium reduction

Biochemistry and Biophysics Reports, 32, 101349 (2022) LITUK PUBMED

16. Boutet, I., Alves Monteiro, H. J., Baudry, L., <u>Takeuchi, T.</u>, Bonnivard, E., Billoud, B., Farhat, S., Gonzales-Araya, R., Salaun, B., Andersen, A. C., Toullec, J.-Y., Lallier, F. H., Flot, J.-F., Guiglielmoni, N., Guo, X., Li, C., Allam, B., Pales-Espinosa, E., Hemmer-Hansen, J., Moreau, P., Marbouty, M., Koszul, R., Tanguy, A. Chromosomal assembly of the flat oyster (*Ostrea edulis* L.) genome as a new genetic resource for aquaculture

Evolutionary Applications, 00, 1–19.

(d) Sequencing Collaboration

17. Yamamoto, K., Yoneda, Y., Makino, A., Tanaka, Y., Meng, X.Y, Hashimoto, Y., Shinya, K., Satoh, N., Fujie, M., Toyama, T., Mori, K., Ike, M., Morikawa, M., Kamagata, Y., and Tamaki H.

Draft genome sequence of Bryobacteraceae strain F-183

Microbiology Resource Announcements, 11(1):e0045321. doi: 10.1128/mra.00453-21. (2022) FUBMED

18. Yamamoto, K., Yoneda, Y., Makino, A., Tanaka, Y., Meng, X.Y, Hashimoto, Y., Shin-ya, K., Satoh, N., Fujie, M., Toyama, T., Mori, K., Ike, M., Morikawa, M., Kamagata, Y., and Tamaki H.

Complete Genome Sequence of *Luteitalea* sp. Strain TBR-22.

Microbiol Resour Announc. 11(2):e0045521. doi: 10.1128/mra.00455-21.

(2022) **PUBMED**

19. Choirunnisa, A. R., Arima, K., Abe, Y., Kagaya, N., Kudo, K., Suenaga, H., Hashimoto, J., Fujie, M., Satoh, N., Shin-ya, K., Matsuda, K., Wakimoto, T. New azodyrecins identified by a genome mining-directed reactivity-based screening

Beilstein J. Org. Chem. 18, 1017–1025.

https://doi.org/10.3762/bjoc.18.102 LINK FUBMED

5. Seminar

1. Dr. Yuuri Yasuoka

Researcher in RIKEN Center for Integrative Medical Sciences

Title: "Tissue-specific expression of carbohydrate sulfotransferases drives keratan sulfate biosynthesis in the notochord and otic vesicles of Xenopus embryos"

Date: October 5th, 2022 - 13:00 to 14:00

Venue: D015

2. Dr. Christopher B. Cameron

Professor in the Department of Biological Sciences at University of Montreal.

Title: "The origin of gills, skeletal ossicles & tubes in the deuterostomes"

Date: November 21st, 2022 – 16:00 to 17:00

Venue: C210