Excursion guide to the radiolarians of the East China Sea near Sesoko Island, Okinawa, Japan: An important research station for living radiolarian studies

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Abstract

The Okinawa Radiolarian Tour, an annual workshop on living radiolarians, has been held at the Sesoko Station, the Tropical Biosphere Research Center of the University of the Ryukyus in Okinawa Prefecture, Japan, since 1997. More than 200 researchers and students have joined this tour to observe living radiolarians. The tours have provided valuable knowledge on living radiolarians, such as faunal characteristics, biological activities, skeletal growth, and molecular phylogeny. In this guide, brief histories of radiolarian biological research and the Okinawa Radiolarian Tour are given. Practical, latest information on oceanographic conditions, travel, safety, and handling and storage procedures for radiolarian studies will be given at the Sesoko Station.

Key words: Okinawa Radiolarian Tour, living radiolaria, East China Sea, Kuroshio Current, Sesoko Station, faunal characteristics, biological activities, skeletal growth, molecular phylogeny, culture experiment

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Introduction

Scientific interest in radiolaria, marine holoplanktonic protists, have goes back at least to Tilesius (1818), who illustrated a living radiolarian cell. As the radiolaria have not only a long geologic range (Cambrian to Recent) but also inhabit all water depths in all open oceans including the Arctic Ocean (e.g., De Wever et al., 2001; Suzuki and Aita, 2011; Suzuki and Not, 2015), they have played an important role in paleontology, geology, and marine ecology. In particular, knowledge of living radiolarian biology has great potential as a source of information to reconstruct the group's biologic history through the Phanerozoic.

One of the authors, Atsushi Matsuoka, has worked with living radiolarians at the Sesoko Station (Fig. 1) of the University of the Ryukyus since 1992. An annual workshop on living radiolarians in Sesoko, called the Okinawa Radiolarian Tour, has been held since 1997. As Table 1 indicates, living specimens recovered from surface waters around Sesoko Island have provided valuable knowledge on living radiolarians, such as faunal characteristics, biological activity, skeletal growth, and molecular phylogeny. The Sesoko Station is in fact one of the most important research stations for living radiolarian studies in the world.

The brief guide to observing radiolarians during this tour has already been published in both Japanese (Matsuoka, 2002) and English (Matsuoka, 2007). In this article, we first provide a brief history of radiolarian biological study. More practical details and latest information such as oceanographic conditions, travel, safety, handling and storage for radiolarian studies will be given at the Sesoko Station.

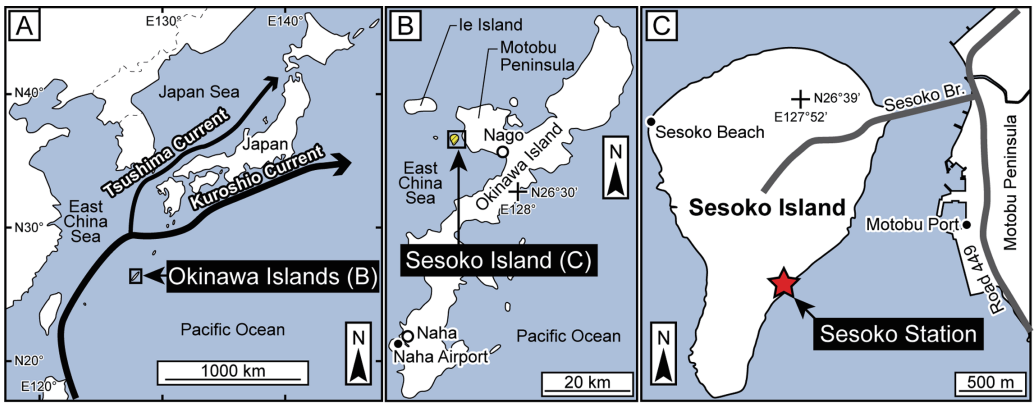

Fig. 1. Index map of the Sesoko Station with major warm ocean currents around the station.

Table 1. Major studies at the Sesoko Station.

Previous studies	Outline
Faunal characteristics	
Matsuoka (1993a)	Report of radiolarian fauna, composed of more than 30 species; comparison of the fauna with living radiolarians of the surface water near Barbados in the Caribbean Sea and indicating their similarities in the taxonomic components
Matsuoka (2009)	Observation of scanning electron microscopic images of radiolarian skeletons by using of sulfuric acid and showing ca. 60 species of the radiolarians
Matsuoka (2017)	Showing live and skeleton images of radiolarians (14 spumellarian and 15 nassellarian species) with transmitted light microscopy and scanning electron microscopy
Activities (Inc. feeding behavior and symbiosis)	
Matsuoka (1993a)	Observation of biological characters of nine species of living radiolarians; description of several biological characters of each species, such as activities of pseudopodia and color and size of symbionts
Suzuki and Sugiyama (2001)	Observation of axopodial activity of *Diplosphaera hexagonalis* Haeckel and recognition of cyclic extension and contraction of axopodia
Takahashi et al. (2003)	Observation of 29 species of living radiolarians to investigate their symbioses by using of epifluorescence microscope
Suzuki (2005)	Observation of axopodial activity of *Rhizosphaera trigonacantha* Haeckel having numerous fine axopodia (Type I axopodia) and few thick axopodia (Type II axopodia); clarification of the differences of these types of axopodia in shape and movement
Matsuoka (2007)	Finding a relationship between feeding behavior and shell morphology based on observation of living radiolarians
Sugiyama et al. (2008)	Observation of pseudopodial activities of *Eucyrtidium hexagonatum* Haeckel, *Pterocorys zancleus* (Müller), and *Dictyocodon prometheus* Haeckel; clarification of the relationships between pseudopodial activities and feeding behavior
Suzuki et al. (2009a)	Discovery of *Haliommilla capillaceum* (Haeckel) with a chain of extracellular cells; description of movement of the extracellular cells and pointed out the similarity to the sporogenesis of the host-specific parasitic dinoflagellate *Duboscquella* sp.
Suzuki et al. (2009b)	Report of distribution patterns of nuclei and symbionts of living radiolarians collected from the surface water around Sesoko Island and the Nansei Islands by using of C16H15N5 (DAPI)
Yuasa et al. (2012)	Observation and molecular anlysis on cyanobacterial symbionts within *Dictyocoryne profunda* Ehrenberg
Suzuki et al. (2013)	Discovery of novelty activity of *Streblacantha* sp. cf. *S. circumtexta* (Jørgensen)
Yuasa and Takahashi (2014)	Observation of repruductive swarmers of *Sphaerozoum punctatum* (Huxley)
Yuasa and Takahashi (2015)	Description *Gymnoxanthella radiolariae* , a symbiotic dinoflagellate from solitary polycystine radiolarians
Yuasa et al. (2016)	Observation of reproductive swarmers of *Didymocyrtis ceratospyris* Haeckel, *Pterocanium praetextum* (Ehrenberg), *Tetrapyle* sp., and *Triastrum aurivillii* Cleve
Skeletal growth	
Ogane et al. (2009)	Use of fluorescent compound $C_{20}H_{23}N_5O_3$ (PDMPO) to reveal skeletal growth of polycystine radiolarians
Ogane et al. (2010)	Applicaton of PDMPO to 50 cells from 22 species; clarification that skeletal thickening growth commonly occurs in polycystine radiolarians and that the patterns of skeletal growth differ by species
Ogane et al. (2014)	Discovery of assimilation of siliceous matter within pseudopodia; suggestion of 'pseudo silica absorption hypothesis'
Molecular phylogeny	
Takahashi et al. (2004)	Examination of the Family Spongodiscidae, including *Dictyocoryne profunda* Ehrenberg, *D. truncatum* (Ehrenberg), and *Spongaster tetras* Ehrenberg, with using of 18S ribosomal DNA sequence
Yuasa et al. (2004)	Examination of the Class Phaeodarea, including *Protocystis xiphodon* (Haeckel), *Challengeron diodon* Haeckel, and *Conchellium capsula* Borgert, with using of 18S ribosomal DNA sequence
Yuasa et al. (2005)	Examination of the Family Spongodiscidae (Class Polycystinea, Order Spumellarida) and the Family Pterocorythidae (Class Polycystinea, Order Nassellarida) with using of 16S ribosomal DNA sequence
Yuasa et al. (2006)	Examination of the Class Phaeodarea, including *Protocystis xiphodon* (Haeckel), *Challengeron diodon* Haeckel, and *Conchellium capsula* Borgert, with using of 18S ribosomal DNA sequence
Yuasa et al. (2009a)	Examination of *Hexacontium pachydermum* Jørgensen, *Cladococcus viminalis* Haeckel, *Arachnosphaera myriacantha* Haeckel, and *Astrosphaera hexagonalis* Haeckel with using of 18S ribosomal DNA sequence
Yuasa et al. (2009b)	Description of a simple method for obtaining 18S ribsomal DNA sequencs from a single radiolarian specimen

Histories of radiolarian biological research and the "Okinawa Radiolarian Tour"

There is a long history of study of radiolarian biology. Biological studies were carried out in Messina between Catania and the Italian Peninsula by German protozoologists as early as the 19th century (Müller, 1859; Haeckel, 1862; Hertwig, 1879), and led to discovery of yellowish brown photosynthetic microbiota, previously called "zooxanthella", for the first time in a marine organism - from the collodarian radiolarian *Collozoum inerme* (Brandt, 1881). Parasites were described from Taxopodia and Acantharia by German protozoologists (Koeppen, 1894; Borgert, 1898) and afterwards the cytological structure and parasites of radiolarians were detailed by French workers at the Villefranche-sur-mer Oceanological Observatory in 1950s–1960s (Hollande, 1953; Hollande and Enjumet, 1953, 1955, 1960; Cachon, 1964). Jean Cachon and Monique Cachon cooperated in clarifying the development of the cytoskeleton and its function in the 1970s (Cachon and Cachon, 1969, 1971, 1972a, 1972b, 1976, 1978, 1980). A Russian cytologist, Igor B. Raikov, summarized available knowledge of the radiolarian and phaeodarian nucleus in 1978, and the English version of this book was published four years later (Raikov, 1982). Despite these studies' progress on radiolarian cytology, little was known about physiological ecology at that time. Experimental physiology was studied mainly by Americans in 1970s to 1990s. Their results and interpretations were summarized in many publications (Anderson, 1978, 1980, 1983, 1984, 1986, 1993, 1994, 2012; Swanberg and Harbison, 1980; Anderson et al., 1983, 1984, 1986, 1989a, 1989b, 1989c; Swanberg, 1983; Swanberg et al., 1985, 1986, 1990; Swanberg, and Bjørklund, 1987; Swanberg and Caron, 1991; Swanberg and Eide, 1992; Caron et al., 1995; Michaels et al., 1995).

In the 1990s, A. Matsuoka and Kazuhiro Sugiyama independently stayed at the Lamont Doherty Geological Observatory of Columbia University and learnt how to work on living radiolarians under the guidance of O. Roger Anderson (Anderson and Matsuoka, 1992; Matsuoka, 1992; Matsuoka and Anderson, 1992; Sugiyama and Anderson, 1997). Just after coming back from the U.S., Matsuoka started making an effort to find suitable marine stations for the study of living radiolarians in Japan. Full-color images of living radiolarians around Japan were reported for the first time by Matsuoka (1993a) who collected these specimens in 1992 from surface waters in the East China Sea around Sesoko Island, Okinawa Prefecture, Japan (Table 2). From 1992 to 1996, Matsuoka prepared research equipment for living radiolarian research with the assistance of Satoshi Funakawa and Katsunori Kimoto. In 1997, he organized the first Observation Tour of Living Radiolarians at the Sesoko Station, the Tropical Biosphere Research Center, the University of the Ryukyus. The participants were K. Sugiyama, K. Kimoto, Katsuo Sashida, Osamu Takahashi, and Hideto Okuda. Since then this "tour" or workshop has been held more than 20 times almost every year. In 2002 the tour could not be organized due to reconstruction of buildings at the Sesoko Station. Most radiolarian workers in Japan have attended this tour and some of them

have become radiolarian biologists. The total number of the participants exceeds 200 people and includes American, Chinese, Filipino, French, and other foreigners.

The "Observation Tour of Living Radiolarians at Sesoko" is occasionally simply called "Okinawa Tour" or "Okinawa Radiolarian Tour". This tour efficiently contributed to living radiolarian and other protistan studies such as planktonic foraminifers and dinoflagellates (Suzuki and Sugiyama, 2001; Takahashi et al., 2003; Suzuki, 2005; Kimoto, 2005; Kimoto and Matsuoka, 2006; Sugiyama et al., 2008; Ogane et al., 2009, 2010, 2014; Suzuki et al., 2009a, 2009b, 2013; Probert et al., 2014; Biard et al., 2015; Takagi et al., 2016). The first molecular phylogenetic data of Spumellaria (exclusive of Collodaria) were also obtained from cells collected near Sesoko Island (Takahashi et al., 2004). After their participation in a couple of the tours, O. Takahashi and his colleague Tomoko Yuasa have regularly visited the Sesoko Station and successfully continue their own research (Yuasa et al., 2004, 2005, 2006, 2009a, 2009b, 2012, 2016; Yuasa and Takahashi, 2014, 2015).

The experience accumulated at the Sesoko Station has been transferred to other marine

Table 2. Participant numbers of the "Okinawa Radiolarian Tour" and major activities in Sesoko.

Year	Month	Tour number	Number of participants	Publication	Note
1992	Nov.		1		Matsuoka's first visit to the Sesoko Station
1993	-	-	-	Matsuoka (1993a)	
1994	-	-	-		
1995	Sept.		2		
1996	Sept.		3		
1997	Sept.	1st	6		Okinawa Radiolarian Tour starts
1998	Oct.	2nd	16		
1999	Sept.	3rd	12		
2000	July	4th	12		
2001	May-Sept.		6		Matsuoka's 5 months stay in Motobu
2002	March		4	Matsuoka (2002)	
	Nov.		6		
2003	May		1	Takahashi et al. (2003)	
	Nov.	5th	12		
2004	May		2		
	Nov.	6th	12		58th Symposium of Society of Science on Form
2005	April		2		
	Nov.	7th	7		
2006	Dec.	8th	11	Kouduka et al. (2006), Yuasa et al. (2006)	
2007	Nov.	9th	24	Matsuoka (2007)	
2008	Nov.	10th	21	Sugiyama et al. (2008)	Symposium for 10th Radiolarian Tour
2009	Nov.	11th	17	Matsuoka (2009), Ogane et al. (2009), Suzuki et al. (2009a, 2009b)	
2010	Dec.	12th	15	Ogane et al. (2010)	
2011	Dec.	13th	17		Japan-France Sympoium on Radiolarians
2012	Dec.	14th	5	Yuasa et al. (2012)	
2013	Dec.	15th	7		
2014	Dec.	16th	5		
2015	Nov.	17th	10		
2016	Oct.	18th	5		
2017	Oct.	19th		Matsuoka (2017)	Excursion of InterRad XV in Niigata 2017

stations in Japan. Matsuoka has carried out living radiolarian research at the Sado Marine Station of Niigata University since 2000 (Matsuoka et al., 2001, 2002; Itaki et al., 2003; Kurihara and Matsuoka, 2004, 2005, 2009, 2010; Kurihara et al., 2006, 2007, 2008). Although living radiolarians have been reported and studied from a variety of sampling locations (e.g., Sashida and Kurihara, 1999; Ishitani et al., 2011, 2012a, 2012b, 2012c, 2014; Ishitani and Takishita, 2015; Decelle et al., 2012a, 2012b, 2012c, 2013, 2014), studies in 2000s–2010s frequently used plankton samples collected around Sesoko Island (Tables 1, 2).

Location, climate, and oceanographic condition

Sesoko Island (26° 38' 46" N, 127° 51' 54"E) is located 600 m west of the Motobu Peninsula of Okinawa Island (Honto). Sesoko Island is a 7.3 km circumference pear-shaped island with a population of 800 people, which is connected with the Motobu Peninsula by the Sesoko Bridge (Sesoko-Oh-hashi; 762 m in total length). The Sesoko Station is located on the east coast of the island (Fig. 1).

Okinawa has a subtropical climate so that the air temperature is hot in summer (av. 28°C, min: 24°C, max: 33°C) and warm in winter (av.: 16°C, min: 10°C, max: 25°C). Late October is presumably 21–23°C on average. From July to early October, typhoons often cross over Okinawa. The temperature of surface water at the sampling site off Sesoko Island on 31 October in 2016 was 28.0° C.

Marine organisms around Sesoko are affected by the Kuroshio Current, a northward-flowing, strong western boundary current derived from the westward-flowing North Equatorial Current off the east coast of the Philippines (Fig. 1A). The Kuroshio Current is characterized by warm, high salinity and low nutrients; as a consequence, the radiolarians around Sesoko Island represent a subtropical fauna.

Travel information: transportation

The capital city of Okinawa, "Naha", is located in the south of the island, as is the Naha Airport. The Naha Airport is a hub airport for Okinawa and neighboring islands so that over 100 domestic flights are available every day. International flights are also available. The connection between the airport and city area of Naha is very easy via the Okinawa Urban Monorail (Yui Rail).

Public transit for Sesoko Island is not so convenient. A bus network exists on Okinawa Island but bus services to Sesoko Island are limited in number. Frequent bus services are available between Naha and Nago, located at the base of the Motobu Peninsula (Fig. 1B). Taking a taxi is the recommended way to get to Sesoko Station from the Nago Bus Terminal.

Life in the Sesoko Station

For the use of the Sesoko Station, you need to submit an application form prior to your visit. Detailed information is available at the following web page: "User Instructions for Sesoko Station, Tropical Biosphere Research Center, the University of the Ryukyus" [URL1].

1. Equipment

Guests can use a laboratory, a lecture room, and a galley in a building upon request. Any equipment such as beakers and Kleenex is not provided by the station (Table 3). Thus, the hosts or group leaders must bring all equipment and facilities including microscopes, bottles and consumables themselves. Furthermore, the hosts or group leaders must receive and dispatch baggage by themselves in the station. Other attendees are responsible only for private daily commodities like extra clothes and shoes.

2. Accommodations

Accommodations for "the Okinawa Tour" are arranged by the host or group leader.

3. Meals

There is no meal supply in the Sesoko Station, so that you need to go out for meals or prepare food by yourself. If your group gets permission to use the kitchen in the station, you can cook by yourself.

The day of sampling is special. In general, sampling will start in the morning (~ 9 o'clock) with the sailing of the boat. The boat will dock again by 11 o'clock. Onshore, the living radiolarians must be picked out from the sampling bottles as soon as possible to keep them healthy. For this reason, there is no time to have lunch, so light meals (e.g., snacks) might be better on sampling day.

From sampling to observations

This chapter explains the processes from preparation of sampling tools before a sampling day to observational methods and techniques for living radiolarians. For each process, an outline is given first, followed by the detailed steps.

1. Before the sampling day: Setup of the laboratory and checking sampling tools

Outline: All equipment and facilities, including a plankton net, should be checked by each sampling group and set up in the laboratory as compactly as possible, before the sampling day.

(1) All equipment and facilities should be set up in the laboratory at least one day before the

Table 3. Example list of equipment for collection and observation of living radiolarians.

Goods	Notes	Prepared by... participant	host
Microscope			
Inverted microscope	4X, 10X, 20X(LWD), 40X(LWD) objective lens		+
Digital video (or camera)	equiped to microscope		+
*Binocular microscope	If you pick up larger radiolarians		+
*Lights for bioncular microscope			+
*Upright microscope with water objective lens			+
USB flash drive	The people who want to bring back the captured photos	+	
*Tool kit	including tools to assemble and disassemble the microscope		+
Pick-up tools			
Adjustable Air Displacement Pipette	P20 (0-20 µL). Using to pick up radiolarians under a microscope		+
Disposable Tip	for your selected air displacement pipette		+
Transfer pipettes	variable sizes		+
Measuring pipettes	Move collected water to a dish. It is preferable to use the pipettes with larger opening at the tip (5 mL type preferable)		+
Glass petri dish	To find radiolairans cells from the collected water. Size is vabile for your purpose and microscopes		+
Plastic petri dish (or cell culture dish)	35 mm in diameter or like. This plastic petrish is used instead of large glass petri dish		+
Observation tools			
Flat bottom cell culture plates (=multiwell inesert system)	6-well (each cell diameter is 3.5 cm) and/or 12-well (2.26 cm), for hading radiolarian cells		+
*Glass bottom dish (= cell imaging dish)	The bottom of the dish is made of cover glass		+
Slide glass			+
Cover glass	18 mm x 18 mm, 24 mm x 32 mm		+
*Imaging plate cover glass (glass bottom well culture plate)	Similar to flat bottom cultureplates but its bottom is made of cover glass		+
Clear glass vials with screw caps (screw vials)	SV-20 is recommended		+
Consumers			
Aluminium foil			+
Dry wipes and cloths	To wipe on the desk, microscope and anywhere with sea water		+
waste bag			+
Sampling tools			
Cotton work gloves	To use on ship		+
Raincoat jacket and pants	For savety, you may not ware a long rain coat. Raincoat jacket and pants should be weared prior to your boarding.	+	
Shoes aviable wet conditions	Slippers, moccasins, ballet flats, sandals, high-heeled, and pump are strongly prohibited on boarding	+	
Plankton nets (hand net) with ropes	The length of rope is roughly 15 m		+
Wide mouth plastic bottle(jars) with screw plastic caps	1 L or 2 L. Keeping each towing sample onboard and in the labolatory		+
Bucket and water tank	Seawater at the sampling point is ready for labolatory work		+
GPS			+
Portable salinity meter, temperture meter, etc.			+
Travel sickness tablets		+	
Extra clothes for change after sampling	The station strongly prohibts to eneter into the building with wetted condition	+	
Towels	See above	+	
Protections for sunlight	sun glass, sunscreen	+	
Sorting tools			
Sieves	ca. 7.5 cm in diameter. 30–50 µm, 63–65 µm, and 1–4 mm openings are recommended		+
Stationery products			+
Chemicals and special equipment			
Ethanol	For DNA analysis		+
*Formalin	To examine stains		+
*Hydrogen peroxide	To revmoe protoplasm		+
*Sulfuric acid	To burn out protoplasm		+
*Millipore membrane filter holder assembly			+
*Manual operated vacuum pump			+
*Millipore membrane filter holder assembly	0.025 µm pore size for DNA and 0.45 µm pore size for other purposes		+
*Microtubes	To keep a single cell		+

planned sampling day.

(2) Size and material of the plankton net will depend on your research purpose. Matsuoka (2007) used 44 or 100 μm mesh plankton nets, while Suzuki et al. (2009) used a 38–43 μm mesh plankton net. We have also used larger cod-ends (the small, tip end) of the plankton nets for collecting living radiolarians.

(3) Sampling tools should also be prepared so that they can be easily transported to the research boat on the sampling day. The boat is so small that hosts and leaders should minimize the duplication of sampling tools, e.g. by sharing among sampling groups.

2. On the sampling day: Decisions and sailing

Outline: Going out or canceling the sampling trip is decided by the boat's captain. If we are given a go, we will put on our life-jackets and board on the boat with our sampling tools and personal bags. While cruising from the port to a sampling location, everyone must remain seated on the floor of the boat.

(1) The host will first discuss the sampling plan, date and hour with the captain of the boat at the Sesoko Station.

(2) The final decision on going or canceling sampling is made by the captain early in the morning of the chosen sampling day. The decision will depend largely on the weather forecast, especially on the sea surface roughness.

(3) As soon as we have the captain's approval to go out, our sampling tools should be brought quickly to the boat. Personal belongings will need to be put in a single waterproof bag.

(4) Shipboard participants should take along a change of clothes as well as a normal pair of shoes for after landing, which should be left somewhere outside of the building. Before boarding, we need to put on a rain jacket, coat, and a pair of shipboard shoes. Bring a drink as needed. It is obvious that while on board that we can become completely soaked with seawater, so that electric devices which are non-water proof such as mobile phones should be wrapped in a plastic bag or left in the building. If you get seasick easily, taking travel sickness tablets is recommended before boarding.

(5) Once the captain arrives, you will receive and put on the life-jacket from the lateral side of the boat (Fig. 2A), and take the sampling tools and your personal bag on board. Before you board on the boat, the safety instructions will be provided. Please follow the instructions to the letter for our safety.

(6) Soon after you board the boat in port (Fig. 2B), (a) you must sit down on the floor; (b) never stand up, never move, never go to the foremost part of the boat (bow); and (c) MUST keep your hand away from the any edge of the boat at all times (in particular, while in port). You may stand up and move when the boat stops at a sampling location.

(7) It may take 20 to 30 minutes to reach the sampling station from the port.

Fig. 2. Photographs of collecting and observational methods of living radiolarians. **A:** Sesoko Station and boat dock. **B:** Research boat for collecting living plankton. **C:** Plankton net streaming in the current. **D:** Pulling up the plankton net. **E:** Transferring plankton-bearing sea water to a bottle. **F:** Picking up radiolarians by using a binocular microscope. **G:** Transfer of a radiolarian individual to a flat bottom cell culture plate.

3. Work at the sampling station: Measuring water characteristics and collecting plankton-bearing seawater

Outline: At the sampling station, we will begin by measuring physical oceanic data. Then each sampling group will do their tows with the plankton net, each in their own working space. After several minutes (typically 5 minutes), each group will pull on the rope of the plankton net and quickly transfer the plankton-bearing seawater from a collecting bucket to a sampling bottle.

(1) When the boat arrives at the sampling station, the captain will stop the engine to avoid rolling the plankton nets together. People who get seasick should sit on the floor near the edge of the boat and try not to get in the way of the sampling. In particular, you must not lean over the boat edge all the time.

(2) At first, physical oceanic data (position, water depth, salinity, water temperature etc.) will be acquired. While this is being done, each sampling group should get their own plankton nets and work spaces ready. The spaces will be assigned by the host. Sampling groups can start sampling when ready. Tie the rope firmly to the boat to avoid losing your plankton net.

(3) Tow your plankton net carefully so as not to get entangled with other nets and ropes. Plankton nets are gently deployed into the sea with the opening facing into the direction of the wind (Fig. 2C). No gear to hoist the net out of the water is available on boat.

(4) The net should be towed from 3 to 7 mins (regularly 5 min) to capture a sufficient volume of plankton. The water depth to tow the net at depends on the sampling plan. During sampling, seawater will be collected in sampling bottles for use at the onshore laboratory.

(5) Recover the net at the end of the towing time by pulling on the rope (Fig. 2D). Before you completely pull it out the water, the interior cod-end of the net should be carefully washed with seawater to recover attached plankton. This attached plankton is handled differently depending on sampling purpose. For estimation of the total biomass, all plankton must be put into a collecting bucket. But for observing healthy radiolarians, one should never use the attached (= damaged) plankton from the net and the cod-end, so for studying healthy radiolarians do not put attached plankton in the collecting bucket.

(6) Soon after the plankton net is drawn back on the boat, the plankton in the collection bucket is gently transferred to the sampling bottle with additional fresh seawater (Fig. 2E), to decrease the planktonic density in the bottle. The bottles are kept in a cold box to maintain the freshness of the samples. The bottles with plankton should be full of seawater.

(7) Repeat towing several times as needed. However, we should go back to the Sesoko Station as soon as possible to begin the separation of specimens, so sampling repetitions should be limited.

4. Return to the Sesoko Station: Flushing seawater before entering the laboratory

Outline: On return the port, everything exposed to saltwater should be washed with freshwater. Participants should also take shower and change clothes.

(1) After we finish sampling, all things should be cleared up promptly as the boat may leave again.

(2) After we return the port, all tools and samples should be brought back to the laboratory. However, bringing things wet with seawater inside is strictly prohibited by station rules so that everything, including the participants, needs to be washed with fresh water before we enter in the building. A few members should be chosen to shower first so that they can bring the samples quickly inside. The remaining people can then bring the tools to the shower room where they are washed with freshwater. We then take our own showers and change to dry clothes and shoes.

(3) The washed but wet items should only be brought into the laboratory after being wiped out completely.

5. First few hours in the laboratory: First sorting and observation

Outline: The first step is to extract the fresh radiolarians from the collected seawater in the sampling bottles using binocular stereomicroscopes and/or inverted microscopes. The radiolarians are transferred to flat bottom cell culture plates.

(1) Living radiolarians will weaken quickly within a few hours under the "plankton soup" conditions that exist in the collecting bottles. Fresh radiolarians must thus be extracted as soon as possible. Due to this reason, we should separate radiolarians from other plankton before the making any observations.

(2) In order to settle the radiolarians at the bottom of the bottle, the sampling bottles are gently placed for 5–10 minutes near the sink for sea water in the laboratory.

(3) While waiting, fill each cell of the flat bottom cell culture plates with the seawater which was collected at the sampling location (2/3 height of each cell is enough). The flat bottom cell culture plates are sterilized, so they don't need to be washed when using a new one. This is true as well for the other observation and picking tools.

(4) To start, bring a glass petri dish or a plastic petri dish to the sink where a bottle is placed, transfer an appropriate volume of plankton soup into the dish, and then wipe off the dish bottom. The bottom of the dish must *always* be kept completely dry.

(5) The dish is carefully placed on the stage of a microscope.

(6) Two types of microscopes are used: the Niigata University research group regularly uses the binocular stereomicroscopes, while the Tohoku University research group uses the inverted microscopes.

6a. Binocular stereomicroscope – Good for picking large radiolarians in a wide area.

6b. Inverted microscope – Good for picking any size of radiolarians but view area is narrow.

(7) Start to pick-out radiolarians (Fig. 2F). If you are interested in polycystines, it is better for you to ignore acantharians because they are easily misidentified as spumellarians. Radiolarians can be picked up with an adjustable air displacement pipette (P20 and yellow disposable tips), transfer pipettes, or other tools.

(8) Once a radiolarian cell has been siphoned into a pipette, quickly transfer it to the flat bottom cell culture plate (Fig. 2G). Effective techniques to avoid damage to the radiolarian cell are as follows. 1) You don't need to check whether you successfully moved a cell or not. 2) You don't need to be concerned if other organisms get transferred along with the target radiolarian specimen. 3) If you use a 6-cell flat bottom cell culture plate, 10–20 radiolarian specimens can put together in a cell while 5–10 radiolarian specimens can be placed in cells of 12-cell flat bottom culture plates. 4) It is not recommended to mix unhealthy-looking specimens with fresh ones. The extraction process continues for several hours until radiolarians in the plankton soup are no longer viable, or nearly dead.

6. After first observation: Second sorting

Outline: If healthy radiolarians could not be found in the first order samples, examine the remaining liquid in the sampling bottle as a second sorting.

(1) If you could not find any healthy specimen in the first order samples, move to the second sorting. Before starting, another flat bottom cell culture plate is prepared with fresh seawater.

(2) Under an inverted microscope, carefully look for radiolarians (10x or 20x objective lenses are regularly used). Radiolarians tend to stay on the bottom of the periphery of a cell in the flat bottom cell culture plate. Very healthy-looking radiolarian specimens sometimes float in the middle or upper level of the seawater, so these specimens may be overlooked under an inverted microscope. In this case, carefully tap the flat bottom cell culture plate to shrink their pseudopodia, which causes them to settle.

(3) In the second sorting, it is important to pipette individuals without contaminant substances.

Important notice on the laboratory work

(1) When handling seawater, take special care not to spill it on the microscope, desk or anywhere else except in the sink. All spills must be quickly wiped up. Once seawater gets into microscopes and electric devices, they will be damaged.

(2) In the first sorting, spherical radiolarians are often found with a lot of attached organic matter. As some radiolarians can shed this attached organic matter, pick them up and transfer them into a separate cell of the flat bottom cell culture plate. These specimens should be kept separated from other "clean" radiolarian cells.

(3) Tiny radiolarians like the Plagiacanthidae, Lophophaenidae, Stephaniidae, and Sethophormidae tend to be passed over in the first sorting not only under the binocular stereomicroscope but also the inverted microscope.
(4) Pylonioidea specimens tend to be wrapped with gelatinous adhesive protoplasm and look immobile.
(5) Several radiolarian species can be only found with phase-contrast imaging.

Concluding remarks

People generally find a fantastic plankton world under the microscope. We also have been fascinated by beautiful radiolarians and have been giving more than a passing thought to the long history of radiolarians and past climates. We believe anyone participating the Okinawa Tour will have this experience. The Okinawa Tour is not only a simple hands-on activity but also is one entrance to an important field in future radiolarian science. Matsuoka (2007) pointed out that research on living radiolarians would provide us with fundamental data for a better understanding of the past marine ecosystems. However, our knowledge on the role of radiolarians, even in modern marine environments, is still limited. As Suzuki and Not (2015) summarized in a recent research perspective on living radiolarians, very basic information on their biology and ecology, such as life cycle and feeding behavior, are still missing. As living radiolarians are found in open oceans just about everywhere in the world, anyone can start to study them. We do hope that this paper contributes to being able to handle living radiolarians as a first step towards your own studies.

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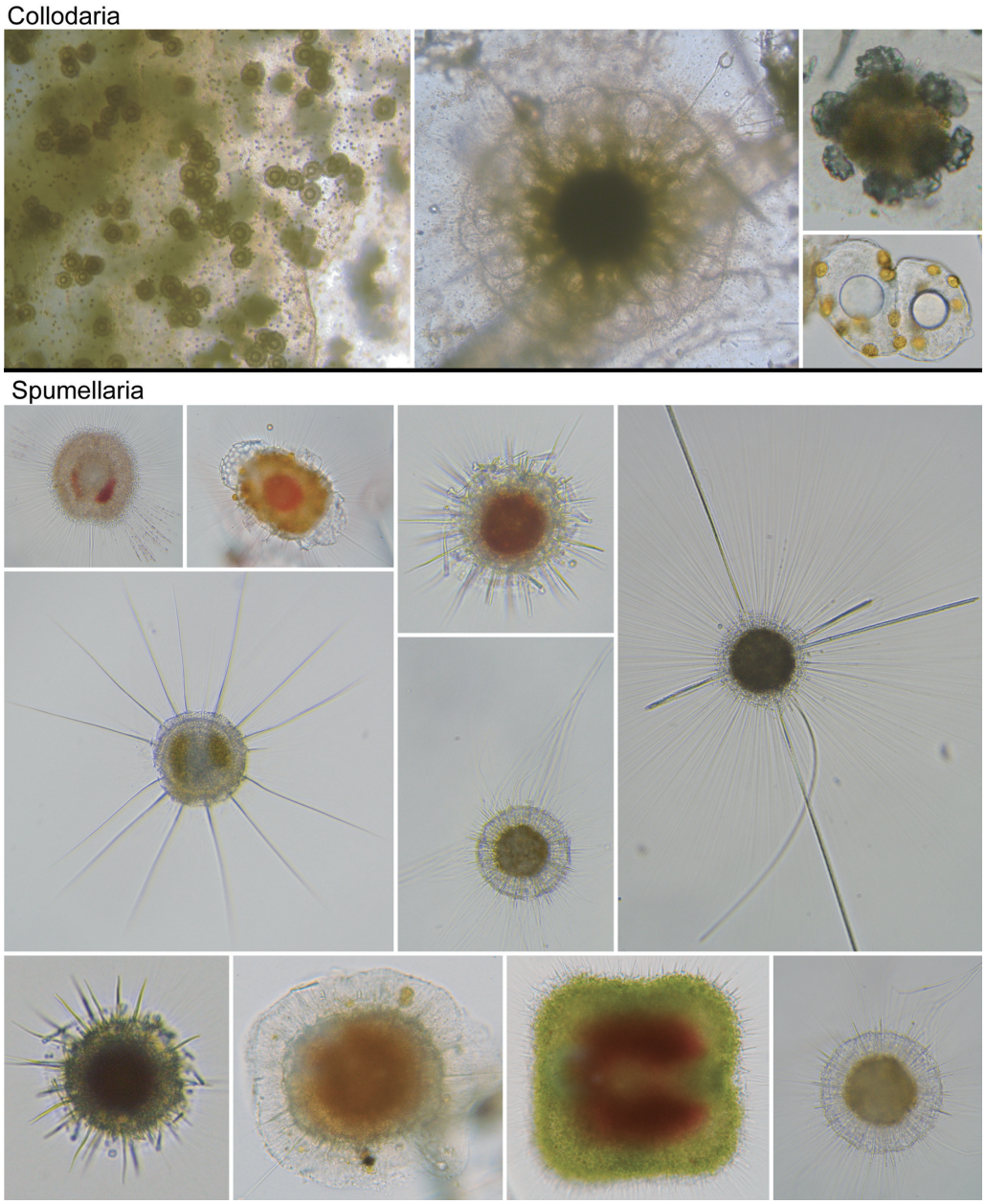
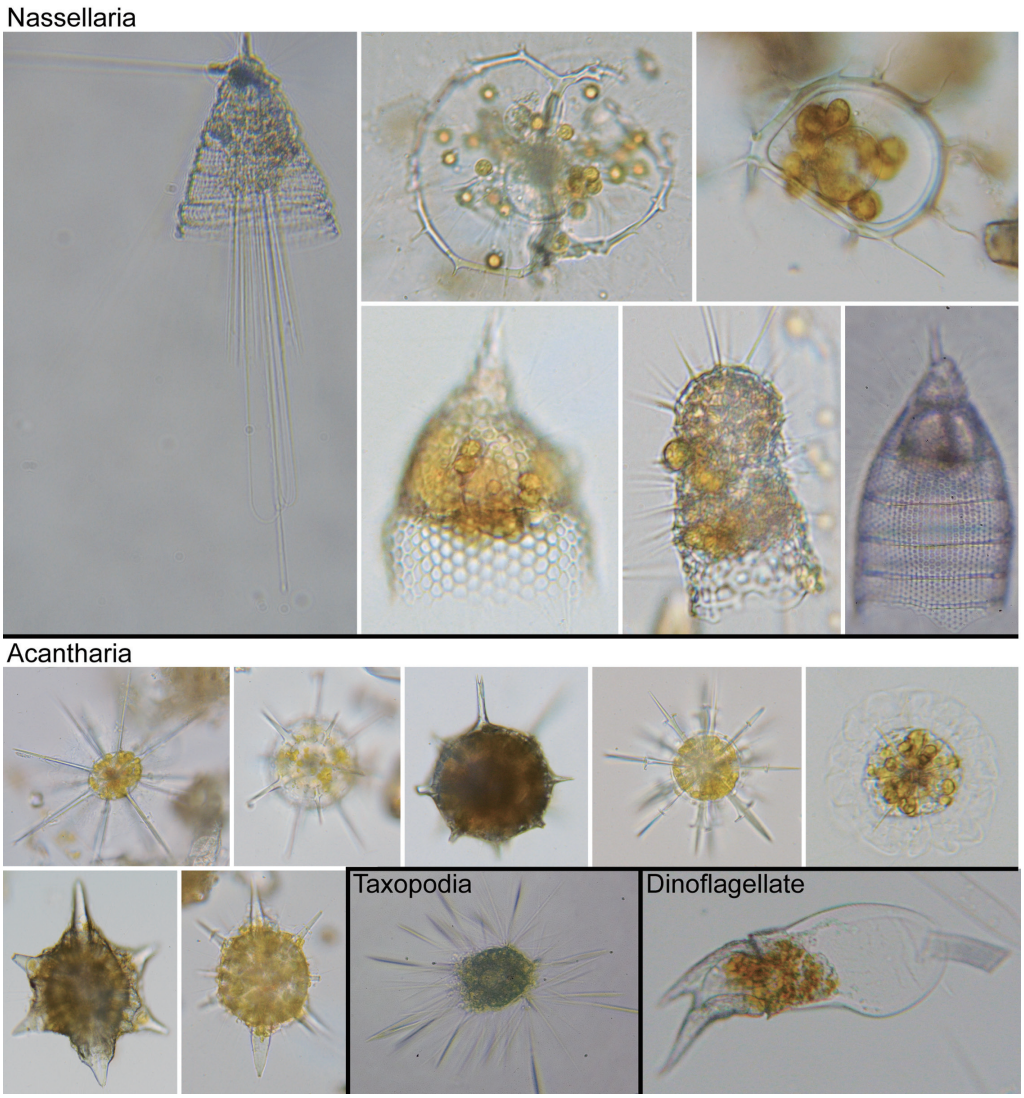
[URL1] User Instructions for Sesoko Station, Tropical Biosphere Research Center, the University of the Ryukyus (http://www.tbc.u-ryukyu.ac.jp/sesoko/user-information).



Appendix A. Photomicrographs of major living radiolarians (Collodaria and Spumellaria) collected from the sea water off Sesoko Island, Okinawa Prefecture, Japan.



Appendix B. Photomicrographs of major living radiolarians (Nassellaria and Acantharia) and other protists (Taxopodia and Dinoflagellate) collected from the sea water off Sesoko Island, Okinawa Prefecture, Japan.