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Foraminifera of the Kuril–Kamchatka Trench area: The prospects of molecular study

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ABSTRACT

Foraminifera remain poorly studied from deep-sea sediment settings, although they often dominate meiofaunal communities and represent an important part of the functional deep-sea diversity. Moreover, there is a striking gap in our knowledge of deep-sea Foraminifera since most of the foraminiferal diversity corresponds to single-chambered monothalamids displaying inconspicuous morphologies. The previous expeditions suggest that the foraminiferal biomass is large in the Kuril–Kamchatka Trench area, including many macrofaunal-size xenophyophoreans and komokiaceans. However, the on-site foraminiferal diversity remains poorly described and was never genetically examined. During the KuramBio expedition, we collected over 1400 specimens representing all major foraminiferal groups, focusing on monothalamids and particularly komokiaceans. From the deep-sea sediments, using four different sampling gears, we sorted, identified and photographed single specimens for DNA (or RNA) extraction. The material we report here will be used in our ongoing research on estimation and visualisation of the diversity of deep-sea monothalamous foraminifera and enigmatic taxa such as the komokiaceans, the origin of which is yet to be determined.

1. Introduction

Foraminifera represent one of the most abundant and diverse group of organisms living in deep-sea sediments. Besides species involved in key biogeochemical processes such as organic matter cycling (Enge et al., 2011) and constituting a substantial food source for macrofauna (Brökeland et al., 2010), the biodiversity and biology of Foraminifera remain poorly known from deep-sea setting. Only few studies have provided descriptions of foraminiferal community from the Kuril–Kamchatka Trench area (Khusid, 1979; Saidova, 1967, 1970). Their biomass has been estimated to reach 10 g/m^2 (Belyaev, 1989; Saidova, 1970) indicating large population turnover, but there is yet no molecular data available to understand the Kuril–Kamchatka Trench diversity.

Single-chambered Foraminifera, or 'monothalamids' constitutes at least 50% of the deep-sea foraminiferal diversity (Gooday, 2002; Gooday et al., 2004b). This is a paraphyletic assemblage of many lineages, forming the basal radiation of early Foraminifera (Pawlowski et al., 2003). Over the past ten years, many new species of deep monothalamids were described (Cedhagen et al., 2009; Gooday et al., 2004a, 2010, 2013, Gooday and Pawlowski, 2004). However, this is still a small fraction of their actual diversity, primarily because abyssal areas are among the world's most undersampled habitats, but also due to the patchy distribution of some species (Gooday and Jorissen, 2012). Many monothalamous Foraminifera are tiny species possessing inconspicuous morphologies and are often overlooked in deep-sea faunal surveys. Yet, some of them, like members of Xenophyophorea may attain giant sizes (Lecroq et al., 2009c; Tendal and Gooday, 1981). Another particularly interesting and heterogeneous group allegedly attributed to the monothalamids are the komokiaceans, which true identity remains enigmatic, despite a lot of described species (Gooday et al., 2007a, 2007b; Tendal and Hessler, 1977).

It is crucial to document the diversity of the Foraminifera living at abyssal depths by sequencing isolated specimens. Our previous studies show that the small subunit (SSU) of the ribosomal RNA gene is highly informative for inferring molecular phylogenies and to support species descriptions (Pawlowski, 2000, Pawlowski and Holzmann, 2014). Moreover, sequencing additional specimens fuels the reference molecular database (http://forambarcoding.unige.ch) with more information and thus ameliorates the taxonomic assignments of environmental DNA sequencing data (Pawlowski et al., in press). Indeed, with a good database and high-throughput sequencing of sediments sampled in contrasting regions, it was

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confirmed that the monothalamids represent the most diverse compartment of the foraminiferal diversity in the deep sea (Lecroq et al., 2011, Pawlowski et al., 2011).

Our participation on the KuramBio expedition offered a unique opportunity to compare and complete the current record of Foraminifera from the abyssal Kuril–Kamchatcka Trench area (KKT). Here, we report the number of foraminiferal specimens collected and identified during the KuramBio expedition, and their distribution per station according to gear deployments and the mode of preservation for molecular work. We underline the most promising outcomes with respect to molecular phylogeny.

2. Material and methods

During the *RV Sonne* cruise leg SO223, we took non-quantitative samples from 12 stations situated at abyssal depths on the KKT (Table 1) in order to isolate Foraminifera specimen for DNA sequencing. We recovered sediment samples using either a multiple corer (MUC) equipped with tubes of 10 cm in diameter, as well as from a box-corer (GKG), an epibenthic sledge (EBS) or an Agassiz trawl (AGT). From the MUC, we obtained at least 3 sediment cores per station, at a rate of one core per deployment. We systematically sieved the top 2 cm of sediments on board and at 4 °C through >63, >125, >250 and >500 μm fractions. Similarly for the GKG, we collected and sieved ca. 5 ml from the 2 cm of surface sediment, or we were allowed to pick large visible Foraminifera specimens. Occasionally, we subsampled ca. 5 ml of material from the >250 μm fraction of EBS and AGT catches that we directly screened for Foraminifera. We kept the sieved sediment fractions in deep-sea water at 4 °C filtered through 0.2 μm-mesh size filters, with c.a. 2 volumes of water for 1 volume of sediments.

Dark-coloured specimens or cells displaying cytoplasmic material were sorted and identified under a stereomicroscope Leica S8AP0. We photographed each collected specimen using a Color CCD camera mounted to the stereomicroscope (Imaging Source Inc.) before immersion in Guanidine isothiocyanate for DNA extraction (Pawlowski, 2000) or in RNAlater (Ambion) for both DNA and RNA extraction of later morphological identification of agglutinated Foraminifera and komokiaceans. We have also dried or preserved in ethanol some large and common Foraminifera specimens for further morphological analyses. We sent all isolated specimens frozen and they are kept at −20 °C in the laboratory (Geneva, Switzerland) prior to DNA and/or RNA extraction and Sanger sequencing.

Table 1
Sampling stations information. *n*: Number of specimen (per station or per deployment).

Station number	Deployment number	Date	Time	Coordinates		Water depth (m)	Gear
1	6	29/07/12	12:13	43°58,14′N	157° 19,90′E	5406	MUC
	7	29/07/12	16:31	43°58,19′N	157°19,81′E	5407	MUC
	8	29/07/12	20:40	43°58,14′N	157°19,78′E	5410	MUC
	11	30/07/12	13:37	43°58,10′N	157°24,06′E	5370	EBS
2	4	2/8/12	0:33	46°14,04′N	155°33,12′E	4869	GKG
	5	2/8/12	4:49	46°14,04′N	155°33,26′E	4871	GKG
	6	2/8/12	8:40	46°13,99′N	155°33,07′E	4871	MUC
	7	2/8/12	12:37	46°14,04′N	155°33,10′E	4869	MUC
	8	2/8/12	16:16	46°13,98′N	155°33,08′E	4872	MUC
	10	3/8/12	5:29	46°10,99′N	155°35,26′E	4797	EBS
3	7	5/8/12	1:16	47°14,33′N	154°42,41′E	4982	MUC
	10	5/8/12	19:14	47°10,22′N	154°36,62′E	5006	AGT
4	6	7/8/12	13:30	46°57,98′N	154°32,53′E	5769	MUC
	8	7/8/12	22:31	46°57,98′N	154°32,52′E	5771	MUC
5	5	10/8/12	9:56	43°35,01′N	153°58,10′E	5378	GKG
	7	10/8/12	18:03	43°35,01′N	153°58,03′E	5377	MUC
	8	10/8/12	22:13	43°34,99′N	153°58,04′E	5379	MUC
6	5	13/08/12	14:36	42°29,04′N	153°59,90′E	5299	GKG
	6	13/08/12	18:48	42°29,01′N	153°59,93′E	5297	MUC
	7	13/08/12	23:01	42°29,03′N	153°59,99′E	5297	MUC
	8	14/08/12	3:11	42°29,01′N	153°59,92′E	5296	MUC
7	5	16/08/12	17:32	43°2,29′N	152°59,15′E	5223	GKG
	6	16/08/12	21:46	43°2,30′N	152°59,12′E	5223	MUC
	7	17/08/12	1:48	43°2,30′N	152°59,00′E	5222	MUC
	8	17/08/12	5:43	43°2,32′N	152°59,15′E	5223	MUC
8	4	19/08/12	17:14	42°14,59′N	151°43,56′E	5127	GKG
	6	20/08/12	1:27	42°14,60′N	151°43,55′E	5125	MUC
	7	20/08/12	5:08	42°14,59′ N	151°43,50′E	5127	MUC
9	4	22/08/12	21:41	40°34,93′N	150°59,90′E	5407	GKG
	5	23/08/12	2:30	40°35,02′N	150°59,98′E	5404	GKG
	6	23/08/12	6:40	40°34,96′N	151°0,05′E	5412	MUC
	7	23/08/12	10:46	40°35,02′N	151°0,03′E	5402	MUC
	8	23/08/12	14:44	40°35,02′N	150°59,98′E	5404	MUC
	12	24/08/12	22:00	40°38,71′N	150°59,72′E	5385	EBS
10	6	26/08/12	5:53	41°12,00′N	150°5,75′E	5250	MUC
	7	26/08/12	9:48	41°11,98′N	150°5,72′E	5252	MUC
	8	26/08/12	13:41	41°12,01′N	150°5,70′E	5249	MUC
	12	27/08/12	20:26	41°8,44′N	150°5,53′E	5209	EBS
11	6	29/08/12	10:19	40°12,85′N	148°5,98′E	5349	MUC
	7	29/08/12	14:01	40°12,90′N	148°6,04′E	5348	MUC
	8	29/08/12	17:53	40°12,96′N	148°6,05′E	5348	MUC
12	3	31/08/12	18:24	39°43,39′N	147°10,03′E	5229	MUC