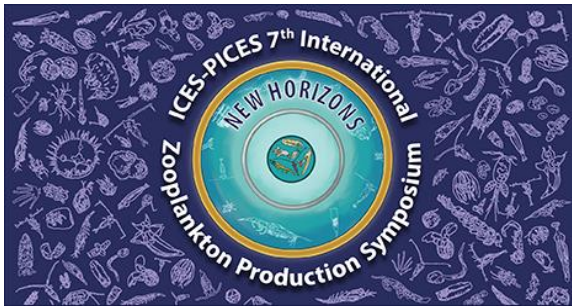


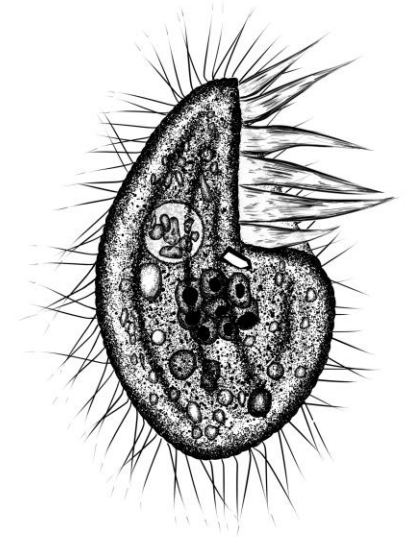
MINJIANG UNIVERSITY

A new *Protocruzia* species (Ciliophora: Protocruziida) isolated from the Mariana Trench area



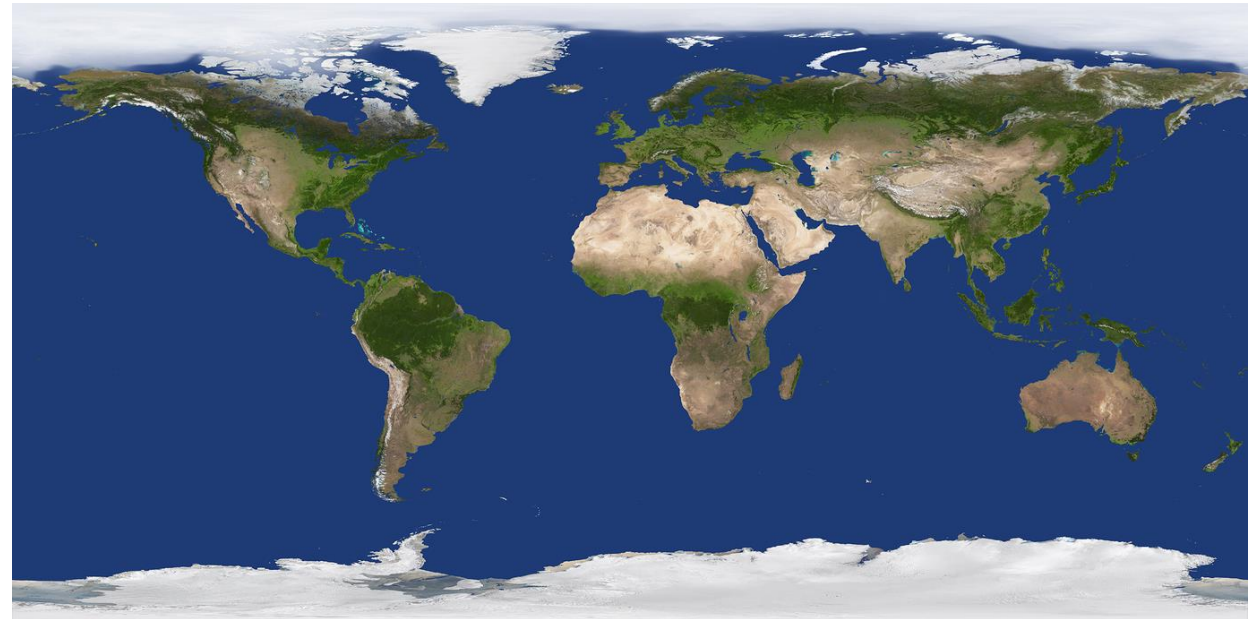
Dr. Chen Liang
College of Geography and Oceanography
Minjiang University

March 22nd, 2024 Hobart



Deep sea (over 200 meters depth)

- Ocean **71%** **Earth's surface**
- >1000 m **65%**
- > 200 m **95%** **Oceans' volume**

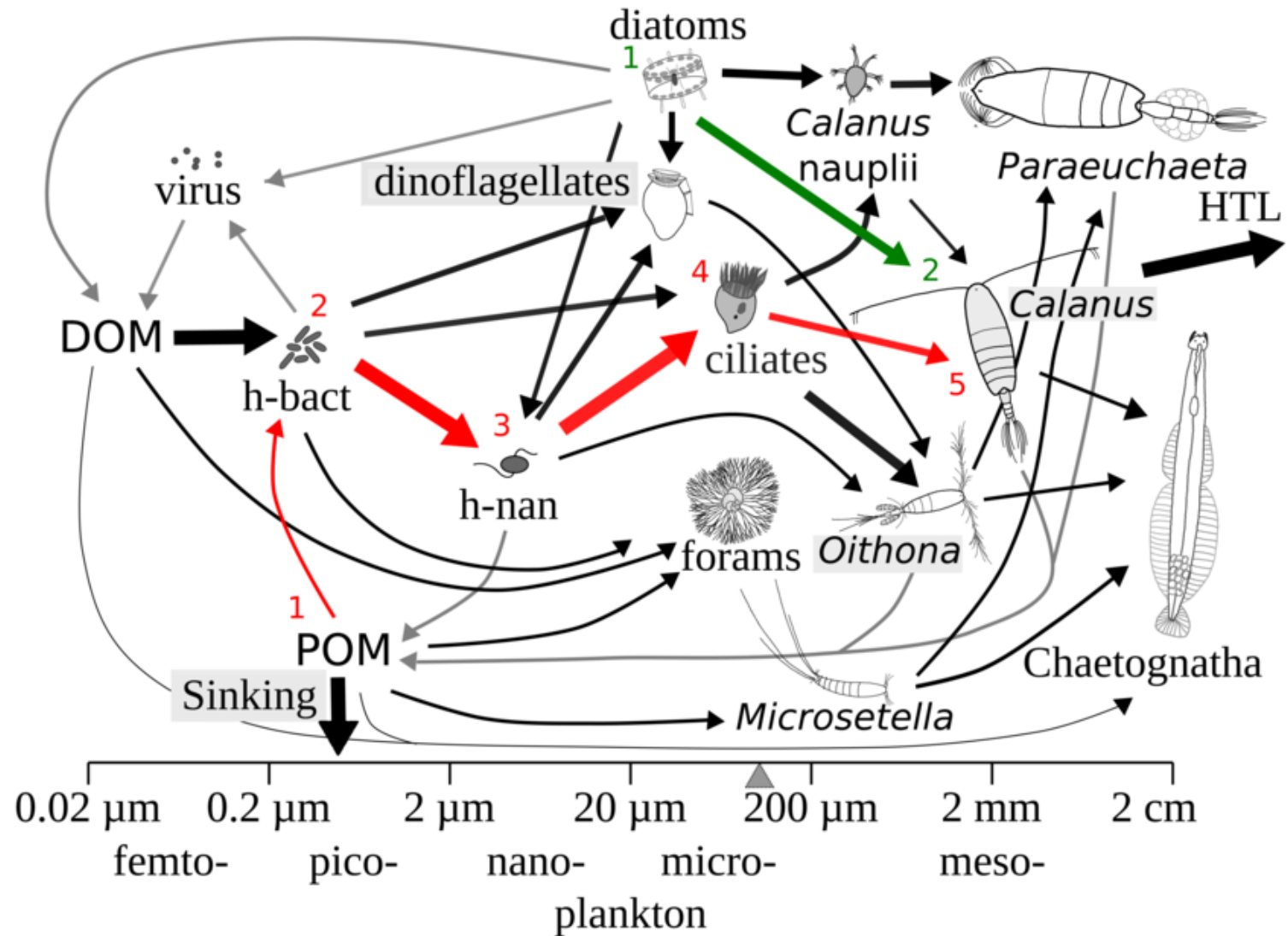


Less than **0.0001%** of the deep ocean's area has been investigated

<https://www.nespmarine.edu.au/>

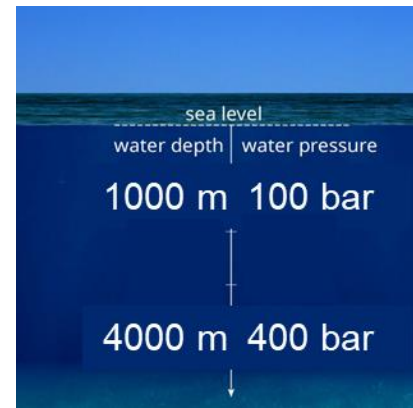
Danovaro et al., 2017

- Ciliates are an important, ubiquitous, and diverse group of microzooplankton.
- These organisms play an important role in microbial food webs in marine ecosystems.



Basedow et al., 2016

- Limited food availability
- Low temperature
- High hydrostatic pressure

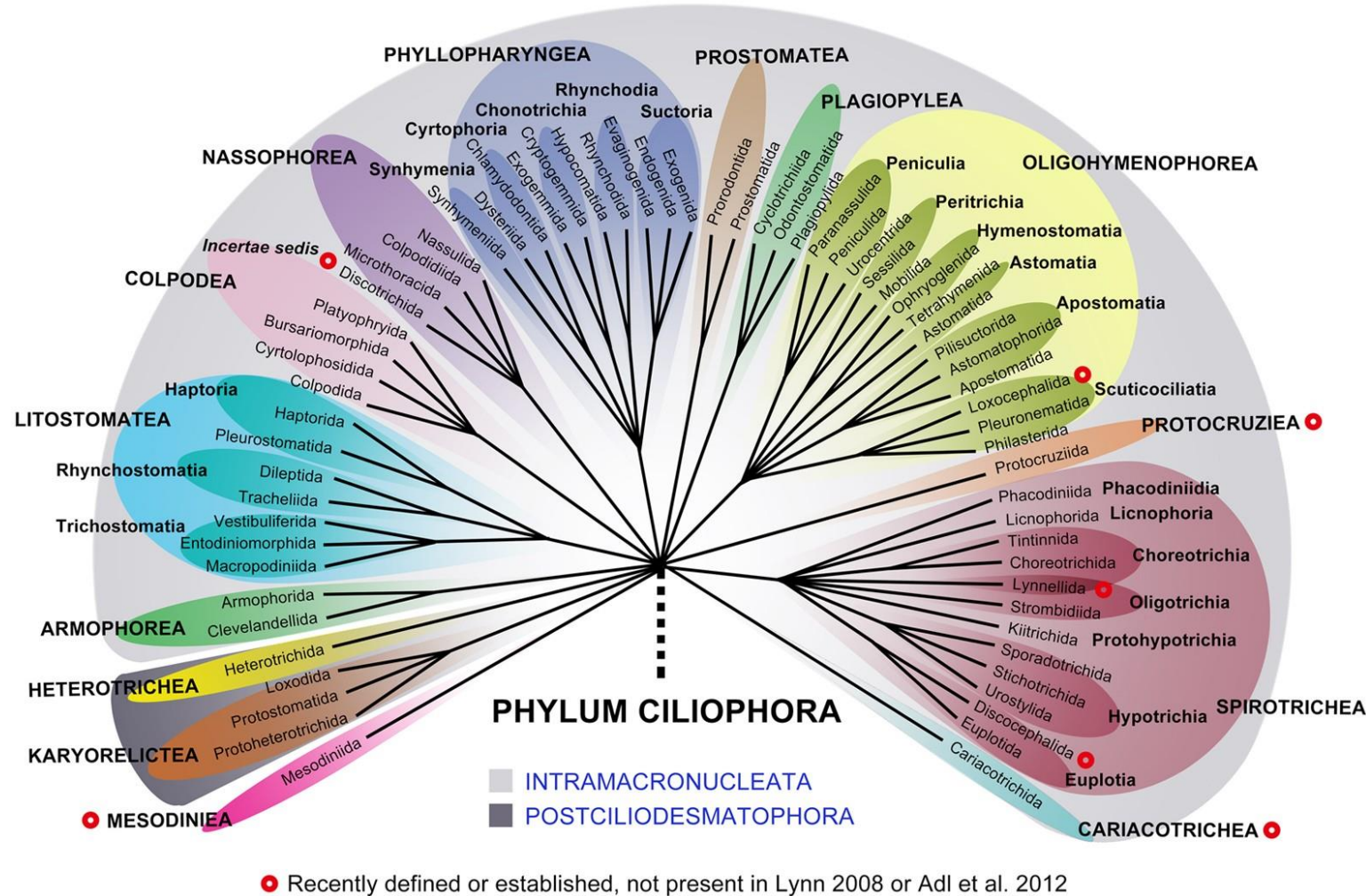


Strain	type	Sample depth (m)	Location	Reference
<i>Aristerostoma</i> sp. HFCC744	sediments	4296	23°33'N 48°5'W Atlantic Ocean	Živaljić et al., 2020b
<i>Euplotes dominicanus</i> HFCC757	sediments	4296	23°33'N 48°5'W Atlantic Ocean	Živaljić et al., 2020a, 2020b
<i>Pseudocohnilembus persalinus</i> HFCC814	sediments	2687	13°58'N 145°30'E Pacific Ocean	Schoenle et al., 2017
Ciliates <i>P. persalinus</i> HFCC822	Sea waters	5276	25°22'N 134°22'E Pacific Ocean	Schoenle et al., 2017
<i>P. persalinus</i> HFCC778	Sediments	4000	15°56'N 68°53'W Caribbean sea	Živaljić et al., 2020b
<i>Protocruzia marianaensis</i>*	Upper layer waters of sediment	3144/3092	11°13'N 139°56'E/11°48'N 140°7'E Pacific Ocean	Liang et al., 2021
<i>Uronema</i> sp. HFCC823	Sediments	5719	10°37'N 148°49'E Pacific Ocean	Schoenle et al., 2017

Only a few ciliate species were successfully cultivated in lab.

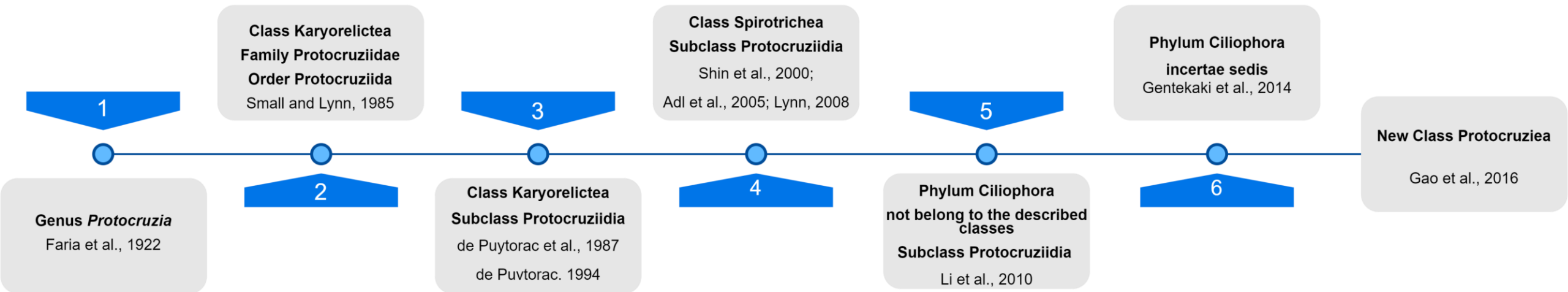
- DNA metabarcoding technology has been widely used in deep-sea investigations.
- Nearly all groups of Ciliophora were reported in deep sea (> 1000 m)

But not for Rhynchodia and Protocruziidia



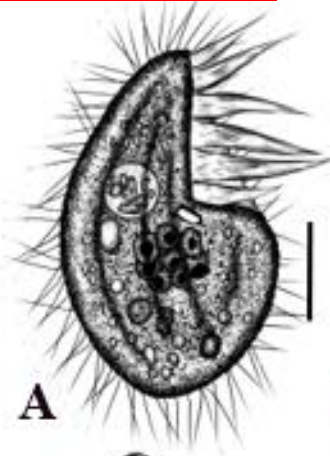
Gao et al., 2016

The systematic status of the ciliate genus *Protocruzia* has been somewhat ambiguous

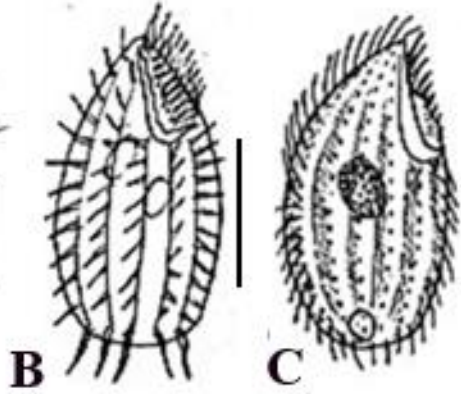


Up to date, only eight nominal species currently belong to this genus

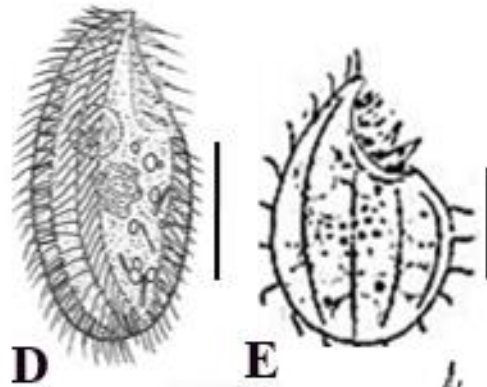
P. marianaensis



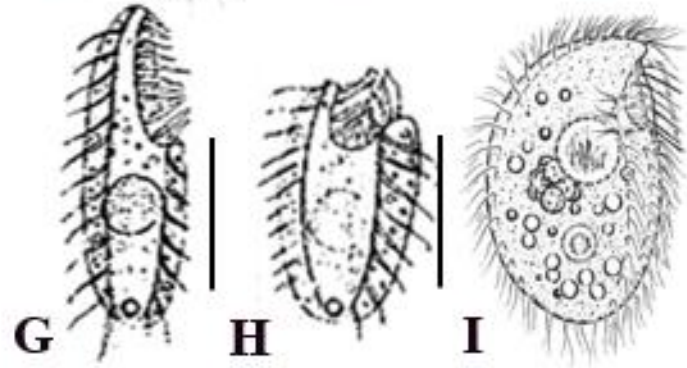
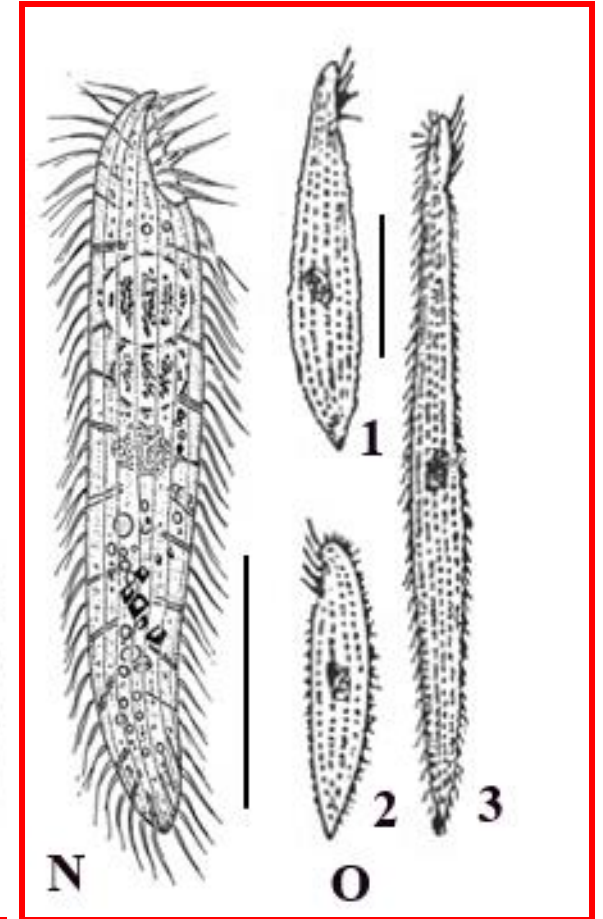
P. adhaerens



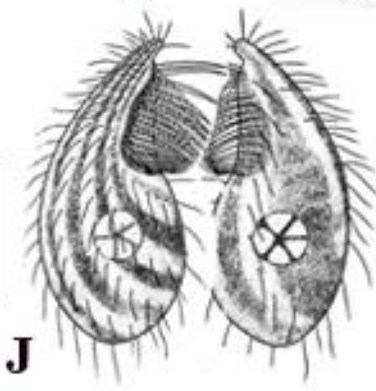
P. tuzeti



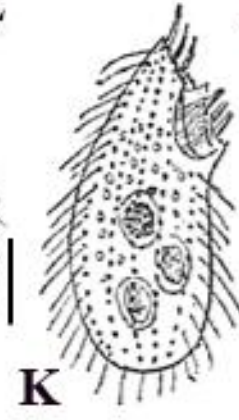
P. granulosa



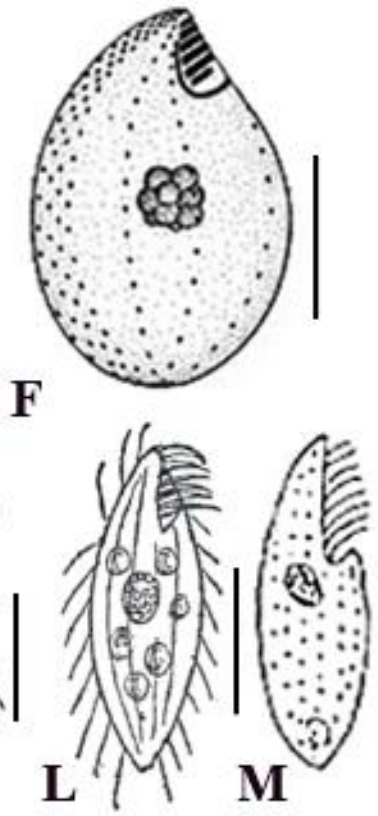
P. contrax



P. depressa

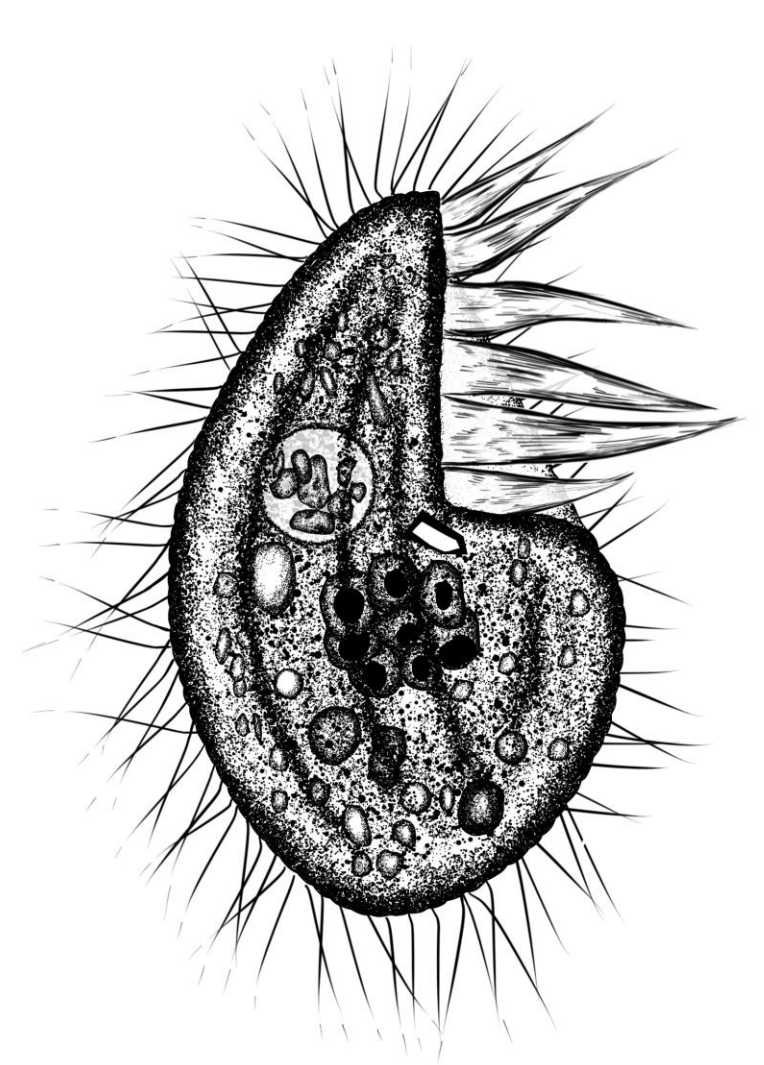


P. labiata

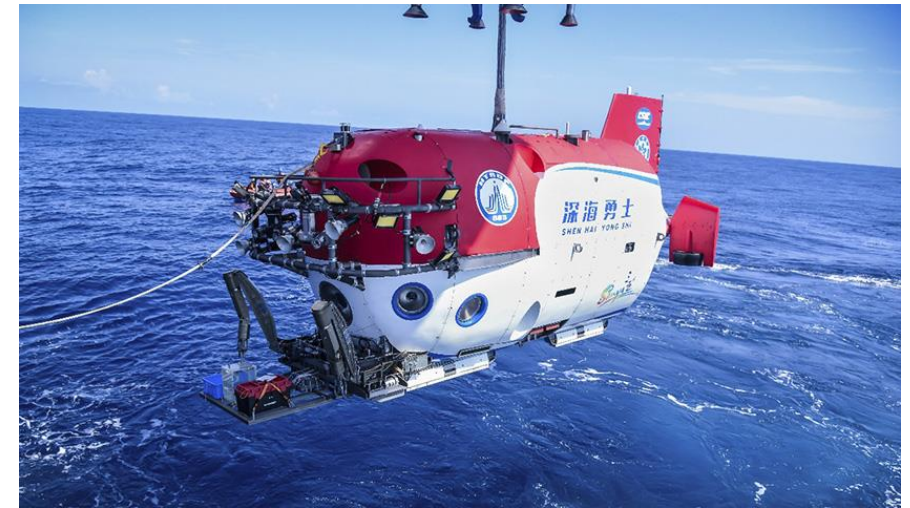
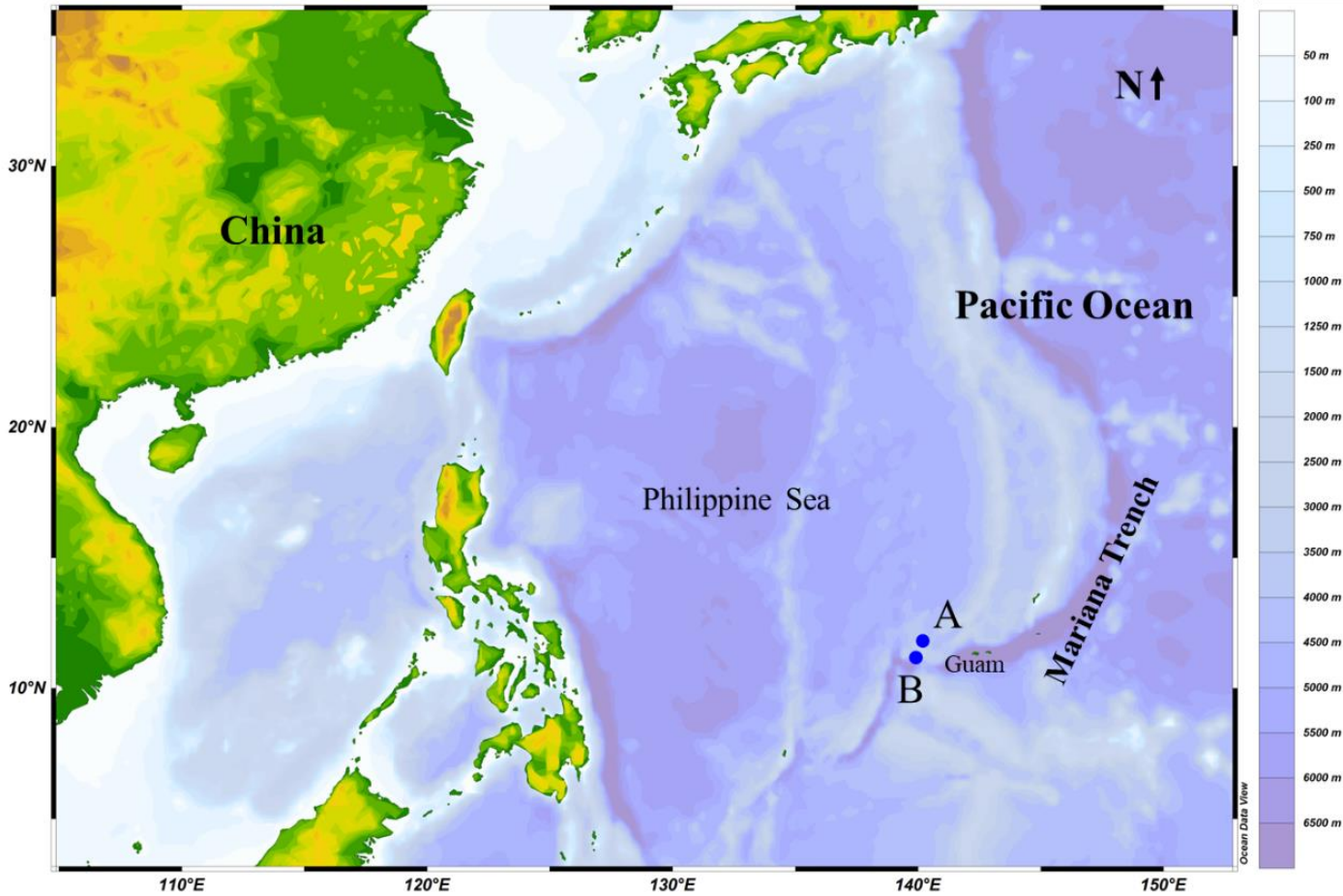


P. pigerrima

- All previous studies on *Protocruzia* species have focused on populations inhabiting relatively shallow nearshore waters.
- This is the first report about *Protocruzia* species as a bathyplankton in abyssal area.
- We succeeded in isolating and cultivating a new *Protocruzia* species from a depth of 3,000 m in the vicinity of the Mariana Trench.



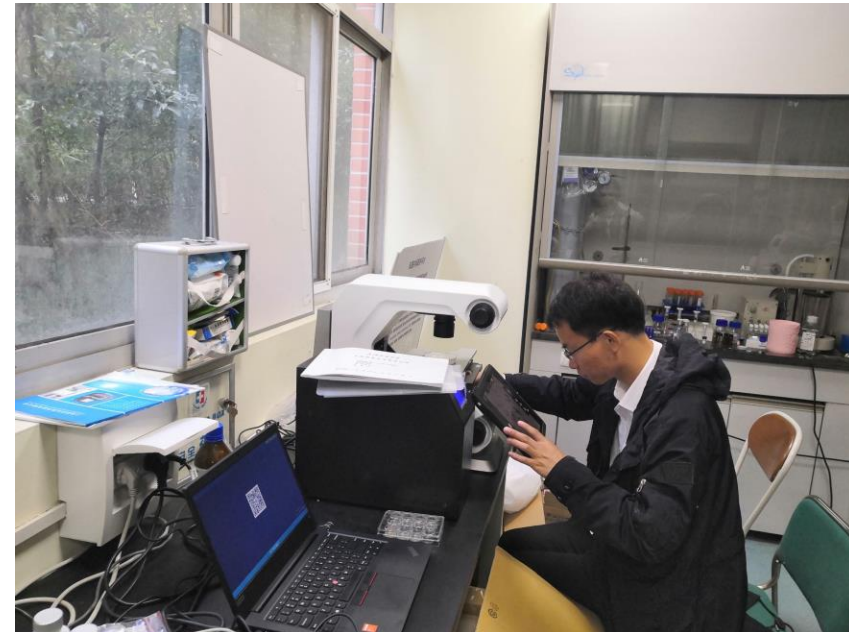
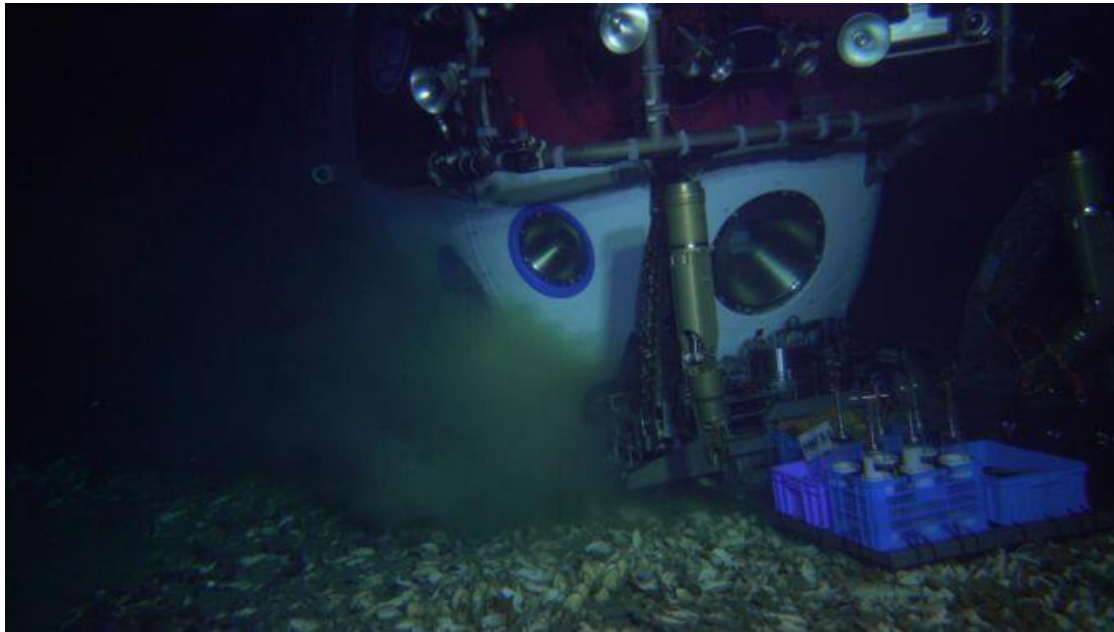
Sampling stations and process



A (11°13'9"N, 139°55'50"E 3144 m)

B (11°47'49"N, 140°7'6"E 3092 m)

- We sampled sediments and upper waters (34 PSU) immediately above the sediments were collected at each station.
- Live cells were observed and isolated under microscope.
- Single-cell cultivation was conducted.



- We added both one autoclaved grain and shrimp bait to promote the growth of bacteria in water.
- *Escherichia coli* and Luria Bertani medium were added according to growth.



E-coli

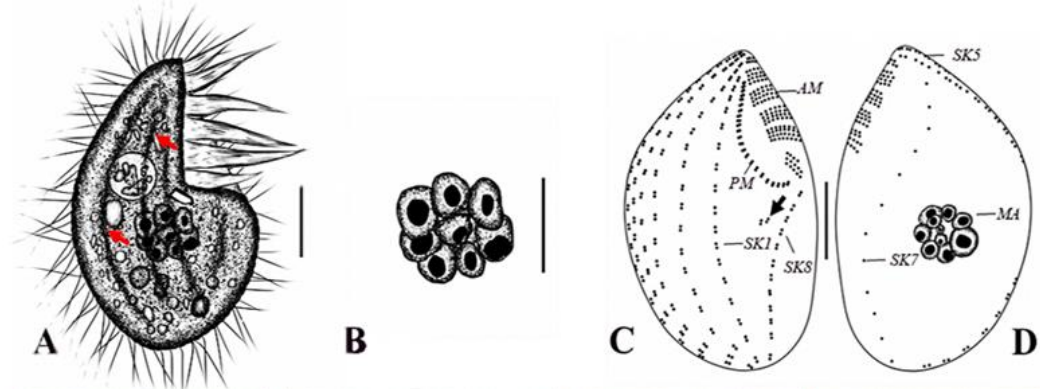


Luria Bertani medium

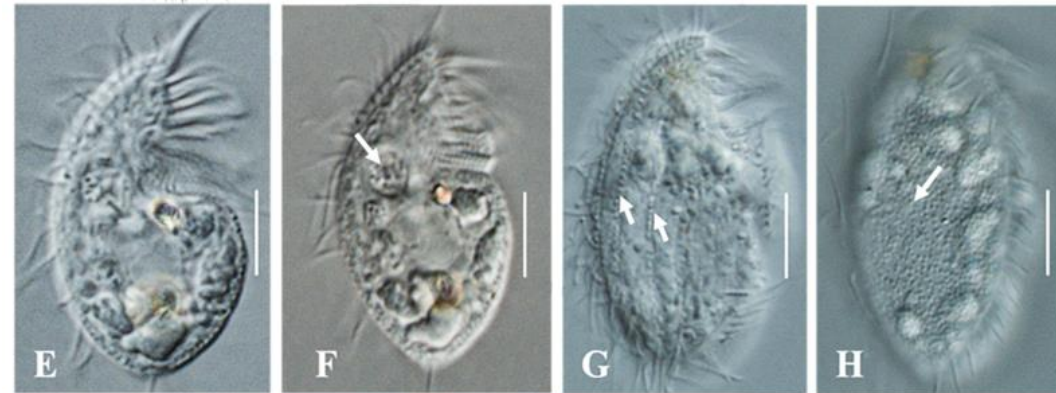
Taxonomy - Morphology

- Cell size *in vivo* approximately 25–32 × 14–17 μm
- Eight somatic kineties.
- One macronucleus located at the center of the cell, composed of 7–11 adjacent globules.

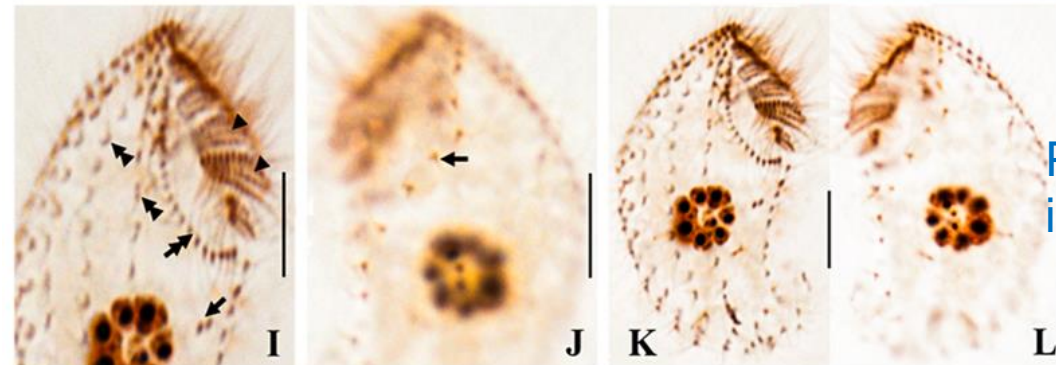
...



Sketch



Live cell



Protargol impregnation

Taxonomy - Morphology

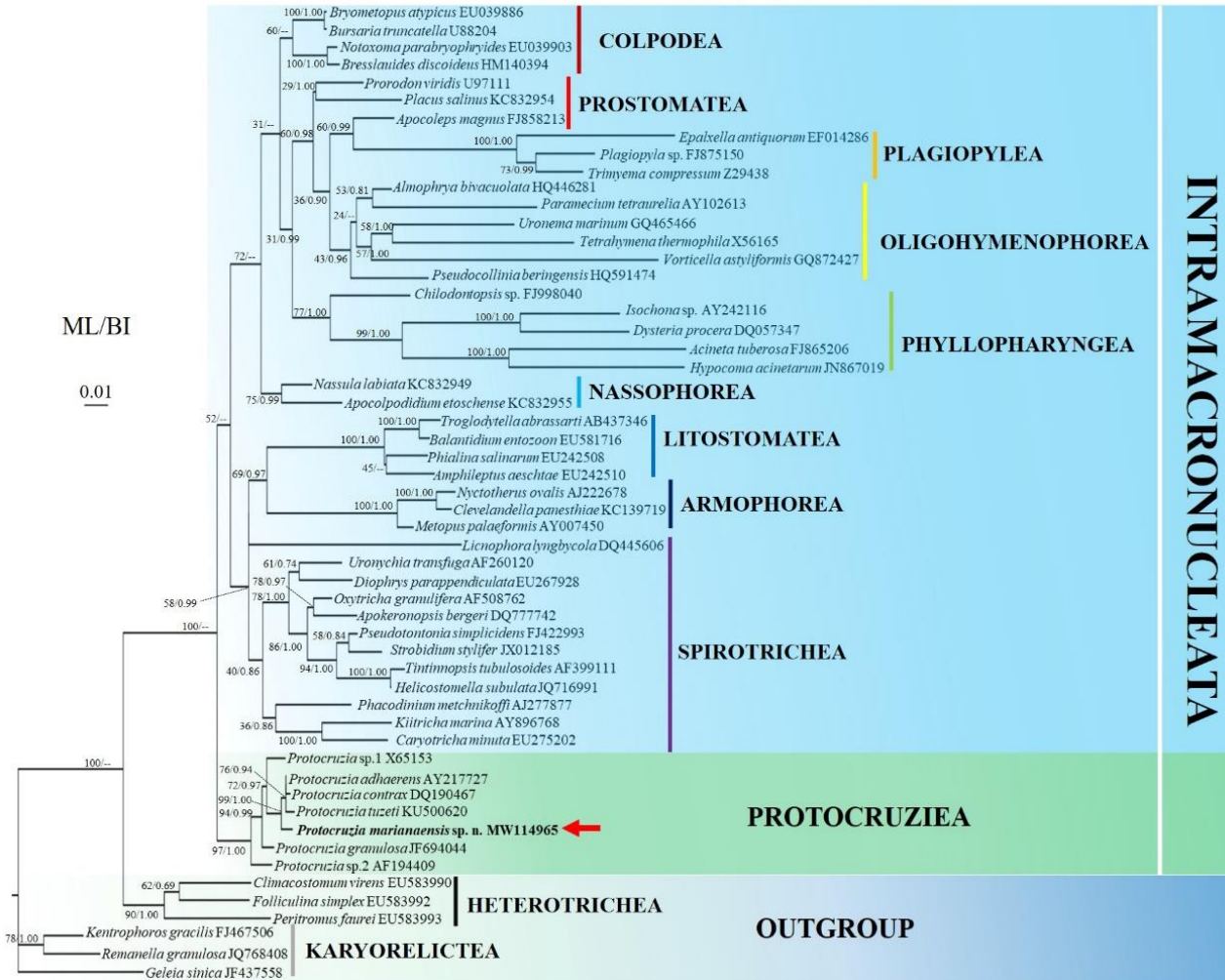
TABLE 1 | Morphometric data of *Protocruzia marianaensis* sp. n. based on *in vivo* and protargol-impregnated specimens.

Characters	Min	Max	Mean	Median	SD	CV	n
Body length (<i>in vivo</i> , μm)	25	32	28.8	28.7	2.1	7.2	20
Body width (<i>in vivo</i> , μm)	14	17	15.7	15.6	0.9	5.6	20
Buccal length (<i>in vivo</i> , μm)	10	12	10.7	10.6	0.7	6.1	20
Ratio of buccal length/body length (<i>in vivo</i> , %)	33	43	36.5	37.0	3.5	9.7	20
Body length (μm)	18	24	21.4	22.0	2.4	11.4	20
Body width (μm)	11	16	13.4	13.0	1.9	14.2	20
Buccal length (μm)	8	13	10.9	11.0	1.6	14.3	20
Ratio of buccal length/body length (%)	35	47	41.9	43.1	4.9	11.7	20
Number of adoral membranelles	6	6	6.0	6.0	0.0	0.0	20
Number of somatic kineties	8	8	8.0	8.0	0.0	0.0	20
Postoral dikinetids	1	3	2.0	2.0	0.7	42.5	20
Number of macronuclear globules	7	11	8.5	8.0	1.3	15.3	20
Macronuclei length (μm)	6	9	7.0	7.0	0.8	11.7	20
Macronuclei width (μm)	5	7	5.8	6.0	0.7	11.8	20

CV, coefficient of variation in%; Max, maximum; Mean, arithmetic mean; Min, minimum; n, number of cells investigated; SD, standard deviation.

Taxonomy – Phylogenetic analysis

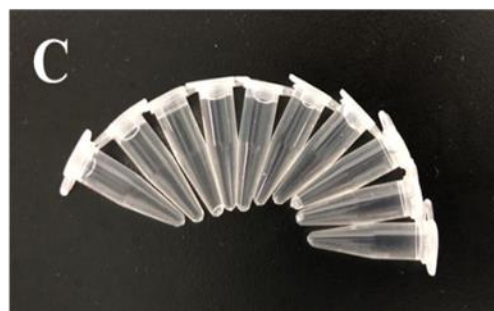
Small Subunit rRNA Gene Trees



- The sequence of the SSU rRNA gene, with length 1,791 bp and a GC content of 44.5%.
- three sequences most closely similar to that of *P. marianaensis* sp. n. are all *Protocruzia* species.

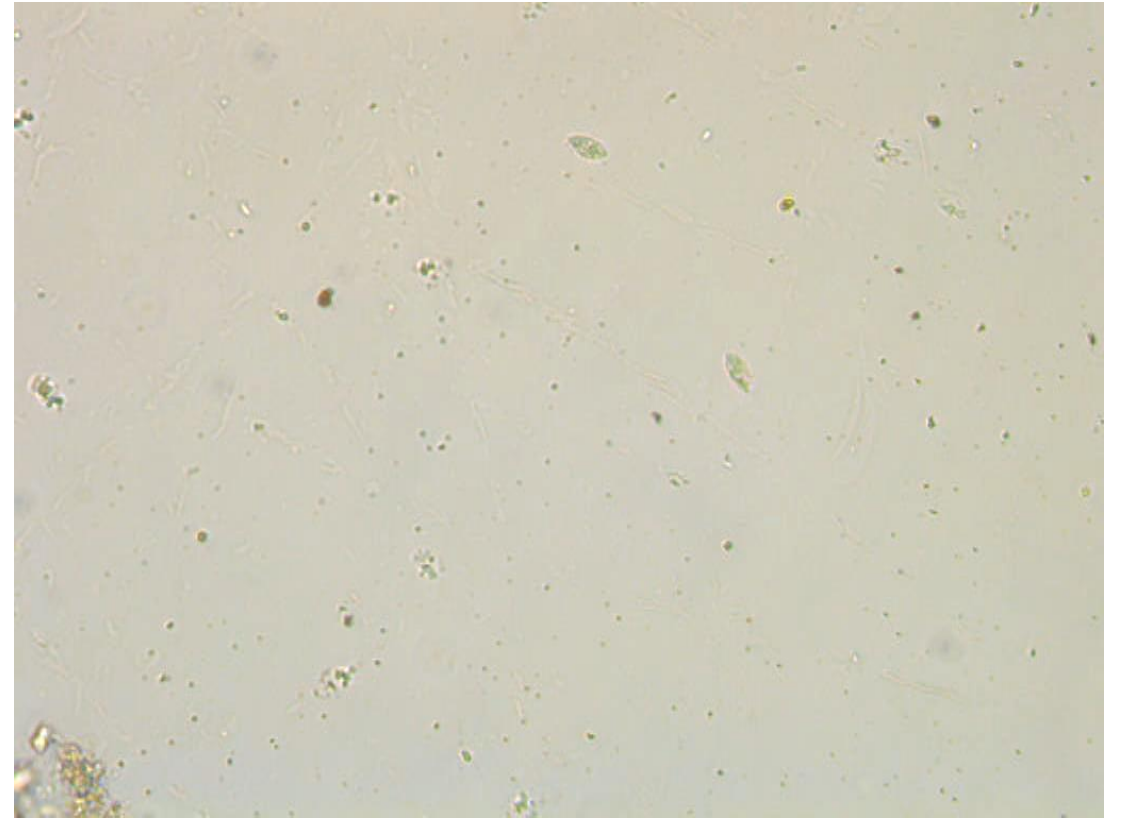
Deep-sea species, or species in deep sea?

- Indigenous species? Contaminated? Cyst?
- For these questions, we conducted a experiment of survival analysis





Video S1 *Protocruzia* cells in atmospheric pressure



Video S2 *Protocruzia* cells after high-pressure treatment

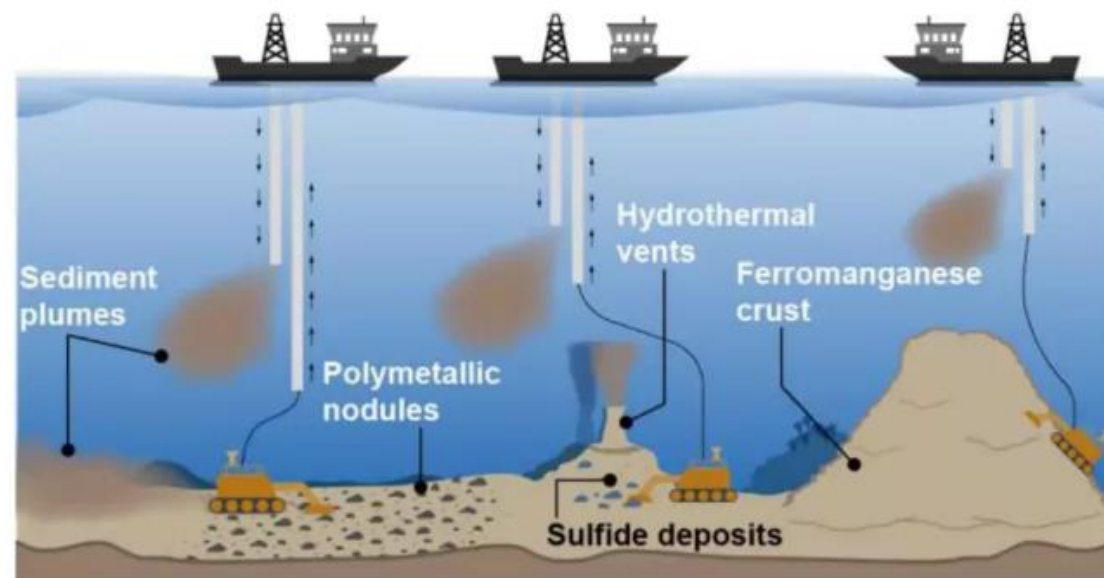
- we found no evidence to indicate cyst formation either in the hydrostatic pressure experiment or in cultures maintained at atmospheric pressure.
- These findings would tend to indicate that *P. marianaensis* sp. n. maintain activity at the depths at which specimens were originally collected.

The most reliable approach to establishing the actual status of ciliates inhabiting deep-sea regions is to microscopically examine *in situ* samples under pressure.

Work in progress and future work

Program:

Protozoan diversity assessment in deep-sea mining areas
and construction of germplasm resource bank (MED202303)



Source: GAO analysis of peer reviewed journal articles. | GAO-22-105507

Marine protozoan germplasm resource bank in Minjiang University



Thank you!



College of Geography and Oceanography
地理与海洋学院
Minjiang University
闽江学院

