Proc. 15th INA Conf., Bohol, Philippines
J. Nannoplankton Res. **36** (2), 2016, pp.133–136 © 2016 International Nannoplankton Association
ISSN 1210-8049 Printed by The Sheridan Press, USA

Xenospheres and anomalous coccospheres from plankton samples of the Southern Indian Ocean

Shramik M. Patil, Rahul Mohan*, Syed A. Jafar, Sahina Gazi
National Centre for Antarctic and Ocean Research (NCAOR), Headland Sada, Vasco-da-Gama, Goa-403804, India; *rahulmohan@ncaor.gov.in

Manuscript received 26th October, 2015; revised manuscript accepted 23rd July, 2016

Abstract New xenospheres and anomalous coccospheres are reported from the plankton samples collected during the austral summer (January–February) of 2010 in the Southern Indian Ocean. A new xenosphere consisting of a *Calcidiscus leptoporus* coccosphere and a *Syracosphaera nodosa* exothecal coccolith is documented. Another xenosphere consisting of different morphotypes of *Emiliania huxleyi* is illustrated. Additionally, an anomalous coccosphere of *Calcidiscus leptoporus* and a coccosphere with very variable sized *Oolithotus fragilis* coccoliths are recorded.

Keywords Xenosphere, coccolithophores, anomalous coccospheres, Southern Indian Ocean

1. Introduction

Coccolithophores predominantly produce coccospheres consistently formed of one or a few types of coccoliths. However, occasional records of combination coccospheres (consisting of two or more types of coccoliths which do not normally co-occur) have been recorded since the beginning of 19th century (Lohmann, 1902; Kamptner, 1940; Lecal-Schlauder, 1961), as reviewed by Cros *et al*. (2000a). These have now been shown to record life-cycle transitions (Cros *et al*. 2000b) and, owing to limited culture studies, the documentation of genuine combination coccospheres in recent years has added significantly to the recognition of life-cycle associations and led to changes in nomenclatural taxonomy based on the rules of priority (e.g. Alcober & Jordan, 1997; Cros *et al*., 2000b; Cros *et al*., 2000a; Cros & Fortuño, 2002). These combination coccospheres are typically heterococcolith-holococcolith combinations (Kleijne, 1991; Thomsen *et al*., 1991; Billard, 1994; Cros *et al*., 2000a; Cortés, 2000; Cortés & Bollmann 2002; Geisen *et al*., 2002; Frada *et al*., 2009) and less frequently heterococcolith-nannolith combinations (Cros *et al*., 2000b; Sprengel & Young, 2000).

During the terminology workshop of the 4th INA Conference (1991) in Prague, the concept of combination coccospheres was discussed. The term 'xenosphere' was proposed by Jackie Burnett and recommended it for the terminology guide that ultimately followed (Young *et al*., 1997). The term xenosphere was defined therein as: "*Anomalous coccosphere containing coccoliths, normally regarded as forming on quite discrete species*". This term was redefined by Young & Geisen (2002): "*Specimens resembling coccospheres but which include coccoliths of discrete species, which are unlikely to have been produced as a result of a life-cycle change or hybridization event*".

Coccolithophores thrive in marine waters co-occurring with a variety of organic (zooplankton, picoplankton, bacteria and virus) and inorganic particles with low gravitational sinking rates but active brownian movement, electrical forces and surface tension. In such a scenario, postmortem and fragmental remains of natural coccospheres float around in close vicinity. Live coccolithophores probably adopt a strategy of certain marine zooplankton, which use hydromechanical/biochemical cues to sense prey, predator, mate, light, etc. (Visser, 2001; Harvey *et al*., 2013). If strange coccoliths are biologically picked up then inserted and arranged in a natural configuration (concave-down convex-up orientation) then biogenic inclusion is a strong possibility (Young & Geisen, 2002: Plate 1, figs. 1–2, *Emiliania huxleyi* and *Gephyrocapsa oceanica*). In contrast, if the strange coccolith is inserted side-ways or in a concave-up convex-down orientation then accidental inclusion in high density preparation material is a strong possibility (Young & Geisen, 2002) and, therefore, such examples are unlikely to be true combination coccospheres and may instead be xenospheres.

2. Material and methods

Xenosphere examples illustrated herein were observed in the plankton samples collected during the 4th Indian Southern Ocean Expedition (January-February, 2010) within the area 30°S–65°S (~48°E and 57.3°E transects).

Vertical water samples were collected using Niskin bottles attached to a Conductivity Temperature Depth (CTD) rig between the surface and 110m water depth. One litre of water was collected in a clean, prewashed plastic bottle and immediately vacuum filtered through Whatman Nuclepore Track-Etched membrane filters (47mm diameter, 0.2μm pore size) using a Pall filtration unit. The filter membranes were further dried in an oven at 45°C for 48 hours and kept in Millipore sterile petri dish until analysis.

In the laboratory at NCAOR (National Centre for Antarctic and Ocean Research), a small piece of filter membrane (~5mm^2) was cut and placed on a double sided carbon tape attached to a 1 cm diameter aluminum stub and sputter coated with platinum (~2nm thick). The sample was inspected under a JEOL JSM-6360LV Scanning Electron Microscope (SEM) at 2,000x magnification using 5–15KV accelerating voltage.

3. Examples

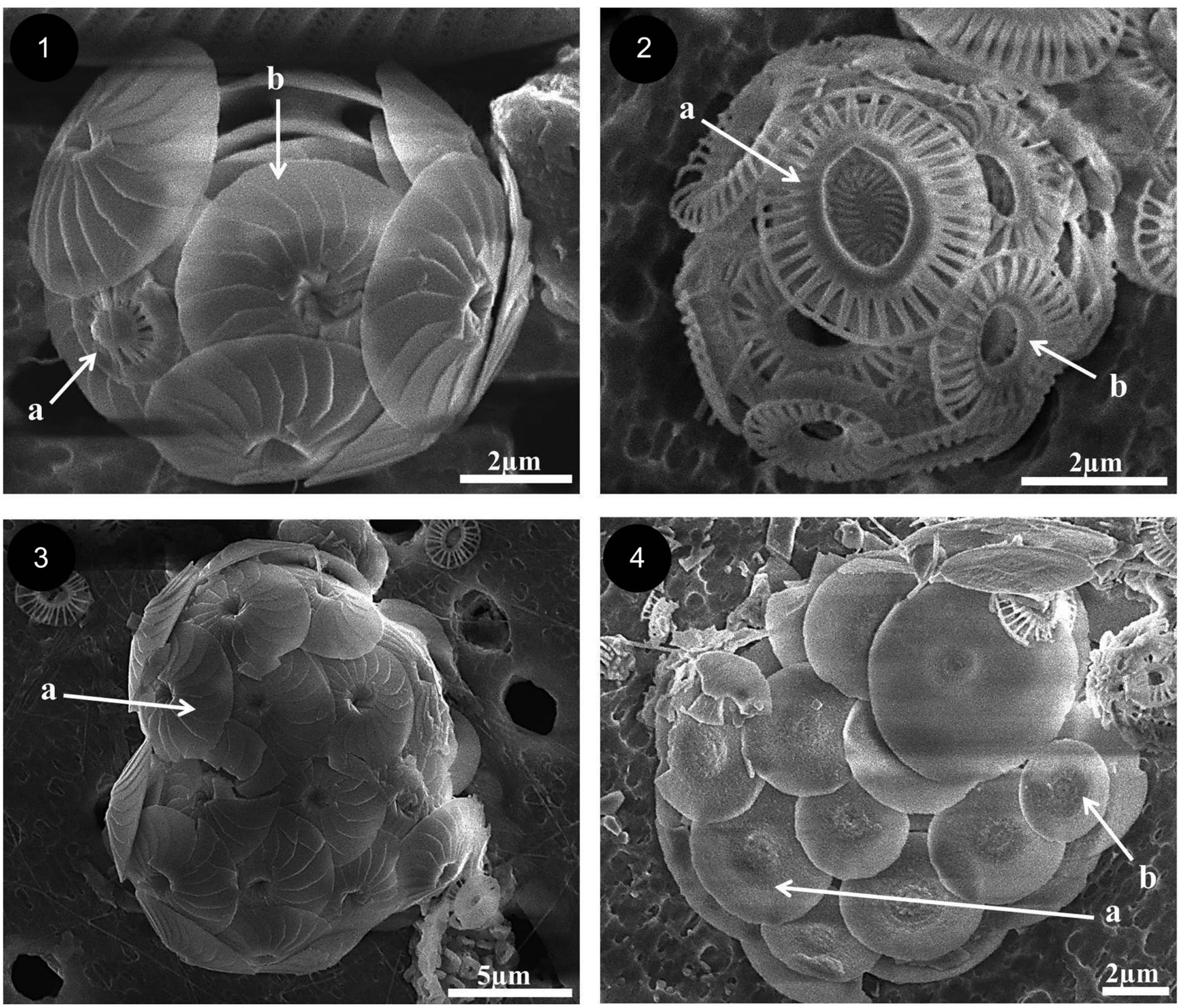
3.1 Xenosphere: *Calcidiscus leptoporus* coccosphere with *Syracosphaera nodosa* exothecal coccolith

This specimen shows numerous *Calcidiscus leptoporus* heterococcoliths and a single smaller wheel-like coccolith. The smaller coccolith is about 2μm across and appears to be planar with a distinct medial cycle of oblique laths. This is almost certainly a *Syracosphaera nodosa* exothecal heterococcolith and, given the simple rim, is identified as *Syracosphaera nodosa* type A *sensu* (Young *et al*., 2003). The *S. nodosa* exothecal coccolith (Pl. 1, fig. 1 [a]) is lying in normal orientation on a *C. leptoporus* coccosphere (Pl. 1, fig. 1 [b]) and is covered by *C. leptoporus* coccoliths on one side, with no sign of forcing.

3.2 Xenosphere: *Emiliania huxleyi* morphotype combination

This specimen shows the combination of morphologically different *Emiliania huxleyi* coccoliths (Pl. 1, fig. 2 [a,b]). This coccosphere consist of numerous coccoliths of *E. huxleyi*

Plate 1



All images were captured with a JEOL JSM- 6360 LV Scanning Electron Microscope at the NCAOR, Goa, India. All plankton samples were collected (0-110m depths) during 4th Indian Southern Ocean Expedition.

Fig. 1. Xenosphere of *S. nodosa* (a) and *C. leptoporus* exothecal coccoliths (b); 44°S, 57.3°E; 60m depth.

Fig. 2. Xenosphere of *E. huxleyi* morphotypes: (a) large (b) normal; 43°S, 57.3°E; 60m depth.

Fig. 3. *Calcidiscus leptoporus* twin coccosphere; 46°S, 57.3°E; Surface.

Fig. 4. *Oolithotus fragilis* morphotypes: (a) large (b) small; 43°S, 57.3°E; 60m depth.

morphotype O (Hagino *et al*., 2011) and a single, heavily calcified *E. huxleyi* morphotype A coccolith. Examination of the coccosphere reveals the presence of various *E. huxleyi* morphotype O coccoliths with different sizes (distal shield length [L] and width [W] was about 2.7 and 2.3μm, respectively). This size range was comparable to other *E. huxleyi* 'O' morphotypes recorded in the same sample (L = 2.6–3.0μm; W = 1.8–2.6μm). The large *E. huxleyi* morphotype A (L = 3.58μm, W = 3.19μm) observed is included in the coccosphere in a perfectly regular fashion and overlapped by normal morphotype O coccoliths. This suggests selection and inclusion onto coccosphere by biological process.

Combinations of *E. huxleyi* and *G. oceanica* were illustrated in the previous studies by Clocchiatti (1971), Winter *et al*. (1979), Young & Geisen (2002: Plate 1, figs 1–2; Plate 2, fig. 3), and also by EMIDAS (http://www.emidas.org).

3.3 Anomalous coccosphere: *Calcidiscus leptoporus* double-cell

In addition to the above mentioned examples of co-occurrence, we also observed occasional abnormally large *Calcidiscus leptoporus* coccospheres (Pl. 1, fig. 3 [a]). Coccospheres of *C. leptoporus* usually consist of about 14–26 placoliths (Young *et al*., 2003) but the present example shows the association of more than 42 placoliths. The formation of twin coccospheres during normal asexual binary division of the cell has previously been described (e.g. Klaveness, 1972), and likely explains this type of coccosphere observed here.

3.4 Anomalous coccosphere: *Oolithotus fragilis* coccoliths

A broken coccosphere of *O. fragilis* consisting of large, medium and small coccoliths (except one, the proximal shield was visible on all *O. fragilis* coccoliths). The diameter of the proximal shield of *O. fragilis* coccoliths varied from 2.8–4.7μm (Pl. 1, fig. 4 [a,b]). The present example shows that the coccosphere formation is independent of the size of the coccoliths and is not caused by accidental clustering but biological process. Studies on *G. oceanica* and *C. leptoporus* report coccolith sizes can be highly variable within cell, although one size of coccolith is more frequent. This needs to be further investigated.

4. Summary

Xenospheres continue to fascinate and challenge us, and experimental evidence (e.g. Harvey *et al*., 2013) is needed to properly understand the mechanisms involved in their formation. Based on the forms documented here and a survey of the relevant literature, we hypothesize that, akin to the strategy adopted by marine zooplankton, coccolithophores may adopt hydromechanical/biochemical cues to sense the postmortem remains of coccoliths and incorporate these into the living coccosphere in the proper orientation. Besides this biological strategy, other xenospheres may represent accidental clustering caused during preparation techniques; however, clustering of coccoliths/coccosphere in faecal pellets are not included in this discussion.

Unlike heterococcolithophore-holococcolithophore combinations, which basically represent the natural two-phase life-cycle, xenospheres are abnormal coccospheres, largely resulting from the accidental inclusion of strange coccoliths, but also possibly by rare involvement of biologic process (see Harvey *et al*., 2013). The anomalous coccosphere combinations represent unusual coccosphere formation with variations in coccolith-coccosphere size and shape.

The selection and elegant incorporation of *G. oceanica* coccoliths into *E. huxleyi* coccospheres, as documented in previous studies, does not appear to be accidental but designed by biological process, thus more rigorous experimental studies of live coccospheres could usefully be carried out in the future.

Acknowledgements

The authors are thankful to the Secretary, Ministry of Earth Sciences (MoES), Government of India and the Director, National Centre for Antarctic and Ocean Research for supporting the Past Climate and Oceanic variability group. We would also like to thank the Chief Scientist and the expedition members of the Southern Ocean cruise for the support extended for our sampling needs. We gratefully acknowledge Captain Satishraja Rangaraja, Ebin James and other crew members of ORV Sagar Nidhi, for their support during the expedition. The authors would like to thank the editor and both reviewers for their valuable comments. Dr. Shramik would like to thank DST Inspire for providing funding. This is NCAOR Contribution no. 31/2016.

References

Alcober, J. & Jordan, R.W. 1997. An interesting association between *Neosphaera coccolithomorpha* and *Ceratolithus cristatus* (Haptophyta). *European Journal of Phycology*, **32**(1): 91–93.

Billard, C. 1994. Life cycles. *In:* J.C. Green & B.S.C. Leadbeater (Eds). *The haptophyte algae*. Clarendon Press, Oxford: 167–186.

Clocchiatti, M. 1971. Sur I'existence de coccosphères portant des coccolithes de *Gephyrocapsa oceanica* et de *Emiliania huxleyi* (Coccolithophoridés). *Compte rendu de l'Acadèmie des sciences*, *Paris*, **273**: 318–321.

Cortés M.Y. 2000. Further evidence for the heterococcolith-holococcolith combination *Calcidiscus leptoporus*-*Crystallolithus rigidus*. *Marine Micropaleontology*, **39**(1–4): 35–37.

Cortés, M.Y. & Bollmann, J. 2002. A new combination coccosphere of the heterococcolith species *Coronosphaera mediterranea* and the holococcolith species *Calyptrolithophora hasleana*. *European Journal of Phycology*, **37**(1): 145–146.

Cros, L. & Fortuño, J.-M. 2002. Atlas of Northwestern Mediterranean Coccolithophores. *Scientia Marina*, **66** (Suppl. 1): 186pp.

Cros, L., Kleijne, A. & Young, J.R. 2000b. Coccolithophorid diversity in the genus *Polycrater* and possible relationship with other genera. *Journal of Nannoplankton Research*, **22**(2): 92.

Cros, L., Kleijne, A., Zeltner, A., Billard, C. & Young, J.R. 2000a. New examples of holococcolith-heterococcolith combination coccospheres and their implications for coccolithophorid biology. *Marine Micropaleontology*, **39**(1–4): 1–34.

Frada, M., Percopo, I., Young, J., Zingone, A., de Vargas, C. & Probert, I. 2009. First observations of heterococcolithophore-holococcolithophore life cycle combinations in the family Pontosphaeraceae (Calcihaptophycideae, Haptophyta). *Marine Micropaleontology*, **71**: 20–27.

Geisen, M., Billard, C., Broerse, A.T.C., Cros, L., Probert, I. & Young, J.R., 2002. Life-cycle associations involving pairs of holococcolithophorid species: intraspecific variation or cryptic speciation? *European Journal of Phycology*, **37**(4): 531–550.

Hagino, K., Bendif, M., Young, J.R., Kogame, K., Probert, I., Takano, Y., Horiguchi, T., de Vargas, C. & Okada, H. 2011. New evidence for morphological and genetic variation in the cosmopolitan coccolithophore *Emiliania huxleyi* (Prymnesiophyceae) from the COX1b-ATP4 genes. *Journal of Phycology*, **47**(5): 1164–1176.

Harvey, E.L., Jeong, H.J. & Menden-Deuer, S. 2013. Avoidance and attraction: Chemical cues influence predator-prey interactions of planktonic protists. *Limnology and Oceanogrraphy*, **58**(4): 1176–1184.

Kamptner, E. 1940. Die Coccolithineen der Südwestküste von Istrien. *Ann. Naturhistorischen Museum Wien*, **51**: 54–149.

Klaveness, D. 1972. *Coccolithus huxleyi* (Lohm.) Kamptn II. The flagellate cell, aberrant cell types, vegetative propagation and life cycles. *British Phycological Journal*, 7(3), 309–318.

Kleijne, A. 1991. Holococcolithophorids from the Indian Ocean, Red Sea, Mediterranean Sea and North Atlantic Ocean. *Marine Micropaleontology*, **17**: 1–76.

Lecal-Schlauder, J. 1961. Anomalies dans la composition des coques de flagelles calcaires. *Bulletin Society Natural History Africa Nord*, **52**: 63–66.

Lohmann, H. 1902. Die Coccolithophoridae: eine Monographie der Coccolithen bildenden Flagellaten, Zugleich ein Beitrag zur Kenntnis des Mittelmeerauftriebs. *Arch. Protistenk*, **1**: 89–165.

Sprengel, C. & Young, J.R. 2000. First direct documentation of associations of *Ceratolitus cristatus* ceratoliths, hoop-coccoliths and *Neosphaera coccolithomorpha* planoliths. *Marine Micropaleontology*, **39**(1–4): 39–41.

Thomsen, H.A., Ostergaard, J.B. & Hansen, L.E. 1991. Heteromorphic life histories in Arctic coccolithophorids (Prymnesiophyceae). *Journal of Phycology*, **27**(5): 634–642.

Visser, A.W. 2001. Hydromechanical signals in the plankton. *Marine Ecology Progress Series*, **222**: 1–24.

Winter, A., Reiss, Z. & Luz, B. 1979. Distribution of living coccolithophore assemblages in the Gulf of Elat (‘Aqaba). *Marine Micropaleontology*, **4**: 197–223.

Young, J. R. & Geisen, M. 2002. Xenospheres- associations of coccoliths resembling coccospheres. *Journal of Nannoplankton Research*, **24**(1): 27–35.

Young, J., Geisen, M., Cros, L., Kleijne, A., Sprengel, C., Probert, I. & Ostergaard, J. 2003. A guide to extant coccolithophore taxonomy. *Journal of Nannoplankton Research Special Issue* **1**: 125pp.

Young, J.R., Bergen, J.A., Bown, P.R., Burnett, J.A., Fiorentino, A., Jordan, R.W., Klijne, A., Niel, B.E. van, Romein, A.J.T., Salis, K. von. 1997. Guidelines for coccolith and calcareous nannofossil terminology. *Paleontology*, **40**: 875–912.