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Verification of the *Algirosphaera robusta–Sphaerocalyptra quadridentata* (coccolithophores) life-cycle association

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ABSTRACT – Living coccolithophores were collected from eight stations along a transect in the gulf of Korthi (southeastern Andros island, Aegean Sea, Eastern Mediterranean) in August 2001. Samples were collected from 0–120 m water depth to determine the cell density, the species composition and the biogeographical (spatial and vertical) distribution of the coccolithophore biocommunities in coastal marine ecosystems. The studies revealed an impressive heterococcolith–holococcolith combination coccosphere (SEM micrograph) involving the species *Algirosphaera robusta* and *Sphaerocalyptra quadridentata.* In addition, a second association was observed by light microscopy. This discovery verifies the suggestions of Kamptner (1941) and provides strong proof on the assignment of these two 'species' in a common life cycle, increasing significantly our knowledge of life-cycle pairings ecology. *J. Micropalaeontol.* **22**(1): 107–111, July 2003.

INTRODUCTION

Coccolithophores are unicellular marine biflagellate golden-brown algae which, at some points in their life cycle, produce minute exothecal calcium carbonate plates called coccoliths. Two structurally different types of coccoliths, heterococcoliths and holococcoliths, formed by different types of biomineralization, are recognizable. Heterococcoliths are formed from complex shaped crystal units and their biomineralization occurs intracellularly (Manton & Leedale, 1969; Westbroek *et al.*, 1989; Pienaar, 1994; Young *et al.*, 1999). Holococcoliths are formed of numerous minute euhedral crystallites, and appear to be calcified extracellularly (Manton & Leedale, 1963; Rowson *et al.*, 1986). The coccoliths make an important contribution to translocation of the inorganic carbon produced in pelagic areas to the ocean floor and thus to the sedimentary archive. Since they are biologically formed and sediment forming, coccoliths play key roles in global biochemical cycles and are extremely valuable for stratigraphic and palaeoceanographic purposes, especially in reconstructing the past productivity of the oceans.

Many coccolithophores have a complex life cycle that involves the sequential production of holo- and heterococcoliths. This was first recognized in cultures of *Coccolithus pelagicus* (Wallich)–*Crystallolithus hyalinus* (Gaarder & Markali) by Parke & Adams (1960). However, the life cycles of coccolithophores are poorly known, primarily because only a limited number of species have been successfully cultured and, in most cases, phase changes occur only sporadically and unpredictably.

Associations of hetero-holococcolithophores on a single cell 'combination coccospheres' have been known for a long time (e.g. Kamptner, 1941), but it is only recently that such associations have been systematically recognized (e.g. Lecal-Schlauder, 1961; Kleijne, 1991; Cros *et al.*, 2000; Cortés, 2000; Geisen *et al.*, 2002; Cros & Fortuño, 2002).

Coccolithophore phase changes are considered to be a relatively fast process, and it is anticipated that combination coccospheres will only be found occasionally (Cros, 2001).

One of the most significant studies is that by Cros *et al.* (2000), where seven well-established associations of heterococcoliths with holococcoliths and five less well-established life-cycle combinations have been documented from the Western Mediterranean. Young *et al.* (1999) and Cros *et al.* (2000) suggested that heterococcolith–holococcolith life-cycle associations must have a common phylogenetic origin and that a consistent relationship of coccolith type and life-cycle stage is likely.

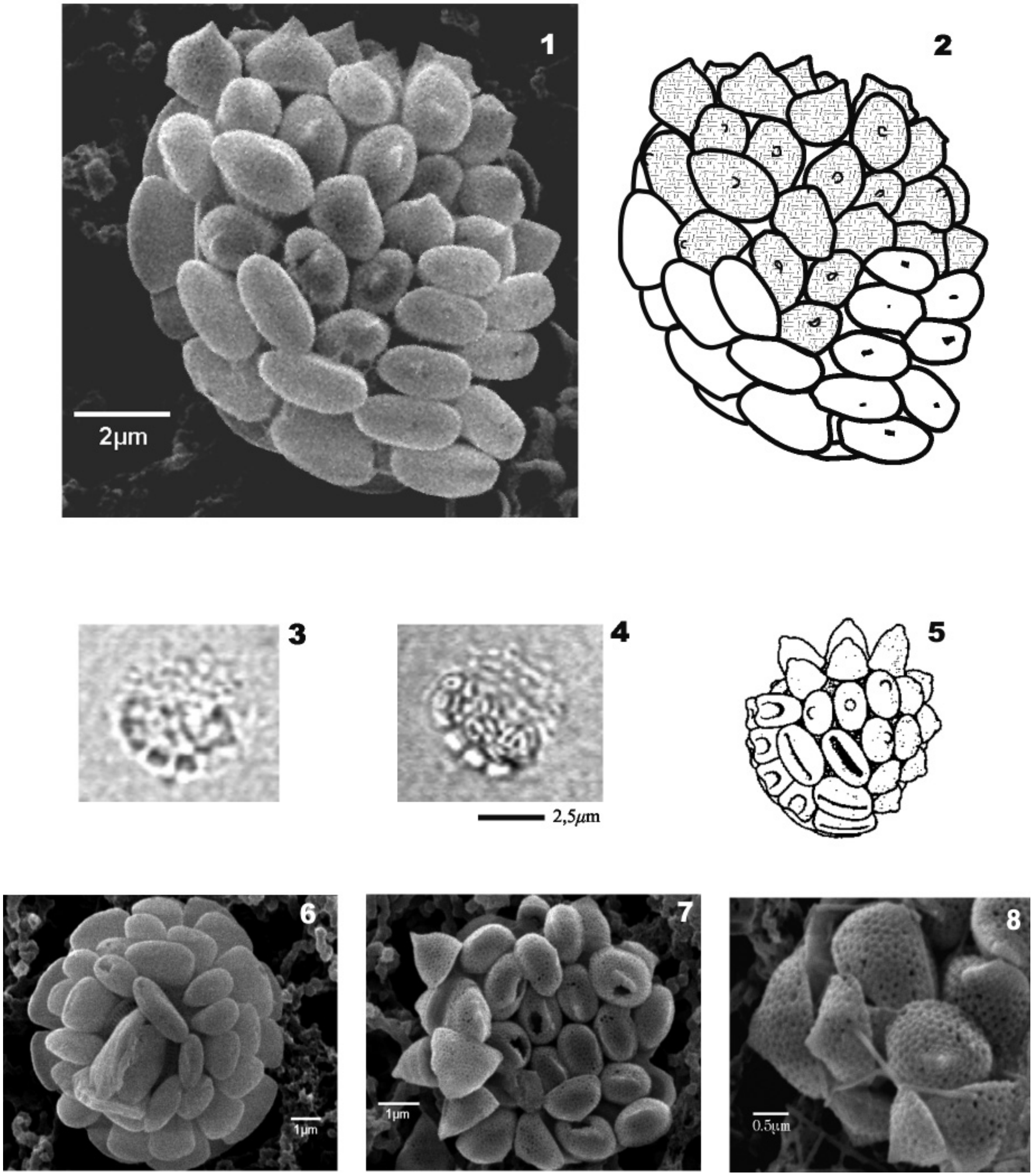
In the present paper we document an unambiguous *Algirosphaera robusta* (Lohmann, 1902) Norris, 1984 – *Sphaerocalyptra quadridentata* (Schiller, 1913) Deflandre, 1952 combination coccosphere from the Eastern Mediterranean (Aegean Sea), verifying the first report (drawing and LM) made by Kamptner (1941, figs 153, 154, pl. XV; see Pl. 1, fig. 5) who recognized five specimens of *Anthosphaera robusta* (=*Algirosphaera robusta*) with *Calyptrosphaera quadridentata* (=*Sphaerocalyptra quadridentata*), from the coastal environments of the Adriatic Sea.

The complete nannoflora recorded during the present study are described in Triantaphyllou *et al.* (2002).

MATERIALS AND METHODS

Water samples were collected for coccolithophore analysis from coastal environments in August 2001. They came from eight stations (Fig. 1, Table 1), along a 7.5 km transect in the Gulf of Korthi (Andros Island, middle Aegean Sea, Eastern Mediterranean). The Aegean Sea surface water circulation during spring and summer is incorporated, in general, into an anti-clockwise gyre system, similar to that in the Adriatic, Ionian, Tyrrhenian and Alboran Seas; see Triantaphyllou *et al.* (2002) for a brief description of the study area.

Water samples were collected from a number of depths (0–120 m), using a single oceanographic Hydro-bios bottle. The sampling was undertaken between 7.00 a.m. and 1.00 p.m. (local time) and the water stratification conditions were excellent. For each sampling depth, 1.5 litres of seawater were filtered on Whatman cellulose nitrate filters (47 mm diameter, 0.45 μm pore size), using a vacuum filtration system. The filters were open dried and stored in plastic Petri-dishes. A piece of each filter approximately 8×8 mm^2 was attached to a copper electron microscope stub using double-sided adhesive tape and coated



Explanation of Plate 1.

figs 1, 2. *Algyrosphaera robusta–Sphaerocalyptra quadridentata* combination: **1,** a single well-developed coccosphere consisting of sacculiform body rhabdoliths of *Algirosphaera robusta* bearing a circular depression or a small nodular structure on their distal part, and ordinary calyptroliths of *Sphaerocalyptra quadridentata*, sample T3-2, 5 m; **2,** index sketch of fig. 1, hachured coccoliths belong to *S. quadridentata.* Note that both types of coccoliths are represented by almost equal numbers on the combination coccosphere. **figs 3, 4.** *A. robusta–S. quadridentata* combination, as observed by light microscopy: **3,** specimen at low focusing, T3-1, 45 m; **4,** specimen at high focusing, sample T3-1, 45 m. **fig. 5.** Analogous specimen figured by Kamptner (1941). **fig. 6.** *Algirosphaera robusta* (Lohman) Norris, coccosphere in apical view, showing the circum-flagellar rhabdoliths closing the flagellar opening, sample T3-1, 90 m. **figs 7, 8.** *Sphaerocalyptra quadridentata* (Schiller) Deflandre: **7,** dimorphic coccosphere, both types of calyptroliths bear the characteristc campanulate form, sample T3-6, 15 m; **8,** detail of body calyptroliths with the microcrystals irregularly arranged and small perforations in-between. Their distal part is characterized by a pointed protrusion, sample T3-2, 5 m.

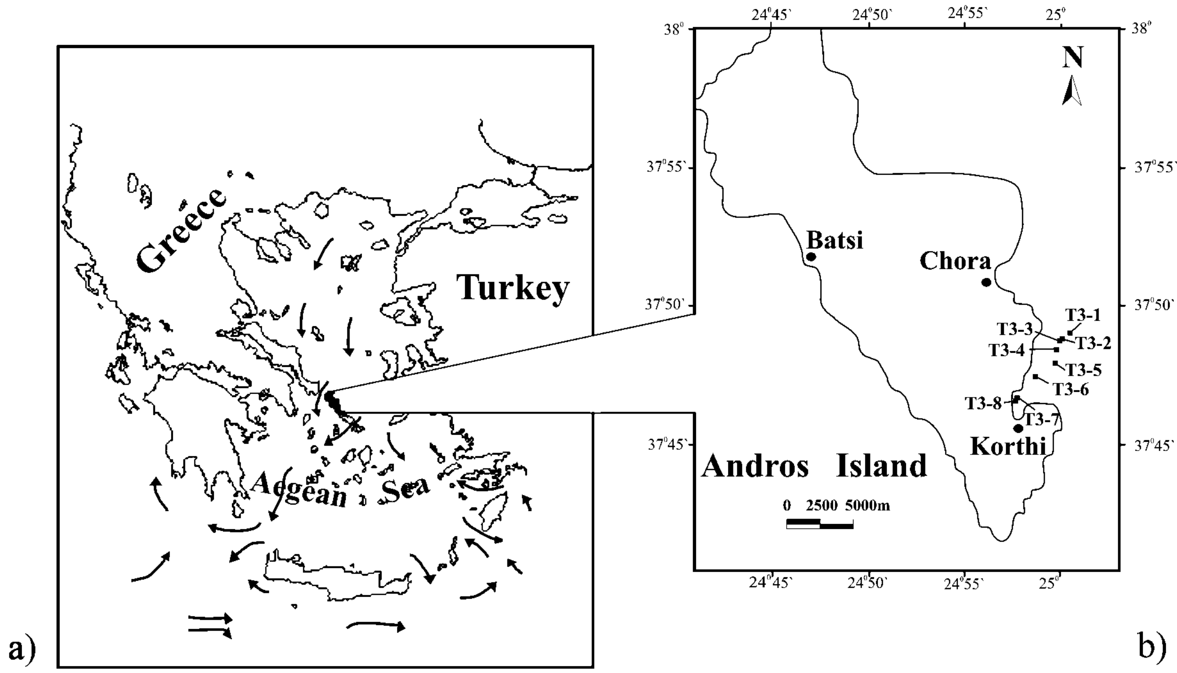


Fig. 1. (a) Generalized current patterns in the Aegean Sea during the summer period (based on Lacombe & Tchernia, 1972). **(b)** Map of the study area (Andros Island) and location of the sampled stations.

with gold. The filters were examined in a JEOL JSM 5600 scanning electron microscope (SEM). To determine the species composition and standing crop the samples were examined mainly with SEM, and the working magnification was 1200× throughout the counting procedure. The absolute abundances of cell densities (cells/l) were calculated following the methodology of Jordan & Winter (2000).

RESULTS

The distribution patterns in the middle Aegean Sea indicate that holococcolithophores may constitute a significant part of the summer flora in the upper photic zone of the coastal waters of Andros Island, providing evidence of vertical differentiation in the water column and showing affinities to shallower environments and rather normal nutrification conditions (Triantaphyllou *et al.*, 2002). Additionally, heterococcolithophore biocommunities are well represented even in shallow coastal environments, showing quite diverse assemblages.

In the present study, *Algirosphaera robusta* (Pl. 1, fig. 6) becomes important at 90 m and 120 m depth (Table 1), making up the typical deep assemblage. However, it must be noted that a few *A. robusta* specimens were recorded at 60 m, 45 m, 25 m and even at 15 m depth in the water column, at several stations (Table 1). This is probably due to the upward displacement of the depth position of the species, correlated with the shallowing coastal environments.

Sphaerocalyptra quadridentata (Pl. 1, figs 7–8) is one of the most common components of the summer holococcolithophore assemblage in the studied water samples. It is dominant in the uppermost layers of the photic zone, especially at 5 m depth, persisting in high frequencies down to 15 m (Table 1). It appears to increase in abundance towards shallower environments (Triantaphyllou *et al.*, 2002).

A single well-developed coccosphere (Pl. 1, figs 1–2) consisting of sacculiform body rhabdoliths of *Algirosphaera robusta* (heterococcoliths) and ordinary calyptroliths of *Sphaerocalyptra quadridentata* (holococcoliths) showing the characteristic campanulate form, was found in sample T3-2 at 5 m depth. Both types of coccoliths are represented by almost equal numbers on the combination coccosphere. The sacculiform body rhabdoliths of *Algirosphaera* bear a circular depression or a small nodular structure on their distal part. Circum-flagellar and labiatiform rhabdoliths (in contrast to Kamptner's observations) of *A. robusta*, as well as stomatal coccoliths of *Sphaerocalyptra* are not visible.

Additionally, one similar coccosphere exhibiting few *A. robusta* coccoliths combined with holococcoliths has been observed by light microscopy in sample T3-1, 45 m (Pl. 1, figs 3–4).

DISCUSSION

Algirosphaera is primarily represented in the living plankton and is known from the North Atlantic, Mediterranean Sea, Red Sea, Indian and Pacific Oceans (Kleijne, 1992). Several species have been described in this genus, all of them regarded as synonyms of the species *Algirosphaera robusta* by Kleijne (1992) and Aubry (1999).

A. robusta is a species with many unusual characteristics, especially of coccolith formation and ultrastructure (Probert *et al.,* 2000). It occupies lower photic zone water (Takahashi & Okada, 2000) and is adapted to low light environments, found between 80 m and 180 m (Okada & Honjo 1973). Samtleben & Schroeder (1992) rarely record it at temperatures above 9 °C, but other authors such as Hallegraeff (1984) and Kleijne (1992) demonstrated that similar specimens are also known from the warm Indian Ocean. However, its distribution seems to

Stations	Sea bottom depth (m)	Longitude (° ’ ”)	Latitude (° ’ ”)	Local time	Water depth (m)	Total standing crop ($\times 10^3$cells l^{-1})	*Algirosphaera robusta* %	*Sphaerocalyptra quadridentata* %
T3-1	130	37 49 01	25 00 31	08:50	0	4.05		14.3
					5	13.43		
					15	12.72		19.5
					45	14.7	0.4	4.8^{+}
					90	6.75	19.6	
					120	1.92	9.4	
T3-2	120	37 48 48	25 00 02	08:18	0	8.70		8.9
					5	12.64		15.4^{+}
					15	11.08		13.9
					45	5.93		2.9
					90	3.36	1.8	
T3-3	124	37 48 46	24 59 58	08:40	0	3.08		3.6
					5	6.70		16.9
					15	13.64	0.8	12.7
					45	7.40	0.9	2.7
					90	3.72	11.1	2.2
T3-4	110	37 48 28	24 59 41	09:11	0	3.70		
					5	8.48		10.9
					15	10.28		10
					45	11.34		1.9
					60	5.94	1.8	3.7
T3-5	75	37 47 56	24 59 20	09:30	0	2.48		
					5	7.30		19.8
					15	5.97		13.7
					45	13.58	2.2	0.5
T3-6	50	37 47 24	24 58 45	10:00	0	8.38		6.9
					5	10.38		27.3
					15	11.17	0.6	22.4
					45	1.99		
T3-7	30	37 46 40	24 57 44	10:30	0	4.24		11.4
					5	8.26		14
					15	8.92		18.5
					25	7.93	1.2	13.1
T3-8	15	37 46 34	24 57 23	11:30	0	7.80		9.9
					5	11.95		28.2
					7	8.32		31.1

+combination coccosphere

Table 1. *Local and temporal position of samples collected on August 21 2001, coccolithophore total standing crop and* A. robusta, S. quadridentata *abundances at all depths.*

be determined by temperature and nutrient levels, making *A. robusta* an indicator of the relatively cold and eutrophic environments, typical of upwelling conditions.

On the other hand, *Sphaerocalyptra quadridentata* lives near the surface and, in particular, it dominates the holococcolithophore assemblages of the uppermost layers (5 m water depth) of the photic zone (Cros, 2001; Triantaphyllou *et al.,* 2002).

Data from Table 1 clearly show the depth preferences of the two involved coccosphere types in the studied samples when they are occurring individually. The documented combination coccospheres have been found between 5 m and 45 m in the water column, where the sea bottom depth at that point ranges between 120 m and 130 m. This suggests that the physical conditions of the area, in particularly the shallow coastal environments, may somehow favour frequent phase changes in the species life cycles.

The *Algirosphaera robusta–Sphaerocalyptra quadridentata* life-cycle stages appear to occupy two different ecological niches in the water column. The heterococcolith stage is adapted to deep waters with a higher nutrient content and the holococcolith prefers normal to oligotrophic surface water environments. It can be suggested, therefore, that the *Algirosphaera robusta–Sphaerocalyptra quadridentata* association is comparable to the life-cycle strategy postulated for the heterococcolithophore–holococcolithophore combination between *Helicosphaera carteri* and *Syracolithus catilliferus* (Cros *et al.*, 2000).

Recently Cros & Fortuño (2002, figs 114A, B) have reported two partially collapsed coccospheres combining *Sphaerocalyptra quadridentata* with *Rhabdosphaera clavigera.* If we consider this association as suggestive, then it is probable that the holococcolithophore species *S.quadridentata* may be related to two different representatives (heterococcolithophores) of the Rhabdosphaeraceae family.

Cros *et al.* (2000) have recorded some examples of holococcolith–holococcolith combination coccospheres, suggesting that these might be caused by intraspecific variation. Additionally, Geisen *et al.* (2002) have described several life-cycle associations involving pairs of holococcolithophore species. They suggested that at least some of them provide strong evidence for heterococcolithophore cryptic speciation.

According to this assumption, a holococcolithophore cryptic speciation might also be reasonable in our case, where a holococcolith (*S. quadridentata*) appears to be associated with two heterococcoliths (*A. robusta* and *R. clavigera*). Apparently, the speciation in the genus *Sphaerocalyptra* is not clearly recognizable from the holococcolithophore morphology, suggesting that 'morphological species' possibly represent an assemblage of several 'genetic species'.

The complexity of different coccolithophore life-cycle stages may cause a widespread revision of nomenclatural taxonomy. Cros *et al.* (2000) suggested that heterococcolith morphology is the most reliable basis for coccolithophore taxonomy. Since it is obvious that coccolithophore phase changes occur only sporadically and as it is possible that life-cycle associations involve pairs of holococcolithophores (Geisen *et al.* 2002), or pairs of heterococcolithophores (suggestive in this study), it is reasonable that longstanding incongruence in the classification may be solved only once coccolithophore life cycles are better understood.

CONCLUSIONS

The present study documents one heterococcolithophore–holococcolithophore combination with a high degree of confidence, proving that *Algirosphaera robusta* and *Sphaerocalyptra quadridentata* represent different phases of the same coccolithophore species, during an ecological life-cycle strategy.

This provides good reason for further research on coccolithophore life-cycle pairings in coastal environments, where ecological variabilities may somehow trigger phase changes in coccolithophore life cycles.

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