Evidence for a possible life-cycle association between *Syracosphaera protrudens* (heterococcolithophore) and *Syracosphaera pulchra* HOL *pirus*-type (holococcolithophore)

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ABSTRACT – An apparently unambiguous combination coccosphere from the Eastern Mediterranean (Aegean Sea, Greece) is documented involving *Syracosphaera protrudens* Okada & McIntyre, 1977 and *Syracosphaera pulchra* Lohmann, 1902 HOL *pirus*-type. This finding is difficult to interpret in terms of the current understanding of *Syracosphaera* taxonomy and adds evidence to the hypothesis of a distinctly complex *Syracosphaera pulchra* life cycle. *J. Micropalaeontol.* 28(1): 1–6, May 2009.

KEYWORDS: living coccolithophores, combination coccospheres, *Syracosphaera*, taxonomy, life cycle

INTRODUCTION

Coccolithophores form a major component of the marine nannoplankton and, at least at some stage in their life cycle, produce minute calcium carbonate plates called coccoliths. Two types of coccoliths, heterococcoliths and holococcoliths, are recognizable (Young *et al*., 1999, 2004; Young & Henriksen, 2003). In culture studies several coccolithophores have been shown to have complex life cycles involving an alternation between a haploid holococcolith-producing phase and diploid heterococcolith-producing phase (Parke & Adams, 1960; Houdan *et al*., 2004). The limited data available from culture studies have been supplemented greatly by the observation of combination coccospheres, representing the moment of life-cycle transition, in field samples (Kamptner, 1941; Lecal-Schlauder, 1961; Kleijne, 1991; Thomsen *et al*., 1991; Alcober & Jordan, 1997; Young *et al*., 1998; Cortés, 2000; Cros *et al*., 2000; Cortés & Bollmann, 2002; Cros & Fortuno, 2002; Geisen *et al*., 2002, 2004; Triantaphyllou & Dimiza, 2003; Triantaphyllou *et al*., 2004). None the less, for the vast majority of extant coccolithophores their life cycle is still unknown, and new observations are much needed (Young *et al*., 2003; Jordan & Winter, 2000).

This paper presents the first description of a combination coccosphere between the heterococcolithophore species *Syracosphaera protrudens* Okada & McIntyre, 1977 and holococcolithophore species *S. pulchra* HOL *pirus*-type [=Daktylethrapirus (Kamptner, 1937) Norris, 1985].

MATERIALS AND METHODS

The *S. protrudens*—*S. pulchra* HOL *pirus*-type combination coccosphere evidenced in this study comes from water samples collected off Andros Island (middle Aegean Sea). The samples were collected on 21 August 2001, from eight stations at selected depths (Fig. 1, Table 1), between 0 m and 120 m, using a single oceanographic Hydro-bios bottle. Additional samplings were performed in the same area during April 2002 and September 2004. For each sampling depth, 1.5 l of sea water was filtered through Millipore cellulose nitrate filters (47 mm diameter, 0.45 µm pore size), using a vacuum filtration system. Salt was removed by washing the filters with about 2 ml of bottled drinking water. The filters were oven dried and stored in plastic Petri dishes. A piece of each filter approximately 8 × 8 mm² was attached to a copper stub using double-sided adhesive tape, and gold-coated. The filters were examined in a JEOL JSM 5600 Scanning Electron Microscope (SEM) and all coccolithophore specimens occurring on the examined filter piece were identified and counted. The working magnification was 1200 × throughout the counting procedure. The absolute abundances of coccolithophores (cells 1⁻¹) were calculated following the methodology of Jordan & Winter (2000).

RESULTS

Coccolithophore biocommunities constitute a significant part of the flora in the upper photic zone of the coastal waters of Andros Island, showing quite diverse assemblages. The complete nannoflora recorded during the present study is described in Triantaphyllou *et al*. (2002) and Dimiza *et al*. (2008).

A single well-developed combination coccosphere (Pl. 1, figs 1–2), consisting of numerous body coccoliths of *Syracosphaera protrudens* (heterococcoliths) and characteristic body holococcoliths of *Syracosphaera pulchra* HOL *pirus*-type (holococcoliths), was found in sample T3-5 at 45 m water depth (37°47.93’ N, 24°59.33’ E). The heterococcoliths (caneoliths) of *Syracosphaera protrudens* have a high, almost vertical, wall with two flanges, and a variable number of teeth protruding centripetally from the distal flange. Both *Syracosphaera protrudens* (Pl. 1, fig. 4) and *Syracosphaera pulchra* HOL *pirus*-type (Pl. 1, fig. 7) were minor components in the assemblages (less than 3% – included in *S. halldalii* abundance – and 6% of the coccosphere standing stock, respectively, Table 1). The filter sample and the SEM micrograph are in the collections of the Museum of Paleontology and Geology in the University of Athens.

DISCUSSION

*Syracosphaera protrudens* is known from the Red Sea, Mediterranean Sea and North Atlantic (Kleijne, 1993). This form was described by Okada & McIntyre (1977) but has often been included in *Syracosphaera halldalii* Gaarder in Gaarder & Hasle, 1971. Recently, Young *et al.* (2003) and Jordan *et al.*...
(2004) reinstated *S. protrudens* as a separate species, but closely related to *S. halldalii*. Triantaphyllou *et al.* (2004) illustrated an unambiguous combination coccosphere of *S. halldalii* hetercoccoliths and holococcoliths of *Calyptrolithina divergens* var. *tuberosa* (Heimdal *in* Heimdal & Gaarder, 1980) Heimdal, 1982. This strongly suggested that these two 'species' are alternate life-cycle stages and they recommended that *C. divergens* var *tuberosa* should be regarded as a junior synonym of *S. halldalii*.

As illustrated in Figure 2, *pirus*-type holococcoliths (= *S. pulchra* HOL, *pirus*-type) and *tuberosa*-type holococcoliths (= *S. halldalii* HOL) are similar in shape. They both have moderately flaring tubes with a distal cover that is slightly recessed in the tube then rises to form a central dome. They differ primarily in that *tuberosa*-type holococcoliths have numerous small pores in the distal cover, whilst *pirus*-type holococcoliths have a few large pores. So in this respect the associations *halldalii*–*tuberosa* and *protrudens*–*pirus* appear unproblematic. However, *pirus*-type holococcoliths are also known to be formed by the very different species *S. pulchra*. As has been well documented (Lecalschlauder, 1961; Cros *et al.*, 2000; Geisen *et al.*, 2002, 2004; Saugestad & Heimdal, 2002), the heterococcolithophore *Syracosphaera pulchra* Lohmann, 1902 forms life-cycle associations with two holococcolithophore coccosphere-types previously assigned to different genera: *Calyptrosphera oblonga* Lohmann, 1902 and *Daktylethra pirus* (Kamptner, 1937) Norris, 1985. Geisen *et al.* (2002) documented both the *S. pulchra*–*C. oblonga* and *S. pulchra*–*D. pirus* associations from a mix of culture observations (one strain showing alternations between *S. pulchra* and *C. oblonga*) and multiple plankton observations of combination coccospheres. They gave an extended discussion on the similarities and differences between *pirus*-type and *oblonga*-type holococcoliths and demonstrated a clear affinity in their coccolith morphology and structure, despite the consistent differences in morphology (Geisen *et al.*, 2002; Young *et al.*, 2003). Geisen *et al.* (2002) also discussed various possible interpretations of these data and concluded that this was most likely a case of pseudo-cryptic speciation, inferring that *S. pulchra* probably comprises two biological species, which are almost impossible to separate in the heterococcolith phase, but which produce quite distinct holococcoliths. Based on these data, Geisen *et al.* (2002) synonymized *C. oblonga* and *D. pirus* with *S. pulchra*; this opinion was followed in the recent taxonomic syntheses of Young *et al.* (2003) and Jordan *et al.* (2004).

The set of relationships suggested by the various combination coccospheres, as summarized in Figure 2, is clearly rather problematic. A number of different hypotheses can be suggested as possible explanations.

1. *Syracosphaera pulchra* and *S. protrudens* are sibling species that have evolved different heterococcolith morphologies but retained similar holococcolith morphologies. The problem with this hypothesis is that the heterococcolith morphologies are totally dissimilar, differing in numerous ways, including; exothecal coccolith form (dome-shaped in *S. pulchra*); body coccolith rim structure (complex in *S. pulchra* with three flanges (Young *et al.*, 2004), simple in *S. protrudens* with two flanges); circumflagellar coccolith spine type (bifurcate in *S. pulchra*, simple in *S. protrudens*). These characters have been inferred to be significant indicators of phylogenetic relationships between the numerous *Syracosphaera* species and so *S. protrudens* and *S. pulchra* have been included in disparate groups in the syntheses of Young *et al.* (2003) and Cros & Fortuño (2002), with the implication that they are related only distantly. So, this interpretation would imply that heterococcolith...
3.

*S. pulchra*

*S. halldalii* (counted together with Table 1.

Possible association between *S. protrudens* and *S. pulchra* HOL *pirus*-type

<table>
<thead>
<tr>
<th>Stations</th>
<th>Sea bottom depth (m)</th>
<th>Longitude</th>
<th>Latitude</th>
<th>Water depth (m)</th>
<th>Total standing crop</th>
<th>Syracosphaera halldalii (cells l(^{-1}))</th>
<th>Syracosphaera pulchra (cells l(^{-1}))</th>
<th>Syracosphaera pulchra HOL <em>pirus</em> (cells l(^{-1}))</th>
<th>Syracosphaera pulchra HOL <em>oblonga</em> (cells l(^{-1}))</th>
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</thead>
<tbody>
<tr>
<td>T3-1</td>
<td>130</td>
<td>37°49’01”-25°00’31”</td>
<td>0</td>
<td>4.05</td>
<td>96</td>
<td>330</td>
<td>96</td>
<td>220</td>
<td>220</td>
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<tr>
<td>T3-2</td>
<td>120</td>
<td>37°48’48”-25°00’02”</td>
<td>0</td>
<td>8.70</td>
<td>330</td>
<td>110</td>
<td>220</td>
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<tr>
<td>T3-3</td>
<td>124</td>
<td>37°48’46”-24°59’58”</td>
<td>0</td>
<td>3.08</td>
<td>308</td>
<td>617</td>
<td>220</td>
<td>220</td>
<td>220</td>
</tr>
<tr>
<td>T3-4</td>
<td>110</td>
<td>37°48’28”-24°59’41”</td>
<td>0</td>
<td>3.70</td>
<td>308</td>
<td>283</td>
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<tr>
<td>T3-5</td>
<td>75</td>
<td>37°47’56”-24°59’20”</td>
<td>0</td>
<td>3.70</td>
<td>308</td>
<td>617</td>
<td>283</td>
<td>283</td>
<td>283</td>
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<td>37°47’24”-24°58’45”</td>
<td>0</td>
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<td>37°46’40”-24°57’44”</td>
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<td>T3-8</td>
<td>15</td>
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<td>3.70</td>
<td>308</td>
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<td>283</td>
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Table 1. Local position of samples collected on 21 August 2001, heterococcolithophore and holococcolithophore total standing crop and *S. protrudens* (counted together with *S. halldalii*), *S. pulchra*, *S. pulchra* HOL *pirus*-type, *S. pulchra* HOL *oblonga*-type abundances at all depths

morphism is much more plastic and less reliable as an indicator of evolutionary relationships than has been assumed.

2. *Syracosphaera pulchra* and *S. protrudens* are related only distantly but have evolved similar holococcolith morphologies as a result of convergent evolution. The problem with this hypothesis is that the *pirus*-type holococcoliths are rather distinctive and the holococcoliths on the *protrudens-pirus* combination coccosphere show the typical morphology. So, if it is an example of convergent evolution it is rather extraordinary.

3. *S. pulchra* and *S. protrudens* are, in fact, not discrete species but rather one species with a very complex life cycle capable of producing multiple coccolith types. Thus, *pulchra*-type and *protrudens*-type heterococcoliths and *pirus*-type and *oblonga*-type holococcoliths may actually all be formed by one polymorphic species. An ambiguous specimen showing coccoliths of both *Syracosphaera pulchra* HOL *oblonga*-type and *Syracosphaera pulchra* HOL *pirus*-type has been reported by Malinverno et al. (2008). The problem with this hypothesis is that culture studies have provided no evidence of this type of polymorphism. So, this interpretation would imply much more complexity in coccolithophore life cycles than has been observed previously.

4. The *S. protrudens-pirus* combination coccosphere may be a xenosphere (sensu Young et al., 1997; Young & Geisen, 2002), i.e. an artefact produced by accidental agglomeration of coccoliths, inclusion of coccoliths from the water column onto a coccosphere or agglutination of coccoliths into the wall of a non-coccolithophore. The problems with this hypothesis are that the two coccolith types are rather rare in the water column, they are separated into different areas on the coccosphere, no other coccolith types are included in the coccosphere and the coccolith arrangement on the coccosphere is rather regular. So, this interpretation implies that combination coccospheres are less reliable indicators of life-cycle associations than has been assumed and perhaps can be produced by mechanisms not considered previously.
CONCLUSIONS

The coccosphere illustrated here, involving *protrudens*-type heterococcoliths and *pirus*-type holococcoliths, looks like an unambiguous example of a combination coccosphere, indicating a life-cycle association between these two coccosphere-types. However, all possible interpretations of the coccosphere pose difficult problems for our understanding of coccolithophore biology or evolutionary relationships. From the available evidence it is not possible to reach a simple conclusion. More significantly, any possible interpretation raises significant

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**Explanation of Plate 1.**

**figs 1, 2.** *Syracosphaera protrudens*–*Syracosphaera pulchra* HOL *pirus*-type combination: 1, a single well-developed coccosphere consisting of numerous body caneoliths of *Syracosphaera protrudens* bearing a high and almost vertical wall with two flanges and with a variable number of teeth that protrude centripetally from the distal flange, and characteristic ordinary areoliths of *Syracosphaera pulchra* HOL *pirus*-type, sample T3-5 at 45 m; 2, index sketch of fig. 1. **fig. 3.** *Syracosphaera pulchra–Syracosphaera pulchra* HOL *pirus*-type combination coccosphere (T1-100, 15 m, September 2004). Numerous elliptical cap-shaped *oblonga*-type holococcoliths associated with *Syracosphaera pulchra* body coccoliths. **fig. 4.** *Syracosphaera protrudens* (T1-2, 60 m, August 2001). Body coccoliths are elliptic with relatively high vertical wall with basal and distal flange; several distinct well-separated long teeth protrude toward the central area, which is spanned by thin radial laths leaving slits in between, with a very small axial structure. **fig. 5.** *Syracosphaera pulchra* (T3-1, 45 m, April 2002). Body coccoliths are elliptical with three well-developed flanges; central area is closed by three concentric cycles of small radial laths, leaving small slits in between. Apical coccoliths are similar, but with more elevated central area and robust bifurcated spine. The exotheal coccoliths are formed by a basal flat rim and an elevated dome-shaped central area, built of laths, which, in the central part, leave slits in between, with a central conical depression. **fig. 6.** *Syracosphaera pulchra–Syracosphaera pulchra* HOL *pirus*-type combination coccosphere (T3-1, 45 m, April 2002). Numerous elliptical cap-shaped with areolate interior *pirus*-type holococcoliths associated with *Syracosphaera pulchra* body coccoliths. **fig. 7.** *Syracosphaera pulchra* HOL *pirus*-type (T3-4, 45 m, August 2001). Holococcoliths are elliptical cap-shaped with areolate interior (areoliths), formed by a tube, a vaulted central area and a proximal ring forming a basal flange. Apical holococcoliths are similar but more elevated and with a well-developed pyramidal spine extending distally. **fig. 8.** *Syracosphaera pulchra* HOL *oblonga*-type (T1-6, 10 m, August 2001). Holococcoliths are elliptical cap-shaped calyptroliths with proximal ring forming a basal flange. Apical holococcoliths are similar, with a well-developed pyramidal spine extending distally.
problems with our current understanding of coccolithophore evolution, coccolithophore life cycles or the significance of combination coccospheres. Evidently, more research is needed in this field and, in particular, more observations from both culture studies and field observations of combination coccospheres.

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