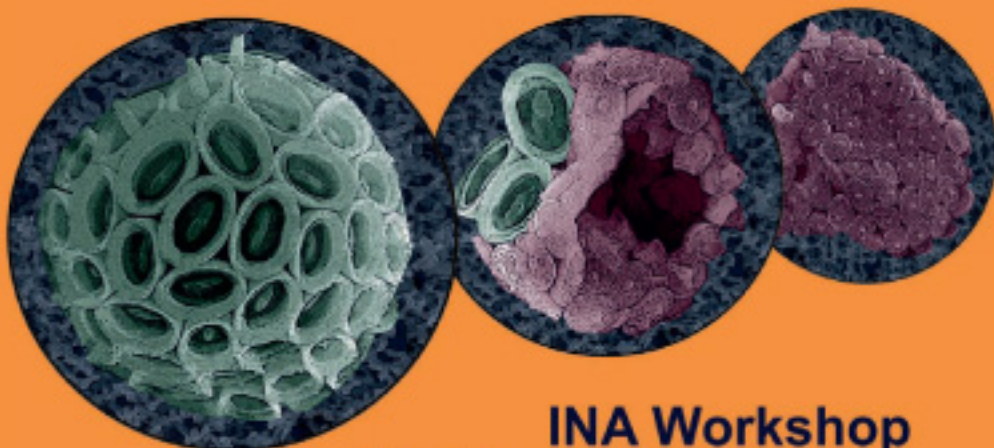


Journal of Nannoplankton Research

volume 34 | special issue | october 2014

COCCOLITHOPHORES 2014



INA Workshop
on Extant Coccolithophores research
5-10 October, 2014
Heraklion, Crete, Greece

coccolithophore biocalcification and Ocean Acidification
paleontological agenda for extant research
phylogeny, species-concepts and molecular genetics
life-cycles, ecology and biogeography

organized by
University of Athens (UoA), Faculty of Geology & Geoenvironment, Assoc. Prof. Maria Triantaphyllou
Hellenic Centre for Marine Research (HCMR), Institute of Oceanography, Dr. Stella Psarra
University College London (UCL), Dr. Jeremy Young

sponsored by
INTERNATIONAL NANNOPLANKTON ASSOCIATION (INA)
THE MICROPALAEONTOLOGICAL SOCIETY (TMS)



HELLENIC REPUBLIC
National and Kapodistrian
University of Athens
Faculty of Geology
& Geoenvironment



International
Nannoplankton
Association

Observations on the life cycle and ecology of *Acanthoica quattrosolina* Lohmann from a Mediterranean estuary.

Luka Šupraha

Department of Earth Sciences, Paleobiology Program, Uppsala University, Villavägen 16, SE-752 36 Uppsala, Sweden; luka.supraha@geo.uu.se

Zrinka Ljubešić

Department of Biology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000, Zagreb, Croatia

Hrvoje Mihanović

Institute of Oceanography and Fisheries, Šetalište I. Meštrovića 63, 21000 Split, Croatia

Jorijntje Henderiks

Centre for Ecological and Evolutionary Synthesis, Department of Biosciences, University of Oslo, Blindern PO Box 1066, N-0316 Oslo, Norway

Abstract: Different life cycle phases of *Acanthoica quattrosolina* life cycle were studied under the scanning electron microscope. Samples were collected during winter 2013 at two stations in the lower reach of the Krka River estuary (Eastern Adriatic Sea, Croatia). An ecological analysis revealed that the species was tolerant to low salinity and reached high abundance at the halocline of the estuary. Examination of holococcoliths showed great variability in the morphology of the central area, indicating that several specimens previously described as *Sphaerocalyptra* sp. represent a variety of the *Acanthoica quattrosolina* holococcolith phase. Furthermore, the observed variability of holococcoliths may represent a distinct mechanism of holococcolith production, with structural similarities to other members of the *Sphaerocalyptra* genus.

1. Introduction

Many coccolithophore species exhibit a heteromorphic life cycle, alternating between morphologically distinct haploid (holococcolith) and diploid (heterococcolith) phases (Young *et al.*, 2005). Great progress in understanding their life cycle has been reached by investigations of both spontaneous (Parke and Adams, 1960; Houdan *et al.*, 2004) and controlled (Nöel *et al.*, 2004) phase changes in culture material. Furthermore, combination coccospheres (bearing both heterococcoliths and holococcoliths of the same species), that are occasionally observed in field samples, have helped establish the taxonomic relationships between previously unrelated heterococcolithophore and holococcolithophore taxa (Thomsen *et al.*, 1991; Cros *et al.*, 2000; Geisen *et al.*, 2002; Triantaphyllou *et al.*, 2004; Malinverno *et al.*, 2008; Frada *et al.*, 2009; Triantaphyllou, 2010).

The family Rhabdosphaeraceae encompasses mostly morphologically well-documented extant species (Kleijne, 1992; Young *et al.*, 2003). However, only a few electron micrographs of their holococcolithophore phases are available to date, including *Rhabdosphaera clavigera* (Cros and Fortuño, 2002), *Algirosphaera robusta* (Triantaphyllou and Dimiza, 2003) and *Acanthoica quattrosolina* (Cros *et al.*, 2000; Cros and Fortuño, 2002; Young *et al.*, 2003).

The heterococcolith life phase of *A. quattrosolina*, the type species of the *Acanthoica* genus, was described by Lohmann (1903) and subsequently examined by Schiller (1913). In a detailed review of the *Acanthoica* genus, Kleijne (1992) recognized several species having the same set of coccolith types as *A. quattrosolina* (e.g. *A. acanthifera*, *A. maxima*, *A. janchenii*) but with different body coccolith morphology. Most subsequent workers have only recorded *A. quattrosolina* so it is unclear how much diversity there is within the genus.

Cros *et al.* (2000) and Cros and Fortuño (2002) reported on combination coccospheres containing heterococcoliths of *A. quattrosolina* and previously undescribed holococcoliths with affinity to *Sphaerocalyptra* sp. Coccospheres bearing only holococcoliths that are morphologically related to ones from combination coccospheres were found in the same study. However, due to the high degree

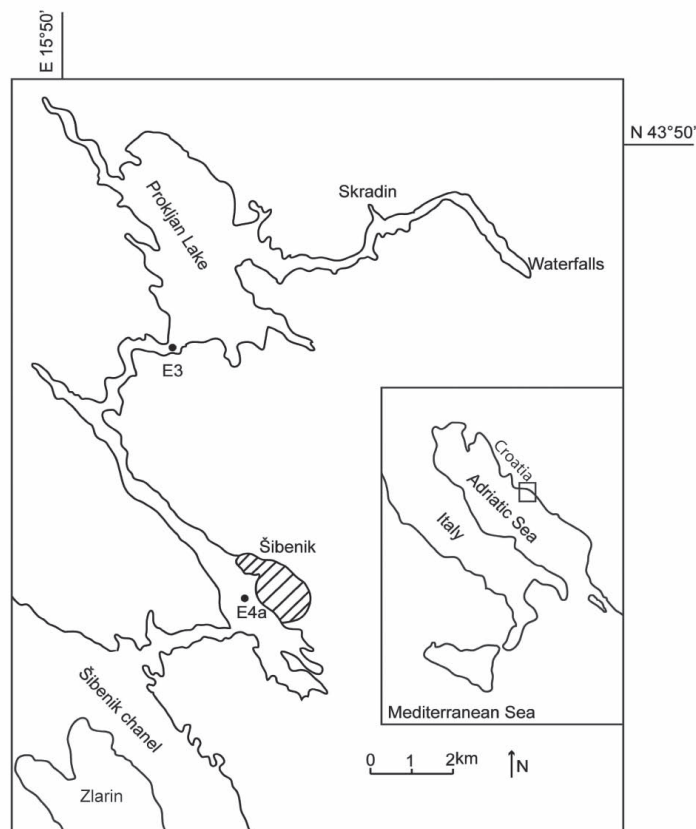


Figure 1. Map of the investigated area with two sampling stations E3 and E4a.

Date	Station	Depth (m)	Salinity (PSU)	Amount filtered (ml)	Volume analysed (ml)	Total coccolithophores (cells/l)	<i>E. huxleyi</i> HET (cells/l)	<i>A. quattrosospina</i> HET (cells/l)	<i>A. quattrosospina</i> COMB (cells/l)	<i>A. quattrosospina</i> HOL (cells/l)
26.2.2013	E3	0	1.4	100	0.83	7.2×10^3	4.8×10^3	0	0	0
26.2.2013	E3	4	2.5	50	0.41	3×10^5	1.2×10^5	3.6×10^4	7.2×10^3	5.1×10^4
26.2.2013	E3	5	22.3	50	0.41	2.6×10^5	1.2×10^5	2.9×10^4	0	3.9×10^4
26.2.2013	E3	7.5	25.9	50	0.41	3.4×10^5	2.8×10^5	4.8×10^3	2.4×10^3	7.2×10^3
26.2.2013	E3	13	27.2	100	0.83	3.1×10^5	2.8×10^5	3.6×10^3	0	0
26.2.2013	E3	20	28.8	100	0.83	8.8×10^4	6.5×10^4	2.4×10^3	0	0
25.2.2013	E4a	0	4.7	200	1.66	9.7×10^3	7.2×10^3	1.2×10^3	0	0
25.2.2013	E4a	3	20.1	100	0.83	5.7×10^4	3.0×10^4	7.2×10^3	0	1.2×10^3
25.2.2013	E4a	5	28.0	200	1.66	5.8×10^4	3.7×10^4	5.4×10^3	0	1.8×10^3
25.2.2013	E4a	20	28.7	200	1.66	9.8×10^4	7.6×10^4	7.2×10^3	0	0
25.2.2013	E4a	35	28.8	200	1.66	2×10^4	1.7×10^4	6×10^2	0	0

Table 1. Sampling data with the abundance of total coccolithophores and life cycle phases of *A. quattrosospina*.

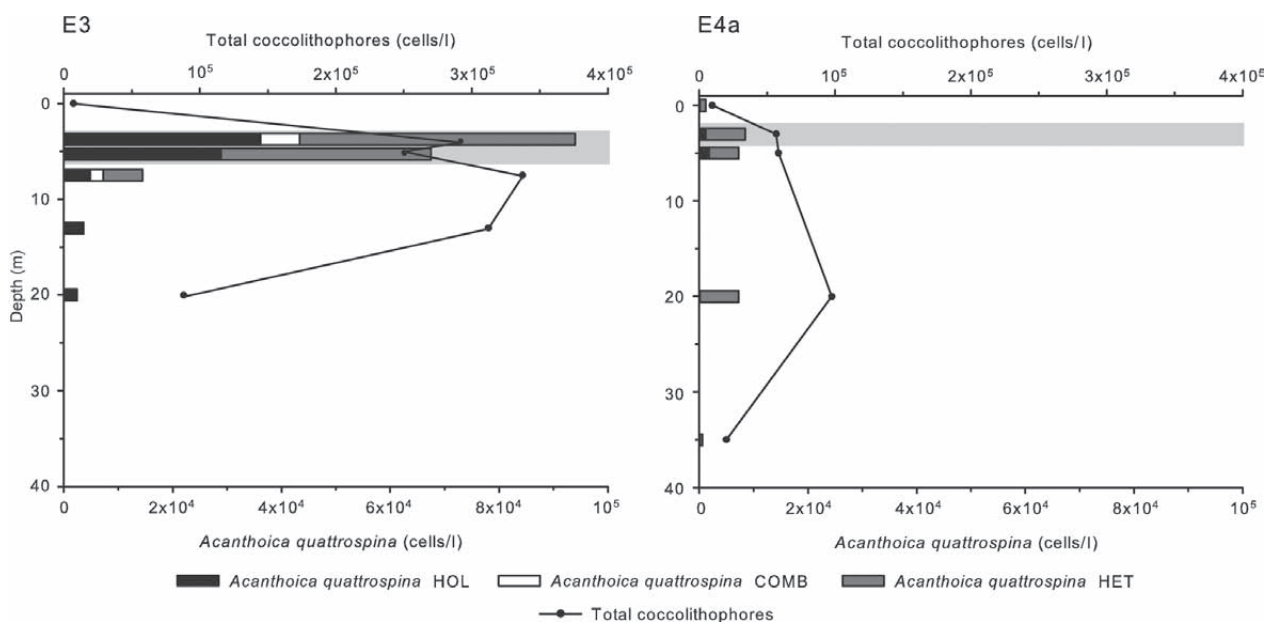


Figure 2. Plot showing the contribution of each of the life phases vs. the total abundance of *Acanthoica quattrosospina* at the sampled depths. Grey area marks the extent of the halocline. Note that the scales for the abundance of total coccolithophores and *A. quattrosospina* are different.

of morphological variability they could not be unambiguously assigned to *A. quattrosospina*, indicating the necessity for more observations on the holococcolith phase of the species.

During the investigation of coccolithophore assemblages of the Krka River estuary (Eastern Adriatic Sea), we detected a high abundance of both life cycle phases of *A. quattrosospina*, as well as combination coccospheres. This allowed us to document the complete morphological variability of the holococcolith phase and put our observations in an ecological context. Our findings provide new

insights into the life cycle of the species, and its particular ecological preferences.

2 Material and methods

Samples used for this study were collected during winter 2013 (25-26 February) at two stations in the lower reach of the Krka River estuary (Fig. 1). Sampling depths (Table 1) were determined after the fine scale examination of temperature, salinity and Chlorophyll *a* fluorescence using a SeaBird 19plus CTD probe (SeaBird Electronics, Inc. Washington, USA). Seawater for the analysis was obtained simultaneously at all depths, using a series of 5L Niskin samplers. For quantitative SEM analysis, a known volume of sea water (Table 1) was filtered using a vacuum pump onto a 25 mm polycarbonate filter (0.8 μ m Cyclo-pore, Whatman). A cellulose nitrate filter of the same size

(0.8 μm Whatman) was placed underneath the polycarbonate filter to ensure the even distribution of material. After filtration, the filter was rinsed with 2ml of bottled drinking water (pH=7.54) and dried in the oven at 50°C. For analysis under a Zeiss Supra35-VP scanning electron microscope (SEM), a piece of filter was mounted on a metal stub and sputter-coated with gold. Quantification of coccolithophores was conducted following the recommendations of Bollmann *et al.* (2002). Standard taxonomic literature (Cros and Fortuño, 2002; Young *et al.*, 2003) was used for the qualitative analysis of coccolithophore assemblages. For description of the specimens, we used the terminology introduced by Kleijne (1992) and Young *et al.* (1997). Since the holococcolithophore phase of *Acanthoica quattropsina* had not been described as a distinct species before the observations on combination

coccospheres, suitable informal name for the morphotype is *Acanthoica quattropsina* HOL, as recommended by Young *et al.* (2003).

3. Results

3.1 Distribution of *A. quattropsina*

A sharp halocline was present at both stations, characterized by the increase in salinity from 2.6 to 31.2 PSU between 3.7-5.3 m depth at the E3 station, and from 6.6 to 36.1 PSU within 1.9-4 m depth at the E4a station (Table 1, Fig. 2). *Acanthoica quattropsina* was abundant in this transition layer (up to 9.4×10^4 cells/l) accounting for maximum 32% of total coccolithophores at the halocline of the E3 station (Table 1, Fig. 2). Peak abundance at the E4a station (8.4×10^3 cells/l) was also reached at the halocline, although with a contribution of only 14% to the total coccolithophore count

(Table 1, Fig. 2). While *A. quattropsina* reached maximum abundances at the halocline, peaks in total coccolithophore abundance (dominated by *Emiliana huxleyi*) were found in the marine layer below the halocline at both stations (Table 1, Fig. 2).

Trends in the vertical distribution of the heterococcolith and holococcolith phases differed between the stations. The two life-cycle phases were equally represented at the halocline of the E3 station, while the holococcolith phase dominated deeper layers. On the other hand, station E4a was dominated by the heterococcolith phase, with minor contributions of the holococcolith phase at the halocline.

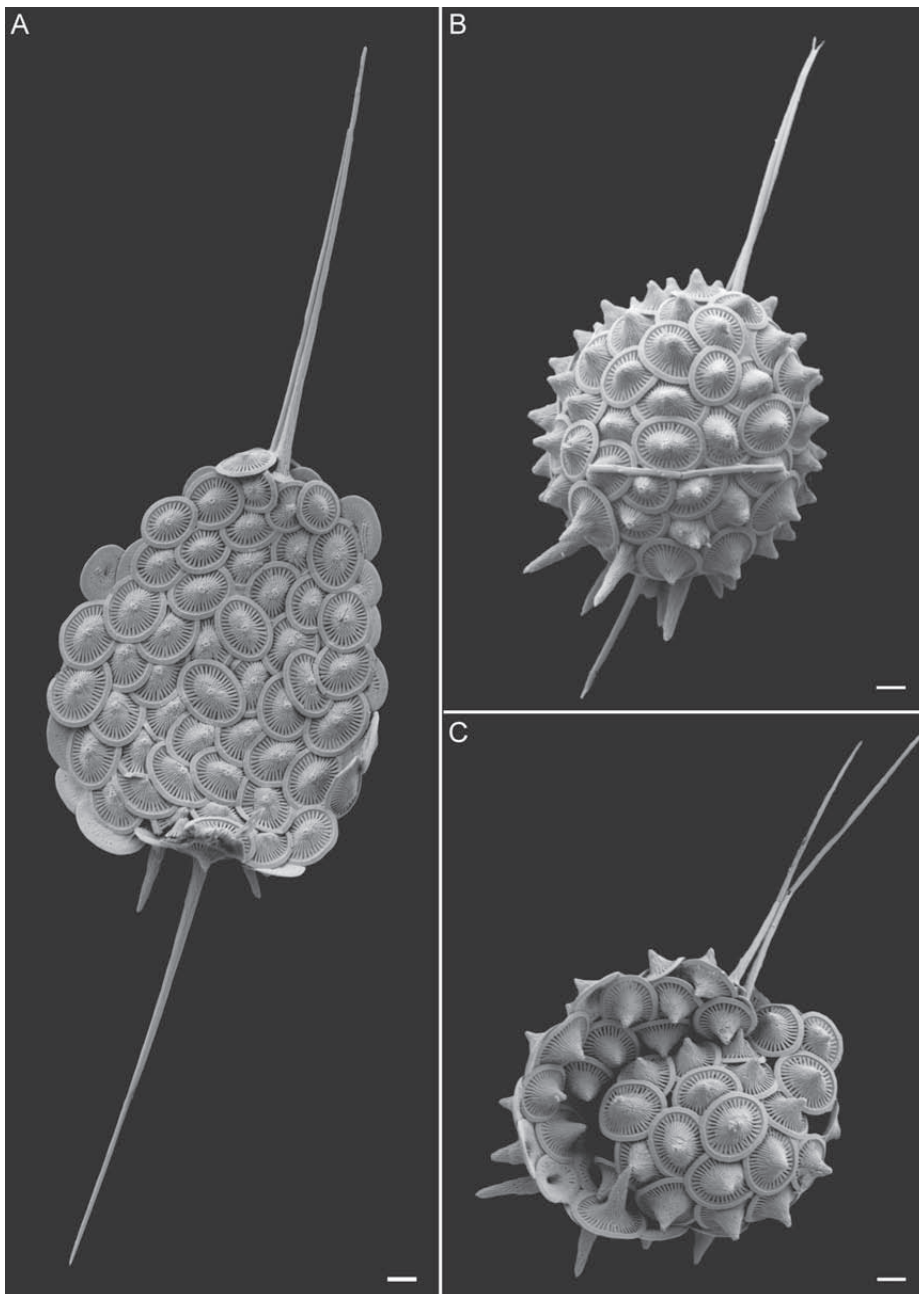


Figure 3. SEM micrographs of the heterococcolith phase of *Acanthoica quattropsina*: A) Typical coccosphere of *Acanthoica quattropsina* with two long spines at the basal end and one long and three short spines at the apical end (E3, 4m). Scale bar=1 μm ; B) *Acanthoica quattropsina* coccosphere with atypical morphology. Note more than three short apical spines and high conical protrusions. (E4a, 0 m). Scale bar=1 μm ; C) Atypical *quattropsina* morphotype with many short apical spines. Long apical spine has fallen off. (E3, 0 m). Scale bar= 1 μm .

Combination coccospheres were found only at the halocline and at 7.5 m depth of the E3 station (Table 1, Fig. 2).

3.2 Heterococcolith phase

The five specimens of *Acanthoica* heterococcolith phase for which we have high resolution SEM images belonged to two distinct morphotypes. Three coccospheres exhibited the characteristic morphology of *A. quattrosospina*,

with two long antapical spines and one long spine on the apical end accompanied by three short spines (Fig. 3A). Body rhabdoliths had relatively flat central area with short conical protrusion and pronounced cuneate cycle in the centre. Coccospheres of this morphotype were on average $13.8 \pm 2.4 \mu\text{m}$ long and $10.9 \pm 1 \mu\text{m}$ wide, built of 83-105 rhabdoliths. Two of the observed specimens differed from the typical *A. quattrosospina* morphology (Fig. 3B, C),

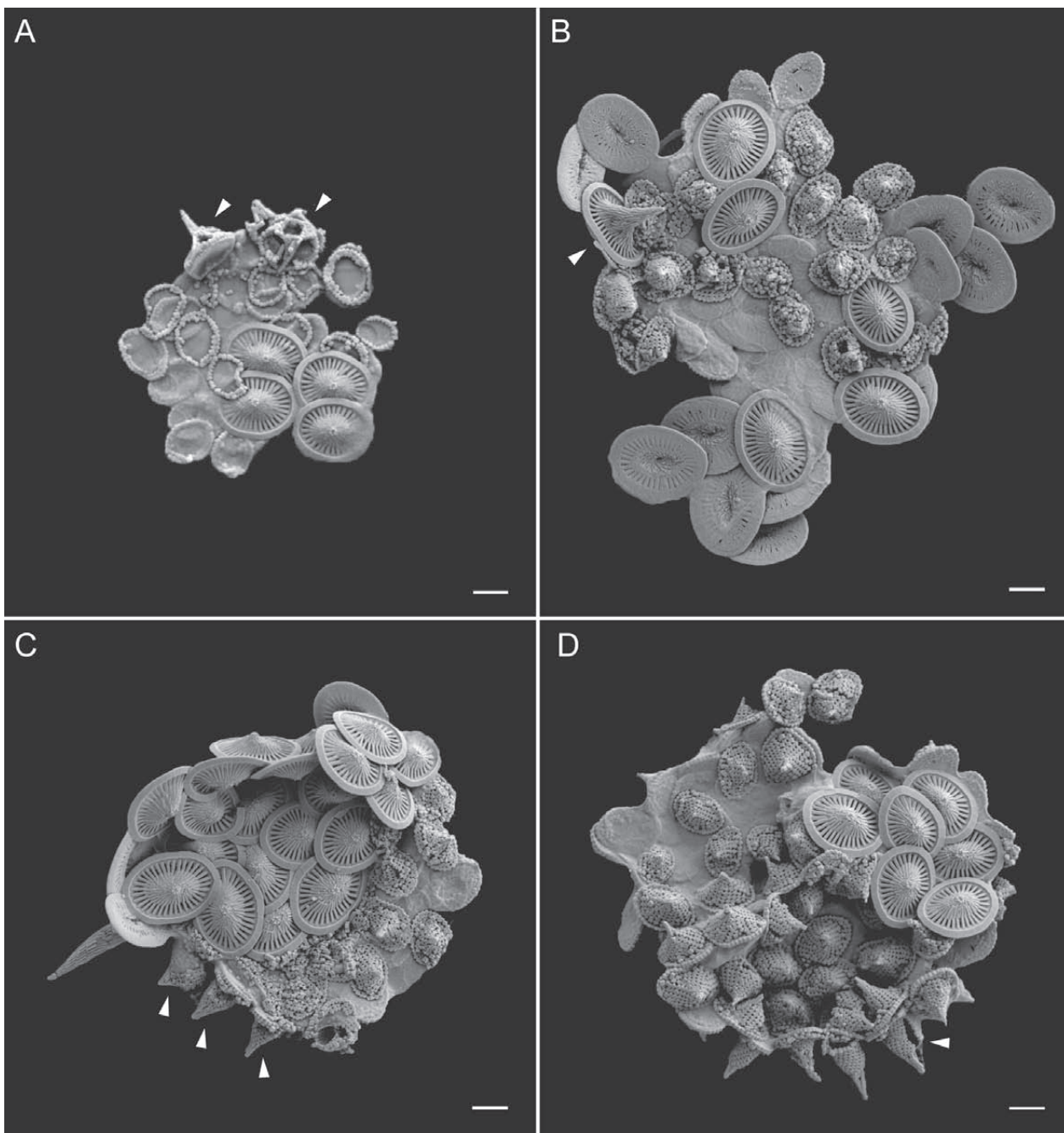


Figure 4. SEM micrographs of combination coccospheres of *Acanthoica quattrosospina*: A) Complete coccosphere in the heterococcolith phase showing two basal spines and one long spine with several short spines on the apical end (E4a, 0 m). Scale bar=1 μm ; A) Combination coccosphere with four body rhabdoliths and body calyptroliths in the early stage of the development. Two circumsflagellar calyptroliths with lines of crystals supporting the production of conical protrusion (arrow) are visible (E3, 4 m), Scale bar=1 μm ; B) Combination coccosphere with the short apical spine (arrow) and organic scales that are clearly visible in the centre of the coccosphere (E3, 4 m), Scale bar=1 μm ; C) Combination coccosphere exhibiting one heterococcolith short apical spine and three circumsflagellar calyptroliths (arrows), (E3, 4 m), Scale bar=1 μm ; D) Combination coccosphere showing fully developed body calyptroliths with high cones and hollow circumsflagellar calyptrolith (arrow), (E3, 4 m), Scale bar=1 μm .

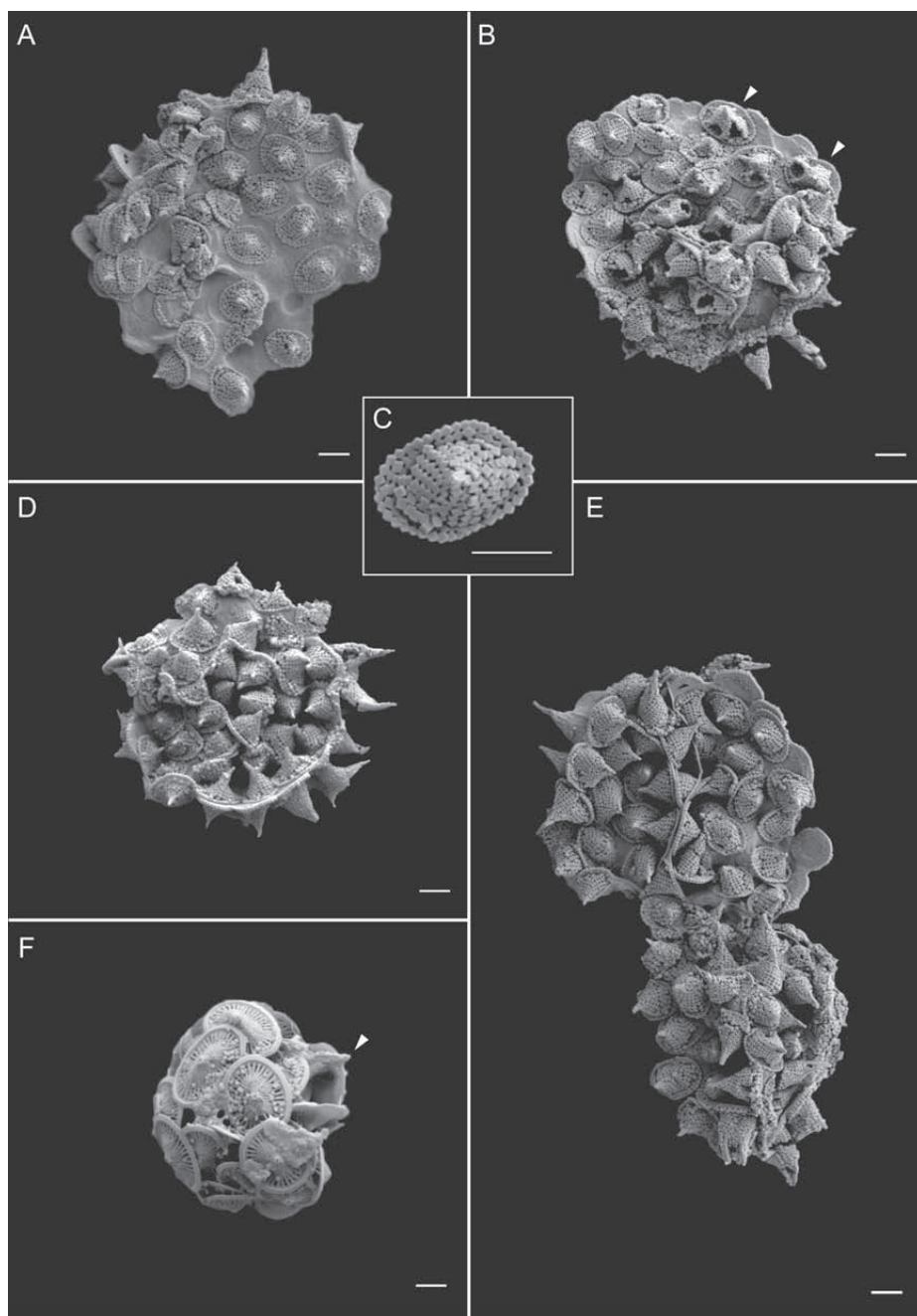


Figure 5. SEM micrographs of *A. quattropsina* in holococcolith phase (A-E) and a combination coccosphere (F): A) Holococcolith phase showing body calyptroliths with small conical protrusion (E3, 4 m), Scale bar=1 μm ; B) Holococcolith phase. Note the building mechanism of conical protrusion (arrows), where the crystallites are added laterally onto the skeletal supporting lines. The flagellar opening is visible, surrounded by large circumflagellar calyptroliths (E3, 4 m), Scale bar=1 μm ; C) Typical body calyptrolith, tipped with a single crystallite (E3, 4 m) Scale bar=1 μm ; D) Typical holococcolith phase with large circumflagellar calyptroliths (E3, 4m), Scale bar=1 μm ; E) Dividing coccosphere with fully developed calyptroliths (E3, 4m), Scale bar=1 μm ; F) Possible combination coccosphere showing the transition from the holococcolith to heterococcolith phase, with short apical spine (arrow) emerging at what seems to be a flagellar opening (E3, 4m), Scale bar=1 μm .

3.3 Combination coccospheres

A total of nine combination coccospheres showing the phase transition were analysed during our study (Fig. 4A-D). Some of the combination coccospheres still possessed the short apical spines of *A. quattropsina*, confirming their taxonomic affiliation (Fig. 4B, C). All heterococcoliths in combination coccospheres belonged to the typical *A. quattropsina* morphotype, and no combination coccospheres were found containing rhabdolites with the high conical protrusion. Holococcoliths on the combination coccospheres showed different levels of structural complexity and high morphological variability. In the most simple form, only the base of the holococcolith was present, composed of a several crystallites wide and one crystallite layer high elliptical ring (Fig. 4A). In some holococcoliths several arches of crystallite units were growing upwards from the edge of the base and meeting distally (Fig. 4A). Finally, most of the holococcoliths had the area between the arches filled with crystallites forming a conical protrusion (Fig. 4D, Fig. 5B). As a result of the addition of crystallites laterally between the arches, conical protrusions were hollow both on the body

having more than three short apical spines and body rhabdolites with a high conical protrusion. A high degree of plasticity was found in body rhabdolites of the morphotype, and the height of their conical protrusion being almost as high as the short apical spines. Average length and width of the coccospheres were $10.4 \pm 0.1 \mu\text{m}$ and $10.1 \pm 0.8 \mu\text{m}$, respectively. Coccospheres were built of 77-85 rhabdolites. All analysed specimens possessed long apical and basal spines, typical of the *Acanthoica* genus, so these atypical specimens may represent an intra-specific variant of *A. quattropsina* or a discrete species.

combination coccospheres were found containing rhabdolites with the high conical protrusion. Holococcoliths on the combination coccospheres showed different levels of structural complexity and high morphological variability. In the most simple form, only the base of the holococcolith was present, composed of a several crystallites wide and one crystallite layer high elliptical ring (Fig. 4A). In some holococcoliths several arches of crystallite units were growing upwards from the edge of the base and meeting distally (Fig. 4A). Finally, most of the holococcoliths had the area between the arches filled with crystallites forming a conical protrusion (Fig. 4D, Fig. 5B). As a result of the addition of crystallites laterally between the arches, conical protrusions were hollow both on the body

and circumflagellar calyptroliths. Minute organic body scales were also observed on some of the combination coccospheres (Fig. 4B).

While most of the combination coccospheres represented the transition from hetero- to holococcolith phase, we interpret that the coccosphere presented in Fig. 5F represents the transition from holo- to heterococcolith phase. The coccosphere possessed only few crystallites scattered over the unusually small heterococcosphere, on which body heterococcoliths and one short apical spine seemed to be emerging from the inside of the cell while the holococcolith cover was being lost. Furthermore, what seems to be the flagellar opening of a holococcolithophore phase was also visible. The opening was placed where the short apical spine was emerging, indicating that circumflagellar holococcoliths develop at the apical end of the coccosphere, which corresponds to a pattern observed on other combination coccospheres (Fig. 4C). However, the flagellar opening of the holococcolith phase was not clearly observed in our material. A structure resembling a flagellar opening was present on only one of our specimens (Fig 5B).

3.4 Holococcolith phase

A great abundance of cells in the holococcolith phase was found in our samples, allowing for the investigation of the morphology of 18 specimens. The spherical coccospheres were on average $8.4 \pm 0.9 \mu\text{m}$ in diameter, composed of 44-95 holococcoliths. Two main types of holococcoliths could be distinguished. The body calyptroliths were flat with a basal plate (on average $1.6 \pm 0.1 \mu\text{m}$ long and $1.2 \pm 0.1 \mu\text{m}$ wide) that was one crystallite layer high, and with a variable central protrusion (on average $1.1 \pm 0.2 \mu\text{m}$ high) tipped with a single crystallite (Fig. 5C). Circumflagellar calyptroliths had a higher conical protrusion (on average $1.9 \pm 0.3 \mu\text{m}$ high) ending in an extended tip (Fig. 5B). All crystallites were arranged in a rhomboid pattern, with the base of conical protrusion not reaching the edge of the calyptroliths base, leaving a basal rim which was one crystallite high in fully developed calyptroliths (Fig. 5D, E). There was a high degree of variability in the morphology of a central protrusion, which can be rather small, with a 3-4 crystallites wide rim area (Fig. 5A). In some specimens it was difficult to distinguish body calyptroliths from circumflagellar calyptroliths due to the high central protrusion of body calyptroliths (Fig. 5E).

4. Discussion

Our data provides the first report of high abundance of *Acanthoica quattrosipina* in an estuarine coccolithophore community. One of the reasons for high counts in our samples could be the species' tolerance to low salinity, which allowed it to outcompete other coccolithophores in the unstable environment of the halocline. The halocline of the Krka River estuary is reported to be the zone of commonly high nutrient accumulation (Legović *et al.*, 1994) and high bacterial production (Fuks *et al.*, 1991).

Nutrient availability at the halocline could have triggered the high abundance of *A. quattrosipina*. Furthermore, the presence of the flagellated holococcolith phase in the bacteria-rich halocline indicates the potential importance of phagotrophic nutrition in this species, as was reported for other coccolithophores (Houdan *et al.*, 2006).

Two distinct heterococcolith *Acanthoica* morphotypes were detected in our samples, one of which differed significantly from the typical *A. quattrosipina* morphology (Fig. 3B-C). Basic body plan and key structural features (location of long and short spines and morphology of body rhabdoliths) suggest that the atypical morphotype may represent a variant of *A. quattrosipina*. However, only typical *A. quattrosipina* morphotype was found in combination coccospheres, indicating that there is a genetic and physiological distinction between the two morphotypes, and that the atypical morphotype may represent a discrete species.

Morphological observations provide useful details on the variability of the holococcolith phase, and help to determine the taxonomical affiliation of specimens presented by other authors. Our findings are in agreement with the micrographs of *A. quattrosipina* HOL found in plate 42, figure 13-15 of Young *et al.* (2003) and specimens presented on plate VI of Cros *et al.* (2000). However, the specimen shown on the figures 9 and 12 of plate 42 in Young *et al.* (2003), identified as sp. aff. *A. quattrosipina* HOL exhibits significantly different morphology of the conical protrusion on body coccoliths compared to our specimens. Cros and Fortuño (2002) reported combination coccospheres containing body rhabdoliths of *A. quattrosipina* and holococcoliths belonging to *Sphaerocalyptra* sp. (Fig 18 in Cros and Fortuño 2002). They also presented a coccosphere identified as *Sphaerocalyptra* sp. 1 (Fig. 104A-B), with similar body coccoliths but significantly higher conical protrusions than the ones found in the combination coccospheres. Our observations show that their *Sphaerocalyptra* sp. 1 represents the holococcolith phase of *A. quattrosipina*, since the variability in the size of conical protrusion of body coccoliths represents the phenotypic plasticity in this species (both in holococcolith and heterococcolith phase). Furthermore, coccospheres on plate 1, figure 4 of Dimiza *et al.* (2005) and identified as *Sphaerocalyptra* sp.1 show very similar morphology to *A. quattrosipina* HOL from our samples, with highly variable size of the conical protrusion.

Several specimens of *Sphaerocalyptra* spp. that were reported in the literature show some morphological similarities with *A. quattrosipina* HOL, but still differ significantly in key taxonomical characters. *Sphaerocalyptra* sp. 1 presented in plate 43, figures 7-8 of Young *et al.* (2003) has similar body calyptroliths as *A. quattrosipina* HOL, but without the basal rim, and circumflagellar calyptroliths are much larger than in *A. quattrosipina* HOL, with crystallites growing spirally towards the top of the conical protrusion. Circumflagellar holococcoliths on the combination coccosphere shown in figure 4A of our manuscript

have some morphological similarities to *Sphaerocalyptra* sp. 4 illustrated by Cros and Fortuño (2002), figure 104C-D, indicating that the holococcoliths on figure 4A could represent a separate form of *A. quattrosipina* HOL. However, body holococcoliths presented in our work do not have developed arches, and the extension of the conical protrusion is not as developed as in *Sphaerocalyptra* sp. 4, suggesting that more observations of that holococcolithophore are needed to prove its affiliation with *A. quattrosipina*. Another interesting specimen with morphology similar to *A. quattrosipina* HOL is *Sphaerocalyptra* cf. *adenensis* shown in figure 103 C-D of Cros and Fortuño (2002). This specimen has almost cylindrical circumflagellar coccoliths and very large central protrusion on body coccoliths lacking the crystallite on top of the protrusion, different from *A. quattrosipina* HOL.

Since the production of holococcoliths was shown to take place outside of the cell (Nöel *et al.*, 2004), the observed variability in body calyptroliths could be a result of incomplete development of a conical protrusion. An interesting aspect of the morphology of this species is the apparent mechanism of holococcolith production that can be inferred from our observations. We assume that the production of conical protrusion begins with the initial construction of several supporting arches meeting in the tip of the cone. Subsequent addition of crystallites laterally between the arches forms the cone and leaves the interior of the cone hollow. Such crystallite arches meeting in the tip of the cone are commonly found in other representatives of the *Sphaerocalyptra* genus (e.g. plate 43 of Young *et al.* 2003, plate 1 of Dimiza *et al.* 2005) and the morphology found in *A. quattrosipina* HOL seems to be an advanced variant of the same coccolith architecture.

A single combination coccosphere that possibly shows the transition from the holococcolith to the heterococcolith phase was found in our material (Fig. 5F). Previous investigations of the phase transitions showed that the holococcolith layer is either lost or a simple crystallite layer is present prior to the production of heterococcoliths, leaving an almost naked cell (Nöel *et al.*, 2004). We therefore suggest that our specimen may represent the formation of a new heterococcolith phase after the phase transition.

5. Conclusions

Our work gives a detailed morphological analysis of the life cycle of *Acanthoica quattrosipina*, with a special focus on its holococcolith phase and combination coccospheres. We showed that several previously detected members of *Sphaerocalyptra* genus represent the holococcolith phase of *Acanthoica quattrosipina*. Morphological observations were put in the ecological context, indicating that species can reach high abundances in estuarine ecosystems. Finally, SEM examinations of combination coccospheres are proved to be an important tool for investigations of coccolithophore life cycle.

Acknowledgements

This research was funded by the Research Council of Norway (FRIMEDBIO project 197823) and the Royal Swedish Academy of Sciences through a grant from the Knut and Alice Wallenberg Foundation (KAW 2009.0287). We thank the crew and the scientific staff on board the RV Hydra of the Hydrographic Institute of the Republic of Croatia for their help during the fieldwork. We are grateful to Jeremy Young, Maria Triantaphyllou, Annelies Kleijne and Sebastian Meier for reading the manuscript and providing valuable comments.

References

- Bollmann, J., Cortés, M.Y., Haidar, A.T., Brabec, B., Close, A., Hofmann, R., Palma, S., Tupas, L. & Thierstein, H.R. 2002. Techniques for quantitative analyses of calcareous marine phytoplankton. *Marine Micropaleontology*, **44**(3): 163-185.
- Cros, L. & Fortuño, J.M. 2002. Atlas of northwestern Mediterranean coccolithophores. *Scientia Marina*, **66**(S1): 1-182.
- Cros, L., Kleijne, A., Zeltner, A., Billard, C. & Young, J. 2000. New examples of holococcolith-heterococcolith combination coccospheres and their implications for coccolithophorid biology. *Marine Micropaleontology*, **39**(1): 1-34.
- Dimiza, M.D., Triantaphyllou, M.V. & Theodoridis, S. 2005. *Sphaerocalyptra dermitzaki* and *Sphaerocalyptra youngii*, two new holococcolithophore species from the Aegean Sea (eastern Mediterranean). *Journal of Nannoplankton Research*, **27**(2): 135-139.
- Prada, 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 (Calcihaptophycidae, Haptophyta). *Marine Micropaleontology*, **71**(1-2): 20-27.
- Fuks, D., Devescovi, M., Precali, R., Krstulović, N. & Šolić, M. 1991. Bacterial abundance and activity in the highly stratified estuary of the Krka River. *Marine Chemistry*, **32**: 333-346.
- Geisen, M., Billard, C., Broerse, A., Cros, L., Probert, I. & Young, J. 2002. Life-cycle associations involving pairs of holococcolithophorid species: intraspecific variation or cryptic speciation? *European Journal of Phycology*, **37**(4): 531-550.
- Houdan, A., Billard, C., Marie, D., Not, F., Sáez, A.G., Young, J.R. & Probert, I. 2004. Holococcolithophore-heterococcolithophore (Haptophyta) life cycles: Flow cytometric analysis of relative ploidy levels. *Systematics and Biodiversity*, **1**(4): 453-465.
- Houdan, A., Probert, I., Zatylny, C., Véron, B. & Billard, C. 2006. Ecology of oceanic coccolithophores. I. Nutritional preferences of the two stages in the life cycle of *Coccolithus braarudii* and *Calcidiscus leptoporus*. *Aquatic microbial ecology*, **44**(3): 291.
- Kleijne, A. 1992. *Extant Rhabdosphaeraceae (coccolithophorids, class Prymnesiophyceae) from the Indian Ocean, Red Sea, Mediterranean Sea and North Atlantic Ocean*. *Scripta Geologica*, **100**: 1-63.
- Legović, T., Žutić, V., Gržetić, Z., Cauwet, G., Precali, R.

- & Viličić, D. 1994. Eutrophication in the Krka estuary. *Marine Chemistry*, **46**(1-2): 203-215.
- Lohmann, H. 1903. Neue Untersuchungen über den Reichthum des Meeres an Plankton und über die Brauchbarkeit der verschiedenen Fangmethoden. Zugleich auch ein Beitrag zur Kenntniss des Mittelmeerauftriebs. *Wissenschaftliche Meeresuntersuchungen Abteilung Kiel*, **7**: 1-87.
- Malinverno, E., Triantaphyllou, M.V., Dimiza, M. & Young, J.R. 2008. New possible hetero-holococcolithophore combinations within the genus *Syracosphaera*. *Journal of Nannoplankton Research*, **30**: 1-8.
- Nöel, M.-H., Kawachi, M. & Inouye, I. 2004. Induced Dimorphic Life Cycle of a Coccolithophorid, *Calyptrorpha Sphaeroidea* (Prymnesiophyceae, Haptophyta)1. *Journal of Phycology*, **40**(1): 112-129.
- Parke, M. & Adams, I. 1960. The motile (*Crystallolithus hyalinus* Gaarder & Markali) and non-motile phases in the life history of *Coccolithus pelagicus* (Wallich) Schiller. *Journal of the Marine Biological Association of the United Kingdom*, **39**(02): 263-274.
- Schiller, J. 1913. *Vorläufige Ergebnisse der Phytoplankton-Untersuchungen auf den Fahrten SMS "Najade" in der Adria*. Hölder.
- Thomsen, H.A., Østergaard, J.B. & Hansen, L.E. 1991. Heteromorphic life histories in Arctic coccolithophorids (Prymnesiophyceae). *Journal of Phycology*, **27**(5): 634-642.
- Triantaphyllou, M.V. 2010. Possible affinities between the holococcolithophores *Syracosphaera pulchra* HOL oblonga-type and *Calyptrolithophora papillifera*. *Journal of Nannoplankton Research*, **31**(2): 114-120.
- Triantaphyllou, M.V. & Dimiza, M.D. 2003. Verification of the *Algirosphaera robusta*-*Sphaerocalyptra quadridentata* (coccolithophores) life-cycle association. *Journal of Micropalaeontology*, **22**(1): 107-111.
- Triantaphyllou, M.V., Dimiza, M.D. & Dermitzakis, M.D. 2004. *Syracosphaera halldalii* and *Calyptrolithina divergens* var. *tuberosa* life-cycle association and relevant taxonomic remarks. *Micropaleontology*, **50**(Suppl 1): 121-126.
- Young, J.R., Bergen, J.A., Bown, P.R., Burnett, J.A., Fiorentino, A., Jordan, R.W., Kleijne, A., Van Niel, B., Romein, A.T. & Von Salts, K. 1997. Guidelines for coccolith and calcareous nannofossil terminology. *Palaeontology*, **40**(4): 875-912.
- Young, J.R., 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*.
- Young, J.R., Geisen, M. & Probert, I. 2005. A review of selected aspects of coccolithophore biology with implications for paleobiodiversity estimation. *Micropaleontology*: 267-288.