

Life-cycle associations involving pairs of holococcolithophorid species: intraspecific variation or cryptic speciation?

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New holococcolith–heterococcolith life-cycle associations are documented based on observations of combination coccospheres. *Daktylethra pirus* is shown to be a life-cycle phase of *Syracosphaera pulchra* and *Syracolithus quadriperforatus* a life-cycle phase of *Calcidiscus leptoporus*. In addition, new observations from cultures confirm the life-cycle associations of *Crystallolithus braarudii* with *Coccolithus pelagicus* and of *Zygosphaera hellenica* with *Coronosphaera mediterranea*. In all four cases previous work has shown that the heterococcolithophorid species is associated with another holococcolithophorid. Two other examples of a heterococcolithophorid being associated with two holococcolithophorids have previously been identified, so this seems to be a common phenomenon. The six examples are reviewed to determine whether a single underlying mechanism is likely to be responsible for all cases. It is concluded that there is no single mechanism but rather that the six examples fall into three categories: (a) in two cases the holococcolith types are probably simply ecophenotypic morphotypes; (b) in two other cases the holococcolith types are discrete and are paralleled by morphometric differences in the heterococcolith types; (c) in the final two cases the holococcolith types are discrete but are not paralleled by any obvious morphological variation in the heterococcolith morphology. We infer that cryptic speciation may be widespread in heterococcolithophorid phases and that study of holococcolithophorid phases can provide key data to elucidate this phenomenon.

Key words: coccolithophorids, cryptic speciation, haptophytes, holococcolith–heterococcolith combinations, intraspecific variation, life-cycles

Introduction

Coccolithophorids are unicellular marine photosynthetic algae in the division Haptophyta (synonym Prymnesiophyta). They form a major component of the oceanic microplankton and are one of the main open ocean primary producers. Traditionally the taxonomy of this group has been based on morphological characters of the minute calcite plates that cover the cell, the coccoliths, of which two major types, heterococcoliths and holococcoliths, can be distinguished. Heterococcoliths are formed of a radial array of complex crystal units of variable shape whereas holococcoliths are formed of numerous minute identical euhedral crystallites. Calcification of the heterococcoliths takes place intracellularly and is consequently under strong cellular control (e.g. Westbroek *et al.*, 1984). In contrast biomineralization of the holococcoliths

apparently occurs outside the cell membrane within an organic ‘skin’ which surrounds the cell (Rowson *et al.*, 1986), but the regulatory mechanisms for this process remain poorly understood (for reviews of coccolith morphology and formation see Leadbeater, 1994; Pienaar, 1994; Young *et al.*, 1999).

The dominant reproductive mode of haptophytes is asexual mitotic division. However, in many haptophytes more complex life-cycles have been documented, with two or more morphologically distinct phases (Fig. 1). Evidence for this has come from two main sources: (1) culture observations of phase transitions (e.g. Parke & Adams, 1960), supported by direct observation of meiosis and syngamy (e.g. Gayral & Fresnel, 1983), and chromosome counts (Rayns, 1962; Fresnel, 1994) in cultured clones; (2) rare observations from natural populations of combination coccospheres bearing different coccolith types interpreted as representing the moment of life-cycle phase transition (e.g. alternation of haploid and diploid phases).

Coccolithophorid life-cycles

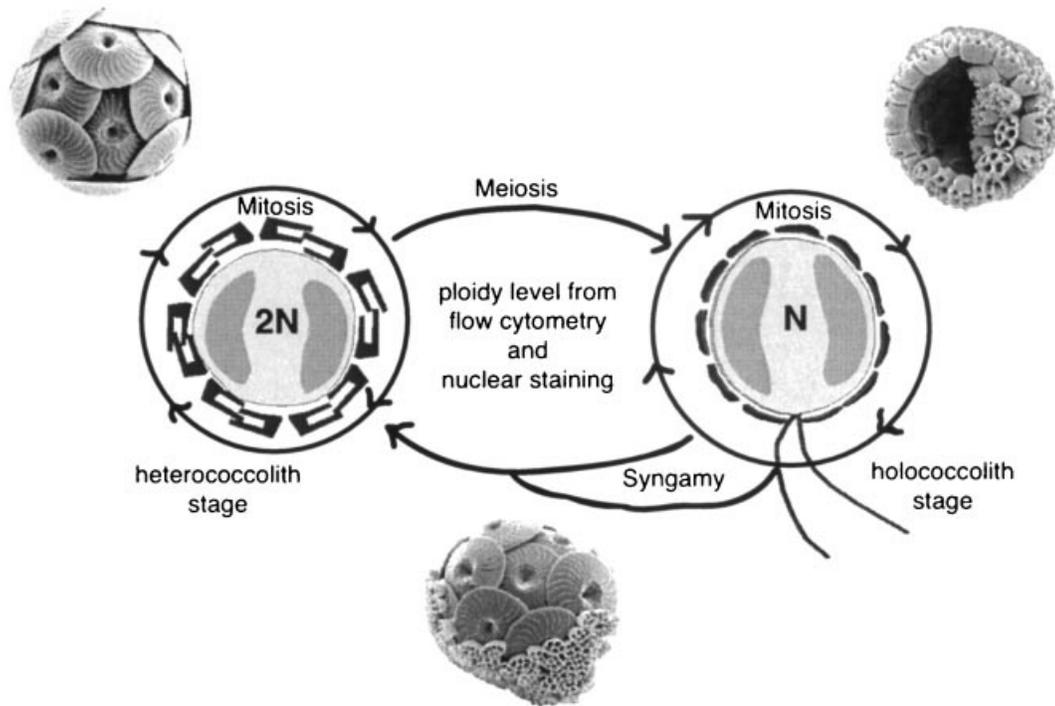


Fig. 1. Schematic representation of coccolithophorid life-cycles. The diploid stage is covered with heterococcoliths which are produced intracellularly, whereas the motile haploid stage is covered with holococcoliths which are produced outside the cell membrane. Most examples given in this paper would represent syngamy of two haploid gametes with both holococcoliths and heterococcoliths being present on a single coccosphere.

Summarizing available data, Billard (1994) inferred that there was a common pattern of alternating haploid and diploid phases, both of which were capable of mitotic reproduction, characterized by consistent differences in their coccolith and scale covers. Of particular relevance, she noted that available evidence suggested that heterococcoliths were characteristic of diploid phases and holococcoliths of haploid phases of coccolithophores. This hypothesis has been directly tested, and supported, by investigation of ploidy level in cultures of *Emiliania huxleyi* (Green *et al.*, 1996) and *Coccolithus pelagicus* (Young *et al.*, 2000).

Combination coccospheres bear two coccolith types which were traditionally regarded as belonging to separate species. Numerous new examples of holococcoliths and heterococcoliths forming combination coccospheres in field samples have recently been observed (Cortés, 2000; Cros *et al.*, 2000; Renaud & Klaas, 2001), indicating that they are alternate phases of the life-cycle of single species. From such data, *associations* of ‘species’ are inferred. *Het-hol associations* involve one heterococcolith species and one holococcolith species. The available examples span the biodiversity of coccolithophorids, strongly supporting the hypothesis that the primitive state for coccolithophorids is to have a diploid heterococcolith-bearing phase and

haploid holococcolith-bearing phase (Bown, 1998; Young *et al.*, 1999, 2000).

Amongst the reported het-hol combinations, three cases of one heterococcolithophorid forming separate combinations with two holococcolithophorids have been reported (see Cros *et al.*, 2000). For each of these cases of *het-hol-hol associations*, Cros *et al.* (2000) concluded that this phenomenon is most likely the result of non-genotypic variation in the degree of calcification of the holococcolithophorid phase. Three new examples of het-hol associations are reported here, and in each case the heterococcolithophorid has previously been found in association with a different holococcolithophorid. There are therefore now a total of six het-hol-hol associations, representing nearly one-third of all associations discovered to date. This increasingly common pattern of one heterococcolith being associated with two holococcoliths is intriguing and suggests a common cause; several possible explanations can be postulated.

The first possibility is **hybridization** occurring between the gametes (i.e. haploid phases) of two closely related species. Hybridization is a widespread phenomenon within higher plant taxa and some macroalgae (e.g. Cosson *et al.*, 1984; Cosson, 1987), though hybridization of marine protists has not been documented. The available evidence

from cultures (e.g. Gayral & Fresnel, 1983) suggests that all haploid cells can act as gametes. Hence hybridization between closely related coccolithophorids would be predicted to give rise immediately after syngamy to a combination coccosphere bearing holococcoliths of the two species.

A second possibility is **complex life-cycles**; phytoplankton life-cycles often include a range of morphologies. Even assuming that the basic het–hol division corresponds to a separation between diploid and haploid phases, it is possible that the haploid phase may have more than one morphotype. Variable cell morphologies have been documented in the haploid phase of the life-cycle of haptophytes such as *Pleurochrysis pseudoroscoffensis* (Gayral & Fresnel, 1983), other species of *Pleurochrysis* (Fresnel & Billard, 1991) and *Phaeocystis* (Lancelot & Rousseau, 1994), although at present there is no indication that coccolith morphology varies within a phase.

A third possibility is **sexual dimorphism**; if a haplo-diplontic life-cycle also involves sexual differentiation, then discrete male and female haploid gametes, potentially with differing holococcolith morphologies, would be formed. This is a well-known phenomenon in other algal classes (e.g. centric diatoms (von Stosch, 1954), cryptomonads (Hill & Wetherbee, 1986) and dinoflagellates (von Stosch, 1972)).

Fourth, these observations may represent **intra-specific variation**, without genotypic control. Holococcolith taxonomy is entirely based on coccolith morphology and since very few holococcolithophorids have been maintained in culture there is little information on the degree of variability in coccolith morphology possible within one species. Cros *et al.* (2000) speculated that intraspecific variation in the degree of calcification of the holococcolith phase was a possible explanation for the het–hol–hol associations they observed, particularly since the holococcoliths involved were morphologically rather similar.

Finally it is conceptually possible for speciation to occur without obvious morphological change (**cryptic speciation**); indeed molecular genetic results for certain protist groups have suggested that this may be a common phenomenon (e.g. Andersen *et al.*, 1998; Darling *et al.*, 2000). As a variant we can imagine that morphological change following speciation may be apparent in the holococcolith phase but cryptic in the heterococcolith phase.

In this paper we present our new evidence of het–hol–hol associations and, for each case, review which of these potential causes can most reasonably be invoked to explain this interesting and increasingly commonly detected phenomenon. Our data come from four separate research groups working on diverse research tasks within the larger

EU-TMR Coccolithophorid Evolutionary Biodiversity and Ecology Network (CODENET); the disparate data are combined here to allow timely synthesis of this topic.

Materials and methods

Taxonomic nomenclature

Traditional coccolithophorid taxonomy was established using the morphological characters of the coccoliths covering the cell, and crystallographic orientation of the component crystal units (e.g. Bown, 1998; Young *et al.*, 1999). This taxonomy has been successfully applied to the fossil record and compares well with findings from other characterization methods such as cell ultrastructure and more recently molecular genetics. The discovery that heterococcolithophorids (HE) and holococcolithophorids (HO) can be formed by single species in different phases of the life-cycle does not invalidate this taxonomy but it does lead to nomenclatural problems. There has been much debate as to how nomenclatural taxonomy should be adjusted to reflect these observations. We strongly agree with Cros *et al.* (2000) and Silva (personal communication) that once such associations are established a single scientific name should be adopted for all phases following the normal rules of botanical nomenclature, with informal terminology used to indicate the phase observed where appropriate. However, since this publication is concerned with establishing such associations, for clarity we use the original 'species' names for newly associated phases; the correct names for future usage, based on nomenclatural priority, are given in the Results and Discussion section.

Field samples

The key specimens reported here were found in samples collected from the Alboran Sea (Western Mediterranean) during a cruise of the Institut de Ciències del Mar (CSIC) on board the R/V *Hesperides* in 1999 as part of the MATER research project (Mater II, from 26 September to 6 October).

During this cruise water samples were obtained at selected depths using a rosette sampler with Niskin bottles attached to a Conductivity, Temperature, Depth (CTD) probe. Depending on the concentration of phytoplankton, up to 1000 ml of seawater were filtered using vacuum filtration. Two types of filters were used: (1) 25 mm cellulose nitrate filters with 0.45 μm retention (Whatman) and (2) 25 mm polycarbonate filters with 0.47 μm pore size (Millipore). Salt was removed by rinsing the filters with mineral water. The filters were oven-dried at 50 °C for 1 h. For scanning electron microscopy (SEM) a part of the filter was mounted on a stub and coated with gold–palladium before examination in a Philips XL-30 FEG or a Cambridge Stereoscan S250 microscope. For light microscopy (LM) a part of the cellulose nitrate filter was mounted with immersion oil on a slide and covered with a coverslip. LM observations were performed using a Zeiss Axioplan with a Hamamatsu CCD video camera. LM was used both for morphological observations and to determine the crystallographic orientation of the con-

Table 1. Sample locations for all combination coccospheres discussed in the text. Where possible the direction of the life-cycle phase transition is given

Combination cells	Cruise	Location	Date	Station	Latitude	Longitude	Depth (m)	Type	Fig.	Transition mode	Notes	BMNH image ref.	Reference
<i>H. carteri</i> / <i>Sl. catilliferus</i>	MESO-96	NW Mediterranean	Jun.–Jul. 1998	G4	41° 08'00" N	02° 45'02" E	70	SEM	Not figured	Hol–het			Cros <i>et al.</i> 2000 – Plate 1, Fig. 4
<i>H. carteri</i> / <i>Sl. catilliferus</i>	FRONTS-95	NW Mediterranean	Jun. 1995	24 W	40° 33'00" N	02° 38'70" E	70	SEM	Not figured	Unknown			Cros <i>et al.</i> 2000 – Plate 1, Fig. 3
<i>H. carteri</i> / <i>Sl. confusus</i>		Mediterranean	1955				75	LM	Not figured	Unknown			Lecal-Schlauder, 1961 – Photo 4, 5
<i>Sl. confusus</i> / <i>Sl. catilliferus</i>	Meteor 36/2	NE Atlantic		178	33° 00'20" N	22° 00'00" W	20	SEM	Not figured	n/a			Cros <i>et al.</i> 2000 – Plate 1, Fig. 6
<i>Sl. confusus</i> / <i>Sl. catilliferus</i>	MESO-96	NW Mediterranean	Jun. 1996	F2	41° 27'02" N	02° 52'00" E	5	SEM	4-Jun	n/a			This publ.
<i>Sl. bannockii</i> / <i>Corisphaera</i> sp. type A	MESO-96	NW Mediterranean	Jun.–Jul. 1998	G6	40° 56'30" N	02° 56'70" E	40	SEM	Not figured	Het–hol			Cros <i>et al.</i> 2000 – Plate 7, Fig. 3
<i>Corisphaera</i> sp. type A/ <i>Z. bannockii</i>	FANS-1	NW Mediterranean	Nov. 1996	127 (141)	39° 52'80" N	00° 54'00" E	5	SEM	Not figured	n/a			Cros <i>et al.</i> 2000 – Plate 7, Fig. 5
<i>Corisphaera</i> sp. type A/ <i>Z. bannockii</i>	Sonne 117	Indian Ocean		20/3	14° 29'70" N	64° 44'40" E	20	SEM	Not figured	n/a			Cros <i>et al.</i> 2000 – Plate 7, Fig. 6
<i>Corisphaera</i> sp. type A/ <i>Z. bannockii</i>	FANS-1	NW Mediterranean	Nov. 1996	123	39° 59'60" N	00° 44'40" E	40	SEM	10	n/a			This publ.
<i>Corisphaera</i> sp. type A/ <i>Z. bannockii</i>	FANS-1	NW Mediterranean	Nov. 1996	127	39° 52'80" N	00° 54'00" E	5	SEM	Not figured	n/a			This publ.
<i>Corisphaera</i> sp. type A/ <i>Z. bannockii</i>	FANS-1	NW Mediterranean	Nov. 1996	127	39° 52'80" N	00° 54'00" E	40	SEM	8, 9	n/a			This publ.
<i>Cl. pelagicus</i> / <i>Cr. hyalinus</i>	Meteor 7	N. Atlantic	Sept. 1985	10	72° 13'00" N	16° 05'00" W	Surface	SEM	Not figured	Hol–het	<i>Cl. pelagicus</i> small morphotype		Samtleben & Schröder, 1992 – Plate 1, Fig. 8
<i>Cl. pelagicus</i> / <i>Cr. hyalinus</i>		N. Atlantic, Norwegian Sea	Jun. 1986	2	65° 30'00" N	00° 08'00" W	15	SEM	13	Hol–het	<i>Cl. pelagicus</i> small morphotype		Samtleben in Winter & Siesser, 1994
<i>Cl. pelagicus</i> / <i>Cr. hyalinus</i>		N. Atlantic, Norwegian Sea	Jun. 1986	2	65° 30'00" N	00° 08'00" W	15	SEM	Not figured	Hol–het	<i>Cl. pelagicus</i> small morphotype		Samtleben & Bickert, 1990 – Plate 1, Fig. 8
<i>Cl. pelagicus</i> / <i>Cr. hyalinus</i>		N. Atlantic, Norwegian Sea	Jun. 1986	2	65° 30'00" N	00° 08'00" W	15	SEM	Not figured	Hol–het	3 observations, <i>Cl. pelagicus</i> small morphotype		This publ.
<i>Cl. pelagicus</i> / <i>Cr. hyalinus</i>	ARK VII/1	N. Atlantic, Norwegian Sea	Jun. 1990	43	70° 45'00" N	05° 30'00" W	Surface	SEM	Not figured	Hol–het	<i>Cl. pelagicus</i> small morphotype		Baumann <i>et al.</i> 1990 – Plate 1, Fig. 1
<i>Cl. pelagicus</i> / <i>Cr. hyalinus</i>		N. Atlantic, Norwegian Sea	Sept. 1988	554	72° 00'00" N	13° 00'00" W	10	SEM	14	Het–hol	2 observations, <i>Cl. pelagicus</i> small morphotype		This publ.
<i>Cl. pelagicus</i> / <i>Cr. hyalinus</i>				OG33A			500	SEM	Not figured	Het–hol	Sediment trap, <i>Cl. pelagicus</i> small morphotype		Andruleit
<i>Cl. pelagicus</i> / <i>Cr. hyalinus</i>		N. Atlantic, Norwegian Sea	Sept. 1988	552	71° 38'00" N	08° 25'00" W	32	SEM	Not figured	Het–hol	<i>Cl. pelagicus</i> small morphotype		This publ.
<i>Cl. pelagicus</i> / <i>Cr. braarudii</i>		Arcachon, SW France					Surface	SEM, LM, TEM	15	Both	Change observed in culture (LK1-4, CF4-5, KL2)		This publ.
<i>Cl. pelagicus</i> / <i>Cr. braarudii</i>		English Channel	Apr. 1985		50° 02'00" N	04° 22'00" W	10	SEM, LM, TEM	Not figured	Both	Change observed in culture (PLY 128)		Parke & Adams, 1960; Rowson, 1986; Manton & Leedale, 1963
<i>Cd. leptoporus</i> / <i>Cr. rigidus</i>	Snellius II	W. Mediterranean	Jul. 1985	GX-192	36° 54'00" N	02° 11'30" E	5	SEM	Not figured	Hol–het	Intermediate morphotype		Kleijne, 1991 – Plate 4, Fig. 4
<i>Cd. leptoporus</i> / <i>Cr. rigidus</i>		WC Atlantic Ocean	May 1991		32° 10'00" N	64° 30'00" W	25	SEM	Not figured	Hol–het	Intermediate morphotype		Cortés, 2000 – Plate 1, Figs 1, 2
<i>Cd. leptoporus</i> / <i>Cr. rigidus</i>		WC Atlantic Ocean	May 1991		32° 10'00" N	64° 30'00" W	1	SEM	Not figured	Hol–het	Unknown morphotype		Renaud & Klaas (2001)
<i>Cd. leptoporus</i> / <i>Cr. rigidus</i>		WC Atlantic Ocean	May 1991		32° 10'00" N	64° 30'00" W	25	SEM	Not figured	Hol–het	Intermediate morphotype		Renaud & Klaas (2001)
<i>Cd. leptoporus</i> / <i>Cr. rigidus</i>		WC Atlantic Ocean	May 1991		32° 10'00" N	64° 30'00" W	25	SEM	Not figured	Hol–het	Intermediate morphotype		Cortés, 2000 – Plate 1, Figs 3, 4
<i>Cd. leptoporus</i> / <i>Cr. rigidus</i>	MATER II	W. Mediterranean	Sept.–Oct. 1999					LM	Not figured	Het–hol	Intermediate morphotype, phase change observed in culture (AS 31)		This publ.

Table 1 (contd.)

<i>Cd. leptoporus/Cr. rigidus</i>	MARA	S. Atlantic Ocean						LM	Not figured	Het-hol	Intermediate morphotype, phase change observed in culture (NS10-2, NS4-2, NS8-2)		This publ.
<i>Cd. leptoporus/Sl. quadriperforatus</i>	MATER II	W. Mediterranean	Oct. 1999	69	39° 25-98' N	02° 25-30' W	5	SEM	21, 22	Hol-het	Large morphotype	MG124-04 to 13	This publ.
<i>Ss. pulchra/D. pirus</i>	MATER II	W. Mediterranean	Sept. 1999	15	35° 55-20' N	01° 20-73' W	5	LM	30	Unknown			This publ.
<i>Ss. pulchra/D. pirus</i>	MATER II	W. Mediterranean	Sept. 1999	15	35° 55-20' N	01° 20-73' W	5	SEM	33, 34	Unknown		MG117-67 to 69	This publ.
<i>Ss. pulchra/D. pirus</i>	MATER II	W. Mediterranean	Sept. 1999	15	35° 55-20' N	01° 20-73' W	5	SEM	Not figured	Hol-het			This publ.
<i>Ss. pulchra/D. pirus</i>	MATER II	W. Mediterranean	Oct. 1999	69	37° 25-98' N	00° 25-30' W	42-5	SEM	31	Hol-het		MG128-4 to 6	This publ.
<i>Ss. pulchra/D. pirus</i>	MATER II	W. Mediterranean	Oct. 1999	69	37° 25-98' N	00° 25-30' W	42-5	SEM	32	?hol-het		MG127-26, 27	This publ.
<i>Ss. pulchra/D. pirus</i>		C. Mediterranean	Mar. 1961	9	37° 47-00' N	11° 23-00' E	100	LM, TEM	Not figured	Het-hol			Saugestad & Heimdal, 2002 – Plate 4, Figs 1 (a-c)
<i>Ss. pulchra/D. pirus</i>		C. Mediterranean	Mar. 1961	9	37° 47-00' N	11° 23-00' E	50	LM	Not figured	Het-hol			Saugestad & Heimdal, 2002 – Plate 4, Figs 2 (a-c)
<i>Ss. pulchra/D. pirus</i>		Mediterranean					Unknown		Not figured	Unknown	1 observation, no photo		Lecal-Schlauder, 1961
<i>Ss. pulchra/D. pirus</i>		C. Mediterranean, off Naples	2000				Surface	SEM	Not figured	Het-hol	Change observed in culture (NAP-10)		This publ.
<i>Ss. pulchra/Ca. oblonga</i>	JGOF5 4	N. Atlantic	Jun. 1990	2	53° 30-00' N	20° 30-00' W	30	SEM	26	Unknown		MG130-6, 7	This publ.
<i>Ss. pulchra/Ca. oblonga</i>	JGOF5 4	N. Atlantic	Jun. 1990	2	53° 30-00' N	20° 30-00' W	30	SEM	27	Unknown		MG130-8 to 10	This publ.
<i>Ss. pulchra/Ca. oblonga</i>	MEDEA-98	NW Mediterranean	Mar. 1998		41° 28-00' N	02° 19-10' E	Surface	LM, SEM	Not figured	Unknown			Cros <i>et al.</i> 2000 – Plate 2, Figs 3, 4
<i>Ss. pulchra/Ca. oblonga</i>	FRONTS-96	NW Mediterranean	Sept. 1996	21	41° 11-70' N	03° 41-60' E	20	SEM	Not figured	?hol-het			Cros <i>et al.</i> 2000 – Plate 2, Fig. 2
<i>Ss. pulchra/Ca. oblonga</i>		C. Mediterranean	?1902				Unknown	LM	Not figured	Unknown	2 observations, drawing		Lohmann, 1902 – Plate 6, Fig. 67, Plate 5, Fig. 54
<i>Ss. pulchra/Ca. oblonga</i>		Adriatic Sea	1926				Unknown	LM	Not figured	Unknown	Some observations on living cells		Kamptner, 1941
<i>Ss. pulchra/Ca. oblonga</i>		Mediterranean	1954				25	LM	Not figured	Unknown			Lecal-Schlauder, 1961 – Photo 2, 3
<i>Cs. mediterranea/Cy. wettsteinii</i>	MESO-96	NW Mediterranean	Jun.–Jul. 1998	12	41° 13-90' N	02° 20-70' E	40	SEM	Not figured	Hol-het			Cros <i>et al.</i> 2000 – Plate 4, Fig. 3
<i>Cs. mediterranea/Cy. wettsteinii</i>		C. Mediterranean, near Rovigno	?1939				Unknown	LM	Not figured	Hol-het	2 observations, drawing		Kamptner, 1941 – Plate 15, Fig. 152
<i>Cs. mediterranea/Cp. hasleana</i>	HOTS	C. North Pacific	late 1996		22° 45-00' N	158° 00-00' W	5	SEM	Not figured	Unknown			Cortés & Bollmann, 2002 – Figs 1, 2
<i>Cs. mediterranea/Z. hellenica</i>		S. Atlantic Ocean	2000				Surface	SEM, LM	38, 39	Het-hol	Change observed in culture (NS 8-5)	MG163-49, 50	This publ.

Ca., *Calyptosphaera*; Cd., *Calcidiscus*; Cl., *Coccolithus*; Cp., *Calyptrolithophora*; Cr., *Crystallolithus*; Cy., *Calyptrolithina*; D., *Daktylethra*; H., *Helicosphaera*; Sl., *Syracolithus*; Ss., *Syracosphaera*; Z., *Zygospaera*; LM, light microscopy; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

stituent crystallites of the holococcoliths using cross-polarized illumination (see Moshkovitz & Osmond, 1989, or Young, 1992, for a description of this technique as applied to coccoliths).

Other specimens were obtained from field samples collected during various cruises in the Mediterranean, the North Atlantic Ocean and the Gulf of Mexico (Table 1).

Culture samples

A large collection of unialgal coccolithophorid strains has been established during the CODENET project, including many species not previously cultured successfully. Cultures discussed here were isolated from plankton net samples collected off Arcachon (SW France), off South Africa (S. Atlantic), in the Alboran Sea (western Mediterranean) and off Naples (Italy) (see also Table 1).

Seawater collection and culture isolation

To obtain concentrated seawater for isolation of coccolithophorids small hand-operated plankton nets with a mesh size of 5 and 10 μm were deployed from ships on station at depths between 5 to 15 m and were left in the water for up to 2 h. Additionally the nets were used to concentrate water from the CTD rosette sampler to sample species from the deep photic zone.

After collection the concentrated seawater samples were filtered through a 64 μm mesh sieve to remove larger zooplankton and transferred into translucent storage containers. Usually two containers were used per sample and GeO_2 (to inhibit the reproduction of diatoms) and nutrients were added to one aliquot. The containers were stored at the respective ambient water temperature either in an incubator with a 16 h light, 8 h dark (16L/8D) cycle or in a room with continuous light, and were opened daily to allow air exchange. Samples were transported back to the laboratory in a cool box as soon as possible.

Culture isolation was performed on an inverted microscope using $\times 80$ magnification and a glass micropipette. Single cells were captured, transferred into fresh medium, picked up again and finally transferred into sterile polystyrene tissue culture microplates with the wells filled with a media series ranging from K/2 to K/10 (Keller *et al.*, 1987). Normally a microplate with 24 wells, each filled with 2 ml medium, was used. After completion the lid of the microplate was sealed with Parafilm to prevent evaporation and the microplate was stored in an incubator. The microplates were checked regularly and growing cultures were then transferred into sterile 75 ml tissue culture flasks filled with 40 ml of medium.

Culture maintenance

Cultures were maintained in exponential growth in an incubator at 17 °C on a 16L/8D cycle. Typically, the cultures were checked with an inverted microscope every 2 weeks and reinoculated into fresh medium using a laminar flow cabinet to prevent contamination. Medium was prepared from seawater collected from the French coast of the English Channel. The seawater was filtered with an ordinary filter paper circle and autoclaved at 120 °C for 15 min. After cooling, nutrients – nitrate (500 μM), phosphate (20 μM), trace metals and vitamins–

were added under a laminar flow cabinet. For the detailed chemical composition of K medium refer to Keller *et al.* (1987).

Results and discussion

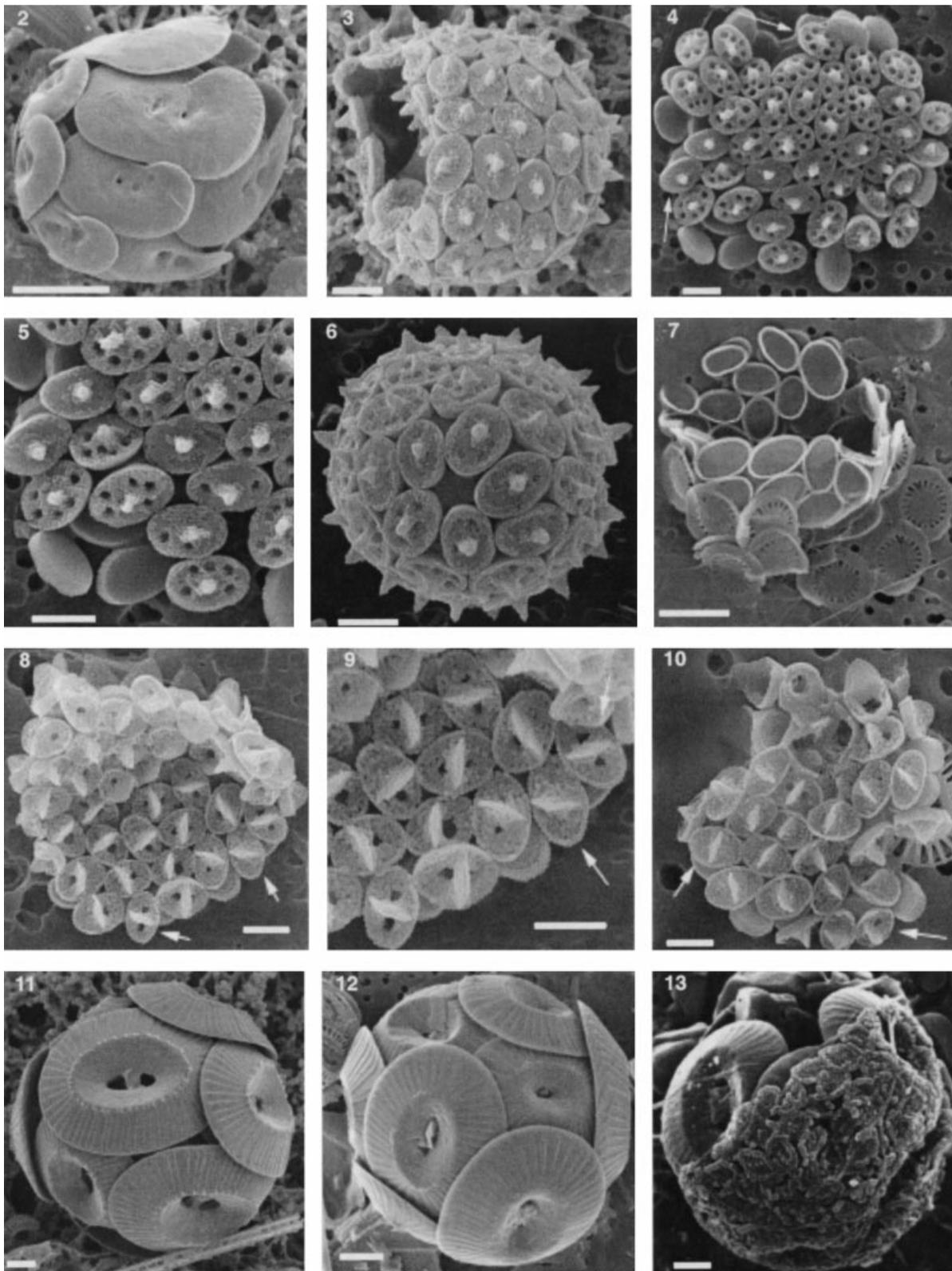
We have additional data for each of three het–hol–hol associations identified by Cros *et al.* (2000), and evidence of three further examples. For clarity the previous observations are briefly summarized here together with our new results. For all discussed examples of combination coccospheres refer to Table 1 for sample details.

Helicosphaera carteri with *Syracolithus catilliferus* and *S. confusus* (Figs 2–6)

Cros *et al.* (2000) illustrated two examples of the heterococcolithophorid *Helicosphaera carteri* (Fig. 2) forming het–hol combination coccospheres with *Syracolithus catilliferus* (Fig. 3). In addition they observed a single holococcolith–holococcolith (hol–hol) combination coccosphere with *S. confusus* and *S. catilliferus*. Finally they reinterpreted a light micrograph of Lecal-Schlauder (1961) as illustrating a combination coccosphere of *H. carteri* with *Syracolithus confusus*.

We have found two further examples of *S. confusus* with *S. catilliferus* (hol–hol) combination coccospheres (Figs 4–6). Unlike the examples shown in Cros *et al.* (2000), these specimens clearly contain transitional morphotypes between these two (morpho-)species.

If hybridization or sexual dimorphism were responsible for this het–hol–hol association we would expect to observe both hol–hol combination coccospheres with discrete holococcolith types, and het–hol–hol combination coccospheres, again with discrete holococcolith types (the latter being a more advanced stage in the process of transition from the haploid to the diploid phase). However, no het–hol–hol combination coccospheres have been discovered and the two additional examples which have been found of hol–hol combination coccospheres include intermediate stages between the two morphologies (Figs 4–6). Hybridization and sexual dimorphism therefore seem very unlikely as causes of this association. The similarity of *S. catilliferus* and *S. confusus* was noted by Kleijne (1991), and Cros *et al.* (2000) predicted that this may be an example of intraspecific variation in the degree of calcification, the two holococcolith types differing essentially in the presence/absence of perforations in the coccolith structure. The additional observations of hol–hol combination coccospheres with intermediate morphotypes validate this prediction. Hence it seems very likely that this is an example of fine-scale, non-genotypic, intraspecific variability in the morphology of the holococcolith-bearing phase



Figs 2–13. Scanning electron micrographs (SEMs) of coccospheres of various species. **Fig. 2.** SEM of a coccosphere of *Helicosphaera carteri*. Water sample, N. Atlantic, R/V *Meteor* 42-4B cruise, station US1B. **Fig. 3.** SEM of a coccosphere of *Syracolithus catilliferus*. Water sample, N. Atlantic, off Canary Islands, R/V *Poseidon* cruise 233, station 3. Image courtesy C. Sprengel, AWI Bremen. **Figs 4–6.** SEMs of two *Syracolithus confusus*–*Syracolithus catilliferus* combination coccospheres. All the figures show the *S. catilliferus* and *S. confusus* coccoliths (arrows) as well as transitional forms on one coccosphere. Fig. 5 shows a detail of Fig. 4. Water sample, NW Mediterranean, MESO-96 cruise, station F2. **Fig. 7.** SEM of a collapsed *Syracosphaera delicatus* coccosphere. Both endo- and exothecal coccoliths can be seen. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 69. **Figs 8–10.** SEMs of two *Zygosphaera bannockii*–*Corisphaera* sp. type A combination coccospheres. Fig. 9 shows a detail of Fig. 8. Both coccospheres show

and all three morphospecies should be referred to as a single species.

Helicosphaera carteri (Wallich, 1877) Kamptner, 1954.

HETEROTYPIC SYNONYMS: *Syracolithus catilliferus* (Kamptner, 1937) Deflandre, 1952; *Syracolithus confusus* Kleijne, 1991.

As discussed in Cros *et al.* (2000), *Helicosphaera carteri* has priority and so is the appropriate name. N.B. *Syracolithus* Deflandre 1952 would have priority over *Helicosphaera* Kamptner, 1954, but the type species of *Syracolithus* is *S. dalmaticus*, which is not known to form associations with *Helicosphaera*. As proposed in Cros *et al.* (2000) informal terms (*H. carteri* HO-solid for *catilliferus* type and *H. carteri* HO-perforate for *confusus* type holococcoliths) should be used to distinguish the respective intraspecific holococcolith morphologies.

Syracosphaera bannockii with *Zygosphaera bannockii* and *Corisphaera* sp. type A. (Figs 7–10)

The holococcolithophorid *Zygosphaera bannockii* was observed by Cros *et al.* (2000) to form both het–hol combination coccospheres with a previously undescribed *Syracosphaera* (Fig. 7) species and hol–hol combination coccospheres with *Corisphaera* sp. type A. (N.B. Since the *Syracosphaera* species was previously undescribed the name *S. bannockii* is now applied to it, as recommended by Cros *et al.*, 2000).

We have found three further examples of the hol–hol combination of *Z. bannockii* with *Corisphaera* sp. type A (Figs 8–10). In all cases these specimens contain transitional morphotypes between these two (morpho-)species.

This example is directly analogous to the *H. carteri* case and so is also interpreted as a result of non-genotypic, intraspecific variation.

Syracosphaera bannockii (Borsetti *et Cati*, 1976) Cros *et al.* 2000.

HOMOTYPIC SYNONYM: *Zygosphaera bannockii* (Borsetti *et Cati*, 1976) Heimdal, 1982.

HETEROTYPIC SYNONYM: *Corisphaera* sp. type A Kleijne, 1991.

Syracosphaera bannockii was proposed by Cros *et al.* (2000) as a new combination, since the heterococcolith morphotype had not previously been

described, while the genus *Syracosphaera* has priority over *Zygosphaera*. By analogy to *H. carteri* an informal classification should be used to distinguish the respective intraspecific holococcolith morphologies: *S. bannockii* HO-bridged and *S. bannockii* HO-solid.

Coccolithus pelagicus with *Crystallolithus hyalinus* and *Crystallolithus braarudii* (Figs 11–15)

Combination coccospheres of *Coccolithus pelagicus* and *Crystallolithus hyalinus* have been illustrated from field-collected samples (Samtleben & Bickert, 1990; Samtleben & Schröder, 1992; Baumann *et al.*, 1997; C. Samtleben personal communication). Unpublished micrographs of specimens from field-collected samples from the N. Atlantic Ocean were made available to us by Christian Samtleben (University of Kiel) and Karl-Heinz Baumann (University of Bremen) (Figs 13, 14); like the published micrographs, these reveal unambiguous combination coccospheres of *C. pelagicus* with *Cr. hyalinus*.

Several of our monoclonal cultures of *C. pelagicus* from Arcachon (SW France) have given rise to the holococcolith-bearing phase, but in each case the holococcolithophorid associated is *Crystallolithus braarudii* (Fig. 15), rather than *Cr. hyalinus*.

The two holococcolith types involved, *Crystallolithus braarudii* and *Crystallolithus hyalinus*, are structurally very similar, hence Cros *et al.* (2000) concluded that the *Coccolithus pelagicus*–*Crystallolithus* combination was another example of variation in the degree of calcification. In this case, however, the two holococcolith morphotypes have not been observed co-occurring on a single coccosphere, and the holococcolith morphology appears to be consistent within monoclonal cultures maintained under a range of environmental conditions.

A review of the literature reveals that *Cr. hyalinus* and *Cr. braarudii* have often been confused. Parke & Adams (1960), who first demonstrated the association in cultures, identified the holococcolith-bearing stage as *Cr. hyalinus* and Rowson *et al.* (1986) maintained this identification. In the original description (Gaarder & Markali, 1956) the central area of *Cr. hyalinus* is described as being filled with calcite rhombohedra arranged in parallel rows, with

coccoliths of *Z. bannockii* and *Corisphaera* sp. type A (arrows) as well as transitional forms. Water sample, NW Mediterranean, FANS 1 cruise, station 127 (Figs 8, 9) and station 123 (Fig. 10). **Figs 11, 12.** SEMs of coccospheres of *Coccolithus pelagicus*. The images display the coccolith size variation between the large temperate (Fig. 11) *C. pelagicus* and the small Arctic morphotype (Fig. 12). Water sample, S. Atlantic, off Namibia, R/V *Meteor* cruise M48-4 (Fig. 11) and N. Atlantic, off Iceland (Fig. 12). **Fig. 13.** SEM of *Coccolithus pelagicus*–*Crystallolithus hyalinus* combination coccosphere. The *C. pelagicus* heterococcolith is of the small morphotype. The *Cr. hyalinus* coccolith shows its typical central area features, with the calcite rhombohedra arranged in parallel rows and covering all the central area. Water sample, N. Atlantic, Greenland Sea. Image courtesy C. Samtleben, University of Kiel. Scale bars represent: Figs 2, 8–10: 1 μm ; Figs 3–7, 11–14: 2 μm .

each crystal lying on one face and partly touching the surrounding crystals at parts of the adjacent faces (compare Figs 13, 14). Two years after the publication of the *C. pelagicus*–*Cr. hyalinus* life-cycle by Parke & Adams (1960), Gaarder (1962) described the new holococcolithophorid *Cr. braarudii*. Whereas the rim structure in this species is similar to *Cr. hyalinus*, the basal layer is incomplete with the crystallites being confined to a few radial spokes and sometimes a central ellipse (compare Fig. 15). The specimens figured in both Parke & Adams (1960) and Rowson *et al.* (1986) clearly resemble *Cr. braarudii* rather than *Cr. hyalinus*. Several of our cultures of *C. pelagicus* (strains LK1, 2 & 3, CF4 & 5, all from Arcachon, SW France) have undergone phase change and in each case examination with transmission electron microscopy and light microscopy revealed the holococcolith *Cr. braarudii* (Fig. 15). All observations made so far from culture material thus seem to display a *C. pelagicus*–*Cr. braarudii* life-cycle. By contrast, the *C. pelagicus*–*Cr. hyalinus* combinations, figured in Samtleben & Bickert (1989), Samtleben & Schröder (1992), Winter & Siesser (1994) and Baumann *et al.* (1997), have only been observed from plankton samples. There is also a biogeographic division between these associations, all plankton observations coming from Arctic waters whilst the cultures in which phase transformations have been observed have all been isolated from temperate waters. It has recently been shown that the temperate and Arctic *C. pelagicus* populations show different ecological adaptations, produce different-sized heterococcoliths (Figs 11, 12), and are genetically differentiated (Baumann *et al.*, 2000; Cachao & Moita, 2000; our unpublished data). It seems likely that these populations represent discrete species, or subspecies. Since in this case the holococcolith differentiation appears to parallel that of the heterococcoliths, there is no support for inferences such as complex life-cycles, hybridization or sexual dimorphism, and intra-specific variation seems unlikely. Instead, it seems that a recent phylogenetic divergence event has occurred with slight qualitative separation of the holococcoliths and quantitative, biometrically measurable, separation of the heterococcoliths (parallel differentiation). We recommend distinguishing the morphotypes as subspecies rather than species, due to the slight morphological differentiation and to minimize nomenclatural confusion.

Coccolithus pelagicus (Wallich, 1877) Schiller, 1930 (type species of *Coccolithus*).

HETEROTYPIC SYNONYMS: *Crystallolithus braarudii* Gaarder, 1962; *Crystallolithus hyalinus* Gaarder *et al.* Markali, 1956 (type species of *Crystallolithus*).

Coccolithus pelagicus has priority over the heterotypic synonyms. Since the type of *Crystallolithus* Gaarder & Markali 1956 is *C. hyalinus* this genus is a junior synonym of *Coccolithus* Schiller 1930. This work indicates *C. pelagicus* consists of two different biological taxa, so it is suggested that *C. pelagicus* subsp. *pelagicus* and *C. pelagicus* subsp. *braarudii* are used for the respective subspecies. Re-examination of the type material collected by Wallich on the *Bulldog* cruise indicates that the smaller, Arctic heterococcolith morphotype is the type form. Hence if the forms are differentiated as subspecies this form must bear the name *C. pelagicus* subsp. *pelagicus*, which is an autonym and so does not need to be formally proposed (ICBN Art 26.3).

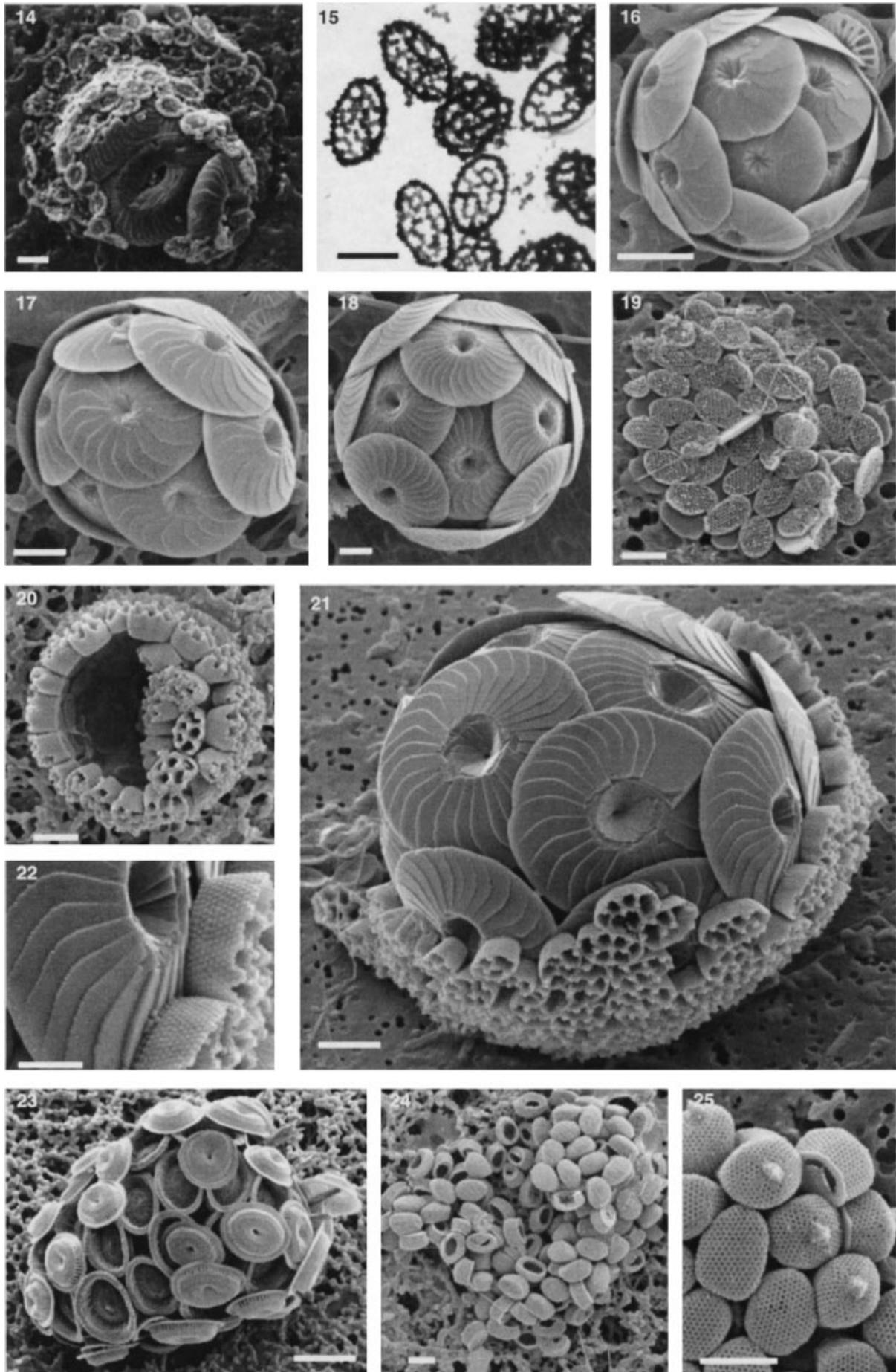
Coccolithus pelagicus subsp. *braarudii* (Gaarder, 1962) Geisen *et al.*, comb. & stat. nov.

BASYNONYM: *Crystallolithus braarudii* Gaarder, 1962 (*Nytt. Mag. Bott.*, **10**, p. 43, pl. 7).

Calcidiscus leptoporus with *Crystallolithus rigidus* and *Syracolithus quadriperforatus* (Figs 16–22)

Calcidiscus leptoporus (Figs 16–18) has previously been shown to be associated with the holococcolithophorid *Crystallolithus rigidus* (Fig. 19) (Kleijne, 1991), an association that has been confirmed by observations from the Bermuda area (Cortés, 2000; Renaud & Klaas, 2001). Recently this observation has been proven by a partial transition in four of our cultures of *Calcidiscus* (AS 31, Alboran Sea, western Mediterranean and NS10-2, NS4-2, NS8-2, S. Atlantic, off South Africa) which have given rise to holococcolithophorids bearing *C. rigidus* coccoliths. We found a single combination cell of the heterococcolith *C. leptoporus* with the holococcolithophorid *Syracolithus quadriperforatus* (Figs 21, 22) at MATER cruise station 69. Although we have only this single coccosphere as evidence of the new association, it is an exceptionally clear specimen with a uniquely well-preserved outer cover of holococcoliths. In our view it is highly unlikely that this specimen could be any form of artefact.

The holococcolithophorids involved, *Crystallolithus rigidus* (Fig. 19) and *Syracolithus quadriperforatus* (Fig. 20), have coccoliths with very different morphologies and structures. The coccoliths of *Cr. rigidus* are essentially plate-like, consisting of two layers of crystallites in a hexagonal array surrounded by a rim three crystallites high. *S. quadriperforatus* coccoliths, by contrast, have a high tube with internal walls which define four to six openings (compare Figs 19 and 20). On the proximal surface there are two or three concentric rings of crystallites and a large central opening usually covered by an organic membrane. These two structures are very different and close affinity



Figs 14–25. Electron micrographs of coccospheres and coccoliths of various species. Fig. 14. SEM of *Coccolithus*

between them has never been predicted. Crystallographically there is more affinity between these structures; the internal walls of *S. quadriperforatus* and the hexagonal meshwork plate of *Cr. rigidus* are both formed of calcite crystallites with vertical c-axes whilst the tube and rim are formed of crystallites with radial c-axes.

C. leptoporus heterococcoliths show considerable variation in size and in certain elements of their morphology. Kleijne (1991) and Knappertsbusch *et al.* (1997) distinguished three morphotypes: (1) small morphotype (Fig. 16) – liths 3–5 μm , 10–20 elements, distal shield sutures often angular and serrated (Kleijne, 1991), sometimes the inner part of the distal shield elements shows a dextral inclination (our observations); (2) intermediate morphotype (Fig. 17) – liths 5–8 μm , 15–30 elements, sutures variable; (3) large morphotype (Fig. 18) – liths 7–11 μm , 20–35 elements, sutures smoothly curved, usually with a zone of obscured sutures around the crest of the tube (Baumann, personal communication; our observations). These morphotypes, particularly the large and intermediate forms, seem to intergrade in morphology and there is no simple pattern to their biogeography (Renaud & Klaas, 2001), but morphometric studies have consistently supported their discrimination (Kleijne, 1991; Knappertsbusch *et al.*, 1997; Baumann & Sprengel, 2000; Renaud & Klaas, 2001). All het–hol associations involving *Cr. rigidus* are with the intermediate-size *C. leptoporus* morphotype (Cortés, 2000; our culture observations). The heterococcoliths of our new combination specimen (*C. leptoporus* with *S. quadriperforatus*) measure 6.7–8.3 μm , on the borderline between intermediate and large morphotypes, but the central area characters indicate that it is the large morphotype.

On the basis of the different morphologies of the holococcoliths involved in the mentioned cases and on the fact that no intermediate holococcolith

morphologies have been observed between the two holococcolithophorid species, intraspecific variation can be ruled out in this case. As with *C. pelagicus*, there seems to be strong evidence of phylogenetic differentiation of biological (sub-)species that show slightly different morphologies in the heterococcolithophorid phase, each associated with a different holococcolithophorid stage.

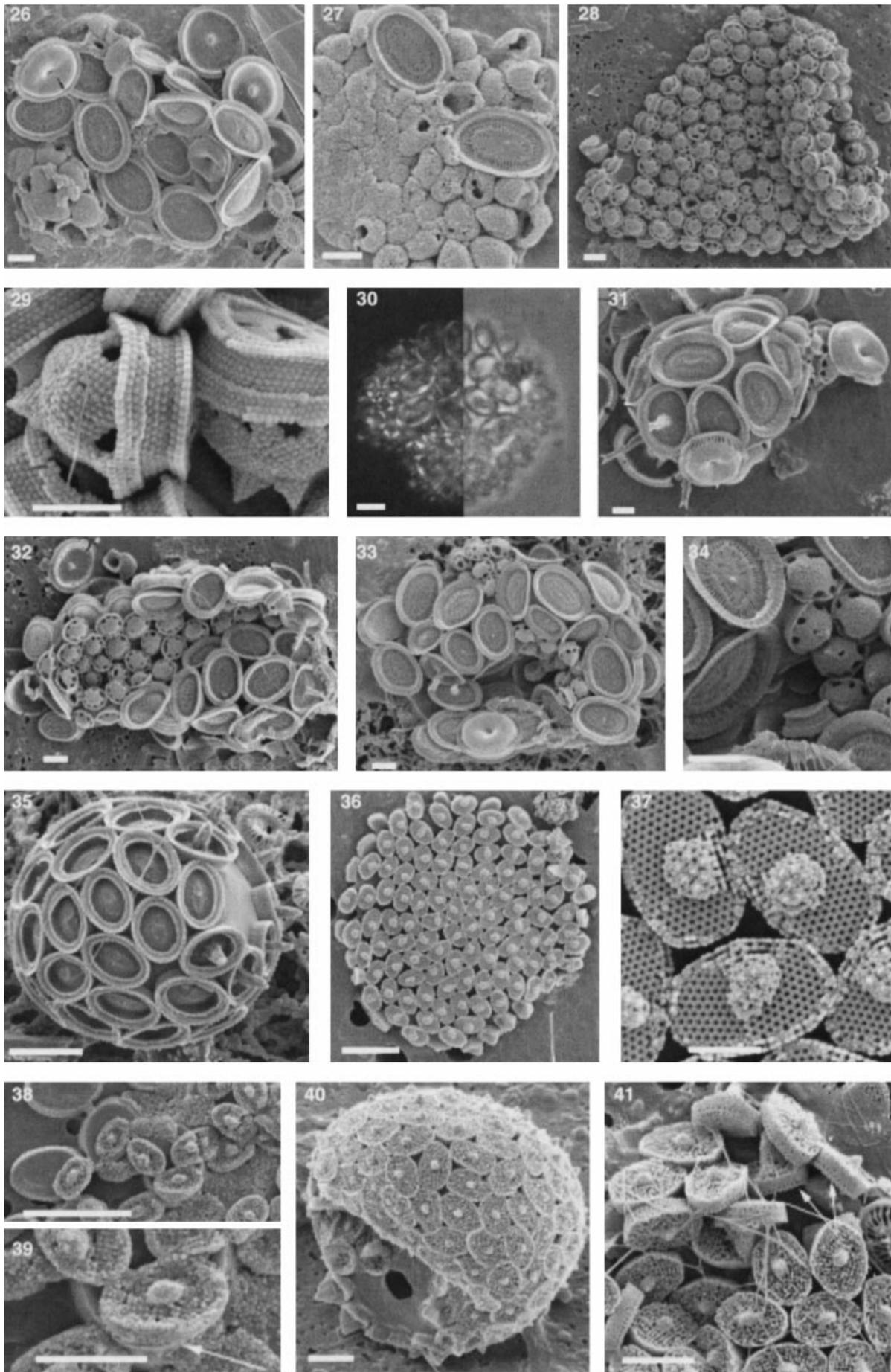
Calcidiscus leptoporus (Murray *et Blackman*, 1898) Loeblich *et Tappan*, 1978.

HETEROTYPIC SYNONYMS: *Crystallolithus rigidus* Gaarder in Heimdal *et* Gaarder, 1980; *Syracolithus quadriperforatus* (Kamptner, 1937) Gaarder in Heimdal *et* Gaarder, 1980.

Calcidiscus leptoporus has priority, but as *C. leptoporus* seems to incorporate three biological subspecies it is appropriate to introduce subspecies. The size range of coccoliths pictured in the type description of *C. leptoporus* is that of the intermediate morphotype, consequently the name *C. leptoporus* subsp. *leptoporus* should be applied to this form. (G. Murray worked at the Natural History Museum London, but we have been unable to locate any coccolith preparations of his, and it seems likely that he used water mounts. Fixed samples of his do exist but these have decalcified. Hence the type illustrations are the only available evidence.) *C. leptoporus* subsp. *leptoporus* is an autonym and so does not need to be formally proposed (ICBN Art 26.3).

The combination with the holococcolith *S. quadriperforatus* bears heterococcoliths of the large morphotype so the name *C. leptoporus* subsp. *quadriperforatus* should be used. As there are no observations of holococcoliths being associated with the small morphotype of *C. leptoporus* it is suggested here that an informal classification *C. leptoporus* subsp. SMALL be used, pending identi-

pelagicus–*Crystallolithus hyalinus* combination coccosphere. The *C. pelagicus* heterococcolith is of the small morphotype. The *Cr. hyalinus* coccolith shows its typical central area features, with the calcite rhombohedra arranged in parallel rows and covering all the central area. Water sample, N. Atlantic, Greenland Sea. Image courtesy of C. Samtleben, University of Kiel. **Fig. 15.** Transmission electron micrograph of *Crystallolithus braarudii* coccoliths. The *Cr. braarudii* coccoliths show the typical central ellipse with radial spokes connecting to the rim. Culture material, SW France, off Arcachon. **Figs 16–18.** SEM of coccospheres of *Calcidiscus leptoporus*. The images display the coccolith and coccosphere size variation between the small (Fig. 16), intermediate (Fig. 17) and large (Fig. 18) morphotype. Water samples, S. Atlantic, off Namibia, R/V Meteor cruise M48-4, station 20 (Figs 16, 17) and Western Pacific Ocean, Miyake-jima island, Japan (Fig. 18). **Fig. 19.** SEM of a coccosphere of *Crystallolithus rigidus*. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 59. **Fig. 20.** SEM of a coccosphere of *Syracolithus quadriperforatus*. Water sample, N. Atlantic, off Canary Islands, R/V *Poseidon* cruise 233, station 2. Image courtesy of C. Sprengel, University of Bremen. **Figs 21, 22.** SEM of a *Calcidiscus leptoporus*–*Syracolithus quadriperforatus* combination. Fig. 22 shows a detail of Fig. 21. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 69. **Fig. 23.** SEM of a coccosphere of *Syracosphaera pulchra*. Both endo- and exothecal coccoliths can be seen. Water sample, N. Atlantic, off Canary Islands, R/V *Poseidon* cruise 233, station 3. Image courtesy of C. Sprengel, AWI Bremen. **Figs 24, 25.** SEMs of *Calyptosphaera oblonga*. Fig. 24 shows a collapsed coccosphere and Fig. 25 shows a detail of the circumflagellar coccoliths. Note the typical hexagonal structure of the calcite rhombohedra and the absence of an offset between base and hood. The circumflagellar coccoliths often have a pointed hood. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 15 (Fig. 24) and station 69 (Fig. 25). Scale bars represent: Fig. 22: 1 μm ; Figs 14–21, 23–25: 2 μm .



Figs. 26–41. SEMs of various coccolithophorids. **Figs 26, 27.** SEM of *S. pulchra*–*C. oblonga* combination coccospheres. Water sample, N. Atlantic, JGOFS leg 4 (1990) cruise. **Figs 28, 29.** Scanning electron micrograph of *Daktylethra pirus*.

fication of the holococcolith phase which will determine the correct subspecies to be used.

Calcidiscus leptoporus subsp. quadriperforatus (Kamptner, 1937) Geisen et al., comb. & stat nov.
 BASIONYM: *Syracosphaera quadriperforata* Kamptner, 1937 (*Arch. Protistenk.*, **89**, p. 302, pl. 15, figs. 15, 16).

Syracosphaera pulchra with *Calyptosphaera oblonga* and *Daktylethra pirus* (Figs 23, 34)

Cros *et al.* (2000) showed one unambiguous and one questionable combination coccosphere involving the heterococcolithophorid *Syracosphaera pulchra* (Fig. 24) and the holococcolithophorid *Calyptosphaera oblonga* (Figs 24, 25), confirming the previous observations of Lohmann (1902) and Kamptner (1941). We have subsequently found two further examples of this association in a sample from the North Atlantic (Figs 26, 27). However, we have also observed several specimens from the Alboran Sea where *S. pulchra* coccoliths are associated on combination coccospheres with the holococcolithophorid *Daktylethra pirus* (Figs 28, 29). Four SEM and one LM specimen from two stations have been observed (Figs 30–34).

One example of a *S. pulchra*–*D. pirus* combination (described as a *S. pulchra*–*C. oblonga* combination) was recorded without illustration by Lecal-Schlauder (1961), and several further examples have been observed in a study of samples from the Tyrrhenian Sea (Saugestad, 1967; Saugestad & Heimdal, 2002).

Recently a phase change has occurred in one of our cultures of *S. pulchra* (NAP-10 from offshore Naples, Italy). The resulting motile phase bears holococcoliths. These are often malformed, but the better-formed specimens are unambiguously identifiable as *D. pirus* with both LM and SEM. As in the case of *C. leptoporus*, the two holococcolith

types associated with *Syracosphaera pulchra*–*Calyptosphaera oblonga* and *Daktylethra pirus* (Figs 23–25, 28, 29) – have been placed in different genera. They are, however, rather close in morphology, so the similarities and the differences in their morphology require some discussion:

Similarities: (1) Both are cavate holococcoliths consisting of a single-layered tube and convex distal cover; (2) LM observations indicate that all crystallites are arranged with their c-axes perpendicular to the surface of the coccolith; (3) in both cases the proximal surface consists of three or four concentric rings of crystallites, with a distinct central opening usually covered by an organic membrane, and the outermost ring protrudes beyond the tube to form a basal flange (Figs 25, 29). None of these three features is uncommon for holococcoliths, but the co-occurrence in these two holococcolith types does suggest close affinity. Moreover, (4) in both coccolith types circumflagellar coccoliths have distinctive pyramidal bosses on the distal surface, a feature not shown by any other holococcolith types (Figs 25, 28, 29).

Differences: (1) In *C. oblonga* coccoliths the tube wall is initially vertical and curves into the distal cover with no obvious break, whereas in *D. pirus* coccoliths the tube wall flares outward and there is a major inflection between the tube and distal cover; (2) in *D. pirus* large pores are present around the distal cover of the coccolith (Fig. 29); (3) *C. oblonga* coccoliths have a perforated hexagonal crystallite arrangement (Fig. 25), whereas *D. pirus* coccoliths have a non-perforate crystallite arrangement without obvious hexagonal pattern (Fig. 29). The affinity in coccolith structure of these two species is clear and their placing within different genera is little more than a historical accident. Nonetheless, the two morphologies are entirely discrete, being separated by multiple independent characters. Moreover, although both species are very common, neither intermediate morphotypes nor co-occurrence of the two coccolith morphologies on a single

Fig. 28 shows a collapsed coccosphere. Note the pointed hood of the circumflagellar coccoliths. Fig. 29 shows a detail of *D. pirus* coccoliths. Note the clear offset between the hood and the base as well as the perforations in the hood. Water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 69. **Figs 30–34.** Light micrographs and SEMs of *Syracosphaera pulchra*–*Daktylethra pirus* combination coccospheres. Fig. 30 displays a combination cell observed with a light microscope. Left, cross-polarized light; right, phase contrast. Figs 31–34 display SEMs of three further combination coccospheres. Fig. 34 shows a detail of Fig. 33. Water samples, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 15 (Figs 30, 33, 34) and station 69 (Figs 31, 32). **Fig. 35.** SEM of a coccosphere of *Coronosphaera mediterranea*. Water sample, South Atlantic, off Namibia, R/V *Meteor* cruise M48-4, station 476. **Figs 36, 37.** SEMs of *Calytrolithophora hasleana*. Fig. 36 shows a collapsed coccosphere and Fig. 37 shows a detailed view of the coccoliths. Note the hexagonal crystal arrangement of the distal cover. Water samples, western Pacific Ocean, Miyake-jima island, Japan. **Figs 38–41.** SEMs of *Zygosphaera hellenica*. Fig. 38 shows coccoliths of both *Coronosphaera mediterranea* and *Z. hellenica* in a culture in partial transition. Fig. 39 displays a detail of Fig. 38. Fig. 40 shows a coccosphere of *Z. hellenica* from a field sample. Note the difference in comparison with the culture material in Figs 38, 39. In Fig. 41 the arrangement of crystals in concentric rings and the ring of pores around the base of the coccoliths can be clearly seen (arrows). Culture material, S. Atlantic, off South Africa (Figs 38, 39) and water sample, western Mediterranean, Alboran Sea, R/V *Hesperides* cruise MATER 2, station 69. Scale bars represent: Figs 29, 37: 1 μm ; Figs 26–28, 31–34, 39–41: 2 μm ; Figs 30, 36: 5 μm .

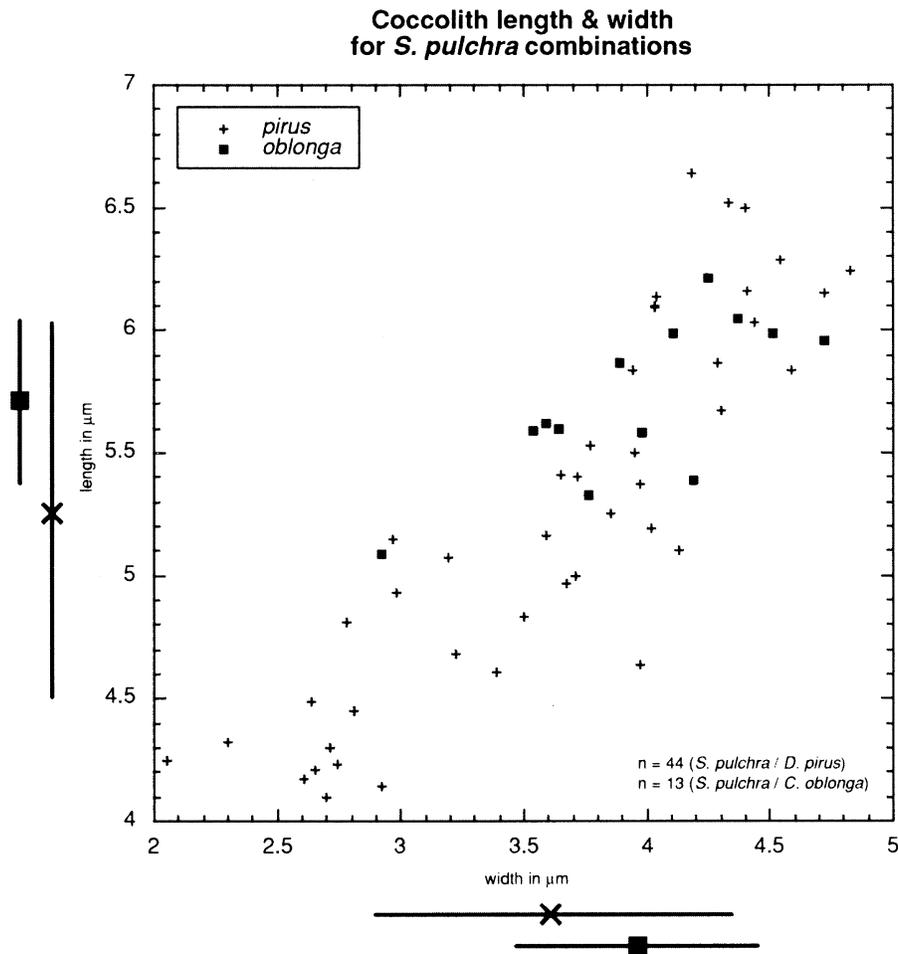


Fig. 42. Length and width of endothecal heterococcoliths of *Syracosphaera pulchra* measured on scanning electron micrographs of seven combination cells. A total of 44 coccoliths on combinations with *Daktylethra pirus* and 13 on combinations with *Calyptosphaera oblonga* were measured. All measurements occupy the same morphospace, with those taken on combination cells with *C. oblonga* showing slightly higher mean coccolith lengths and widths. The means and the standard deviation for the measurements on the two types of combination cells are displayed near the axis.

cell have ever been reported. The *S. pulchra* heterococcoliths on the cells involved in the combination coccospheres show normal morphologies, including both endothecal and exothecal coccoliths. Although *S. pulchra* coccoliths have unusually complex morphologies, we have not been able to detect any consistent differences in morphology between the coccoliths occurring on combination coccospheres with *C. oblonga* and those occurring with *D. pirus*. Measurements of the length and width of all suitably oriented endothecal coccoliths on the combination coccospheres (Fig. 42) showed intriguingly that the *C. oblonga*-associated heterococcoliths had a narrower range of sizes and higher mean size than the *D. pirus*-associated heterococcoliths. However, there is complete overlap between the datasets, and we have too few observations to be able to conclude that these size variations will prove consistent. Equally, size measurements of *S. pulchra* coccoliths from Holocene sediments show a clear monomodal distribution pattern (Fig. 43), indicating that a morphology-based species discrimination of *S.*

pulchra in the sedimentary record will prove difficult.

In this case intraspecific variation can be ruled out as the morphology of the two holococcoliths involved is significantly different and no cells have been observed bearing both holococcolith types or intermediate stages. By analogy to *C. pelagicus* and *C. leptopus*, we believe the most likely cause of this het-hol-hol association is genotypic differentiation, but with only the holococcolith phase having changed morphologically, i.e. a case of cryptic speciation in the heterococcolith phase. An alternative hypothesis of a complex life-cycle with two holococcolith phases cannot be ruled out, however. Obviously it will be interesting to study this case further, particularly with molecular genetics.

Syracosphaera pulchra Lohmann, 1902.

HETEROTYPIC SYNONYMS: *Calyptosphaera oblonga* Lohmann, 1902 (type species of *Calyptosphaera* Lohmann, 1902); *Daktylethra pirus* (Kamptner, 1937) Norris, 1985.

Contour lines 1 - max @ 2 intervals.
Contours < 6 are dashed.

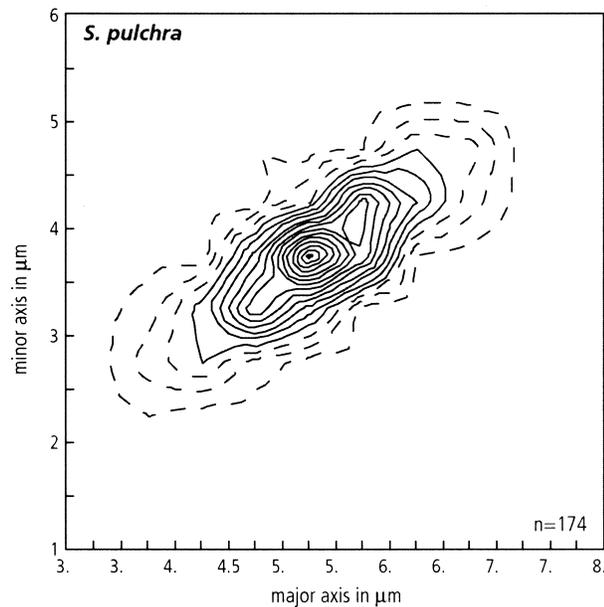


Fig. 43. Density plot of *Syracosphaera pulchra* endotheal coccoliths measured on 174 coccoliths from a number of Holocene samples. The plot shows the monomodal distribution of lengths and widths in the samples.

Neither *S. pulchra*, the type species of *Syracosphaera* Lohmann, 1902, nor *C. oblonga* has clear priority since they were described in the same publication. On the grounds of nomenclatural stability Cros *et al.* (2000) have recommended use of *S. pulchra*. We recommend here the use of *S. pulchra* HE for the heterococcolith phase (until future research allows separation of the heterococcolith subspecies within *S. pulchra*) and the informal *S. pulchra* HO *oblonga*-type and *S. pulchra* HO *pirus*-type for the respective holococcolith phases.

Coronosphaera mediterranea with
Calyptrolithophora hasleana, *Calyptrolithina*
wettsteinii and *Zygosphaera hellenica* (Figs 35–41)

Coronosphaera mediterranea (Fig. 35) has previously been shown to be associated with the holococcolithophorid *Calyptrolithina wettsteinii* (Kamptner, 1941; Cros *et al.*, 2000). Subsequently a single cell of *C. mediterranea* has been observed with the holococcolithophorid *Calyptrolithophora hasleana* (Figs 36, 37) in a field-collected sample from the Pacific (Cortés & Bollmann, 2002).

We have not found further examples of these associations in plankton samples, but we have recently been successful in isolating a culture of *C. mediterranea* from a water sample collected in the South Atlantic (NS 8–5). This culture has subsequently undergone a partial transition to the holococcolithophorid phase. The holococcoliths

produced by this culture are generally not well formed and tend to collapse into unidentifiable mounds of crystallites in SEM preparations. A limited number of intact holococcoliths have, however, now been observed (Figs 38, 39) and these are unambiguous specimens of a third holococcolith species, *Zygosphaera hellenica* (Figs 40, 41). Identification criteria include the arrangement of crystallites in numerous concentric rings, and the presence of a ring of pores around the base of the coccolith.

The available data in this case are limited but each combination appears very convincing and the fact that three holococcolith species are apparently involved, rather than two, gives it particular interest. All three holococcolithophorids are dimorphic, and have similar-shaped coccoliths. In each case the body coccoliths are flat-topped tubes with an irregular distal boss whilst the circum-flagellar coccoliths have an elevated transverse bridge. They differ, however, in numerous other characteristics. *C. wettsteinii* coccoliths are cavate, i.e. they have large central openings, the tube is non-perforate and the distal cover is broken by several large openings. *C. hasleana* coccoliths are also probably cavate but the tube and distal cover both have perforate hexagonal crystal arrangements and there are no large openings in the cover (Figs 36, 37). *Z. hellenica* coccoliths by contrast are non-cavate with usually the entire coccolith being filled by concentric layers of crystallites; the tube wall is predominantly non-perforate but there is always a row of perforations around the base and variable numbers of perforations above this (Figs 38–41). The *Z. hellenica* coccoliths appear very different to the others; however, it is noticeable that some *C. hasleana* coccoliths show partial development of the concentric layered structure and that some *Z. hellenica* coccoliths show perforate hexagonal wall structure. So the morphologies are perhaps less different than they appear initially. Nonetheless the differences between these three holococcolith types are sufficiently large and consistent to make it unlikely that these morphotypes result from non-genotypic variation.

By contrast there is no obvious differentiation of the heterococcoliths of *Coronosphaera mediterranea*. As with *S. pulchra* these are morphologically complex coccoliths which appeared to define a very clear morphospecies. However, despite the large number of available morphological characters we cannot find any distinctive features which separate the heterococcoliths associated with *C. wettsteinii* from those associated with *C. hasleana* or *Z. hellenica*. We therefore conclude that this case is analogous to that of *S. pulchra*, i.e. speciation has occurred but that this is only obviously reflected in the morphology of the holococcolith phase, even

though in this case genotypic differentiation has occurred twice.

Coronosphaera mediterranea (Lohmann, 1902) Gaarder in Gaarder et Heimdal, 1977.

HETEROTYPIC SYNONYMS: *Calyptrolithina wettsteinii* (Kamptner, 1937) Kleijne, 1991 (type species of *Calyptrolithina* Heimdal, 1982); *Calyptrolithophora hasleana* (Gaarder, 1962) Heimdal, in Heimdal et Gaarder, 1980 (type species of *Calyptrolithophora* Heimdal in Heimdal et Gaarder, 1980; *Zygosphaera hellenica* Kamptner, 1937 (type species of *Zygosphaera* Kamptner, 1936 by subsequent designation of Loeblich & Tappan, 1963).

The species *Coronosphaera mediterranea*, the type species of *Coronosphaera* Gaarder in Gaarder et Heimdal, 1977, has priority over the three associated holococcolith species. Strictly, the genus *Zygosphaera* Kamptner 1937 has priority over *Coronosphaera* Gaarder 1977. However, the genus *Coronosphaera* is much more widely used and better established than the genus *Zygosphaera* and use of *Coronosphaera* would involve fewer new combinations. We are preparing a submission to the ICBN to conserve the name *Coronosphaera* and suppress the name *Zygosphaera*. Pending this appeal we recommend use of the genus *Coronosphaera*.

As this work suggests that the heterococcolith phase of *Coronosphaera mediterranea* consists of three morphologically indistinguishable biological species or subspecies, we recommend the use of *C. mediterranea* HE for the heterococcolith phase (until future research allows separation of the heterococcolith species in *C. mediterranea* spp.) and the informal names *C. mediterranea* HO *wettsteinii*-type, *C. mediterranea* HO *hasleana*-type and *C. mediterranea* HO *hellenica*-type for the respective holococcolith phases.

Conclusions

In total only about 20 het–hol associations have been discovered. However, since these span the evolutionary biodiversity of coccolithophorids we predict that this will prove to be a common pattern, and that the infrequency of such observations may be a result of the temporally and spatially sporadic nature of most sampling and the fact that syngamy and meiosis are likely to be rapid processes that occur infrequently in the natural environment. Rather surprisingly, in six of these cases, i.e. nearly a third of the total, the heterococcolithophorid involved has been shown to form associations with not one, but two or three holococcolithophorids. Despite the limited number of het–hol–hol combinations observed, a close inspection of each case allows certain conclusions on the possible causative factors to be drawn.

Het–hol–hol associations thus seem to fall into three groups (Fig. 44):

(1) *Helicosphaera carteri* with *Syracolithus catilliferus* and *S. confusus*; and *Syracosphaera bannockii* with *Zygosphaera bannockii* and *Corisphaera* sp. A. In these cases the holococcolith ‘species’ appear to be intraspecific morphotypes, as demonstrated by the occurrence of intergradational morphotypes and co-occurrence of the two morphotypes on single coccospheres. Holococcolith morphology thus appears to be more plastic than heterococcolith morphology, perhaps unsurprisingly given the relative large number and simple arrangement of crystals and the observation that holococcoliths are formed outside the cell membrane.

(2) *Coccolithus pelagicus* with *Crystallolithus hyalinus* and *Cr. braarudii*; and *Calcidiscus leptoporus* with *Crystallolithus rigidus* and *Syracolithus quadriperforatus*. In these cases qualitative differentiation in holococcolith morphology is paralleled by morphometric differentiation in the heterococcoliths. The holococcolith differentiation thus provides strong support for previous inferences of genotypic diversification. In the case of *C. pelagicus*, there is clear evidence that the two subspecies occupy different geographic ranges, suggesting that allopatric speciation has occurred. For *C. leptoporus*, however, there is no evidence of present or past spatial isolation of populations, suggesting that the inferred speciation was sympatric, i.e. a result of ecological niche separation within the same geographical zone. At present there are not enough data to determine whether the different *C. leptoporus* subspecies occupy distinct niches in contemporary oceans. A detailed study of the seasonal and depth distribution of these taxa in relation to variation in physico-chemical parameters would clearly be of interest.

(3) *Syracosphaera pulchra* with *Calyptosphaera oblonga* and *Daktylethra pirus*; and *Coronosphaera mediterranea* with *Calyptrolithina wettsteinii*, *Calyptrolithophora hasleana* and *Zygosphaera hellenica*. In these cases the holococcolith differentiation provides compelling evidence for previously unsuspected cryptic speciation within the heterococcolith species. An interesting theoretical explanation for this phenomenon can be postulated. Protection against the expression of deleterious mutations is often cited as a potential advantage of diploidy over haploidy (for a review see Valero *et al.*, 1992). It can be hypothesized that a corollary of this is that in diploid cells potentially advantageous mutations are not necessarily expressed, depending on the relative dominance of alleles. Any gene mutation will necessarily be expressed in a haploid cell, and even though the rate of evolution of genes may not differ between the phases, one might expect

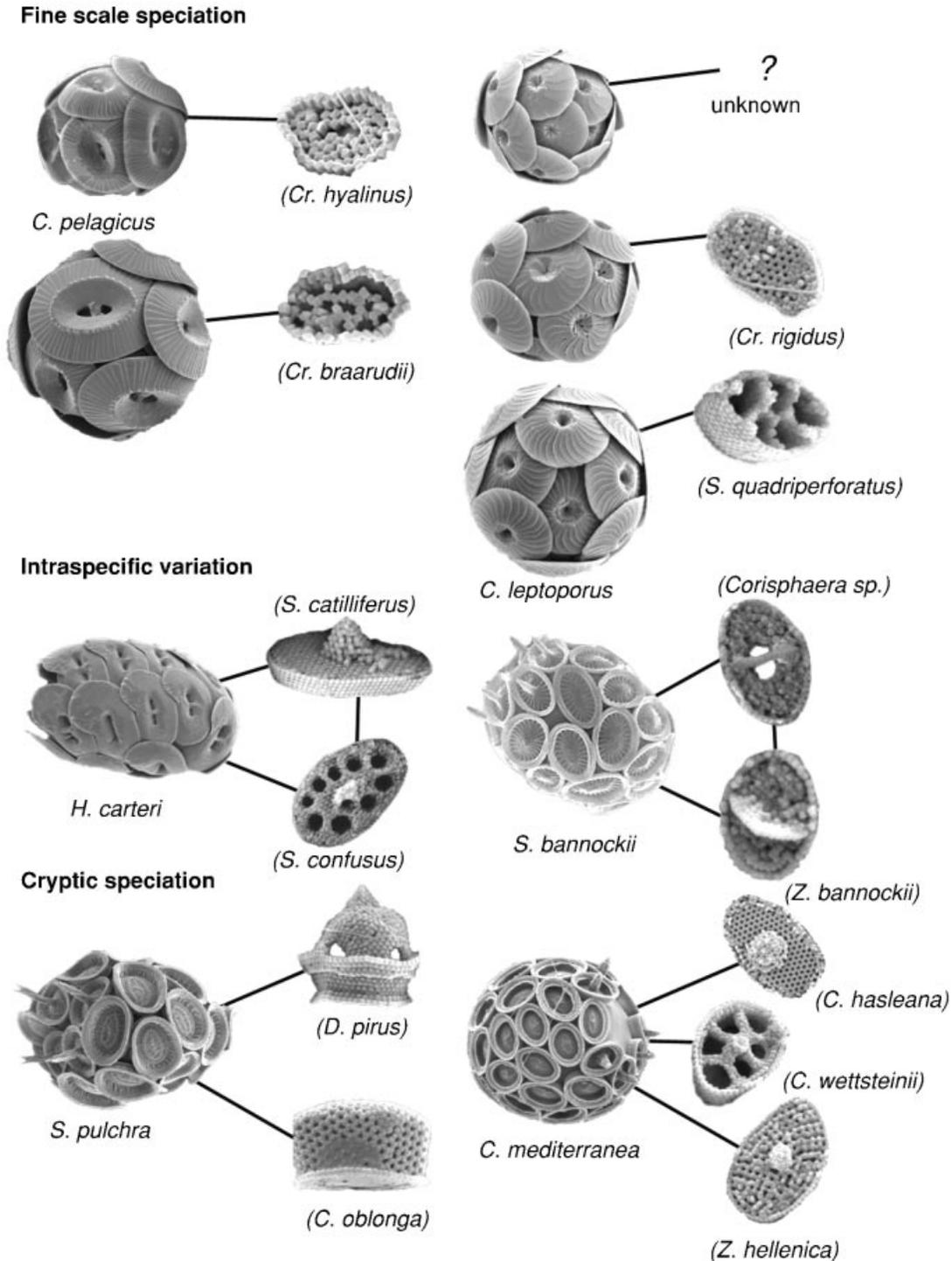


Fig. 44. Summary of life-cycle associations of sets of holococcolithophorids with a single heterococcolithophorid. The lines represent observed combination coccospheres. Intraspecific variation, with transitional morphotypes in the holococcolithophorid phase, is a likely cause for both *Helicosphaera carteri* and *Syracosphaera bannockii* and fine-scale speciation is seen as the likely cause for both *Coccolithus pelagicus* and *Calcidiscus leptoporus*. Two or more discrete holococcolithophorids in combination with one heterococcolithophorid species as observed in *Coronosphaera mediterranea* and *Syracosphaera pulchra* makes cryptic speciation the likely cause. (See Table 1 for abbreviations.)

to preferentially observe the result of gene mutations which cause neutral or advantageous changes in haploid cells. Following a speciation event, therefore, the rate of morphological evolution in haploid cells would be predicted to be greater than that of diploid cells. In any case it

appears clear that holococcolith morphology is more readily variable than heterococcolith morphology and is thus a more sensitive indicator of fine-scale variation, but less useful for identifying phylogenetic relationships.

Het-hol associations are known in five hetero-

Table 2. Comparison of numbers of holococcolithophorid species and of heterococcolithophorid species in families known to form associations

	Total	In combinations	Not in combinations
Holococcolithophorids	59	18	41
Heterococcolithophorids			
Rhabdosphaeraceae	20	1	19
Syracosphaeraceae (including <i>Coronosphaera</i> and <i>Calciosolenia</i> , but not <i>Alisphaera</i>)	50	8	42
Coccolithaceae	10	4	6
Zygodiscaceae	8	1	7
Total heterococcolithophorids	88	14	74

Numbers of taxa are based on the taxon list of Jordan *et al.* (1994) with the addition of undescribed taxa known to the authors. NB: The Papposphaeraceae and likely associated holococcoliths are not included here as they appear to form discrete consistently identifiable groups (14 heterococcolith species and 10 holococcolith species have been described and 5 combinations recognized). The Noelaerhabdaceae, Pleurochrysidaceae and Hymonomonadaceae are excluded since they are known to be non-calcifying in the haploid phase.

coccolithophorid families (Cros *et al.*, 2000). Since this association must be a primitive feature derived from a common ancestor (it is highly unlikely that the complex calcification mode of holococcoliths evolved independently on more than one occasion), it might be predicted that all members of these families will ultimately be shown to have an holococcolithophorid phase. An obvious implication is that there is a shortage of holococcolithophorid species; as shown in Table 2 approximately 59 holococcolithophorid species are known but there are some 88 heterococcolithophorid species in the relevant families. When species in known associations are removed then the discrepancy becomes stronger – 41 holococcolithophorid species versus 74 heterococcolithophorid species. We conclude that het–hol–hol associations, which are an increasingly commonly discovered phenomenon, are the result of intraspecific variation in the degree of calcification of the holococcolithophorid phase or result from non-cryptic or cryptic speciation. This suggests that this discrepancy will widen further as more het–hol–hol associations are discovered. From this shortfall it might be inferred that at least 30–50% of the heterococcolith-forming species in these families have secondarily lost the ability to calcify in the haploid phase, or have become asexual. However, Cros (2001) has illustrated a large number of undescribed, rare holococcolith morphotypes in field samples and we suspect that the most likely explanation is that many species have only short-lived holococcolith phases. It should be noted also that the reverse case, one holococcolithophorid being associated with two heterococcolithophorids, cannot be ruled out.

Finally we note that the production of different biomineralized periplasts rich in phylogenetic data within two phases of the life-cycle of coccolithophorids gives the group special potential for studies of microevolutionary pattern and process.

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