Running head: Differential dissolution in coccolithophores

Species-specific differential dissolution morphology of selected coccolithophore species: an experimental study

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500-character summary

Coccolithophores are important marine $CaCO_3$ producers and their biominerals, the coccoliths, partly dissolve in the upper water column where dissolution is unexpected. Studying coccolith dissolution in field samples is hampered by a paucity of experimental studies describing dissolution morphologies. Here we fill this gap by experimentally dissolving different coccolithophores and applying our results to field samples.

Highlights

- Experimental studies on biogenic $CaCO_3$ dissolution provide novel insights into field sample observations and biomineralization processes
- Experimental data aid the interpretation of aberrant coccolith morphology in field samples
- In *C. braarudii* partial dissolution reveals a nanostructure in the distal shield
- The nanostructure in *C. braarudii* requires adjustments in biomineralization models

Abstract

Coccolith dissolution in the water column is an important process in the marine carbon cycle. Identifying dissolution in water column samples has been difficult due to a lack of experimental reference datasets showing dissolution morphologies. We conducted a laboratory $CaCO_3$ dissolution experiment to detect differential dissolution morphologies of three selected coccolithophore (abundant marine calcareous phytoplankton) species, *Coccolithus braarudii*, *Helicosphaera carteri*, and *Scyphosphaera apsteinii*. These species were selected because they are ecologically and biogeochemically important (significant contributors to $CaCO_3$ production) and have been less studied than *Gephyrocapsa*. Muroliths of *S. apsteinii* dissolve faster than lopadoliths, which in turn dissolve as fast as *H. carteri* but faster than *C. braarudii*. In *S. apsteinii* lopadoliths, dissolution rate depends on the crystallographic orientation of the crystals. Comparison with field samples shows that experimental data are helpful when interpreting field samples. For example, we identify dissolution in water and sediment samples reported in the literature. In *C. braarudii* dissolution reveals a nanostructure on the proximal side of the distal shield, an observation that has implications for coccolith biomineralization models, which do not currently account for the formation of such a structure. This nanostructure features "units" of ca 50-100 nm and resembles the nanostructure well known from extracellular calcifiers such as molluscs and foraminifera. Whether this resemblance is underpinned by a similar formation mechanism remains unknown, but we think this unlikely.

1) Introduction

59 Present anthropogenic CO_2 concentration changes, both atmospheric and marine,
60 cannot be fully understood without considering marine calcium carbonate, largely
61 produced by calcifying organisms (Broecker and Peng 1982, Morse and Mackenzie
62 1990). These calcifying organisms influence air-sea CO_2 exchange in several ways, e.g.
63 through particulate inorganic- and organic carbon production in the surface ocean but
64 also through export of calcium carbonate to the deep ocean (Morse and Mackenzie
65 1990). The most productive marine calcium carbonate ($CaCO_3$) producers are pelagic
66 organisms, with coccolithophores contributing ca. 90% of global pelagic $CaCO_3$
67 production (Ziveri et al., 2023) and ca. 50% of $CaCO_3$ sedimentation (Milliman 1993,
68 Broecker and Clark 2009). Dissolution of $CaCO_3$ in the photic zone is an important
69 process in the marine $CaCO_3$ cycle (Ziveri et al. 2023; Subhas et al. 2022; Sulpis et al.
70 2021). The importance of dissolution for the marine C-cycle has two main aspects.
71 Firstly, dissolution of $CaCO_3$ releases two moles of alkalinity per one mole of dissolved
72 inorganic carbon, thereby shifting the seawater C-system towards higher pH values
73 (Zeebe and Wolf-Gladrow, 2001). Secondly, the loss of ballast minerals reduces carbon
74 export efficiency thereby influencing the C-cycle long-term (Klaas and Archer 2002). In
75 addition to occurring in the open ocean photic zone, dissolution of carbonates in
76 general, and coccoliths in particular, may also occur in sediments and coastal CO_2 vent
77 sites (Honjo 1975, Ziveri et al 2014).

78 Assessing coccolith dissolution in these diverse settings can be challenging, but partial
79 dissolution morphologies as identified in electron micrographs have proved a useful tool
80 (e.g. Langer et al 2007, Ziveri et al 2014). Knowledge of such differential dissolution
81 morphologies will aid interpretation of field samples, e.g. the degree of dissolution in
82 one species will inform inferences about the degree of dissolution in other species.
83 More fundamentally, knowledge about dissolution morphologies will enable us to
84 accurately distinguish malformation / under-calcification from dissolution, which is not
85 necessarily an easy task (Young 1994). Finally, dissolution might reveal informative
86 structural features (Langer et al 2007). The main goal of our study is to provide a
87 dissolution-morphology reference dataset which can be used to identify dissolution in
88 water column samples. The applicability of our data to sediment samples might be more
89 limited as discussed below. The interpretation of field samples is difficult, however,
90 because the degree, and even the mere fact, of dissolution often need to be inferred from
91 the micrographs alone, without precise knowledge of the physico-chemical conditions

leading to the observed morphology. For example, in surface sediments *Calcidiscus leptoporus* coccoliths (placoliths characterized by two shield-like plates connected by a central tube) lacking proximal shields have been taken as a sign of heavy dissolution (Roth and Berger 1975), which has been proposed as a proxy for dissolution in the sedimentary record (Matsuoka 1999). Only an experimental study could show that separation of the shields is the first observable dissolution feature occurring at less than 8% mass loss (Langer et al 2007) revealing the "weak spot" at the proximal end of the tube (the position of the proto-coccolith ring, Young et al 2004).

Despite the importance of experimental studies showing graded dissolution of coccoliths, only a few such studies have been conducted (McIntyre & McIntyre 1971, Burns 1977, Kleijne 1990, Henriksen et al 2004, Langer et al 2006b, Holcová and Scheiner 2023), with a focus on *Gephyrocapsa spp*, in particular *G. huxleyi*, a widely used model species (Wheeler et al 2023).The degree of dissolution in *G. huxleyi* water column samples is difficult to assess as there are variations of progressive dissolution patterns with e.g. warm- and cold-water phenotypes (Burns 1977). The latter author pointed out that a tropical *G. huxleyi* loses the central grille in the first stages of dissolution, while a cold-water phenotype does not. Calcite removal from the long margin of the radial elements is a sign of early dissolution in the cold-water phenotype but not in a heavily calcified phenotype. These and similar observations made by Burns (1977) show that assessing the degree of dissolution in *G. huxleyi* is not an easy task and morphotype-specific assessments are required. While *G. huxleyi* is numerically the most abundant coccolithophore in present oceans, its contribution to coccolithophore $CaCO_3$ production is rivalled by some genera with larger coccoliths, such as *Calcidiscus* and *Coccolithus* (Wheeler et al 2023). The relatively recent appearance of *G. huxleyi* in the fossil record implies that this species is not applicable to deep time sediment core studies (Henderiks et al 2022). It is therefore worthwhile also studying genera with larger coccoliths and mass, biogeochemically important (Ziveri et al., 2004), and with a more extensive evolutionary history, e.g. *Coccolithus*, *Helicosphaera*, and *Scyphosphaera* (Henderiks et al 2022). The latter genera are not as abundant as *G. huxleyi* but play an important role in coccolithophore $CaCO_3$ production and export in modern oceans (Baumann et al., 2004; Daniels et al., 2014, 2016; Gafar et al., 2019; Ziveri et al., 2007).

Based largely on field sediment studies, it is accepted that some coccolith forms dissolve faster than others. While *G. huxleyi* and *Umbilicosphaera sibogae* are among the fast-dissolving placolith bearing species, *G. oceanica*, *C. leptoporus*, and *C. pelagicus* are comparatively slow-dissolving (McIntyre & McIntyre 1971, Berger 1973, Roth and Coulbourn 1982). These studies have not assessed how dissolution morphologies of different species relate to each other. In other words, which dissolution morphology of species x corresponds to a given dissolution morphology of species y?

Coccoliths contain crystals of different orientations, sizes, and shapes. A typical feature, for example, is the presence of crystals with radial c-axis orientations (R-units) and others with vertical c-axis orientations (V-units, Young et al 1992). It might therefore be hypothesized that different crystals display different structural features, not only on the micrometre but also on the nanometre scale. Some of these features might only be discernible in partially dissolved specimens.

In this study we selected laboratory cultures of *Coccolithus braarudii*, *Helicosphaera carteri*, and *Scyphosphaera apsteinii*, and performed a dissolution experiment to follow their differential dissolution morphologies by means of sequential sampling for SEM analysis. Here we analyse two important aspects of dissolution. Firstly, the selective dissolution of different species relative to each other. Secondly, the evolution of morphology of a given species with progressive dissolution. We hypothesize that dissolution morphologies will be different from malformations (Bianco et al 2025, Langer et al. 2006, Langer et al 2021, Gerecht et al 2015, Meyer et al 2020) and therefore a dissolution reference dataset will enable us to unambiguously identify dissolution in field samples. The experimental setup chosen here is ideally suited to analyse sequential dissolution morphology with nanometric resolution. This enables the identification of different dissolution stages in field samples, providing additional information over and above the mere distinction of dissolution features and malformations.

Experimental dissolution studies provide a good source of information on the evolution of morphology with dissolution, without confounding factors from field studies such as variance in the primary biomineralization morphology.

2) Material and Methods

2.1) *Culture conditions*

Clonal cultures of *Coccolithus braarudii* (strain RCC1198), *Scyphosphaera apsteinii* (strain RCC3598), and *Helicosphaera carteri* (strain RCC1323) were grown in aged (3 months), sterile-filtered (Stericup-GP Sterile Vacuum Filtration System, 0.22 µm pore size, polyethersulfone membrane, Merck) natural surface seawater sampled in the English Channel off Roscoff, France, enriched with 288 µM nitrate, 18 µM phosphate, and silicate, trace metals, and vitamins as in Keller et al (1987) with the following modifications: Cu and TRIS are omitted, $NiCl_2*6H_2O$ 0.00314 µM is added, KH_2PO_4 is used as P source. All strains were obtained from the Roscoff Culture Collection (http://www.roscoff-culture-collection.org).

Cultures were grown under a 16:8 h light:dark cycle at a light intensity of 50 µmol photons m^{-2} s^{-1} in temperature-controlled culture incubators. *Coccolithus braarudii* RCC1198 was grown at 15°C, while *Scyphosphaera apsteinii* RCC3598 and *Helicosphaera carteri* RCC1323 were grown at 20°C. Cells were grown in dilute batch cultures, ensuring a quasi-constant seawater carbonate system over the course of exponential growth (Hoffmann et al. 2015). Cell densities were determined by flow cytometry immediately after sampling. Cultures used in the dissolution experiment were initially checked by light and scanning electron microscopy to ensure that coccosphere morphology was normal (as observed in light microscopy) and the percentage of coccolith malformations was below 15% (as determined by SEM analysis, Langer and Bode 2011). The latter is a very low percentage of malformations in cultures (in which values up to 90% have been reported, Langer et al 2006, Langer et al 2013), enabling this study to focus on normal coccoliths and their dissolution morphologies, as opposed to the dissolution features of malformed coccoliths (Langer and Bode 2011, Langer et al 2013, Langer et al 2023). We chose not to analyse the dissolution morphology of malformed coccoliths because results are intended to be applicable to field samples, in which the percentage of malformed coccoliths is typically only ca. 2% (Langer et al 2006, Langer et al 2013). Analysis of the dissolution morphologies of malformed coccoliths would require a different experimental setup, with cultures displaying high proportions of malformed coccoliths. Such an approach would be interesting in itself, but does not fall within the scope of the present study (Langer et al 2006).

2.2) *Dissolution experiment*

To study differential dissolution morphologies accurately, the selected species were combined in a single 2.7L bottle (Holcová and Scheiner 2023), in which case only one calcite saturation state (omega) value can be selected. In pre-experiments we found that *Gephyrocapsa huxleyi* coccoliths dissolved more than 10x faster than coccoliths of *Coccolithus braarudii*, *Helicosphaera carteri*, and *Scyphosphaera apsteinii*, meaning it was not possible to include *G. huxleyi* in our experiment. The three other species, *C. braarudii*, *H. carteri*, and *S. apsteinii*, displayed broadly similar dissolution kinetics and were therefore suited for our purpose.

To start the dissolution experiment, living cells were transferred into a 2.7L bottle containing culture medium that was acidified using calculated amounts of HCl (3.29M) immediately prior to cell transfer. We used acidification to manipulate omega calcite because it is more representative of dissolution scenarios in the field than changes in Ca concentration. Omega calcite is the saturation state of seawater with respect to calcite, with omega < 1 indicating dissolution and omega > 1 potential crystal growth. This decision is important because the manipulation of omega calcite via acidification is more effective than via Ca concentration decrease (Hassenkam et al 2011). We chose to work with living cells, as opposed to isolated coccoliths for the following reasons. Firstly, we wanted our results to be useful for comparison with various dissolution scenarios such as whole cells in copepod and micro-grazer guts, whole cells in marine snow aggregates, whole cells in ocean acidification-affected corrosive waters. Secondly, we wanted to analyse effects of dissolution on coccospheres, as opposed to only on individual coccoliths. Thirdly, removing coccoliths from cells is not always an easy task and often requires chemical or heat treatment which might alter structural integrity and organic content (Manuela Bordiga, personal communication, 2025). Since this is a pilot study, we wanted to keep the experimental setup as straightforward as possible. Follow up studies should deal with modified setups to explore additional factors influencing dissolution patterns. The culture medium prepared using natural surface seawater sampled off Roscoff, France has a typical dissolved inorganic carbon, DIC, of ca 2000 $\mu mol\ kg^{-1}$ (Johnson et al 2022). We used this value for DIC and measured pH (NBS) = 6.44 to calculate omega calcite = 0.033 using the program CO2SYS (Pierrot et al 2011). The calculated value for omega calcite (0.033) was therefore approximate. However, DIC variability of natural surface seawater sampled off Roscoff, France is low, therefore introducing only a negligible inaccuracy in calculated omega calcite in the context of

the present study, i.e. an error of ±0.005 is expected (Johnson et al 2022). The present study was not designed to analyse dissolution kinetics precisely (such as in Subhas et al 2018), meaning an approximate determination of the carbonate system is sufficient. We used a Cyberscan 500 pH meter (Eutech Instruments, UK) equipped with a Mettler Toledo InLab 413/ID67 electrode to determine pH on the NBS scale.

Experimental dissolution over a very short space of time (on the order of seconds as in Yang et al 2021) only allows for comparatively low-resolution light micrographs that would have been insufficient for our purpose. The advantage of a short experiment duration, however, is that DIC uptake and gas exchange with the atmosphere, and therefore carbonate system variability, is negligible. Our dissolution experiment, conducted over a duration of 11 hours, was carried out in the dark at 4°C to ensure that cellular metabolism (including photosynthesis and coccolith production) was severely restricted over the course of the experiment. Cell densities were 711 cells/mL for *C. braarudii*, 665 cells/mL for *H. carteri*, and 586 cells/mL for *S. apsteinii*. The resultant low overall cell density of 1963 cells/mL contributed to ensuring a quasi-constant carbonate system over the course of the experiment (Langer et al 2006, Langer and Bode 2011, Hoffmann et al 2015). Physico-chemical conditions over the course of the experiment were additionally homogenized by regular mixing, i.e. keeping the cells in suspension. No aggregation of cells occurred and no sedimentation of cells or coccoliths took place. The pH did rise by ca 0.1 over the course of the experiment, but this corresponds to an increase in omega calcite of only ca. 0.005, i.e. the same magnitude as the minor uncertainty introduced by our choice of DIC value (see above).

After the dissolution experiment was completed, cells were transferred into normal culture conditions as specified above. All three species, *C. braarudii*, *H. carteri*, and *S. apsteinii*, resumed cell division and coccolith production as confirmed by optical inspection using light microscopy. We did not quantify coccolith morphology in re-calcifying cells, but noted that initially coccoliths seemed to display more malformations than prior to the dissolution experiment. This assessment is based on an informal analysis by means of light microscopy; no images were taken.

Multiple (15) sequential samples for detailed morphological analysis were taken over the 11 hour duration of the experiment. Samples for SEM analysis were filtered onto polycarbonate filters (0.8 µm pore-size), dried in a drying cabinet at 50°C for 24 h, then sputter-coated with gold–palladium using a Cressington 108 sputter coater (Cressington

Scientific Instruments, Watford, UK). Imaging was performed with a Phenom Pro desktop SEM at the Station Biologique de Roscoff, France, and an El SEM Zeiss Merlin at UAB, Barcelona, Spain. All the morphological features described in this study are discernible using the Phenom Pro desktop SEM. We used the Zeiss Merlin FE-SEM only to produce images showing the nanostructure on the proximal side of the distal shield of C. braarudii because the latter microscope has a higher resolution. This nanostructure, however, was discovered using the Phenom Pro desktop SEM. An average of ~350 coccoliths was analysed per sample (Langer and Benner 2009). To describe dissolution morphologies, we selected conspicuous features that could be easily followed over the course of the experiment to ensure robust results and to facilitate application to field samples. In *C. braarudii* and *H. carteri* we analysed dissolution features of coccospheres in addition to dissolution features of coccoliths. In *S. apsteinii* only dissolution features of coccoliths were analysed because coccospheres in this species lack the mechanical stability needed to consistently withstand the mechanical forces experienced in SEM preparation (Langer et al 2023). The following morphological features were used to describe dissolution. In *C. braarudii*: 1) etching of the inner tube, 2) etching of the distal shield, 3) central area bar missing, 4) coccoliths broken, 5) gaps in coccospheres, 6) coccospheres collapsed, 7) nanostructure visible (on proximal side of distal shield). In *H. carteri*: 1) etching, 2) coccoliths broken, 3) coccospheres collapsed. In *S. apsteinii* lopadoliths: 1) etching of base, 2) etching of barrel, 3) rim serrated, 4) lopadoliths broken, 5) isolated lopadolith V units. In *S. apsteinii* muroliths: 1) centre missing, 2) etching, 3) muroliths broken. Scanning electron micrographs of all of these features are shown in Figs 1-5. The most important dissolution features for practical purposes are summarised in Table 1.

3) Results and Discussion

3.1) Differential dissolution: general observations

We subjected living cells of three common coccolithophore species, namely *Coccolithus braarudii*, *Helicosphaera carteri*, and *Scyphosphaera apsteinii*, to seawater undersaturated with respect to calcite, i.e. omega calcite ca. 0.033 (see Methods). The duration of the experiment was 11 hours, at the end of which only a few isolated distal shield elements of *C. braarudii* remained (Fig 6). Our observation that *C. braarudii* is

more dissolution resistant than *H. carteri* tallies well with conclusions drawn from studying Atlantic Ocean floor sediments (Berger 1973). Information on *S. apsteinii* in differential dissolution studies is rare, with this species either only mentioned but not discussed or not mentioned at all (McIntyre & McIntyre 1971, Berger 1973, Roth and Coulbourn 1982). From our data we conclude that *S. apsteinii* lopadoliths display dissolution kinetics similar to *H. carteri*, while *S. apsteinii* muroliths dissolve faster. In *S. apsteinii*, R-units, which are smaller and radially oriented, dissolve conspicuously faster than the larger, vertically oriented V-units (Figs 6, 7, see also Drescher et al 2012). Since lopadoliths contain calcite only, as opposed to e.g. aragonite (Walker et al 2024), the latter observation illustrates that differential dissolution kinetics of biogenic calcium carbonate cannot be inferred from the polymorph only (Langer and Ziveri 2025). Both etching and broken coccoliths appear simultaneously in *S. apsteinii* lopadoliths and *H. carteri* (Figs 6, 7). In *C. braarudii* etching of the inner tube occurs simultaneously with etching in *H. carteri* and *S. apsteinii*, but etching of the *C. braarudii* distal shield appears later, possibly because the latter features the largest crystals (Figs 6, 7). Relatively slow dissolution of the distal shield compared to the tube/central area was also observed in *C. leptoporus* and might be a general feature of Coccolithales placoliths (Langer et al 2007).

3.2) Comparison with field samples

Identification of dissolution in field samples:

As noted in the introduction coccolith dissolution in the water column is being highlighted as a key process, greatly affecting the export production of coccolith $CaCO_3$ to the bottom sediment. Our experimental results on the sequence of dissolution stages might usefully be applied to study of field samples in order to analyse and track water column dissolution. As a proof of concept we show here (Fig 8) images of *Coccolithus braarudii* and *Helicosphaera carteri* coccoliths from sediment trap samples and of coccoliths from water column samples, in both cases showing dissolution features directly comparable to those we observed experimentally. It is also noteworthy that the nanostructure seen in the experimental samples is visible in the field samples (Fig 8) showing that it is not an experimental artefact. Comparable dissolution features have also been illustrated in the literature, for example by Cubillos et al (2012) and Kleijne (1990), although in some cases they have been ascribed to malformation.

It is striking that in the three species studied here dissolution morphologies are clearly different from malformations. The latter do not resemble etching as described here (Figs 1-5). This is remarkable considering that it has typically been difficult to distinguish dissolution from malformation, and even fracture, in *G. huxleyi* (McIntyre & McIntyre 1971, Burns 1977, Kleijne 1990, Holcová and Scheiner 2023, Young 1994, Langer et al 2006b, Langer and Benner 2009, Langer et al 2011). These difficulties in identifying dissolution morphology in *G. huxleyi* are particularly conspicuous in morpho-type B/C, but are clearly noticeable in type A as well (own observations, unpublished). It might be speculated that dissolution is easier to identify in type R because the latter features fused distal shield elements which makes the overall morphology more similar to the one of the species studied here. This conjecture is supported by *G. huxleyi* morphotype-specific dissolution morphologies described in water column samples (Burns 1977). It will be worthwhile studying different *G. huxleyi* morphotypes in greater detail. Species-specific dissolution features such as the serrated rim in *S. apsteinii* lopadoliths are also dissimilar to malformations such as type R (Langer et al 2021, Langer et al 2023). The nanostructure on the proximal side of the distal shield of *C. braarudii* is hardly visible in normal as well as malformed coccoliths, whereas it is clearly visible in partially dissolved coccoliths. In *C. braarudii* a concentric hole sometimes appears in malformed coccoliths (Langer et al 2021). This hole is clearly different from etch pits. A typical feature of more severe malformations in placolith bearing species is the distorted architecture of the shields (Bianco et al 2025, Langer et al 2006, Langer and Benner 2009, Langer et al 2011, Langer and Bode 2011, Langer et al 2012, Langer et al 2013, Langer et al 2021, Langer et al 2023, Kottmeier et al 2022, Gerecht et al 2015, Milner et al 2016, Johnson et al 2022) which does not occur as a result of dissolution.

Do the conditions under which dissolution occurs influence dissolution morphologies?

As a caveat we will say that dissolution morphologies might well depend on the conditions under which dissolution occurs. For example, the presence or absence of an organic coating around coccoliths results in slightly different dissolution morphologies as seen in high resolution AFM imaging (Henriksen et al 2004). Since we did not remove the organic coating, our results should be best applicable to water samples (with organic coating) as opposed to sediment samples (in which the organic coating might be degraded). That said, the organic coating of coccoliths can still slow down dissolution

after 70Ma in the sediment (Sand et al 2014). Whether dissolution morphologies of these ancient coccoliths would be similar to those of cultured specimens remains to be tested. A good candidate would be *C. pelagicus* because it first appeared in the fossil record more than 60 Ma (Henderiks et al 2022). Another aspect to consider is the way undersaturation is achieved. Dissolution kinetics in low-Ca solutions are different from those in low-pH solutions (Hassenkam et al 2011). It is an open question whether dissolution morphologies would differ too. In addition, pressure-driven undersaturation might be relevant for deep-sea sediment samples. All of these issues are amenable to experimental testing and should be the focus of future studies.

3.3) Structural integrity of the coccosphere

An interesting difference between *C. leptoporus* (Langer et al 2007) on the one hand and *C. braarudii / H. carteri* (this study) on the other hand is the structural integrity of coccospheres under dissolution. The earliest feature of dissolution in *C. leptoporus* dissolved at an omega calcite of 0.5, is the separation of the shields resulting in coccosphere collapse (Langer et al 2007). By contrast, in *C. braarudii* and *H. carteri* the earliest dissolution feature is etching leaving the coccospheres intact. Only when coccoliths break due to more pronounced etching do coccospheres collapse in these species (Fig 7). This means that living *C. leptoporus* cells are more vulnerable to dissolution than *C. braarudii / H. carteri* because all three species need a coccosphere to live (Walker et al 2018a, Bianco et al., 2025). While a coccosphere comprised of coccoliths produced by the very cell itself is essential for survival in monospecific cultures of these species, mixed-species coccospheres in natural assemblages indicate that coccosphere integrity can be re-established or modified through incorporation of foreign coccoliths (Johns et al 2023). This might mean that a coccosphere compromised through dissolution or malformation might be repaired by incorporating foreign coccoliths. The protective efficacy of such hybrid coccospheres remains to be tested experimentally. However, the vulnerability sequence described above differs from what would be expected based on species specific coccolith solubility as inferred from sediment samples, which do not suggest that *C. leptoporus* is more vulnerable than *H. carteri* (Berger 1973). Note that we cannot be entirely sure that *C. leptoporus* coccoliths would break faster than *H. carteri* coccoliths when subjected to the same omega calcite because the *C. leptoporus* experiment was conducted at an omega calcite of 0.5 (Langer et al 2007) as opposed to the ca 0.033 used here. Nevertheless, considering the very

early appearance of separated shields in *C. leptoporus* (Langer et al 2007) and the comparatively late appearance of broken coccoliths in *H. carteri*, it is highly likely that coccosphere collapse in *C. leptoporus* would occur earlier than in *H. carteri* (at a given omega calcite). Although bulk surface waters in most parts of the global ocean are currently supersaturated with respect to calcite, ongoing ocean acidification drives the calcite saturation state towards undersaturation which will be reached in some areas, e.g. the Southern Ocean, around the year 2100, posing a threat to calcifying organisms including coccolithophores (Langer and Ziveri 2025). Please note that most surface waters will remain supersaturated with respect to calcite, so that our results are most directly relevant to locally undersaturated conditions (e.g. upwelling regions, eddies, sea-ice melt, or pore waters). Regardless of the actual threat posed by corrosive waters to living coccolithophores, the argument we are making here centres on relative vulnerability of different species in case of calcite undersaturation.

3.4) *A nanostructure in C. braarudii biomineral*

A nanostructure on the proximal side of the distal shield in *C. braarudii* became visible 3 hours into the experiment (Fig. 6). The individual "units" of this nanostructure are ca. 50-100 nm in diameter. The distal side of the distal shield does not show this nanostructure. Differences between the proximal and distal sides of the distal shield have previously been reported (Henriksen et al 2004, Young et al 2004). Whereas the distal side of the distal shield consists of crystallographic a-faces, the proximal side seems to be more profoundly regulated by the cell and does not show crystallographic faces (Young et al 2004). The nanostructure shown here is what was described as "tuberculate surface" by Henriksen et al. (2004). The latter authors conclude that the tubercles are part of the calcite structure. We confirm this conclusion which is illustrated particularly well by a side view of these tubercles (Fig. 3D). We can only speculate what effect this nanostructure might have on the dissolution resistance / susceptibility of the distal shield elements. Considering that the distal shield elements of *C. braarudii* are the only coccolith parts of all three species that are still present at the end of the experiment (Fig. 6), it seems clear that they are comparatively dissolution resistant. Whether this resistance stems from the nanostructure or some other feature remains an open question but it is fair to say that the nanostructure does not make coccolith crystals highly susceptible to dissolution. The importance of micro- and nanostructures in differential dissolution behaviour of various biominerals has been recently highlighted in the

context of vulnerability to ocean acidification (Langer and Ziveri 2025). It is conceivable that the nanostructure in *C. braarudii* slows down etching and / or provides structural reinforcement. This scenario would be plausible if the nanostructure was an organo-mineral composite structure as opposed to being composed of calcite only (Walker and Langer 2021). A nanostructure of similar size in $CaCO_3$ biominerals is widespread in extracellular calcifiers, where it is a central indicator of a layered growth mechanism featuring particle accretion which is believed to be non-operative in coccolithophores (Kadan et al 2021, Walker and Langer 2021). It remains, however, an open question whether the nanostructure in *C. braarudii* is similar to that in extracellular calcifiers i.e. whether it is also an organo-mineral composite structure (Walker and Langer 2021). This question is pertinent to coccolithophore biomineralization because an extracellular-like nanostructure in coccoliths would call into question widely held views about crystallization of coccolith crystals (Walker and Langer 2021). However, even if the tuberculate nanostructure in *C. braarudii* should turn out to be extracellular-like, it would still be unclear how it is possible that the distal side of the distal shield is different, i.e. shows crystallographic a-faces and no nanostructure. The standard biomineralization model explaining the nanostructure in extracellular calcifiers cannot account for the difference between the two sides of the distal shield in *C. braarudii*, and neither can the standard model of coccolith biomineralization (Young et al 2004, Walker and Langer 2021). This difference between the proximal and the distal side of the distal shield shows how finely tuned morphogenesis in *C. braarudii* is. We can only speculate how this fine tuning is achieved, but the composition of the organic coating might play a role. The composition of coccolith associated polysaccharides is known to be species specific, but we speculate that it might also be site specific within the coccolith vesicle (Walker et al 2018b).

4) Conclusions

In summary, our results show that dissolution experiments complement field studies and contribute to a deeper understanding of both coccolith structure and the ecological impact of seawater undersaturation with respect to calcite. We conclude that

1) the most dissolution-resistant species is *C. braarudii*, followed by *H. carteri* and *S. apsteinii*;

2) structural integrity of the coccosphere under dissolution is highest in *C. braarudii*, followed by *H. carteri* and *S. apsteinii*, with *C. leptoporus* probably showing the weakest coccosphere;

3) we identify dissolution in published field data where it was not recognised;

4) lopadolith R-units dissolve faster than V-units, illustrating that different microstructures in the same coccolith have different dissolution kinetics despite containing the same mineral;

5) the nanostructure in the distal shield of *C. braarudii* points to a fine-tuning in coccolith morphogenesis that is not accounted for by our current model of coccolith biomineralization.

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Competing interests

The authors declare no conflict of interests.

Author contributions

GL: conception, experiments, analysis, writing, IP: experiments, writing, JRY: analysis, field samples, writing, PZ: writing.

Data availability

All data are available in the Supplement.

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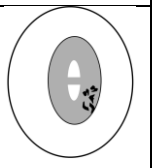
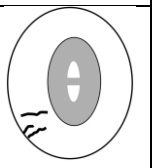
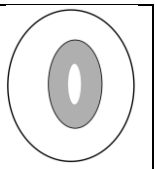
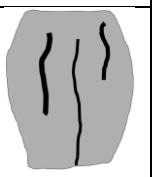
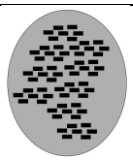
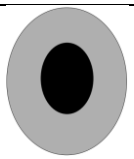
669

670 TABLES

671 Table 1. Summary of important dissolution features.

672

coccolith	dissolution feature	sketch
C. braarudii	etching of inner tube	
C. braarudii	etching of distal shield	

C. braarudii	central area bar missing	
H. carteri	etching	
S. apsteinii lopadoliths	etching of barrel	
S. apsteinii lopadoliths	serrated rim	
S. apsteinii muroliths	etching	
S. apsteinii muroliths	centre missing	

673

674

675 FIGURE CAPTIONS

676 Fig 1 *Coccolithus braarudii*

677 A) coccosphere at t0, no dissolution. Scale bar 5µm. B) coccosphere; etching (e) of tube
678 and distal shield and central area bar missing (b). Scale bar 5µm. C) broken coccoliths
679 (bc), etching of tube, central bar missing and gaps in coccosphere. Scale bar 5µm. D)
680 collapsed coccosphere (c), also showing etching (e) of tube and distal shield and central
681 area bar missing (b). Scale bar 5µm. E) coccolith, etching (e) of tube and distal shield,
682 and central area bar missing (b). Note that the etching consistently occurs by opening of
683 sutures between elements rather than by dissolution of element surfaces. Scale bar 3µm.

Fig 2 *Coccolithus braarudii*

A) broken coccolith (bc) distal shield in distal view. B) broken coccolith proximal view of distal shield showing nanostructure (ns); the arrow indicates isolated distal shield elements in distal view, from another coccolith, not displaying nanostructure (nns) on distal and vertical surfaces. All scale bars 2µm.

Fig 3 *Coccolithus braarudii*

A) broken coccolith distal shield in proximal view showing nanostructure (ns). Scale bar 2µm B) proximal view of distal shield elements showing nanostructure (ns). Scale bar 500nm. C) proximal view of distal shield elements showing nanostructure (ns); individual "tubercles“ (t) of the nanostructure are ca 50-100nm, Scale bar 200nm. D) isolated distal shield elements showing nanostructure "tubercles“ (t) in vertical side view (arrow). Scale bar 200nm.

Fig 4 *Helicosphaera carteri*

A) coccosphere at t0, no dissolution. Scale bar 5µm. B) coccosphere displaying coccoliths with severe etching (e) and a broken coccolith (bc). Scale bar 5µm. C) collapsed coccosphere including broken coccoliths (bc). Scale bar 5µm. D) coccoliths in distal view with etching (e) in flange and blanket. Scale bar 3µm. E) coccolith in distal view with etching in flange and blanket. Scale bar 2µm. F) coccolith in proximal view with etching (e) in flange. Scale bar 5µm.

Fig 5 *Scyphosphaera apsteinii*

A) coccosphere at t0, no dissolution B) lopadolith base etching (be); murolith centre missing (cm) C) lopadolith barrel etching (re) and serrated rim (sr) D and E) broken lopadoliths F) isolated V-units G) murolith at t0, no dissolution H) murolith with etching (me) I) lopadolith in distal view showing R- and V-units (R- and V arrows, Young 2008); and broken murolith (bm). All scale bars 5µm.

Fig 6 Timelines of dissolution. Bars indicate the period during which the respective feature can be observed. Shaded backgrounds indicate the onset points of major features. Pink backgrounds indicate the onset of etching, blue backgrounds indicate the onset of breakage/collapse. For example-images of each feature see Figs 1-5.

Fig 7 Quantification of the observations illustrated in Fig 6. Plotted is the percentage of each analysed feature versus time in hours from start of experiment. A) *Scyphosphaera apsteinii* lopadoliths B) *Scyphosphaera apsteinii* muroliths C) *Helicosphaera carteri* D) *Coccolithus braarudii*

Fig 8 Field samples showing etching patterns comparable to those seen in the experimental samples (arrows). All scale bars 2 µm.

Coccolithus braarudii: A) Lower surface of a broken piece of distal shield showing nanostructure. B) Central area of distal shield showing early stage dissolution. C) Proximal surface showing advanced dissolution.

Helicosphaera carteri: D) Distal Surface showing early stage dissolution. E) Proximal surface showing advanced dissolution.

A, B - sediment trap samples from 3200m, N. Atlantic; C - surface water sample, NW Atlantic; D- sediment trap sample, Canaries, 200m; E plankton sample from 120m, Gulf of Mexico.

Fig 1

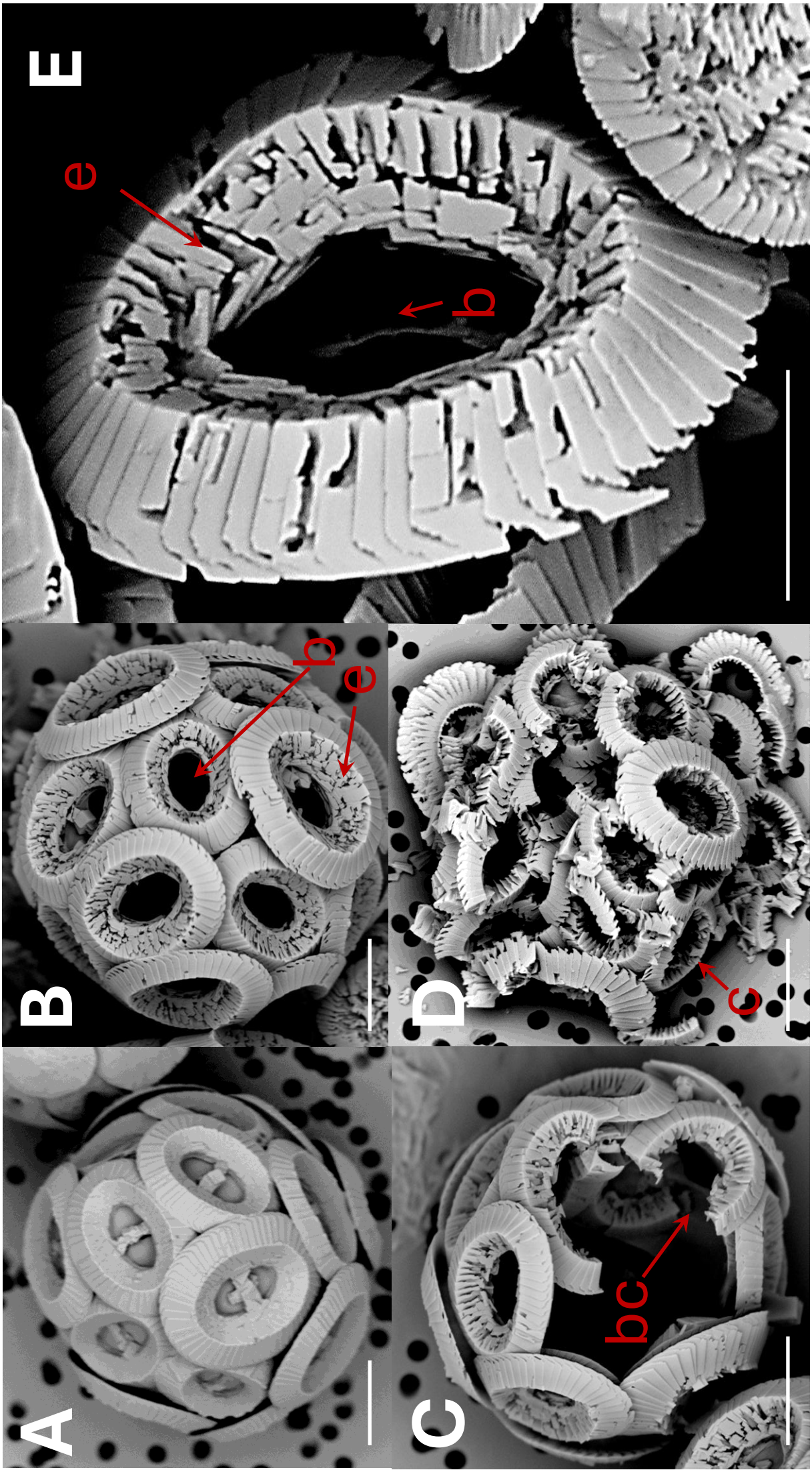


Fig 2

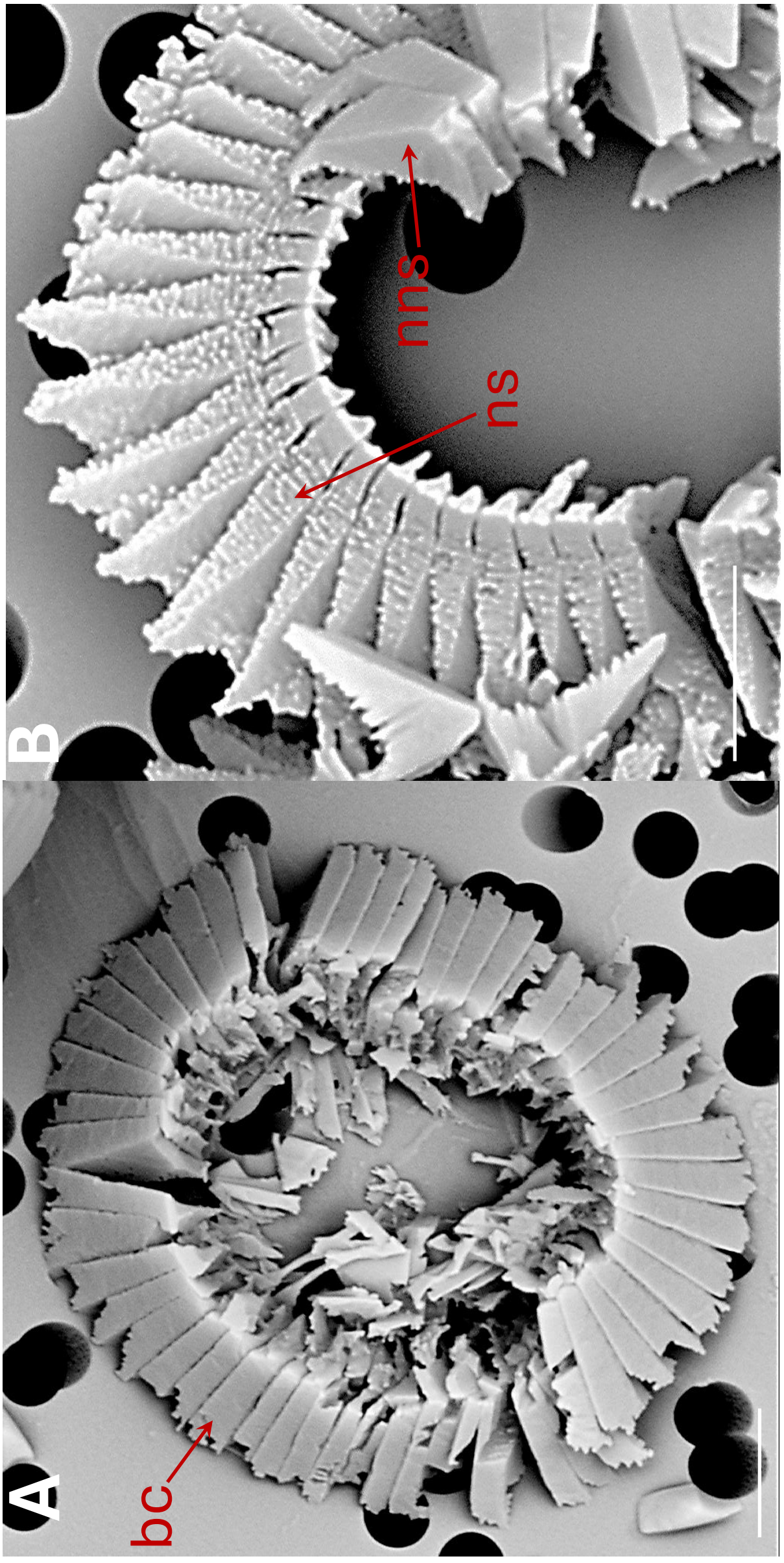


Fig 3

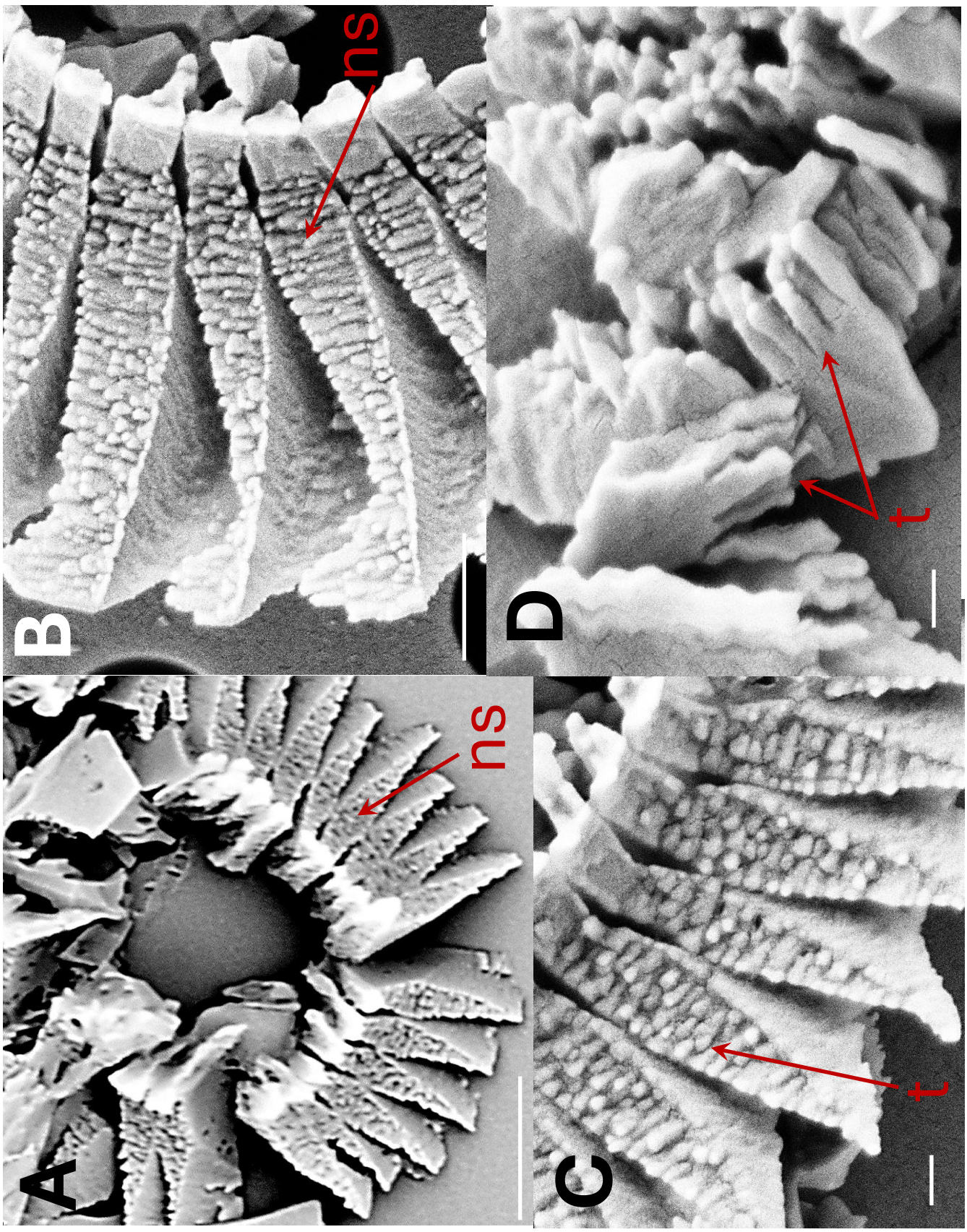
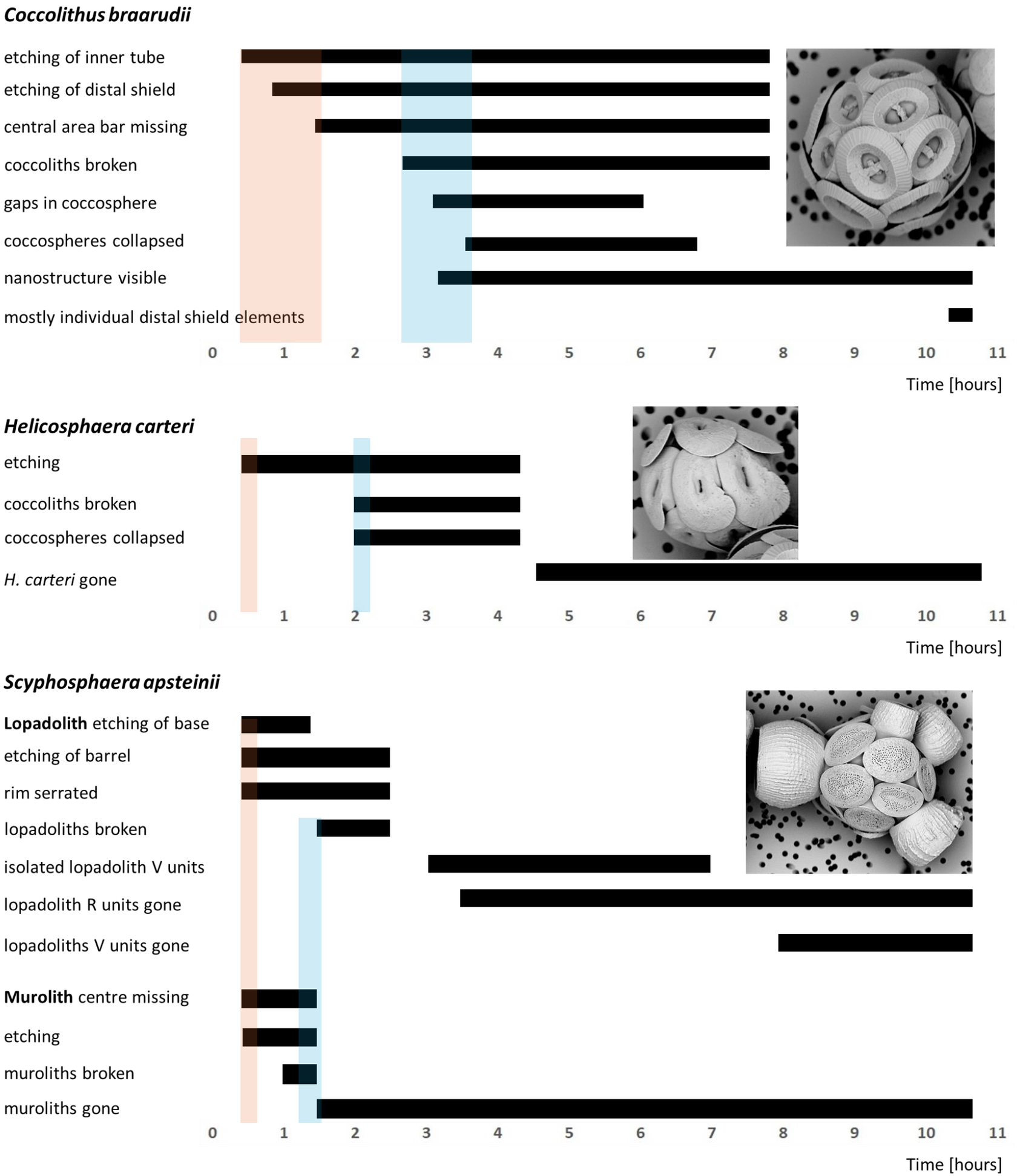


Fig 4

Fig 5

Fig 6


Coccolithus braarudii
etching of inner tube
etching of distal shield
central area bar missing
coccoliths broken
gaps in coccosphere
coccospheres collapsed
nanostructure visible
mostly individual distal shield elements
0 1 2 3 4 5 6 7 8 9 10 11
Time [hours]
Helicosphaera carteri
etching
coccoliths broken
coccospheres collapsed
H. carteri gone
0 1 2 3 4 5 6 7 8 9 10 11
Time [hours]
Scyphosphaera apsteinii
Lopadolith etching of base
etching of barrel
rim serrated
lopadoliths broken
isolated lopadolith V units
lopadolith R units gone
lopadoliths V units gone
Murolith centre missing
etching
muroliths broken
muroliths gone
0 1 2 3 4 5 6 7 8 9 10 11
Time [hours]

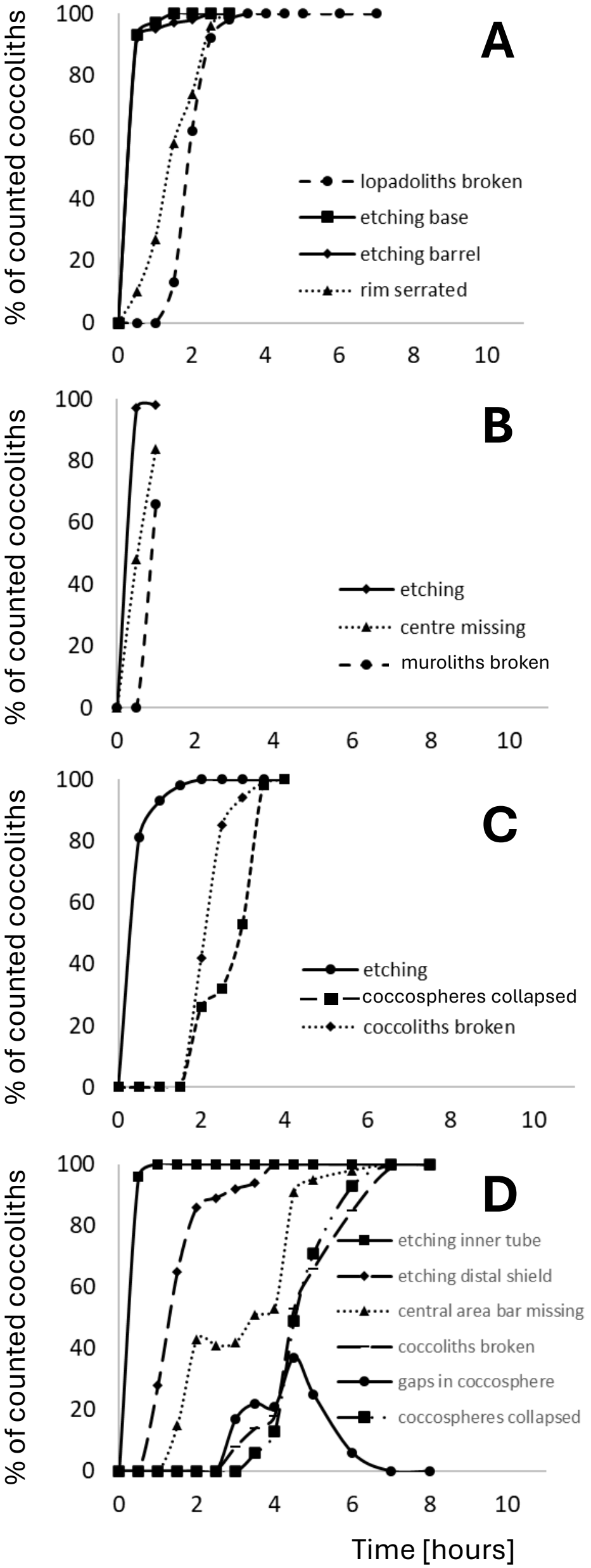

A
% of counted coccoliths
100
80
60
40
20
0
0
2
4
6
8
10
lopadoliths broken
etching base
etching barrel
rim serrated
B
% of counted coccoliths
100
80
60
40
20
0
0
2
4
6
8
10
etching
centre missing
muroliths broken
C
% of counted coccoliths
100
80
60
40
20
0
0
2
4
6
8
10
etching
coccospheres collapsed
coccoliths broken
D
% of counted coccoliths
100
80
60
40
20
0
0
2
4
6
8
10
etching inner tube
etching distal shield
central area bar missing
coccoliths broken
gaps in coccosphere
coccospheres collapsed
Time [hours]

Fig 8