Research paper

Fine structure of silicoflagellate double skeletons

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ABSTRACT

Silicoflagellate double skeletons are commonly considered to be pre-division stages, even though their life cycle is only partially resolved, especially with respect to reproduction. Double skeletons of the modern silicoflagellate genera *Dictyocha* Ehrenberg, *Distephanus* Stöhr, and *Octactis* Schiller are for the first time examined in detail by scanning electron microscopy in order to improve our understanding of how skeletal morphology relates to paired skeletons. A number of genus-specific mechanisms enable sibling skeletons to be held together at their abbasal surfaces, including a zig-zag design of the basal ring achieved via apical structure, strut attachment and pike rotation (in *Distephanus* and *Dictyocha*), and the presence of organic material binding the generally planar basal rings (in *Octactis*). Contrary to what is generally understood, the siblings are not mirror images of one another. Instead, the triple junctions formed by the skeletal elements of each apical structure are transposed across the middle of the dividing cell to produce a copy with the same rotation. Thus, two dome-shaped skeletons represent halves of a more spherical design, which suggests that the role of the silicoflagellate basal ring is to enable double skeleton formation, but the full implications of this have yet to be explored.

Although the purpose of double skeleton formation in silicoflagellates remains unclear, observations from the fossil record indicate that differences in the relative alignment of doublet members can have a high significance for phylogeny. Differences in the doublet structure of living silicoflagellates call for a combined biological and geological perspective of the utility of maintaining *Dictyocha*, *Distephanus* and *Octactis* as separate genera.

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1. Introduction

Silicoflagellates (Class Dictyochophyceae P.C. Silva) are marine unicellular pigmented heterokonts (Daugbjerg and Henriksen, 2001) that have a fossil record extending to at least 115 million years (myr) ago, i.e., to the Early Cretaceous (McCartney et al., 1990, 2010b, 2014b). The group has a wide biogeographic distribution, but their biology is poorly understood mainly because their life cycle has been resolved only partially (Henriksen et al., 1993). Multiple life cycle stages have been identified in natural and cultured populations, only one of them being skeleton-bearing (Henriksen et al., 1993), but it is unknown which is the prevalent form. Furthermore, the naked and skeleton-bearing stages have been linked relatively recently (Jochem and Babenerd, 1989; Moestrup and Thomsen, 1990), and therefore the ecological importance of silicoflagellates in marine ecosystems may be underestimated.

An intriguing and largely overlooked aspect of silicoflagellate biology is the formation of double skeletons (also termed doublets or paired skeletons) that are widely regarded as pre-division stages (Boney, 1981; Moestrup and Thomsen, 1990). These are usually presented as chance discoveries without in-depth discussion, although they have been used as productivity indicators by Takahashi and Blackwelder (1992)

and Takahashi et al. (2009). Both the purpose and mechanisms underlying double skeleton formation have received little attention in studies on natural and cultured populations, and the few published illustrations (listed in Table 1) provide insufficient details. Therefore, this study aims to provide the first detailed examination of double skeleton structure for all modern silicoflagellate genera. In addition, we review the silicoflagellate skeletal morphology with a particular focus on double skeleton structure, expecting that an improved understanding of silicoflagellate biology and skeletal morphology will help increase their utility in various fields of Earth sciences.

2. Taxonomy

The general morphology of siliceous skeletons (Figs. 1A–E and 2A–D) has been the basis for silicoflagellate taxonomy with genera commonly distinguished by the presence or absence and/or complexity of the apical structure. This paleontological approach has been criticized (Van Valkenburg and Norris, 1970), but given the lack of data on naked stages in most of the living taxa, skeletal morphology-based taxonomy remains the only plausible method of species identification. The skeleton-bearing life cycle stages of extant silicoflagellates are characterized either by an apical bridge (*Dictyocha* Ehrenberg) or apical ring (*Distephanus* Stöhr and *Octactis* Schiller).

There are, however, significant differences in the taxonomic interpretation of the group by biologists and paleontologists. Biologists generally recognize three modern species often combined into the genus *Dictyocha* (Moestrup and Thomsen, 1990; Henriksen et al., 1993), while paleontologists and oceanographers typically see a larger diversity of both genera and species in the Holocene (Poelchau, 1976).

Biologists generally place skeletons with apical rings in *Dictyocha*, based in part on nomenclatural grounds, as the name *Distephanus* Stöhr 1880 is a junior homonym of *Distephanus* Cassini 1817, a terrestrial plant (Moestrup and Thomsen, 1990). Silicoflagellates with apical bridges (*Dictyocha*) and apical rings (*Distephanus*), however, have long and distinct geologic histories dating back at least to the Eocene (~56–34 myr ago). Therefore, paleontologists interpret these morphologies as belonging to separate genera and thus have continued to use '*Distephanus*' despite the taxonomic invalidity (e.g., Malinverno, 2010; Rigual-Hernández et al., 2010). *Distephanopsis* Dumitrică (1978) has come into some recent use in place of *Distephanus* (e.g. Desikachary and Prema, 1996; Dumitrica, 2014), although it is currently illegitimate, and besides there has not been a new combination offered for *Distephanus speculum*. In this paper, we use *Distephanus*, but a potential

taxonomic alternative will be presented in the near future in a separate communication.

An additional reason for the application of *Dictyocha* as the only extant silicoflagellate genus by phycologists is due to the results of early culturing experiments. Van Valkenburg and Norris (1970) concluded that clonal cultures of *Dictyocha fibula* Ehrenberg produced skeletal morphologies of three genera (*Dictyocha, Distephanus* and *Cannopilus* Haeckel) as used by paleontologists. Most of the skeletons illustrated in Van Valkenburg (1970), however, appear teratoid and often lack the essential symmetries of silicoflagellates commonly observed in the fossil record or in living natural populations. General taxonomic conclusions should not be based on obviously aberrant morphologies.

In addition to skeletal morphology, Chang et al. (2012) provided preliminary molecular data which suggested that silicoflagellates with an apical bridge (*Dictyocha*) and apical ring (*Distephanus*) may indeed represent distinct genera of the Dictyochales. The molecular phylogeny in Chang et al. (2012) indicated that *Distephanus speculum* (*Dictyocha speculum* in Chang et al., 2012) and *Octactis pulchra* (*Dictyocha octonaria* in Chang et al., 2012; Chang, pers. comm., 2014) are more closely related, and may be classified within a single genus (Chang, pers. comm., 2014; see also Ling and Takahashi, 1985). Despite this, we treat *Octactis* Schiller as a separate genus, as there are significant morphological differences between skeletons of this taxon and *Distephanus,* which include the placement of the strut attachments and the absence of pikes as will be presented below.

3. The silicoflagellate skeleton

Although out of necessity the skeleton serves as the basis for the taxonomy of extant silicoflagellates, few studies on its formation are available (Preisig, 1994). The few transmission electron microscope (TEM) studies of *Distephanus speculum* have not addressed skeleton formation to any extent (e.g., Moestrup and Thomsen, 1990). TEM photographs of *D. fibula* (Van Valkenburg, 1970, 1980) suggest skeleton secretion to be internal, as sectioned cells revealed four large vacuoles with thick walls. As a consequence, it was speculated that these were skeleton-forming vacuoles (Van Valkenburg, 1970, 1980). By contrast Moestrup and Thomsen (1990) concluded that the *Distephanus* skeleton is external. McCartney and Loper (1989, 1992), based on optimization models of the skeletal morphologies of three silicoflagellate genera, interpreted the skeleton as supporting the cell boundary to a shape that might reduce surface tensional forces.

Studies on silicoflagellate skeletons lack a consistent terminology. The terminology used here (Fig. 2) incorporates terms proposed by

Table 1
Previously published illustrations of silicoflagellate double skeletons.

Author (year)	Illustration	Genus	Illustration
Haeckel (1887)	pl. 101, fig. 12	*Dictyocha*	line drawing
Möbius (1887)	pl. 8, figs. 50, 52	*Distephanus, Dictyocha*	line drawing
Gemeinhardt (1930)	fig. 53e	*Distephanus*	line drawing
Hovasse (1932)	fig. 3	*Distephanus*	line drawing
Hovasse (1946)	figs. 1a, 4a, 4b, 4c	*Dictyocha, Distephanus*	line drawings
Deflandre (1950)	figs. 18–24, 40–41	*Distephanus, Mesocena?*	line drawings
O'Kane (1970)	fig. 11	*Distephanus*	line drawing
Tampieri (1972)	pl. 2, figs. 1, 3; pl. 3, fig. 1	*Dictyocha*	SEMs
Boney (1976)	fig. 1d–f	*Distephanus*	line drawings
Poelchau (1976)	pl. 5, fig. b	*Dictyocha*	LM
Haq (1978)	fig. 3	*Distephanus*	SEM
Ling and Takahashi (1985)	pl. 1, fig. 5; pl. 2, figs. 1, 2, 4	*Octactis*	SEMs
Moestrup and Thomsen (1990)	pl. 7, figs. 30–33; pl. 9, fig. 39	*Distephanus*	LMs
Takahashi and Blackwelder (1992)	figs. 3-2, 3-3, 3-5	*Distephanus, Octactis*	LMs
Hallegraeff (2005)	fig. 4.2d	*Distephanus*	SEM
Takahashi et al. (2009)	pl. 2, figs. 3, 5, 14	*Distephanus*	LMs
Davidson et al. (2010)	fig. 4f	*Distephanus*	SEM
Cefarelli et al. (2011)	fig. 4o	*Distephanus*	SEM
Onodera and Takahashi (2012)	fig. 5	*Distephanus*	LMs
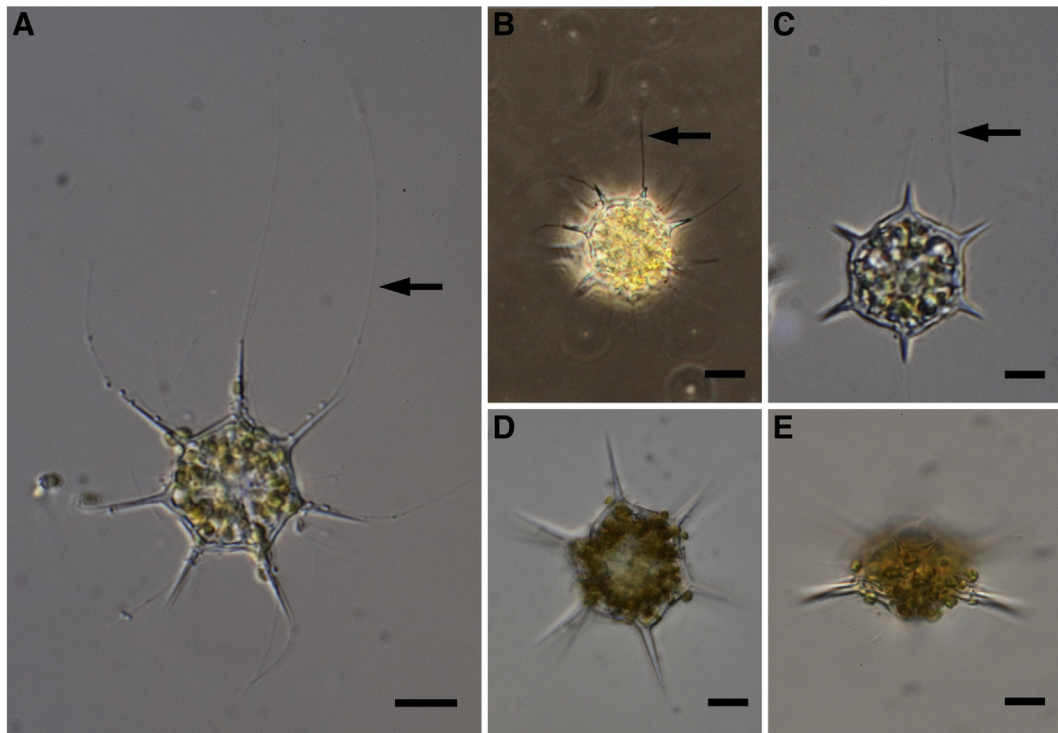
Fig. 1. Light micrographs of live cells of the skeleton-bearing phase of *Distephanus speculum*. Material from East Greenland. Arrows indicate tentacles (= pseudopod in Marshall, 1934) in A and B, and the arrow in C indicates the single emergent flagellum. A. Cell showing long tentacles, which are aligned along the basal spines. Notice that this cell possesses seven spines emerging from the basal ring. B. Cell in phase contrast to illustrate tentacles. C. Cell with the typical six basal spines in this species. Notice the single emergent flagellum. Nomarski interference contrast. D, E. Cells revealing a high number of circular chloroplasts. Some are positioned outside the basal and apical rings. Tentacles were not observed in these cells.
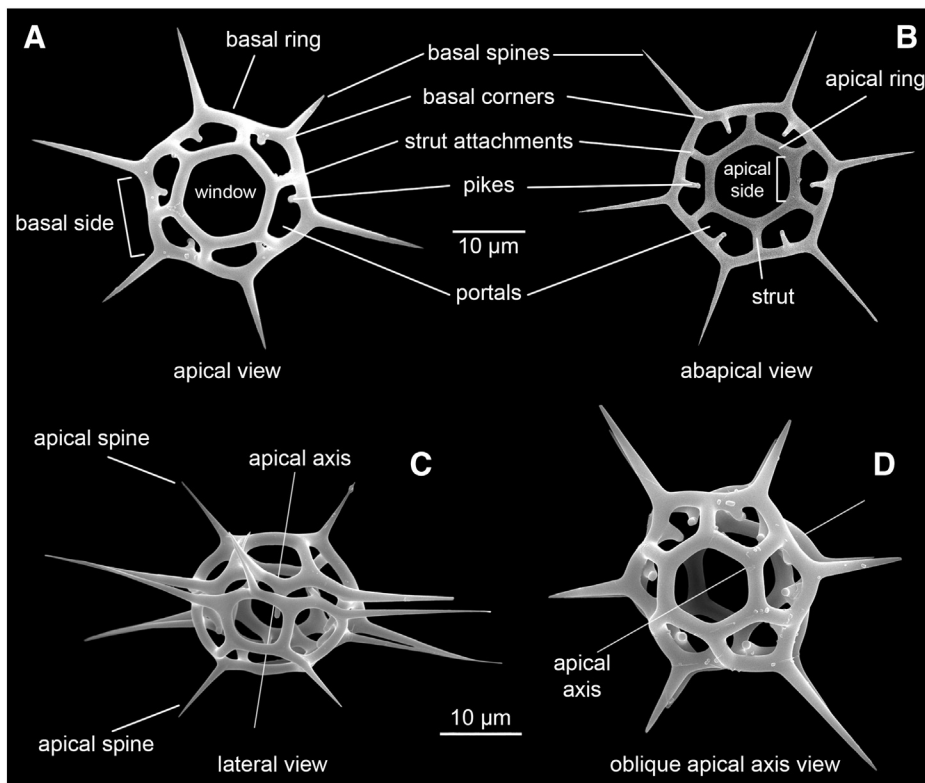
Fig. 2. SEM photographs of *Distephanus speculum* to show descriptive morphological terminology. Note that for double skeletons we use apical "axis" view to note that there are both apical and abapical skeletons. A. Single skeleton from apical view, Chilean coast. B. Single skeleton from abapical view, Greenland coast. C. Double skeleton from lateral view, Indian Ocean. D. Double skeleton from apical axis view, Chilean coast.

Bukry (1976) and McCartney and Loper (1989). The skeleton is made of rod-shaped "elements" which make up the basal ring and apical structure. The terms apical, abapical and basal are here used by convention (Lemmermann, 1908; Marshall, 1934; and subsequent literature) and do not refer to the orientation of a living cell (see comments in Moestrup and Thomsen, 1990). The apical structure is attached to the basal ring by struts, one to each basal side between the corners of the polygonal basal ring. The apical ends of the struts can meet at an apex (the fossil genus *Corbisema* Hanna), support a bridge (*Dictyocha*), apical ring (*Distephanus*) or an extensively subdivided apical structure (the fossil *Cannopilus*).

In addition to the elements, which are joined at both ends, are "spines" and "pikes" that connect with elements at one end and terminate distally. Spines and pikes are distinctly different features of the silicoflagellate skeleton and have different orientations. Spines typically occur at the corners of the basal ring (basal spines) within the plane of the basal ring, may be present on the apical structure (apical spines) and generally radiate from the middle of the basal plane. Where the basal ring has a major axis, spine lengths are proportional to the relative dimensions of the basal axes. Pikes are typically smaller, occur on the underside of the basal ring and point in an abapical direction. The shape of pikes can vary but that of the spines does not.

The "basal plane" (McCartney et al., 2011) goes through the middle of the skeletal elements that meet at the corners of the basal ring, and would be parallel to the plane of the apical ring if present. We use the terms "abbasal surface" for the surface of the basal ring furthest from the apical structure and "abbasal plane" for the plane that connects the points of contact between the sibling skeletons at the basal corners. We propose "apical axis" for an axis perpendicular to the basal plane that goes through the geometrical center of the apical structure and basal ring (Fig. 2D). The middle of the abbasal surface should represent the center of the double-skeletoned cell.

The structural design of the silicoflagellate skeleton appears to almost universally abide by simple rules (McCartney and Loper, 1989, 1992). Foremost among these is that the skeletal latticework consists of elements that meet at triple junctions with obtuse, usually similar and near 120° angles between the elements, especially on the apical structure. Where only two elements meet at an angle, a spine or pike may serve as the third part of the triple junction. The near 120° angles generally observed on the apical structure do not necessarily apply where the apical structure attaches to the basal ring. While no detailed mathematical work has been done on this aspect of skeletal construction, elements of larger mass appear to sometimes be less deflected at the junctions than thinner elements such as those of the apical structure.

One feature that commonly occurs on fossil and recent silicoflagellate skeletons is a sinistral movement of the strut attachments with rotation of the apical structure, when observed from apical view (Poelchau, 1976) (Fig. 3A–C). The evolution of this morphology in modern four-sided *Dictyocha* occurred in the early Pliocene (~4–5 myr ago) when the apical

bridge, previously parallel to the major basal axis, became inclined with a change in the strut attachment positions (McCartney et al., 1995). The pikes moved dextrally along the basal side towards the other corner. Dextral rotations occur elsewhere in the silicoflagellate record and show the need for all silicoflagellate illustrations to be labeled according to apical or abapical view.

Double skeletons are connected to one another primarily at the abbasal surface of the basal ring beneath the basal corners and spines. They are commonly observed in modern water samples (Boney, 1973) but rarely in sediments due to disarticulation before or after deposition, or during the process of sample preparation. The published record of modern double skeletons is shown in Table 1. Double skeletons have been recently observed from the Cretaceous (McCartney et al., 2010a, b), which indicates that double skeleton formation is an inherent feature of this group since early in its evolutionary history.

4. Materials and methods

The modern silicoflagellate double skeletons examined and illustrated in this study came from a number of localities (Tables 2 and 3), including Lake Grevelingen in the Netherlands; Indian Ocean; Pacific Ocean; Seto Inland Sea, Japan; fjords along the Chilean coast; East Greenland coast; North Adriatic; East Australian Current; Arctic Ocean and the Southern Ocean. These include lateral views and show the detailed position of pikes that have not previously been illustrated.

Double skeletons were generally extracted from water samples, and examined by means of the scanning electron microscope (SEM) at a number of scientific facilities. For detailed information on sampling procedures and microscope techniques, see Appendix S1 in the online Supporting Information.

5. Results

5.1. Distephanus speculum

Fifty-six *Distephanus speculum* double skeletons were examined. Observed specimens generally had the sibling basal spines in close contact over their entire lengths (Fig. 4A–K), although a few were slightly disarticulated. The spine lengths varied from ~50% to ~150% of the basal ring diameter. The basal spines of sibling skeletons were generally of the same length, but cases where one skeleton had shorter spines occurred (Figs. 2D and 4G). Where spine lengths were equivalent, the thickness of one spine near the basal ring may have been significantly lower than the other (Fig. 4G). A single observed specimen (not illustrated) with five basal sides also had basal spines in close contact.

The sides of the basal ring were not linear when seen from oblique or lateral view, but consisted of three elements bounded by the basal corners, strut attachments and pikes (Fig. 2C). The basal elements inclined apically towards the strut attachment and abapically towards the pike
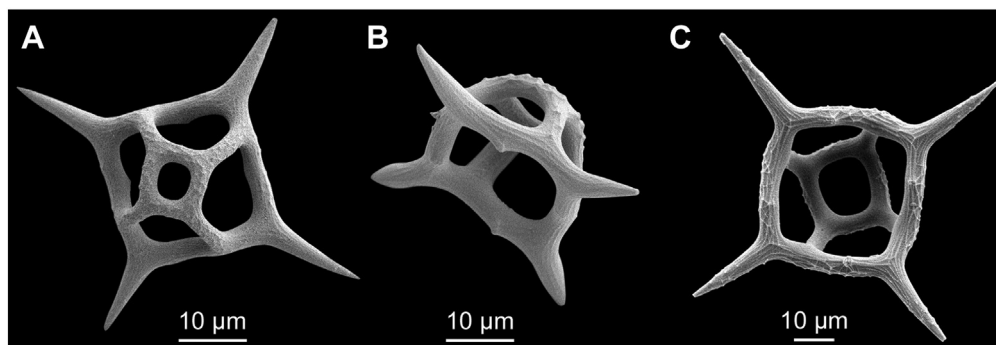

Fig. 3. SEM photographs of *Distephanus crux* (Ehrenberg) Haeckel that show sinistral rotation (defined from apical view) of strut attachments and apical structure. Specimens are from the late Eocene, Oamaru, New Zealand. A. Apical view. B. Lateral view. C. Abapical view.

Table 2
Localities of observed silicoflagellate double skeletons (McCartney, Witkowski et al.).

Locality	Specimens	Skeletal morphologies (genus)	Photographer
North Adriatic (Eastern Mediterranean)	3	*Dictyocha* (1 aberrant)	Elisa Malinverno
East Australian Current eddy, offshore Sydney	3	*Dictyocha* (2), *Octactis* (1)	Gustaaf Hallegraeff
Antarctic waters	1	*Distephanus*	Susan Campbell, via G. Hallegraeff
Arabian Sea	2	*Octactis* (1 aberrant)	Magali Schweizer
Bering Sea, R/V *Mirai*	1	*Distephanus*	Susumu Konno
Chilean coast, R/V *Mirai*	16	*Distephanus*	Hiroya Kano
Chukchi Sea (Arctic), R/V *Mirai*	1	7-sided pseudofibulid *Distephanus*	Susumu Konno
Hawaii, Central North Pacific Gyre	2	*Dictyocha* (1), *Octactis* (1)	Magali Schweizer
Indian Ocean, R/V *Hakuho Maru*	5	*Distephanus* (4, 1 aberrant), *Octactis* (1)	Ayaka Shiozawa
Lake Grevelingen, The Netherlands	12	*Distephanus* (2 aberrant, 1 five-sided)	Kevin McCartney, Manfred Ruppel
Seto Inland Sea, *Bosei Maru*	14	*Dictyocha* (1), *Octactis* (14)	Kenta, Abe, Yuko Fukunaga
Southern Ocean, south of Australia *L'Astrolabe*	7	*Dictyocha* (1), *Distephanus* (6, 1 seven-sided)	Fiona Scott
Southwest Pacific	6	*Distephanus* (1 with two windows)	Elisa Malinverno, Manfred Ruppel
Arctic waters	3	*Distephanus*	Jeremy R. Young
Antarctic waters	5	*Distephanus*	Jeremy R. Young
North Pacific, R/V *Kilo Moana*	2	*Dictyocha*	Jeremy R. Young

to form triple junctions that combined to form a zig-zag design around the basal ring. The sibling sides did not necessarily conform to the same contours and were not in as close contact as the basal spines.

Triple junctions of the apical structure appeared to be transposed across the cell to produce sibling skeletons with equivalent sinistral rotations of the apical structure. Thus, the sibling skeletons were not mirror images as stated by Boney (1976) and other workers, since the skeletons were not reflections of one another across a mirror plane. Rather, the triple junctions of the apical structure appeared to be transposed across the middle of the dividing cell to the sibling skeleton, which produced a copy that had the same rotation. This rotation is also shown in the position of the strut attachment on the basal ring. Seen in apical view and following the strut to the attachment (e.g. Fig. 2A), the pike occurred dextrally on each basal side.

The location of the pikes in comparison to the sibling basal ring varied. The pikes could be located in close proximity to the strut attachment of the sibling (Figs. 2C and 4C, D) or at a greater distance (Fig. 4E, F). The pikes trended briefly towards the interior of the basal ring of the sibling skeleton before deflecting away from the basal plane (Fig. 4C–F). Moestrup and Thomsen (1990) similarly observed these "inwardly bent hook-shaped" pikes. Near the point of this deflection, the pike tapered distally to ~50% of its thickness near the basal side (Fig. 4A, B). The pikes projected into the region of the sibling skeleton where they often trended around the basal side or strut (Fig. 4C–F). The pikes had blunt terminations in all specimens examined in this study.

Table 3
Dates and coordinates for illustrated modern double skeletons (McCartney, Witkowski et al.).

Figure	Date	Coordinates	Locality
2C	14-Feb-08	65°10′14″S, 46°55′14″E	Indian Ocean
2D	16-Mar-09	41°55.1745′S, 73°20.0973′W	Chilean Coast
4A-B	29-Mar-09	53°23.7246′S, 70°37.2311′W	Chilean Coast
4C-D	24-Mar-09	53°5.8645′S, 73°20.2427′W	Chilean Coast
DE-F	21-Mar-09	47°45.0031′S, 76°2.265′W	Chilean Coast
4G	15-Mar-09	35°47.7897′S, 73°19.6298′W	Chilean Coast
4H	1-Jan-05	67°27.87′S, 178°12.42′E	Southwest Pacific
4I-J	30-May-11	51°43′N, 3°60′E	Lake Grevelingen
4 K	4-Mar-09	45°07.0011′S, 146°56.0114′E	Southern Ocean
5A	4-Mar-09	45°07.0011′S, 146°56.0114′E	Southern Ocean
5B	May-05	22°75′N, 158°0′E	Hawaii
5C	May-81	35°40′S, 150°30′E	East Australian Current
5D	Aug-Sep-94	22°45′N, 158°0′W	Hawaii
5E	15-Feb-12	45°42′03″N, 13°42′36″E	North Adriatic
6A, C-F	1-May-08	34°32.5′N, 135°10′E	Seto Inland Sea
6B	1-Feb-08	39°6′50″S, 25°5′15″E	Indian Ocean

For nearly all of the specimens examined in this study, the general morphologies of sibling apical structures were similar. The only noteworthy exception was a pair with a teratoid skeleton that had an incomplete basal ring that lacked a basal side but whose existing five struts supported a five-sided apical ring in contrast to the six-sided sibling. Other specimens displayed differences in the diameters of the apical ring. The *Distephanus* specimens examined here generally had a slight sinistral rotation of the strut attachments that was more easily observed in a comparison of the two sibling skeletons (Fig. 4I). The pikes were, however, generally offset to a greater degree and are thus located closer to the basal corners (Fig. 4A, I).

5.2. Dictyocha messanensis

Nine *Dictyocha* double skeletons were examined, five of which are illustrated here (Fig. 5A–E; note that E is slightly disarticulated). All are believed to fall within the range of variation of *D. messanensis* Haeckel. This species has the strut attachments and apical bridge rotated sinistrally with respect to the midpoint of each basal side when seen from apical view, so that the bridges of the double skeleton show an "X" pattern when viewed down the apical axis. The skeletal elements in double skeletons of *D. messanensis* were transposed across the center of the cell in a similar manner as for *Distephanus speculum*.

In *D. messanensis*, the pikes appeared to go up the underside of the sibling struts (Fig. 5A–D), which is different from *Distephanus speculum*, where the pikes were offset to the side of the strut closer to the basal corner (Fig. 4A–K). Also, in *D. messanensis* the overall deflection of the basal elements that form the side was lower at the point where the pike connected to the basal ring (Fig. 5A, C, E), so that the basal sides did not have as pronounced a zig-zag pattern relative to *Distephanus speculum* (Fig. 2C). This, however, may be a function of the angle at which the specimens are viewed.

5.3. Octactis pulchra

Seventeen *Octactis* double skeleton specimens were available for this study (Fig. 6A–F). The apical ring elements of *Octactis* were of much smaller thickness than generally observed in *Distephanus*. The basal ring elements were generally more massively constructed in *Octactis* and lack pikes. Three of the observed specimens (see Fig. 6C for one of these) had one skeleton with incomplete struts that terminated distally without apical ring elements. Those skeletons with incomplete struts also had shorter basal spines, which suggests they were daughter members of a doublet. Such incomplete apical structures were not observed on any of the *Distephanus* and *Dictyocha* double skeleton specimens.
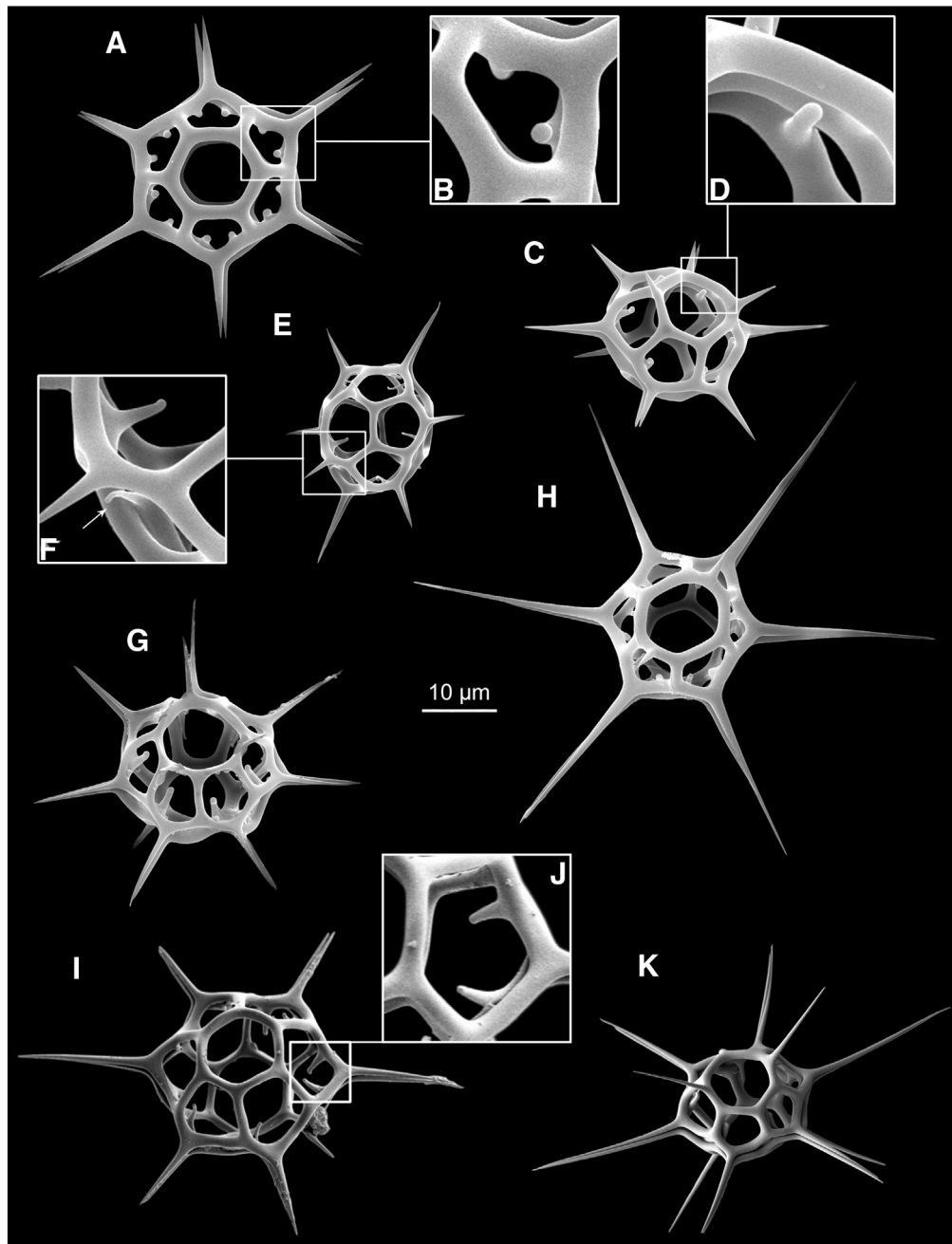
Fig. 4. SEM photographs of *Distephanus speculum* double skeletons. "Apical axis view" means view is down the axis of a double skeleton, with near skeleton being apical and far skeleton abapical view. A, B. Apical axis view, Chilean coast, with magnification showing pikes of apical and abapical siblings. C, D. Oblique axis view, Chilean coast, with magnification showing pike of abapical sibling inside the basal ring of the apical sibling. E, F. Oblique axis view, Chilean coast, with magnification showing pike of apical sibling inside the basal ring of the abapical sibling. G. Oblique axis view, Chilean coast. H. Apical axis view Southwest Pacific Ocean. I, J. Two views of a specimen from Lake Grevelingen, the Netherlands. I: Oblique axis, the abapical sibling appears to have an incomplete basal ring on the lower right basal side; J: magnification showing pikes of apical (top) and abapical (bottom) siblings. K. Oblique axis view, Southern Ocean, South of Australia.

The strut attachments for *Octactis* were located at the midpoints of each basal side without the sinistral rotation typical of modern *Distephanus*. The attachment positions tended to be located in the middle of the basal side when the apical ring was of smaller diameter, but were near the outer edge of the basal ring when the apical and basal rings were of more equal diameters (Fig. 6B). The thickness of the struts diminished markedly from the strut attachment towards the apical ring. The thickness of the apical ring elements may have varied more between sibling skeletons (Fig. 6E) than was observed for *Distephanus*. No apical spines or pikes were observed on the specimens of this study, although small apical spines are known to occur (see Ling and Takahashi, 1985, pl. 1, figs. 7 and 9).

Octactis double skeletons included in this study were usually at least slightly disarticulated; other more disarticulated doublets were observed but are not counted. Where the sibling skeletons were still connected with the basal spines aligned (Fig. 6B, E, F), struts and strut attachments also appeared to be generally aligned, although there are exceptions to this (see the far sides of Fig. 6D). While individual skeletons seen from abapical view appeared to have a planar abbasal surface, examination of double skeletons in oblique or lateral view (Fig. 6D,
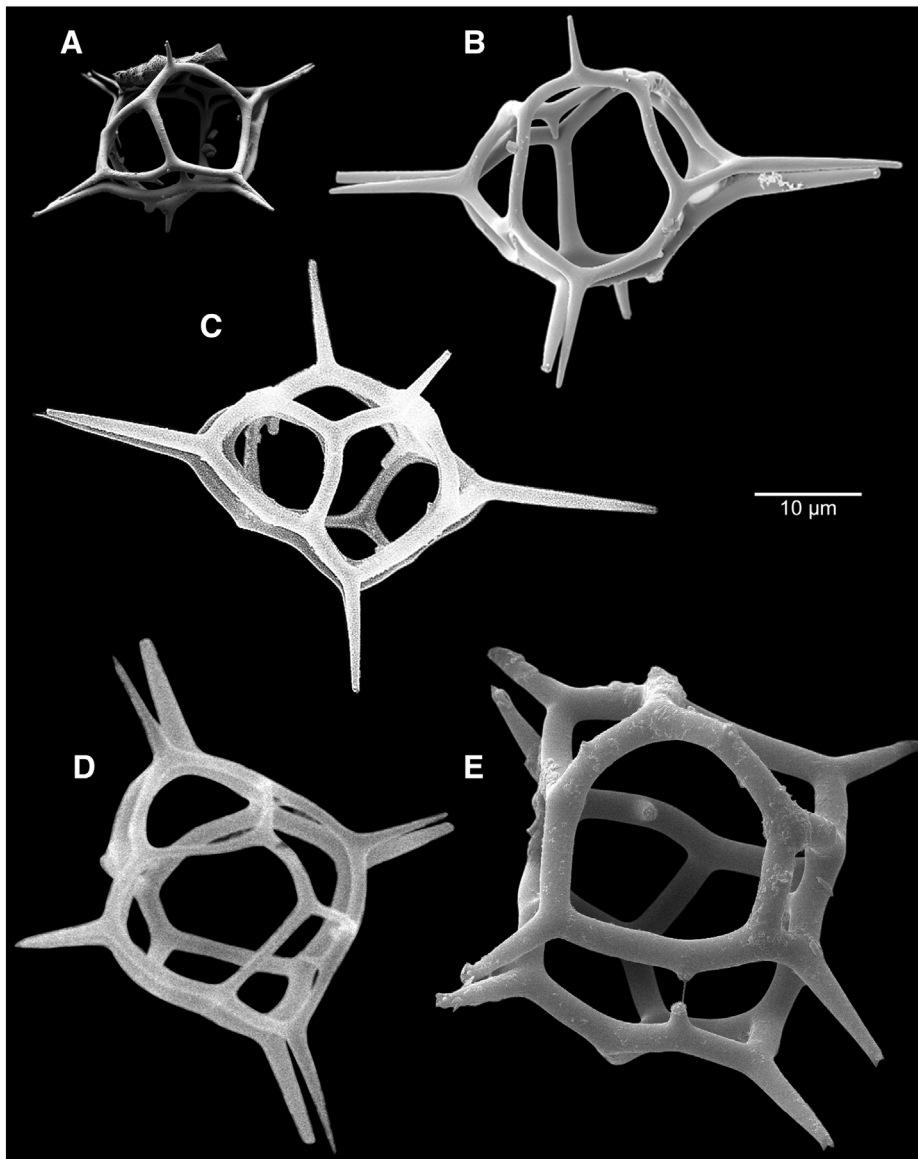
Fig. 5. SEM photographs of *Dictyocha messanensis* double skeletons. A. Oblique view, somewhat disarticulated, North Adriatic. B. Oblique view, Northwest Pacific. C. Oblique view, East Australian Current off Sydney. D. Oblique view, Hawaii. E. Oblique view, Southern Ocean, south of Australia.

F) often showed a gap between the strut attachments. This shows that, as with *Distephanus* and *Dictyocha,* the main points of contact between the sibling skeletons are between the basal corners and spines. *Octactis* lacks pike-like structures of any kind, and the zig-zag basal design. Since the strut attachments were located midway between the corners, there was no apical rotation. The individual basal spines of a sibling pair varied, with one spine being sometimes more curved than the other and not as closely attached as in *Distephanus.*

6. Discussion

Regularly occurring polygonal patterns, such as those associated with soap bubbles, result from the mathematical optimization of one or more variables (Almgren and Taylor, 1976). Thompson (1942) proposed a similar optimization as an explanation for radiolarian and silicoflagellate skeletal structure. Successful mathematical optimization models for silicoflagellates produced by McCartney and Loper (1989, 1992) were based on the apical surface area being minimized for

enclosed volume, and showed that skeletons with apical rings reduce the apical area but use more skeletal material. But if the minimization of surface area is of major importance to the silicoflagellate organism, as the mathematical models suggest, then why do the skeletons not take a more spherical shape typical of radiolarians and ebridians? The presence of two distinct skeletal structures, an apical dome and undivided basal ring, suggests that each serves a separate purpose (McCartney and Loper, 1989). We propose that the purpose of the basal ring is to allow for the construction and binding of double skeletons, with the sibling apical structures forming a spherical structure that optimizes the surface area for the doublet cell.

The purpose of silicoflagellate double skeletons remains uncertain. Borgert (1891) and Schulz (1928) considered the double skeletons to be products of sexual reproduction, while Gemeinhardt (1930) believed they represent halves of a complete skeleton. Hovasse (1932) was the first to argue in detail that the double skeletons were a stage of reproduction prior to division, and witnessed and described the growth of the daughter skeleton. A number of these early investigators reported
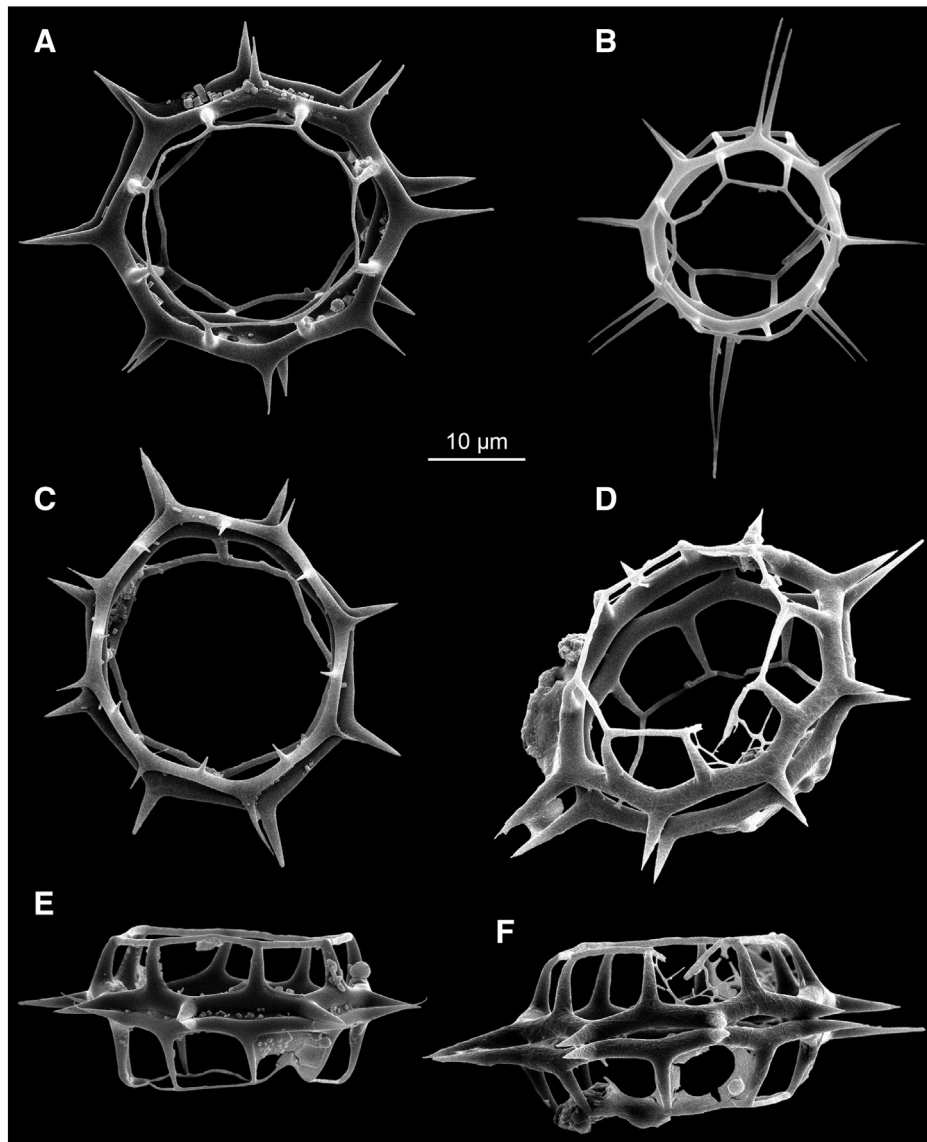
Fig. 6. SEM photographs of *O. pulchra* double skeletons from the Indian Ocean (B) and Seto Inland Sea (A, C-F). A. Apical axis view. B. Apical axis view. C. Apical axis view with the apical structure of the near (daughter) skeleton being incompletely formed. D. Oblique view. E, F. Lateral view. The same specimen is illustrated in D and F.

that the daughter skeleton formed in association with an already completed initial skeleton. Schulz (1928) reported that the daughter skeleton might have thinner elements and be less refrigent, and Hovasse (1932) and Deflandre (1950) have illustrated such pairs, but this was not supported by photographic evidence. Hovasse (1932) also reported that the two cells may separate before the development of the daughter skeleton is complete.

One question that needs greater study is the sequential timing of the daughter skeleton in relation to cellular division. Is the daughter skeleton formed by the parent cell prior to division or by the daughter cell concurrent with the divisional process? The more spheroidal shape provided by the double skeleton may provide the additional internal space required for the divisional process. Also uncertain are the organic structures that hold the skeletons together. That such organic structure must exist is shown by *O. pulchra* and various fossil morphologies that lack the zig-zag basal sides and pikes that appear to help hold the sibling skeletons in place.

Numerous heterokont lineages (e.g. diatoms and chrysophytes) are known to reproduce both asexually and sexually (Andersen, 2004); Chang et al. (2012) document the sexual phase in *Vicicitus*, a member of the Dictyochales as suggested by molecular evidence, despite its total lack of siliceous skeleton. Since sexual reproduction was never observed in the skeleton-bearing Dictyochales (Henriksen et al., 1993), more modern studies of living silicoflagellates assumed that double skeleton formation is associated with mitotic division of the cell (Moestrup and Thomsen, 1990). Boney (1981) observed up to 27% of *Distephanus* cells as double skeletons in North Atlantic blooms. Mitosis has been documented for both naked and skeleton-bearing life cycle stages (Henriksen et al., 1993). However, it is unknown whether double skeleton formation is the only mode of asexual reproduction in skeleton-bearing silicoflagellates, or whether this process can proceed without forming doublets.

Pikes have also received little attention in studies on silicoflagellate skeletal morphology. The pikes appear to be specialized structures for the purpose of binding the two skeletons together. Boney (1976) and Moestrup and Thomsen (1990), both of whom studied the six-sided *Distephanus* morphologies, have noted that the temporary interlocking of the paired skeletons is maintained by the pikes. The angular structure of each basal side, caused by the triple junctions of basal elements with the strut and pike, allows the two skeletons to interlock and be bound

together firmly enough to remain joined after extensive centrifugation (Boney, 1976), yet doublets can easily disarticulate during cellular division.

There is uncertainty about whether *Distephanus* and *Octactis* represent one or two genera. Ling and Takahashi (1985) noted that the thin apical ring elements thought to be characteristic of *Octactis* also occur in *Distephanus speculum tenuis* (Bukry, 1982), which is used as a biostratigraphic zonal marker for the equatorial late Miocene (McCartney et al., 1995). However, there appears to be no direct evolutionary link between these two taxa. Examination of the double skeleton structure provides further evidence that *Distephanus* and *Octactis* might be regarded as separate taxa. *Octactis* skeletons lack the sinistrally rotated apical structures with dextrally offset pikes and basal elements that zig-zag apically and abapically around the basal ring observed in modern *Distephanus*. While both *Dictyocha* and *Distephanus* have prominent pikes that reach into the sibling skeleton and apparently hold the skeletons together, doublets of *Octactis* are seemingly held together only by organic material. Double skeletons offer an important tool for interpreting the early evolution of silicoflagellates. McCartney et al. (2010a) have shown that in addition to the basal "corner-to-corner" pairs documented for the first time in detail here, Cretaceous and some Paleogene double skeletons have the sibling basal rings arranged in a "Star-of-David" configuration in which the corners are aligned with the middle of the sibling basal sides. Double skeletons in the Star-of-David configuration are observed as late as the Eocene (McCartney et al., 2014a), but are not observed in more modern silicoflagellates. The presence of two double skeleton configurations has profound consequences to the silicoflagellate phylogeny, as it implies that some of the taxa previously viewed as monophyletic might represent paraphyletic groupings.

Skeletal variability is one of the distinguishing characteristics of silicoflagellates, and it too can be interpreted in the context of double skeleton formation. Various observers of both fossil and modern double skeletons (Gemeinhardt, 1930; Bukry and Foster, 1973; Boney, 1976; Takahashi et al., 2009) have illustrated six-sided *Distephanus* double skeletons where one sibling has a divided window. Malinverno (2010) also noted variant morphologies of *Distephanus speculum*. Boney (1981) reported that about 3% of double skeletons had an aberrant sibling. Therefore, the transposition of triple junctions across the middle of the cell prior to division may cause the triple junction locations to vary from being at the exact opposite side of the cell, which favors increased morphological variability.

Thus double skeletons, previously understudied, may provide a basis for new interpretations of silicoflagellates. A more detailed examination of silicoflagellate double skeletons is needed from both phycological and geological perspectives. Clearly, there is much more to be gained from a better knowledge of silicoflagellate life cycle, reproduction, cellular division and expression of skeletal morphology.

7. Conclusions

A detailed examination of silicoflagellate double skeletons helps in the understanding of various features of the skeletal design. For *Dictyocha* and *Distephanus*, the sinistral rotation of the strut attachments, with dextral displacement of the pikes, creates a basal ring made of elements that trend apically towards the strut and abapically to the pike. The zig-zag design probably serves to help hold the skeletons together during pre-divisional stages and presumably projects the pikes into the cytoplasm of the sibling cell. During the divisional process, we believe that the organic material that helps to connect the two sibling skeletons dissolves, thus allowing the double skeleton to disarticulate.

For *Octactis*, the basal ring is more planar, although the sides arch slightly in an apical direction between the corners in at least some specimens. *Octactis* skeletons lack pikes and the strut attachments of sibling skeletons are generally aligned. The higher number of basal corners and

spines may increase the number of points of connection between the sibling skeletons, which are thought to be held together by organic material. The differences between the basal rings of *Distephanus* and *Octactis* support their separation at the generic level.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marmicro.2014.08.006.

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