New observations on the Antarctic Asteromphalus darwinii/ hookeri diatom species-complex (Asterolampraceae)

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Abstract

Antarctic diatom populations of *Asteromphalus hookeri* and related species such as *A. hyalinus* and *A. parvulus* exhibit a highly variable number of hyaline rays ranging from 3 broad + 1 narrow (3 + 1) in the smallest valves, with 4 + 1 (27%) and 5 + 1 rays (35%) most common, and 6 + 1, 7 + 1, and rarely 8 + 1 rays only in larger cells. During December 1959 to April 1960 in the southern sector of the Atlantic Ocean, 6% of valves occurred as “double forms” with epitheca and hypotheca of the same cell exhibiting 4 + 1/3 + 1, 5 + 1/4 + 1, 6 + 1/5 + 1 and 7 + 1/6 + 1 ray combinations. Smaller cells (3 + 1, 4 + 1) always exhibited jagged separation lines in the central area, but larger cells (7 + 1, 8 + 1) had mostly smooth lines, and either jagged or smooth separation lines occurred in intermediate 5 + 1 and 6 + 1 forms, respectively. Epitheca and hypotheca of one and the same cell always exhibited jagged or smooth separation lines, but never mixtures. Observations of silica deposition during October to November 2011 around the Kerguelen Island plateau using the PDMPO fluorescent marker suggest that *Asteromphalus* separation lines play a key role in silica cell wall development. We discuss implications for taxonomy and our understanding of ecophysiology of what we designate as two highly variable and often confused and overlapping diatom taxa, *A.darwiniii* (jagged separation lines; synonyms *A. beaumontii, A. hyalinus, A. leboimei*, *A. parvulus, A. rossii*) and *A. hookeri* (smooth separation lines; synonym *A. antarcticus, A.buchii, ?cuvierii, ? humboldtii*).

Introduction

The centric diatom genus *Asteromphalus* Ehrenberg (*Astero* = star; *omphalos* = navel; “Strahlenschild” sensu Ehrenberg 1844) comprises discoid valves strikingly ornamented by a variable number of hollow tubes (hyaline rays, now termed ordinary rays), supplemented by a single narrow ray (now singular ray), both alternating with areolate sectors to form a bilaterally symmetrical spider-like pattern. We here use the designation 4 + 1 to indicate a valve with 4 ordinary and 1 singular rays, as distinct from the radially symmetrical genus *Asterolampra* Ehrenberg where all rays are equal. Ehrenberg (1844) simultaneously described and illustrated from Antarctic plankton samples, provided to him by Hooker and Darwin, seven new species *A. darwinii, A. rossii, A. hookeri, A. buchii, A. beaumontii, A. humboldtii* and *A. cuvierii*, which differed in valve diameter, number of rays (4 + 1 to 8 + 1), and branching of the separating (“umbilical”) lines of the central area (zigzag in *A. beaumontii, A. rossii, A. darwinii*; straight in the others). While originally spelled as *hookerii*, according to the International Code of Nomenclature (Turland et al. 2018; rule 60.8.a), when a species name is taken from the name of a man (Hooker), it should be formed by adding *ii*, except when the name ends in *-er* when *i* is added. The latter applies and the spelling *A. hookeri* is also adopted by AlgaeBase (Guiry and Guiry 2021). Van Landingham (1967) considered that only three of Ehrenberg’s species were valid, *A. darwinii, A. hookeri* and *A. beaumontii*, while the other four were relegated to be synonyms. Subsequent workers, including Greville (1860) and Rattray (1889) introduced other diagnostic characters such as size of the central area compared to valve diameter, shape of the narrow singular ray within the central area, shape of the areolated sectors, and density of areolae. Later Antarctic plankton workers, such as Castracane (1886), Karsten (1905) and Manguin (1960) continued to describe further new Antarctic *Asteromphalus* species, such as *A. antarcticus* Castracane, *A. challengerensis* Castracane, *A. ovatus* Castracane, *A. wyvillii* Castracane, *A. hyalinus* Karsten, *A. ornithopus* Karsten, *A. parvulus* Karsten, *A. regularis* Karsten and *A. leboimei* Manguin. Boyer (1927) designated the chronologically first described species *A. darwinii* Ehrenberg as the lectotype, but when discussing *A. hookeri* did not cover the discriminating characteristics of *A. darwinii.* Fryxell and Hasle (1974) produced the first scanning electron micrographs of labiate processes present at the end of all hyaline rays (illustrated for *A. hookeri* from the Weddell Sea in their Fig. 5). The application of combined light and electron microscopic characters for this diatom genus was expanded by Hernández-Becerril (1991,1992,1995) and Tiffany and Hernández-Becerril (2005). Hernández-Becerril (1991) newly described in *Asteromphalus* an indentation in the valve margin close to the singular ray, but absent in *Asterolampra.* Hernández-Becerril (1995) also reinstated the genus *Spatangidium* Brébisson for *A. arachne* (Brébisson) Ralfs based on the presence of 1–3 rimoportulae near the central portion of the valves, so far found only in that single taxon. Tiffany and Hernández-Becerril (2005) pointed to species-specific areolae patterns and structures of the cribra covering the loculate areolae albeit subject

to maturational cell wall development, while Priddle and Fryxell (1985) emphasized branching of the separation lines as a diagnostic character.

AlgaeBase (Guiry & Guiry 2021) currently lists 85 accepted species names for the genus *Asteromphalus*, as well as 22 intraspecific names, well demonstrating the uncertainties of taxonomy and understanding of species variability of this distinctive diatom genus. At present, only a single 18S SSU molecular sequence for Northern Hemisphere *Asteromphalus* sp. TN-2014 has been published (Nakov et al. 2015), with its genetically closest relatives being *Coscinodiscus* and *Stellarima* species. The genus does show up in Antarctic waters in the Tara oceans miTAG 16S 18S data base (Vernette et al. 2021) but failed to be detected in a recent Antarctic sediment DNA study (Armbrecht et al. 2022). To the best of our knowledge no culture studies have ever been published. While species of *Asteromphalus* are common (Ocean Biodiversity Information System OBIS lists 4770 global records), they are rarely abundant. An opportunity to study morphological variation in prolific Antarctic populations of the *A. hookeri* complex occurred in December 1959 to March 1960 from a series of seawater pump samples collected during a voyage by the Netherlands whale-factory ship MS *Willem Barendsz*. A preliminary account of variation of diatoms in this material using light microscopy was presented by Van der Spoel et al. (1973), but in the present work *Asteromphalus* diatoms from this same material were re-examined by scanning electron microscopy and interpreted against the background of changing taxonomic interpretations. Complementary observations on silica deposition in Antarctic *Asteromplalus* were made during October to November 2011 around the Kerguelen Island plateau.

Material And Methods

Plankton net samples were collected by Prof S. van der Spoel and Dr W.L. van Utrecht during a voyage of MS *Willem Barendsz* entering the Atlantic sector of the Southern Ocean in December 1959-January 1960, conducting its whaling operations throughout January and March 1960, and exiting March-April 1960 (Figs. 1A,B). Dutch whaling operations were discontinued after 1964. Samples were collected by running seawater from the ship's seawater system, pumped from about 4m below surface and a flow rate of 13 L/min, through 40 µm plankton gauze. Samples were preserved in 5% formaldehyde, and prepared for light microscopy after potassium permanganate-hydrogen peroxide oxidation and mounting in Styrax. Some 629 *Asteromphalus* cells were examined by oil immersion light microscopy with an Olympus microscope and later bright-field and differential interference contrast using an Axioskop 2 Plus Zeiss microscope with Zeiss Axiocam HR digital camera. Samples for scanning electron microscopy (over 150 valves examined) were thoroughly rinsed with distilled water, mounted on nucleopore filters on aluminium stubs, coated with platinum-palladium (5–20 nm layer thickness) and examined with a Hitachi SU70 scanning microscope (SEM) at 1.5–25 kV.

Complementary observations on silica deposition in Antarctic *Asteromphalus* cells were made from 8 October to 22 November 2011 around the Kerguelen Island plateau, during KEOPS2 cruise on board the RV *Marion Dufresne* (Fig. 1C). Niskin and phytoplankton net samples were collected at various stations during the Antarctic spring diatom bloom. Samples were stained with PDMPO (LysoSensor Yellow/Blue DND-160), a fluorescent probe marking new Si deposition over a 24h incubation, for epifluorescence microscopy analyses (Leblanc and Hutchins 2005). Seawater samples of 125 ml were incubated in flow-through deck incubators with a 0.125 µM final concentration PDMPO. After 24h, a smaller volume was filtered onto a black 0.8 µm polycarbonate filters depending on cell density in order to avoid cell overlap. The filters were mounted on a glass slide, covered with a drop of immersion oil, and a round coverslip and stored at -20°C. Filters were examined at the laboratory on an epifluorescence Nikon TE-2000 microscope equiped with a long pass DAPI filter cube (λex 330–380 nm, λdichroic 400 nm, λem 435 nm) and a Nikon DS-5Mc camera.

Results

The diatom genus *Asteromphalus* was omnipresent in MS *Willem Barendsz* 1959–1960 samples and typically comprised 10–30% of total Thalassiosiraceae and Coscinodiscaceae. *Asteromphalus* abundance followed the pattern of < 1°C

surface seawater temperatures, in austral summer being mainly confined to the area south of 51°S (Figs. 2A,B; 217 valves studied), but they were more abundant in autumn (Figs. 2C,D; 412 valves studied) when their populations reached further north up to 40°S. In austral summer surface seawater temperatures < 1°C reached northwards to 51°S (from station 131 southwards) but in austral autumn < 1°C temperatures reached further to 50 °S (from station 592 southwards). The lowest seawater temperatures recorded were − 1.4 to -1.2°C at stations 558, 495 and 469 near the Antarctic ice edge.

Morphology

Figures 3–14 illustrate via a combination of light and scanning electron microscopy the morphology in our Antarctic material of 8 + 1, 7 + 1, 6 + 1, 5 + 1, 4 + 1, and 3 + 1 ray forms, arranged from large to small cells. The number of hyaline rays was rarely 8 + 1 (Fig. 3), 7 + 1 (Fig. 4), or 6 + 1 (Figs. 5,6) and these occurred only in larger cells (39–97 µm diameter), with 5 + 1 rays (35%; Figs. 7–10) and 4 + 1 (27%: Figs. 11–13) the most common) and 3 + 1 representing the smallest valves (14–31 µm diameter; Fig. 14). Larger cells (6 + 1, 7 + 1, 8 + 1) always had smooth separation lines (arrowed in Fig. 4) while smaller cells (3 + 1; 4 + 1; 14–36 µm diameter) always contained jagged (zig-zag) separation lines in the central area (arrowed in Figs. 10, 13). In the intermediate size range of 5 + 1, 6 + 1 and rarely 7 + 1 ray forms, smooth separation lines occurred in larger cells (Fig. 8) but jagged spokes occurred in smaller 5 + 1 forms (Fig. 10). Similar to the variable number of rays or branched separation lines, the feature of straight rays (5 + 1 to 8 + 1) or curved rays (3 + 1, 4 + 1) was correlated with cell diameter. Furthermore, the size of the central area also varied with cell diameter, being proportionally smaller in the largest 6 + 1, 7 + 1, and 8 + 1 rays forms (0.33–0.50 of diameter), larger (0.55–0.64) in the smaller 3 + 1 and 4 + 1 rays forms (e.g. Figure 12), and intermediate in the 5 + 1 ray form (0.5–0.75). Again, with the same 4 + 1 ray morphs, larger cells had a proportionally smaller central area compared to smaller cells (compare Figs. 11 and 12).

Figures 15–33 illustrate fine structural characters used for *Asteromphalus* species discrimination. Indentation of the valve margin was conspicuous, located to the left of the singular ray when viewed internally from the bottom of the valve (Fig. 15; arrow), but located to the right of the singular ray when viewed externally from the top of the cell (Fig. 16; arrow. The endings of the broad rays were always rounded in all ray forms (Fig. 15). Areolae occurred in rows parallel, or nearly so, to the margin, with larger areolae on the inner border of the segments (Fig. 15), 6 in 10 µm, and decreasing to 10 in 10 µm at the margin of the valves. The external (tympanum covering ray hole; Figs. 18–20) and internal rimoportulae details (flat kidney shaped labiate process; Fig. 21) of the ordinary ray holes were identical for all ray morphs. Similarly the external (more elongate ray hole ; Fig. 22) and internal (Fig. 23) rimoportulae details of the singular ray were uniform for all ray morphs. Large 8 + 1 and 7 + 1 ray morphs exhibited a quincunx cribrum structure (type D of Hernández-Becerril 1991; Fig. 24), but mixtures of quincunx to circular cribrums occurred in intermediate 6 + 1 and 5 + 1 ray morphs (Figs. 25–27), and exclusively circular cribrum pore patterns were observed in 4 + 1 (Fig. 28) and 3 + 1 ray forms (Fig. 29). The nature of the separation lines was revealed to be clearly related to the partitioning of the central area into chambers. Smooth lines are demonstrated by SEM and LM, respectively, for 5 + 1 ray morphs (Figs. 30, 31) and jagged lines for 4 + 1 ray morphs (Figs. 32, 33). Partially formed sibling cells (Fig. 34) or broken cells (Fig. 33) revealed that the separation lines were exclusively located in the roof of the central area (Fig. 35, arrows).

Silification Deposition Patterns

Samples from the KEOPS2 cruise from the Kerguelen Plateau in October - November 2011 similarly contained abundant *Asteromphalus* cells represented by 9 + 1, 8 + 1, 7 + 1 and 6 + 1 ray forms (Figs. 36–41). The use of the fluorescence probe PDMPO allowed us to identify cellular regions of active Si deposition. Active Si deposition was consistently associated with the central separation lines (Figs. 36,3 7, arrows), which were smooth in large 8 + 1 ray forms, but in the case of jagged separation lines in 7 + 1 and 6 + 1 ray forms also associated with the bulbous bend in those lines (Figs. 39, 41, arrows).

Double Forms

Typically, the epitheca and hypotheca of an *Asteromphalus* valve are identical, except that the tube-shaped rays of one cell half are offset by one half of an areolated segment relative to the underlying valve. Figures 42–45 illustrate this for two recently divided, still adhering 6 + 1 rays cells comprised of four identical valves each rotated relative to the other. A surprise observation, first reported 50 years ago by Van der Spoel et al. (1973), was that of the 629 *Asteromphalus* cells studied a total of 37 (6%) exhibited intervalvar variation with the designated hypotheca always having one ray less than the epitheca. Such 'double forms' were most common for the 4 + 1/3 + 1 and 5 + 1/4 + 1 ray morphs, rarer for 6 + 1/5 + 1, with only a single detection of a paired 7 + 1/6 + 1 double form, and no 8 + 1/7 + 1 couple observed. Double forms were more common and more widespread in autumn (10–20% of total cells in individual stations) than in summer when they were confined to two stations only (Figs. 2C,D). Figures 47–50 illustrate two recently divided, still adhering cells, in the sequence 7 + 1/6 + 1; 7 + 1/6 + 1 rays, that is a double form generating a replica double form. A double form of 5 + 1/4 + 1 ray morphs is illustrated in Figs. 52–54. All ray morphs always exhibited identical arrangements of jagged (Figs. 42–45; 52–53) or smooth separation lines (Figs. 47–50) between epitheca and hypotheca of the same cell and between parent and sibling cells.

Figure 55 summarises the total number of *Asteromphalus* cells studied from summer and autumn samples and their partitioning into the 8 + 1, 7 + 1, 6 + 1, 5 + 1, 4 + 1, and 3 + 1 ray forms, indicating the widespread occurrence of "double forms". The relationship between cell diameter and ray forms and the disappearance of jagged separation lines in large cells are indicated. The *Willem Barendsz* Antarctic material of 629 valves examined by oil immersion light microscopy and over 150 valves studied by scanning electron microscopy exhibited a gradient of ray morphs with no clear discontinuities discernible except for intermediate 5 + 1 and 6 + 1 ray forms where overlapping patterns of separation lines and cribrum structure were evident.

Discussion

The nature of the "double forms"

The incidence in our material of 4 + 1/3 + 1, 5 + 1/4 + 1, 6 + 1/5 + 1, and 7 + 1/6 + 1 "double forms" provides strong circumstantial evidence that the ray morphs form part of the life cycle of one or more highly variable species. One possibility is that they could be a form of endogenous resting spore, resulting from unequal cell divisions and with some resting spores known to be heterovalvate (Johansen et al. 1985; McQuoid and Hobson 1996). The fact that the internal valves had identical fine structure and were not heavily silicified argues against this. The clear correlation between ray number and cell size (Fig. 54) makes it more likely that they form part of the normal process of vegetative cell divisions and resulting MacDonald-Pfitzer cycle of size reduction (MacDonald 1869, Pfitzer 1869). The cycle would start with the largest sized 8 + 1 and 7 + 1 forms, gradually decreasing in diameter down to the smallest 3 + 1 forms. The finding of sibling cells with incompletely formed cribra (Fig. 16) supports that these are cell division stages. The largest number of 4 + 1/3 + 1 double forms coincided with the highest population density of 4 + 1 forms, largest number of 5 + 1/4 + 1 forms with 5 + 1, and 6 + 1/5 + 1 forms with high 6 + 1 population density (Fig. 45). Castracane (1886, Plate 9, Fig. 2) previously illustrated from Antarctic material a teratological malformation (forma "*monstrosa*") of a dividing cell of *A. challengerensis* Castracane, comprising of 8 + 1 rays on the left and 5 + 1 rays on the right. He stated that "if this admitted (a frustule with a smaller number of radii than the other) then it is clear that no importance should be placed on the number of radii in specific determinations ". Priddle and Fryxell (1985) also suggested this monstrosity to be a synonym of *A. hookeri*. An anomalous form with two incompletely formed ordinary rays of the *A. hookeri* 5 + 1 ray form was illustrated by Hustedt (1958; his Plate 8, Fig. 90). A malformed cell of *Spatangidium (Asteromphalus) arachne* with 3 + 1 instead of 4 + 1 rays has also been reported (Tiffany and Hernández-Becerril 2005, Plate 26, Fig. 2). The closest observation to our double forms, was made by Wood (1959) who illustrated from Antarctic samples a "peculiar diatom", observed on two occasions in the

same sample, with an *Asteromphalus* 8 + 1 rays epitheca combined with, what he believed to be, a *Coscinodiscus* hypotheca, and suggested to be mutations or crosses between two genera. We suggest that, alternatively, this could have been an *Asteromphalus* valve in the process of formation. At present, we have no knowledge of any such mechanism of hybridisation between different diatom species. Hence, we avoided the use of the term hybrid cells and instead refer to them as “double forms” until their precise status has been clarified. Except for the infrequent observation of teratological *Asteromphalus* cells, it remains surprising that no other reports of “double forms” are available for other *Asteromphalus* taxa.

Species taxonomy

Figure 56 summarises type illustrations of Antarctic *Asteromphalus* taxa described by Ehrenberg (1844), Castracane (1886), Karsten (1905) and Manguin (1960). Table 1 compiles morphometric data used by various workers to discriminate Antarctic species of the *Asteromphalus darwinii* / *hookeri* species-complex. Species have been arranged according to the presence of jagged (*darwinii, rossii, beaumontii, parvulus, hyalinus, leboimei)* versus smooth separation lines (*hookeri, buchii, humboldtii)* and clustered according to ray morphs (3 + 1, 4 + 1, 5 + 1, 6 + 1, 7 + 1, 8 + 1) and valve diameter. Smallest valve diameters (14–36 µm) were always confined to the 3 + 1 and 4 + 1 ray morphs which also consistently have jagged separation lines and cribra with circular pore patterns. Largest valve diameters (up to 60 to 98 µm diameter) always had 7 + 1 or 8 + 1 ray morphs, smooth separation lines and quincunx cribrum patterns. Overlapping valve diameters between the two species groups occurred with the 5 + 1 and 6 + 1 ray forms. Density of areolae overlapped between the two species groups (7–10 in 10 µm) and size of the central area relative to cell diameter was strongly correlated with valve diameter (0.5–0.6 of diameter in small cells ranging to 0.3–0.4 in large cells).

Table 1

Morphometric data of the *Asteromphalus darwinii / hookeri* species-complex from Antarctic waters as interpreted by various workers. Species have been arranged according to the presence of jagged (*darwinii, rossii, beaumontii, parvulus, hyalinus, leboimei)* versus smooth separation lines (*hookeri, ?buchii, ?humboldtii)* and clustered according to ray morphs (3 + 1, 4 + 1, 5 + 1, 6 + 1, 7 + 1, 8 + 1) and valve diameter.

Species	Number of ordinary + singular rays	Valve Diameter (µm)	Size central area relative diameter	Sector areolae in 10 µm; cribrum fine structure	Shape of Separation lines	Curvature of ordinary rays	locality	Authority
darwinii	4 + 1	32	0.30*	No data	**jagged**	straight	Antarctic	Ehrenberg 1844, plate June, Fig. 1
ibid.	4 + 1 (constant)	63–72	0.30	7–10; quincunx	jagged	Angled, bent	Yezzo Natanai (Japan)	Hernández-Becerril 1991
rossii	5 + 1	60	0.40*	No data	jagged	straight	Antarctic	Ehrenberg 1844, plate June, Fig. 2
beaumontii	6 + 1	47	0.40*	No data	jagged	straight	Antarctic	Ehrenberg 1844, plate June, Fig. 5
parvulus	5 + 1	30–48	0.5–0.75	“coarse”	jagged	straight	Antarctic	Karsten 1905, Plate 8, Fig. 14
ibid.	5 + 1; 6 + 1	30–50 (55)	no data	no data	jagged	no data	Antarctic	Priddle and Fryxell 1985
ibid.	5 + 1	22–48	0.5–0.75	8–10	“broken”	straight	Global	Hasle and Syvertsen 1997
ibid.	5 + 1 to 6 + 1	33.5–38.5	0.50	7–8; cribrum pores in circular pattern	branched		Antarctic	Ferrario et al. 2021
	5 + 1 to 6 + 1	30–38	0.50–0.56	8; Circular or star-shaped pore patterns	jagged	straight	Antarctic	**Present Work**
hyalinus	4 + 1, curved	22–32	0.5	“fine”	jagged	curved	Antarctic	Karsten 1905 Plate 8, Fig. 15
ibid.	4 + 1	20–30	no data	6–9	jagged	no data	Antarctic	Priddle & Fryxell 1985
ibid.	2 + 1? to 4 + 1	15–32	0.5	8–12	genu-flexed; branched	curved	Global	Hasle & Syvertsen 1997

Species	Number of ordinary + singular rays	Valve Diameter (µm)	Size central area relative diameter	Sector areolae in 10 µm; cribrum fine structure	Shape of Separation lines	Curvature of ordinary rays	locality	Authority
ibid.	3 + 1 to 6 + 1	15.5–35.8	0.5	8–10; cribrum pores in circular pattern	branched		Antarctic	Ferrario et al. 2021
	4 + 1	**14–36**	0.55–0.64	7.5–10; cribrum pores in circular pattern	jagged	Straight /slightly curved	Antarctic	**Present Work**
leboimei	3 + 1	20		10	Zigzag with hooks	curved	Antarctic	Manguin, 1960, Plate 3, Fig. 44
	3 + 1	**14–31**	0.53–0.63	9; cribrum pores in circular pattern	jagged	slightly curved	Antarctic	**Present Work**
hookeri	5 + 1	87	0.32*	No data	**smooth**	straight	Antarctic	Ehrenberg 1844, plate June, Fig. 3
ibid.	5 + 1 to 8 + 1	25–60	0.33–0.50	5–9?	smooth		Global	Hasle and Syvertsen 1997
ibid.	6 + 1 to 10 + 1	no data	no data	no data	smooth, bifurcate if > 6 + 1	no data	Antarctic	Priddle and Fryxell 1985
ibid.	8 + 1	38–98	0.48	8	smooth	straight	Antarctic	**Present Work**
ibid.	7 + 1	39–98	0.53	8	smooth	straight	Antarctic	**Present Work**
ibid.	6 + 1	34–79	0.47	9; quincunx cribrum	smooth	straight	Antarctic	**Present Work**
ibid.	5 + 1	(22) 27–74	0.48–0.53	8–9; quincunx cribrum	Smooth in larger cells	straight	Antarctic	**Present Work**
ibid.	5 + 1 to 8 + 1	25–60	0.33–0.50	5–9?	smooth		Global	Hasle and Syvertsen 1997
ibid.	6 + 1 to 10 + 1	no data	no data	no data	smooth, bifurcate if > 6 + 1	no data	Antarctic	Priddle and Fryxell 1985
ibid.	8 + 1	38–98	0.48	8	smooth	straight	Antarctic	**Present Work**

Species	Number of ordinary + singular rays	Valve Diameter (µm)	Size central area relative diameter	Sector areolae in 10 µm; cribrum fine structure	Shape of Separation lines	Curvature of ordinary rays	locality	Authority
buchii	6 + 1	60	0.34*	No data	smooth	straight	Antarctic	Ehrenberg 1844, plate June, Fig. 4
humboldtii	7 + 1	82	0.44*	No data	smooth	straight	Antarctic	Ehrenberg 1844, plate June, Fig. 6
ibid.	7 + 1 to 10 + 1	91–149	0.33	6-6.5	smooth	straight	Antarctic	Hernández-Becerril 1995
ibid.	5 + 1; 6 + 1	43–78; 40-73.5	0.33–0.50; 0.50	7–8; 8–10; quincunx; cribrum of central pores surrounded by elongate pores	angled, straight-curved	Straight or slightly curved	Antarctic	Hernández-Becerril 1991; Ferrario et al. 2021
cuvierii	8 + 1	82	0.48*	No data	smooth		Antarctic	Ehrenberg 1844, plate June, Fig. 7

In the present study, we newly point to the important role the separation lines play in silica uptake (Figs. 36–41). While the number of hyaline rays appears to be highly variable in an as yet unexplained mechanism, even between epitheca and hypotheca of sibling cells (Figs. 42–53), the feature of jagged versus smooth separation lines appeared consistent also between epitheca and hypotheca of sibling cells.

The chronologically first described Antarctic species *A. darwinii* Ehrenberg 1844 (reproduced in Fig. 56I) was designated the lectotype of the genus by Boyer (1927). The only report in contemporary literature (Hernández-Becerril 1991) of *A. darwinii* relates to fossil material from Japan (Yezzo Natanai), not from the Antarctic type locality. These Japanese cells consistently had 4 + 1 rays, jagged separation lines and a type G cribrum of hyaline areas without poroids and flat areas with poroids. We argue that the 6 + 1 ray and 5 + 1 ray morphs with jagged separation lines, described as *A. beaumontii* Ehrenberg 1844 (Fig. 56F) and *A. rossii* Ehrenberg 1844 (Fig. 56G) are synonyms of *A. darwinii*. Hasle and Syvertsen (1997) also already raised possible conspecificity with *A. darwinii* and *A. rossii*. The 4 + 1 form with jagged separation lines described as *A. hyalinus* (Karsten 1905; present Fig. 56J) closely resembles *A. darwinii* (4 + 1 rays), except for variations in size of the central area (0.3–63 of diameter) and curvature of the ordinary rays. We here argue that this constitutes part of the variability of the 4 + 1 morphs (Figs. 11–13). Hasle and Syvertsen (1997) already raised the problem that *A. parvulus* (5 + 1 and 6 + 1 forms; present Fig. 56H) and larger specimens of *A. hyalinus* (4 + 1) could not be readily distinguished. We support this view, also reiterated by Ferrario et al. (2021). The smallest 3 + 1 form with jagged separation lines which was first illustrated by Hustedt (1958, Plate 8, Fig. 85; as *A. hyalinus*), but described as a new species *A. leboimei* by Manguin (1960; present Fig. 56K), here also is proposed as a synonym of *A. darwinii.*

With regard to the Antarctic *Asteromphalus* with smooth separation lines, the chronologically first described species was *A. hookeri* Ehrenberg 1844 (type illustration reproduced in Fig. 56D) should have priority. We argue that this 5 + 1 ray morph is

synonymous with the 6 + 1 ray morph described as *A. buchii* Ehrenberg 1844 (Fig. 56C). *Astereomphalus antarcticus* Castracane was described from a sample with numerous *A. darwinii*, and differentiated as having 5 + 1 rays but straight umbilical lines (Castracane 1886, Plate 16, Fig. 11; present Fig .56 E). We believe this species is a synonym of *A. hookeri.* Hernández-Becerril (1995) presented arguments that *A. humboldtii* (more robust and larger, commonly more than 7 + 1 up to 10 + 1 rays), always with smooth separation lines, should not be a synonym of *A. hookeri.* We note that the *A. hookeri* cells illustrated by Hernández-Becerril (1991; Plate 24) include 5 + 1 and 6 + 1 ray morphs, but also forms with both jagged and smooth separation lines, and a quincunx cribrum fine structure. This suggests the inclusion of two different taxa. These two taxa, *A. darwinii* (synonyms *A. beaumontii, A. hyalinus, A. leboimei*, *A. parvulus, A. rossii*) and *A. hookeri* (synonyms *A. antarcticus, A.buchii, ?cuvierii, ?humboldtii*) were also confused in our earlier account on the Willem Barendsz material (Van der Spoel et al. 1973)

We hope that the present observations to unravel two highly variable and often confused Antarctic diatom species, *A.darwiniii* (jagged separation lines) and *A. hookeri* (smooth separation lines), encourage future *Asteromphalus* culture studies to elucidate the fascinating valve development processes how the hollow rays form and pursue molecular sequences of hand-picked cells to confirm the revisions suggested here. Correct taxonomy of Antarctic diatoms is critical to document any future impacts from climate change. The validity of putative species distribution records of *A. hookeri, A. parvulus* and *A. hyalinus* in cold-water Northern Hemisphere habitats similarly calls for scrutiny (Hendey 1964, Hasle and Syvertsen 1997).

Declarations

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The authors have no competing interests to declare that are relevant to the content of this article.

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Figures

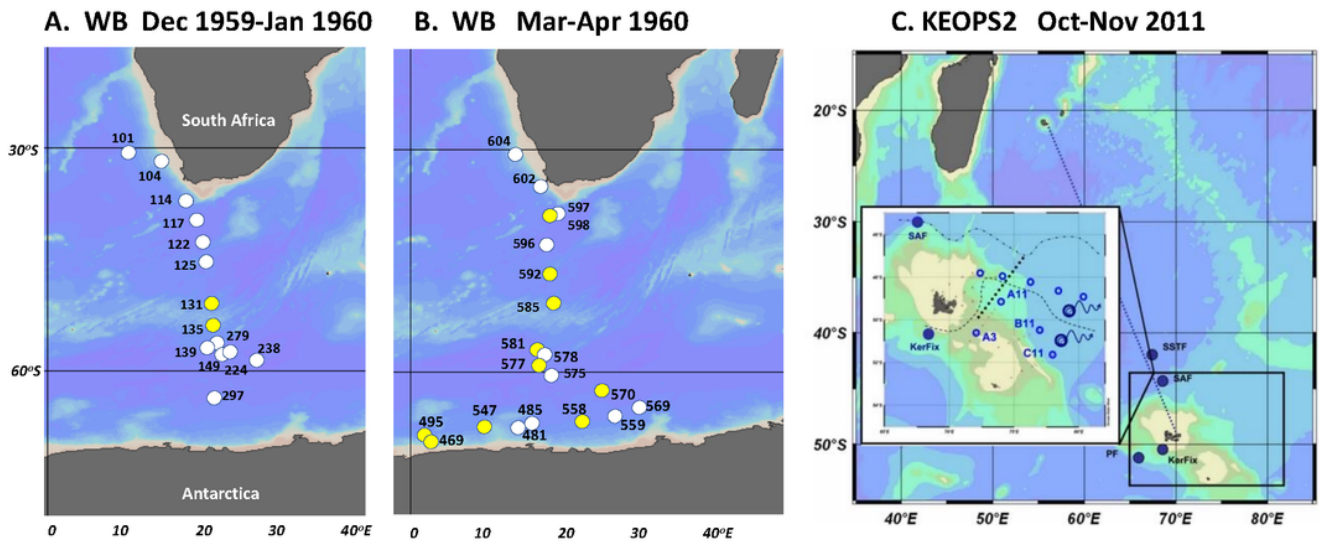

Figure 1

Cruise tracks by MS *Willem Barendsz* entering the Atlantic sector of the Southern Ocean in (A) December 1959-January 1960, conducting its whaling operations throughout January and March 1960, and (B) exiting March-April 1960; C. KEOPS2 cruise track by RV *Marion Dufresne* from 8 October to 22 November 2011 around the Kerguelen Island plateau.

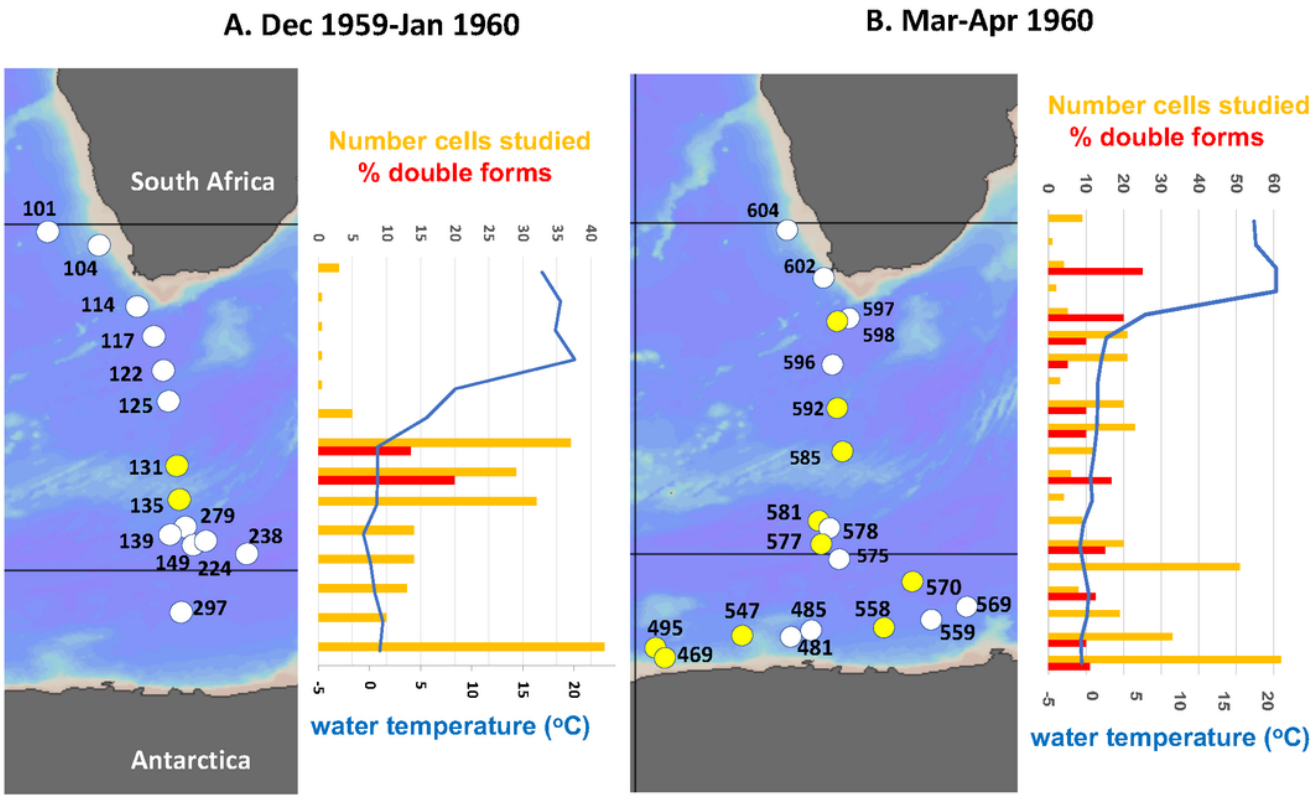

Figure 2

Cruise track of MS *Willem Barendsz* entering the Atlantic sector of the Southern Ocean in December 1959 (A, B) and exiting March-April 1960 (C, D). Station numbers where *Asteromphalus* "double forms" were observed are indicated in yellow. Surface seawater temperatures (blue line), the total number of *Asteromphalus* cells studied (orange bars) and % "double forms" (red bars) are indicated (B, D).

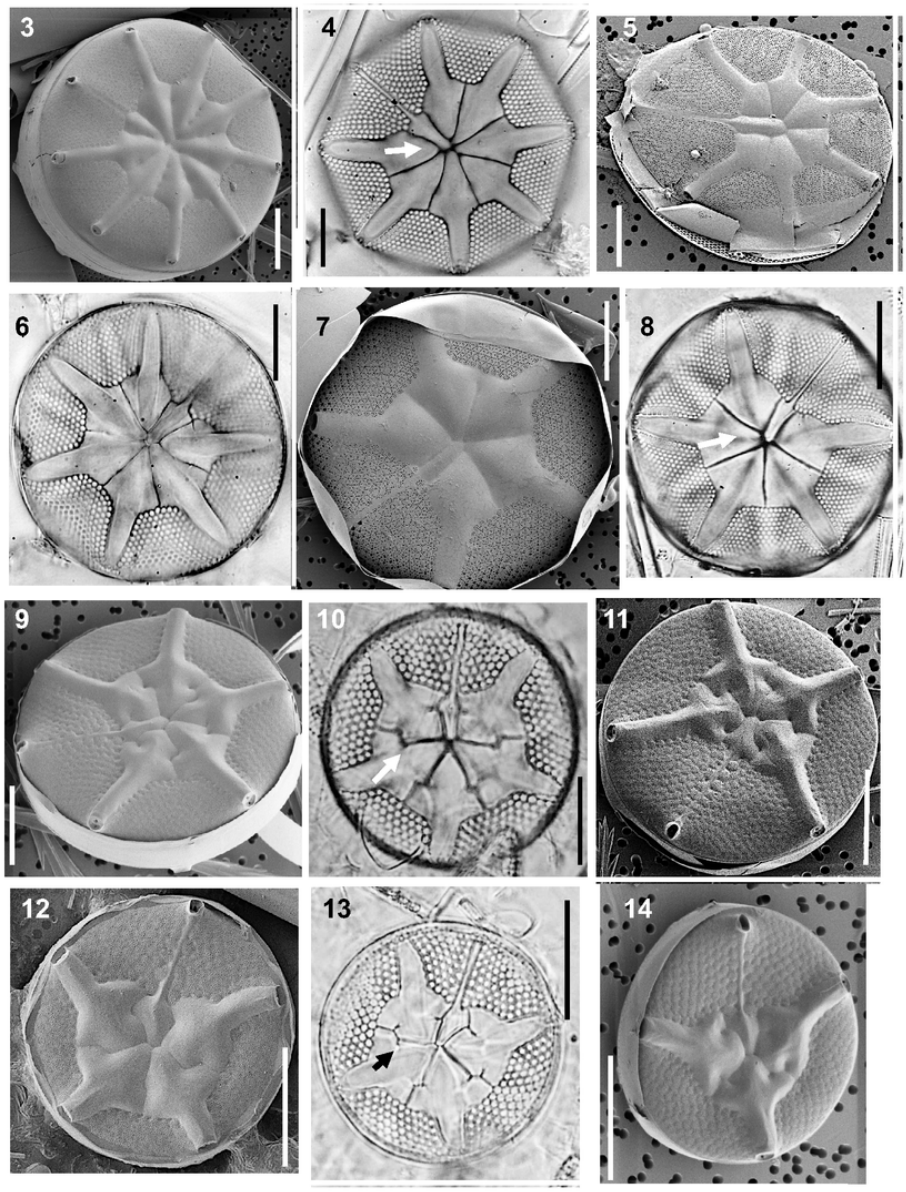

Figure 3

3-14. Light and scanning electron micrographs of 8+1 (Fig. 3), 7+1 (Fig. 4), 6+1 (Figs 5, 6), 5+1 (Figs 7-10), 4+1 (Figs. 11-13), and 3+1 (Fig. 14) ray morphs of *A. hookeri/ darwinii*, arranged from large to small forms. The highly variable 5+1 forms include cells with smooth (Figs 7,8) and with jagged separation lines (arrows; Figs. 9,10), best visible by LM but appearing as harmonica-like folds of the central area by SEM. Both 5+1 and 4+1 forms exhibit considerable variation in the size of the central area relative to diameter (compare Figs 7 and 9; 11 and 12). We identify the 8+1 ray morph in Fig. 3 as *A. humboldtii*, the 7+1, 6+1 and 5+1 ray morphs with smooth separation lines in Figs 4-8 as *A. hookeri,* and the 5+1, 4+1 and 3+1 ray morphs with jagged separation lines in Figs 9-14 as *A. darwinii*. All scale bars 10 µm.

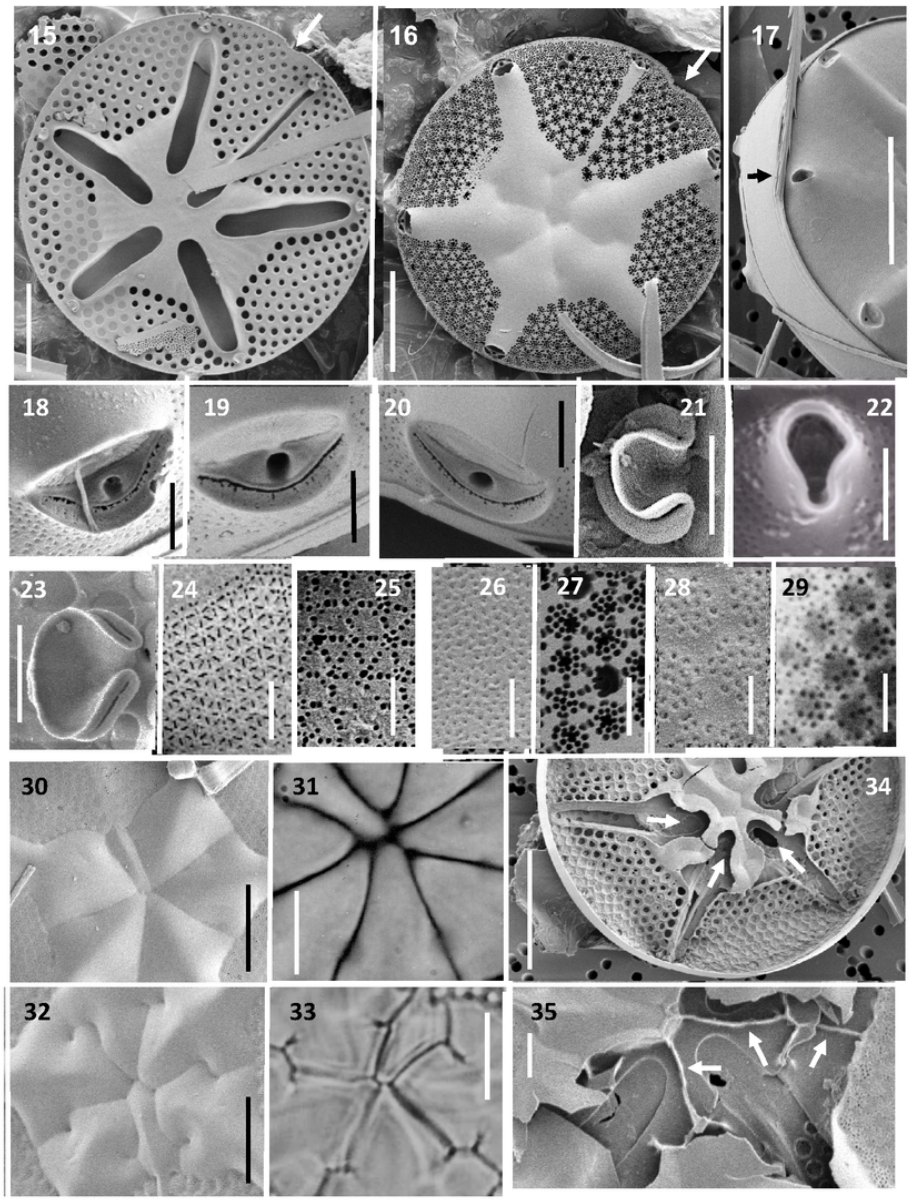

Figure 4

15-33. Fine structure of *A. hookeri/ darwinii* valves. Fig 15. Internal view of 5+1 ray morph showing the rounded ends of the broad rays (hollow tubes) and the linear singular ray. The marginal indentation (arrow) is located to the left of the singular ray. Tangential lines of areolae with internal foramina are shown, with slightly larger areolae surrounding the central areal; Fig. 16. External view of an immature 5+1 sibling valve with quincunx external cribrum in various stages of development. The marginal indentation (arrow) is located to the right of the singular ray; Fig. 17. Detail of valve margin of a 4+1 ray morph showing the keyhole shaped external opening of the rimoportulae of the singular ray (arrow) flanked by two external openings of the rimoportulae of the broad rays; Figs 18-20. Details of the external opening (ray hole) of rimoportulae of the broad rays of the 6+1, 5+1 and 4+1 ray morphs, respectively; Fig. 21. Internal lip-shaped rimoportula (labiate process) of a broad ray; Fig. 22. Key-hole shaped external ray hole of singular ray; Fig. 23. Internal lip-shaped rimoportula (labiate process) of a singular ray; Figs 24-29. Detail of areolation. Fig.24. Quincux external cribrum of mature valve (6+1 ray form in Fig.5, smooth separation lines); Fig. 25. External cribrum of immature sibling cell 5+1 ray form in Fig.7, smooth

separation lines.; Fig.26. Cribrum of 6+1 ray form with jagged separation lines; Fig. 27. External cribrum of immature sibling cell 5+1 ray form in Fig.16 (jagged separation lines); Fig. 28 Cribrum of 4+1 ray form; Fig.29. Cribrum of 3+1 ray form; Figs. 30-33. Details of separation lines; Figs 30-31. Smooth lines of 5+1 ray morph by SEM (Fig.30) and LM (Fig.31), respectively; Figs 32-33. Jagged lines of 4+1 ray morph by SEM (Fig.32) and LM (Fig.33), respectively. Fig. 34. Sibling cell with incompletely formed roof (arrows) of the central area of 5+1 morph; Fig. 35. Broken roof of central area of 4+1 morph showing string-like separation lines (arrows), and ordinary ray pattern on bottom of the chamber. All scale bars 10µm, except Figs 18-29 (1 µm).

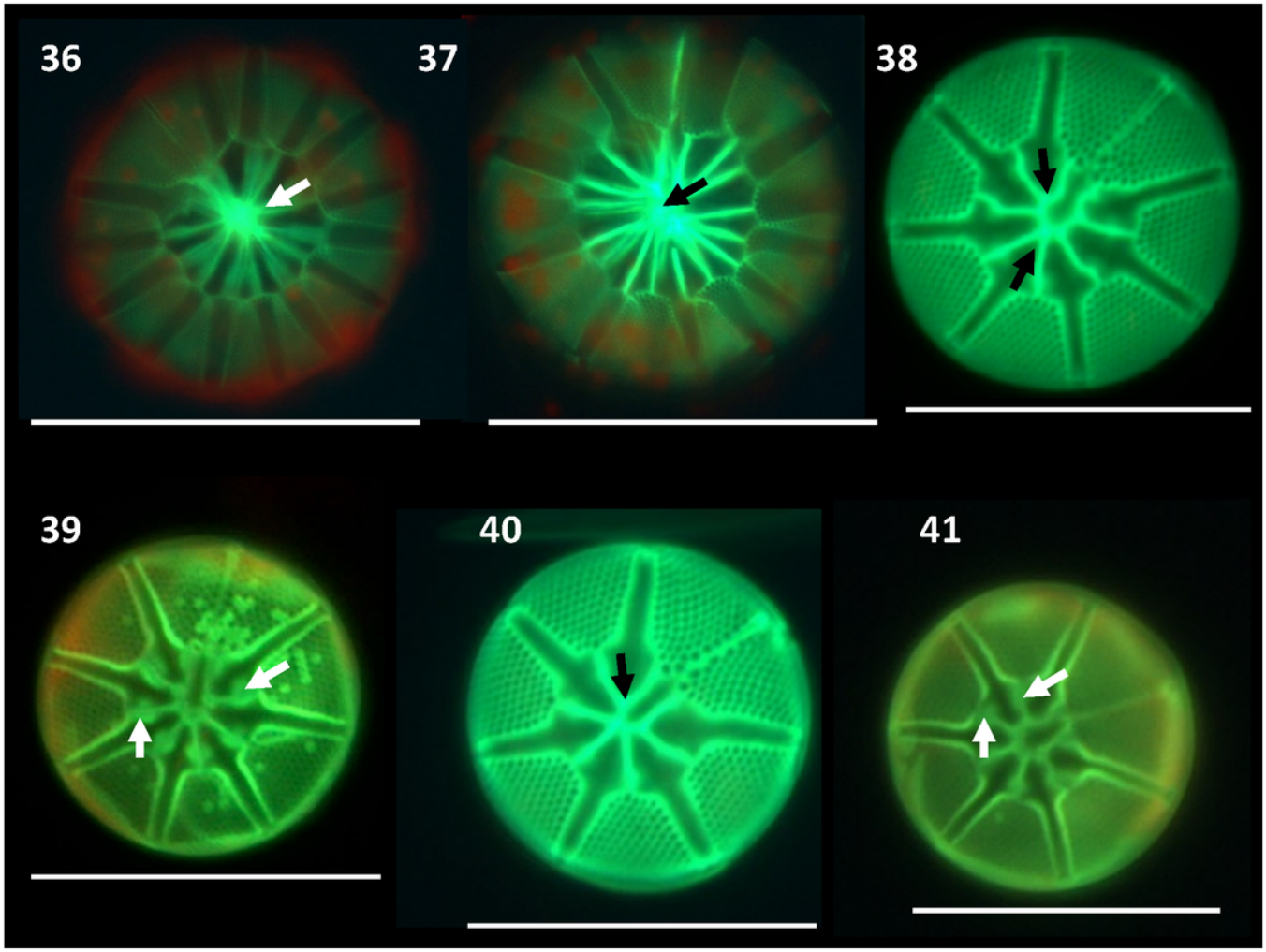

Figure 5

36-41. PDMPO fluorochrome stained *Asteromphalus* cells from the Kerguelen Plateau in October -November 2011 represented by 8+1, 7+1 and 6+1 ray forms. Active Si deposition was associated with the central separation lines (Figs 36, 37, arrows), which were smooth in large 8+1 ray forms *A.humboldtii*, but in the case of jagged separation lines in 7+1 and 6+1 ray forms (*A. darwinii*) also associated with the bulbous bend in those lines (Figs 39, 41, arrows).

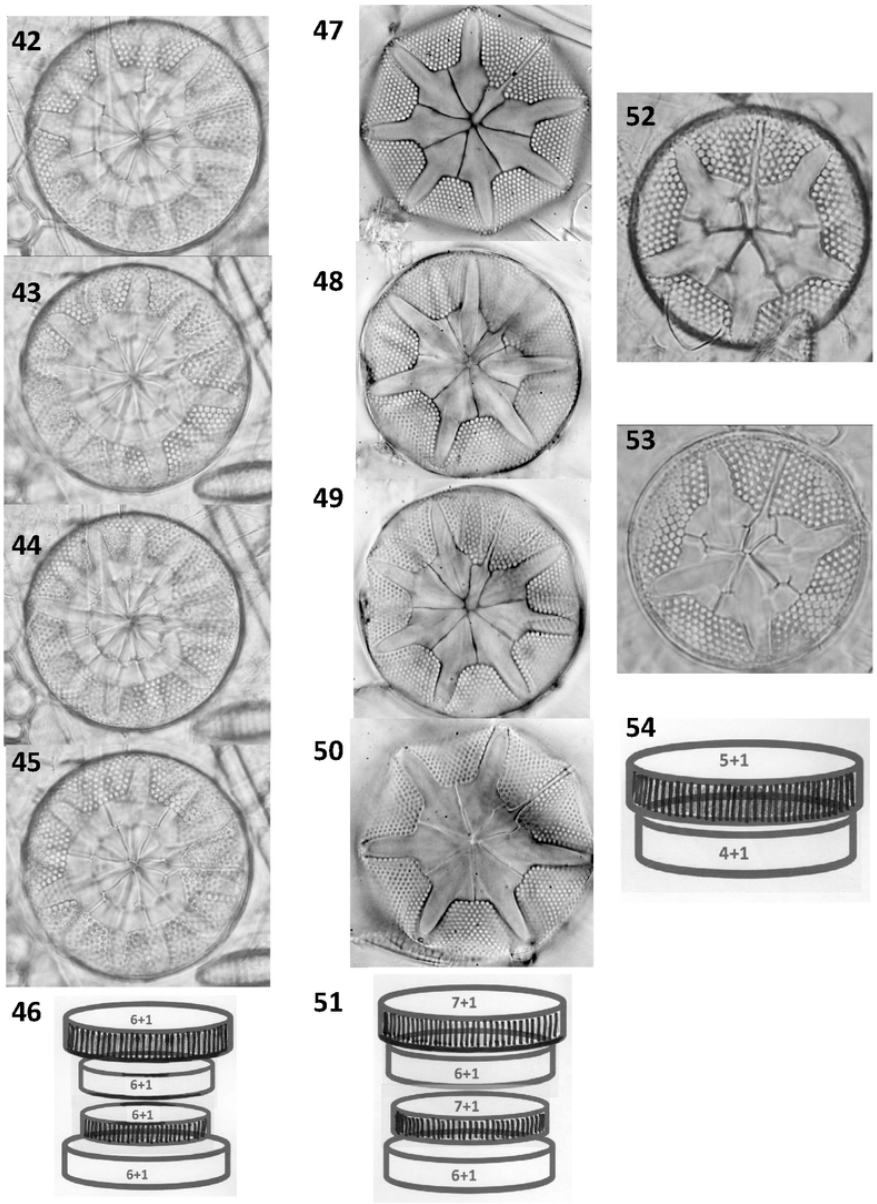

Figure 6

42-54. Various scenarios of *Asteromphalus* cell division stages. Figs 32-36. Four 6+1 ray morph valves in a parent epitheca/sibling hypotheca/sibling epitheca/parent hypotheca arrangement, diagrammatically summarised in Fig. 46; Figs 47-51. “Double form” arrangement of 7+1 parent epitheca/6+1 sibling hypotheca/7+1 sibling epitheca/6+1 parent hypotheca arrangement, diagrammatically summarised in Fig.51; Figs 52-54. “Double form of 5+1 epitheca/ 4+1 hypotheca, diagrammatically summarised in Fig. 54. Note that all ray morphs exhibit identical arrangements of jagged (Figs 42-45; 52-53; *A. darwinii*) or smooth separation lines (Figs 47-50; *A. hookeri*) between parent and sibling cells.

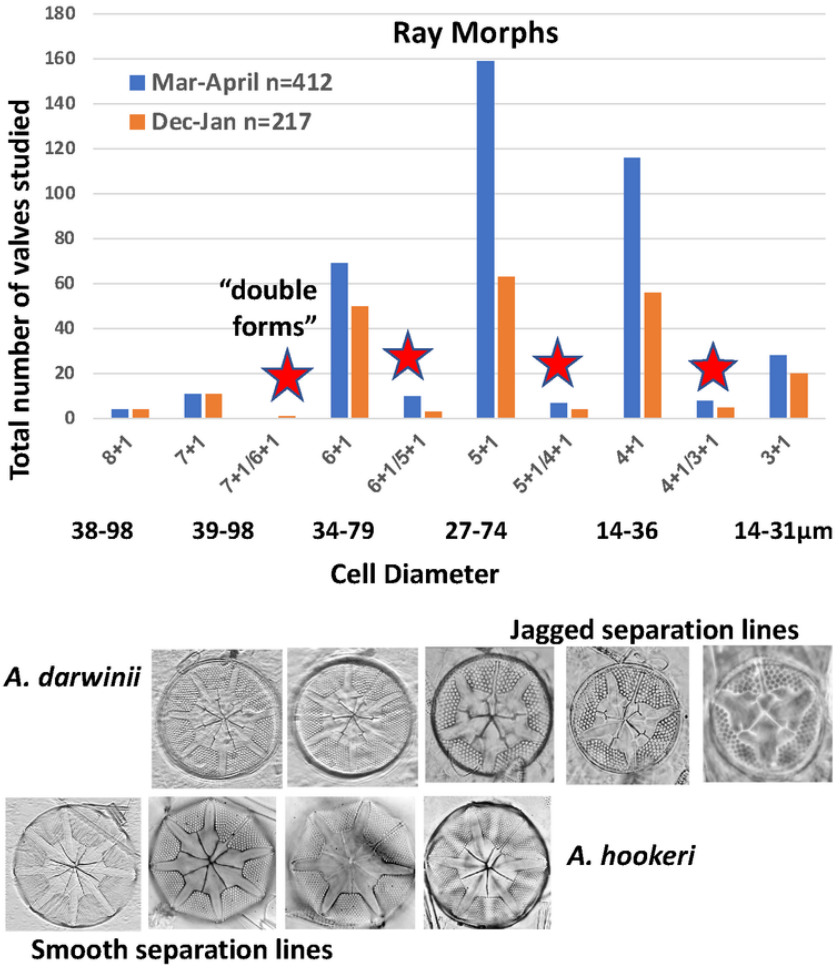

Figure 7

Partitioning of the number of *Asteromphalus* cells counted from summer and autumn samples into the 8+1, 7+1, 6+1, 5+1, 4+1, and 3+1 ray forms. The number of hyaline rays varied from 3 broad + 1 narrow rays (designated 3+1) in the smallest valves (14-31 µm diameter), with 4+1 (27%) and 5+1 rays (35%) the most common, and 6+1, 7+1, and rarely 8+1 rays present only in larger cells (39-97 µm diameter). Smaller cells (3+1; 4+1; 14-36 µm diameter) commonly contained jagged (zig-zag) separtation lines in the central area, identified as *A. darwinii*. Larger cells (6+1, 7+1, 8+1) most commonly had smooth separation lines, identified as *A.hookeri.* In the intermediate size 5+1 and 6+1 ray forms , both jagged and smooth spokes overlapped. Similar to the variable number of rays and size of the central area, the feature of curved (3+1, 4+1) or straight rays (5+1 to 8+1) was correlated with cell diameter.

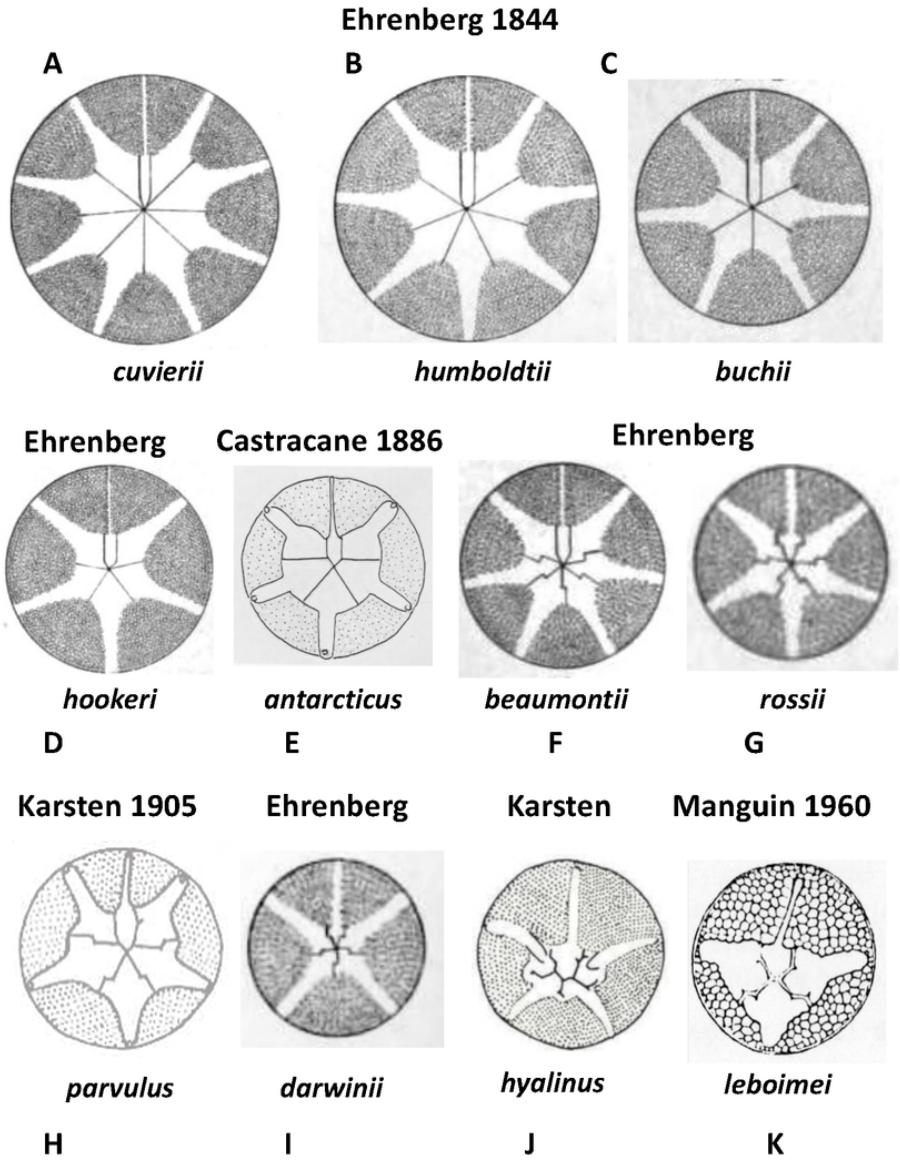

Figure 8

Type illustrations (line drawings) of Antarctic *Asteromphalus*species reproduced from Ehrenberg 1844 (*Ber. Bekanntm. Verh. Königl. Preuss. Akad. Wiss. Berlin*, Plate June, Figs 1-7); Castracane 1886 (*Rep. Sci. Res. Voyage H.M.S. Challenger 1873-1876*, Botany 2, Plate 16, Fig.11); Karsten 1905 (*Wiss. Ergebn. Deutschen Tiefsee-Expedition auf dem Dampfer 'Valdivia' 1898-1899*, Plate 8, Figs 14-15); and Manguin 1960 (*Ann. Sci. Nat. Bot.* série 12 , Plate 3, Fig. 44). Cells have been rearranged according to a sequence from 8+1 rays (*A. cuvierri*), 7+1 (*A. humboldtii*), 6+1 (*A. buchii),* 5+1 (*A. hookeri, A. antarcticus)* all with smooth separation lines; and 6+1 (*A. beaumontii*), 5+1 (*A. rossi, A. parvulus*), 4+1 (*A. darwinii, A. hyalinus*) to 3+1 rays (*A. leboimei*), all with jagged separation lines. This same sequence reflects a decrease of valve diameter from 97 down to 20 µm (compare Table 1).