Phycologia (2000) Volume 39 (6), 527–546 Published 5 March 2001

Spatial and temporal stability of demes in *Diploneis smithii/D. fusca* (Bacillariophyta) supports a narrow species concept

STEPHEN J.M. DROOP[1], DAVID G. MANN[1]* AND GIJSBERT M. LOKHORST[2]

[1]*Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, UK*
[2]*RijksHerbarium/Hortus Botanicus, P.O. Box 9514, 2300 RA Leiden, The Netherlands*

S.J.M. DROOP, D.G. MANN & G.M. LOKHORST. 2000. Spatial and temporal stability of demes in *Diploneis smithii/D. fusca* (Bacillariophyta) supports a narrow species concept. *Phycologia* 39: 527–546.

An earlier study demonstrated the presence of 11 morphologically distinct demes ('morphotypes') within the *Diploneis smithii/D. fusca* species complex in a single sample from Ganavan near Oban on Scotland's west coast. Two of these demes were subsequently shown to occur elsewhere around the British coastline and to keep their identity wherever they occurred together. However, the conclusion that the demes were sufficiently distinct and widespread for them to be described as separate species has been criticized on the grounds that no evidence exists that there are reproductive barriers between them which could provide independent evidence for their relationships. It has not been possible, thus far, to grow the species in culture (a prerequisite for controlled breeding experiments), and so independent evidence has been sought elsewhere. It is shown here that nine of the 11 demes from Ganavan also occur in Cumbrae (in the Firth of Clyde, some 80 km SSE of Oban), not only in recent samples but also in a sample collected in 1858. A detailed morphometric and ultrastructural analysis shows that the demes are stable over 80 km and 140 years. This is discussed in the context of the length of diatom life cycles, and it is concluded that at least several generations of size reduction and restitution must have occurred since 1858. In most diatoms, size reduction and restitution accompany allogamous sexual reproduction, so it is inferred that the demes within the *D. smithii/D. fusca* complex are probably true sexual species.

INTRODUCTION

It has been shown elsewhere (Droop 1994, 1995) that *Diploneis smithii* (Brébisson) Cleve and *D. fusca* (Gregory) Cleve comprise several different morphotypes. At one locality, a sandy beach at Ganavan near Oban on the west coast of Scotland, the complex exists as 11 distinct entities (Droop 1994), each of which shows a wide size range like that present in most species of diatoms. In diatoms, a wide range of size is usually a corollary of a sexual life cycle, involving slow size reduction during the vegetative phase and more rapid size restitution via a sexually produced auxospore (Drebes 1977; Mann 1988; Round *et al.* 1990). Each morphotype also exists at other localities around the coast of Britain (Droop 1994); two have been studied in detail and shown to remain distinct from each other wherever they occur together (Droop 1995).

While morphological distinctness and occurrence over a wide geographical range would normally be enough evidence to justify description of a separate species, more proof is required by some diatomists, perhaps because of the subtlety and complexity of the variation in the *D. smithii/fusca* aggregate, which carries with it the implication of huge numbers of undescribed diatom species (Mann & Droop 1996). For example, Cox (1997) has pointed out that the patterns of variation in morphotypes of *D. smithii/D. fusca* observed within a single sample could be 'a phenotypic effect of ecological differentiation', i.e. that the population of each morphotype (exhibiting size reduction) could be nothing more than a clone derived mitotically from a single individual. Each population would thus be equivalent to one individual of a sexually reproducing multicellular species and would be expected to differ from other 'individuals' (populations) if the phenotype was in any way plastic in response to differences in the environment between the populations. Such a model would predict that the morphotypes would be sexually compatible with each other, and it is the lack of evidence refuting this that Cox bemoans.

Despite numerous efforts to bring morphotypes of *D. smithii/fusca* into cultivation, it has not been possible to produce clonal cultures; isolated cells fail to divide yet can survive for months. An alternative approach is needed, therefore, if it is to be shown that the Ganavan morphotypes (Droop 1994, 1995) are not mere ecological variants perpetuated clonally but are instead (by most criteria) true species. The project reported here grew out of a chance observation that what appeared to be the Ganavan morphotypes of *D. smithii/fusca* were also present in material collected in the 1850s by Dr Roger Hennedy from the Island of Cumbrae, some 80 km south of Ganavan. Subsequent detailed morphometric and ultrastructural analysis has shown that most of the Ganavan morphotypes are indeed present, not only in Hennedy's old sample from the Island of Cumbrae, but also in material collected recently from Cumbrae. The morphotypes have remained stable with respect to distance (*c.* 80 km) and time (*c.* 140 years) and exhibit size reduction in each sample. It is argued, therefore, that the diatoms of a particular morphotype from each of the different samples cannot be members of a single clone; size-reducing clones do not last that long.

We consider that the evidence presented in this paper is strong enough to justify recognizing the morphotypes as true species. However, since it is based on pattern rather than process, we prefer to adopt a cautious approach. Eventually, we

* Corresponding author (d.mann@rbge.org.uk).

Table 1. Material used in the analyses.

Provenance	Sample	Collection date	Locality	LM slides prepared from sample	SEM stubs prepared from sample
Old Cumbrae	Walker Arnott 583/ Van Heurck Type no. 104	2 Aug. 1858	Isle of Cumbrae; 'washings of sand'	BM 11836; Collection J. Deby; A. Walker Arnott no. 583; Cumbrae; Hennedy	
				BM s/n; Dr Henri Van Heurck; *Types du Synopsis des Diatomées de Belgique* no. 104; Angleterre	VH104/1–3
Recent Cumbrae	E 2673.2	3 May 1997	Bell Bay, Isle of Cumbrae	E 2673.2/1	E 2673.2/1–3
	E 2679.2	3 May 1997	Fintry Bay, Isle of Cumbrae	E 2679.2/1	E 2679.2/1–3
	E 2682.2	3 May 1997	Kames Bay, Isle of Cumbrae	E 2682.2/1	E 2682.2/1–3
Ganavan	E 1014	1 Mar. 1991	Ganavan Bay, nr Oban	E 1014/1	E 1014/1–4
	E 1016	1 Mar. 1991	Ganavan Bay, nr Oban	E 1016/1	E 1016/1–3

would like to be able to conduct breeding experiments that would show whether or not there were barriers to gene flow between the morphotypes. Until we find such evidence, we use the neutral term 'deme' (Gilmour & Heslop-Harrison 1954) to indicate informal entities, used without any prefix to avoid prejudging the cause of discontinuities between them (see Mann 1984, 1989, 1999).

MATERIAL AND METHODS

Table 1 summarizes the material examined for this paper. Although several samples are involved, they fall into three groups (provenances): Hennedy's sample from Cumbrae in 1858 and the slides and scanning electron microscopy (SEM) stubs prepared from it are referred to collectively as 'old Cumbrae', the three samples collected from different localities in Cumbrae in 1997 (and material prepared from them) are referred to collectively as 'recent Cumbrae', and the two samples collected from different parts of Ganavan Bay in 1991 (and material prepared from them) are referred to collectively simply as 'Ganavan'.

Hennedy's sample from Cumbrae was collected as 'washings from sand'. It was sent to Professor George Walker Arnott in Glasgow, who incorporated it into his collection as nos 583, 622 and 623 (according to Walker Arnott's notebook kept at the Natural History Museum in London, no. 583 is a coarse fraction, while no. 623 is a lighter fraction). Four slides prepared by Walker Arnott from two of these subsamples now reside in the herbarium at the Royal Botanic Garden Edinburgh (E); unmounted material also found its way to other diatomists, and at least four slides made from Walker Arnott 583, 622 and 623 are to be found in the collections of Deby and Greville at the Natural History Museum in London (BM). It was decided to base the study on only one of these subsamples (no. 583) for two reasons. Firstly, the best slide for morphometric analysis prepared from Hennedy's material is BM 11836, prepared from subsample 583. This slide was prepared as a Canada Balsam mount (many of the others were air mounts), and the density and cleanness of the diatoms are such that a relatively high proportion are measurable (see below). The second reason is that unmounted material of it was available in the collection of Henri Van Heurck in Antwerp. This material had been used to prepare no. 104 in the exsiccata set '*Types du Synopsis des Diatomées de Belgique*' (Van Heurck 1880–1885). The bottle from which type no. 104 was prepared has Van Heurck's label pasted on top of a blue label with the inscription 'Walker Arnott 583'. Some of this material was recleaned in 70% nitric acid for our analysis, and stubs were prepared from it for study with SEM. Slides were also prepared from it for light microscopy (LM), but these were not of a high enough quality to use for measurements and were used only to confirm the identity of the sample.

The other samples examined were collected by an adaptation of the Eaton & Moss (1966) lens tissue method, cleaned by boiling in a mixture of concentrated nitric acid and sulphuric acid and mounted in Naphrax for LM, or dried onto coverslips for SEM. A Reichert Polyvar 2 photomicroscope was used for LM, and digital images were taken using a Kodak MegaPlus 1.4 camera linked to a computer. The morphometric data were collected using the image analysis software Optimas v5.2 (Media Cybernetics, L.P., 8484 Georgia Avenue, Silver Spring, MD 20910, USA). A Zeiss DSM 962 was used for SEM, operated at 15 kV; the stage was tilted to 40°. Photographs were taken on Kodak Technical Pan film. Some of the SEM pictures are printed in reverse to facilitate comparison.

One problem usually encountered in detailed morphological work of this sort involving both LM and SEM is matching diatoms between the two instruments, since LM and SEM reveal different suites of features. However in this species complex, there is enough variation in the characters visible with both instruments for matching to be relatively straightforward. Where a match cannot be made on the basis of qualitative (presence–absence) characters, measurements of quantitative characters suffice.

Two sets of data (see the following two paragraphs) were analysed with principal components analysis (PCA) using Minitab (version 7.0), and graphs were prepared using SigmaPlot, both as detailed in Droop (1994). The stereo pairs ('frog-spawn plots') were also created using SigmaPlot, with a rotation of 6° between individual plots of a pair. It is best to view the plots through a stereoscopic viewer. If this is not possible, the graphs may be viewed by diverging the eyes, so that the left hand graph of each pair is viewed with the left eye, and the right hand graph with the right eye. If the graphs

are superimposed by squinting cross-eyed at them, then the perspective (and the front–back depth of points and clusters) will be reversed, relative to the description in the text.

ANALYSIS 1: The main morphometric analysis was carried out on 500 randomly selected valves, 100 from each of five slides: E 1014/1 from 'Ganavan', E 2673.2/1 and E 2679.2/1 from 'recent Cumbrae', and BM s/n and BM 11836 from 'old Cumbrae' (Table 1). Valves had to satisfy the following criteria for inclusion: each must (1) belong to the *smithii/fusca* complex; (2) lie flat, with the external surface uppermost; and (3) have a clearly identifiable unbroken outline, free from excessive overlying debris (i.e. have a perfect outline after only minimal editing of the digital image). Slides were scanned systematically, and the first 100 valves (meeting the above criteria) to cross the field of view were included in the study. The macro facility of Optimas was programmed to measure length, width, rectangularity and stria density and to prompt the user to score the shape of the central raphe endings and the structure of the striae (all as detailed in Droop 1994, 1995). The only change from the protocol of Droop (1994) was that the measurement of the shape of the central raphe endings was made more precise: instead of just three character states for each individual raphe ending (0, 1 or 2, allowing five states when the two raphe endings are averaged for a valve), five were recognized for each raphe ending (0, 0.5, 1, 1.5, 2, allowing nine when averaged for a valve). Although all the valves in the species complex on slide E 1014/1 had already been measured by Droop (1994), except 'morphotype 7' which was randomly sampled, these measurements were not used for this study (1) to reduce the possibility of between-provenance differences arising from subtle changes in operator practice over the intervening five years and (2) because the raphe ending character had been redefined. Instead, the slide was treated just like the others and new measurements were made of 100 randomly selected valves.

ANALYSIS 2: Because of the random choice of valves in Analysis 1, the proportion of demes measured on each slide reflected the uneven frequency of each in the sample, so some demes were only poorly represented. To combat this and to provide a large enough sample of rare demes, a complete search was made of the five slides, together with E 2682.2/1 ('recent Cumbrae') and E 1016/1 ('Ganavan'). Demes #5, #6, #8, #9, #11 and #13 were poorly represented in Analysis 1 (fewer than an arbitrary 20 valves each), and all measurable valves of these demes were found on each slide and included in Analysis 2. In addition, 14 individual valves were found that did not match any of the demes seen in Analysis 1, nor any in the analysis made by Droop (1994). These were provisionally grouped into demes #12, #15, #16, #17, #18 and #19. In total, 218 valves were used in Analysis 2, including 34 from Analysis 1.

RESULTS

Morphometric analysis 1

The results of the PCA of the first set of data (500 valves measured randomly, 100 from each of five slides) are shown in Fig. 1; it shows the first three dimensionless principal components (pc) as a series of stereo pairs. The top pair shows the cube (comprising the axes of pc1–3) more or less in the plane of pc2 and pc3; the middle and bottom pairs show the cube rotated around pc2 by 15° and 57°, respectively, relative to the top pair. The Eigenanalysis of the correlation matrix (Table 2) shows that the first three principal components account for 90% of the total variation in the analysis. Length and width are the main components of pc1, so that large valves appear in the foreground of the top pair of plots (Fig. 1) and smaller valves nearer the back. Rectangularity and the shape of the central raphe endings account for most of pc2, so that valves with high rectangularity and bilaterally symmetrical central raphe endings tend to appear towards the left of the top pair of plots. The structure and density of the striae account for most of pc3: valves with low stria density tend to appear towards the top of the top pair of plots, while those with a '*fusca*-type' structure tend to appear towards the bottom.

The morphological variation in these 500 randomly selected valves from three provenances is discontinuous, irrespective of any empirical judgements as to their identity. At least six discrete clusters can be easily detected in the top pair of plots in Fig. 1, resolving into at least seven as the 3-D viewpoint is rotated (middle and bottom pairs). Altogether, 10 clusters can be seen after persistent viewing of each pair of plots in turn.

Most of the clusters in Fig. 1 correspond to the demes from Ganavan illustrated and described by Droop (1994). Figure 2 is a plot of pc2 vs pc3 that shows the same data as Fig. 1, but with the individual points differentiated to show their relationship to the Ganavan demes identified by Droop (1994). The numbering used is the same as in Droop (1994, figs 2–30, 33–38), with the addition of #13 and #14 for two demes that occur on Cumbrae but not at Ganavan. Many of the overlaps between clusters in Fig. 2 can be resolved when individual points or clusters are viewed in three dimensions (Fig. 1: the top pair of plots shows the same data in the same orientation as Fig. 2). For example, the individual of deme #5 lying within the cluster of deme #7 in Fig. 2 can be seen in Fig. 1 (top and middle pairs) to be lying some distance in front of the cluster of deme #7. Similarly, the individual of deme #2 lying next to deme #13 in Fig. 2 is in quite a different plane in Fig. 1. Demes #4 and #14, which overlap in Fig. 2, are clearly shown to be separate when the 3-D viewpoint is rotated, as in the bottom pair of plots in Fig. 1. All 13 demes are morphologically distinct, in that envelopes drawn around the points of each do not overlap with any other in pc1–3 space (only pc2 vs pc3 is illustrated in Fig. 2).

Nine of the 11 demes from Ganavan (Droop 1994, figs 2–30, 33–38) also occur in 'old Cumbrae' and 'recent Cumbrae': these are demes #1–#4, #6–#8, #10 and #11. Although deme #10 occurs in 'recent Cumbrae', it does not occur in the two slides from that provenance used for Analysis 1. Deme #5 occurs in 'Ganavan' and 'old Cumbrae', but not in 'recent Cumbrae'. Deme #9 from Ganavan was not found in any other sample in this analysis, although it has been found in samples from the Outer Hebrides (north-west Scotland) and near St Andrews (east Scotland) (our unpublished observations). Demes #13 and #14 are both new to this analysis, being absent from Ganavan. Both occur in only one provenance: deme #13 in 'old Cumbrae', deme #14 in 'recent Cumbrae'. (See Analysis 2 below for deme #12.)

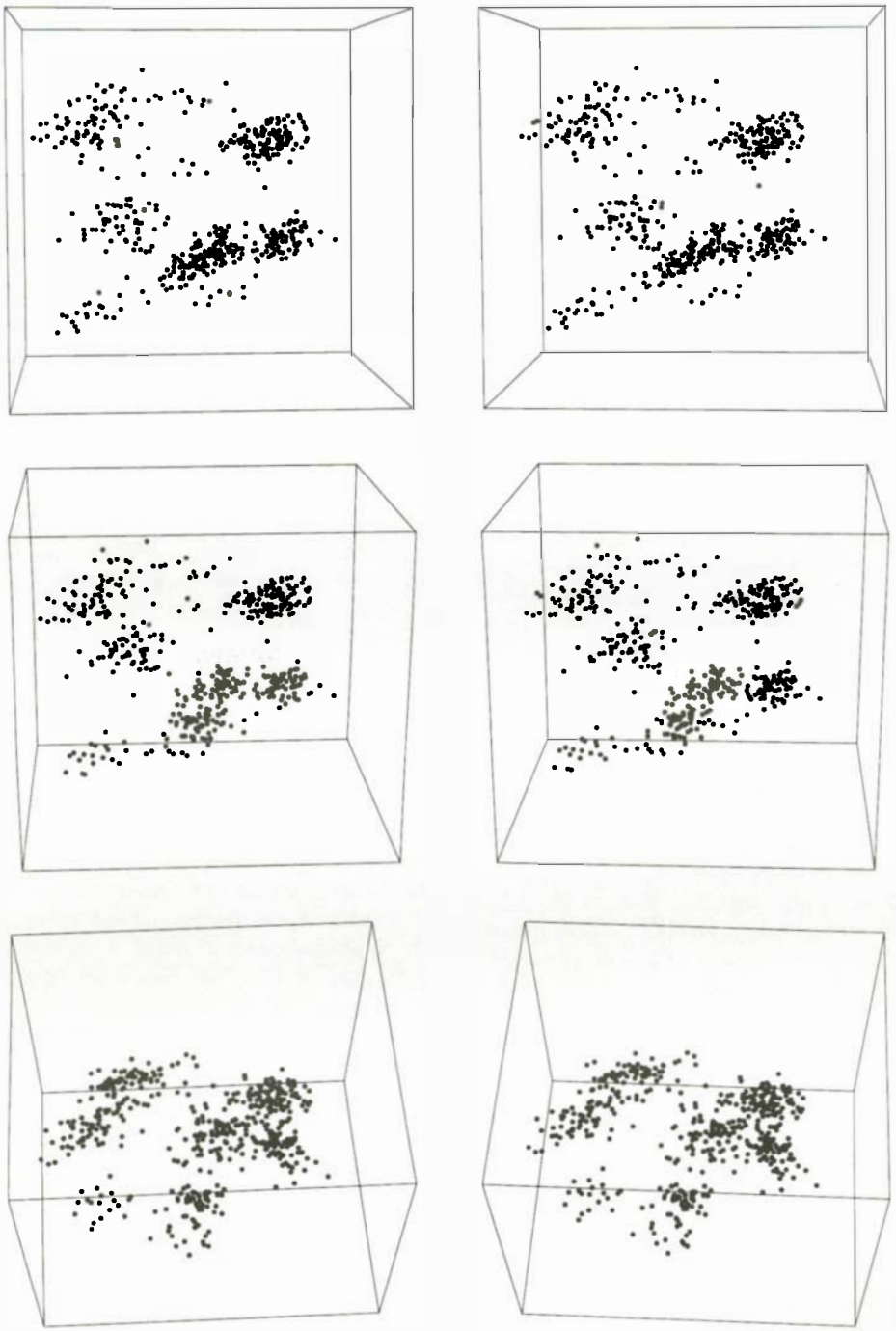


Fig. 1. Analysis 1: principal components analysis of 500 valves of *Diploneis smithiilfusca* from three provenances: 'Ganavan' (collected 1991), 'recent Cumbrae' (collected 1997), 'old Cumbrae' (collected 1858). Stereo pairs of pc1–3; the top pair is in the plane of pc2 (*x*-axis) and pc3 (*y*-axis), with pc1 more or less perpendicular to the paper; the middle and bottom pairs are rotated around pc2 relative to the top pair. See Material and Methods for viewing instructions.

In Fig. 3, envelopes have been added to indicate the provenance of each point within each of the 10 demes that occur in more than one provenance. Individual points are not shown, except in the five cases where the envelopes contain only one point. The PCA has placed members of the same deme near to each other, irrespective of their provenance. In the common demes (#1–#4, #7), in particular, the overlap of envelopes is very close: half or more of the smallest envelope in each deme overlaps with both of the others. In the less well-represented demes, however, the overlap is less close, and in demes #5, #8 and #11 there is no overlap at all between the sets of individuals of different provenances. These demes are represented better in Analysis 2 (in which there is shown to be overlap between the envelopes for individuals of different provenances for demes #8 and #11).

The data for four of the characters included in the PCA are

Table 2. Analysis 1: Eigenvalues of the correlation matrix.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Eigenvalue	2.8343	1.4753	1.0944	0.3557	0.1966	0.0437
Proportion	0.472	0.246	0.182	0.059	0.033	0.007
Cumulative	0.472	0.718	0.901	0.960	0.993	1.000
Variable						
Length	0.558	0.091	0.044	0.478	−0.146	−0.655
Width	0.537	0.290	−0.071	0.152	−0.362	0.685
Stria density	−0.397	−0.069	−0.646	0.222	−0.602	−0.095
Rectangularity	0.163	−0.712	−0.269	0.430	0.382	0.251
Shape of central raphe endings	−0.364	0.556	−0.017	0.616	0.404	0.125
Stria structure	0.289	0.296	−0.709	−0.366	0.421	−0.122

summarized as box plots in Figs 4–7 (combined with the data relating to the rare demes from Analysis 2). Table 3 gives the total number of valves measured of each morphotype from each provenance (Analyses 1 and 2 combined). Within most demes, there is a very close match for each character between the data from different provenances, whereas the differences between demes are often considerable. In Fig. 4, for example, the stria densities of deme #1 (all three provenances) are very tightly clustered around about 6.4 striae in 10 μm, whereas those of deme #2 are slightly less tightly clustered around 7 striae in 10 μm. Such small but consistent differences in stria density illustrate the need for greater precision in measurement than is often undertaken (Droop 1993). The difference between demes #1 and #2 is even more striking in rectangularity (Fig. 5), where there is only a slight overlap between the demes but considerable overlap between provenances within a deme. The most obvious difference between demes #1 and #2 is in the shape of their central raphe endings (Fig. 6); those of deme #1 are bilaterally symmetrical (scored as 0 in Fig. 6), whereas those of deme #2 are hooked to one side (scored as 2 in Fig. 6 – see also Figs 33–35). The difference in stria structure between demes #1 and #2 is also quite well marked (Fig. 7), especially because between-provenance variability is so low. On the other hand, the between-provenance overlap is less distinct in some of the less well represented demes discussed in Analysis 2, below.

Each of the 12 demes represented by more than one valve in Analysis 1 (Fig. 2) shows variation in length and width that is consistent with a normal life cycle involving size reduction and size restitution. For example, Fig. 8 is a plot of length vs width from the Analysis 1 data for three of the demes (#3, #4 and #7), showing a greater proportional decrease in length than in width, as in other pennate diatoms (Round *et al.* 1990). All three clusters are well defined, even though they include valves from all three provenances. There is a tendency for there to be a curvilinear relationship between width and length, width reducing relatively more slowly in the earlier parts of the size reduction sequence (when the cells are larger).

LIGHT MICROSCOPY: The demes represented in Analysis 1 are illustrated in Figs 9–29. Individuals of a deme appear to be

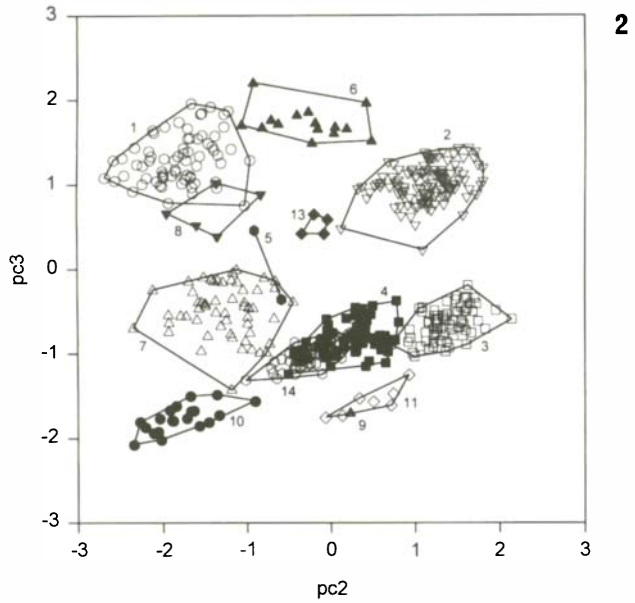


Fig. 2. Analysis 1: pc2 vs pc3, showing the identity of each valve represented. The numbers are the same as those used in Droop (1994); demes #13 and #14 are new to this study.

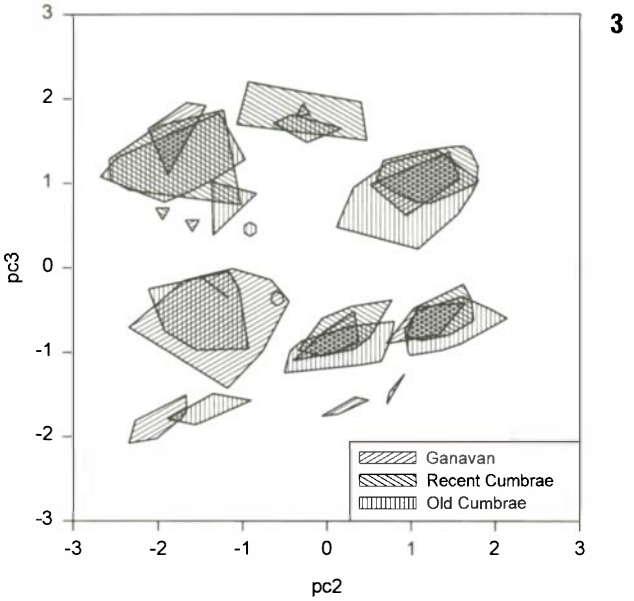
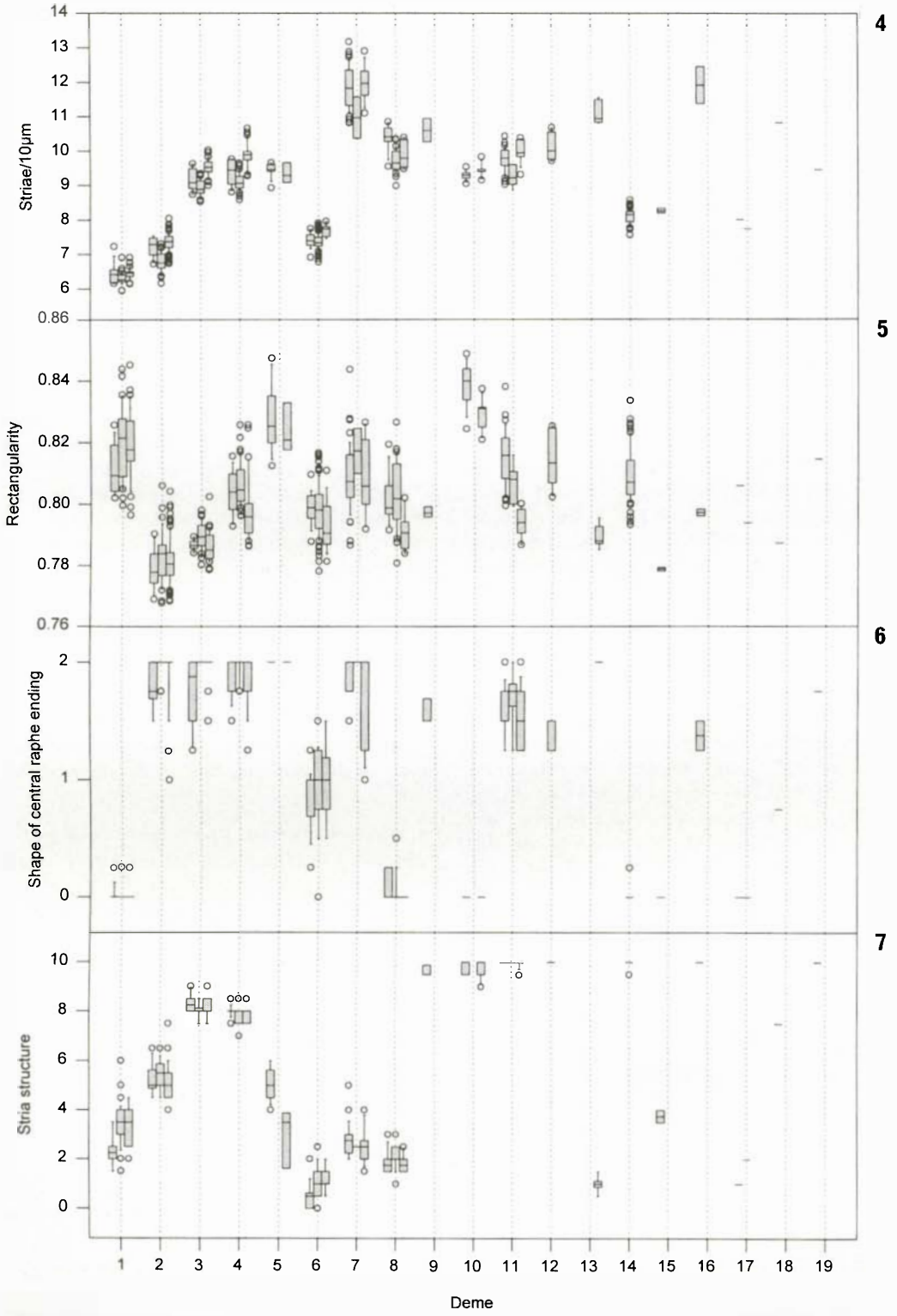


Fig. 3. Analysis 1: pc2 vs pc3, as in Fig. 2, but showing only those demes that occur in more than one provenance. Envelopes surround the points of each deme from each provenance (but symbols are used where only one valve of a deme is included from any provenance).



Figs 4–7. Box plots of the data (except length and width) used in Analyses 1 and 2 combined (684 valves), arranged by character, deme and provenance. The boxes bound the 25th to 75th percentiles (with a line at the 50th); caps indicate 10th and 90th percentiles, with individual points beyond. The provenance is indicated by the position of each box relative to the dotted line for each deme: data from 'Ganavan' are to the left of the line, data from 'recent Cumbrae' are at the line, and data from 'old Cumbrae' are to the right of the line. Table 3 gives the numbers of valves measured.

Fig. 4. Stria density vs deme (striae in 10 µm).

Fig. 5. Rectangularity (the area enclosed by the diatom outline as a proportion of the area of the rectangle defined by the apical and transapical axes of the diatom) vs deme.

Fig. 6. Shape of central raphe endings vs deme (0 = bilaterally symmetrical, 1 = turned slightly to the side, 2 = hooked to the side).

Fig. 7. Stria structure (the extent of *fusca*-type structure on the valve, as a proportion of the total area of the striae distal to the longitudinal canals, × 10) vs deme.

Table 3. Numbers of valves measured (Analyses 1 and 2 combined).

	Deme																		
Provenance	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Ganavan	10	9	6	10	9	13	44	8	3	13	31	—	—	—	2	2	1	1	1
Recent Cumbrae	32	31	29	36	—	74	2	26	—	—	5	6	—	60	—	—	1	—	—
Old Cumbrae	27	79	35	27	3	11	9	6	—	7	10	—	5	—	—	—	—	—	—

identical, regardless of provenance: Figs 9–20 show individuals of four demes from each of the three provenances (there is insufficient space to illustrate every deme from every provenance in which it occurs).

Deme #1 is shown in Figs 9–11, one valve from each of 'Ganavan', 'recent Cumbrae' and 'old Cumbrae', respectively. The similarity between the valves is striking: all three are oblong and coarsely striate, and only distal to the raphe do the striae comprise a double row of alternating pores (the adaxial portions are divided into more or less square chambers like those usually associated with *D. fusca*). The longitudinal canals are narrow (compared to those of demes #2–#4: Figs 21–23), and their adaxial edges are almost straight (contrast those of deme #6, which are undulate: Figs 12–14). The distance between the proximal raphe endings is smaller than in other, similarly sized demes (Figs 12–14, 21–26), and the raphe endings themselves are bilaterally symmetrical. This feature, although clearly visible in LM, is out of focus in Figs 9–11 (but shown in Droop 1994, fig. 39). This is because the focus chosen for all the LM photographs is on the abaxial edge of the longitudinal canals and the valve architecture of deme #1 is such that the central part of the valve is always out of focus, more so than in any other deme (Figs 12–29).

Deme #6 is shown in Figs 12–14. The three valves, from 'Ganavan', 'recent Cumbrae' and 'old Cumbrae', respectively, are very similar to each other and consistently different from the other demes: they are more rounded in outline than those of demes #1 (Figs 9–11) and #10 (Fig. 25) but less pointed than deme #2 (Fig. 21). Each stria comprises a double row of alternating pores, which extends from close to the longitudinal canals as far as the valve margin (*smithii*-type structure); all other demes have at least some *fusca*-type structure (striae divided into square or rectangular chambers) near the longitudinal canals. This deme is also unique in the extent to which the adaxial edges of the longitudinal canals undulate. The proximal raphe endings are slightly hooked and the distance between them is large.

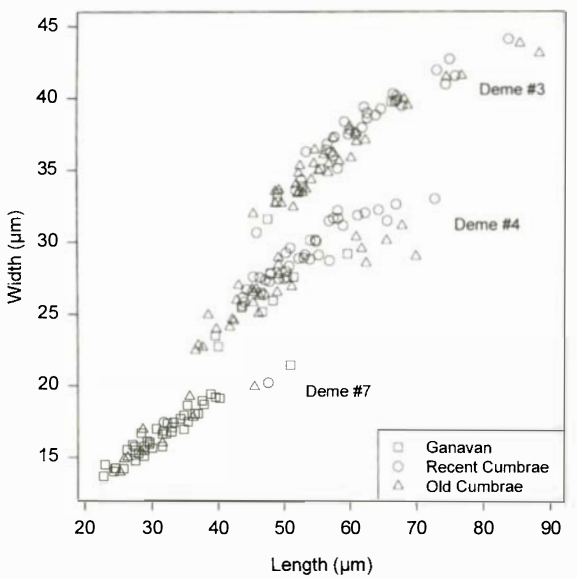


Fig. 8. Width vs length for three demes (#3, #4, #7), each from all three provenances.

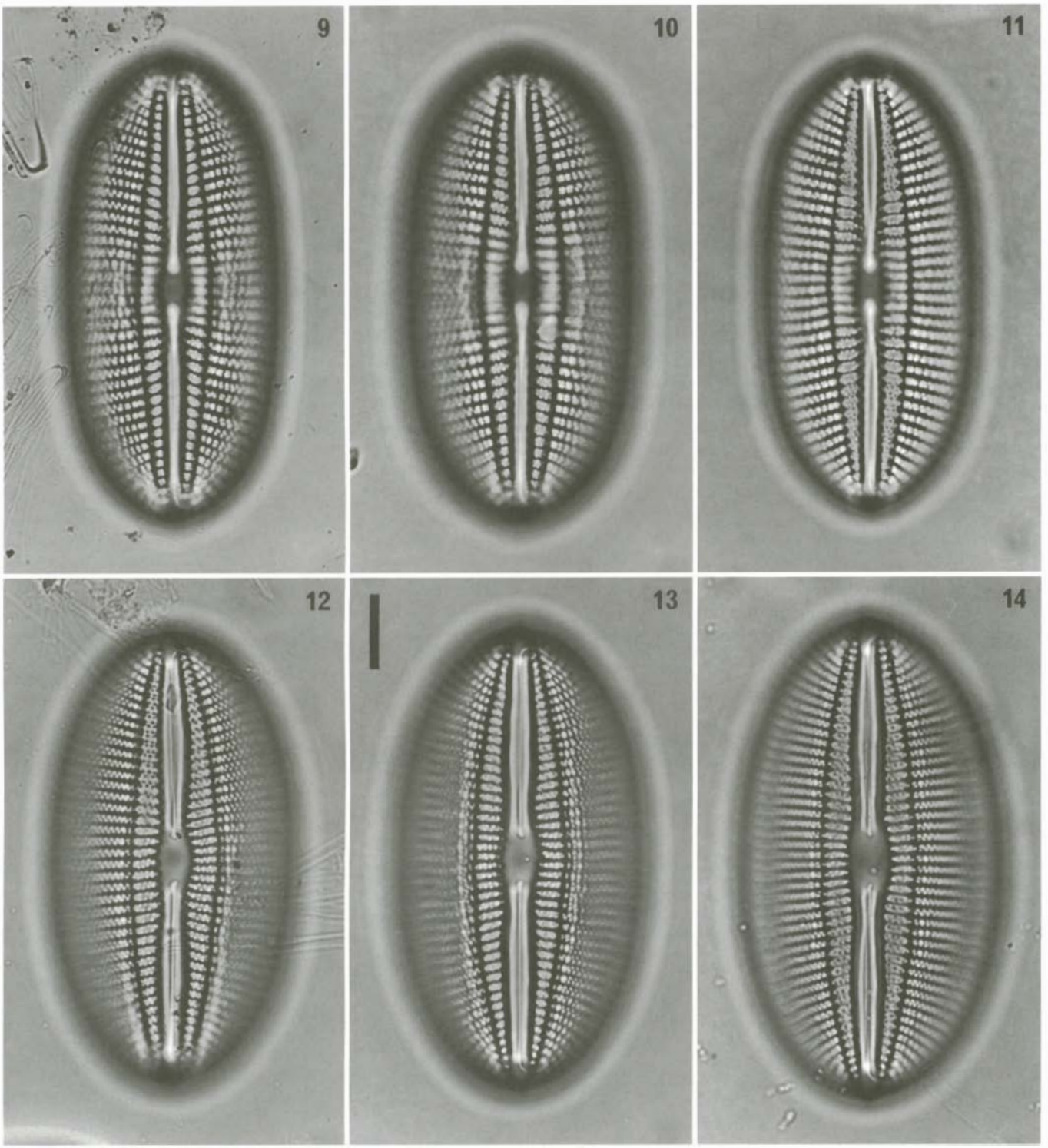
Demes #7 and #8 are shown in Figs 15–17 and 18–20, respectively, one valve of each from each provenance. Again, there is a conspicuous similarity of the valves within each deme (valve size and shape, density and arrangement of striae, and shape of longitudinal canals and of central raphe endings), but consistent differences between the two, especially in the shape of the valve outline and raphe endings. Deme #8 is more elliptical, has simple central raphe endings and simply hooked terminal raphe endings; deme #7 is more oblong, has hooked central raphe endings and recurved terminal raphe endings.

The other demes featured in Analysis 1 are illustrated in Figs 21–29, all from Cumbrae material (except #9, Fig. 27, which has not been found there). There is a close match between each of these valves and equivalent valves from Ganavan, which were illustrated in Droop (1994): compare Figs 21–26 with Droop (1994, figs 5–17, 33–38). Demes #13 and #14 (Figs 28, 29) are new.

A subset of this data has been used by Mann in a review of species concepts in diatoms, and comparative photographs of demes #2 and #10 from all three provenances are included (Mann 1999, figs 13–18). In addition, demes #3 and #4 have already been compared between several localities, not including Cumbrae (Droop 1995, figs 16–38).

SCANNING ELECTRON MICROSCOPY: Examination of the demes with SEM further highlights the close similarity between individuals of a deme from different provenances, and also the consistent differences between the demes. All demes examined with the scanning electron microscope for this analysis are unique and recognizable on the basis of either a single diagnostic character or a combination of characters. Many of the diagnostic details discussed below were not included in the morphometric analysis, either because they were not visible in LM or because they were too difficult to score meaningfully. In all, 289 photographs were taken of 40 valves, representing 10 of the 13 demes included in Analysis 1, from six different samples from the three provenances. The 'old Cumbrae' material has deteriorated (through dissolution) since its collection, but the main structures can still be seen, and even some of the finest detail can be inferred from what is left (e.g. Figs 41, 46, 50).

Of the nine demes that are known to occur in all three provenances, five were seen from all three in SEM, and the



Figs 9–14. *Diploneis smithii/fusca*: demes #1 and #6, one valve of each from each provenance. LM, brightfield. All valves to the same scale; scale bar = 10 μm.

Figs 9–11. Deme #1.

Fig. 9. E 1014 (Ganavan).

Fig. 10. E 2673.2 (recent Cumbrae).

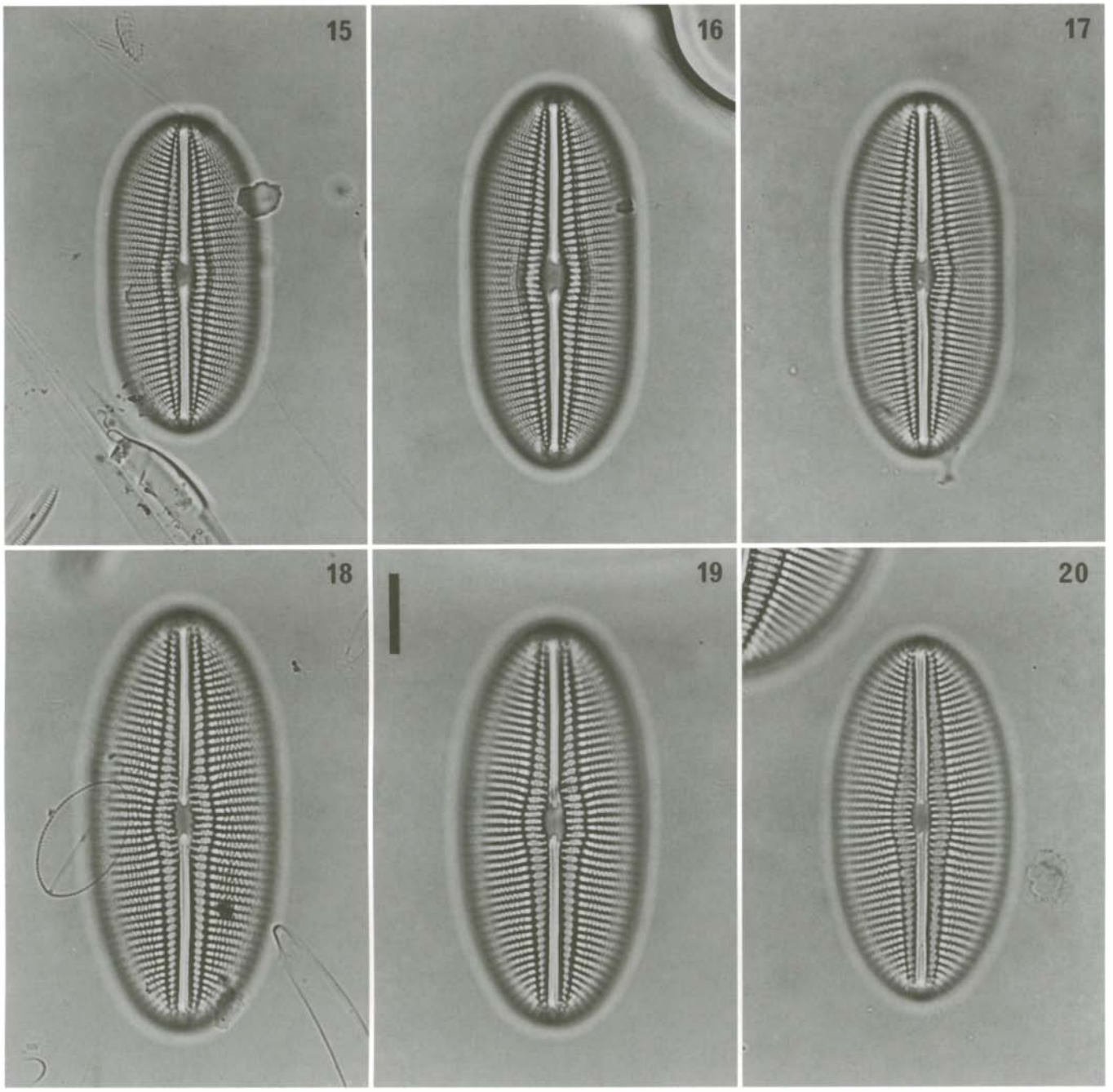
Fig. 11. BM s/n (old Cumbrae).

Figs 12–14. Deme #6.

Fig. 12. E 1016 (Ganavan).

Fig. 13. E 2673.2 (recent Cumbrae).

Fig. 14. BM 11836 (old Cumbrae).

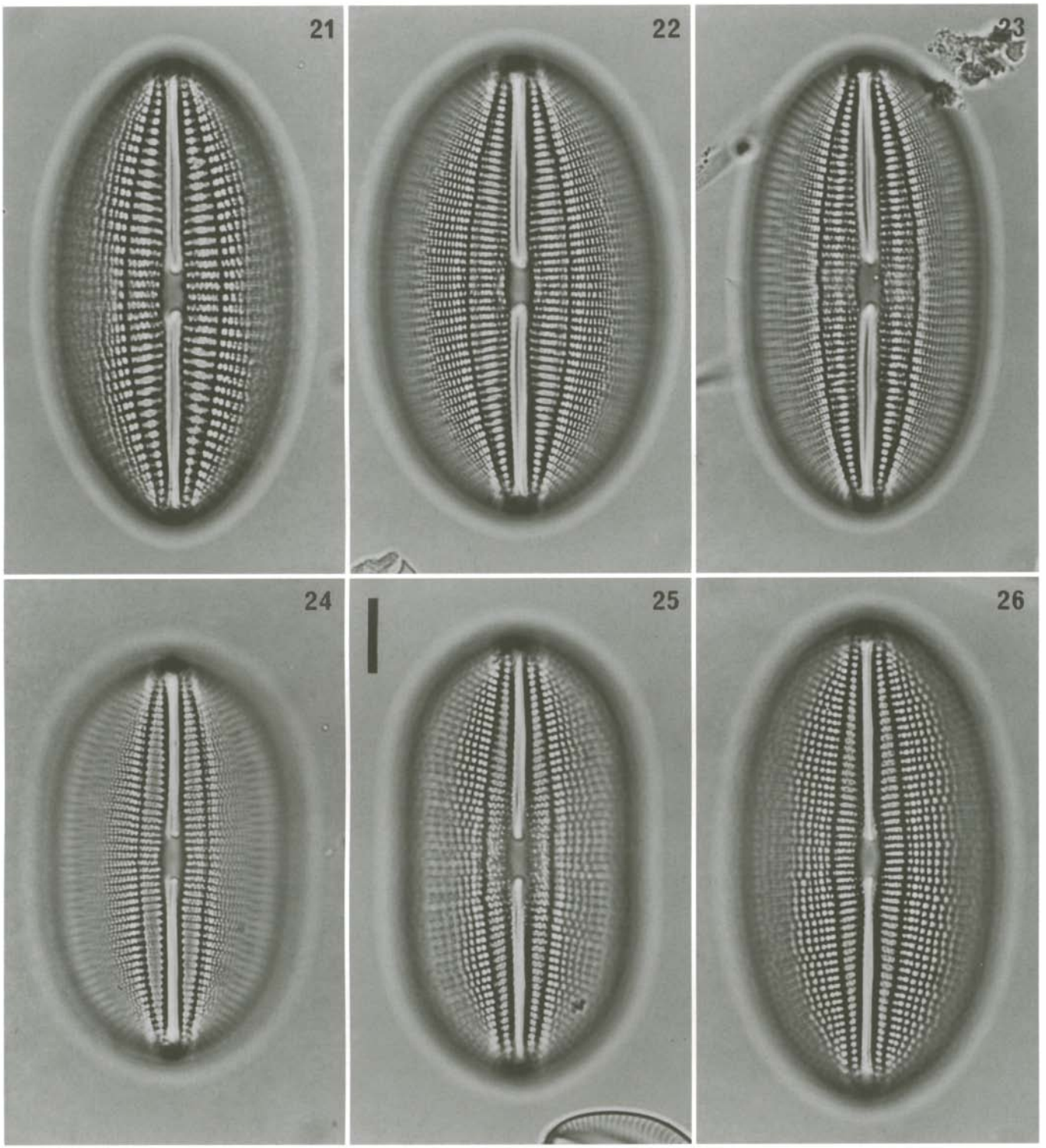


Figs 15–20. *Diploneis smithii/fusca*: demes #7 and #8, one valve of each from each provenance. LM, brightfield. All valves to the same scale; scale bar = 10 μm.

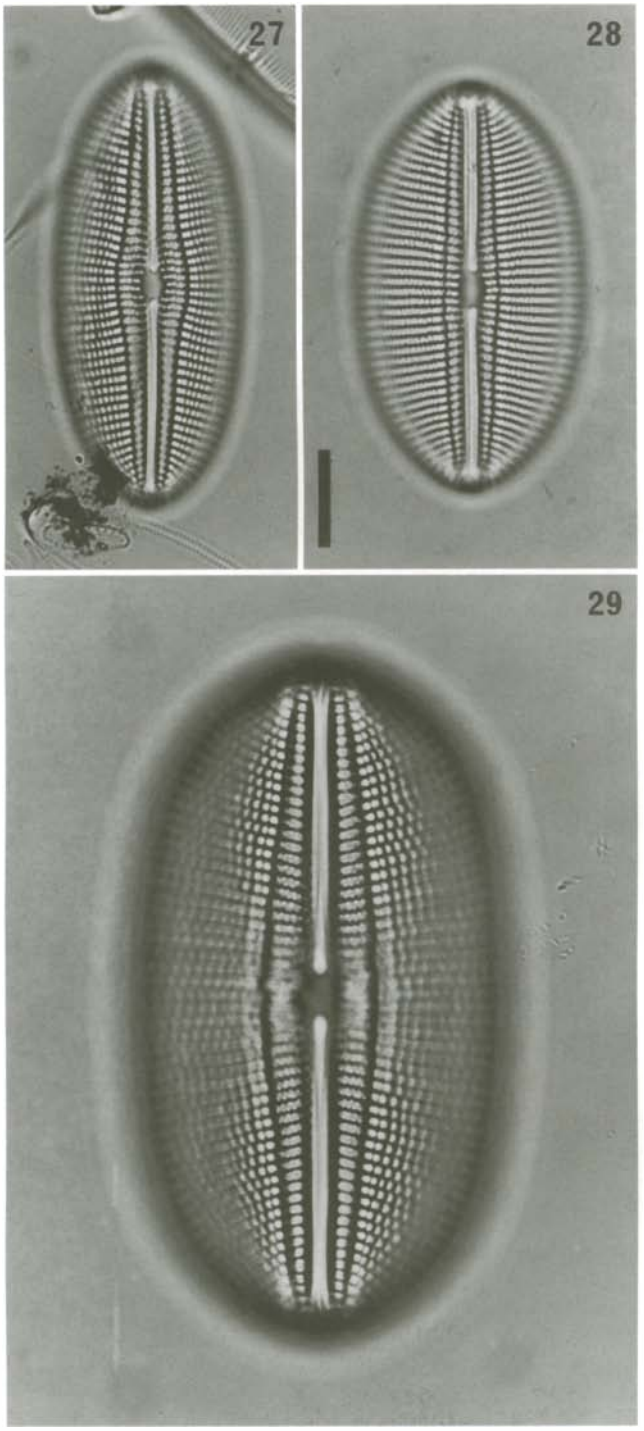
Figs 15–17. Deme #7.
Fig. 15. E 1014 (Ganavan).
Fig. 16. E 2673.2 (recent Cumbrae).
Fig. 17. BM 11836 (old Cumbrae).
Figs 18–20. Deme #8.
Fig. 18. E 1014 (Ganavan).
Fig. 19. E 2673.2 (recent Cumbrae).
Fig. 20. BM 11836 (old Cumbrae).

remaining four were seen from two out of three. Because of space limitations, they cannot all be illustrated fully (nor, indeed, described fully). Selected details of six demes are described below, each illustrated by comparative views from at least two provenances (Figs 30–50). The similarity between individuals of a deme from different provenances is evident, as are the differences between demes, although these are not discussed.

Individuals of deme #2 from different provenances are shown in Figs 30–35, which illustrate the valve pole (left-



Figs 21–26. *Diploneis smithii/fusca*: demes #2–#5, #10, #11. LM, brightfield. All valves to the same scale; scale bar= 10 μm.
Fig. 21. Deme #2, E 2679.2 (recent Cumbrae).
Fig. 22. Deme #3, E 2679.2 (recent Cumbrae).
Fig. 23. Deme #4, E 2673.2 (recent Cumbrae).
Fig. 24. Deme #5, BM s/n (old Cumbrae).
Fig. 25. Deme #10, E 2682.2 (recent Cumbrae).
Fig. 26. Deme #11, E 2673.2 (recent Cumbrae).



Figs 27–29. *Diploneis smithii/fusca*: demes #9, #13 and #14. LM, brightfield. All valves to the same scale; scale bar = 10 μm.
Fig. 27. Deme #9, E 1014 (Ganavan).
Fig. 28. Deme #13, BM s/n (old Cumbrae).
Fig. 29. Deme #14, E 2673.2 (recent Cumbrae).

hand pictures) and the central area (right-hand pictures). In all three valves, the polar raphe endings are sickle shaped and the marginal pores characteristically large (Figs 30–32, arrow in Fig. 31). Marginal pores are found in the external valve surface of many species of *Diploneis*. They are not visible on the interior valve surface, but they connect with the intercostal chambers (e.g. Idei & Kobayasi 1989, fig. 17). The central raphe endings are hooked, and the pores of the longitudinal canals are sunken relative to the valve surface (Figs 33–35).

The central raphe endings of the three valves of deme #10 (Figs 36–38) are very similar to each other (straight, in a rather deep, bilaterally symmetrical groove) – likewise the arrangement of the pores of the longitudinal canals, which are hardly sunken at all in this deme.

In deme #1 (Figs 39–41) the polar raphe endings are simple hooks (Figs 39–41), and the marginal pores are small (arrows in Figs 39, 41).

The central raphe endings in deme #7 (Figs 42–44) are strongly hooked and the end of each fissure is raised relative to the rest of the raphe. In most other demes the end of each fissure lies in a groove. Unique to deme #7 is a groove (Fig. 42, arrow) on either side of the central nodule, apparently comprising an irregular row of sunken pores and situated over part of the abaxial wall of each longitudinal canal. The groove is also discernible in Figs 43 and 44, though less clearly. The absence of pores in the valve surface abaxial to the longitudinal canals is also characteristic of this deme.

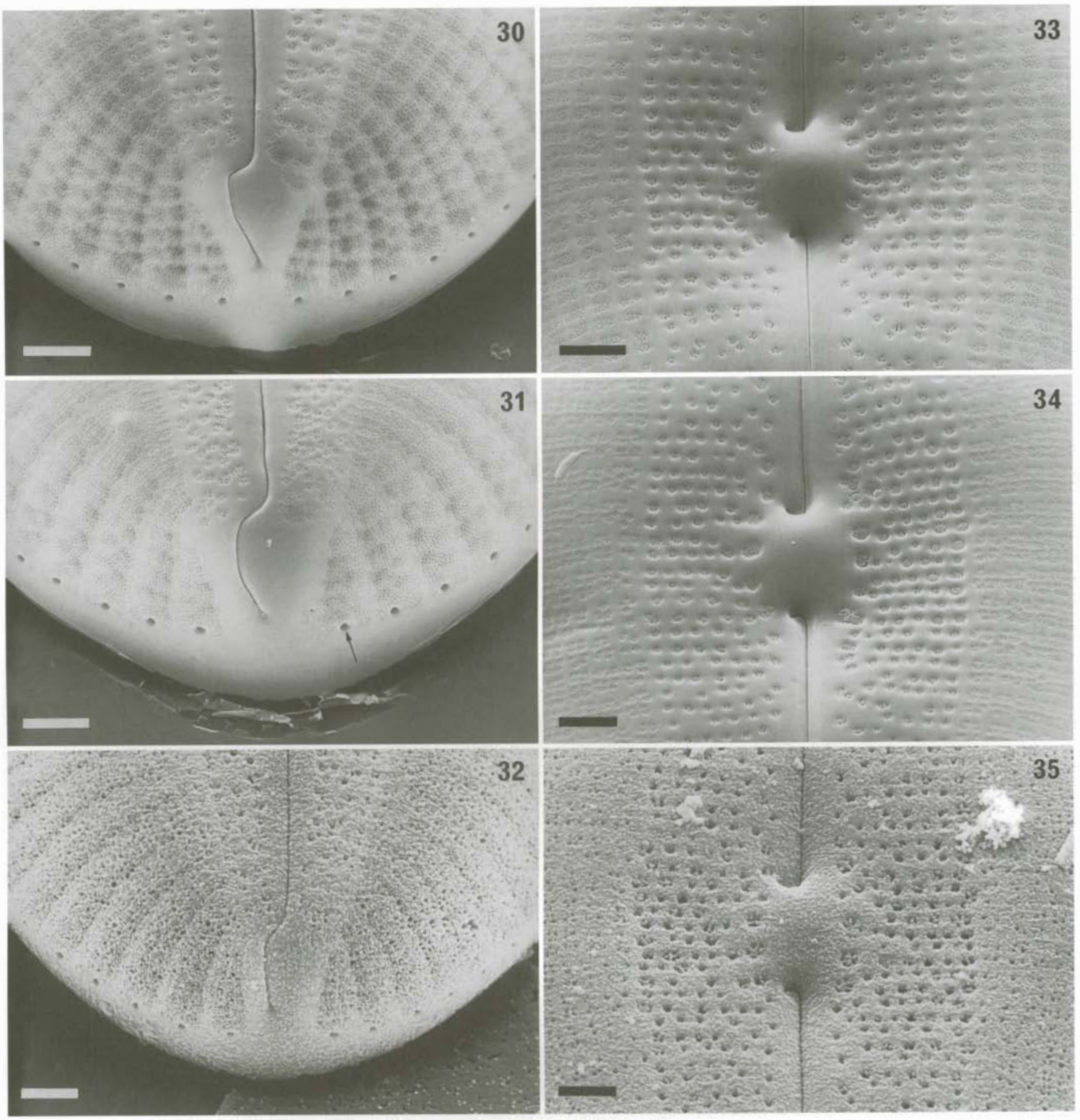
Both valves of deme #8 illustrated (Fig. 45 from 'recent Cumbrae', Fig. 46 from 'old Cumbrae') have more or less bilaterally symmetrical raphe endings rather similar to those of deme #10 (Figs 36–38), but in deme #8 the channel is less deep, and there is a short groove around the raphe ending that is not present in deme #10.

Deme #6 (Figs 47–50) is unique in several respects, but all the details are shared by individuals from 'recent Cumbrae' (top pair, Figs 47, 49) and 'old Cumbrae' (bottom pair, Figs 48, 50). The central raphe endings are weakly hooked, as are the polar raphe endings, which stop well short of the valve margin. Marginal pores are absent (Figs 49, 50).

Morphometric analysis 2

The results of Analysis 2, dealing with the rarer demes of the *D. smithii/fusca* complex, are shown in Figs 51–53 (and also contribute to Figs 4–7). As in Analysis 1, the undifferentiated data points do not form a single coherent group in pc hyperspace but form more or less discrete clusters on the basis of the morphology of the valves represented (Fig. 51). Three main clusters are present, but with several outlying points and/or additional clusters. Table 4 shows the results of the Eigenanalysis; as in Analysis 1, more than 90% of the total variation is expressed by the first three principal components, but the contribution of characters to each component is very different from Analysis 1 (Table 2). The first component comprises mostly dimensions and stria density; the second, rectangularity, the shape of the central raphe endings and stria structure; and the third, rectangularity and the shape of the central raphe endings.

Most of the clusters in Fig. 51 represent demes already encountered in Analysis 1, but with more individuals. Figure 52 is a plot of pc1 vs pc2 that shows the same data as Fig. 51, but from a different angle and with the individual points differentiated with respect to their identity. A number of valves were found during Analysis 2 that did not match any morphotype previously found at Ganavan or Cumbrae, and they are introduced here as demes #12 (Fig. 54) and #15–#19 (Figs 55–59, respectively). Otherwise, the numbering used is



Figs 30–35. *Diploneis smithii/fusca*: deme #2, from all three provenances, SEM. Scale bars = 2 μm.
Figs 30–32. Pole.
Fig. 30. E 1016 (Ganavan).
Fig. 31. E 2673.2 (recent Cumbrae) – arrow indicates a marginal pore.
Fig. 32. VH 104 (old Cumbrae).
Figs 33–35. Central area.
Fig. 33. E 1016 (Ganavan).
Fig. 34. E 2673.2 (recent Cumbrae).
Fig. 35. VH 104 (old Cumbrae).

the same as in Analysis 1. Demes #18 and #19 are represented by one valve only; the others are represented by two or more valves. The uppermost cluster in Fig. 51 (consisting mostly of quite large diatoms with *fusca*-type structure and hooked central raphe endings) can be seen in Fig. 52 to comprise several overlapping or nearly overlapping demes. The lower/left-hand cluster in Fig. 51 is deme #6 (Fig. 52), but with the two valves of deme #17 embedded within it. Demes #9, #13,

#15, #16 and #18 are separate in Fig. 52 (although the separation of #9 and #16 is minimal). Two-dimensional plots were also prepared for all combinations of pc1–4, but only one is illustrated (Fig. 52). They show that all pairs of demes can be distinguished using pc1–3 (i.e. the envelopes around the individuals of each deme do not overlap in at least one plot), with the following exceptions: one or more valves of deme #17 are included within the envelope for deme #6 in all plots involving any of pc1–4; demes #5, #11 and #12 are not distinguished using pc1–3, although #5 can be distinguished from the other two using pc4; demes #11 and #12 cannot be distinguished using pc1–4; deme #19 cannot be distinguished from #11 using pc1–4.

Of course, PCA can only distinguish between demes if they differ in the characters included in the analysis. Demes #11 and #12 exemplify this problem (Figs 26, 54). They are very similar in many respects: they are both medium-sized diatoms with *fusca*-type striae, similar stria density and rectangularity, and hooked central raphe endings. However, they differ in at least three characters that were not included in the analysis because of difficulties in quantifying or scoring them: the longitudinal canals in deme #12 (Fig. 54) are much broader along most of their length than in deme #11 (Fig. 26), and there is a hint of lateral asymmetry in deme #12 that is not present in deme #11: the longitudinal canal on the secondary side of the valve in deme #12 – on the right-hand side of the valve in Fig. 54 – has slight angles in its abaxial wall (arrows) at about one quarter and three quarters of its length, perhaps corresponding to Voigt discontinuities. In addition, the cross-walls of the chambers in the striae of #12 are aligned longitudinally over much of the valve, giving it a very regular appearance (Fig. 54), whereas those in deme #11 are not so arranged (Fig. 26). The same three characters relate deme #12 (Fig. 54) to deme #16 (Fig. 56) and to a lesser extent deme #19 (Fig. 59). Deme #19 seems more or less intermediate between demes #11 and #12 on the basis of these characters, although the PCA was able to distinguish them on the basis of their different sizes and stria densities. The true relationship between demes #12, #16 and #19 is unclear (also between demes #19 and #11), since they have been seen so rarely, but it seems unlikely that they are the same deme; demes #16 and #19 are sympatric, occurring in the same Ganavan sample (E 1016).

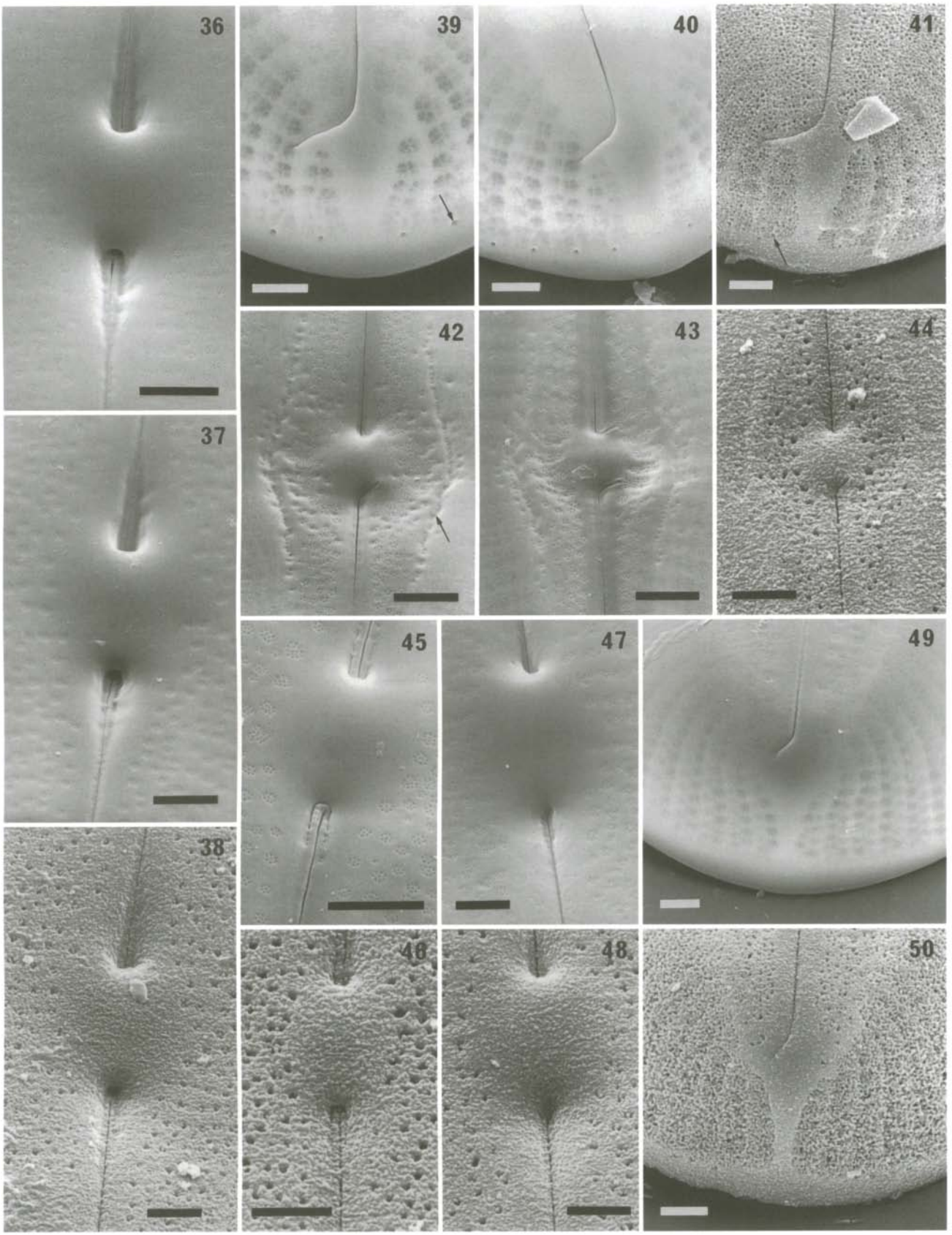
Only three of the demes from Analysis 2 occur in all three provenances: #6, #8 and #11. Two others occur in two provenances: #5 occurs in 'old Cumbrae' and 'Ganavan', and #17 occurs in 'recent Cumbrae' and 'Ganavan' [although not in the sample investigated by Droop (1994)]. Figure 53 is based on Fig. 52, with envelopes inserted to indicate the provenance for each point within each of the demes that occur in more than one provenance. The PCA has placed members of the same deme together, irrespective of their provenance (Fig. 53). Deme #6 is well represented in Analysis 2 (having been classed as 'rare' in Analysis 1), and all three polygons overlap; this indicates that the morphology of deme #6 (as measured for the analysis) is essentially unchanged over 80 km and 140 years (see also Figs 12–14). The same is true to a lesser extent for demes #8 and #11 in which one polygon overlaps both the others, although there is no point where all three coincide. The polygons in deme #5 lie close together but do not overlap. Deme #17 is represented by only two valves (one from each of two provenances).

The incomplete overlap of polygons from different provenances in demes #5, #8 and #11 is due to slight differences in morphology between provenances. The data on which the analysis is based are summarized as box plots in Figs 4–7, combined with the Analysis 1 data. The two populations of deme #5 differ most in stria structure (Fig. 7), those from 'old Cumbrae' scoring lower than those from 'Ganavan' (it is possible that this discrepancy is due to recording error, since the structure of individual striae varied widely within single valves of this deme, making it difficult to estimate structure for the whole valve). There were slight differences in width, stria density (Fig. 4) and rectangularity (Fig. 5) between the populations of deme #8 from different provenances, those from 'Ganavan' being slightly narrower and with slightly higher stria density than the others, while those from 'old Cumbrae' had slightly lower rectangularity than the others. There were differences in dimensions, stria density (Fig. 4) and rectangularity (Fig. 5) between the populations of deme #11 from different provenances, those from 'recent Cumbrae' being bigger and with lower stria density than the others; all three provenances differed from each other in rectangularity. However, despite these slight differences, the identity of the valves is not in doubt, since the characters that delimit the demes do not vary to the same extent (e.g. Figs 18–20 for deme #8).

Of the demes included in Analysis 2, only demes #6, #8, #11 and #12 have been seen with SEM. Demes #6 and #8 have been briefly described above under Analysis 1 (Figs 45–50). Two valves of deme #11 have been seen in SEM (one from each of 'Ganavan' and 'old Cumbrae') but are not illustrated here; they appeared to be almost identical. One valve of deme #12 has been seen in SEM; the longitudinal alignment of the stria chambers was particularly clear (seen in LM, Fig. 54). The other demes from Analysis 2 are known only from the very few specimens seen in LM. Demes #15 and #17 are similar in some respects to demes #1 and #6, having coarse striae and bilaterally symmetrical central raphe endings (Figs 55, 57). However, deme #15 is much smaller than demes #1, #6 and #17 and has a very low rectangularity (Fig. 5); its longitudinal canals are even narrower than those of deme #1. Deme #17 has broad longitudinal canals like those of deme #6, but they lack the undulations of the adaxial walls of the longitudinal canals that characterize deme #6 (Figs 12–14, 57). Deme #18 (Fig. 58) seems to be most similar to deme #9 (Fig. 27) but is smaller, with narrower longitudinal canals and less regular longitudinal alignment of stria pores.

DISCUSSION

The above results show that 10 of the 11 demes identified previously in a sample collected from Ganavan (Droop 1994) also occur in an old sample from Cumbrae, some 80 km to the south and collected in 1858. Nine also occur in one or more samples collected from Cumbrae in 1997. Most are virtually identical in all samples on the basis of overall morphology and ultrastructure, and an objective morphometric analysis clusters individuals of each together, irrespective of the sample from which they originated. A few demes differ slightly between provenances, but the differences do not affect the identifications. Does this and other available evidence sup-


36
39
40
41
42
43
44
37
45
47
49
38
46
48
50

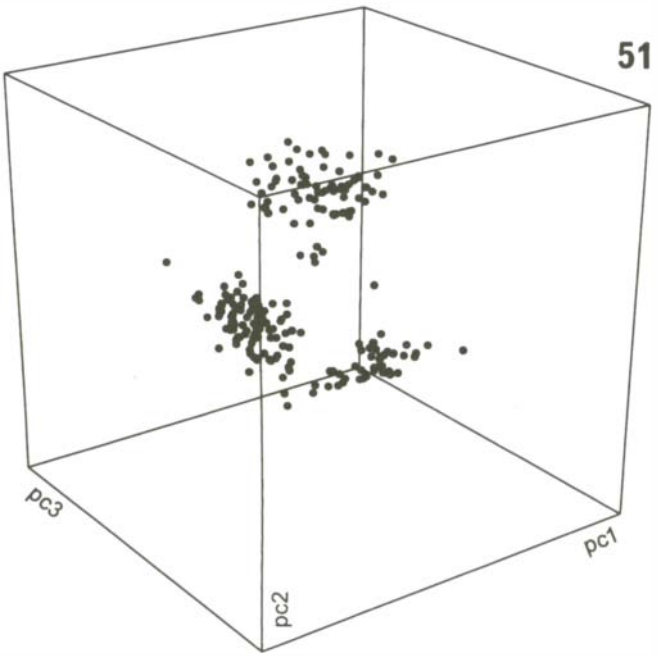


Fig. 51. Analysis 2: principal component analysis of 218 valves (belonging to demes that were poorly represented in Analysis 1) from three provenances: 'Ganavan' (collected 1991), 'recent Cumbrae' (collected 1997) and 'old Cumbrae' (collected 1858). The orientation was chosen to achieve the greatest visual separation between the three main clusters.

port recognition of the demes as species, or are they best interpreted as infraspecific genotypic variants, or is the variation pattern caused by some, as yet, poorly understood process involving phenotypic plasticity? Ideally, these questions would be answered by experimentation with clonal cultures, either by changing environmental conditions or by testing for reproductive isolation. In addition, information on genotypic differentiation might be gained by sequencing parts of the genome, which could also clarify the evolutionary history of the group. However, as mentioned previously, it has not yet been possible to obtain clonal cultures of *D. smithii/fusca* demes. Amplifying DNA from single *Diploneis* cells may soon be possible, but so far this has been achieved (within the diatoms) only in fairly large centric species (e.g. *Coscinodiscus* species: Droop, unpublished). Sherbakova *et al.* (1998) extracted DNA from 300 wild-collected cells of *Aulacoseira skvortzowii* Edlund, Stoermer & C.M. Taylor, but this approach is impractical for living cells of *D. smithii/fusca* demes, owing to their small size and, in some cases, the difficulty of distinguishing them in low-magnification LM.

Cox (1997, pp. 15, 24) has suggested that it would be premature to interpret the morphologically distinct, sympatric populations of *Diploneis smithii/D. fusca* found by Droop (1994) as separate species without evidence of breeding barriers or ecological differentiation. She suggested an alternative interpretation of Droop's (1994) data, in which the different morphotypes (demes) would represent phenotypic variants of the same species that have differentiated in response to ecological factors; each morphotype would represent a clone. The sample used by Droop (1994) represents a single point in time and space, and many of the cells in it could be from the same clone (since diatom populations consist mostly of cells produced mitotically). Cox's explanation would thus be quite reasonable if Droop's (1994) results were the only evidence available. Its plausibility is lessened, however, by the fact that what appear to be the same demes occur in widely separated sites (Droop 1994, 1995), which Cox (1997) appears to have overlooked. The stability of the demes with respect to locality [alluded to in Droop (1994) and the main thesis of Droop (1995)] and time (evidence presented in this paper) is in fact central to the debate as to whether or not they have any significance beyond mere phenotypic plasticity. To continue to interpret the demes as a phenotypic effect of ecological differentiation (e.g. Cox 1997, p. 15) in the face of evidence of their spatial and temporal stability would require either (1) that all the individuals of a deme are clonally derived from a single cell, even if the populations are separated in time and/or space, or (2) that the same morphology can arise independently and abruptly in different populations, presumably in

←

Figs 36–50. *Diploneis smithii/fusca*: demes #1, #6–#8 and #10 from at least two provenances, SEM. Scale bars= 2 μm.
Figs 36–38. Deme #10, central raphe endings.
Fig. 36. E 1016 (Ganavan).
Fig. 37. E 2682.2 (recent Cumbrae).
Fig. 38. VH 104 (old Cumbrae).
Figs 39–41. Deme #1, pole.
Fig. 39. E 1016 (Ganavan) – arrow indicates a marginal pore.
Fig. 40. E 2673.2 (recent Cumbrae).
Fig. 41. VH 104 (old Cumbrae) – arrow indicates a marginal pore, visible despite erosion.
Figs 42–44. Deme #7, central area.
Fig. 42. E 1014 (Ganavan) – arrow indicates groove overlying part of abaxial wall of longitudinal canal.
Fig. 43. E 2679.2 (recent Cumbrae).
Fig. 44. VH 104 (old Cumbrae).
Figs 45, 46. Deme #8, central raphe endings.
Fig. 45. E 2679.2 (recent Cumbrae).
Fig. 46. VH 104 (old Cumbrae).
Figs 47, 48. Deme #6, central raphe endings.
Fig. 47. E 2673.2 (recent Cumbrae).
Fig. 48. VH 104 (old Cumbrae).
Figs 49, 50. Deme #6, pole.
Fig. 49. E 2679.2 (recent Cumbrae).
Fig. 50. VH 104 (old Cumbrae).

Table 4. Analysis 2: Eigenanalysis of the correlation matrix.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Eigenvalue	2.6071	2.0713	0.7319	0.3404	0.1824	0.0669
Proportion	0.435	0.345	0.122	0.057	0.030	0.011
Cumulative	0.435	0.780	0.902	0.958	0.989	1.000
Variable						
Length	−0.527	0.227	0.242	0.471	−0.471	0.410
Width	−0.577	0.164	−0.220	0.0383	0.028	−0.764
Stria density	0.549	0.230	0.040	0.129	−0.691	−0.388
Rectangularity	−0.015	0.508	0.757	−0.331	0.210	−0.126
Shape of central raphe endings	−0.097	0.568	−0.521	−0.535	−0.176	0.281
Stria structure	0.281	0.537	−0.217	0.599	0.475	0.048

response to the same ecological conditions. We will examine both possibilities.

Could each deme be derived mitotically from a single cell?

For each of our demes to have been derived mitotically from a single cell, at least some lineages of cells must have survived without reproducing sexually for at least 133 years and be capable of being transported 80 km in that time. Long-distance transport of diatoms is certainly possible (see discussion in Mann 1999). However, it is very unlikely that our demes have been exclusively mitotic for 140 years (the time between the two Cumbrae collections), as we argue below.

It is clear that the demes reduce in size according to the pattern typical of diatoms (i.e. a pattern that is entirely consistent with the MacDonald–Pfitzer rule: MacDonald 1869; Pfitzer 1869). Figure 8 shows valve dimensions for three demes from Analysis 1 data, with provenance indicated by different symbols. With the three provenances pooled, valve length in deme #3 ranges from 45.3 to 88.3 μm; deme #4, 36.7–72.6 μm; and deme #7, 22.8–51.0 μm. Since the valves measured for Analysis 1 only represent a small sample of each deme from each provenance, it is likely that the size ranges given above are underestimated. Indeed, a search of all the slides made from the five samples included in Analysis 1 extended the size ranges for each of the three demes, yielding ranges for deme #3 of 42–98 μm; deme #4, 31–84 μm; and deme #7, 22–56 μm.

Size reduction is clearly unsustainable in the long term without some form of periodic size restitution. In pennate diatoms, size restitution is almost always mediated by sexually produced auxospores. On the other hand, a few diatoms are known that do not reduce in size with succeeding mitotic divisions and which have never been seen reproducing sexually. The 'lanceolate' deme of *Sellaphora pupula* (Kützing) Mereschkowsky, for example, always exhibits a much narrower size range than the other five demes in Blackford Pond (Mann & Droop 1996), based on measurements during *c.* 16 years,

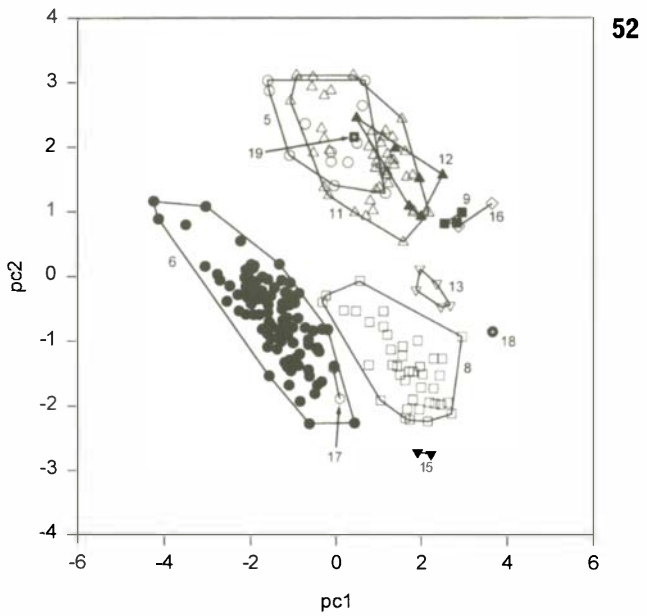


Fig. 52. Analysis 2: pc1 vs pc2, showing the identity of each valve represented. Deme numbers up to 11 are the same as those used in Droop (1994); numbers greater than 11 are new to this study.

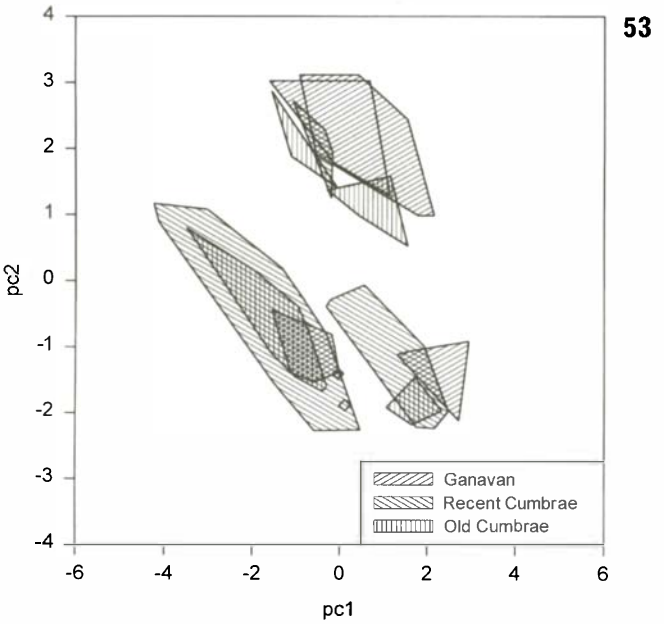
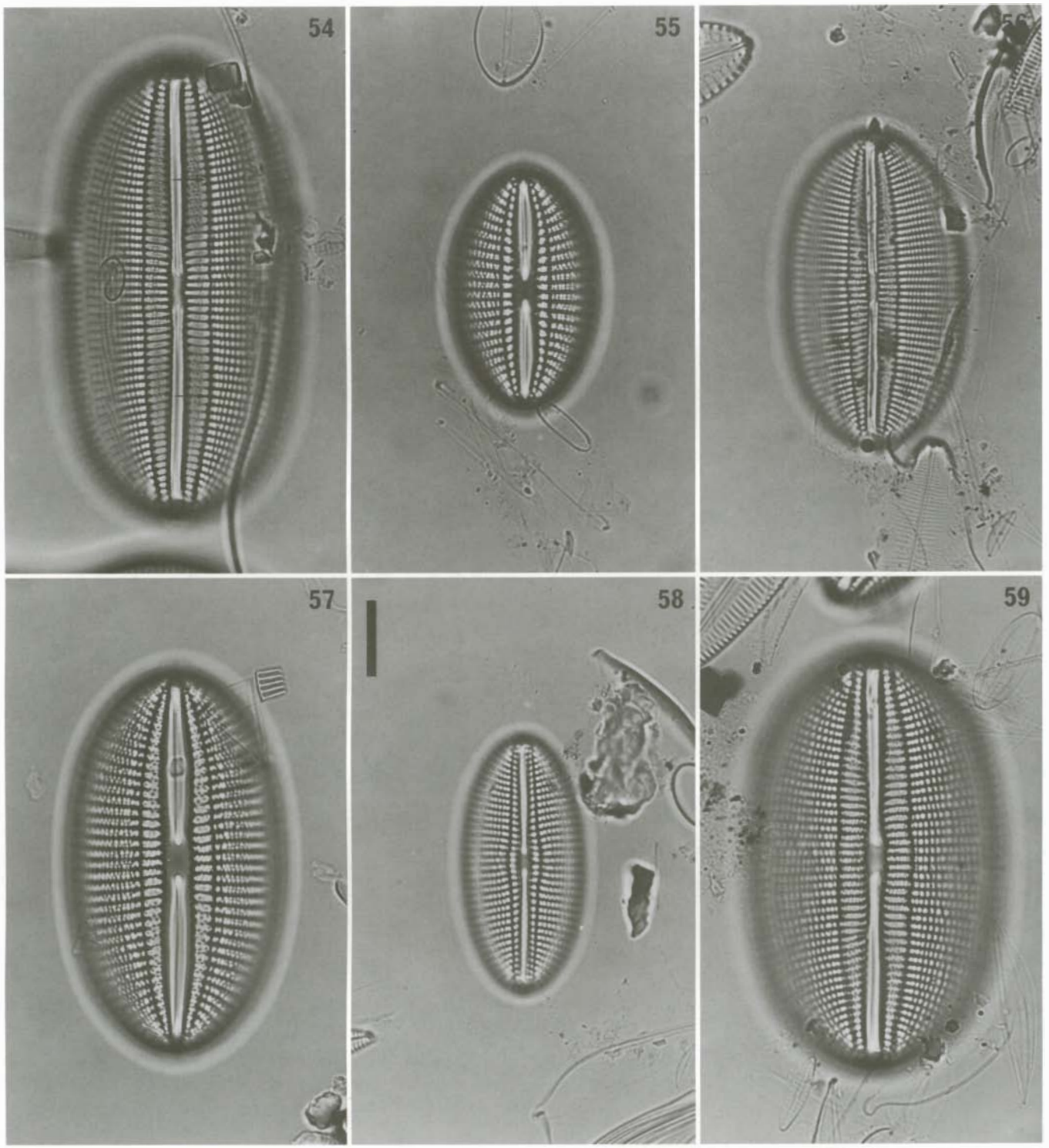


Fig. 53. Analysis 2: pc1 vs pc2, showing only those demes that occur in more than one provenance (#5, #6, #8, #11, #17). Envelopes surround the points of each deme from each provenance (but symbols are used where only one valve of a deme is included from any provenance).



Figs 54–59. *Diploneis smithii/fusca*: demes #12, #15–#19. LM, brightfield. All valves to the same scale; scale bar = 10 μm.
Fig. 54. Deme #12, E 2682.2 (recent Cumbrae) – arrows indicate the slight angle in the abaxial wall of the longitudinal canal on the secondary side of the valve.
Fig. 55. Deme #15, E 1016 (Ganavan).
Fig. 56. Deme #16, E 1016 (Ganavan).
Fig. 57. Deme #17, E 2682.2 (recent Cumbrae).
Fig. 58. Deme #18, E 1016 (Ganavan).
Fig. 59. Deme #19, E 1016 (Ganavan).

and no evidence has been found for sexual reproduction. The same can be said of *Caloneis amphisbaena* (Bory) Cleve in Blackford Pond (Mann, unpublished observations). Furthermore, where reduced sexuality has been observed during auxosporulation (e.g. in *Gomphonema constrictum* Ehrenberg, *Cocconeis placentula* Ehrenberg and *Cymbella ventricosa* Agardh: Geitler 1973), it is restricted to individual populations within species complexes whose other members are allogamous (Geitler 1957, 1973; Drebes 1977), and so the reduction of sexuality in these groups can be interpreted as a fairly recent evolutionary event. No genus or group of species is known in diatoms in which all or even most species are apomictic or asexual. Hence, since all the *D. smithii/fusca* demes exhibit size reduction, it is most parsimonious to assume that they are sexual and allogamous.

The rate of size reduction cannot be measured directly in a sample of prepared (and therefore dead) diatoms, but an estimate can be made by measuring the relative lengths of the valves of individual frustules. Slide BM 424 ('old Cumbrae') contains several whole frustules lying in girdle view, in which the length of the individual valves can be measured. A sample of 11 of these (five of deme #1, three of deme #4, three of deme #7) had an average difference in length between the epivalve and the hypovalve of 0.89 μm. Assuming a growth rate of one cell division per day [Eppley (1977) gives maximum growth rates between 0.7 and 4.0 divisions per day for diatoms] and using the above figure of 0.89 μm between the length of the epi- and hypovalves, each clone would reduce in size at an average rate of 0.445 μm per day (only one daughter cell of each mitotic division is smaller than the mother cell). At this rate of size reduction, a cell alive in 1858 that divided mitotically to give rise to even the smallest valve of deme #3 seen in the Ganavan sample (42 μm, collected in 1991) would have had to have been 21.6 mm long! Even taking the much lower growth rate of one division every two weeks, the 1858 cell would have had to have been 1.5 mm long.

In addition, there is no evidence from other diatom species for life cycles as long as 133 years. Mann (1988) and Jewson (1992b) have summarized and interpreted available data on the length of diatom life cycles. The longest life cycle recorded is for *Aulacoseira* [= *Melosira*] *islandica* var. *helvetica* (O. Müller) Simonsen, studied from the Zürichsee by Nipkow (1927), which Mann (1988) interpreted as having a life cycle of 40 years. Jewson (1992a) interpreted the same data differently, revising the estimate to four to six years.

It is therefore reasonably safe to infer that diatoms in the recent Cumbrae samples are not clonally related to those collected 140 years ago and that cells in the recent samples have been through at least one cycle of size reduction and allogamous sexual reproduction (probably several) since the old Cumbrae material was collected in 1858.

Have demes arisen independently several times?

The alternative explanation that would fit Cox's interpretation (Cox 1997) of our results is that variation arises in response to different ecological conditions but is constrained morphogenetically in such a way that only a limited number of morphologies are possible, in much the same way that Mendel's peas were either smooth or wrinkly. This explanation would allow for the same morphology to arise independently in any population in which the ecological conditions were the same. There are two points in the life cycle at which morphology can change: during valve formation and during auxosporulation.

New valves are formed immediately inside the cell walls of mitotic mother cells, and so the size and shape of the daughter cells are physically constrained. But there is no constraint on the pattern or ornamentation of the valves, even in those species with interactive cell division (Mann 1994), where the large-scale surface sculpturing of each new valve complements that of the other. Changes in morphology (valve ornamentation) during the mitotic part of the life cycle have been documented for several species [summarized by Cox (1993), Droop (1994) and Mann (1999)]. Recently, McBride & Edgar (1998) described a population of *Gomphonema angustatum* (Kützing) Rabenhorst containing a high proportion of Janus cells (in which the valves are morphologically different). The authors show that the occurrence of Janus cells in *G. angustatum* need not be environmentally controlled, but can be entirely explained as the independent expression in each valve of a randomly and continuously varying morphogenetic system. Stoermer (1967) described a population of *Mastogloia grevillei* W. Smith and *M. elliptica* var. *dansei* (Thwaites *ex* W. Smith) Cleve that contained a small proportion of Janus frustules with one valve of each 'species'. The valves differed in stria structure and density but were apparently almost identical in all other morphological features.

No Janus cells have been seen in the populations of *D. smithii/fusca* from Ganavan or Cumbrae, although they might be expected to be very rare in a population if switching between morphologies is controlled by a finely tuned and rarely activated environmental trigger: high proportions of Janus cells would then occur only if the environment fluctuated repeatedly through the trigger (McBride & Edgar 1998). However, the greatest argument against morphogenetic switches operating to produce the demes of *D. smithii/fusca* discussed here is that most of the demes have significantly different sizes and shapes, so that it would be physically impossible for valves of different demes to be accommodated in the same frustule. Figures 9–14 and 21–26 are all printed to the same magnification and show values of similar length, but the differences in shape (summarized by the rectangularity character in Analyses 1 and 2 above) would make the formation of Janus cells impossible. The rather oblong valves of deme #1 (Figs 9–11) could not coexist in the same frustule as those of deme #6 (Figs 12–14), nor could those of #7 and #8 (Figs 15–17 and Figs 18–20, respectively). Possible exceptions are demes #5 and #10 (Figs 24, 25, respectively), which have more or less the same size and shape, and demes #2 and #11 (Figs 21, 26, respectively). It is conceivable that these two pairs of demes represent different morphological expressions of the same genotype. However, though possible, this is extremely unlikely, since the differences in morphology within each pair are far greater than have been described so far for Janus cells in any other pennate diatoms. Not only do stria density and stria structure differ between #5 and #10 and between #2 and #11, but so also does raphe structure: the central external raphe endings in #5 are hooked (Fig. 24), while those in #10 are bilaterally symmetrical (Fig. 25); the polar raphe

endings of #2 are reflexed (Figs 21, 30–32), while those of #11 are hooked (Fig. 26).

The part of the life cycle where shape and size are not directly constrained by the dividing parent cell is during auxosporulation: here the expansion of the auxospore and its final shape are dictated by a combination of genotype and environment. If the effect of the environment were large, differences in environmental conditions could produce morphological differences between auxospores and hence between the clones derived mitotically from them. For example, Schmid (1979) found that initial cells of *Surirella peisonis* Pantocsek grown in different salinities differed from each other to the extent that they could be identified as four different varieties of *S. ovalis* Brébisson and *S. ovata* Kützing. If, in addition, the expression of such plasticity was constrained morphogenetically so that only a limited number of discrete morphologies was possible, then it is conceivable that several demes of the sort described above for *D. smithii/fusca* could be produced and could coexist. The occurrence of the same deme elsewhere or at different times would simply indicate auxosporulation events that had occurred under similar environmental conditions. With this model there would be no need to invoke long life cycles, since the same conditions might recur at intervals independently, and since the differences between the demes would be mostly phenotypic rather than genotypic, there would be no reproductive incompatibility between the demes.

However, there is no evidence that diatom morphology is constrained morphogenetically in this way. Nor is there evidence that details of ultrastructure like those that distinguish the demes of *D. smithii/fusca* can be modified by changing environmental conditions artificially – changes in morphology during auxosporulation have only been shown to affect valve size and shape and pattern periodicity.

In contrast, there is mounting evidence from other diatom taxa that the kind of variation observed here (including the spatial stability of demes, if not the temporal) has a real genetic basis and is often accompanied by reproductive barriers between demes. This was first demonstrated by Lothar Geitler (extensively referenced by Mann 1989, 1999), and further illustrations are given by Mann (e.g. Mann 1984, 1989, 1999; Mann & Droop 1996; Mann *et al.* 1999). The list of genera in which reproductive isolation between subtly different demes has been documented now includes *Amphora* Ehrenberg *ex* Kützing, *Neidium* Pfitzer, *Caloneis* Cleve, *Achnanthes* Bory, *Cocconeis* Ehrenberg, *Cymatopleura* W. Smith and *Sellaphora* Mereschkowsky. Most recent work has concentrated on *Sellaphora pupula* (Mann & Droop 1996; Mann *et al.* 1997, 1999), which we are using as a model to investigate several aspects of systematics and reproductive biology. As well as showing reproductive incompatibility between sympatric clones of different demes of *S. pupula,* we have now been able to demonstrate reproductive compatibility between allopatric clones of the same deme and have successfully crossed populations of the 'capitate' deme from Ukraine and Scotland, and populations of the 'rectangular' deme from three different Scottish lochs (Droop & Mann 1998; Mann 1999; D.G. Mann & V.A. Chepurnov, in preparation). In addition, molecular sequencing has revealed several base pair differences in small subunit ribosomal DNA (a relatively conservative part of the genome), which supports the idea of long-term evolutionary divergence between demes (Droop & Mann 1998; Mann 1999).

Conclusions

We have shown that morphologically distinct demes of *D. smithii/fusca* in western Scotland are stable with respect to distance and time. Although our evidence is indirect, the most plausible and parsimonious explanation of the variation pattern is that the demes are separate allogamous sexual species. Alternative explanations of the demes – that they are clonal variants or reflect switching between different morphogenetic pathways during the cell cycle or life cycle – are not credible. Nevertheless, direct evidence from breeding studies and molecular genetic data concerning their nature and evolution is desirable but will be difficult to obtain unless ways are found to maintain *D. smithii/fusca* demes in clonal culture.

ACKNOWLEDGEMENTS

We are very grateful to staff at BM, ANSP and AWH for loan of material and, in particular, to Geraldine Reid, Koen Sabbe, Charles Reimer and Dr G. Van Steenbergen for spending time looking for it. D.G.M. thanks Barry Leadbeater for kindly agreeing to arrange reviews of this paper after his official term as Associate Editor had ended.

REFERENCES

Cox E.J. 1993. Diatom systematics – a review of past and present practice and a personal vision for future development. *Nova Hedwigia, Beiheft* 106: 1–20.

Cox E.J. 1997. Assessing and designating diatom taxa at or below the species level – a consideration of current status and some suggested guidelines for the future. *Nova Hedwigia* 65: 13–26.

Drebes G. 1977. Sexuality. In: *The biology of diatoms* (Ed. by D. Werner), pp. 250–283. Botanical Monographs 13. Blackwell Scientific Publications, Oxford.

Droop S.J.M. 1993. Research note: the "Striatometer" – a new device for measuring striation densities. *Diatom Research* 8: 195–198.

Droop S.J.M. 1994. Morphological variation in *Diploneis smithii* and *D. fusca* (Bacillariophyceae). *Archiv für Protistenkunde* 144: 249–270.

Droop S.J.M. 1995. A morphometric and geographical analysis of two races of *Diploneis smithii/D. fusca* (Bacillariophyceae) in Britain. In: *Proceedings of the 13th International Diatom Symposium* (Ed. by D. Marino & M. Montresor), pp. 347–369. Biopress Ltd., Bristol.

Droop S.J.M. & Mann D.G. 1998. Species concepts in diatoms: the saga continues. In: *Abstracts: 15th International Diatom Symposium, 28th September–2nd October 1998, Perth, Western Australia,* p. 144. Curtin University of Technology, Western Australia.

Eaton J.W. & Moss B. 1966. The estimation of numbers and pigment content in epipelic algal populations. *Limnology and Oceanography* 11: 584–595.

Eppley R.W. 1977. The growth and culture of diatoms. In: *The biology of diatoms* (Ed. by D. Werner), pp. 24–64. Botanical Monographs 13. Blackwell Scientific Publications, Oxford.

Geitler L. 1957. Die sexuelle Fortpflanzung der Diatomeen. *Biological Reviews of the Cambridge Philosophical Society* 32: 261–295.

Geitler L. 1973. Auxosporenbildung und Systematik bei pennaten Diatomeen und die Cytologie von *Cocconeis*-Sippen. *Österreichische Botanische Zeitschrift* 122: 299–321.

Gilmour J.S.L. & Heslop-Harrison J. 1954. The deme terminology and the units of micro-evolutionary change. *Genetica* 27: 147–161.

IDEI M. & KOBAYASI H. 1989. The fine structure of *Diploneis finnica* with special reference to the marginal openings. *Diatom Research* 4: 25–37.

JEWSON D.H. 1992a. Life cycle of a *Stephanodiscus* sp. (Bacillariophyta). *Journal of Phycology* 28: 856–866.

JEWSON D.H. 1992b. Size reduction, reproductive strategy and the life cycle of a centric diatom. *Philosophical Transactions of the Royal Society of London* B 335: 191–213.

MACDONALD J.D. 1869. On the structure of the diatomaceous frustule and its genetic cycle. *Annals and Magazine of Natural History,* Series 4, 3: 1–8.

MANN D.G. 1984. Observations on copulation in *Navicula pupula* and *Amphora ovalis* in relation to the nature of diatom species. *Annals of Botany* 54: 429–438.

MANN D.G. 1988. Why didn't Lund see sex in *Asterionella*? A discussion of the diatom life cycle in nature. In: *Algae and the aquatic environment* (Ed. by F.E. Round), pp. 384–412. Biopress Ltd., Bristol.

MANN D.G. 1989. The species concept in diatoms: evidence for morphologically distinct, sympatric gamodemes in four epipelic species. *Plant Systematics and Evolution* 164: 215–237.

MANN D.G. 1994. The origins of shape and form in diatoms: the interplay between morphogenetic studies and systematics. In: *Shape and form in plants and fungi* (Ed. by D.S. Ingram & A.J. Hudson), pp. 17–38. Academic Press, London.

MANN D.G. 1999. The species concept in diatoms. *Phycologia* 38: 437–495.

MANN D.G. & DROOP S.J.M. 1996. Biodiversity, biogeography and conservation of diatoms. *Hydrobiologia* 336: 19–32.

MANN D.G., CHEPURNOV V.A., DROOP S.J.M., SLUIMAN H.J. & GUIHAL C. 1997. A total evidence study of diatom speciation in a model system: the Waltonian species concept. *Phycologia* 36(supplement): 70.

MANN D.G., CHEPURNOV V.A. & DROOP S.J.M. 1999. Sexuality, incompatibility, size variation, and preferential polyandry in natural populations and clones of *Sellaphora pupula* (Bacillariophyceae). *Journal of Phycology* 35: 152–170.

MCBRIDE S.A. & EDGAR R.K. 1998. Janus cells unveiled: frustular morphometric variability in *Gomphonema angustatum. Diatom Research* 13: 293–310.

NIPKOW F. 1927. Über das Verhalten der Skelette planktischer Kieselalgen im geschichteten Tiefenschlamm des Zürich- und Baldeggersees. *Zeitschrift für Hydrologie, Hydrographie und Hydrobiologie* 4: 71–120.

PFITZER E. 1869. Ueber den Bau und die Zelltheilung der Diatomeen. *Botanische Zeitung* 27: 774–776.

ROUND F.E., CRAWFORD R.M. & MANN D.G. 1990. *The diatoms. Biology & morphology of the genera.* Cambridge University Press, Cambridge. 747 pp.

SCHMID A.-M.M. 1979. Influence of environmental factors on the development of the valve in diatoms. *Protoplasma* 99: 99–115.

SHERBAKOVA T., KIRILCHIK S., LIKHOSHWAY YE. & GRACHEV M. 1998. Phylogenetic position of some diatom algae of the genus *Aulacoseira* from Lake Baikal following from comparison of sequences of 18S rDNA gene fragments. *Molekularnaya Biologiya* 32: 735–740.

STOERMER E.F. 1967. Polymorphism in *Mastogloia. Journal of Phycology* 3: 73–77.

VAN HEURCK H. 1880–1885. *Synopsis des Diatomées de Belgique.* Anvers. Atlas: pls 1–132, suppl. pls A–B (1880–1881); table alphabetique: 120 pp. (1884); texte: 235 pp. (1885). Published privately.

Accepted 8 October 2000