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A LIGHT MICROSCOPICAL INVESTIGATION AND MULTIVARIATE STATISTICAL ANALYSES OF HETEROVALVAR CELLS OF *CYCLOTELLA*-SPECIES (BACILLARIOPHYCEAE) FROM LAKES OF THE BERLIN-BRANDENBURG REGION

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113 randomly selected frustules of *Cyclotella* taxa, taken from the plankton of four lakes, have been investigated with respect to variation between the two thecae of each frustule and also variation within and between populations occurring in the different lakes. Multivariate statistical analyses of phenetic similarity of the 226 thecae were based on a data matrix recording the status of 11 characters drawn from the structure of the siliceous wall. From the cluster analysis, 55 % of the frustules could be characterized as hereovalvar. In these cases the phenetically dissimilar thecae of a single frustule were allocated to different clusters. Principal component analyses showed that the variation in the relative length of the marginal striae, and also the number of marginal striae on the entire valve face, are correlated with valve circumference. The arrangement of depressions on central valve and the development of the central valvar undulation appear to be unrelated to valve size. Due to the existence of different patterns of markings within one frustule ("*Cyclotella kuetzingiana* var. *radiosa*"-pattern, "*C. kuetzingiana* var. *planetophora*"-pattern, "*C. ocellata*"-pattern, "*C. comensis*"-pattern), these patterns have litmited or no taxonomic value. Thus, as far as one can judge from LM observation alone, it seems reasonable to assign all the investigated frustules to the same species, which thus shows great phennotypic plasticity in cell wall structure.*

INTRODUCTION

The taxonomic delimitation of some *Cyclotella* taxa (*C. krammeri* Håkansson, *C. kuetzingiana* var. *planetophora* Fricke, *C. kuetzingiana* var. *radiosa* Fricke, *C. ocellata* Pantocsek, *C. comensis* Grunow) is difficult, because of the polymorphism of the valve. While it has been common in the past to keep these taxa separate, some authors have emphasized the phenotypic similarity and have noted morphological transitions; it is possible that all are part of the same species (Cleve-Euler 1951, Klee & Steinberg 1987, Håkansson 1990, 1993, Håkansson in Krammer & Lange-Bertalot 1991).

In the course of comparative studies of the phytoplankton of lakes and ponds in the Berlin and Brandenburg regions of Germany, *Cyclotella* cells were found that could be assigned to two different

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* The results of this paper were presented on a poster at the 8th Meeting of German speaking Diatomists at Bremerhaven 25-27.3.1994.

taxa since the epi- and hypotheca of one cell had different structures. Frustules having valves with either different or similar surface markings are here called “heterovalvar” and “isovalvar” respectively. This use of heterovalvy has not the same meaning as that in Round, Crawford & Mann 1990. The phenomenon of hereovalvar sells is dealt with in this paper.

My aim is to describe the variability of morphological characters between the thecae of a single frustule, between the frustules in each population and between the frustules of different populations. This is done via the multivariate statistical analyses.

MATERIAL AND METHODS

Material was taken from Kiessee in Berlin-Mühlenbeck/Mönchsmühle; Rosinsee, Parsteiner See and Großer Plagesee in Brandenburg, near Brodowin, north of Eberswalde (morphometric, physical and chemical data in Table 1).

Table 1. Morphometric*, average annual physical and chemical data of the lakes from which the *Cyclotella* populations were taken (mean values and standard deviation, samples were taken from a depth of 0.5m).

	Kiessee	Rosinsee	Parsteiner See	Gr. Plagesee
max. lake depth [m]	4.5	9	27	5
lake area [ha]	20	20	1100	78
secchi depth [m]	1.4 ± 0.3	3.3 ± 1.7	3.8 ± 1.0	1.3 ± 0.4
pH	8.2 ± 0.1	8.2 ± 0.4	8.2 ± 0.4	8.3 ± 0.3
alkalinity [meq/l]	2.2 ± 0.3	2.3 ± 0.1	1.9 ± 0.1	1.2 ± 0.1
conductivity [mS/m]	90.8 ± 2.3	54.1 ± 1.4	48.0 ± 1.3	27.9 ± 9.1
total nitrogen [µmol/l]	71 ± 19	89 ± 13	68 ± 8	101 ± 12
total phosphorus [µmol/l]	1.43 ± 0.5	0.98 ± 0.2	0.80 ± 0.3	1.13 ± 0.5
soluble reactive silicon [µmol/l]	35.7 ± 25.4	18.4 ± 10.0	7.1 ± 6.1	5.3 ± 5.4

* data from Anwand 1973

The frustules studied were derived from monthly phytoplankton samples, taken in 1992. The samples contained an abundance of *Cyclotella* cells (Kiessee: March/April/June; Rosinsee: March; Parsteiner See: March/April; Großer Plagesee: October). The study was based exclusively on light microscopy (LM), using positive phase-contrast optics.

For preparing permanent slides, samples were treated gently to keep both thecae of each frustule together. For this reason the plankton samples (fixed with Lugol’s solution and stored at 10°C for not longer than a year) were cleaned by warming in hydrogen peroxide (for 7 hours, up to 60°C, twice) and then in a mixture of hydrogen peroxide and acetic acid (in a ratio 2:1; again for 7 hours), and finally washed and mounted in naphrax.

From the four lakes 113 cells were selected at random for investigation. Multivariate statistical analyses of phenetic similarity between the 226 thecae were based on a data matrix of variation in 11 valve characters, which are listed in Table 2. The number of marginal fultoportulae (Håkansson & Carter 1990) was not included as a character since it could not be determined for every valve and so would have left gaps in the data matrix. Marginal fultoportulae are difficult to observe in LM and often were identifiable only in one theca of a frustule of only around a part of the margin of the valve (compare Figs 12a, 16a, 21a, 24a/b; see also Håkansson 1990, p. 266).

Table 2. Eleven characters selected for the estimation of phenetic resemblance between thecae (LM-observation). These were the basis for the data matrix subjected to the mulrivariate statistical analysis.

running number	multistate character	character states
1	symmetry of arrangement of large puncta (depressions) in the central area	trigonal symmetrical arrangement (state 0) non-trigonal – radial arrangement (state 1) irregular arrangement (state 2) central area without structure (state 3)
2	number of large puncta (depressions in the central area)	no depressions, one or two; three; four; five; more than five depressions (state 0 to 4)
3	type of puncta arrangement in the central area (depressions and openings of valve face fultoportulae)	no puncta; puncta in the form of single point; double or three points; points and grooves; grooves (state 0 to 4)
4	relative length of marginal striae	average length of striae relative to the radius of valve (division into intervals) (state 0 to 3)
5	structural delimitation between the margin from the central area (depending on the regularity of the length of the marginal striae)	regular circular; moderately irregular; strongly irregular (star-shaped) states (state 0 to 2)
6	number of shortened marginal striae on the entire valve face	none to more than 3 shortened striae (state 0 to 3)
7	number of striae on the entire valve face	striae frequency in relation to a fixed angle (360°), (division into intervals) (state 0 to 3)
8	degree of valvar undulation	central area flat to radially undulate (state 0 to 3)
9	moulding of papillae	none, weakly or moderately formed (state 0 to 2)
10	valve shape	valve hemispherically inflated, slightly dome-shaped; valve face planar (drum-shaped frustule) (state 0 to 2)
11	valve diameter	division into intervals of different sizes (state 0 to 4)

A non-standardized original matirx, based on ordinal data with similar ranges of values for each character was used for the hierarchical cluster analyses (gamma distance [Goodman-Kruskals], ward linkage; using computer program SYSTAT (Version 5, SYSTAT, Inc., USA). The data were divided *a priori* into two subsets, consisting of trigonal and nontrigonal valves, respectively (see Figs 19-21 for contrasted trigonal and nontrigonal valves), to make it easier to demonstrate the heterovalvy of frustules.

A standardized matrix of both ordinal and metric data (correlation matrix) was used for all principal component analyses (PCA), using computer program STATGRAPHICS (Version 5, STSC, Inc., USA). The extraction of variables in result from PCA has not been carried out since only four lakes have been investigated and consequently the question arises as to what extent the 226 thecae show the full range of variation.

RESULTS

The morphological characters chosen for analyses were those already known or suspected to vary between the two thecae of a single individual and also between thecae of different frustules (see multistate characters 1-11 in Table 2).

The question which needs posing is: how con one illustrate the morphological variability within single frustules in *Cyclotella*?

Since the characters are considered as a whole (Geissler 1970) and have not been weighted *a priori* with respect to their supposed taxonomic relevance in the multivariate statistical analysis (Sneath & Sokal 1973), it is difficult to find a criterion allowing the unambiguous indentification of frustules as either isovalvar or heterovalvar (apart from the trigonal-nontrigonal distinction made before clustering was applied). An estimate of the quantitative ratio of isovalvar to heterovalvar cells can be obtained, however, based on the results of the heirarchical cluster analyses (Fig. 1). For 55 %

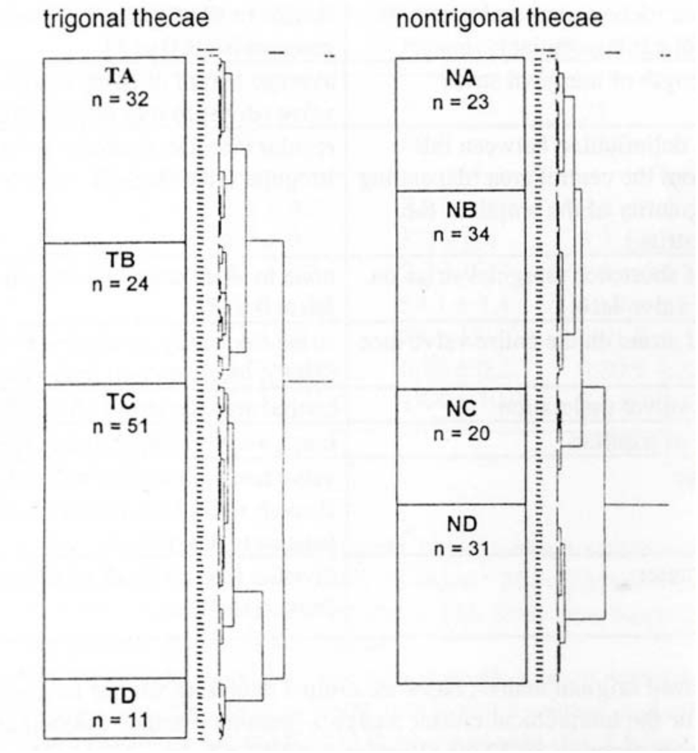


Fig. 1. The two heirarchical cluster analysis dendrograms of trigonal thecae and nontrigonal thecae show eight clusters (TA-ND, the morphological characteristics of the clusters are given in Table 3) and the number of thecae (n) per cluster. The clusters are used to illustrate the morphological variability between thecae of single frustules. For 55 % of the total number of cells (113 frustules) the two phenetic dissimilar thecae of one frustule can be allocated to two different clusters. The Fig. 2 shows examples which clusters are linked together for each lake through heterovalvy. The remaining frustules are isovalvar.

Table 3. The morphological characteristics of the eight clusters (TA-ND) distinguished through hierarchical cluster analysis, based on 11 selected characters (Table 2). The relative frequency of the thecae of the four populations in the different clusters is shown in Fig. 1. Figs in the cluster column illustrate the morphological descriptions.

morphological description of clusters				cluster
trigonal symmetry	mostly larger thecae ((8-)14-21 µm), often with marginal striae that extend in to 2/3 from the margin to the centre central area mostly flat, sometimes with moderately formed papillae	mostly less than 2 shortened striae on the entire valve face, frustules always drum-shaped		**TA** Fig. 11a below
		mostly with 3 or more shortened striae on the entire valve face, some valves slightly dome-shaped or hemispherically inflated		**TB** Fig. 14a
	mostly smaller thecae (8-14 µm), often with marginal striae that extended only halfway to the centre, usually with fewer than 2 shorteded marginal striae on entire valve face in most cases the central area lacks any formed papillae	central area flat or weakly radially undulate		**TC** Fig. 11a above
		central area strongly radially undulate		**TD** Fig. 23a
non-trigonal symmetry	mostly larger thecae (8-19-(22) µm) of drum-shaped frustules, arrangement of puncta (depressions) almost always non-trigonal but radially symmetrical	central area flat, seldom with papillae, usually more or less circular (seldom star shaped)	thecae 8-22 µm, often with marginal striae extended up to 2/3 of the middle on valve face area, partly dome-shaped	**NA** Fig. 17a
			thecae 8-14 µm, often marginal striae extended only to ¼ of the middle on valve face area	**NB** Fig. 18b
		thecae 8-14 µm, central area often strongly radially undulate, without any papillae, usually more or less irregular in outline (star shaped)		**NC** Fig. 23b
	mostly smaller thecae (6-14 µm), central area flat to strong radial undulated with irregularly arranged puncta (depressions), valves often dome-shaped			**ND** Fig. 29b

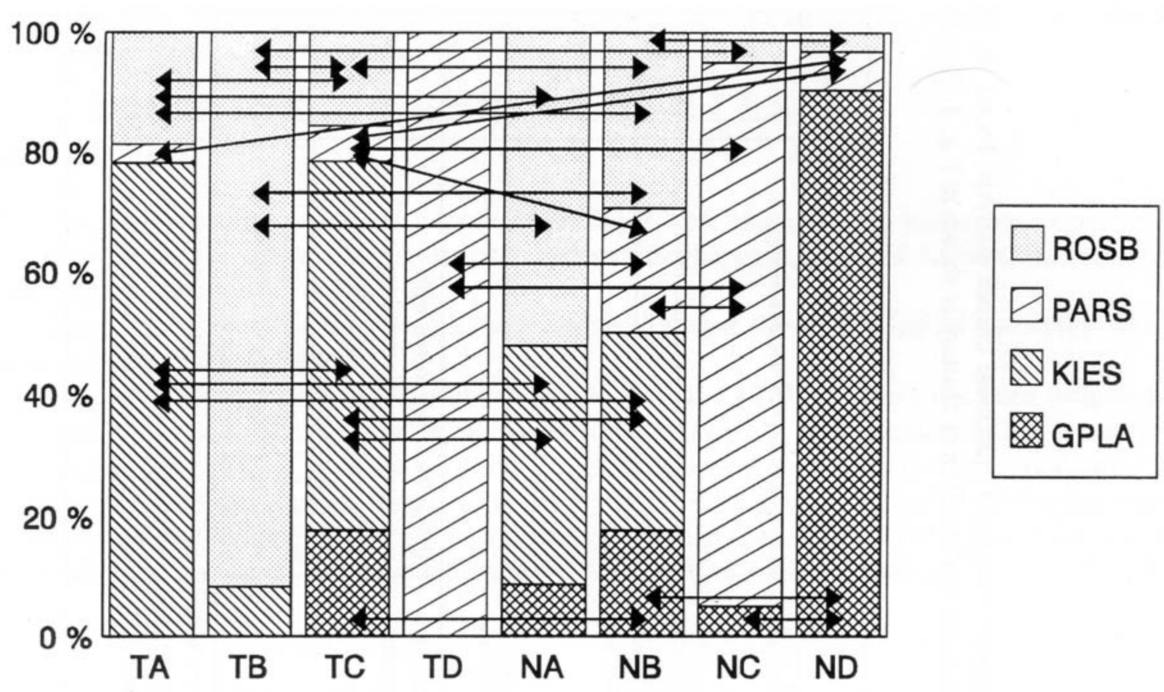


Fig. 2. Relative frequency of thecae of the four populations in the clusters detected by hierarchical cluster analyses (Fig. 1.). The morphological characteristics of the clusters (TA-ND) are given in Table 3. The 24 arrows indicate qualitatively which clusters are linked together for each lake through hetervalvy (ROSB-Rosinsee, PARS-Parsteiner See, KIES-Kiessee, GPLA-Großer Plagesee).

of the total number of cells (i.e. in 113 frustules) the two thecae of the same frustule were allocated to different clusters. In this sense these cells could be characterized as heterovalvar. In Fig. 2. connections between the eight different clusters (Table 3) are shown; these represent examples of heterovalvy. The clusters of phenotypically different thecae thus have no taxonomic significance.

Another convenient comparative method of estimating phenetic resemblance is a principal component plot for the distribution of 226 thecae (cf. Theriot & Stoermer 1984, Theriot *et al.* 1988, Komarek & Marvan 1992). Since the eigenvalues of 2nd and 3rd component are at the same level, it is appropriate to plot both against the 1st component (Fig. 3a, b). In this way, 56 % of total variance is considered. Fig. 3 shows that the points representing the thecae from each of the four lake populations cluster together in principal components 1-3 space. Each population tends to have its own morphological characteristics, but overlap, so that, considered together, there is a continuum of variation.

Figs 4 and 5a make it possible to analyse in more detail the variation and co-variation of different characters (cf. Henrion & Henrion 1988).

The vectors for characters 11 and 7, and to a lesser extent 4, are similar, indicating the correlation between them. The stria number on the entire valve face is strongly related to valve size, as might be expected (characters 7 and 11 in Figs 4, 5a). Thus, the numger of striae per fixed valve circumference (10 μm) is almost constant, although, a weak tendency could be found for smaller valves to have a higher number of striae (number of striae per 10 μm (in brackets the mead valve circumference): Großer Plagesee 17,6 ± 2,2 (8,6 μm), Parsteiner See 17,8 ± 1,4 (12,0 μm), Rosinsee 15,7 ± 2,3 (14,1 μm), Kiessee 13,0 ± 1,3 (16,3 μm)) (corresponding to the variation of interstria number in *Stephanodiscus hantzschii* Grunow by Geissler 1986).

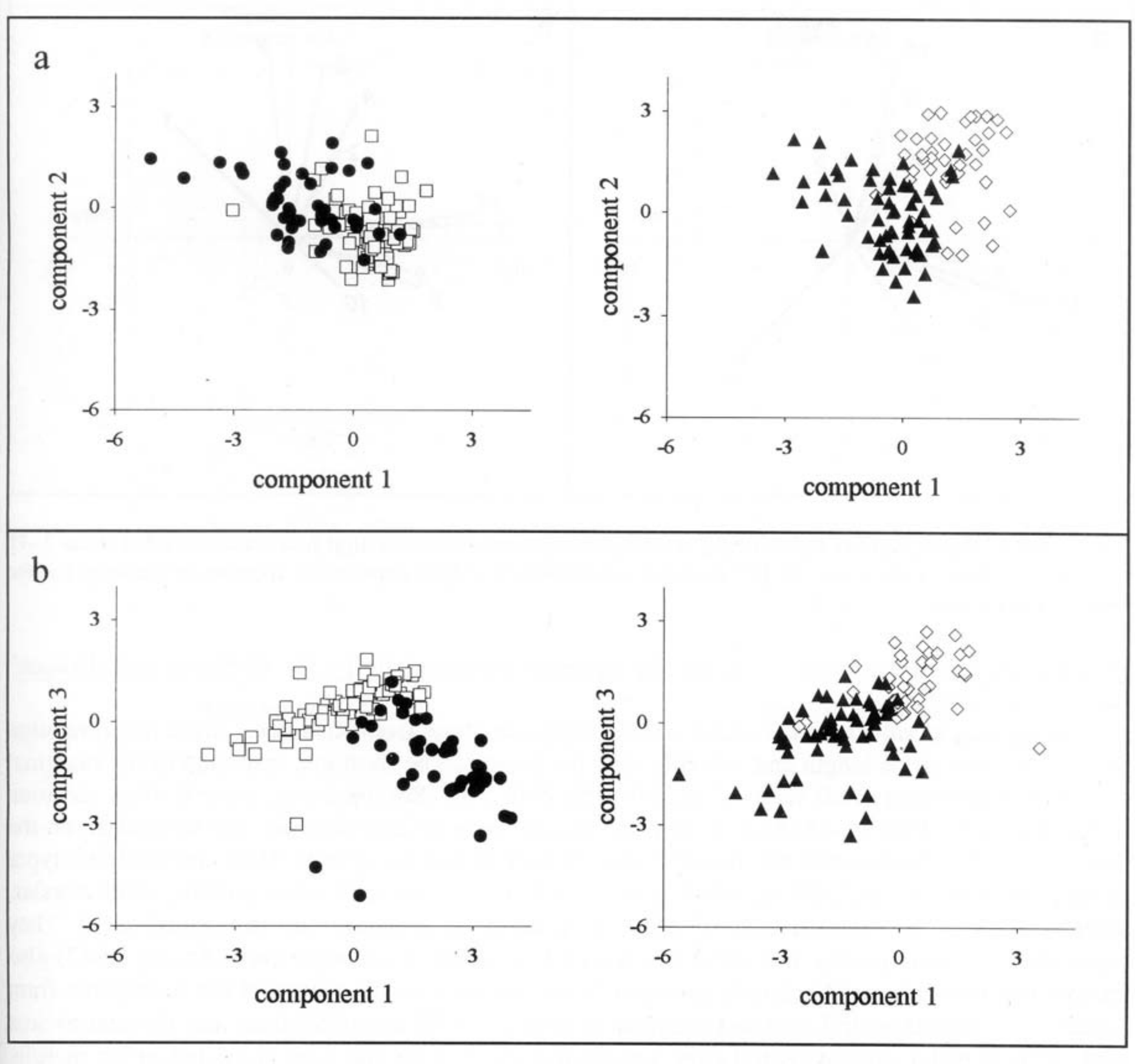


Fig. 3. Plot of the second (a) and third (b) principal component versus the first for distribution of all 226 thecae from four populations. Every symbol represents one theca from Kiessee (squares), Großer Plagesee (circles), Rosinsee (triangles), Parteiner See (rhombs). For clarity the results are shown in two displays (right and left); the percent of variance for the first three components was 27%+15%+14%; the corresponding plots showing the variables (character 1-11) are shown in Fig. 4.

The correlation between stria length and valve size might not have been predicted (characters 4 and 11).

Correlation between valve diameter (size) and relative striae length is especially strong in Kiessee and Gr. Plagesee (Fig. 5a). In smaller valves the striae are generally restircted to the marginal area (this was observed in *Cyclotella ocellata* by Hustedt; in Huber-Pestalozzi 1942). Consequently the relative length of marginal striae seems to be inappropriate as a diabgnostic character for taxonomic delimitation of these *Cyclotella*-species. This character can thus be assigned to the group

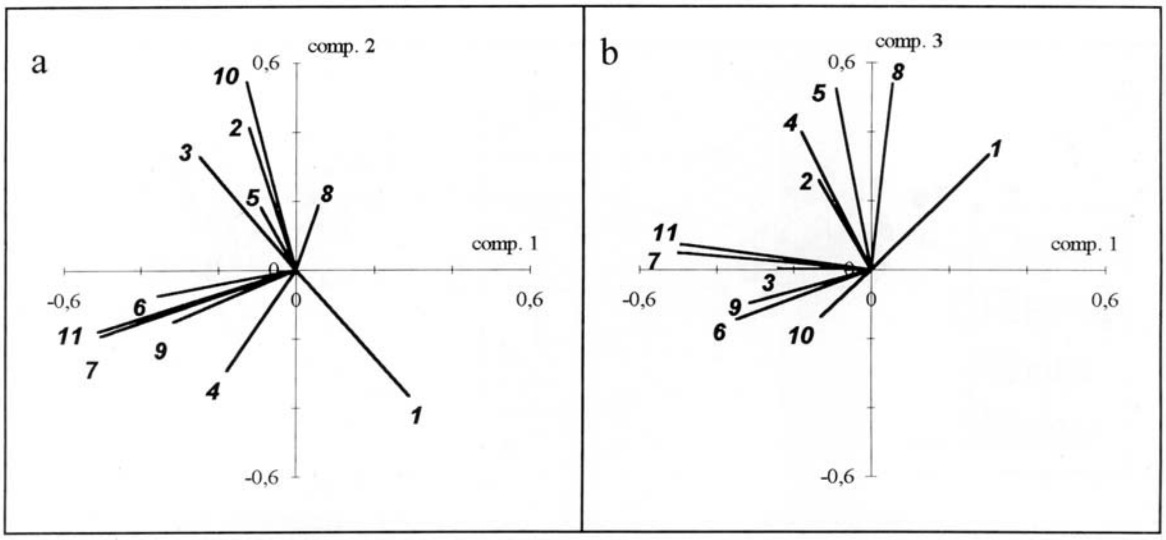


Fig. 4. Plot of the second (a) and third (b) principal components versus the first four variables (characters 1-11 of Tab. 2) in view of the whole of 113 frustules (=226 thecae) of four populations (percent of variance for the first three components in Fig. 3).

of characters whose variation is caused "by reproduction especially by the modus of cell division" (Geissler & Jahn 1986).

In contrast to valves from Kiessee and Gr. Plagesee, those from Rosinsee exhibit no correlation between relative striae length and size (Fig. 5a); the striae can be short and restricted to the marginal area both in large and small valves (Figs 14b, 15b, 16b, 17b, 18b). However, there is often variation within a frustule. Figs 14-18 show short on one valve of each frustule, but long stiae on the other. The same phenomenon was mentioned by Schultz & Trainor (1969). They observed two types of valve in their *Cyclotella*-clone, which they described as "*C. meneghiniana* pattern" (with regular, short striae) and "*C. cryptica* pattern" (with irregular striae extended into the central area). They found that "not infrequently, one valve of each type occurred on the same cell". Straub (1987) also showed that two different *Cyclotella comensis*-forms can exist in one sample at the same time: form A had a star-shaped central area and irregular extensions of the marginal striae into the central area while form B had a circular central area, and regular, short striae that were restricted to the margin. These two types of *C. comensis* may well have been produced by the same cells.

A possible explanation for the different lengths of marginal striae in the two thecae of one frustule could be that, at the moment of the fixation epitheca and hypotheca of one frustule have attained different stages of silification, as a result of being at different stages of their development (A.-M. M. Schmid, personal communication, Pickett-Heaps, Schmid & Edgar 1990).

Some characters, e.g. characters 1 and 8, are not correlated with valve size (Figs 4 and 5a).

The arrangement of large depressions on the central area – trigonal-symmetrical (e.g. Figs 9a/b, 23a), nontrigonal-radial-symmetrical (e.g. Figs 23b, 24a/b), or irregular (e.g. Figs 13a, 25b) – was equally variable in each of four populations (character 1 in Fig. 5a; see also *Cyclotella cf. comensis* given by Kling & Håkansson 1988). Variation between two thecae of one frustule and between thecae of different frustules (e.g. Figs 12a/b, 13a/b, 22a/b) seems to be at random and demonstrates well the extent of phenetic plasticity. The arrangement of depressions in the central area can thus be evaluated as taxonomically meaningless.

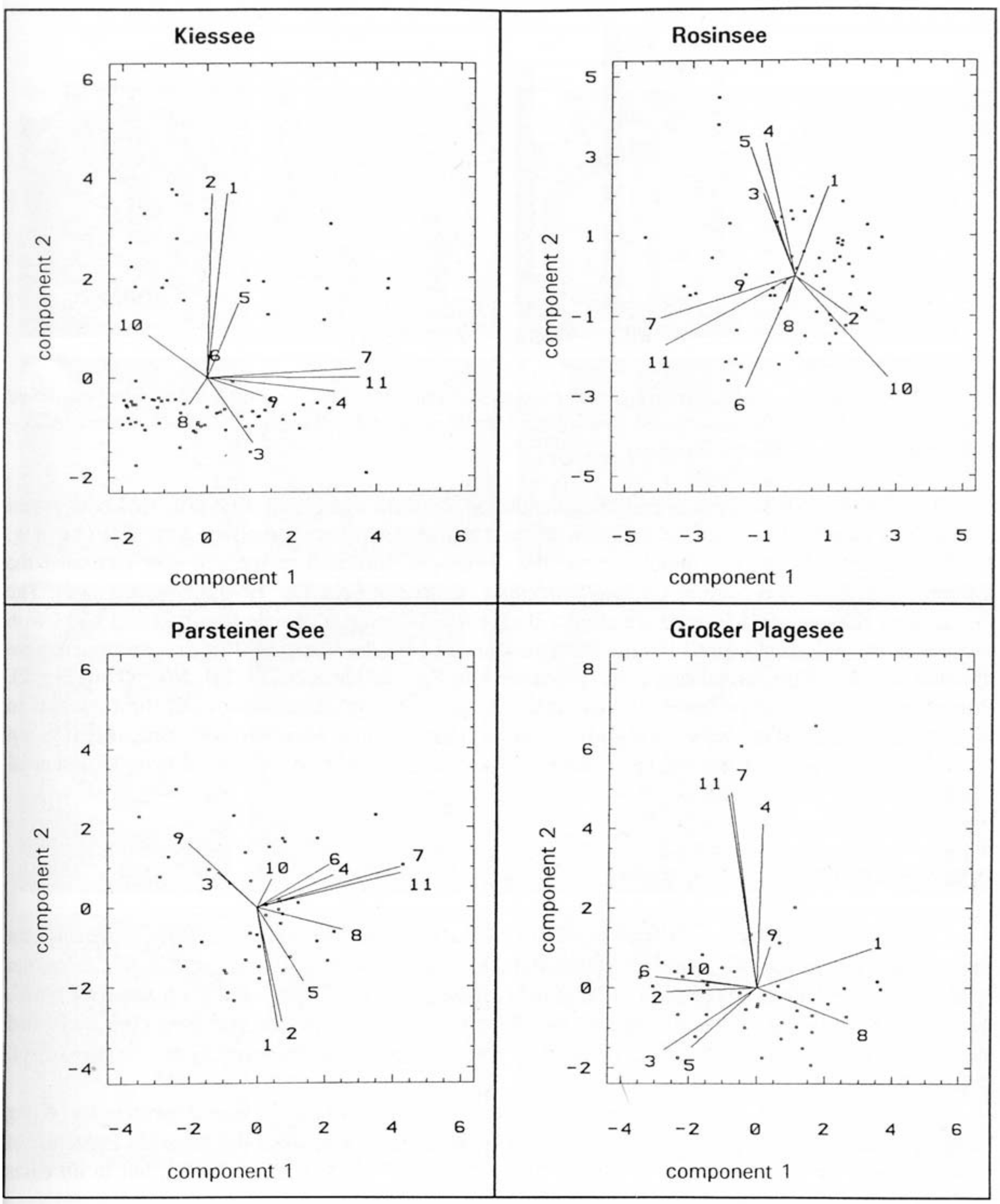


Fig. 5a. Plot of the first two principal components for variables (characters 1-11 cf. Tab. 2) and distribution pattern of thecae for each of the four populations separately (each PCA is based on a data matrix of only one population; percent of variance for the first two components: Gr. Plagesee 28 %+23 %, Kiessee 26 %+20 %, Parsteiner See 24 %+18 %, Rosinsee 23 %+18 %).

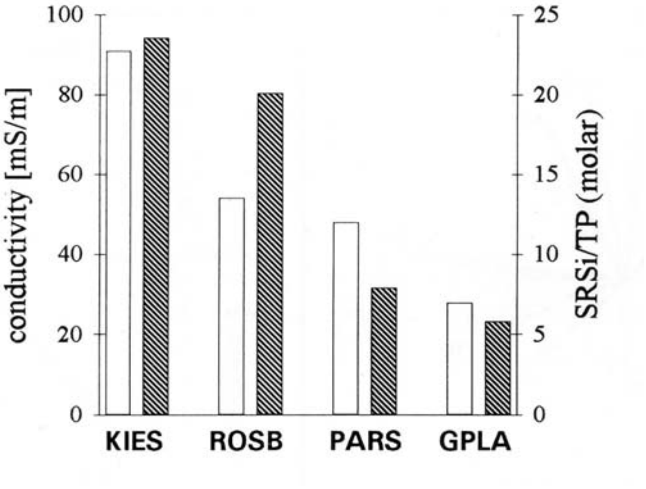


Fig. 5b. Bar charts showing the average annual conductivity (unshaded area) and the soluble reactive silicon/ total phosphorus-ratio (SRSi /TP: shaded area) of the four lakes (ROSB – Rosinsee, KIES – Kiessee, PARS – Parsteiner See, GPLA – Großer Plagesee).

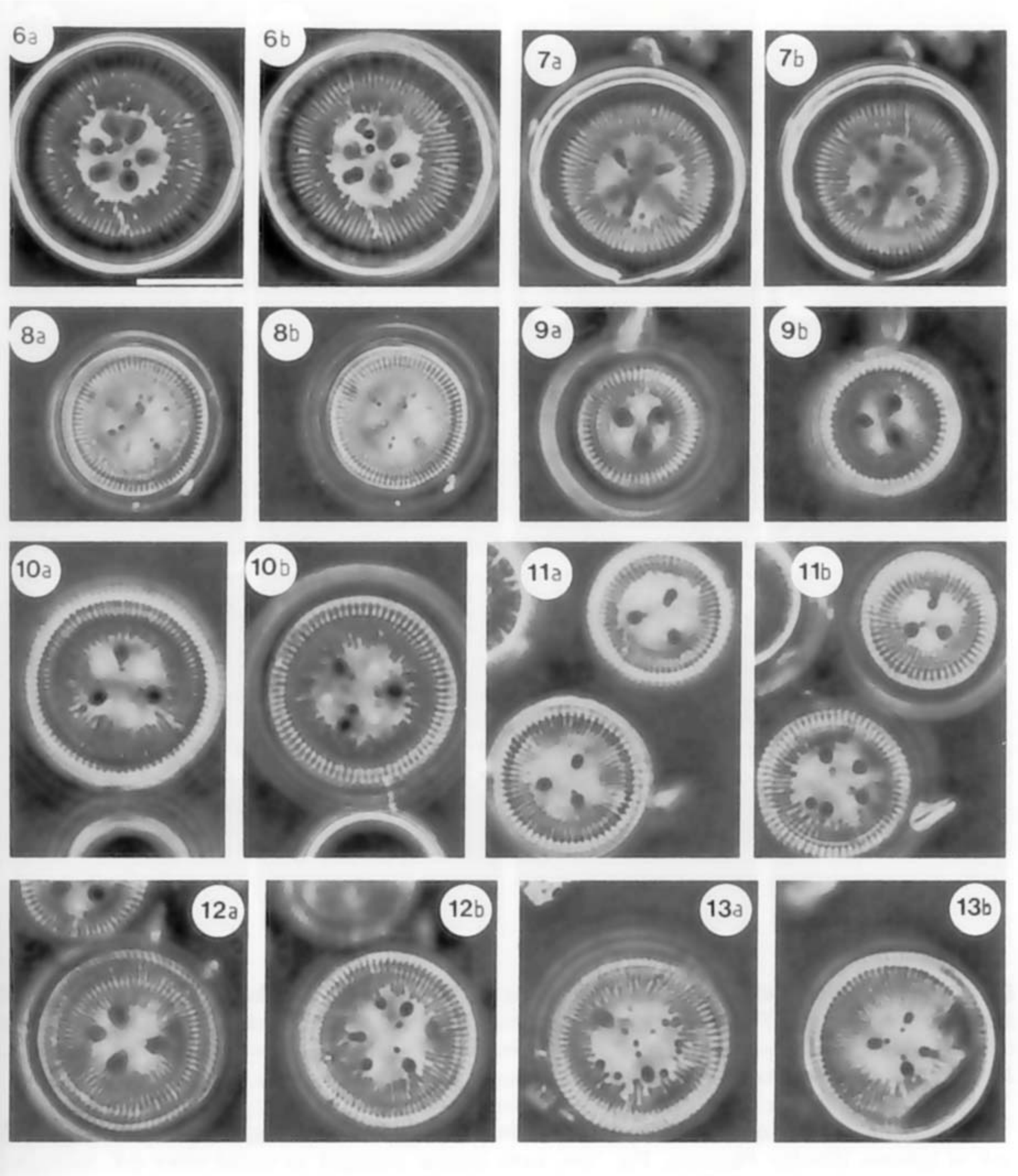
The vectors for characters 8 and 11 in the plot of the third against the first principal component (in Fig. 4b) clearly show that the variation in the form of central area, ranging from flat (e.g. Fig. 11a/b) to a strongly radially undulate (e.g. Fig. 23a/b), is unrelated to the cell size (see also the different central areas present in *Cyclotella ocellata*: Casper & Scheffler 1990, pl. 4, figs 1, 3). The thecae from Kiessee and Rosinsee are almost always flat. In contrast, thecae from the two lakes with lower conductivity and lower Si:P ratio, Parsteiner See and Großer Plagesee (Fig. 5b), are much more variable and often have undulate valves (character 8 in Fig. 5a; clusters TC, TD, NC, ND in Fig. 2). Regarding this, it would be interesting to subject a larger number of populations investigated it is not possible to determine to what extent the degree of undulation could be influenced by environmental conditions.

DISCUSSION

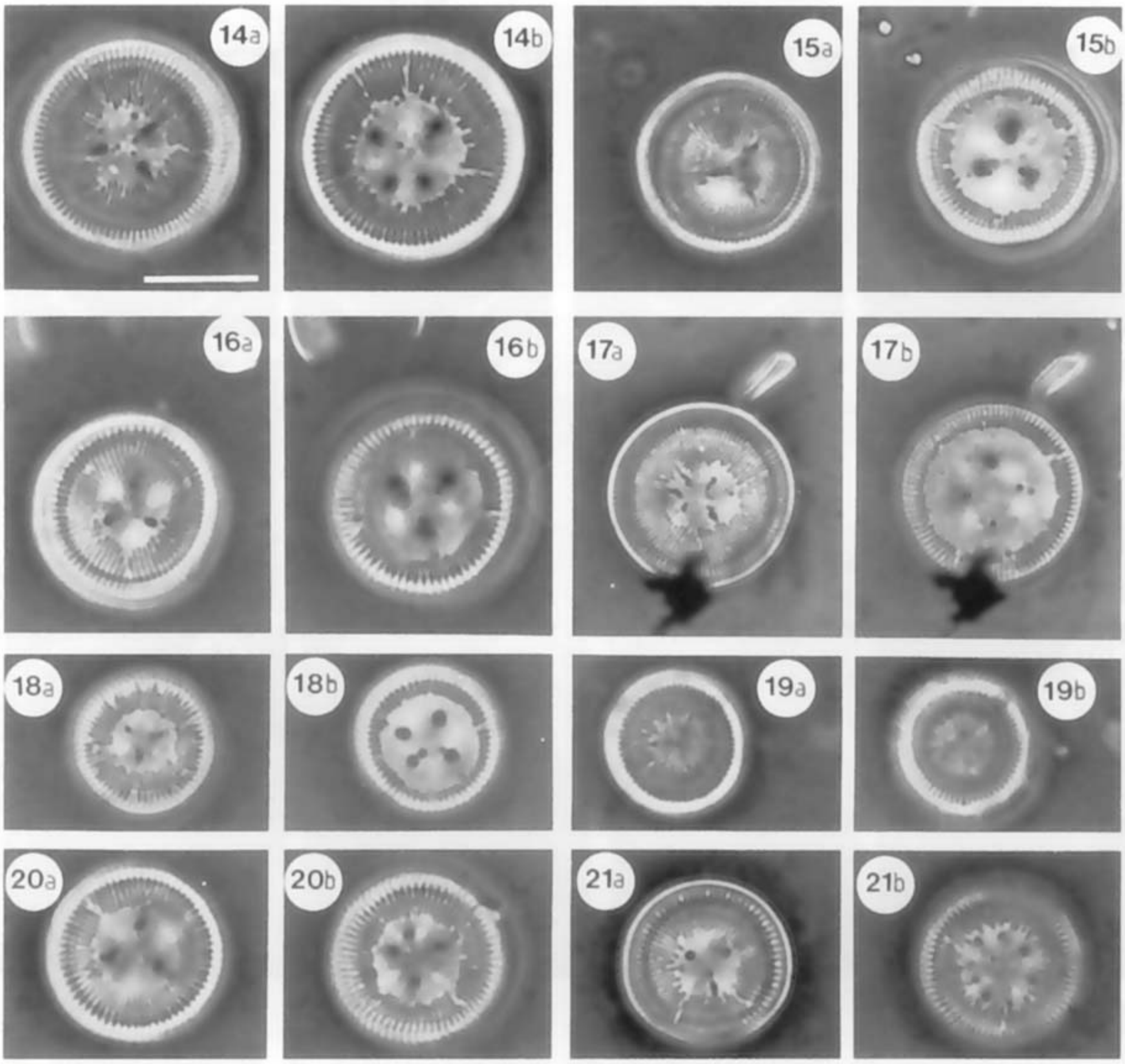
The taxonomic delimitation of *C. krammeri, C. kueztingiana* var. *planetophora, C. kuetzingiana* var. *radiosa, C. ocellata, C. comensis* has proved difficult. For example, the separation of *C. ocellata* (Håkansson 1990, 1993, Håkansson in Krammer & Lange-Bertalot 1991) and *C. comensis* from *C. kuetz*. var. *planetophora* was not supported by Cleve-Euler (1951). Klee & Steinberg (1987) claimed to have found intermediates between *C. ocellata* and *C. kuetzingiana* respectively to *C. comensis* (cf. Klee, Schmidt & Müller 1993).

Some authors have described the morphological plasticity of these *Cyclotella* species (e.g. Kling & Håkansson 1988, Håkansson 1990, 1993), while others have discussed the possible existence of different forms or morphotypes (e.g. Straůb 1987, Klee et al. 1993, Schmidt 1993), but in all cases analysis has been at the level of single thecae.

In view of pleomorphy in wall structure demonstrated previously between the two thecae of a frustule and within thecae of different populations (Hasle, Heimdal & Fryxell 1971, Haworth 1983), it is clearly impossible to base the taxonomic description on one o a few valves that seem to have a particularly characteristic morphology.

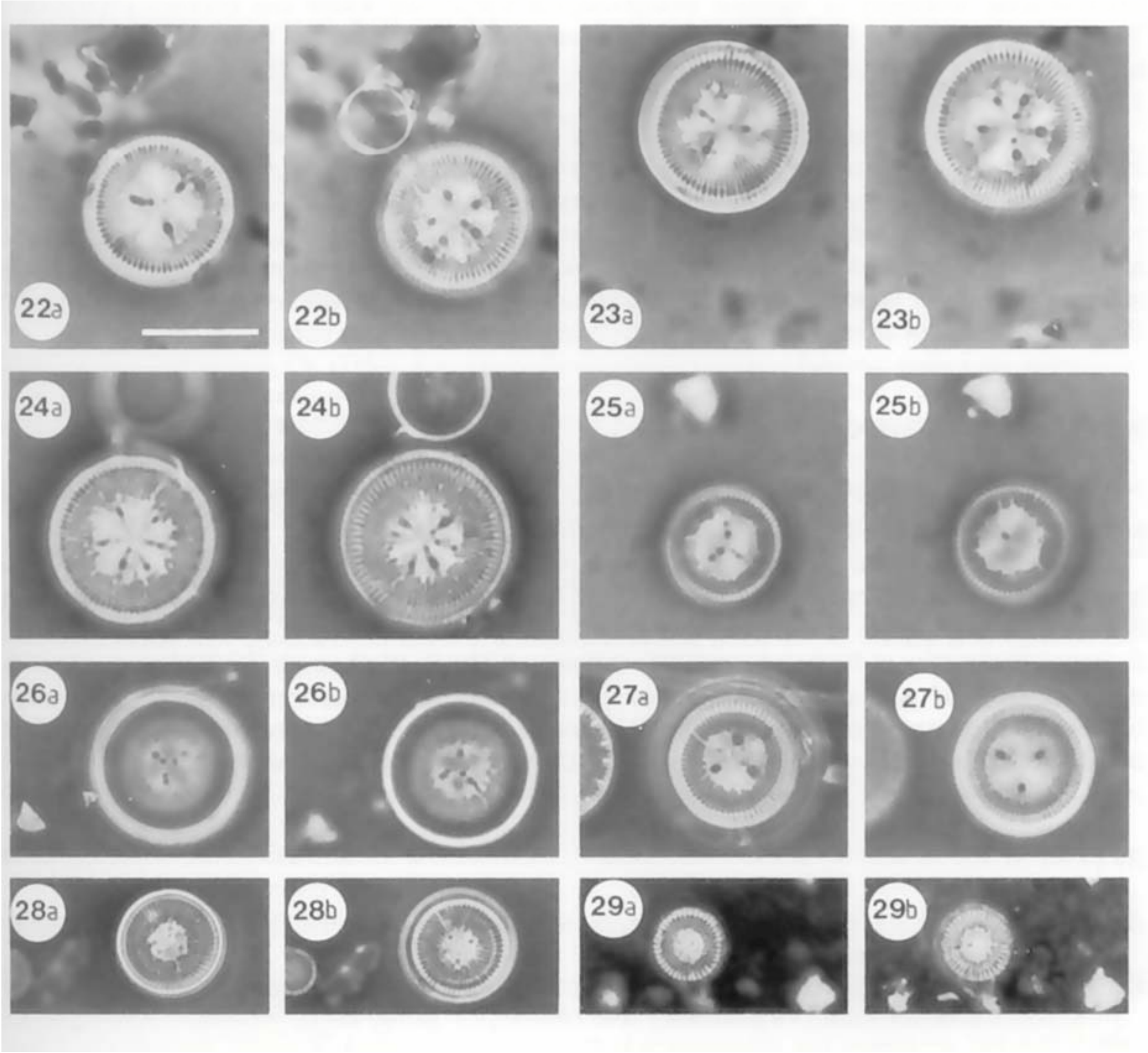


Figs 6-13. *Cycotella*, Kiessee population, LM. Scale bar (Fig. 6a) = 10 µm. In each case the two thecae (a + b) of a frustule are shown. Figs 6, 7. Dome shaped initial cells (?) at a stage of cell wall development with "*C. kuetzingiana* var. *radiosa*"-pattern. Figs 9, 10a, 11a, 11b (above). "*C. ocellata*"-pattern. Figs 11b (below), 12, 13b. "*C. ocellata-C. kuetz*. var. *planetophora*"-pattern. Fig. 13a "*C. kuetzingiana* var. *planetohpora*"-pattern.



Figs 14-21. *Cyclotella*, Rosinsee population, LM. Scale bar (Fig. 14a) = 10 µm. The two thecae (a + b) of each frustule are shown, as in Figs 6-13. Figs 14-18 show obvious differences in striae length between the two thecae of one frustule. Figs 19-21. The “a”-thecae show trigonal symmetry, in contrast to the “b”-thecae, Fig. 20b shows a slight hint of the “*C. comensis*”-pattern.

The dome shaped initial cells (?) found in plankton samples both in spring (Kiessee, Figs 6, 7) and in autumn (Großer Plagesee, Fig. 26), have puncta arranged in radial rows (cf. multistate character 3 in Table 2) and could be identified therefore as “*Cyclotella kuetzingiana* var. *radiosa*” (cf. Fricke in Schmidt’s Atlas 1900, fig. 8). Thecae that deviate from trigonal or radial symmetry (cf. multistate character 1 in Table 2) culminating in irregular arrangement of structures (e.g. Fig. 13a) could be referred to “*C. kuetzingiana* var. *planetophora*” (cf. Hustedt in Huber-Pestalozzi 1942, fig. 491). Some thecae with a flat central area could be characterized as “*C. ocellata*” or as “*C. ocellata-*



Figs 22-29. *Cyclotella*, Parsteiner and Großer Plagesee populations, LM. Scale bar (Fig. 22a) = 10 µm. The two thecae (a + b) of each frustules are shown, as in Figs 6-13. Figs 22-25. Parsteiner See population. Figs 22-24 show more or less radially undulate valve surfaces. Figs 23b, 24 show nontrigonal symmetry with "*C. comensis*"-pattern. Fig. 25 shows a frustule with weakly tangentially undulate valve surfaces. Figs 26-29. Großer Plagesee population. Fig. 26. Dome-shaped initial cell (?) at a stage of cell wall development with "*C. kuetzingiana* var. *radiosa*"-pattern.

C. kuetz. var. *planetophora*" (Figs 9a/b, 10a, 11a/b, 12a/b, 27a, 18b,) and those with radially undulate central area as "C. comensis" (Figs 23b, 24a/b) (cf. Van Heurck 1882, Fricke in Schmidt 1900, Pantocsek 1902, Hustedt in Huber-Pestalozzi 1942, Cleve-Euler 1951). By studying frustules, rather than separated valves, and by examining both thecae of each frustule, it is possible to examine variation in a new way. On the one hand, one can search for maximal phenotypic dissimilarity between the thecae of single frustules; on the other hand, if one of the two thecae of one frustule is very similar to one of the thecae of a different frustule, this provides a basis for linking variants and demonstrating continuity of variation (morphological transitions) within and between populations. The latter approach can be demonstrated, for example, by linking certain of the thecae illustrated here, to make the following series: Figs 9a/b-16b - 16a-23a - 23b-24a/b, 11a(below) – 11b (below)-12b – 12a-13b – 13a.

With the proviso that these observations have been carried out exclusively with LM (Håkansson 1990, 1993) and may therefore present an incomplete picture of variation, it can be concluded that the thecae of all four populations belong to the same, polymorphic species. This is demonstrated by variation both within individual frustules and between different frustules. Morphological variants may represent different stages in cell wall development or different stages of life cycle, or may reflect growth under various environmental conditions. The decision which name should be applied to this entity calls for an re-investigation of the type material of all taxa concerned.

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A LIGHT MICROSCOPICAL INVESTIGATION AND MULTIVARIATE STATISTICAL ANALYSES OF HETEROVALVAR CELLS OF *CYCLOTELLA*-SPECIES (BACILLARIOPHYCEAE) FROM LAKES OF THE BERLIN–BRANDENBURG REGION

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113 randomly selected frustules of *Cyclotella* taxa, taken from the plankton of four lakes, have been investigated with respect to variation between the two thecae of each frustule and also variation within and between populations occurring in the different lakes. Multivariate statistical analyses of phenetic similarity of the 226 thecae were based on a data matrix recording the status of 11 characters drawn from the structure of the siliceous wall. From the cluster analysis, 55% of the frustules could be characterized as heterovalvar. In these cases, the phenetically dissimilar thecae of a single frustule were allocated to different clusters. Principle component analyses showed that the variation in the relative length of the marginal striae, and also the number of marginal striae on the entire valve face, are correlated with valve circumference. The arrangement of depressions on central valve and the development of the central valvar undulation appear to be unrelated to valve size. Due to the existence of different patterns of markings within one frustule ("*Cyclotella kuetzingiana* var. *radiosa*"-pattern, "*C. kuetzingiana* var. *planetophora*"-pattern, "*C. ocellata*"-pattern, "*C. comensis*"-pattern), these patterns have limited or no taxonomic value. Thus, as far as one can judge from LM observation alone, it seems reasonable to assign all the investigated frustules to the same species, which thus shows great phenotypic plasticity in cell wall structure.*

INTRODUCTION

The taxonomic delimitation of some *Cyclotella* taxa (*C. krammeri* Håkansson, *C. kuetzingiana* var. *planetophora* Fricke, *C. kuetzingiana* var. *radiosa* Fricke, *C. ocellata* Pantocsek, *C. comensis* Grunow) is difficult, because of the polymorphism of the valve. While it has been common in the past to keep these taxa separate, some authors have emphasized the phenotypic similarity and have noted morphological transitions; it is possible that all are part of the same species (Cleve-Euler 1951, Klee & Steinberg 1987, Håkansson 1990, 1993, Håkansson in Krammer & Lange-Bertalot 1991).

In the course of comparative studies of the phytoplankton of lakes and ponds in the Berlin and Brandenburg regions of Germany, *Cyclotella* cells were found that could be assigned to two different

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* The results of this paper were presented on a poster at the 8th Meeting of German speaking Diatomists at Bremerhaven 25–27.3.1994.

taxa since the epi- and hypotheca of one cell had different structures. Frustules having valves with either different or similar surface markings are here called "heterovalvar" and "isovalvar" respectively. This use of heterovalvy has not the same meaning as that in Round, Crawford & Mann 1990. The phenomenon of heterovalvar cells is dealt with in this paper.

My aim is to describe the variability of morphological characters between the thecae of a single frustule, between the frustules in each population and between the frustules of different populations. This is done via the multivariate statistical analyses.

MATERIAL AND METHODS

Material was taken from Kiessee in Berlin-Mühlenbeck/Mönchsmühle; Rosinsee, Parsteiner See and Großer Plagesee in Brandenburg, near Brodowin, north of Eberswalde (morphometric, physical and chemical data in Table 1).

Table 1. Morphometric*, average annual physical and chemical data of the lakes from which the *Cyclotella* populations were taken (mean values and standard deviation, samples were taken from a depth of 0.5m).

	Kiessee	Rosinsee	Parsteiner See	Gr. Plagesee
max. lake depth [m]	4.5	9	27	5
lake area [ha]	20	20	1100	78
secchi depth [m]	1.4 ± 0.3	3.3 ± 1.7	3.8 ± 1.0	1.3 ± 0.4
pH	8.2 ± 0.1	8.2 ± 0.4	8.2 ± 0.4	8.3 ± 0.3
alkalinity [meq/l]	2.2 ± 0.3	2.3 ± 0.1	1.9 ± 0.1	1.2 ± 0.1
conductivity [mS/m]	90.8 ± 2.3	54.1 ± 1.4	48.0 ± 1.3	27.9 ± 9.1
total nitrogen [µmol/l]	71 ± 19	89 ± 13	68 ± 8	101 ± 12
total phosphorus [µmol/l]	1.43 ± 0.5	0.98 ± 0.2	0.80 ± 0.3	1.13 ± 0.5
soluble reactive silicon [µmol/l]	35.7 ± 25.4	18.4 ± 10.0	7.1 ± 6.1	5.3 ± 5.4

* data from Anwand 1973

The frustules studied were derived from monthly phytoplankton samples, taken in 1992. The samples contained an abundance of *Cyclotella* cells (Kiessee: March/April/June; Rosinsee: March; Parsteiner See: March/April; Großer Plagesee: October). The study was based exclusively on light microscopy (LM), using positive phase-contrast optics.

For preparing permanent slides, samples were treated gently to keep both thecae of each frustule together. For this reason the plankton samples (fixed with Lugol's solution and stored at 10°C for not longer than a year) were cleaned by warming in hydrogen peroxide (for 7 hours, up to 60°C, twice) and then in a mixture of hydrogen peroxide and acetic acid (in a ratio 2:1; again for 7 hours), and finally washed and mounted in naphrax.

From the four lakes 113 cells were selected at random for investigation. Multivariate statistical analyses of phenetic similarity between the 226 thecae were based on a data matrix of variation in 11 valve characters, which are listed in Table 2. The number of marginal fultoportulae (Håkansson & Carter 1990) was not included as a character since it could not be determined for every valve and so would have left gaps in the data matrix. Marginal fultoportulae are difficult to observe in LM and often were identifiable only in one theca of a frustule or only around a part of the margin of the valve (compare Figs 12a, 16a, 21a, 24a/b; see also Håkansson 1990, p. 266).

Table 2. Eleven characters selected for the estimation of phenetic resemblance between thecae (LM-observation). These were the basis for the data matrix subjected to the multivariate statistical analysis.

running number	multistate character	character states
1	symmetry of arrangement of large puncta (depressions) in the central area	trigonal symmetrical arrangement (state 0) non-trigonal – radial arrangement (state 1) irregular arrangement (state 2) central area without structure (state 3)
2	number of large puncta (depressions in the central area)	no depressions, one or two; three; four; five; more than five depressions (state 0 to 4)
3	type of puncta arrangement in the central area (depressions and openings of valve face fultoportulae)	no puncta; puncta in the form of single point; double or three points; points and grooves; grooves (state 0 to 4)
4	relative length of marginal striae	average length of striae relative to the radius of valve (division into intervals) (state 0 to 3)
5	structural delimitation between the margin from the central area (depending on the regularity of the length of the marginal striae)	regular circular; moderately irregular; strongly irregular (star-shaped) states (state 0 to 2)
6	number of shortened marginal striae on the entire valve face	none to more than 3 shortened striae (state 0 to 3)
7	number of striae on the entire valve face	striae frequency in relation to a fixed angle (360°), (division into intervals) (state 0 to 3)
8	degree of valvar undulation	central area flat to radially undulate (state 0 to 3)
9	moulding of papillae	none, weakly or moderately formed (state 0 to 2)
10	valve shape	valve hemispherically inflated, slightly dome-shaped; valve face planar (drum-shaped frustule) (state 0 to 2)
11	valve diameter	division into intervals of different sizes (state 0 to 4)

A non-standardized original matrix, based on ordinal data with similar ranges of values for each character was used for the hierarchical cluster analyses (gamma distance [Goodman-Kruskals], ward linkage; using computer program SYSTAT (Version 5, SYSTAT, Inc., USA). The data were divided *a priori* into two subsets, consisting of trigonal and nontrigonal valves, respectively (see Figs 19–21 for contrasted trigonal and nontrigonal valves), to make it easier to demonstrate the heterovalvy of frustules.

A standardized matrix of both ordinal and metric data (correlation matrix) was used for all principal component analyses (PCA), using computer program STATGRAPHICS (Version 5, STSC, Inc., USA). The extraction of variables in result from PCA has not been carried out since only four lakes have been investigated and consequently the question arises as to what extent the 226 thecae show the full range of variation.

RESULTS

The morphological characters chosen for analyses were those already known or suspected to vary between the two thecae of a single individual and also between thecae of different frustules (see multistate characters 1–11 in Table 2).

The question which needs posing is: how can one illustrate the morphological variability within single frustules in *Cyclotella*?

Since the characters are considered as a whole (Geissler 1970) and have not been weighted *a priori* with respect to their supposed taxonomic relevance in the multivariate statistical analysis (Sneath & Sokal 1973), it is difficult to find a criterion allowing the unambiguous identification of frustules as either isovalvar or heterovalvar (apart from the trigonal-nontrigonal distinction made before clustering was applied). An estimate of the quantitative ratio of isovalvar to heterovalvar cells can be obtained, however, based on the results of the hierarchical cluster analyses (Fig. 1). For 55%

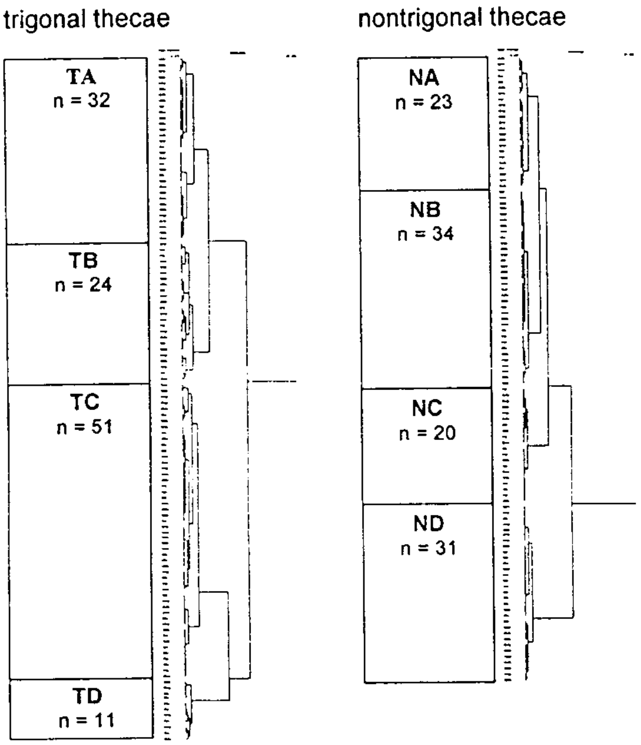


Fig. 1. The two hierarchical cluster analysis dendrograms of trigonal thecae and nontrigonal thecae show eight clusters (TA–ND, the morphological characteristics of the clusters are given in Table 3) and the number of thecae (n) per cluster. The clusters are used to illustrate the morphological variability between thecae of single frustules. For 55% of the total number of cells (113 frustules) the two phenetic dissimilar thecae of one frustule can be allocated to two different clusters. The Fig. 2 shows examples which clusters are linked together for each lake through heterovalvy. The remaining frustules are isovalvar.

Table 3. The morphological characteristics of the eight clusters (TA–ND) distinguished through hierarchical cluster analysis, based on 11 selected characters (Table 2). The relative frequency of the thecae of the four populations in the different clusters is shown in Fig. 1. Figs in the cluster column illustrate the morphological descriptions.

morphological description of clusters				cluster
trigonal symmetry	mostly larger thecae ((8–)14–21 µm), often with marginal striae that extend in to 2/3 from the margin to the centre central area mostly flat, sometimes with moderately formed papillae	mostly less than 2 shortened striae on the entire valve face, frustules always drum-shaped		**TA** Fig. 11a below
		mostly with 3 or more shortened striae on the entire valve face, some valves slightly dome-shaped or hemispherically inflated		**TB** Fig. 14a
	mostly smaller thecae (8–14 µm), often with marginal striae that extended only halfway to the centre, usually with fewer than 2 shortened marginal striae on entire valve face in most cases the central area lacks any formed papillae	central area flat or weakly radially undulate		**TC** Fig. 11a above
		central area strongly radially undulate		**TD** Fig. 23a
non-trigonal symmetry	mostly larger thecae (8–19–(22) µm) of drum-shaped frustules, arrangement of puncta (depressions) almost always non-trigonal but radially symmetrical	central area flat, seldom with papillae, usually more or less circular (seldom star shaped)	thecae 8–22 µm, often with marginal striae extended up to 2/3 of the middle on valve face area, partly dome-shaped	**NA** Fig. 17a
			thecae 8–14 µm, often marginal striae extended only to 1/4 of the middle on valve face area	**NB** Fig. 18b
		thecae 8–14 µm, central area often strongly radially undulate, without any papillae, usually more or less irregular in outline (star shaped)		**NC** Fig. 23b
	mostly smaller thecae (6–14 µm), central area flat to strong radial undulated with irregularly arranged puncta (depressions), valves often dome-shaped			**ND** Fig. 29b

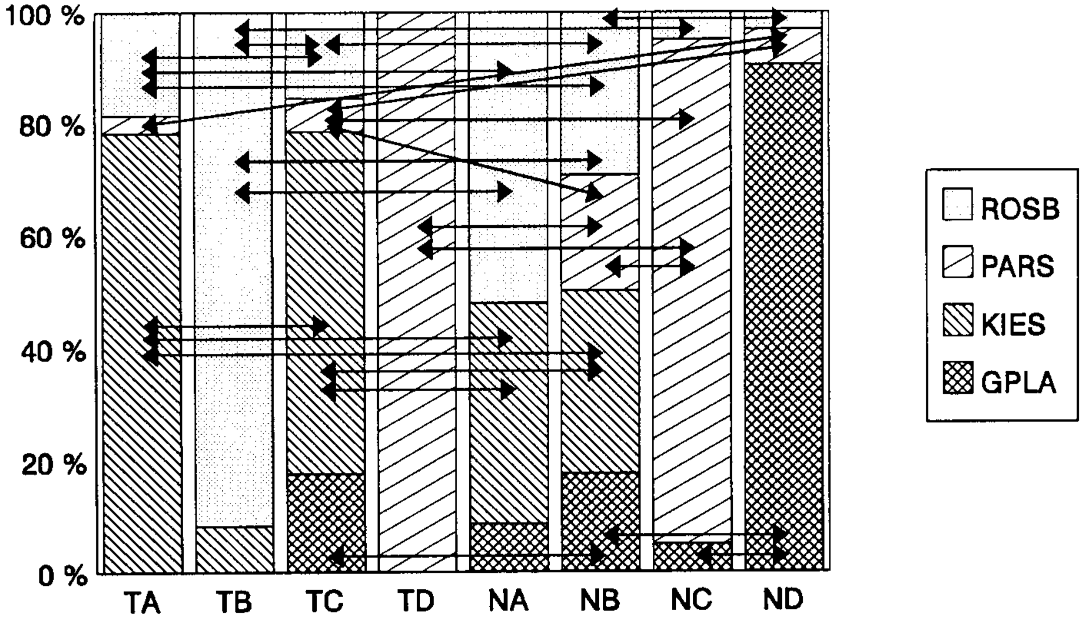


Fig. 2. Relative frequency of thecae of the four populations in the clusters detected by hierarchical cluster analyses (Fig. 1). The morphological characteristics of the clusters (TA–ND) are given in Table 3. The 24 arrows indicate qualitatively which clusters are linked together for each lake through heterovalvy (ROSB–Rosinsee, PARS–Parsteiner See, KIES–Kiessee, GPLA–Großer Plagesee).

of the total number of cells (i.e. in 113 frustules) the two thecae of the same frustule were allocated to different clusters. In this sense these cells could be characterized as heterovalvar. In Fig. 2. connections between the eight different clusters (Table 3) are shown; these represent examples of heterovalvy. The clusters of phenotypically different thecae thus have no taxonomic significance.

Another convenient comparative method of estimating phenetic resemblance is a principal component plot for the distribution of 226 thecae (cf. Theriot & Stoermer 1984, Theriot *et al.* 1988, Komarek & Marvan 1992). Since the eigenvalues of 2nd and 3rd component are at the same level, it is appropriate to plot both against the 1st component (Fig. 3a, b). In this way, 56% of total variance is considered. Fig. 3 shows that the points representing the thecae from each of the four lake populations cluster together in principal components 1–3 space. Each population tends to have its own morphological characteristics, but overlap, so that, considered together, there is a continuum of variation.

Figs 4 and 5a make it possible to analyse in more detail the variation and co-variation of different characters (cf. Henrion, Henrion & Henrion 1988).

The vectors for characters 11 and 7, and to a lesser extent 4, are similar, indicating the correlation between them. The stria number on the entire valve face is strongly related to valve size, as might be expected (characters 7 and 11 in Figs 4, 5a). Thus, the number of striae per fixed valve circumference (10 µm) is almost constant, although, a weak tendency could be found for smaller valves to have a higher number of striae (number of striae per 10 µm (in brackets the mean valve circumference): Großer Plagesee 17,6 ± 2,2 (8,6 µm), Parsteiner See 17,8 ± 1,4 (12,0 µm), Rosinsee 15,7 ± 2,3 (14,1 µm), Kiessee 13,0 ± 1,3 (16,3 µm)) (corresponding to the variation of interstria number in *Stephanodiscus hantzschii* Grunow by Geissler 1986).

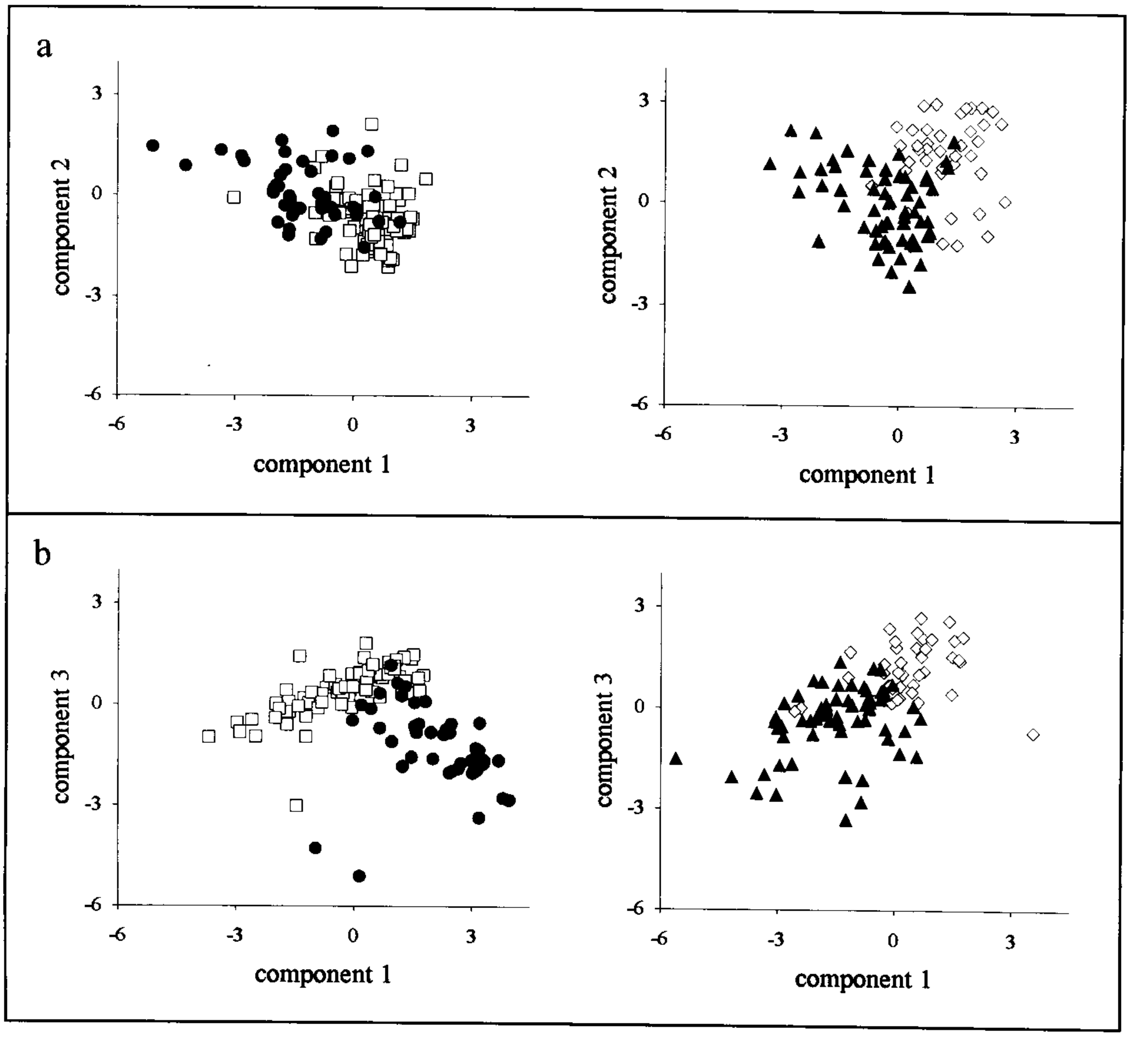


Fig. 3. Plot of the second (a) and third (b) principal component versus the first for distribution of all 226 thecae from four populations. Every symbol represents one theca from Kiessee (squares), Großer Plagesee (circles), Rosinsee (triangles), Parsteiner See (rhombs). For clarity the results are shown in two displays (right and left); the percent of variance for the first three components was 27%+15%+14%; the corresponding plots showing the variables (character 1–11) are shown in Fig. 4.

The correlation between stria length and valve size might not have been predicted (characters 4 and 11).

Correlation between valve diameter (size) and relative striae length is especially strong in Kiessee and Gr. Plagesee (Fig. 5a). In smaller valves the striae are generally restricted to the marginal area (this was observed in *Cyclotella ocellata* by Hustedt; in Huber-Pestalozzi 1942). Consequently the relative length of marginal striae seems to be inappropriate as a diagnostic character for taxonomic delimitation of these *Cyclotella*-species. This character can thus be assigned to the group

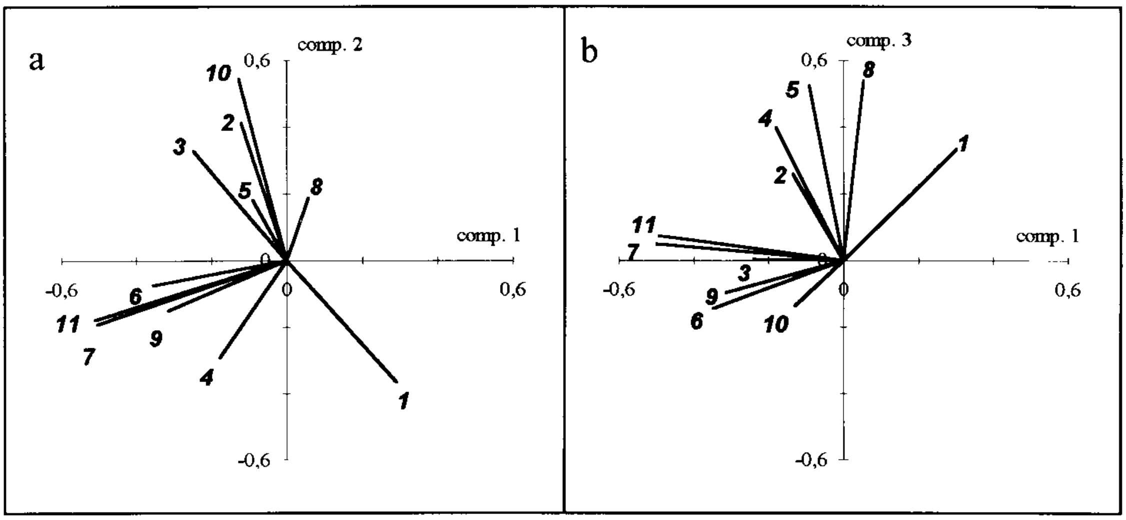


Fig. 4. Plot of the second (a) and third (b) principal component versus the first four variables (characters 1–11 of Tab. 2) in view of the whole of 113 frustules (=226 thecae) of four populations (percent of variance for the first three components in Fig. 3).

of characters whose variation is caused "by reproduction especially by the modus of cell division" (Geissler & Jahn 1986).

In contrast to valves from Kiessee and Gr. Plagesee, those from Rosinsee exhibit no correlation between relative striae length and size (Fig. 5a); the striae can be short and restricted to the marginal area both in large and small valves (Figs 14b, 15b, 16b, 17b, 18b). However, there is often variation within a frustule. Figs 14–18 show short striae on one valve of each frustule, but long striae on the other. The same phenomenon was mentioned by Schultz & Trainor (1969). They observed two types of valve in their *Cyclotella*-clone, which they described as "*C. meneghiniana* pattern" (with regular, short striae) and "*C. cryptica* pattern" (with irregular striae extended into the central area). They found that "not infrequently, one valve of each type occurred on the same cell". Straub (1987) also showed that two different *Cyclotella comensis*-forms can exist in one sample at the same time: form A had a star-shaped central area and irregular extensions of the marginal striae into the central area while form B had a circular central area, and regular, short striae that were restricted to the margin. These two types of *C. comensis* may well have been produced by the same cells.

A possible explanation for the different lengths of marginal striae in the two thecae of one frustule could be that, at the moment of the fixation epitheca and hypotheca of one frustule have attained different stages of silification, as a result of being at different stages of their development (A.-M. M. Schmid, personal communication, Pickett-Heaps, Schmid & Edgar 1990).

Some characters, e.g. characters 1 and 8, are not correlated with valve size (Figs 4 and 5a).

The arrangement of large depressions on the central area – trigonal-symmetrical (e.g. Figs 9a/b, 23a), nontrigonal-radial-symmetrical (e.g. Figs 23b, 24a/b), or irregular (e.g. Figs 13a, 25b) – was equally variable in each of four populations (character 1 in Fig. 5a; see also *Cyclotella cf. comensis* given by Kling & Håkansson 1988). Variation between two thecae of one frustule and between thecae of different frustules (e.g. Figs 12a/b, 13a/b, 22a/b) seems to be at random and demonstrates well the extent of phenetic plasticity. The arrangement of depressions in the central area can thus be evaluated as taxonomically meaningless.

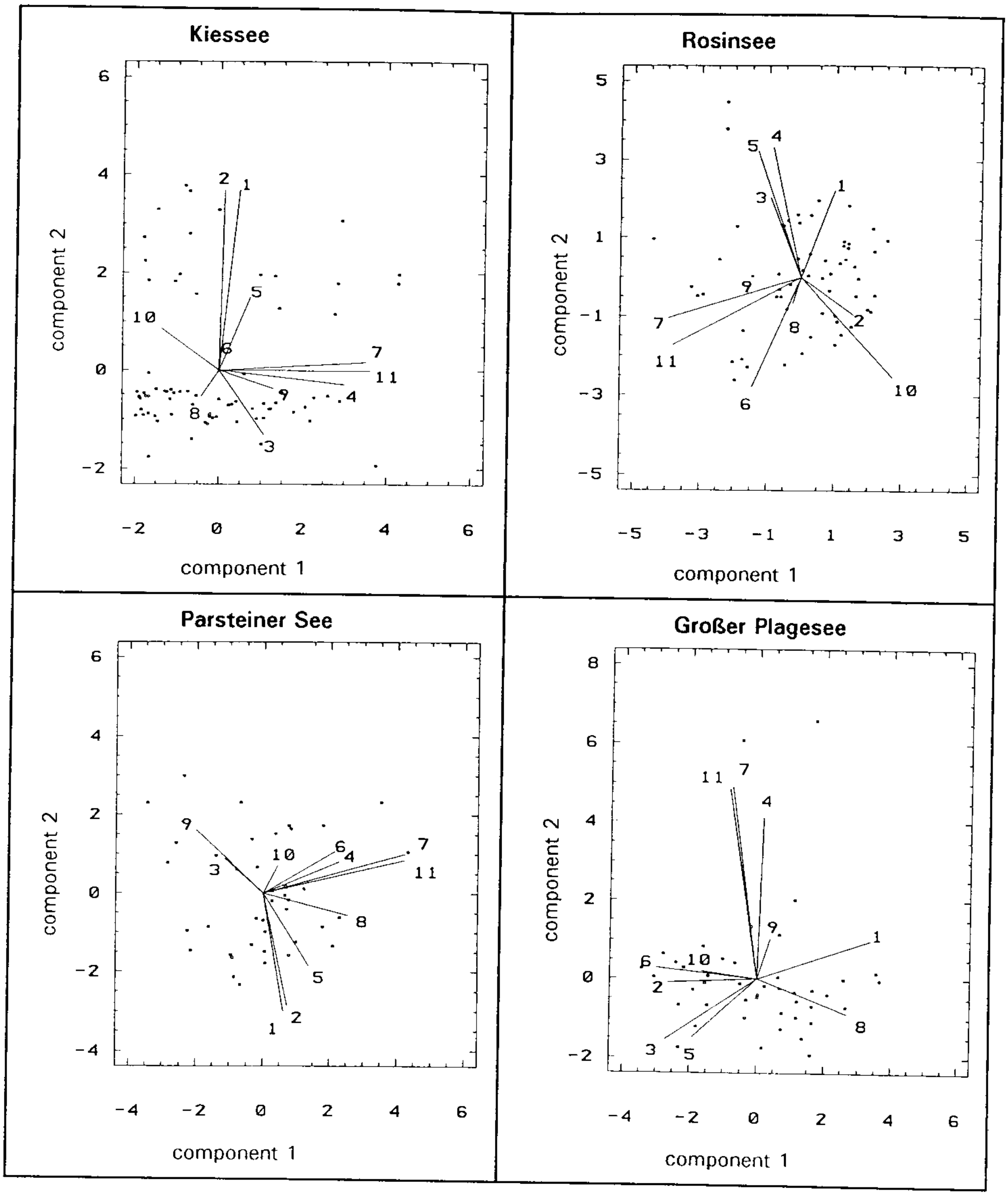


Fig. 5a. Plot of the first two principal components for variables (characters 1–11 cf. Tab. 2) and distribution pattern of thecae for each of the four populations separately (each PCA is based on a data matrix of only one population; percent of variance for the first two components: Gr. Plagesee 28%+23%, Kiessee 26%+20%, Parsteiner See 24%+18%, Rosinsee 23%+18%).

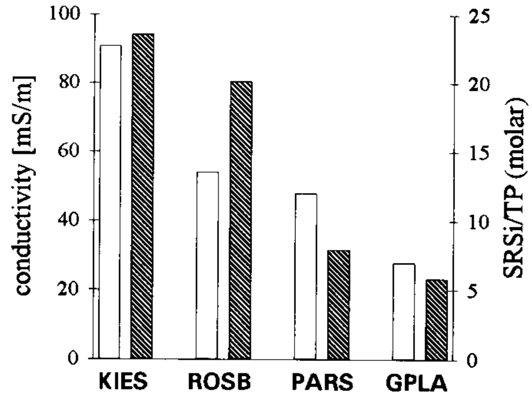


Fig. 5b. Bar charts showing the average annual conductivity (unshaded area) and the soluble reactive silicon/ total phosphorus-ratio (SRSi/TP: shaded area) of the four lakes (ROSB – Rosinsee, KIES – Kiessee, PARS – Parsteiner See, GPLA – Großer Plagesee).

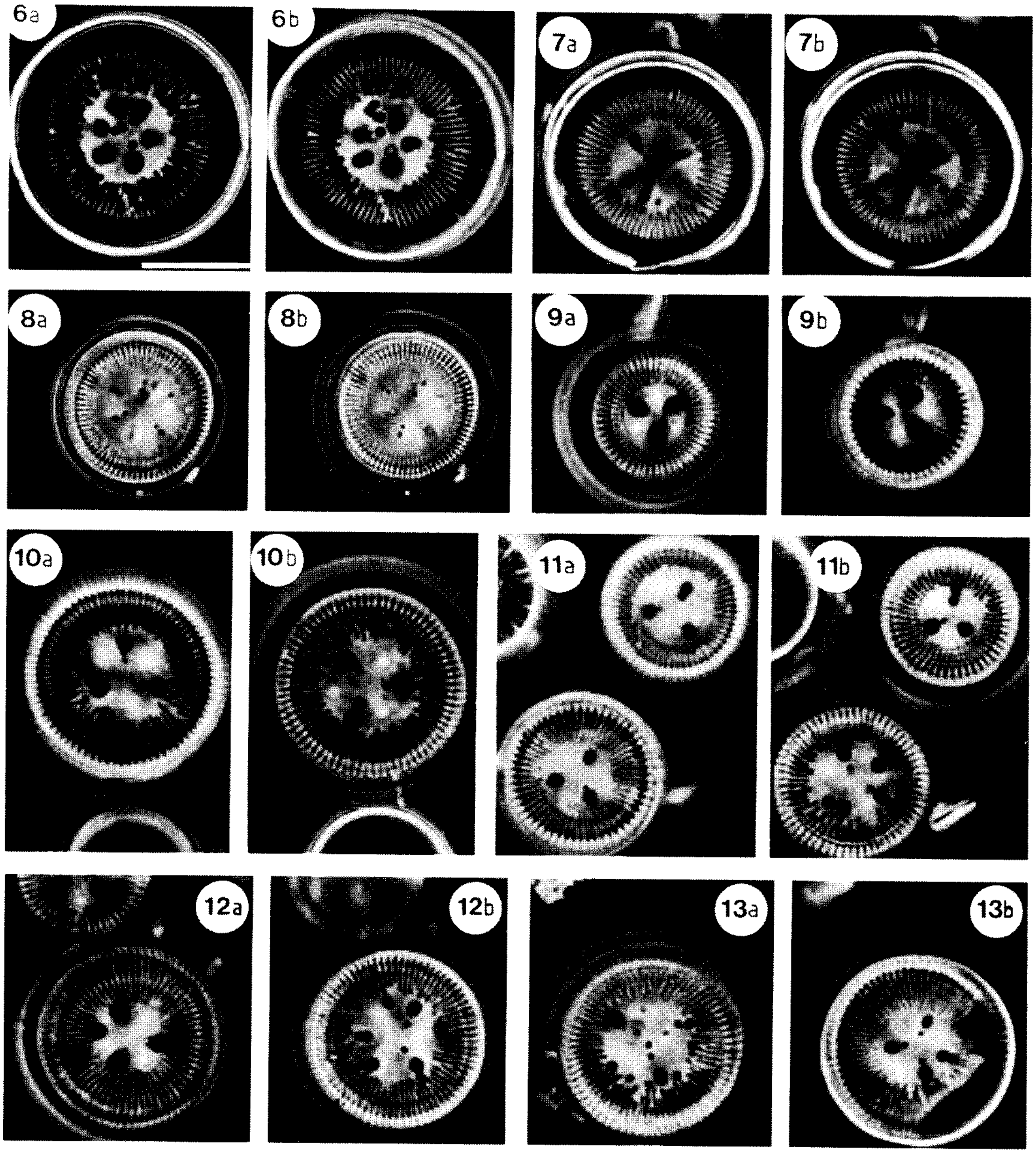
The vectors for characters 8 and 11 in the plot of the third against the first principal component (in Fig. 4b) clearly show that the variation in the form of central area, ranging from flat (e.g. Fig. 11a/b) to a strongly radially undulate (e.g. Fig. 23a/b), is unrelated to the cell size (see also the different central areas present in *Cyclotella ocellata:* Casper & Scheffler 1990, pl. 4, figs 1, 3). The thecae from Kiessee and Rosinsee are almost always flat. In contrast, thecae from the two lakes with lower conductivity and lower Si:P ratio, Parsteiner See and Großer Plagesee (Fig. 5b), are much more variable and often have undulate valves (character 8 in Fig. 5a; clusters TC, TD, NC, ND in Fig. 2). Regarding this, it would be interesting to subject a larger number of populations of these species to multivariate statistical analysis or to study a culture; from the four populations investigated it is not possible to determine to what extent the degree of undulation could be influenced by environmental conditions.

DISCUSSION

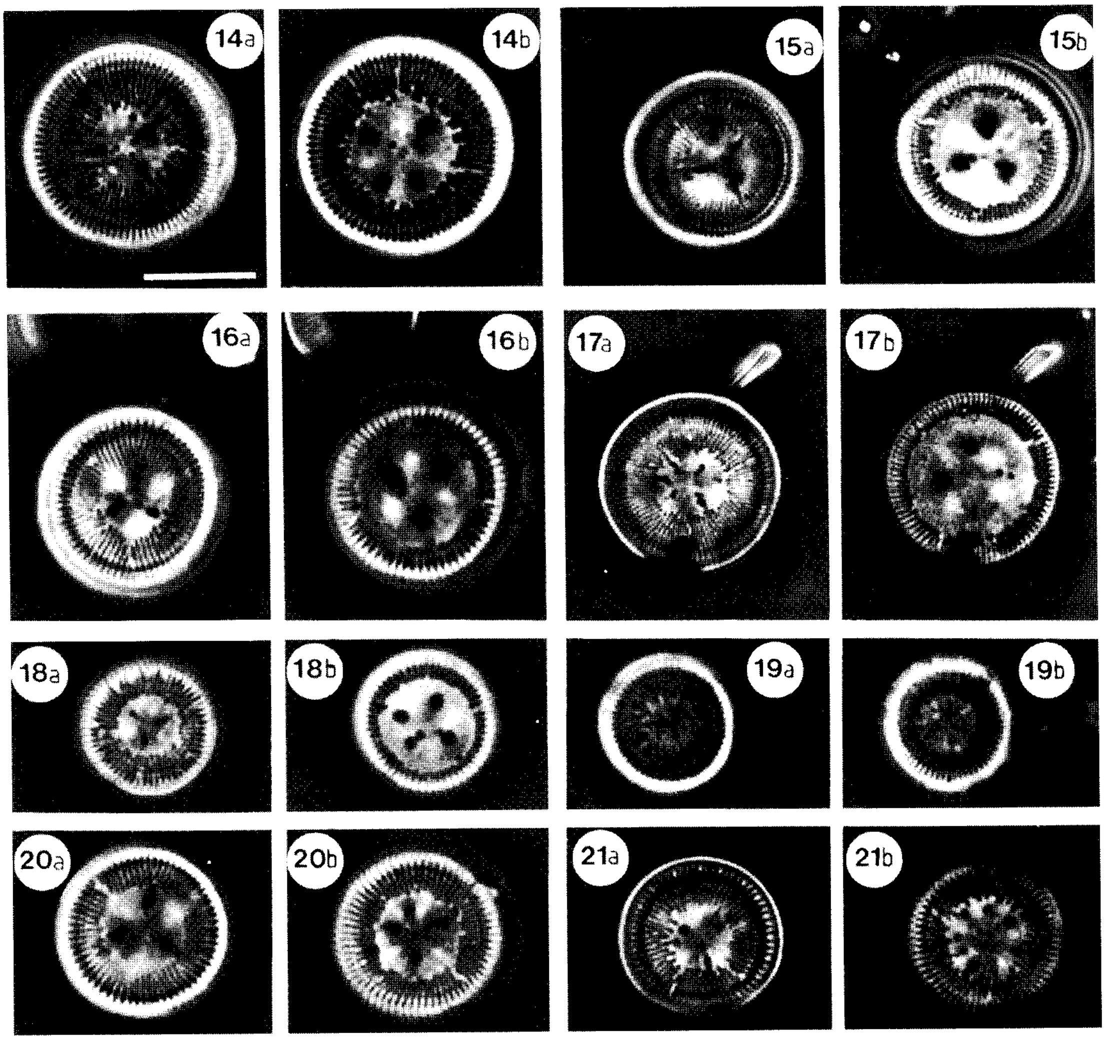
The taxonomic delimitation of *C. krammeri*, *C. kuetzingiana* var. *planetophora*, *C. kuetzingiana* var. *radiosa*, *C. ocellata*, *C. comensis* has proved difficult. For example, the separation of *C. ocellata* (Håkansson 1990, 1993, Håkansson in Krammer & Lange-Bertalot 1991) and *C. comensis* from *C. kuetz.* var. *planetophora* was not supported by Cleve-Euler (1951). Klee & Steinberg (1987) claimed to have found intermediates between *C. ocellata* and *C.kuetzingiana* respectively to *C.comensis* (cf. Klee, Schmidt & Müller 1993).

Some authors have described the morphological plasticity of these *Cyclotella* species (e.g. Kling & Håkansson 1988, Håkansson 1990, 1993), while others have discussed the possible existence of different forms or morphotypes (e.g. Straüb 1987, Klee *et al.* 1993, Schmidt 1993), but in all cases analysis has been at the level of single thecae.

In view of pleomorphy in wall structure demonstrated previously between the two thecae of a frustule and within thecae of different populations (Hasle, Heimdal & Fryxell 1971, Haworth 1983), it is clearly impossible to base the taxonomic description on one or a few valves that seem to have a particularly characteristic morphology.

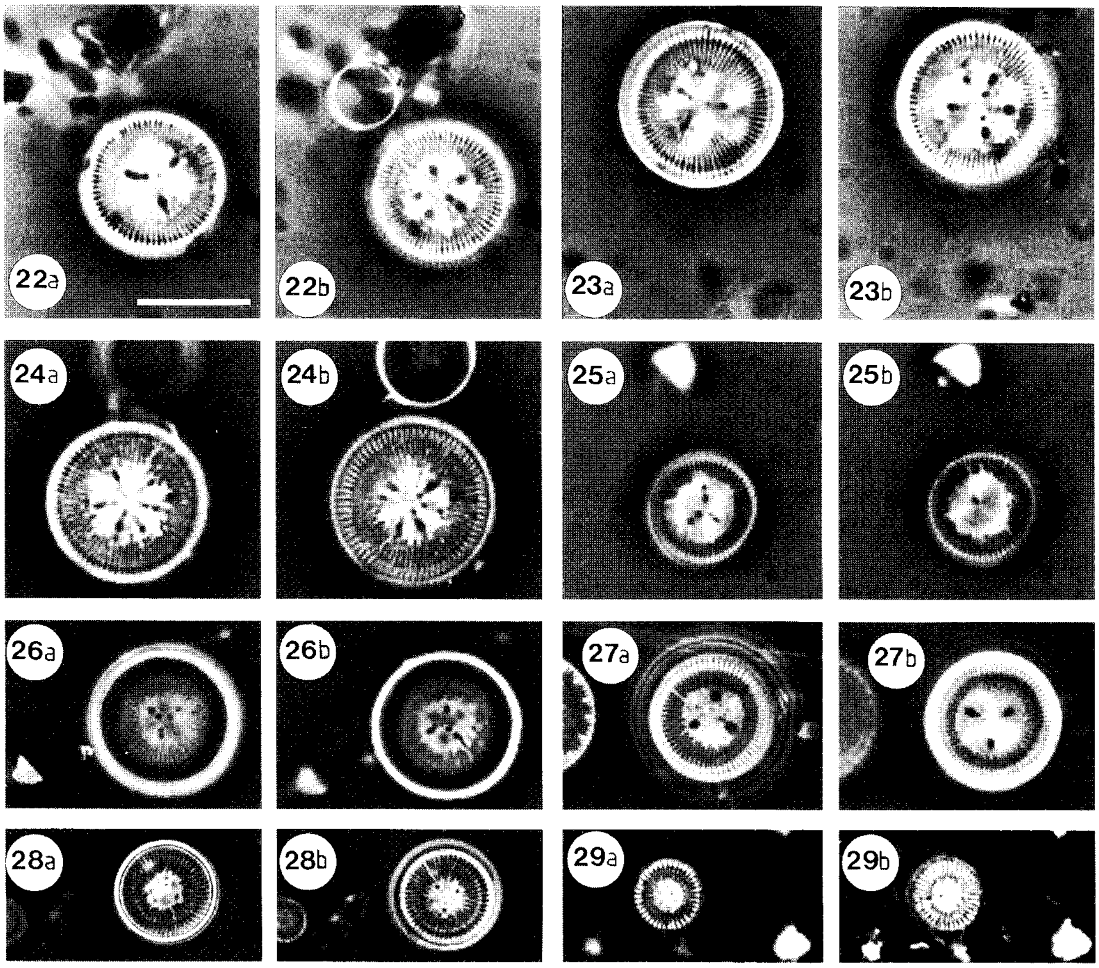


Figs 6–13. *Cyclotella*, Kiessee population, LM. Scale bar (Fig. 6a) = 10 μm. In each case the two thecae (a + b) of a frustule are shown. Figs 6, 7. Dome shaped initial cells (?) at a stage of cell wall development with "*C. kuetzingiana* var. *radiosa*"-pattern. Figs 9, 10a, 11a, 11b (above). "*C. ocellata*"-pattern. Figs 11b (below), 12, 13b. "*C. ocellata-C. kuetz.* var. *planetophora*"-pattern. Fig. 13a "*C. kuetzingiana* var. *planetophora*"-pattern.



Figs 14–21. *Cyclotella*, Rosinsee population, LM. Scale bar (Fig. 14a) = 10 µm. The two thecae (a + b) of each frustule are shown, as in Figs 6–13. Figs 14–18 show obvious differences in striae length between the two thecae of one frustule. Figs 19–21. The "a"-thecae show trigonal symmetry, in contrast to the "b"-thecae, Fig. 20b shows a slight hint of the "*C. comensis*"-pattern.

The dome shaped initial cells (?) found in plankton samples both in spring (Kiessee, Figs 6, 7) and in autumn (Großer Plagesee, Fig. 26), have puncta arranged in radial rows (cf. multistate character 3 in Table 2) and could be identified therefore as "*Cyclotella kuetzingiana* var. *radiosa*" (cf. Fricke in Schmidt's Atlas 1900, fig. 8). Thecae that deviate from trigonal or radial symmetry (cf. multistate character 1 in Table 2) culminating in irregular arrangement of structures (e.g. Fig. 13a) could be referred to "*C. kuetzingiana* var. *planetophora*" (cf. Hustedt in Huber-Pestalozzi 1942, fig. 491). Some thecae with a flat central area could be characterized as "*C. ocellata*" or as "*C. ocellata-*



Figs 22–29. *Cyclotella*, Parsteiner and Großer Plagesee populations, LM. Scale bar (Fig. 22a) = 10 μm. The two thecae (a + b) of each frustule are shown, as in Figs 6–13. Figs 22–25. Parsteiner See population. Figs 22–24 show more or less radially undulate valve surfaces. Figs 23b, 24 show nontrigonal symmetry with "*C. comensis*"-pattern. Fig. 25 shows a frustule with weakly tangentially undulate valve surfaces. Figs 26–29. Großer Plagesee population. Fig. 26. Dome-shaped initial cell (?) at a stage of cell wall development with "*C. kuetzingiana* var. *radiosa*"-pattern.

C. kuetz. var. *planetophora*" (Figs 9a/b, 10a, 11a/b, 12a/b, 27a, 18b,) and those with radially undulate central area as "*C. comensis*" (Figs 23b, 24a/b) (cf. Van Heurck 1882, Fricke in Schmidt 1900, Pantocsek 1902, Hustedt in Huber-Pestalozzi 1942, Cleve-Euler 1951). By studying frustules, rather than separated valves, and by examining both thecae of each frustule, it is possible to examine variation in a new way. On the one hand, one can search for maximal phenotypic dissimilarity between the thecae of single frustules; on the other hand, if one of the two thecae of one frustule is very similar to one of the thecae of a different frustule, this provides a basis for linking variants and demonstrating continuity of variation (morphological transitions) within and between populations. The latter approach can be demonstrated, for example, by linking certain of the thecae illustrated here, to make the following series: Figs 9a/b–16b – 16a–23a – 23b–24a/b, 11a(below) – 11b (below)– 12b – 12a–13b – 13a.

With the proviso that these observations have been carried out exclusively with LM (Håkansson 1990, 1993) and may therefore present an incomplete picture of variation, it can be concluded that the thecae of all four populations belong to the same, polymorphic species. This is demonstrated by variation both within individual frustules and between different frustules. Morphological variants may represent different stages in cell wall development or different stages of life cycle, or may reflect growth under various environmental conditions. The decision which name should be applied to this entity calls for an re-investigation of the type material of all taxa concerned.

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