A CONTRIBUTION TO THE TAXONOMY OF STEPHANODISCUS HANTZSCHII GRUNOW, A COMMON FRESHWATER PLANKTONIC DIATOM

Hannelore Håkansson

Department of Quaternary Geology, Lund University
Tornavägen 13, 223 63 Lund, Sweden

A. E. Bailey-Watts

Institute of Freshwater Ecology, Edinburgh Laboratory,
Bush Estate, Penicuik, Midlothian EH26 OQB, Scotland

Water samples taken from Loch Leven, Scotland, contained unicellular centric diatoms dominated by a taxon we called Stephanodiscus hantzschii Grunow. It existed as several morphotypes, and these are described and compared with the type material of S. hantzschii from the Rabenhorst exsiccate 1104. The morphological features of material subjected to experimental nutrient enrichment are also briefly described. Two very distinct morphotypes and an additional three morphotypes, with more or less constant features of valve pattern have been distinguished. In addition, the presence of hemispherical valves made it possible to describe stages in the life cycle of this very common centric diatom, and an amended description of S. hantzschii is given.

INTRODUCTION

One of the few, more or less regular events in the development of the phytoplankton in the shallow, eutrophic Loch Leven, Scotland (latitude 56° 10'N, longitude 3° 30'W), and a feature of many temperate lakes, reservoirs and slow-flowing rivers, is the late winter-early spring pulse of diatoms, usually dominated by an assemblage of unicellular centric diatoms (Bailey-Watts 1988a). The assemblage recorded in 1981 in Loch Leven (Bailey-Watts 1988b) was dominated by Stephanodiscus hantzschii Grunow, although the following were also found: Cyclotella pseudostelligera Hustedt, C. radiosa (Grunow) Lemmermann (syn. C. comta Kützing), Stephanodiscus parvus Stoermer & Håkansson, S. minutulus (Kütz.) Cleve & Möller, Cyclostephanos invisitatus (Hohn & Hellerman) Theriot, Stoermer & Håkansson and C. dubius (Fricke) Round. The development of this crop, and accompanying changes in, e.g. temperature and nutrient levels, have been described in detail elsewhere (Bailey-Watts 1988b), and a further paper investigates the effects of factorial nutrient enrichments on diatom yields (Bailey-Watts & Håkansson, in prep.).

During the course of these studies, a number of cells were found with features not considered in previous debates on the taxonomy of Stephanodiscus hantzschii Grunow. The present paper discusses these. The Loch Leven material also contained hemispherical valves which have provided an opportunity to describe the morphological development of this diatom from the auxospore formation through the large primary valve stage to the smallest specimens. For completeness, we compare our findings with the original material of S. hantzschii from the Rabenhorst exsiccate 1104.
Since Nipkow (1921 and 1927) published his special investigation on *Stephanodiscus hantzschii*, numerous investigations on species of the genus *Stephanodiscus* Ehrenb. have been reported especially during the last decade (e.g. Anderson 1990, Casper et al. 1987, Geissler 1982, 1986, Häkansson 1986, Häkansson & Ehrlich 1987, Häkansson & Hickel 1986, Häkansson & Stoermer 1984a,b, Häkansson & Kling 1990, Hickel & Häkansson 1993, Kobayasi & Kobayashi 1985, Round 1981, 1982 a,b, Stoermer & Häkansson 1984, Theriot et al. 1987; see also Kalbe 1972, 1973). However, difficulties still exist due to unclear, incomplete or misleading information (e.g. Casper & Klee 1992, Häkansson & Stoermer 1984a, Theriot et al. 1987). Even though the importance to taxonomy of the stability of certain morphological features has been discussed (Geissler 1970, 1982, 1986, Round 1982a, Theriot et al. 1987), more reliable information is still needed (Häkansson & Kling 1990, Genkal & Häkansson 1990). The present paper aims to resolve some of the confusion over the identity of *S. hantzschii*.

The paper deals mainly with cells taken from Loch Leven on 31 March 1981, when the population density was ca. 20 x 10^3 cells ml^{-1}, 17 days after the maximum crop density of 37.3 x 10^3 ml^{-1} was recorded. However, cells subjected to additions of different combinations of nitrate nitrogen (N), phosphate phosphorus (P) and silicate silica (SiO_2), and left in polythene containers in the laboratory for 6 days (see Bailey-Watts & Häkansson, in prep.) were also examined for features of taxonomic relevance.

**MATERIAL AND METHODS**

The material had been fixed with Lugol's iodine, and this is known to distort silica structures in some instances. For example, cells of *Asterionella formosa* Hassall may become bent and then resemble *A. formosa* var. *acaroides* as described by Lund (1961). However, the frustule features discussed in the present paper are not thought to have been affected by the fixative, except that the thinner part covering the internal openings of the areolae (cribrum) has disappeared. The iodine was first removed by addition of sodium thiosulfate, washed several times with distilled water, then heated at 90°C for 4 hours in 30% H_2O_2 and finally re-rinsed several times in distilled water (see Bailey-Watts 1988b). Slides for light microscopy (LM) and scanning electron microscopy (SEM) were made from 8 separate samples taken of water collected at the start of the enrichment experiment, and from 8 repeat samples taken at the end of the test corresponding to "initial" and "final" material in the duplicate "control" containers and the 7 pairs of containers to which the ecologically relevant additions of the following nutrients or nutrient combinations were made: N, P, SiO_2, N + P, N + SiO_2, P + SiO_2, and N + P + SiO_2 (further detail will be reported in Bailey-Watts & Häkansson, in prep.). The morphological features and diameters of 100 centric frustules were noted on each slide, although this paper is confined primarily to the results on cells assigned to the *S. hantzschii* complex. The terminology on frustule features, follows that suggested by Anonymous (1975) and Ross et al. (1979).

**RESULTS**

**Loch Leven material.**

**Morphotype I (mI).** Figs 2-4, 6a, 7, 16a-17, 21a-24, 26, 28

This was the most abundant "morphotype". Under the light microscope it appears that the valve is flat (or nearly flat), with a diameter of 5.2 to 18.5 μm (Figs 2-4, 6a, 7). Within these size limits, 3
Fig. 1. Diagram showing the size distribution of the morphotype I in the 8 "initial samples"
size groups are distinguishable (Fig. 1): (i) 5.5 µm to 8.5 µm with a peak at 8.5 µm; (ii) 9.0 µm to 12.0 µm, a peak at 11.0 µm, and (iii) 13.5 µm to 16.0 µm, the peak at 14.5 µm. There was one specimen measuring 18.5 µm which is very near the diameter of the hemispherical valves (19.5 to 22 µm, see below). The ornamentation is distinct, i.e. single rows of areolae starting in the centre of the valve and becoming bi- to tri-seriate (seldom tri- to quadri-seriate) halfway towards the junction of the valve face and the mantle. Between these rows (fascicles) are the unstructured interfascicles, which become more pronounced (slightly domed) towards the valve face margin. All interfascicles, but one, are subtended by a spine at the face/mantle junction (Figs 2-4, 8). These spines can, however, be broken or only the base is visible. Instead of the missing spine, an opening with a thickened rim or sometimes a very short tubulus was noted (slightly towards the center of the valve face). This is assumed to be the external opening of the rimoportula (Figs 2-4, 8, arrowed); it is neither typical of that described previously for *Stephanodiscus* nor of the simple opening found in species of *Cyclorella*. In several specimens of this morphotype from Loch Leven an annulus is visible in the central part of the valve face (Figs 2-4).

The density of fascicles ranged from 9 to 15 in 10 µm, depending on the valve diameter. No valve face fultoportula is visible. It appears that there are two girdlebands, i.e. one on each valve (Figs 11, 14, 15).

The SEM investigation showed that the vegetative valves of this morphotype are (almost) flat, with a regular structure (Figs 16, 17, 21-24), no valve face fultoportula, and a fairly high mantle with finer areolae (Figs 18, 22, 24). However, the valve face areolae are not always quite round. As can be seen from Fig. 24, they can be fused or angular. The distance between them can also differ slightly. All specimens had one rimoportula, and this is sessile and orientated slightly diagonal to the interfascicles (Figs 38-40) and situated slightly towards the valve centre replacing a spine (Figs 17 & 22b arrowed, 18, 22-24). There seems, however, to be a phenomenon which gives the impression of two openings of the rimoportulae. In some specimens the marginal fultoportulae are thick, short tubulae with thinner spines not placed exactly above these openings (Figs 21a [one and two arrows], 22 arrowed “a”). The external opening of this rimoportula usually had a thickened rim (Fig. 21 a) which could be deformed (Figs 18, 24), or a very short tubulus (Figs 19, 26). The marginal fultoportulae occur directly beneath every second to third (seldom fourth or even fifth) spine (Figs 19, 21); they have three satellite pores internally (Figs 20, 37-40). Interestingly, the areolae on the mantle often form parallel rows (Figs 20, 22-24, 39, 40).

**Morphotype II (mII).** Figs 12b, 13, 21b, 34-36

Under the light microscope the morphotype described here as morphotype II appears more delicately silicified than morphotype I. The diameter ranged from 6.9 to 15.9 µm, i.e. within that described for mII; however, most specimens measured between 10.2 and 13.7 µm. The areolae seem to be finer than those in morphotype mI, and are mostly 3- to 4-seriate and occasionally 5-seriate in the fascicles near the valve face/mantle junction. The separating interfascicles, 9 to 10 in 10 µm, are thin and often curved. This morphotype always possesses an annulus in the central region of the valve face, but equally in this material, no valve face fultoportula.

SEM micrographs show the typical curved interfascicles and, towards the valve face/mantle junction, more rows of areolae between the interfascicles than mI (Figs 34-36) Also here the areolae on the valve mantle are slightly finer. The central annulus surrounds several areolae. The marginal fultoportulae have 3 satellite pores and occur directly beneath every second to third (seldom fourth or even fifth) spine. Some specimens of this morphotype had occasionally a short tubulus of the rimoportula (Fig. 35).

This description generally corresponds to the second morphotype described by Håkansson & Stoermer (1984a). In their description, however, nothing is mentioned about the appearance of the external opening of the rimoportula. Those authors also found the annulus “in most” specimens investigated, while the present investigation suggests that this is a constant feature.
Figs 2-15. LM, scale bar in Fig. 2 = 10 μm. Figs 2-4, 7. *Stephanodiscus hantzschii*, morphotype I; different size; rimoportula arrowed. Fig. 5. Morphotype V. Fig. 6. a = morphotype I, b = morphotype IV. Fig. 7. Morphotype I and morphotype III (a). Figs 9 and 10. Different focus. The impression is that these could belong to three different species, but they all belong to *Stephanodiscus hantzschii*; it is difficult to decide to which morphotype. Fig. 11. Morphotype IV and two cells in girdleband view. Fig. 12. a = morphotype V, b = morphotype II. Fig. 13. Morphotype II. Fig. 14. Mantle view with girdle band. Fig. 15. Two cells in girdle band view.
Morphotypes III-V (mIII-mV). Figs 5, 6b, 7a, 11, 12a, 25?, 30, 32

While morphotypes I and II were the most common in this material, an additional 3 "morphotypes" have been distinguished. It is possible that they are not true morphotypes, because they seem to be based on anomalies, appearing everywhere in the development of the valve pattern.

Type mIII is a very hyaline-structured form, or the structure was difficult to identify, particularly where the spines are long or thick and sometimes bent (Figs 7a, 25?, 30).

The form classified here as mIV has a denser structure than the other types, giving the impression of a double structure (Figs 6b, 11); (no specimens of mIV were found under the SEM).

The final morphotype distinguished here (mV), is characterized by an irregular structure and spine-insertion (Figs 5, 12a, 32).

Hemispherical valves. Figs 27, 41-43

Hemispherical valves represent stages associated with auxosporulation. The appearance of these was the most interesting aspect of the material. They had a diameter between 19.5 µm and 22 µm in several slides (Figs 27, 41-43). These valves are known to be the initial valves, which are laid down in an auxospore, which is formed by sexual reproduction, when size reduction through successive division is no longer possible (Schmid 1987). Nipkow (1921) had reported on auxospore formation of Stephanodiscus hantzschii in samples from Lake Zürich, Switzerland. Round (1982) recorded this phenomenon in a population of Stephanodiscus in material from Farmoor Reservoir, Oxford, England. The valves recorded in the Leven material have already formed the rows of areolae, the interfascicles, and the marginal fultoportulae with the satellite pores (Fig. 43). In the central part of the valve there is an annulus. All these features are, however, not yet "settled" in their final (vegetative cell) position on the valve.

Material from nutrient enrichment experiments.

All of the morphotypes described above, were found in each of 8 duplicate subsets of water subjected to various nutrient enrichments and kept in the laboratory for 6 days under natural light and ambient temperatures. However, the relative abundances of the types varied. While material from the whole series of experiments on samples collected from the loch between mid-February and mid-April 1981 remains to be analyzed, the following features were observed. Firstly, an increasing number of short chains of cells were observed in the material to which phosphate plus silicate had been added; the cells had very strongly silicified, sometimes bent spines. In the material treated with nitrate plus silicate, only a few cells appeared paired, but they also seemed to have longer spines. Finally, in the material treated with nitrate plus phosphate, strongly silicified specimens were more notable.

LM- and SEM-observation on type material of S. hantzschii

A re-examination of 100 specimen of the Type material, revealed the three morphotypes described by Håkansson & Stoermer (1984a). The data on cell diameter (5.6 µm to 15.6. µm) as well as on the density of the interfascicles (8-9 in 10 µm) was confirmed. In addition, however, slight differences in the appearance of the marginal fultoportulae and the rimoportula were noted. Specimens with one to two interfascicles between the mantle fultoportulae were dominant – as illustrated, but not mentioned by Håkansson & Stoermer (1984a, figs 9, 11 and 12). Also the external opening of the rimoportula was in most cases simply an opening with a thickened rim or a very short tubulus. This was already visible in the LM micrographs in Håkansson & Stoermer (op.
Figs 16-20. SEM, scale bars in Fig. 19 = 1 μm, Figs 17, 20 = 2 μm, Figs 16, 18 = 5 μm. Fig. 16. a, morphotype I; b, morphotype I with forked spines; c, valve face view with occluded areolae and some forked spine. Fig. 17. Morphotype I with spine thickened at the base and parallel rows of areolae on the mantle. The position of the rimoportula is arrowed. Fig. 18. Detail of a valve with some spines and the deformed (and thickened) external opening of the rimoportula. Fig. 19. Mantle view of some specimens with “bent” spines and directly beneath some of them the openings of the marginal fultoportulae (often as a short tubulus or having a thickened rim around the opening). Fig. 20. Inside view of a valve with the domed cribrum over the areolae.

cit. figs 3 and 8) but was neither mentioned nor discussed in that paper. Interlocking pairs were also recorded.

The second morphotype found in the type material, the “tenuis”-form, appears to be identical with that described by Håkansson & Stoermer (1984a).

The above described two morphotypes are the same as those found in the Loch Leven material.
DISCUSSION

We assume that the taxon with the morphotypical characters found in Loch Leven is *S. hantzschii* as described, albeit briefly, by Grunow (in Cleve & Grunow, 1880). Our investigations, however, have highlighted some morphological features in *S. hantzschii* not considered in the literature available, but which are of interest.

Round (1981) and Håkansson & Hickel (1986) considered that several morphological characters, e.g. the type of spination, the ornamentation of areolae, and the length of the external opening of the marginal fultoportulae, are not suitable for species separation within *Stephanodiscus*, because they are variable within a population. Geissler (1978, 1986) and her students also showed that some characters within *S. hantzschii* can vary with respect to valve size and/or environmental conditions, such as phosphorus levels (Krankemann 1974), nitrate concentration (Randig 1974) and salt-concentration (Kruke 1974, Geissler 1978, 1986). Round (1981) considered that “the depth of the mantle, the proximity of the fultoportulae to the spines and to the mantle edge, the periodicity of the fultoportulae and rimoportulae are all diagnostic features”. Round & Håkansson (1992) summarized the characters of some genera in the family Thalassiosiraceae: *Stephanodiscus*, *Cyclostephanos*, and *Cyclotella*. Our findings on the Leven material, especially the appearance of the external opening of the rimoportula and the appearance of an annulus need to be considered in relation to this work.

Theriot et al. (1987) stated that the rimoportula in species of *Stephanodiscus* has a long external tube; those authors continue: “...Thus rimoportula morphology can be sufficient, if not necessary, evidence for classification of a species as either *Cyclostephanos* or *Stephanodiscus* ...”. Håkansson & Stoermer (1984) made the taxon with an annulus they found in the original material of *S. hantzschii* a forma *tenuis*. It was their view that, because of its abundance in special environments, its occurrence could reflect certain distinct ecological conditions. There are, however, other investigations reported in the literature which are somewhat confusing.

The investigation made with LM and SEM by Kobayasi & Kobayashi (1985) on *S. hantzschii* fo. *tenuis* shows three taxa differing in the extent of silicification (both hemispherical and vegetative valves) and other aspects of their appearance. The finely silicified valves (p. 295, figs 1-10), as well as the moderately silicified valves (figs 11-18) both have an annulus and an external tubulus of the rimoportula. On the other hand, the heavily silicified specimens had “lost” the annulus or it was not readily visible due to the thicker silicification, and the external opening of the rimoportula has merely a thickened, slightly deformed, rim. These specimens also have occluded areolae. There are other features of interest. Figures 4, 5, 7, 8 and 18 (in Kobayasi et al., op.cit.) show a diatom with a flat cribrum, whereas figs 25 and 27 show a domed cribrum. Also, the direction of the rimoportula differs slightly between these two types. One might question whether two different species or even different genera are involved.

Figs 21-26. SEM, scale bars in Fig. 21 = 5 μm; Figs 23, 25 = 2 μm; Figs 22, 24, 26 = 1 μm. Fig. 21. a) morphotype I; b) morphotype II; c) inside view of a valve. Rimoportula arrowed. On the left side of Fig. 21 a is the opening of the rimoportula (one arrow), on the right side might be a second rimoportula (two arrows)? The explanation to this phenomenon is given in the following figure. Fig. 22. Morphotype I. The areolae are not always quite circular. There are thick, short tubulae of the marginal fultoportulae, the spines above these openings are finer and sometimes not quite above (arrowed “a”). The opening of the rimoportula has only a thickened rim (arrowed “b”) and the central annulus is not visible. Fig. 23. Morphotype I with circular areolae, very distant spines and the central annulus, parallel rows of areolae on the mantle and a thickened opening of the rimoportula. Fig. 24. A coarse structured form of morphotype I. Also here the areolae are not quite circular, they sometimes fuse. The spines above the marginal fultoportulae are finer, sometimes shorter. The opening of the rimoportula is deformed. Fig. 25. A very fine structured morphotype (III?) with thick silicified spines. Fig. 26. Detail of a valve with a short external tubulus of the rimoportula.
Kling (1992, and see also Håkansson & Kling 1990) found that in culture, clones of *S. hantzschii* formed valves with occluded areolae at a particular time during the life-cycle; she also stated that, at the same point in the life-cycle, the external opening of the rimoportula is reduced to only a thickened opening. She continues that “only fully developed frustules should be used in species diagnosis, as the state of the tubular structure may depend on the growth stage of the cell at the time of sampling”. In the present study specimens with occluded areolae, were observed only occasionally. Meanwhile the rimoportula on nearly all specimens (regardless of morphotype) had a thickened opening; only occasionally was a short tubular opening observed. However, a great number of specimens with occluded areolae were found in the original Rabenhorst type material. In most cases the external opening of the rimoportula in this material was either a short tubulus or a thickened rim regardless of whether specimens had occluded or ordinary areolae. In the present investigation, which concerned size and silicification, and illustrated different morphotypes including initial valves, a long tubulus of the rimoportula was seldom found. More research is needed to clarify the form of the rimoportula in relation to different nutrient, temperature and light availability.

It is known that silicification proceeds centrifugally from what is termed a “pattern centre” or “annulus” (see von Stosch 1977, Schmid et al. 1981, and discussion in Round & Crawford 1981, Mann 1984, and in Picket-Heaps et al. 1990). At one point we thought that the presence of an annulus occurred on valves at a certain time during the life cycle; the more finely silicified forms with an annulus being the first vegetative valves after auxospore-formation. However, it seems that the annulus is present in all specimens, although sometimes it is ill-defined (Round & Crawford 1981, Mann 1984).

Kalbe (1972, 1973) studied plankton samples collected monthly from lakes in Mecklenburg (north-east Germany), where different *Stephanodiscus* species were dominant. In his study on *S. hantzschii* in these samples there were peaks of cells with different valve diameters. He concluded that these must be different races. He found discontinuities in the diameter frequency distributions. Indeed, specimens in the size band of 9-10 μm were missing and he used this fact as a good criteria to separate the variety *pusillus* from the nominate variety.

Working with cultures of centric diatoms including *S. hantzschii*, Geissler (1982) and her students (Kuke 1974; Krankemann 1974; and Randig 1974) observed a decrease in the number of specimens in the same diameter group (9-10 μm). They thought this could be due to cells having divided rapidly through this size band. Although the size distribution of the Loch Leven material shows 3 size modes, the morphological appearance is otherwise the same. It is thus concluded here, that all specimens belong to the same species.

**CONCLUSION**

This investigation has shown that the species *S. hantzschii* is somewhat variable and may even display a degree of polymorphism. It is characterized by a more or less flat valve face without a fultoportula, a fairly high mantle, marginal fultoportulae positioned directly beneath the spines, and one rimoportula. Morphotypes I and II, as defined here, can occur together either as single cells or in short chains of cells. In addition, the number of striae in a fascicle can differ, as can the

Figs 27-32. SEM, scale bars in Figs 28, 29 = 1 μm; Figs 27, 30, 32 = 5 μm; Fig. 31 = 10 μm. Fig. 27. Internal view of a thin silicified morphotype nearly “inside” an initial valve (also inside view). Fig. 28. a) external and b) internal view of morphotype I; c) Cyclotella radiosa (internal view). Fig. 29. Very indistinct structure of the valve face, but thick silicified spines, some of them forked, others shorter. A distorted external opening of the rimoportula (arrowed). Fig. 30. Morphotype II. Indistinct structure of rows of areolae; long spines with thickened base and a short tubulus of the rimoportula (arrowed). Fig. 31. Partly occluded structure on the valve face. Fig. 32. Morphotype V.
occurrence of the marginal fultoportulae, the areolation on the mantle, the external opening of the rimoportula, and the presence or absence of the annulus in the central part of the valve.

It is our conclusion that the description of *S. hantzschii* should be amended, as follows:

*S. hantzschii* has short cylindrical cells with circular, flat (or nearly flat) valves and a diameter between ca 5 to 18 μm. The valve face has rows of areolae arranged in fascicles, single in the centre but 2- to 4-seriate (sometimes 5-seriate) towards the valve face/mantle junction. An annulus in the centre of the valve face is not always clearly visible. In between the fascicles are the structureless interfascicles, which become more or less domed towards the valve face margin. The number of
Figs 37-40. SEM, scale bars in Fig. 37 = 1 μm; Figs 39, 40 = 2 μm; Fig. 38 = 5 μm. Internal view with the marginal fultoportulae with three satellite pores. The marginal rows of areolae are mostly parallel, the interfascicles are not costae-like.

fascicles in 10 μm depends on the size of the specimen; in a small diatom there can be up to 15 interfascicles in 10 μm, but in larger specimens there may be only 8 in 10 μm. Alternate interfascicles are subtended by a spine, which can be long, finely or strongly silicified or bent and sometimes divided at the tip (as in Fig. 33); the base of a spine can vary in thickness. At the end of the single interfascicle not bearing a spine a thickened opening or a very short tube can be seen, slightly towards the centre of the valve: this is the external opening of the rimoportula. There is one rimoportula and no valve face fultoportula.

The electron micrographs reveal the following features:

- a marginal fultoportula can be found directly below every, or every second to fourth spine;
Figs 41-45. SEM, scale bars in Figs 41, 43 = 5 μm; Fig. 45 = 2 μm; Figs 42, 44 = 1 μm. Fig. 41. Initial valve, external view. Fig. 42. Detail with some of the external marginal openings of the fultoportulae. Fig. 43. Internal view of an initial valve with the marginal fultoportulae. Fig. 44. Girdleband view of cell with long spines, slightly bent. Fig. 45. Two cells forming a short chain.

- the mantle areolae are slightly finer and denser than the valve face areolae, and sometimes orientated in parallel rows. The rows on the mantle are sometimes modified towards the mantle edge;
- the marginal fultoportulae have an external, thickened rim or a very short tubulus, and are internally furnished with three satellite pores;
- the external opening of the rimoportula is either an opening with a thickened rim, a short or longer tubulus;
- and internally, it is sessile and orientated slightly diagonally to the line taken by the interfascicles.

This investigation shows that greater detailed morphological variation should be taken into consideration when describing diatom species. More morphological differences are visible in the scanning electron microscope than in the light microscope, and must be taken into account with
caution and discernment in modern studies, subtle differences at any scale of magnification could simply represent variability within a species or the presence of "anomalies".

ACKNOWLEDGEMENTS

We are very grateful to Dr. U. Geissler for the loan of her three students examination papers and her valuable comments on an early draft of the manuscript. The financial support by Naturvetenskapliga Forskningsrådet for HH is gratefully acknowledged.

REFERENCES


