

Discovering diatom species: is a long history of disagreements about species-level taxonomy now at an end?

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Background – Now and in the past, species discovery in diatoms begins, and often ends, with a survey of morphological variation to find breaks in the variation pattern that allow diagnosable entities to be defined and named. For this process to be effective, it needs to be informed by an understanding of the mechanisms that generate variation and many mistakes were made in the early 19th century because of poor knowledge of the diatom life cycle and phenotypic plasticity; some taxonomically important life-cycle characteristics were not properly documented until 1932. Acceptance of the Darwinian view of species as taxa arbitrarily set along a continuum of divergence was accompanied in the late 19th and early 20th century by description of many varieties and forms; most recently described taxa, on the other hand, have been species. The neo-Darwinian emphasis on reproductive isolation as an important factor in speciation, introduced during the ‘New Synthesis’ of the 1940s, did not become influential in diatom taxonomy until the 1970s. It has since been a source of controversy, some seeing it as having no place in taxonomy, others regarding it as a useful aid to the detection of species boundaries, alongside character-based approaches, both morphological and molecular.

Review – This paper discusses changes in how species have been discovered and circumscribed in diatoms, and seeks to establish whether there is a basis for consensus in future work in this field.

Conclusion – Whereas morphology is currently still the primary tool for discovering diatom species diversity, molecular methods may be more cost-effective in future and are the only practical means of exploring cryptic (including pseudocryptic) diversity, which appears to be widespread. By treating species as separately evolving metapopulation lineages, as recommended by de Queiroz, different approaches can be accommodated (including tests of reproductive compatibility), providing a framework within which conflicting results can be analysed and reconciled.

Keywords – Bacillariophyta, diatoms, discovery, history, metapopulation lineages, species definition, taxonomy.

INTRODUCTION: EARLY HISTORY, FROM 1700 TO 1859

This review, which was developed from a presentation to the Van Heurck Centenary Symposium “Diatom taxonomy in the 21st Century”, held in Brussels in August 2009, focuses on how diatom species have been discovered historically, recent controversies, and a possible way to accommodate different viewpoints. Alverson (2008) has provided a detailed consideration of diatom species from a molecular genetic perspective and I have tried to avoid covering the same ground.

Diatoms were discovered and illustrated at the beginning of the 18th century by an unknown English microscopist. His description and illustrations (Anonymous 1703) show chains of cells that can be confidently identified as a *Tabellaria* (fig.

1A). Each chain was “compos’d of rectangular oblongs and exact squares ... joyn’d together... these rectangular parallelograms are all of the same size ... and the texture of every one is nearly the same ... after they had lain a day or two dry on a Glass Plate they alter’d not their Figure, and upon the addition of new Water (warm or cold) they had still the same appearance and cohesion.” Though more than 300 years old, these sentences record aspects of diatoms that have been important ever since for taxonomy, namely the size, shape and surface pattern (‘texture’) of cells, and the retention by dead cell walls of many of the morphological characteristics of the living organism. The anonymous analysis of *Tabellaria* is paradigmatic in another respect, in that it shows the value of having information about diatom populations: the constancy

of particular features could be judged because cells failed to separate after division and remained connected in a chain.

Linnaeus included no diatoms in his classifications, although contemporary microscopes were more than adequate to reveal them (e.g. Baker 1753), and no species were named until Müller described several species, including *Vibrio paxillifer* O.F.Müll. (now *Bacillaria paxillifera* (O.F.Müll.) Hendey: see Jahn & Schmid 2007). The first flowering of diatom taxonomy took place in continental Europe between 1820 and 1850, particularly through the work of C.A. Agardh (e.g. 1830–1832), F.T. Kützing (e.g. 1833, 1844) and C.G. Ehrenberg (e.g. 1838). The discovery and description of species then continued apace, with major contributions by several British diatomists including W. Smith (1853, 1856), W. Gregory (e.g. 1857) and R.K. Greville (e.g. 1857). The method of discovery was to use the light microscope to search through preparations of cleaned cell walls (often as isolated valves) for specimens that differed morphologically from any species that were already known. If the new specimens could be connected to an existing species by a chain of intermediate forms, or could be linked to an existing species in some other way (e.g. as developmental stages), then the circumscription of that species would be expanded. If not, a new species was described. However, communication was a major constraint (literature could take a long time to be distributed and illustrations were often few and poor) and similar forms were often discovered more or less simultaneously and given different names through ignorance of what other taxonomists had published.

Early 19th century diatomists recognized that individuals within species might differ from each other as a consequence of life cycle changes and the effects of the environment, but neither set of factors was then well understood. In Kützing's treatise of 1833, several individuals are illustrated for most species and in each case the cells are very alike in size and shape (fig. 1D). By the 1850s, however, it was accepted that individuals of a species could vary markedly in size, following the discovery that diatom cells copulated and gave rise to much enlarged cells (Thwaites 1847). For example, Griffith (1855) noted that "great differences of size and shape may exist in forms of one and the same species". Nevertheless, the life cycle was misinterpreted for two further decades, to the detriment of taxonomy, at least partly because the cysts of amoebae such as *Arachnula* (e.g. Dobell 1913) were mistaken for stages in diatom reproduction. Smith (1856: xv) observed such cysts containing small frustules of a cymbelloid diatom (fig. 1E, 'V'), which he interpreted as having been produced by division of the contents of enlarged *Cymbella* cells. Smith's interpretation of the life cycle was: (1) conjugation between normal-sized cells (fig. 1E & I) is accompanied by dehiscence and sloughing off of the old cell walls and leads to the formation of enlarged cells (fig. 1E, II). (2) The large cells likewise cast off their walls, forming a cyst in which numerous very small cells are produced (fig. 1E, III–V), and the large cells formed after copulation are therefore to be interpreted as sporangia. (3) The small 'embryonic' cells "burst the membrane which contains them, escape from the cyst, and in a definite, but unascertained period, reach the mature form and size of the ordinary frustule". (4) The 'mature' cells produced in this way then begin to divide, each "necessarily

stereotyping the shape with which it commences, [producing] multitudes of frustules slightly deviating from the normal form ... so that the observer, judging from a single gathering, may be led to fix upon a variety as representing the typical form and size of the species." Smith believed, therefore, that the shape and size of cells might change continuously (and might get bigger) during the transition from embryonic to mature 'normal' cells, but that there would be a clear distinction between any of these embryonic or normal cells and the 'sporangial' cells (which we now refer to as 'initial cells'). The latter were supposed to be uniquely large within each species and might also differ from the normal cells in valve pattern. For example, Smith (1853) interpreted a large elliptical diatom that we now classify in *Lyrella* as the 'sporangial valve' of a *Diploneis* (at that time called '*Navicula elliptica*') (fig. 1F): no intermediates between the two were expected or found (and of course, as we now know, none exist).

A lack of understanding of the diatom life cycle is also evident in other contemporary works, such as the flora by Rabenhorst (1853). Many times, Rabenhorst shows members of a species varying in size but possessing similar shape and pattern (in this case, 'similar' can be taken in the strict geometrical sense). For example, his pl. 6, fig. 31 (reproduced here as fig. 1B) shows two valves in valve view of '*Pinnularia limosa*' [*Caloneis silicula* (Ehrenb.) Cleve sensu Krammer & Lange-Bertalot 1986]. They resemble each other closely in shape, with a triundulate outline produced by expanded poles and an expanded centre. However, they differ profoundly in size and, as is now clear (Geitler 1932: see under 'Modernist phase'), could not be produced during the life cycle of a single clone. The same is true of the diatoms Rabenhorst called '*Pinnularia dicephala*' and '*Navicula amphioxys*' (fig. 1C).

These examples illustrate that assessing the significance of differences between the phenotypes of individual organisms often depends on extra information, such as how morphology or other characteristics change during the life cycle, and the extent to which the phenotype can alter in response to environmental conditions. Furthermore, detecting the gaps in the variation pattern that suggest the existence of species (or monophyletic groups of species) is not straightforward in small, character-poor organisms like diatoms. For instance, Gregory (1855) suggested that an array of naviculoid diatoms from fresh- and marine waters that had hitherto been classified in several species in two different genera (*Navicula* and *Pinnularia*) and possessed different sizes, outlines and striation patterns, might all represent a single highly variable species, which he called '*Navicula varians*' (fig. 2). The reason given was his perception that there was "a very large number of intermediate or transitional forms" and that "in many species at least, the shape or outline is subject to endless variations", so that "form, shape, or outline cannot be regarded as a trustworthy specific character". His opinion had been formed after observations of an abundance of material (in his paper, he lists the provenances of the samples studied and for each several or many slides were certainly made and examined, as shown by collections of his slides and notes now in the Natural History Museum, London, and the Royal Botanic Garden, Edinburgh), and the more material he examined, the more he doubted the existence of separate species in this group of organisms: "...the more the Diatomaceae are stud-

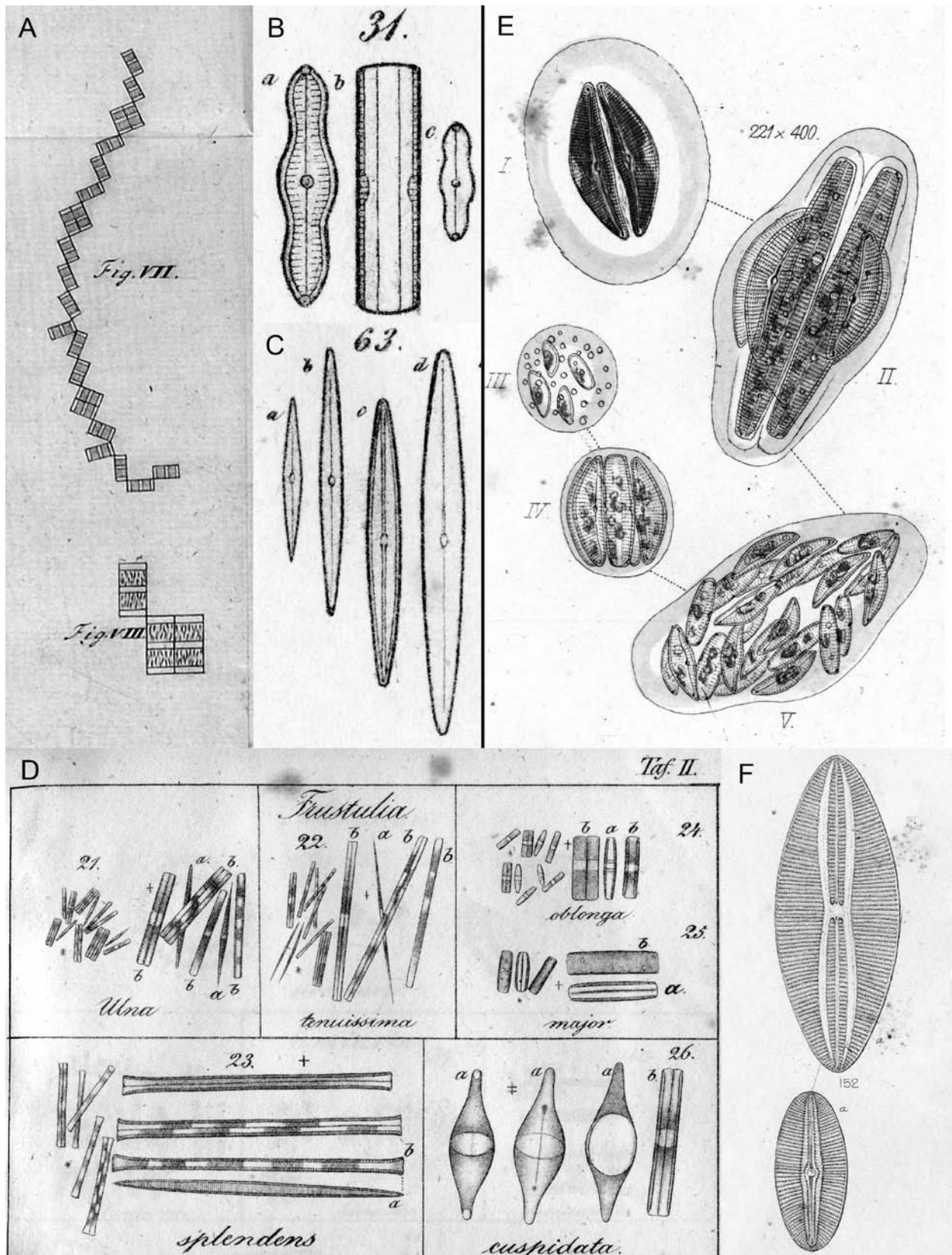


Figure 1 – A, the first published illustration of a diatom, from Anonymous (1703) (Image © The Royal Society, reproduced by permission); B & C, '*Pinnularia limosa*' and '*Navicula amphioxys*' from Rabenhorst (1853, pl. 6, figs 31 and 63, respectively); D, six '*Frustulia*' species illustrated by Kützting (1833, pl. 2): each species (except *cuspidata*) is shown at two magnifications; E, hypothesized life cycle of *Cymbella*, from Smith (1856, pl. C); F, normal (below) and sporangial valve of '*Navicula elliptica*', from Smith (1853, pl. 17, fig. 152).

ied, the more do we perceive that, in many species at least, the shape or outline is subject to endless variations'. There is now consensus, however, based on much further study and better knowledge of the life cycle, that Gregory's '*Navicula varians*' does in fact comprise many different species (e.g. Krammer & Lange-Bertalot 1986).

Nevertheless, although Gregory and other early 19th century diatomists might have found it difficult to decide whether or not a particular set of specimens represented a single species or several, they did not question the ultimate reality of species. I can find no evidence that early 19th century diatomists held views significantly different from the contemporary higher plant taxonomist G. Bentham, who stated that species have a "distinct existence in nature as a group of individuals" (1832) and that "A species comprises all individual plants which resemble each other sufficiently to make us conclude that they are, or may have been, all descended from a common parent" (1858). The reason for diatom species' existence was clearly stated by Smith (1856) when he said that although "The determination of genera ... [is] mainly an artificial mode of ... conveniently grouping together forms possessing in common important and obvious characteristics of structure or function ... it is far otherwise with the determination of species. *We here seek to discover the distinctions which have been impressed by nature upon every individual derived by reproduction or by self-division from the original product of the creative act*" (italics mine). In other words, species were real in a way that genera and higher categories are not, because each species represented a separate act of creation and possessed its own special characteristics: it was uniquely diagnosable.

This view of species as the fundamental, created units of diversity is reflected in the structure of early 19th century diatom classifications: the lowest taxa recognized are species, with no formally named infraspecific taxa (e.g. Ehrenberg 1838, Kützing 1844, Rabenhorst 1853, Smith 1853, 1856). Special morphologies, such as sporangial valves, were indicated as such (e.g. "Var. (Sporangial?) from Poole Bay": Smith 1853: 48, under '*Navicula elliptica*'), and other variants were sometimes referred to via Greek letters, to differentiate them from the 'typical' form (designated 'α'). For example, in *Diatoma elongatum* W. Smith, Smith (1853) distinguished three varieties, β, γ and δ, which "must be regarded as varieties ... [of the species] dependent upon the stages of its growth, or the circumstances which attend its development."

TRANSITIONAL PHASE, 1860–1885

The theory of evolution by natural selection, given substance by Darwin in 1859, seems to have had no immediate impact on diatom taxonomy. Diatom papers in the 1860s by Greville, Arnott, Donkin and others do not mention evolution and there appear to have been no diatomists among the major protagonists in debates about the reality or mechanisms of evolution. However, by the 1880s, changes in diatom taxonomy had occurred – in particular, the recognition of many named varieties and forms within existing or newly described species – that seem to owe their origin to the influence of evolutionary thinking. One effect of Darwin's theory was to undermine the idea that species were a 'special' category, distinct from va-

rieties in their nature and origin. Instead, gradual divergence between lineages was to be expected and would create all degrees of difference between groups of individuals, from barely detectable differentiation in one or a few characters to correlated differences in many. In a famous paragraph from the 'Origin of Species', Darwin (1859: 52) wrote "... it will be seen that I look at the term species, as one arbitrarily given for the sake of convenience to a set of individuals closely resembling each other, and that it does not essentially differ from the term variety, which is given to less distinct and more fluctuating forms. The term variety, again, in comparison with mere individual differences, is also applied arbitrarily, and for mere convenience sake." Hence, although they were arbitrary conveniences, varieties assumed importance as incipient species. And where the significance of morphological differences was unclear or where variants seemed to be linked, albeit tenuously, by a chain of intermediates, taxonomists could simultaneously show caution and imply an evolutionary explanation for the difficulties they encountered by using a hierarchy of categories within species (including forms, varieties and subspecies), reflecting successive stages in speciation.

Taxonomic practice in diatoms c. 1860 can be illustrated best in the work of the Austrian, Albert Grunow. Grunow earliest contributions (1860, 1862, 1863) deal with variation in much the same way as Smith (1853, 1856). He used 'varieties' to flag unusual or noteworthy variants, which were designated via a Greek letter and (usually) a diagnostic descriptor in Latin. For example, short, elongate and normal valves of *Navicula rhynchocephala* Kütz. were referred to as the varieties 'α brevis', 'β elongata' and 'δ genuina' (Grunow 1860: 529, 530). Grunow's (1863) comments on diatom taxonomy also agree well with those of earlier authors, e.g. Gregory (1855) and Smith (1856), in emphasizing the underlying reality of species and also the need for an understanding of the growth and reproduction of diatoms, which Grunow regretted was largely absent.

In 1885, however, when Van Heurck wrote the text accompanying the plates of the *Synopsis des diatomées de Belgique* (published from 1880 onwards), he openly doubted that species could be defined with any certainty and that species had any more reality than varieties, genera or other taxa: "c'est par l'étude attentive de nombreuses diatomées qu'on voit bien que les êtres forment une chaîne continue, que nous brisons artificiellement..." He considered that it was desirable, at least for didactic purposes, for diatoms to be classified into a relatively small number of 'principal types', recognized as species, which were subdivided into 'secondary types', recognized as varieties. This philosophy is clearly consistent with Darwin's view that the distinction between species and varieties is one of convenience and it appears to have been influential in causing the proliferation of diatom varieties and forms (i.e. the 'secondary types') in the late 19th and early 20th centuries; this trend is already evident in Grunow's later work (e.g. in Cleve & Grunow 1880). Even more than previously, taxonomists now argued about the status of taxa, moving them from species to variety or vice versa according to their perception of the importance of particular characteristics, or the distinctness of taxa.

Another vital advance during the ‘transitional phase’ (1860–1885) was elucidation of the diatom life cycle, principally by Ernst Pfitzer, who showed that diatoms get smaller during the vegetative phase of the life cycle and who expounded a mechanism (Pfitzer 1869, more or less simultaneously with MacDonald 1869) to account for this phenomenon (fig. 3A). At the same time, he demonstrated the significance of the size restitution phase, inventing the term ‘auxospore’ (Pfitzer 1871) to replace the misleading ‘sporangial cell’. Although Braun (1851) had already suggested that diatom cells decrease in size during the vegetative phase, it was Pfitzer’s work that provided the first real guide for taxonomists to interpret size variation. However, although diatoms were now known to alternate between a long phase in which size decreases and a short phase in which size is restored via an auxospore, many features of the life cycle remained unknown, including the significance of the cycle in terms of genetic recombination and (more immediately important for taxonomists) the changes in shape and pattern that often accompany size reduction.

MODERNIST PHASE, 1885–1980

For nearly a century after 1885, taxonomic philosophy changed very little in diatoms, largely ignoring the arguments and counterarguments about the nature of species that began during concerted attempts to use population genetics insights to address evolutionary questions in the Neo-Darwinian ‘New Synthesis’ (or ‘Modern Synthesis’) (Dobzhansky 1941, Mayr 1942). Consequently, there was no fundamental change in the way diatom species were recognized and delimited. Just as before, informal analyses of variation in morphology were used to reveal gaps in the variation pattern. These were taken to indicate the boundaries of species or varieties or forms, according to whether the gaps were more or less obvious, or involved characters regarded as more or less important, or more or less liable to change during the life cycle or in response to the environment. For example, as inspection of more samples seemed to blur the boundaries between taxa in the *Sellaphora pupula* (Kütz.) Mereschk. (= *Navicula pupula*) group, existing species and varieties were demoted to varieties and forms, or abandoned altogether (Hustedt 1930, 1927–1966, Ross 1963).

However, even if there were no fundamental changes in taxonomic approach during the period, there were nevertheless important advances in understanding and knowledge, which greatly aided the discovery and definition of species. Particularly significant was the classic study by Lothar Geitler (1932) of phenotypic plasticity and morphological change during the life cycles of pennate diatoms. Critical parts of Geitler’s work were carried out using clonal cultures, following the development of culture techniques for diatoms (see Mann & Chepurnov 2004), and showed that each clone or species exhibits a fairly well defined (and therefore characteristic) range of size, from the initial cells to the gametangia or smallest viable cells (for detail, see Chepurnov et al. 2004). In addition, Geitler showed that, during the life cycles of pennate diatoms, shape and pattern exhibit similar trends in different species: valve width changes much less than length, both proportionally and absolutely, and valve outline

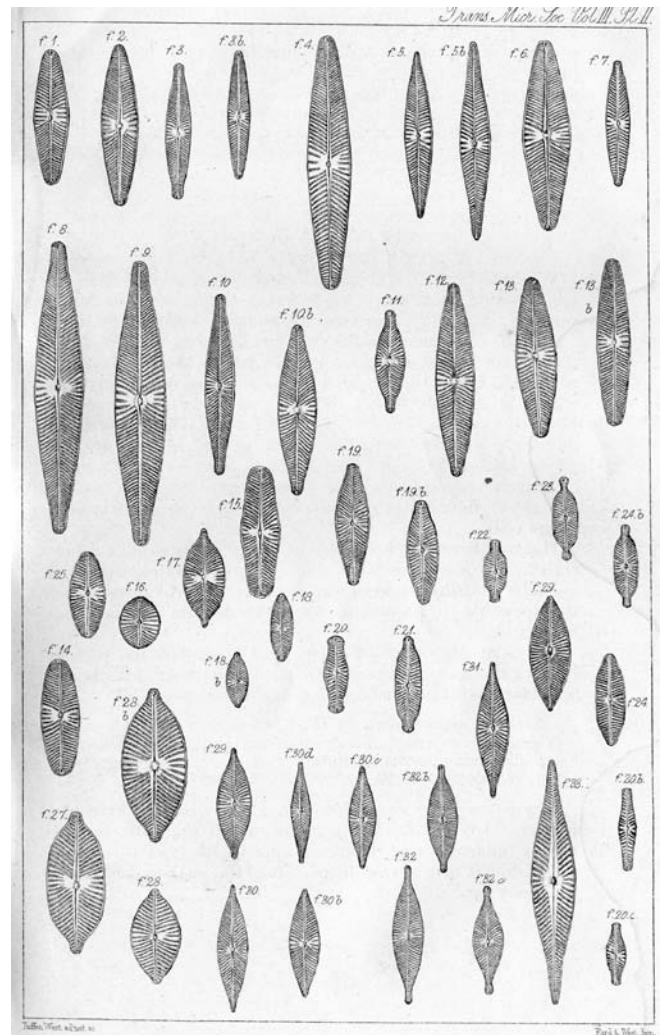


Figure 2 – ‘*Navicula varians*’ from Gregory (1855).

becomes progressively simplified. These ‘rules’ have allowed better interpretation of variation in natural populations and underlie the doubts I expressed earlier about the homogeneity of Rabenhorst’s (1853) ‘*Pinnularia limosa*’, ‘*P. dicephala*’ or ‘*Navicula amphioxys*’. This is not to say that taxonomists *always* misinterpreted shape variation before Geitler’s work. For example, Schmidt’s 1876 drawings of ‘*Navicula yarrensis*’ (fig. 3C, from Schmidt et al. 1874–1959) are plausibly one species; by contrast, those of ‘*Navicula viridis*’ (fig. 3B) are not, because of the contrast in width between the left and right pairs of figures.

There were also significant technological advances between 1880 and 1980. By the end of the 19th century, conventional light microscopes (using incoherent light sources) could be made that were near-perfect in terms of resolution, and the later introduction of phase contrast and interference contrast optics added considerably to the ease with which cell wall detail could be observed. In the 1970s, scanning electron microscopes (SEMs) became widely affordable, offering considerably enhanced resolution and new insights into the structure of the cell wall. However, the main impact of SEM studies was not so much on species delimitation and

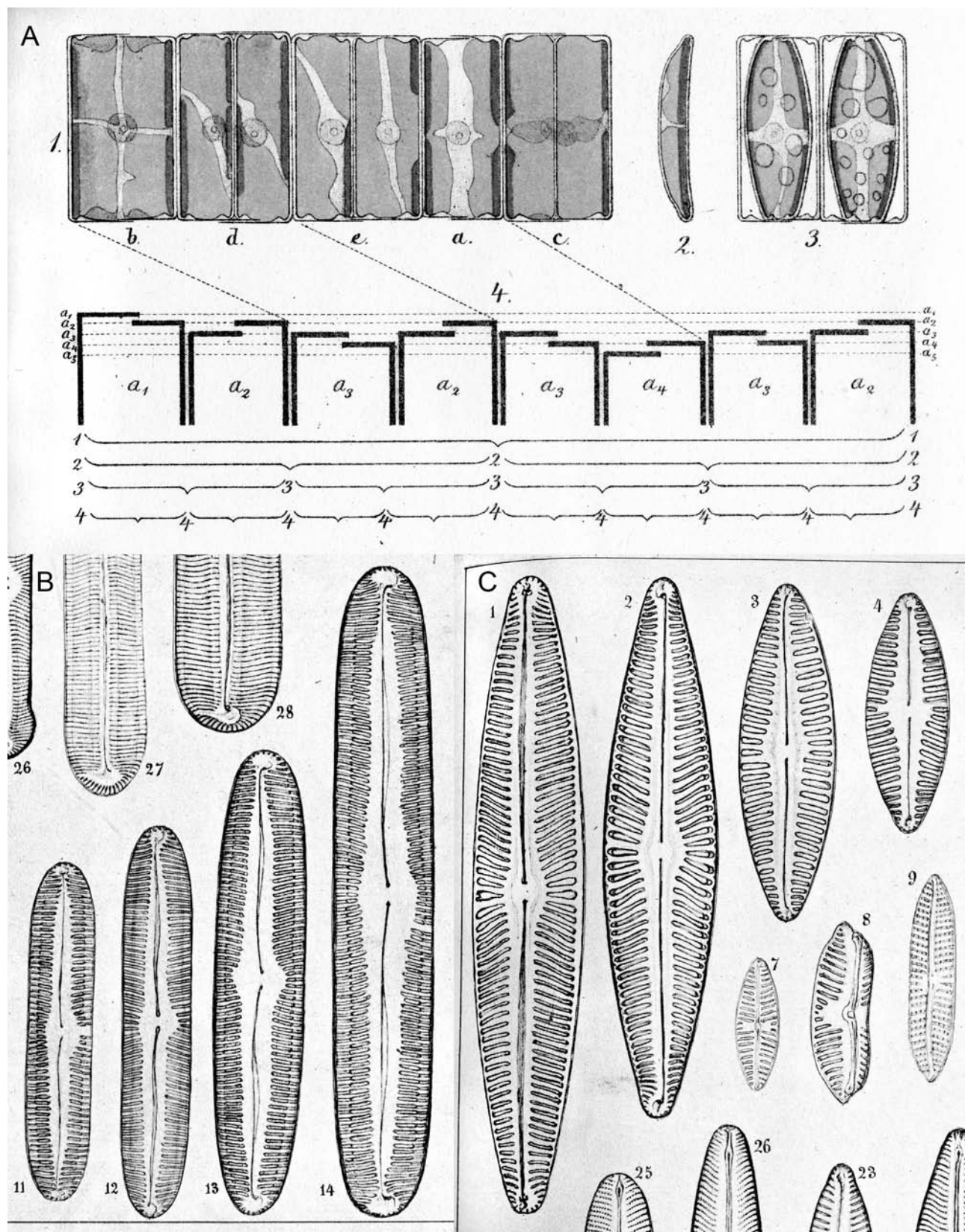


Figure 3 – A, the mechanism of cell size reduction in diatoms, illustrated with reference to a chain-forming *Eunotia* species by Pfitzer (1871, pl. 6, figs 1–4); B & C, ‘*Navicula viridis*’ and ‘*N. yarrensensis*’ valves illustrated by Schmidt (in Schmidt et al. 1874–1959, pl. 42, figs 11–14 and pl. 46, figs 1–4): the discrepancy in width between the two pairs of *N. viridis* valves (figs 11, 12 and figs 13, 14) is incompatible with the ‘rules’ of shape change developed by Geitler (1932), whereas *N. yarrensensis* exemplifies the rules perfectly.

discovery as on the grouping of species into genera and families (e.g. Round et al. 1990). Even in small delicate diatoms, such as *Nitzschia* sect. *Lanceolatae*, light microscopy has remained dominant in species taxonomy (e.g. Lange-Bertalot 1976, 1980), partly because many applications of diatoms, e.g. in palaeoecology or biomonitoring, depend on counting and identification in LM, even if SEM is used for extra checks.

Another technological advance, easily overlooked because it was so universal in its effects, was in the dissemination of information: text could be type-set more easily, illustrations reproduced more effectively and cheaply, copy printed and bound more rapidly and consistently and in greater numbers, and the final publications sent to any part of the 'developed' world within a few days or weeks. Some of the major taxonomic works of the 19th century (e.g. Ehrenberg 1838, Schmidt 1874–1959) have always been rare and are now antiquarian collectors' items. There was little excuse for this to become true of any work of the middle or late 20th century, and reprint exchanges have further eroded the earlier provinciality of diatom taxonomy. The publication of catalogues (e.g. VanLandingham 1967–1979, now partly superseded by the online catalogue of the California Academy of Sciences at <http://research.calacademy.org/research/diatoms/names>) and the development of Biological Abstracts (and subsequently "Web of Science") and similar databases have made it much easier to find and access the literature needed for taxonomic checks. However, a significant proportion of the new diatom taxa described in the last 15 years have been published 'off-line', in books that are not readily accessible to the majority of researchers. I hope that ways can be found to mitigate this problem without infringing the publishers' copyright, e.g. through construction of web-based iconographs or floras illustrating the new species.

With greater availability of taxonomic literature for identification and better characterization of diatom habitats, e.g. in terms of water chemistry, came the first attempts to define the ecological tolerances of species semi-quantitatively, for example via the halobion system developed by Kolbe (1927) and Hustedt (1953) for saline waters, or the pH classification of freshwaters by Hustedt (1937–1939). These and other classifications have in turn allowed the development of palaeoecological research programmes and biomonitoring. There has also been a reciprocal benefit for taxonomy, since many species have been discovered during applied research and many taxonomic revisions have been initiated because of difficulties encountered during routine identification.

One aspect that did not improve during this period was the availability of taxonomically important material (slides and preserved samples), relative to the demand for it. The rules of botanical nomenclature have imposed ever clearer and stricter requirements for naming, describing and typing taxa, and meeting these requirements often demands re-examination of original or authenticated material. Unfortunately, significant collections have been lost (e.g. of C. Mereschowsky, B.V. Skvortzow), or are unavailable (e.g. owing to the lack of a curator), or are too fragile to be sent to those needing to study them. Nevertheless, re-evaluation of type material became a major focus in the latter half of the 20th century (e.g. Schoeman & Archibald 1976–1980, Lange-

Bertalot 1976) and excellent illustrated catalogues of the types of F. Hustedt, H. Heiden and R.K. Greville were made by Simonsen (1987, 1992) and Williams (1988).

DISAGREEMENTS ABOUT THE SPECIES CONCEPT AND THE INTRODUCTION OF MOLECULAR SYSTEMATICS

The first discussion of reproductive isolation as a factor in diatom speciation and as a basis for differentiating between supraspecific and infraspecific variation seems to have been made by Comber (1897), who went against much contemporary thinking (e.g. as summarized by Romanes 1895), which regarded species boundaries as arbitrarily set along a continuum of differentiation in inherited characters, from low (varieties and forms) to high (genera to classes and phyla). Comber suggested that species are real, arguing that sterility between members of different species would prevent the formation of intermediates between them, and so "cut up the originally continuous chain of related forms into separate sections, ...[which] are what appear to me to be natural species, sufficiently distinct and separable from each other, without any reference to special creation". I have previously reviewed this work (Mann 1999: 442), which seems to have had no followers.

The New Synthesis of the late 1930s onwards brought together population genetics and systematic and biogeographical data, mainly for animals, to suggest that species arise through restriction to gene flow between populations, allowing divergence through adaptation and genetic drift. The corollary was that individual species might be recognized by their reproductive isolation from other species and the consequent absence of gene flow. Two definitions of 'biological species' were offered by Mayr (1942: 120): (1) "A species consists of a group of populations which replace each other geographically or ecologically and of which the neighbouring ones intergrade or interbreed wherever they are in contact or which are potentially capable of doing so (with one or more of the populations) in those cases where contact is prevented by geographical or ecological barriers"; or more briefly (2) "species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups." Mayr admitted the difficulties of applying these definitions to populations separated by space or time (allopatric or allochronic populations), and said that "reproductive isolation is ... an immediate, practical test only for sympatric, synchronically reproducing species" (Mayr 1942: 121). For practical purposes, discontinuities in the pattern of variation (of any inherited characteristics: morphological, cytological, physiological, biochemical, molecular genetic) were to be taken as markers of reproductive isolation, but "to the adherent of a morphological species concept, any clear-cut morphological difference is a species difference", whereas "to the supporter of a biological species concept, the degree of morphological difference is simply considered as a clue to the biological distinctness and is always subordinated in importance to biological factors" (Mayr 1942: 121).

Bonik & Lange-Bertalot (1978) accepted Mayr's biological species definition and discussed its application to diatoms, particularly to *Nitzschia*. They carried out morphometric

studies that suggested to them that variation was more or less continuous within *Nitzschia* sect. *Lanceolatae* and concluded from this that biological species could not be distinguished within the group (Bonik & Lange-Bertalot 1978: 131: “Die lanceolaten Nitzschien lassen sich aufgrund der traditionellen Merkmale nicht in verschiedene Biospecies auftrennen, da alle diese Merkmale kontinuierlich variabel sind.”). Bonik (1982) suggested further that gene flow is practically universal among sect. *Lanceolatae* (which therefore comprise a ‘syngameon’), but took place at variable rates and mostly within local populations, allowing the formation of a myriad local ecotypes. No information about gene flow was then available for any diatoms, and it was not even known whether most *Nitzschia* species had biparental sexual reproduction. The recent study of *N. palea* (Kütz.) W.Smith by Trobajo et al. (2009) supports the idea that variation can appear continuous in some *Nitzschia*. However, this work indicates that (1) morphologically and genetically different demes exist within this species complex, (2) more than one deme sometimes coexist in the same locality, implying barriers to free gene exchange even when there is no geographical separation (3) what appears to be the same deme, judging by morphological and LSU rDNA similarity, can occur in sites as far apart as Spain and Japan, indicating high dispersal capacity, and (4) some groups of clones are apparently reproductively isolated from others, although morphologically and genetically similar clones from widely separated localities (Spain, Paraguay and Brazil) can sometimes mate, as required by the syngameon idea. An earlier study (Trobajo et al. 2006) showed that some *Lanceolatae* are automictic, which would also argue against the general validity of the syngameon model. Nevertheless, Bonik’s basic idea, that the appallingly difficult taxonomy of *Nitzschia* sect. *Lanceolatae* may reflect an unusual and complex reproductive biology, could yet prove correct (suggestion of R. Trobajo, IRTA, Spain, pers. comm.).

In 1984, reproductive isolation was detected between sympatric demes of *Sellaphora* and *Amphora* in rough cultures of epipelon from a small pond in Edinburgh (Mann 1984), leading to a series of papers exploring the relationship between morphological similarity and crossability, and later also between these and DNA sequence variation (e.g. Mann 1989, 1999, Behnke et al. 2004, Mann & Chepurinov 2005, Amato et al. 2007, Casteleyn et al. 2007, Vanormelingen et al. 2008). The claim made explicitly or implicitly by these papers is that information about reproductive isolation and gene flow is helpful in the discovery of species, or for confirming the validity of species that have already been described. Other diatomists, however, have rejected Mayr’s biological species definitions and any other species definitions (e.g. Hennig’s: see Meier & Willmann in Wheeler & Meier 2000: 30–43) that claim to rely on a distinction between reticulate relationships (where sexual reproduction leads to gene flow and recombination) and hierarchical relationships (where genetic novelties are restricted to the lineage in which they first arose or in which they were fixed following speciation). Thus, Williams & Reid (2009: 186–188) said that there was “little or no evidence” that morphological differentiation reflects reproductive isolation, and that systematics (taxonomy) should be an almost entirely separate discipline from population genetics: in their view, taxonomy may provide or allow

research programmes for population geneticists, but taxa are to be defined by “a unique combination of character states”, not a hypothesized process (reproductive isolation) by which this uniqueness may be maintained. In another critique of ‘biological’ approaches, Mishler & Theriot (in Wheeler & Meier 2000: 123–126) noted that reticulation and the presence of barriers to mating are usually inferred from morphological or other phenotypic evidence, rather than being demonstrated directly; that biological definitions are useless for organisms that are not sexual (and they suggested that “although diatoms are technically gamospecies, sexuality is extremely rare”, which is misleading in my view but needs a separate review), each individual being reproductively isolated from all others; and that biological definitions such as Mayr’s short version (1942) cannot be applied in any meaningful sense to allopatric populations. For Mishler & Theriot, species are no more or less ‘real’ than any other taxa (in Wheeler & Meier 2000: 183): all taxa are real if they are monophyletic. Others disagree (e.g. Mann 1999), arguing that species have a unique status in sexual organisms (and most diatoms do seem to be sexual), because they mark the fuzzy boundary between reticulate and hierarchical patterns of variation.

The “real question” according to Williams & Reid (2009), is “how might ... taxa, species ... be discovered in the first place”, rather than whether or not morphologically distinct taxa interbreed. However, in advocating their preferred approach [“we define species as the smallest aggregation of (sexual) populations or (asexual) lineages diagnosable by a unique combination of character states”] and in defending the “long and commendable record in diatom systematics and taxonomy” based on morphology, I think Williams & Reid overstate the importance of ‘systematic theory’ and understate the extent to which insights from cytology, reproductive biology and other sources do and should inform taxonomic decisions based on morphological data. The morphological characteristics of each individual diatom valve are the products of unique interactions between a particular genotype and epigenotype and the environment, but it is only the *heritable* characteristics of the organism that are relevant to taxonomy. So, how do we work out which are the heritable characteristics that we can use for assessing relationships when all we may have are dead specimens and no direct knowledge of the processes involved in their formation? Only, I suggest, by interpreting differences and similarities in the light of a general understanding of valve ontogeny (e.g. Pickett-Heaps et al. 1990), the life cycle (e.g. from Pfützer 1871, Geitler 1932, etc), phenotypic plasticity (e.g. Jahn 1986, Trobajo et al. 2009) and programmed polymorphism (e.g. of valve pattern or symmetry relationships, like those documented by Stoermer 1967, or Mann 1980), and also, more mundanely, the artifacts produced by dissolution and specimen preparation. For example, it seems to me that it is information about how pennate diatoms change size and shape during the life cycle, and the general constancy of stria densities within a clone whatever the environment or life cycle stage, that makes it plausible that the four specimens of *Colliculoamphora edgarensis* G.Reid & D.M.Williams illustrated by Williams & Reid (2009: figs 4–6, 8) belong to the same species and are separate from *C. reniformis* G.Reid & D.M.Williams (op. cit.: figs 7, 9). Otherwise, in the absence of information about

whether the valves illustrated by Williams & Reid represent all of those studied or extremes selected to illustrate the range of variation, we might reasonably group the large specimens of both (figs 4, 9) into one taxon and the smaller ones (figs 5–8) into another.

Furthermore, Williams & Reid seem to have overlooked the taxonomic context of some of the diatom studies published since 1984 (listed above) that have advocated use of some version of the ‘biological species concept’ for the discovery or confirmation of species. Most of these studies concerned either nominal species that had been claimed to be continuously variable, such as *Sellaphora pupula* (explicit claim by Ross 1963) or *Neidium ampliatus* (implicit claim by Krammer & Lange-Bertalot 1986), or groups such as *Pseudo-nitzschia* in which very few morphological characters are available. For *Sellaphora pupula*, Ross (1963) wrote that “when a large number of populations are examined... the various forms are found so to intergrade that there seems little justification for the taxonomic recognition of them at any level”; and as Mishler & Theriot say (in Wheeler & Meier 2000: 125), “if all features are truly continuous, there are no characters, and empirically there are not multiple species, but simply one species. Attempts to force parts of a morphocline into multiple species is not a fault of any species concept ... it is simply bad taxonomic practice...”. So, how can we determine whether variation is truly clinal, as Ross believed, or not? My observations of mating patterns among particular localized populations of *S. pupula* (e.g. Mann 1984) suggested that Ross’s morphocline is more apparent than real, since sympatric populations that have only subtly different morphologies cannot interbreed. Subsequent studies revealed small but consistent morphological differences in shape and striation, corroborated by molecular differences (Mann et al. 2004, Evans et al. 2007, 2008), leading to the description of several new species. Could mating data have been by-passed and species erected instead purely from morphological analysis? Probably yes, but more likely the validity of the ‘morphoclines’ would have remained untested, just as it already had been for decades (see Mann 1984). In *Nitzschia palea*, there appears to be a morphocline between clones with broad, coarsely striated valves and those with narrow, finely striated valves (Trobajo et al. 2009); the taxonomic ‘solution’ had previously been to include all in a single highly variable species (Krammer & Lange-Bertalot 1988). However, Trobajo et al. (2009) have shown that (1) individual clones vary little in width and stria density in laboratory cultures and remain distinct from other clones; (2) molecular data indicate phylogenetic structure within the *N. palea* complex, including some groupings of clones that are also similar morphologically; and (3) there seem to be reproductive barriers between some of the clones or populations. In this case, there is still no clear answer as to the best taxonomic treatment and further analysis (e.g. using other molecular markers) is needed, but morphology alone seems to have reached its limits and there are apparently no unique combinations of morphological character states that could be used to break this complex into diagnosable morphospecies.

The example of *N. palea* is just one of many recent studies that have used gene sequence data to investigate species diversity (earlier studies were listed by Mann & Evans 2007).

Problems that are inherent to morphology-based taxonomy, such as incorrect assessment of homology, also afflict molecular systematics, and the problems of convergence are worse with nucleotides (the end-product of evolution of T→A or C→A or G→A or A→A at a particular site is A, whereas convergence in morphological characters could probably always be detected if the character complex is broken down into its constituent parts, e.g. the genes and genetic controls that create it). Molecular methods have become attractive for species discovery and definition in diatoms because, given a suitable gene (in the rate and mode of evolution), many characters can be scored unambiguously, quickly and cheaply. Currently, only one or a very few genes are analysed in each study, which could be misleading if relationships among the organisms studied are reticulate because of hybridization and gene exchange. The assumption being made in most cases, though it may not be stated, is that if the level of divergence between two gene clades exceeds a particular threshold, then those clades deserve species status, though this is sometimes then ‘checked’ by looking for accompanying morphological differences (e.g. Sarno et al. 2005) or evidence of reproductive isolation (Amato et al. 2007); molecular divergences that are less than the threshold are regarded as ‘intraspecific’. However, there is no reason to assume that molecular, morphological and reproductive characters will always evolve in tandem. For example, two sets of populations that are reproductively isolated from the other, and therefore qualify as ‘biological’ species, may not differ with respect to a particular molecular marker that always separates ‘biological’ species in other diatom groups; and it is almost axiomatic that individuals within a ‘biological’ species display some degree of heritable variation in morphology or physiology, and in neutral genetic markers – otherwise there is no basis for adaptive or random evolutionary change. Thus, whatever the data-set used to construct the initial taxonomy, each species remains a hypothesis that is liable to be overturned as more information becomes available from new sources or new material. For cryptic species (see the next section), of course, there is no substitute for molecular methods or mating experiments (e.g. Quijano-Scheggia et al. 2009): here, morphology is by definition inadequate for species discovery and identification.

THE FUTURE: A POSTMODERNIST AGREEMENT TO DIFFER OR ACHIEVING CONSENSUS?

At the moment, diatomists espouse a number of different species definitions – phylogenetic definitions of one sort or another (e.g. Mishler & Theriot in Wheeler & Meier 2000), biological definitions modified or not from the New Synthesis versions (e.g. Bonik & Lange-Bertalot 1978, Mann 1999), or practical definitions based on the degree of morphological difference. In practice, the outcomes are probably often similar (although this can rarely be judged, since different taxonomists generally avoid working on the same problems at the same time) and so one solution to disagreements about definitions is the postmodernist one of bypassing them, in effect treating all definitions as valid despite their contradictions. This is easy but is unlikely to produce convergence towards a stable taxonomy: the history of the last forty years shows huge swings in taxonomic practice, from narrow to

wide species concepts and back again to narrow, and there has also been less tendency than hitherto to describe new taxa as varieties or forms. These changes have not been explained or justified (see Mann 1999) and, until they are, there be no confidence that they will not be reversed when a different generation of diatomists sets the fashion. A second possibility is to seek, through argument, to eliminate support for all except the one true way of discovering and delimiting species. This seems to be the ambition of Williams & Reid (2009), who suggest that species discovery should be based solely on character distributions, with no place for breeding data.

A third option is not to ignore differences between different approaches, nor to argue that only one (biological? character-based? morphological?) can be correct, but to explore the extent to which they are complementary – different ways to evaluate whether or not speciation has occurred. This is the way forward suggested by de Queiroz (2005, 2007), who points out that the various features used to distinguish species – morphometric difference, morphological diagnosability (i.e. that there is an unbridged gap in the morphological variation pattern), reciprocal monophyly of molecular markers, reduced fertility of hybrids, mating preference, full reproductive isolation, ecological differentiation, etc. – are achieved at different times and in a different order in different lineages. All are aspects of the process of speciation, in which one metapopulation lineage splits into two (there is no implication of equality – budding and dichotomous branching both count as splits), a metapopulation lineage being “a metapopulation extended through time” and a metapopulation an “inclusive population made up of connected subpopulations” (de Queiroz 2007). An individual species is therefore “a separately evolving metapopulation lineage” (de Queiroz 2005: 252, 256). The link between this concept and New Synthesis ideas is strong, since the most obvious and usual meaning of the ‘connectedness’ of populations is via dispersal and interbreeding. However, the metapopulation concept avoids saying that the *only* reproductive isolation relevant to species definition is an *intrinsic* inability of organisms to mate with those of another species and produce viable offspring (as in Mayr’s short 1942 definition). It also avoids demanding that species *must* have their own ecological niche, or *must* be morphologically distinct, or *must* have one or more autapomorphies, etc. These and other properties will probably in time be acquired by all species, but they are not all acquired simultaneously, and the order in which they appear will vary from lineage to lineage.

A corollary to the metapopulation lineage concept is that “any property that provides evidence of lineage separation is relevant to inferring the boundaries and numbers of species” (de Queiroz 2007: 883). Williams & Reid (2009) say that “species, like any other taxon, should be regarded as testable hypotheses” in agreement with “the scientific process”, and I agree. But these taxonomic “hypotheses” can be erected according to any of the features that arise during speciation, be they morphological differences or ecological separation, or divergence in selected molecular markers, or reproductive isolation, etc. Some of these features are character-based (e.g. from morphology or gene sequences), as Williams & Reid prefer, whereas others are not (e.g. ecological characteristics or reproductive compatibility, though all of these features

ultimately reflect the genetically controlled physiology and biochemistry of the organisms). And however they are first erected, hypotheses of species’ existence can be tested using evidence of any kind – the kind used originally or a different kind. If the new evidence supports a different hypothesis of species boundaries, then, as Williams & Reid (2009) point out, both the original data-set and the new one need to be re-evaluated. The conflict between them may arise because, although the species being compared are indeed separately evolving metapopulations, they have not yet acquired all the properties that such metapopulation lineages will eventually possess. On the other hand, the conflict may be because populations *are* connected and *are not* separately evolving; they may even represent parts of the same life cycle. Or, what was thought to be a single metapopulation may in fact be a composite of several metapopulations.

However, although any marker of speciation can potentially be used for species discovery, currently the first steps in exploration almost always involve morphology, and those of us who advocate use of molecular and mating data may not have emphasized this enough. The identification and discovery of diatom species, now as for the last 200 + years, almost always begins with the light microscope (because this is the most practical way to screen the diversity within a sample), and taxonomic problems are usually selected for study because the pattern of *morphological* variation is puzzling in some way: a new morphology is observed, morphologies formerly thought to be distinct seem instead to be connected by an unbroken chain of intermediates, or conversely, variation previously thought to be clinal seems on closer examination to be organized in discrete ‘packets’. But in future, morphology may not always be the primary screen in the assessment and discovery of species diversity. Exploration using molecular genetic methods (e.g. via ‘DNA barcode’ genes, e.g. Evans et al. 2007, Moniz & Kaczmarek 2010) is now possible and becoming inexpensive, especially when compared to the cost of training, employing and equipping skilled microscopists. Furthermore, cases are already appearing in diatoms where speciation is apparently uncoupled from morphological divergence (e.g. Quijano-Scheggia et al. 2009), or is accompanied by such small qualitative or quantitative differences as to make morphological separation nearly impossible. For example, after twenty years of intermittently intense study in British lakes, we described *Sellaphora auldreekie* D.G.Mann & S.M.McDonald as a new species (Mann 1984, Mann et al. 2004; fig. 4A, B). Recently, we found and isolated similar specimens in Australian and British lakes (fig. 4C–N), which we initially identified as *S. auldreekie*. In order to check the identifications, we obtained partial *cox1* and *rbcL* sequences (P. Vanormelingen et al., in prep.), applying the DNA barcode approach of Evans et al. (2007). These revealed that some of the new clones from Britain and Australia were indeed identical to true *S. auldreekie*, but most (again from both Britain and Australia) were not and belonged to at least five further molecular clades. Each of these five fulfils Williams & Reid’s (2009) species criterion of diagnosability, since they have unique nucleotide characters, but they cannot be identified reliably on the basis of morphology (fig. 4) because the ranges of variation in metric characters (e.g. stria density, valve width) overlap and no unique presence/absence characters

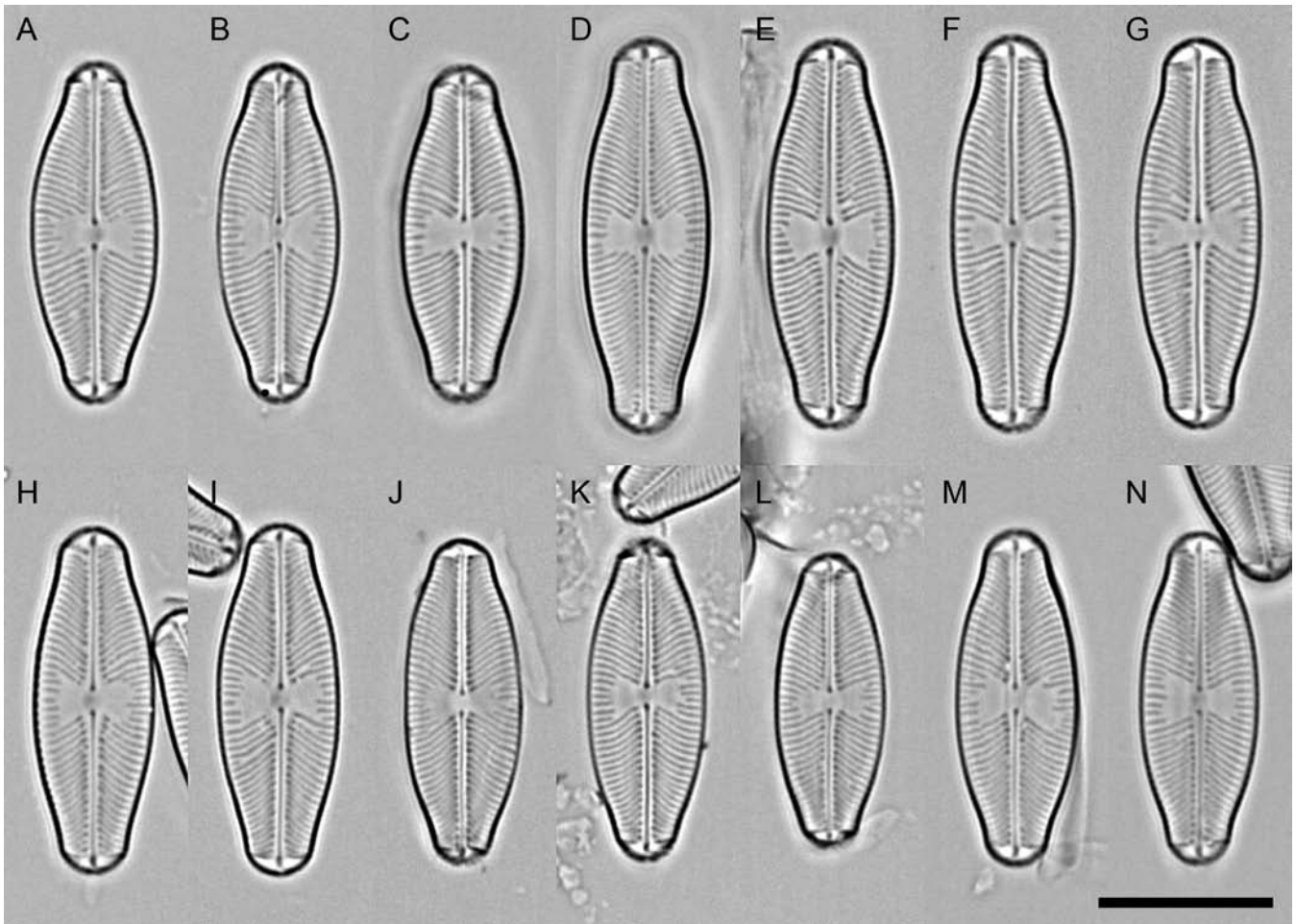


Figure 4 – The *Sellaphora auldreekie* complex: selected valves representing six molecular clades (*cox1* and/or *rbcL*: Vanormelingen et al., in preparation), three of which (*auldreekie*, clade 2 and clade 5) have been tested for reproductive compatibility and found to be isolated from each other. Voucher slides are held in the diatom herbarium at E. A & B, *Sellaphora auldreekie* from Inverleith Pond, Edinburgh, Scotland (clone SEL776INV, voucher E4289); C–E, clade 2, from Kew Billabong, Victoria, Australia (C, clone SEL620K, voucher E4154; D & E clone SEL642K); F & G, clade 3, from Lochend Loch, Edinburgh, Scotland (clone LE D35, voucher E3823); H & I, clade 4, from Lake Mumblin, Victoria, Australia (clone MM 111, voucher E4469); J–L, clade 5, from Kew Billabong, Victoria, Australia (J & K, clone SEL629K, voucher E4163; L, clone SEL624K, voucher E4158); M & N, clade 6, from Streeton Pond, Victoria, Australia (clone Str 13, voucher E3762). Scale bar = 10 μ m.

have been found. Two of the new clades have been tested for compatibility with *S. auldreekie* and found to be reproductively isolated from it (P. Vanormelingen et al., in prep.). Cryptic and pseudocryptic species such as these ‘auldreekies’ have the potential to degrade or invalidate discussion of biogeography, ecological monitoring and palaeoecological reconstruction (cf. Pouličková et al. 2008) and should not be ignored.

In many cases, of course, species hypotheses will remain untested for a very long time and, especially after passage of the species description into floras and databases, the whole idea of a ‘testable hypothesis’ begins to seem somewhat esoteric, and we certainly lack the resources to examine every species in detail. Only if the diatom is perceived to be important (e.g. because of unusual abundance, or as an indicator of particular conditions, or because it is toxic), or occurs somewhere interesting (e.g. in a lake known for endemism in other groups of organisms), is it likely that species hy-

potheses will be examined critically. Hasle’s (1965) *Pseudonitzschia* taxonomy, based on some of the most careful light and transmission electron microscopical studies undertaken in the 20th century, would not have been revisited and probed so vigorously (e.g. Lundholm et al. 2006, Amato et al. 2007, Casteleyn et al. 2007) if members of the genus had not been found to produce toxins. However, although we can’t re-test every species hypothesis, the de Queiroz approach to species definition suggests two things that *can* be done: we can (1) understand and respect the contributions that different kinds of evidence and approach can make, and (2) attempt right from the outset to construct robust hypotheses that are likely to survive testing, if anyone has opportunity to do it. In relation to the second of these, “although presence of a single property provides evidence for lineage separation, a highly corroborated hypothesis of ... the existence of separate species ... requires multiple lines of evidence ...” (de Queiroz 2007). This was a point I have tried to make previously

(Mann 1999: 438–441) amidst attempts to encourage diatom taxonomy away from *exclusive* reliance on valve morphology and to take account also of other types of information, including insights derived ultimately from population genetics. I am wholly unapologetic for these attempts. On the other hand, I do apologize if anything I have written, here or elsewhere, implies that microscopical studies of morphological variation are a waste of time or that the “long and commendable record in diatom systematics” (Williams & Reid 2009) does not exist.

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