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## THE SPECIES PROBLEM IN CILIATES - A TAXONOMIST'S VIEW

**Riassunto** — Il problema della specie nei ciliati - Il punto di vista di un tassonomo. Il problema della specie nei ciliati è determinato da due fattori: la natura stessa dei ciliati e la mancanza di protozoologi capaci di approfondirlo. I fatti attualmente noti tendono a favorire l'ipotesi che la maggior parte, se non tutte, le specie di ciliati sono in realtà dei complessi costituiti da molte specie criptiche.

Questi problemi sono discussi prendendo in considerazione Paramecium, Euplotes e Vorticella.

**Abstract** — The species problem in ciliates is caused by two factors - the nature of the ciliates themselves and the failings of protozoologists. Evidence tends to support the view that most, if not all, ciliate species are really complexes of several sibling species. The problems are discussed with reference to *Paramecium, Euplotes* and *Vorticella*.

Key words - Species problem / Paramecium / Euplotes / Vorticella.

The ciliated protozoa are widely recognized as being a taxonomically difficult group. Indeed, in Britain two recent government sponsored working parties, the Aquatic Microbiology Working Party set up by the Natural Environment Research Council and the Advisory Board for Research Councils Review Group on Taxonomy both specifically identified the urgent need for more taxonomic work to be carried out on ciliates and some other protozoa. Why should they be regarded as being difficult and what are the problems? I believe the answer is twofold — firstly it is due to the nature of the animals themselves but secondly it is also partly due to us — protozoologists both past and present. There is a real lack of suitable keys available to enable one to identify ciliates to the generic and species levels

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and the 50 year old keys written by KAHL (1930-35) remain the standard works. This means it is extremely difficult for anyone to be sure whether or not what he has under the microscope has already been described. Descriptions are scattered throughout a wide and diverse range of literature and it is easy to understand why so many previously described species are re-erected as new species simply because the biologist was not able to make as through a search of the literature as was required. This would not be a problem if we all revised a genus with which we familiar at some stage in our careers. Revisionary taxonomy is not as glamorous as some other areas of protozoology but it should be remembered that accurate identification is fundamental to any biological research, yet protozoologists have failed to supply the keys to enable the biologist to do this. Couple this fact with the problems of the animals themselves and you can see we have the perfect formula for taxonomic chaos.

In spite of the recommendations of the International Code for Zoological Nomenclature, type material is rarely placed in a recognised stable collection. Anyone attempting to review the species of a genus of ciliates quickly discovers that the chances of being able to examine previously described material are remote and soon realises that he has to make his taxonomic judgements on descriptions and diagrams that are often inadequate. All too often this is simply reduced to making personal, subjective judgements. This is not usually the case in other animal groups where it is possible to gather together collections of original material from Museums all over the world thus making it possible to make direct comparisons and hence to be rather more objective. The protozoologist's excuse for this, is that it is difficult to make permanent preparations of ciliates - but that is only partly true. It is possible, indeed the British Museum (Natural History) has in its care slides of ciliates made by the Swiss microscopist Eugene Penard that are some 80 years old and they are still in perfect condition. The lack of type material is compounded by those who having prepared slides fail to put them into a stable collection where they will be both cared for and made available to interested scientists. We are all mortal and few surviving relatives understand the potential scientific value of those dusty pieces of glass they have found in your effects. Editors and referees of papers could play an important role in solving some of these problems simply by asking where the type specimen is to be kept and suggest that its location and accession numbers are published alongside the description. There is also a general failure for protozoologists to adhere to the International Code in other ways and this naturally causes problems. CORLISS (1960) has done much to clear up some of the major historical problems but many still remain and this is part of the job of the revisionary taxonomist.

Let us now turn our attention to a consideration of the animals themselves. The species problem in ciliates is mainly due to two factors. According to the discrimination grid published by MAYR (1969) there can be the situation where there are two specimens that are apparently morphologically identical. However on closer inspection, and particularly after mating experiments, it is found there are two, not one, biological species. That is two separate groups of interbreeding natural populations that are reproductively isolated from each other. This discovery was of course initially made by SONNEBORN (1937) who then spent the rest of his highly productive career building the foundations of much that we understand about what were initially known as varieties, then syngens and finally sibling species of the Paramecium aurelia complex. While Sonneborn immediately recognised that he was dealing with different biological species he actively refused all attempts to persuade him to give the 14 sibling species binomial names until nearly 40 years later (Sonneborn, 1975) when methods for their independent identification had been developed. This should serve as an example to us all on how to make sensible interpretations of the International Code. Sonneborn of course knew that he should not give them binomial names unless 'accompanied by a differential diagnosis' the rules until 1961 when the Code was modified to state unless 'accompanied by a statement that purports to give characters differentiating the taxon'. Sonneborn's argument was that until other techniques could be developed, the only method of identification was to compare unknowns against his own stocks by mating experiments and that this could hardly be described as a differential diagnosis. However by 1975 various methods had been developed and so he gave the 14 sibling species latin names in such a way as to cause the least possible taxonomic confusion. The name Paramecium aurelia was declared a nomen dubium since it is not possible to know precisely which sibling MULLER (1773) actually first described. The siblings were then each given a latinised name based on the numerical system used for the syngens, - primaurelia, biaurelia etc. This meant that the name *Paramecium aurelia* has now disappeared as a taxonomically valid name. Even so, the species remain particularly difficult for the ecologist to identify and he still has the problem of what to call the organisms he finds. He could designate the taxon for example as Paramecium sp. or better as a member of the Paramecium aurelia complex or perhaps make use of the supraspecies concept introduced by GÉNERMONT and LAMOTTE (1980). This latter term (not to be confused with superspecies), sets out to taxonomically link together any group of species difficult to distinguish from one another. Thus we would have Paramecium suprasp. aurelia. This seems an excellent and highly practical solution but there still remains a problem with the Paramecium aurelia complex because of the necessity to have a nominate species within each supraspecies. That is to say the latin name of the nominate supraspecies must be identical to the name of one, presumably the oldest, species included in the group. However there is no longer a Paramecium aurelia and so there cannot be a Paramecium suprasp. aurelia aurelia. Géner-MONT and LAMOTTE (1980) suggest that the solution to this is that Sonneborn's name primaurelia be rejected and replaced with aurelia. This seems a reasonable approach but as CORLISS and DAGGETT (1983) pointed out would require a petition to the International Commission on Zoological Nomenclature for consideration before it could become taxonomically valid so to do.

While the majority of this work has been carried out on the P. aurelia complex the presence of sibling species has been identified in other species of Paramecium, in Tetrahymena, Euplotes, Stylonychia and several others so it may well be that all the ciliate taxa currently described as species are really complexes of sibling species. If that is true then the ecologists are the most likely to be affected since biochemists, physiologists, electron microscopists will already be working on cultures and should be able to establish the species. However the ecologist usually needs to identify many taxa over extended periods of time and cannot be expected to culture every population to check the identities of the sibling species. Clearly the ecologist would only be able to identify to complex rather than species level but would this really affect the potential quality of the ecological results? My initial reaction was one of dismay but on reflection perhaps we should not worry too much since we must consider the problem in relation to the accuracy of the other methods being used. Sibling species are, by definition, virtually morphologically identical, although their biochemistry and physiology may well be different. However in most cases it is likely that they feed, respire and divide at approximately the same rates as each other although we should be careful since there is experimental evidence (SONNEBORN, 1957) to show that the maximum growth rates of sibling species in *Paramecium* do vary. If this is true then it seems unlikely that any differences would be detected and while they may add to the general scatter of the data obtained it is doubtful if the effects would be significant. Until our methods are sufficiently good to be able to sample the aquatic habitat in a really adequate manner it seems rather pointless to worry unduly over sibling species unless of course the object of the exercise is to study the distribution or population dynamics of the species within any particular complex.

The second major reason why there is a species problem in the ciliates is also illustrated in MAYR's (1969) discrimination grid. In this case there can be two apparently morphologically dissimilar ciliates that can be shown to interbreed. If the pair are sympatric then they represent a single species showing intraspecific variation but if they are allopatric then they could be superspecies. The wellknown ciliate genus Euplotes has been cited as an example of both possibilities. Initially most species of Euplotes were described purely on the basis of external morphology. Characters included the numbers and distributions of cirri and the macronuclear shape but TUFFRAU (1960) added the dorsal argyrome patterns that could be seen in silver-impregnated specimens. In the early 70's the present author decided to gather the published data on Euplotes and to attempt to construct a key. At the time interbreeding experiments were few and far between which meant that taxonomic judgements had to be made on purely morphological grounds. The system initially set up by TUFFRAU (1960) was extended by defining six groups of species within the genus which possessed a particular type of dorsal argyrome pattern. At the time it was generally thought that the patterns were stable but subsequent work by GATES and CURDS (1979) showed that they vary to such an extent that one is now forced to regard the double-eurystomus and double-patella patterns to represent the extremes of what is actually a continuum of interkinetal ratios that exist within most large populations of double dorsal argyrome Euplotes. Furthermore, some multiple dorsal argyrome clones yield a few specimens having a complex dorsal argyrome suggesting that this latter is only a variant of the former. In view of this GATES and CURDS (1979) proposed to modify Tuffrau's original classification along the more descriptive lines suggested earlier by CURDS (1975) and call them 'single', 'double' or 'multiple'. The first of these groups includes six small marine oval species all with 10 fronto-ventral cirri. Originally CURDS (1975) keyed them out on a basis of the numbers of dorsal kineties. At the time the numbers of dorsal kineties were known to vary in other species of Euplotes - indeed the species known to do so with their ranges of variation were listed (CURDS, 1975) but there was no information concerning the variability of that character in the single group. While the author wished to synonymise the six species, there was no real evidence to do so and the only way to distinguish them was based on the dorsal argyrome. Of course NOBILI (1964) had already shown that E. minuta, E. crassus and E. vannus would conjugate but produced infertile offspring and this tended to support the existence of at least three of the six species. However a year after the publication of CURDS (1975) key Génermont, Machelon and Tuffrau (1976) published the results of their interbreeding experiments with three of the six species involved. They found that whereas crassus and mutabilis would interbreed, crassus and vannus were infertile. This of course conclusively proved that their strains of *crassus* and *mutabilis* were of the same biological species. Furthermore, they concluded that the variability of characters based on the dorsal argyrome and numbers of cirri was sufficient to result in the three morphotypes being indistinguishable from each other. Later, results by GATES (1978) agreed with their findings. He used a highly sophisticated morphometric analytical approach based on the distribution of the ventral cirri. He reported that on this basis the morphotypes of vannus, crassus, mutabilis and minuta were indistinguishable from each other and stated that all marine Euplotes having a single daryrome pattern and 10 cirri should be referred to Euplotes vannus the oldest name. While the present author believes that Gates is probably correct the case has not been proven completely in practice, GATES (1978) did not, for example, work on any clones of E. cristatus or E. balticus and so he can only extrapolate possibilities. So where does this rather confusing situation leave us?

Let us first deal with proven facts. We certainly have the six binomial names which have arisen from observations on a few individuals. New 'species' have been erected when one or two characters were slightly different to those in previously described specimens. Figure 1 illustrates graphically the known variability in numbers of caudal cirri and dorsal kineties derived from the literature up to 1974 superimposed upon the experimental data given by GÉNERMONT, MACHELON and TUFFRAU (1976). It can be seen that it would be difficult to draw boundaries between discrete clusters.



Fig. 1 - Numbers of caudal cirri and dordal kineties in six species of Euplotes. ●
- vannus, ▶ - crassus and mutabilis from Génermont et alia (1976). • Ba - balticus,
• Cs - crassus, • Ct - cristatus, • Mi - minuta, • Mu - mutabilis, • Va - vannus, lines indicate ranges of reported variation. Data derived from Curds (1975).

That is to say intraspecific variation is apparently sufficient to account for these so called 'species' which are statistically speaking morphologically indistinguishable. Furthermore, DINI and LUPORINI (1979) have demonstrated that some strains will interbreed while others will not and that there are multiple mating types in this group of species. So much for the facts now to move into areas of uncertainty. It would appear that the situation here is analogous to the *Paramecium* and *Tetrahymena* problems of the past except that intraspecific variability has been imposed to add to the confusion. There may be a single *Euplotes vannus* species complex of many sibling species although the position is still from clear. There is a wide range of morphological variation and in some morphotypes this has been wrongly interpreted as evidence for species. Little progress can be made with the purely morphological approach. A return to the classical methods of Sonneborn must be made, that is first establish the syngens and only then worry about giving them binomial names. If it does turn out to be a single species complex then it would become the 'vannus' complex and the other five names currently in use should become synonyms and new ones would be needed. This would prevent taxonomic confusion with earlier records. However there is still the distinct possibility that more than one species complexes are involved and that would result in a much bigger nomenclatural problem.

A different aspect of the species problem can be illustrated by the peritrich genus Vorticella. Anyone who has had to identify a peritrich will know that they are usually simple to identify to genus but much more difficult at the species level and in Vorticella this is a gross underestimation of the problem. Even after the taxonomic revision of NOLAND and FINLAY (1931) about 180 described species remained if both marine and freshwater forms are included. One of the classical methods of creating a key for their identification is based on the outline shape of the bell-like cell and it was this character that ROBERTS, WARREN and CURDS (1983) investigated. Initially 33 specimen drawings were selected from the literature to represent the range of outline shape that had been described for Vorticella. These fell into the five 'shape groups' shown in Figure 2. The outline shape was defined by taking the axis of symmetry down the length of the zooid, standardising it to a common length and the distances from it to the body edger were measured at 22 equidistant points. With the outline quantified in this way it is then possible to compare the shapes mathematically and if necessary one can automatically carry out other methods of standardisation within the computer. In Figure 2 the five shape groups are drawn to a standard scale, a standard length, a standard peristome width and a standard maximum body width. The numerical data defining shape could then be treated mathematically where the object of the mathematical treatment was to reduce the information matrix to one or two characteristics so that different individuals may be more easily compared. Two methods were used for the comparison, Principal Components Analysis (PCA) and the Fourier transform. The variously standardised outline shapes shown in Figure 2 were compared to each other using PCA but in all cases good separation was



Fig. 2 - Five species of Vorticella representing five shape groups. Va - V. aequilata (cylindrical), Vp - V. patellina (triangular), Vc - V. campanula (campanulate), Vm - V. microstoma (microstomatous), Vg - V. globularia (globular). A. entire zooids drawn to scale; B. half profile of zooids drawn to standard length; C. profiles drawn to standard peristomial lip width; D. profiles drawn to standard maximum body width.

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not obtained. In the analysis of shape data by width measurements alone, each measurement was treated independently and not as a series forming a profile as the human eye and brain would do. To take account of this, the gradients between adjacent width measurents were calculated and analysed by PCA. Using this approach the best result was obtained from data based on standardisation of body length and maximum body width, some inter-group overlapping was still obtained but the five groups could be resolved if one examined the third component in a 3-dimensional figure plotted by the computer. Information gained from PCA indicated that the most significant gradients occurred at the ends and centre of each data set and implied that the shape could be reasonably represented by the use of just the two angles shown in Figure 3



Fig. 3 - Characterisation of Vorticella profile by the two angles  $\alpha$  and  $\beta.$ 

and a simple plot of one angle against the other resulted in as good a degree of separation as any of the methods previously mentioned. Only three groups could really be distinguished but this approach does have some practical potential as the two angles could be measured fairly easily by the observer.

Another way of handling profiles is by treating the data set as a wave form which can then be processed by Fourier transform analysis. The data were first made into a wave by creating and join-



Fig. 4 - An example of *Vorticella* profile that has been repeated in reverse order and arranged around the mean of the data set in preparation for Fourier analysis.



Fig. 5 - Scatterplot of Fourier analysis of 75 Vorticella species based on standardisation to maximum body width.

ing a mirror image of the outline data at the stalk end of the zooid as is shown in Figure 4. The Fourier data can then be analysed by PCA. On the whole Fourier data resulted in a more even spread of points on the graph and looked encouraging but when the data set was expanded to 75 species of Vorticella a continuum of shape variation could be detected (Fig. 5). However the plot did make reasonable sense as there was a gradual gradation across the space from globular to campanula to triangular shape types. So again we have this problem of a continuum in a character. In the case of *Vorticella* there is still a major taxonomic problem; some fairly obvious synonyms were detected from this study but they would probably have been detected using the more usual subjective approach. There are still about 90 species left and a key is in the final stages of preparation. This should help but we are still left with the problem of knowing precisely what a species might be in the case of Vorticella. There is apparently no published information concerning the presence or absence of syngens but the reason for this is more likely to be due to the lack of a good method for their cultivation rather than an indication that there actually are no syngens.

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