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STATISTICAL METHODS AND SPECIES DELIMITATION IN THE GENUS OTIDEA

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(With three Tables, five Text-figures)

The aim of this study is to show how some simple statistical techniques introduced into routine taxonomical work renders it possible for the species to be delimited with greater exactness and to be identified with more reliability. The study was based on the genus *Otidea*.

For several years I paid attention to the genus *Otidea* and made extensive collections. The number of species in this genus is neither too large nor too small for a preliminary study with the help of statistical methods. Several different patterns of variation can be found in the genus and, although Nannfeldt (*1966*) calls *Otidea* a much neglected genus, his own study and papers by other authors (Kanouse, *1949*; Maas Geesteranus, *1967*) have thrown enough light on this group to try to produce an inductive classification.

This study is based in its essential part on the collections of the Mycological Herbarium of the Institute of Zoology and Botany of the Academy of Sciences of the Estonian SSR (TAA). Several specimens sent to the author for identification or lent from other herbaria were investigated, too. The range of geographical distribution of the material studied covers all the U.S.S.R. Also several Indian collections and some older collections from western Europe were studied. Unfortunately there was no time to borrow American material for biometric study and only Kanouse's type specimens were consulted.

Sections of fruitbodies made by hand were soaked in a drop of 5 % KOH solution, then covered with coverslip and studied under a MBI-6 light microscope. All measurements of microscopic characters were made using a 40x apochromate objective a 7x compensation ocular at the magnification 700x with ocular micrometer scale. 20 spore lengths and 10 spore widths were measured of each fruitbody studied to calculate individual mean values.

The necessary computations were carried out in the Tartu State University Computing Center on an Ural-4 computer and in the ETKVL Computing Center on a Minsk-22 computer. At first nine characters were considered: height of fruitbody, diameter of fruitbody, spore length, spore width, ascus length, ascus width, width of the paraphyses, width of their tip, and width of the hyphae of the medullary excip-

* Paper read at the Symposium "Taxonomy of operculate Discomycetes" held at the First International Mycological Congress, Exeter, 1971.

ulum. The ectal excipular details were found to be strongly correlated with fruitbody colour and rataer difficult to code. Eventually the study was restricted to spore dimensions because the other characters added comparatively little to the distinction of the species.

Since the study was planned as inductive, all specimens were studied and measured first, then grouped into species. For this step no computer aid was required, since experience showed that the species are sufficiently clear-cut to be distinguished graphically from scatter diagrams, using so-called indicator characters. For the species of *Otidea* the indicator characters are spore length and spore width. In addition a third indicator character was used: the material was divided into dark excipled species (like *O. bufonia*) and light excipled ones (including medium brown species like *O. leporina*). Species with regular cup-shaped apothecia were not included in this study. The advantage of the scatter diagram method lies also in the fact that possible errors of measurement can readily be detected. The results of the clustering technique are shown in Figs. 3 and 4.

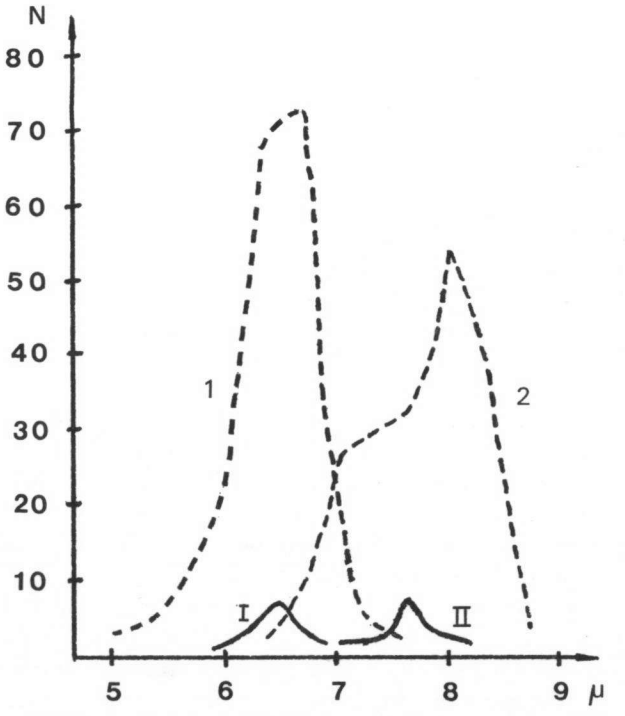

FIG. 1. Spore width distribution curves of *O. onotica* (1, I) and *O. leporina* (2, II). 1 and 2 are compound distributions based on measurements of all spores from all individuals studied. I and II are distributions of individual mean values. The far higher discrimination power of the latter distributions is evident.

The analysis of spore dimensions and other microscopic meristic characters was based on the following principles. Spore dimensions are one of the basic characters of the fungus species. They are usually presented in descriptions by their total range of variability. The total range of variability of single spore dimensions has, however, some unfortunate properties which do not permit its effective use for distinction between species whose spore dimensions are comparatively close together. There is a better way to show the difference between species on the basis of spore dimensions, as can be seen from the following considerations.

Each species has a statistical entity as a set of individuals which are imagined as points in multidimensional space according to the number of characters involved. It is important to emphasize that the distribution of a character, not the character itself, describes the species. The points for single characters, e.g. fruitbody diameter, are determined by a single measurement. Each measurement always has an error but it is usually so insignificant that it may be ignored in the following analysis. Spore and ascus dimensions and other microscopic meristic characters are multiple characters: they can be measured practically in very large numbers in each individual. The distribution of such characters within an individual is more or less normal and characterized by two parameters—mean value and standard deviation.

The mean value of a multiple character j in an individual i determines the point x_{ij} for this individual. The confidence limits of a mean value can be considered analogous to the error of measurement of single character. Practically the mean value is approximated by the arithmetical mean $\bar{x}$ with an error $\varphi \pm \frac{s}{\sqrt{n}}$. It is important for the exactness of the following analysis to reduce the confidence limits of the arithmetical mean to the measurements error ε with an ocular micrometer scale,

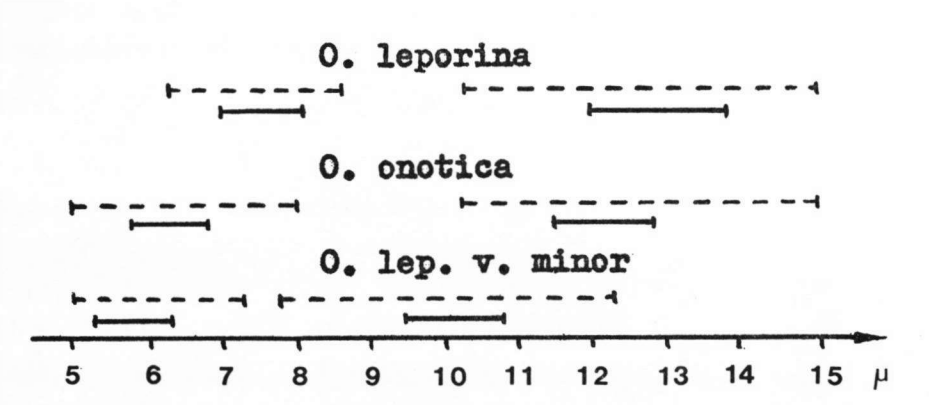

FIG. 2. Comparison of total ranges of variability (dotted line) and ranges of variability of individual mean values of spore width (left) and spore length (right) in three species of *Otidea*. Species indistinguishable in the basis of total ranges of variability can be separated by the ranges of variability of individual mean values.

i.e. to find a sufficient number of single measurements of a character from the equation $n = \frac{(t.s)^2}{\varepsilon}$ where t can be found from the Student's t-table, ε is the wanted minimum value for the error, and standard deviations s has to be found by probe analysis. In the *Otidea* species and in the majority of Discomycetes n varies between 20 and 30 for spore length and does not exceed 10 for the spore width.

Thus spore length and other meristic multiple characters can be presented by two different distributions: (1) the distribution of single spore lengths, and (2) the distribution of individual mean values. The former distribution is based on the spores, the elements of the individual, and can be used for the description of the individual. The species is characterized by the second distribution, which is based on the individuals, the elements of species. The commonly used total range of variability is in fact based on compound distribution summed up from several unequal individual distributions and so is a rather inexact way to describe a species, since it involves a jump over a level of organization, introducing considerable background noise. The correct way to present the multiple character data even in routine taxonomic work should be via specimen mean values. The main advantage of this method—good discrimination between species—can be seen in Figs. 1 and 2. The distribution of specimen mean values can be described in practical taxonomic work by its range of variability or, more correctly, by its tolerance limits, which can be found from the corresponding tables (Owen, *1962*).

The total range of variability, the specimen mean values range of variability, and the tolerance limits for the latter are given for some species of *Otidea* in Table 1. The tolerance limits are given with $P = 90$ which means that at least 90 % of the individual mean values of the given species fall in a given range, ant with $\gamma = 90$ which means that this statement is correct with 90 % probability since the limits are calculated from statistical data.

Turning to the analysis of spore dimension data, graphically presented in Figs. 3 and 4, let us consider first the light-excipled species in Fig. 3. This group is, in fact, distinctly heterogeneous as regards the colour of the apothecia, and should be divided into five subgroups: (1) species with rust-brown or reddish brown apothecia—*O. leporina* (Fr.) Fuck., *O. caligata* (Nyl.) Sacc., and a species for which I have no better name than *O. leporina var. minor* (Rhem) Sacc. sensu Kanouse; (2) species with very light cream-coloured apothecia—*O. alutacea* (Pers.) Massee, *O. rainierensis* Kanouse, and *O. kauffmanii* Kanouse. *Otidea rainierensis* is excluded from this study, since the material available was insufficient, but it may be noted that *O. alutacea var. microspora* Kanouse is identical, while its spore dimensions evidently overlap those of *O. concinna*; (3) light ochraceous to yellowish brown species represented in this study by *O. felina* (Pers.) Bres.; (4) bright ochraceous species with rosy hymenium like *O. onotica* (Fr.) Fuck., which seems to be unique in this group; (5) externally bright lemon-yellow species such as *O. concinna* (Fr.) Sacc., which seems to be unique too. The colour differences between some species are very notable in fresh apothecia but the exact colour is often difficult or even impossible to determine

TABLE 1. — Spore dimension data of some *Otidea* species

	Spore length in microns			Spore width in microns		
	Total range of variability	Range of variability of individual mean values	Tolerance limits for individual maen values $P = 90$, $\gamma = 90$	Total range of variability	Range of variability of individual mean values	Tolerance limits for individual mean values $P = 90$, γ 90
1. *O. leporina* v. *minor*	7.8–12.3	9.5–10.8	9.3–11.0	5.0– 7.3	5.3– 6.6	5.1– 6.9
2. *O. onotica*	10.3–15.0	11.5–12.8	11.5–13.0	5.0– 8.0	5.8– 6.8	6.0– 6.9
3. *O. leporina*	10.3–15.0	12.0–13.8	12.0–13.8	6.3– 8.6	7.0– 8.1	7.0– 8.3
4. *O. caligata*	16.6–21.8	17.8–20.4	17.2–21.4	8.3–12.0	9.5–12.0	9.0–11.8
5. *O. alutacea*	14.1–19.1	15.5–16.8	14.6–17.6	6.6– 8.3	7.5– 8.0	7.5– 8.5
6. *O. felina*	12.0–17.0	13.3–15.8	13.6–16.0	6.0– 7.6	6.3– 7.1	6.3– 7.3
7. *O. kauffmanii*	10.8–13.6	11.5–13.1	10.7–13.4	4.5– 6.0	4.8– 5.3	4.6– 5.5
8. *O. concinna*	9.6–12.3	10.5–11.3	10.1–11.5	5.0– 6.3	5.1– 5.6	5.0– 6.0
9. *O. smithii*	11.5–16.0	13.0–14.4	11.6–15.5	5.5– 7.5	5.8– 6.8	5.3– 7.3
10. *O. bufonia*	12.3–17.3	14.6–16.3	13.8–16.4	5.6– 8.0	6.1– 7.0	6.0– 7.3

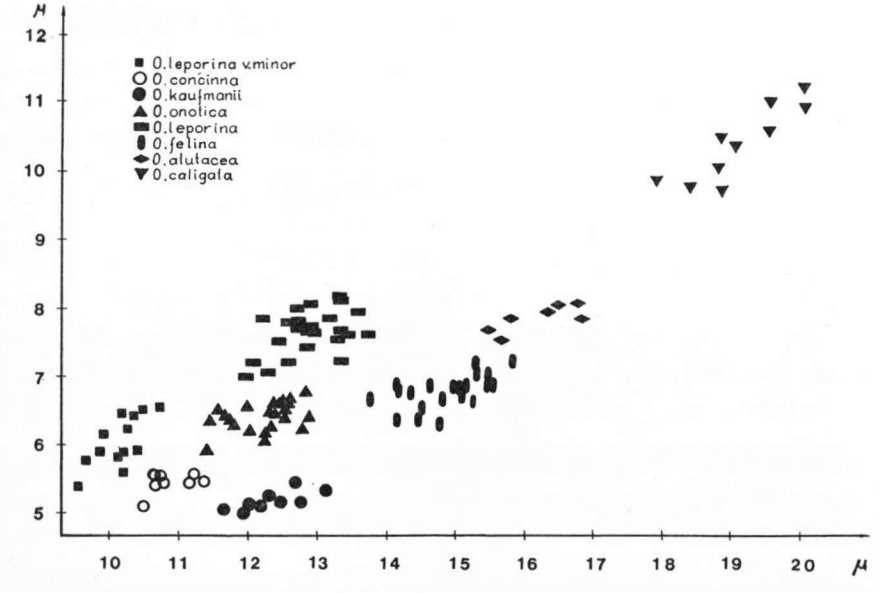

FIG. 3. Spore dimension scatter diagram of light-coloured species of *Otidea*.

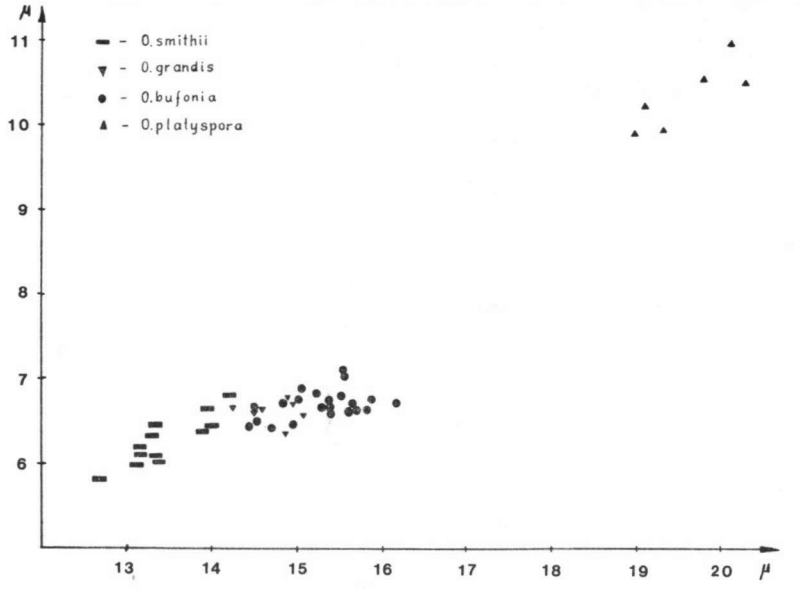

FIG. 4. Spore dimension scatter diagram of dark-coloured species of *Otidea*.

TABLE 2. — D^2 values based on spore length and spore width among 10 species of *Otidea*

	D^2									
	1									
1. *O. caligata*	XXX									
		2								
2. *O. felina*	143	XXX								
			3							
3. *O. alutacea*	67	14	XXX							
				4						
4. *O. kauffmanii*	319	36	93	XXX						
					5					
5. *O. concinna*	342	53	105	16	XXX					
						6				
6. *O. leporina*	147	44	42	93	51	XXX				
							7			
7. *O. onotica*	218	25	52	27	10	17	XXX			
								8		
8. *O. bufonia*	152	1	17	34	60	58	33	XXX		
									9	
9. *O. smithii*	195	59	34	16	24	38	10	8	XXX	
										10
10. *O. lep.* v. *minor*	188	51	45	26	7	14	10	61	34	XXX

TABLE 3. — F values among 10 species of *Otidea* calculated from D^2 values in Table 2.

	N	F								
		1								
1. *O. caligata*	10	XXX								
			2							
2. *O. felina*	22	459	XXX							
				3						
3. *O. alutacea*	7	120	32	XXX						
					4					
4. *O. kauffmanii*	10	713	116	167	XXX					
						5				
5. *O. concinna*	8	670	145	168	31	XXX				
							6			
6. *O. leporina*	26	565	251	108	283	147	XXX			
								7		
7. *O. onotica*	22	701	131	128	87	27	97	XXX		
									8	
8. *O. bufonia*	24	503	5.48	43	113	168	347	181	XXX	
										9
9. *O. smithii*	16	552	26	75	45	58	179	43	37	XXX
										10
10. *O. lep.* v. *minor*	15	517	214	97	72	17	63	42	267	123	XXX

in dried material, which justifies the inclusion of all these species in one group.

The difference between species based on spore dimensions which is evident in scatter diagram can be proved using Mahalanobis' D^2 technique (Rao, *1952*) connected with Hotelling's T^2 and test of significance (Reyment, *1969*). D^2 values between 10 species of *Otidea* are given in Table 2, and variance ratio F values, calculated from D^2 by means of the following formula

$$F = D^2 \frac{N_1 N_2 \quad (N_1 + N_2 - p - 1)}{(N_1 + N_2)\,[p\,(N_1 + N_2 - 2)]}$$

where p is the number of variables, are given in Table 3. The majority of F values are far above the significance level and even in the case of considerable overlapping of spore dimensions (*O. felina* and *O. bufonia*) the difference between two species is significant. For the 2 and 44 degrees of freedom the critical value at the 99 % level is $F = 5.12$ whereas the computed $D^2 = 1.054$ converts into $F = 5.48$, which proves significant difference between the two groups.

For the dark-coloured species (Fig. 3) the picture is essentially the same as for the light-coloured species with the exception of considerable overlapping of *O. bufonia* and *O. grandis* sensu Boud. These species have, however, completely different hymenial colours when fresh, so they cannot be confused.

It becomes evident that closely related species, well distinguished by qualitative characters (colour, gross morphology of fruitbody) usually have very similar spores, the differences between which can be shown only by statistical methods; on the

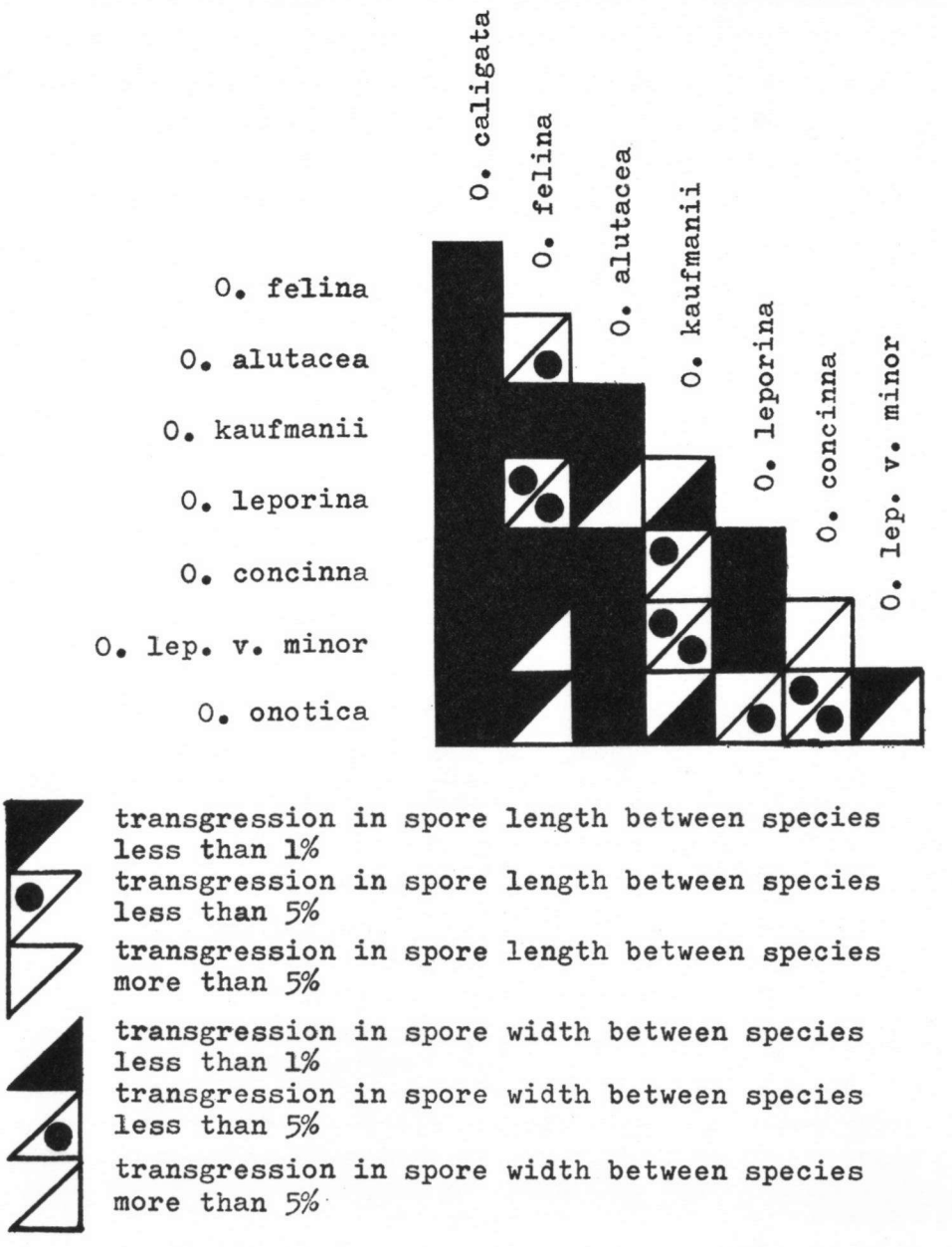

FIG. 5. Evaluation of spore length and spore width as key characters in light-coloured species of *Otidea*.

other hand, species very similar in qualitative characters are completely different in spore dimensions. This phenomenon also proved to occur in the genus *Discina* (*Neogyromitra*), where three species had distinctly different spore width (Raitviir, *1970*).

The next step in this study was the construction of a key to the species of *Otidea* based predominantly on spore dimensions. For this purpose, spore length and spore width were evaluated as key characters by means of Lubishchev's (*1959*) discrimination coefficient $K = \frac{(\bar{x}_1 \quad \bar{x}_2)^2}{S_1^2 + S_2^2}$, which is in fact Student's t squared. The critical values K = 18, K = 11, and K = 5.8 correspond to total absence of linear transgression, 1 % transgression and 1 % probability of error, and 5 % transgression and 5 % probability of error. The results of this analysis are shown in Fig. 5. It may be seen that spore dimensions fail to distinguish between species only in a single instance: between *O. leporina var. minor* and *O. concinna*, but in this case the spore length-width ratio can be used as a key character. This key and data on the geographical distribution of the species studied will be published elsewhere.

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