Morphological variation of Daphnia pulex Leydig (Crustacea: Cladocera) and related species from North America

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Abstract

Several species of *Daphnia* closely related to *D. pulex* occur commonly in North America. These species continue to be difficult to identify because of unsystematic descriptions and the continual appearance of intermediate forms. Uni-and-multivariate analyses of the morphological variation of 18 characters for each of 351 animals from 33 populations fails to demonstrate any significant clusters of populations. None of the characters, singly or in combination, serves to separate the populations into unambiguous groups. A cluster analysis of the similarity of populations suggests that if distinct species do exist, they are not those presently recognized. The data support either the view that the *D. pulex* species group is one widespread and variable species, or that it is comprised of a much larger number of species than presently recognized.

Introduction

Daphnia (subgenus *Daphnia*) are among the most common and most widely distributed freshwater plankton. They are also among the most difficult to identify at the species level. There is still confusion as to the number of species of holarctic *Daphnia,* even though they have been studied in great detail for over 200 years (Hrbácek & Hrbáckova-Esslová 1966). This confusion may well derive from the hitherto unquestioned assumptions that *Daphnia* populations are representatives of a small number of morphologically distinct groups, and that the taxonomic confusion can be cleared up by more careful observation and description.

Daphnia taxonomists seem unable to decide whether they are dealing with a very few distinct biological species (in the sense of Mayr, 1963) which are polymorphic, or with a large number of biological species which, except for the phenomenon of the annual cyclomorphic variation are more or less monomorphic. For example Hellich (1877) classified holoarctic *Daphnia* as belonging to as many as 35 species. Concerning the explosion of names, Wagler (1912) wrote 'seit 1860 sprossen die Arten wie Pilze aus der Erde'. Brooks (1957) presented the opposite view that '. . . there are a few who, because of the difficulty of distinguishing between some '*pulex*' and some '*longispina*' [the two major species of *Daphnia* (*Daphnia*)], would lump both into one species'. A slightly less extreme approach has been to recognize the two holarctic species '*pulex*' and '*longispina*' in the subgenus *Daphnia* and to distinguish morphological variants with tri-and-quadrinomials (Kiser, 1950; Birge, 1918), following the European precedent.

The basic problem with *Daphnia* taxonomy is that while populations in a limited geographic area seem to fall into distinct morphological species or forms, it is also true that each population is morphologically unique (Rylov 1935). Also, while at any one time a population typically shows little variation in its unique morphology, that morphology can change drastically with an annual cycle (Rylov 1935).

Taxonomic treatments of *Daphnia* have been

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based on samples from a small geographic area. Even the excellent monograph of Brooks (1957) works best for animals from the northeastern USA. In my experience, populations outside the area of a particular study, or even new collections from within the study area, produce intermediates in form relative to the described species. That is, *Daphnia* species tend to be nondimensional (Mayr 1963); the addition of the temporal or spatial heterogeneity of new samples makes the classification seem ambiguous. For example, Brooks (1957b), Uéno (1971), Meijering (1975) and Bushnell & Byron (1979) have all found intermediates between *D. pulex* and *D. middendorffiana* in northern and arctic North America, and the intermediates were interpreted as overlapping forms or introgressive hybrids. However, hybridization seems unlikely since many of the ponds lacked males. Asexual populations are very common in the Arctic (Edmondson 1955), and possibly over the whole range of the *D. pulex*-like species (Hebert 1980). An added problem here is that when males have been reported from *D. middendorffiana* populations, they have invariably been *D. pulex* males. (This suggests Brooks (1957) may have erred in his description of the *D. middendorffiana* male.)

The phenomenon of intermediates is especially troublesome when European types and descriptions are used to identify North American animals. Although American authors did describe new endemic species or forms (for example, see Forbes 1893; Herrick 1895; Birge 1918; Kiser 1950) the European precedent was largely adhered to until Brooks (1957).

Daphnia are morphologically depauperate, not in the complexity of their anatomy, but in their lack of large or distinct morphological characters useful in separating the described species. The perceived but subtle and gradual differences of shape or form of the body have proven very difficult to describe, much less use taxonomically. There are few binary (present or absent) characters. For example Brooks (1957) has only one binary character (presence or absence of pigmentation) in his description of the four species of the '*pulex*' group. Thus, any *Daphnia* taxonomic work uses large numbers of drawings and few or no quantitative descriptions.

Studies using novel characters have so far failed to produce useful characters. Edwards (1980) in her study of the anatomy of *Daphnia* mandibles, found the species of the *pulex* group for the most part very similar to one another when compared to other *Daphnia* species. However, within the *pulex* group the populations with the most similar mandibles were not always the same species (her Fig. 11), and no mandible character emerged which would help subdivide the *pulex* species group.

An obvious but tedious approach to the Daphnia species problem is to perform systematic genetic crosses followed by morphological analysis of the reproductive isolates, as Price (1958) did for the *Cyclops vernalis* group of Cyclopoid Copepods. Unfortunately, genetic crosses in *Daphnia* are extremely laborious (Banta & Wood 1939). Of the 2 existing reports of genetic crosses in *Daphnia,* Agar (1920) did not test the fecundity of his pulex x obtusa hybrids, and Hebert & Ward (1972) studied only Fl hybrids within *D. magna.* In some cases genetic crosses are impossible since many populations are completely asexual (Hebert 1980). It may be a long time before the genetic nature of *Daphnia* species is understood.

This present study looks for morphologically distinct sets of populations, without considering whether such sets represent biological species. The '*pulex*' species group was analyzed because they are a widespread distinct group of large easily-dissected animals. The null hypothesis is that there are no morphologically distinct sets of populations within the '*pulex*' group. This hypothesis is tested by quantitative and statistical techniques of numerical taxonomy.

Materials and methods

This study is based on measurements of *Daphnia* from populations distributed over North and Central America (Table 1). The 30 populations of *Daphnia pulex* species group (Brooks, 1957a) were chosen to give as wide as possible a geographic range. Some populations were sampled at different seasons or in different years in order to increase temporal heterogeneity. Samples far apart in time and space should maximize the chance of finding groups of morphologically distinct populations. Populations 29, 30, 31 and 32, Table 1, were derived from one population which was cloned; subclones were grown in duplicate at two different temperature and light regimes (Figs, 1, 2, and 3 are line

Table 1. The 'species' of daphnia. c = *D. catawba;* f = *D. middendorffiana;* p = *D. pulex;* pc = *D. pulicaria;* (m) = minnehaha form of *D. pulex;* (s) = schødleri form of *D. pulicaria;* (s?) = schødleri form of *D. pulex,* but with short tail spine.

Group	Population number	Species	Location
A.	10	p(s?)	Utah, Bear's Ears Pond
	14	p	Colo., Gothic, Pond K2
	32	p	Colo., Gothic clone 21 °C, PCBD pond
	29	p	Colo., Gothic clone 7.5 °C, PCBD pond
	30	p	Colo., Gothic clone 7.5 °C, PCBD pond
	12	p	Wisc., Picnic Pt., Pond, RS*
	31	p	Colo., Gothic clone 21 °C, PCBD pond
	15	p	Colo., Gothic, Pond K2
	26	p	Wisc., Prairie Pond West, SC*
B.	13	p(m)	Mass., small lake #21, FB*
	23	p	Colo., G.M. 15, Aug. '69, pond
	24	p	Colo., G.M. 15, July '69, pond
	20	c	Mass., small lake #20, FB*
	21	c	Penn., Lake Lacawac, CG*
C.	1	p	Alaska, Barrow, W, marsh
	5	pc	Wisc., Lake Mendota, May
	17	f	Calif., Yosemite, small lake, GV*
	6	pc(s)	Wisc., Long Lake, BT*
	27	pc	Wisc., Peter Lake, JK*
	9	pc(s)	Wisc., Lake Mendota, July
	7	pc	Guatemala, Lake Atitlàn
	2	f	Alaska, Barrow, pond 13
	16	f	Alaska, Barrow, pond 8
	33	f	Manitoba, Churchill, Esk. Pt. Pond 7, PDNH*
D.	3	f	Colo., Gothic, Mex. Cut pond 8
	4	f	Colo., Gothic, Mex. Cut pond 9
	18	f	Colo., Gothic, Mex. Cut pond 10
	11	p(m)	Wisc., Bavaria Sausage Pond
	22	p(m)	Colo., G.M. 15, Aug. '69, pond
	19	f	Manitoba, Churchill, Rankin Inlet pond, PDNH*
	25	P(s?)	Colo., G.M. 4, '79, pond
	8	pc(s)	Wisc., Lake Mendota, Sept.
	28	p	Colo., G.M. 4, '69, pond

* Contributors of samples were, other than S. I. Dodson: F. Burchsted (FB), Scott Cooper (SC), P. D. N. Hebert (PDNH), J. Kitchell (JK), B. Torke (BT), R. Smith (RS), Gary Vinyard (GV) and C. Goulden (CG).

drawings of a representative animal from each of the populations.)

A number of people contributed samples to this study (Table 1). The 2 Manitoba samples were killed with alcohol and later fixed with dilute formalin. All the other samples were fixed with dilute formalin. Populations showing distortion due to fixation were not used in this study.

I identified the populations using as a base Brook's (1957) treatment with several modifications. *Daphnia pulicaria* was separated from *D. pulex* following Brandlova's *et al.* (1972) description, except that the rostrum reticulation pattern was not used. Some populations (see Fig. 3A, C. F) that have several characteristics of *D. pulex* were called *D. pulicaria* mostly because they occurred in large lakes. My *D. pulicaria* also includes Brook's *D. schodleri,* following the suggestion of Torke

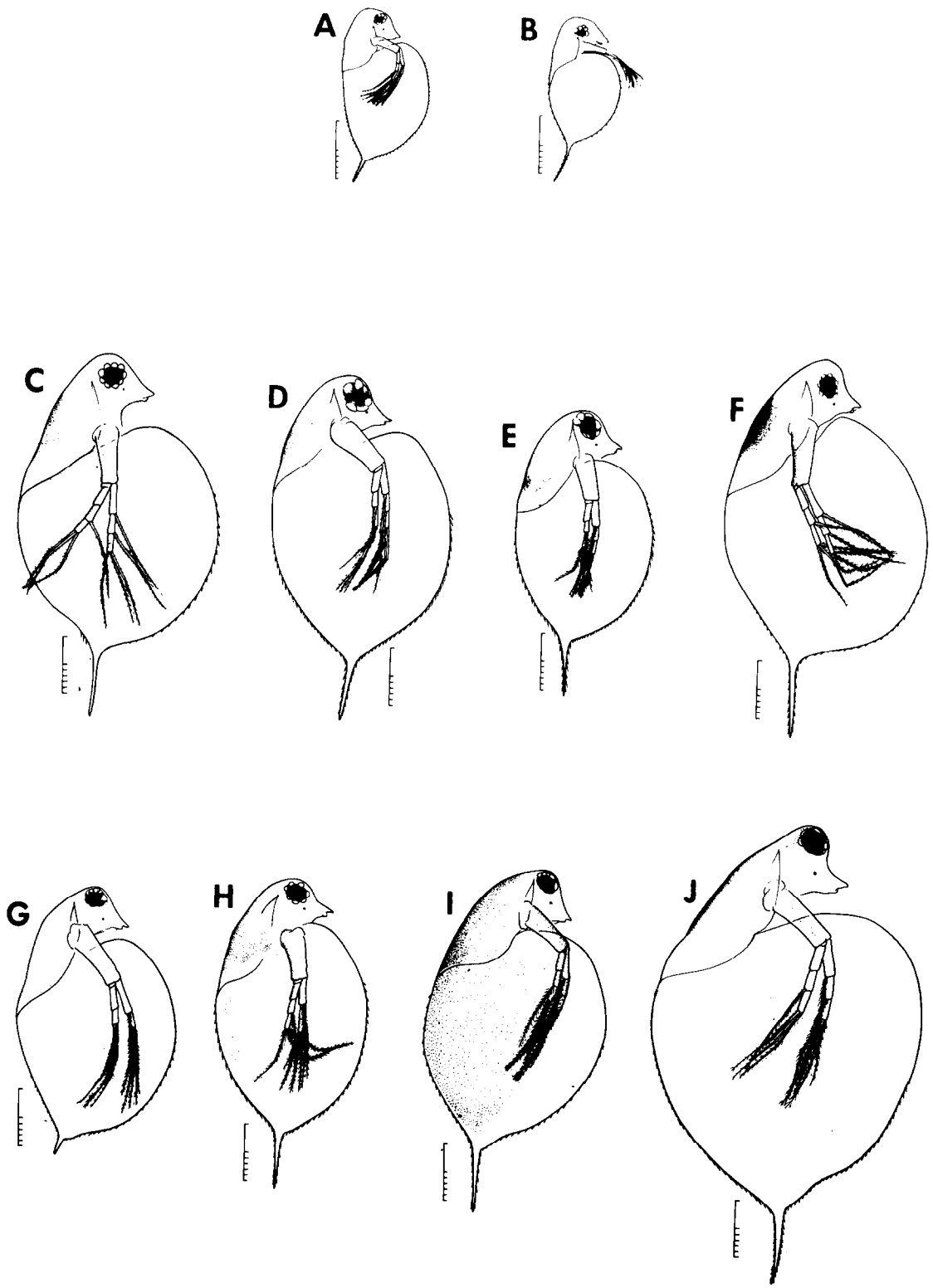

Fig. 1. D. catawba: A(20), B(21); *D. middendorffiana*: C(2), D(3), E(4), F(16), G(17), H(18), I(19), J(35). Population no. in parentheses: see Table 1. The length of the scale bar is 0.26 mm.

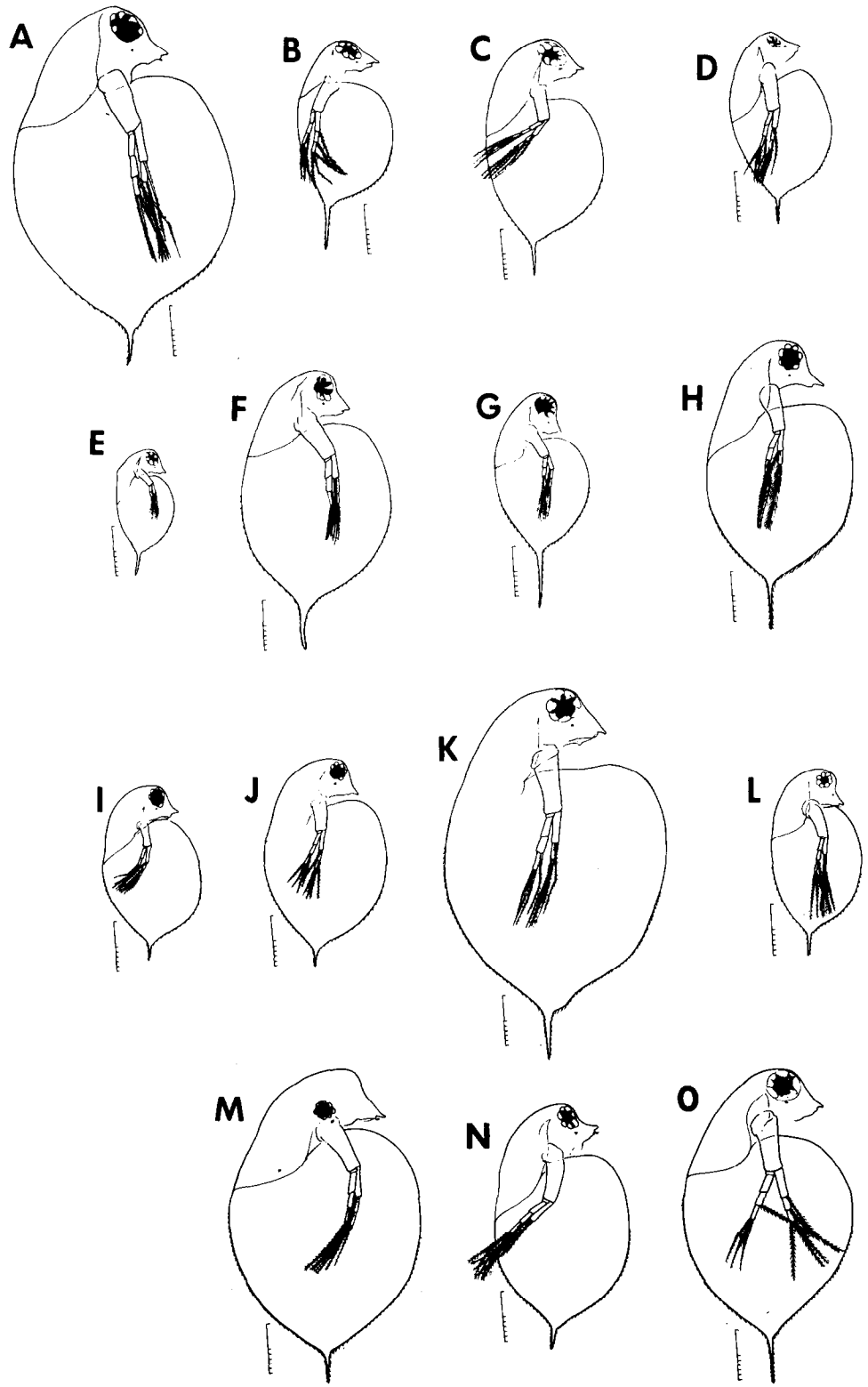

Fig. 2. D. pulex: A(1), B(10), C(11), D(12), E(13), F(14), G(15), H(22), I(23), J(24), K(25), L(26), M(28), N(30), 0(32). Population no. in parentheses: see Table 1. The length of the scale bar is 0.26 mm.

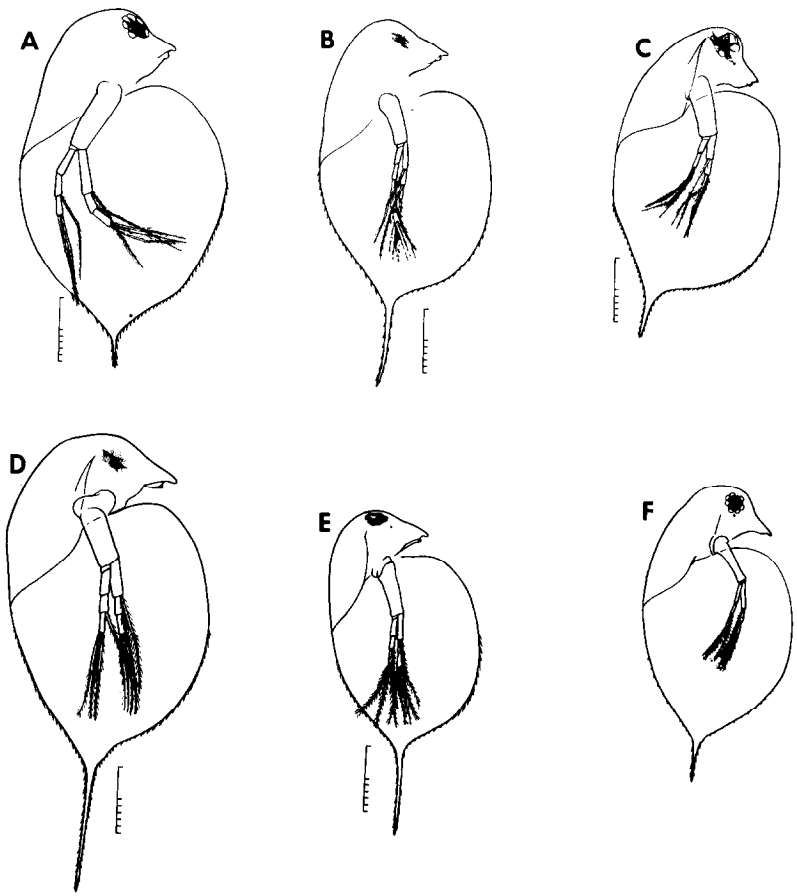

Fig. 3. D. pulicaria: A(5), B(6), C(7), D(8), E(9), F(27). Population no. in parenthesis: see Table 1. The length of the scale bar is 0.26 mm.

(pers. comm.) and Grogg (1977). The *minnehaha* form of *D. pulex* is included in *D. pulex* for the reasons given in Krueger and Dodson (1981). All pigmented populations were named *D. middendorffiana*. Thus, there are 4 species: *D. catawba* (Fig. 1), *D. middendorffiana* (Fig. 1), *D. pulex* (Fig. 2) and *D. pulicaria* (Fig. 3). Since the purpose of this study is the investigation of the morphological relatedness of populations, the specific names used herein should be regarded as conveniences without any particular morphological connotation.

The 351 individuals of this study were adult nonephippial females with intact tail spines. Four characters (Fig. 4A: HEADL, BODYL, COREL and TAILL) were measured with the undissected animal lying in a small drop of dilute Hoyer's mounting medium, using a dissecting microscope with an ocular micrometer (1 division = 0.0397 mm at 120X). The animal was then dissected. The body was arranged on its left side, after the thorax and abdomen had been removed. The first 3 left thoracic legs were then separated from the thorax and oriented with their outside (anterior) sides facing up (Fig. 4B, D, F). The third leg was folded as shown in Fig. 4F. The abreptor was laid on its left side (Fig. 4E). After the Hoyer's had dried enough to avoid movement of the parts, more Hoyer's and a cover glass were added. After the slide dried, data were taken using a compound microscope and eyepiece micrometer (1 unit = 0.00242 mm at 250X, 0.00151 mm at 400X). The characters 6SPNL and 67SPNL (on the ventral margin of the carapace, Fig. 4C), 1LFLA and 1LSPS (1st thoracic leg, Fig. 4B) and 2 LEGB (2nd thoracic leg, Fig. 4D) were measured or counted at 400X: the remaining characters at 250X. In a pilot study, 12 additional characters were measured or counted, mostly at 400X the lengths of setae B and F on leg 1 (Fig. 4B) and at 1000X oil immersion the number of microsetules in patches 7 and 9 on leg 1 (Fig. 4B) and the number in patches a,

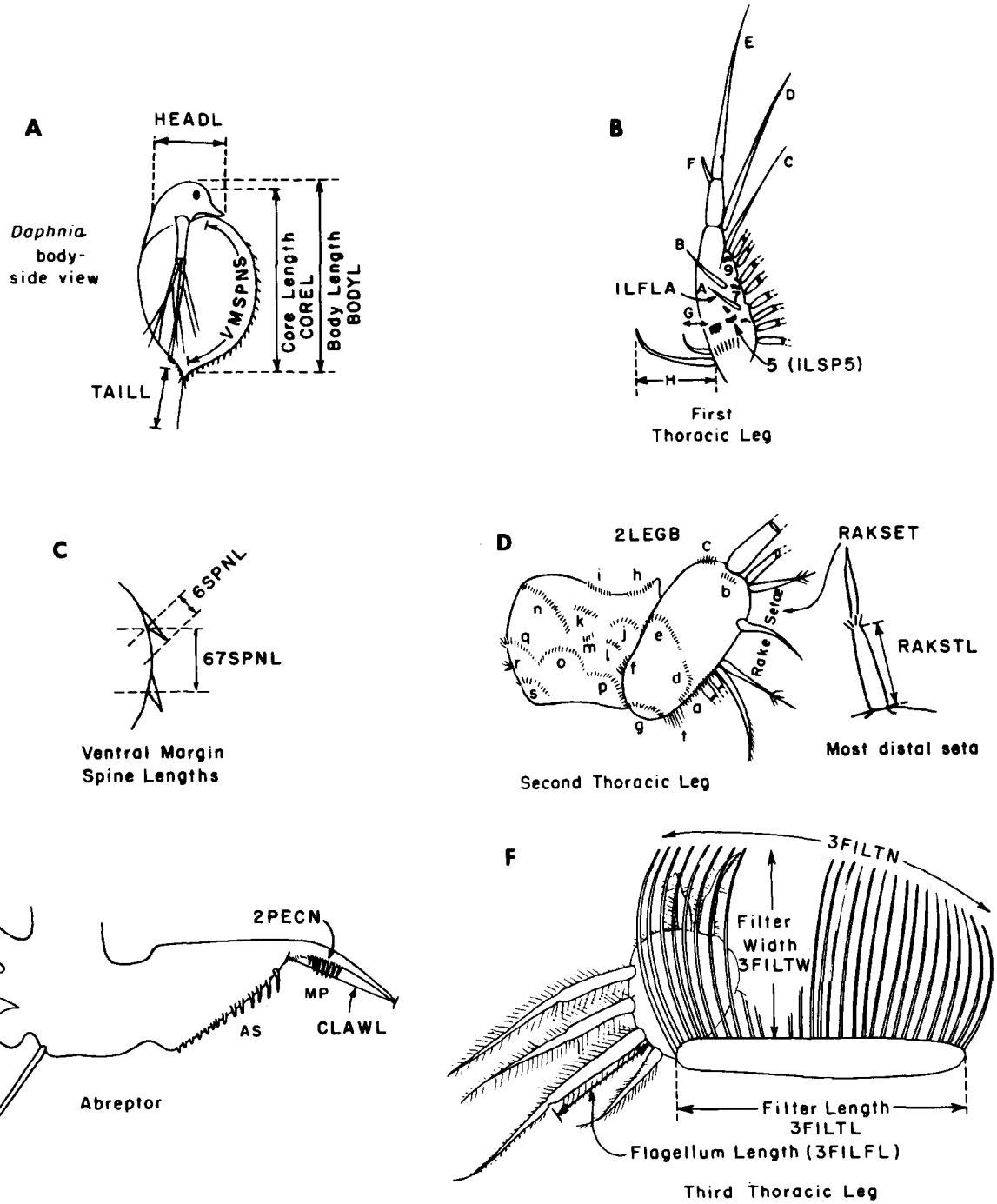

Fig. 4. The 18 characters used in this study are indicated by the 5 or 6 character acronyms. Oil immersion characters are indicated by single numbers or letters. The arrows show how characters were measured.

c, d, e, h, i, j and k on leg 2 (Fig. 4D). A high correlation was found among lengths or numbers on each leg with the result that these laboriously-gathered data added nothing to the analyses. For this reason only the 18 characters (acronyms of Fig. 4) were used. These 18 characters include characters correlated with the discarded characters.

The number of animals measured per population

varied from 8 to 16, but was mostly 11 or 12. There seems to be no quantitative way of estimating the optimal sample size for the analyses used. Brooks (1957) recommends that at least 5 or 6 mature females be used in an identification. Frey (1980) favors 3–29 individuals per instar in his analysis of *Chydorus* (Cladoceron) forms. In all, 351 animals were measured representing 33 populations (including the 4 clonal populations).

Pairwise F ratios and correlation coefficients were calculated to investigate univariate relationships among the characters. These analyses should indicate which single characters are best in separating the populations. The calculations were performed by the CANCOV computer program described by Kowal *et al.* (1976: F tests) and the CORREL program of the BMDP program 1R (Dixon & Brown 1979).

The *Daphnia* populations were also analyzed using canonical analysis, a multivariate statistical technique: '. . . the technique simplifies an unwieldy mass of data by eliminating error variation and by concentrating only on differences among groups' (Kowal *et al.* 1976). Kowal's method allows the use of one or more characters as covariates. However, since using the body length as a covariate caused no significant changes in any of the analyses, and made the results harder to interpret, no covariates were used. Populations are separated using linear combinations (canonical variate) of the characters. Characters are weighted according to the F-ratio, so that the most heavily weighted are those with the smallest within population variance compared to the pooled between population variance. In this case, the 18 characters allow 18 independent linear combinations to be calculated. These 18 combinations can be thought of as an 18 dimensional space in which lie the 33 populations. The first canonical variate best separates the populations, so that most of the separation of populations can be shown in a 2-dimensional graph of the first and second canonical variates. The units of distance in the hyperspace are measured by the pooled within-populations standard deviation of each canonical variate. The 95% confidence limits around the mean point of each population is calculated using equation 10 of Kowal *et al.* (1976).

The distance between the means of 2 populations in the hyperspace is the Mahalonobis distance, also measured in units of the pooled within-populations standard deviation. The pairwise Mahalanobis distances can then be used to construct a dendrogram which shows the hierarchical relationship of the populations. Of Wishart's (1978) several methods available for constructing a dendrogram, single linkage, average linkage and centroid suffered from excess chaining while complete linkage and Ward's method produced a more dicotomus hierarchy, with Ward's method showing the best separation of groups.

Results and discussion

Univariate analyses:

There is some redundant information in the 18 characters of Fig. 4. Correlation coefficients calculated from all 351 individuals show many characters are correlated. Most of the seta, claw lengths and body lengths show a strong degree of correlation, illustrated by Fig. 5 for the length of the post abdominal claw and body length (BODYL). That is, many of the characters used in distinguishing *Daphnia* species show variation related mainly to variation in body size. Several characters are independent of BODYL: the length of both the tail spine (TAILL, Fig. 6) and sixth ventral margin spine (6SPML); the distance between the sixth and seventh marginal spines (67SPNL), the number of spines in patch 5 on the first leg; the number of rake setae (RAKSET) and spines in patch B (2LEGB) on the second leg; and the number of filter setae (3FILTN) on the third leg. RAKSET and 2LEGB are weakly correlated with 3FILTN, but not with each other. Therefore, in future work, measuring time could be saved, with little loss of information, by using instead of the 18 characters only 9: BODYL, TAILL, VMSPNS, 6SPNL, 67SPNL, 1LSPN5, RAKSET, 2LEGB, and 3FILTN.

The rostrum reticulation pattern has been offered as a good character for distinguishing *D. pulex* and *D. pulicaria* (Hrbácek 1959: Brandlova *et al.* 1972). This character was not quantified, but its state was noted for each of the populations (Table 2, Fig. 7). A line drawn from the ocellus to the ventral margin of the head should pass through 6 to 8 rows of polygons many times longer than wide with pulicaria or through no elongated poly-

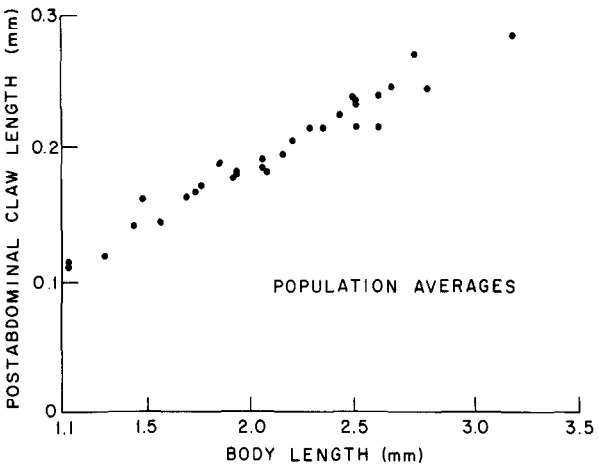

Fig. 5. The positive correlation between the mean length of the postabdominal claw (CLAWL, Fig. 4C) and the mean body length (BODYL, Fig. 4A). The pattern of distribution of the means strongly reflects that of the individuals.

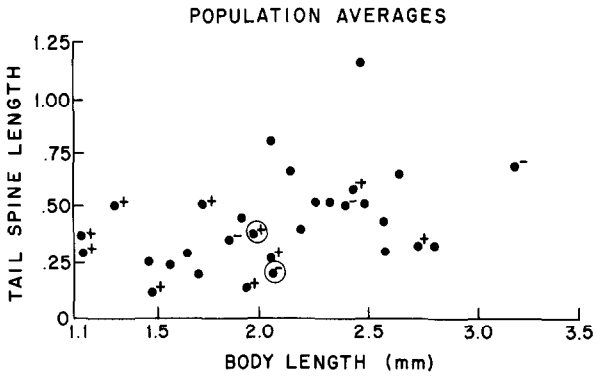

Fig. 6. The relationship between the mean length of the tail spine (TAILL, Fig. 4A) and the mean body length (BODYL, Fig. 4A). The + or − signs indicate statistically significant ($p < .05$) within population correlation coefficients as well as the direction of the correlation. The two circled points are the combined replicates for the two treatments of the *D. pulex* clone (pops. 29 + 30 and 31 + 32).

gons for pulex. About half of my 33 populations, including 3 provisional *pulicaria,* lacked patterns sufficiently distinct for an evaluation (Fig. 7J, K). Of the 8 populations with the *pulicaria* pattern (Fig. 7A, B), 3 would otherwise be called *pulex* and 2 were *middendorffiana.* The *pulex* pattern (Fig. 7E-G) seemed restricted to *pulex* populations. Several populations had rostrum patterns intermediate between the *pulex* and *pulicaria* patterns (Table 2, Fig. 7H, I). This character seems generally unhelpful and often could lead to confusion. It doesn't seem to reliably separate *D. pulex* from *D. pulicaria* at least for widely spread populations in North America.

There have been no systematic studies of the ecological amplitude of these morphological characters. That is, it is not known to what degree the morphological variation seen in nature reflects

Table 2. Reticulation pattern of the head near the rostrum.

Reticulation pattern	Pop.	Species	Reticulation pattern	Pop.	Species
Pulicaria	1	p	near *pulex*	3	f
	5	pc			
	8	pc(s)	faint or	6	pc(s)
	9	pc(s)	absent	7	pc
	18	f		10	p(s?)
	19	f		13	p(m)
	22	p(m)		14	p
	25	p(s?)		15	p
				16	f
Near *pulicaria*	2	f		17	f
	4	f		20	c
	12	p		21	c
	33	f		23	p
				24	p
pulex	11	p(m)		26	p
	29–32	p		27	pc
				28	p

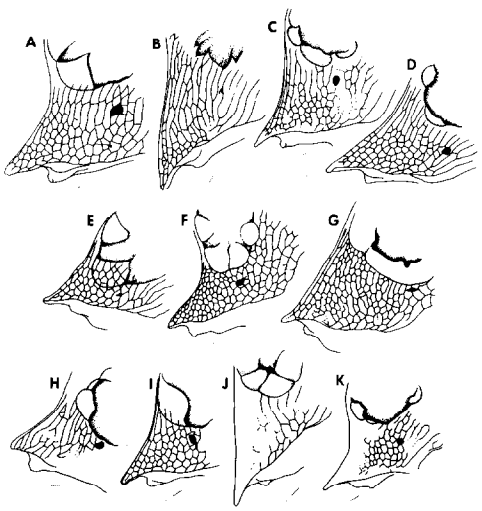

Fig. 7. The pattern of reticulation on the ventral part of the head. *Daphnia pulicaria* pattern: A(1), B(8), C(18), D(22); *D. pulex* pattern: E(11), F(20), G(32); near *D. pulicaria* pattern H(2); near *D. pulex* pattern I(3);pattern too faint for classification J(6) and K(23). Population no. in parentheses: see Tables 1 and 2. The length of the scale bar is 0.024 mm.

a single genotype with phenotypic variations induced by different environmental factors. To find out if any such phenomenon exists I grew one clone of a Colorado *D. pulex* (from the PCBDP site also used in Krueger & Dodson 1981 in duplicate under two different sets of conditions: 7.5 °C with 6 hrs light and 21 °C with 18 hrs light, in constant temperature incubators. Several characters showed large differences between the means for the two conditions (Figs. 2N, O and 7F, G). Notice also the distinct difference in general body shape, especially in the head region for the the representatives of populations 30 and 32. If we use the t test as a guide, there are 3 characters with $t > 2.74$ ($p < .01$, $N = 24$, 24): TAILL, RAKSET, VMSPNS; and 2 with $t > 2.03$ ($p < .05$): the number of teeth in the middle pecten of the postabdominal claw (2 PECN) and 6SPNL. TAILL, which produced the largest t value, nearly doubled in size (Figs. 6, circled dots; 2N, O) from the cold to the hot condition, while the correlation coefficient, significantly different from zero in both cases, shifted from negative to positive. The differences found for TAILL, VMSPNS, and 2PECN in this one clone approach those used to distinguish species (see Brooks 1957).

The variances as well as the means of characters may be effected by different habits. The variances of 17 of the 18 characters had an average, non-significant F ratio of 1.51 (Standard Error = 0.010) in a comparison of the hot versus cold treatments. One character, 3FILTN, had an F ratio of 5.55, implying a very significantly different response by the clone to the two treatments.

Thus, *Daphnia* show morphological variation, both non-genetic and genetic, caused by different habitats, cyclomorphosis, and predator induction. Are there nevertheless aspects of *Daphnia* morphology which can be used to distinguish morphological species which transcend the intraspecific variability? The sampling program of this study was designed so as to contain as much geographic and seasonal variation as possible in order to firmly establish morphological species if they exist.

The single best discriminate character as calculated by Kowal's *et al.* (1976) CANCOV program is 3FILTL, the second best character is 6SPNL. These characters have the smallest within population variance compared to their between population variance. The correlation coefficient for the two characters is 0.087 ($N = 351$) suggesting that they are independent. 3FILTL is strongly correlated with BODYL ($cc = 0.91$) but 6SPNL is not ($cc = 0.10$). Figure 8 shows the means for each population. For these two characters, the coefficient of variation is about .10. Some of the means are shown surrounded by a 1 standard deviation limit. If the means of two populations (both nomally distributed with the same variance) are separated by X standard deviations, then the dividing line will be X/2 SD from each mean and p of the individuals will be misclassified (p is the two-tailed fraction of the normal distribution X/2 standard deviations from the mean). For such populations 2 SD apart, 16% of all individuals will be misclassified; at 3SD, 6.7%; at 4SD, 2.3%; at 6SD, .13%. If either of the two best characters defined morphologically distinct groups, one would expect to see clusters of populations, with the peripheral populations of each cluster separated by four of five SD from the peripheral populations in the nearest adjacent cluster. By inspection of the populations of Fig. 8, none is more than 3SD from its nearest neighbor. Assuming the assumptions of this model are approximately true, then more than 6.7% of adjacent populations would be misclassified using either of the two best single characters alone. The other characters given even less satis-

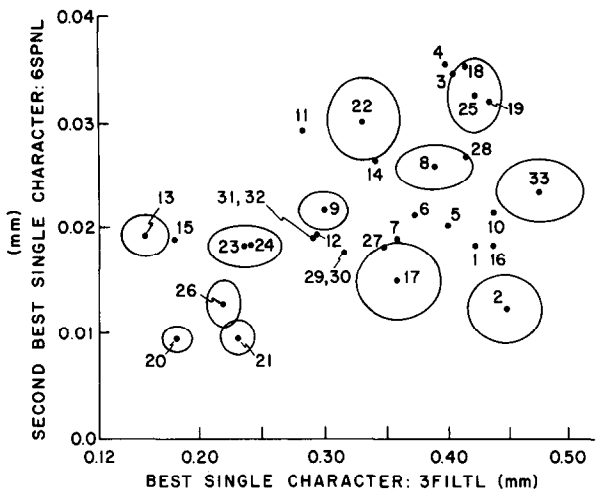

Fig. 8. The relationship between the within-population means of the two most discriminating characters: the length of the filter comb base on the 3rd leg (3FILTL, Fig. 4F) and the length of the 6th spine on the ventral margin of the carapace (6SPNL, Fig. 4C). The ellipses represent a limit of 1 standard deviation around each population mean. Only a few limits were drawn in order to reduce clutter.

factory results. Thus, none of the characters can be used singly to reliably distinguish the various populations or species. The impression of Fig. 8 is of a morphological continuum in which extreme forms are the ends of an array of intermediate forms.

Multivariate analyses

Although no single character separates the populations into distinct morphological groups, it is still reasonable to ask whether some combinations of all the characters might serve to define distinct groups. Figure 9A shows the 33 populations graphed using the first two canonical variates. Together, these two dimensions account for about 66% of the total separation between populations; additional variates would give increasingly less resolution. The impression of Fig. 9A is much that of Fig. 8. In Fig. 9A the populations are adjacent to one another with some overlap. They show no

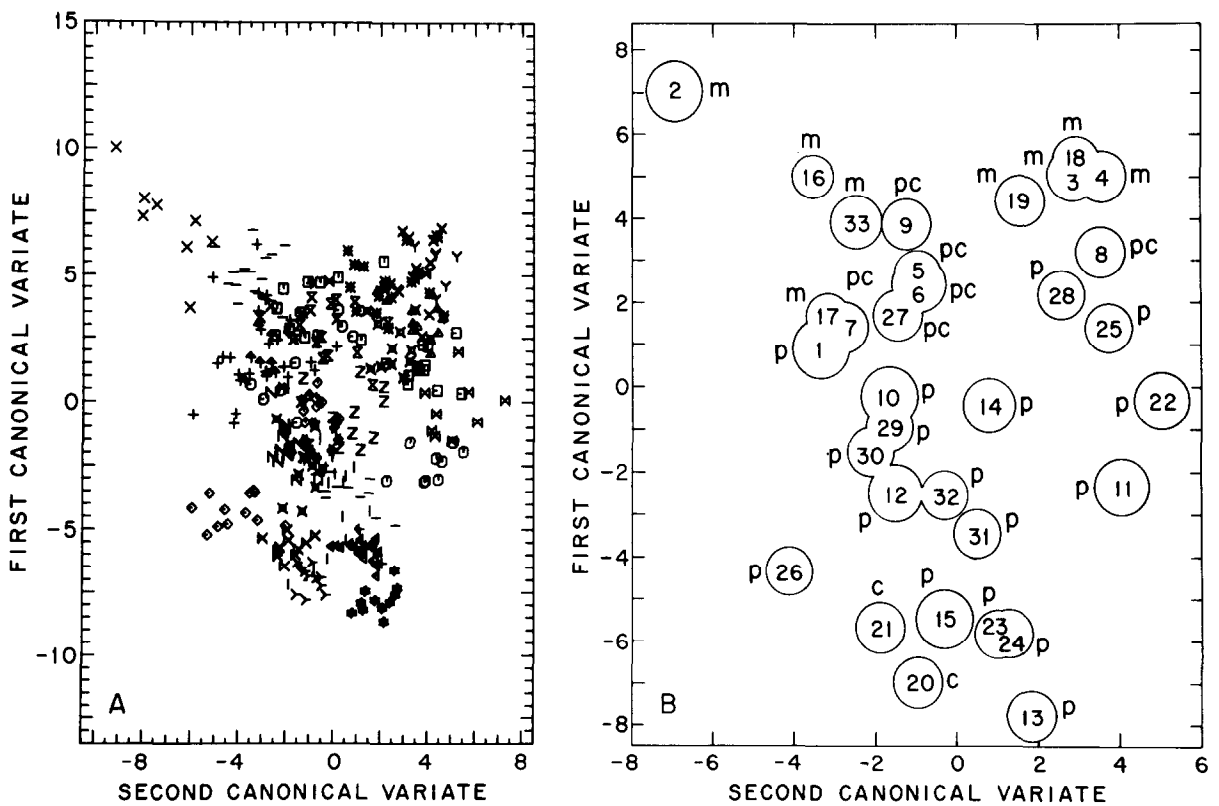

Fig. 9. Two aspects of the canonical analysis of the 33 populations. A: the position of each individual relative to the first 2 canonical variates. Populations are indicated by the different symbols, with some symbols being used for 2 populations. B: the position of the mean of each population relative to the first 2 canonical variates. Each mean is surrounded by a 95% confidence limit. The letters and numbers indicate the species designations and populations numbers of Table 1.

tendency to form clusters of populations that are separated by five or six SD. Instead, the populations seem to be members of a continuum. Figure 9B shows the provisional species designations, the population means, and the means' 95% confidence intervals for the individuals of Fig. 9A. The body lengths are arranged along a gradient more or less parallel to the first canonical variate, from the smallest animals (nos. 13 and 20 at 28.9 mm BODYL) to the largest (no. 33 at 80.6 mm). The array is not structured along a gradient of habit or geographical location. The species names are grouped, but they seem to be distributed along the body size gradient as along a spectrum and not in distinct clusters.

The means of the populations do tend to be significantly distinct. While this result may be the result of too few populations, it does corroborate the observation of Rylov (1935) that while each *Daphnia* population seems distinct, still an exact

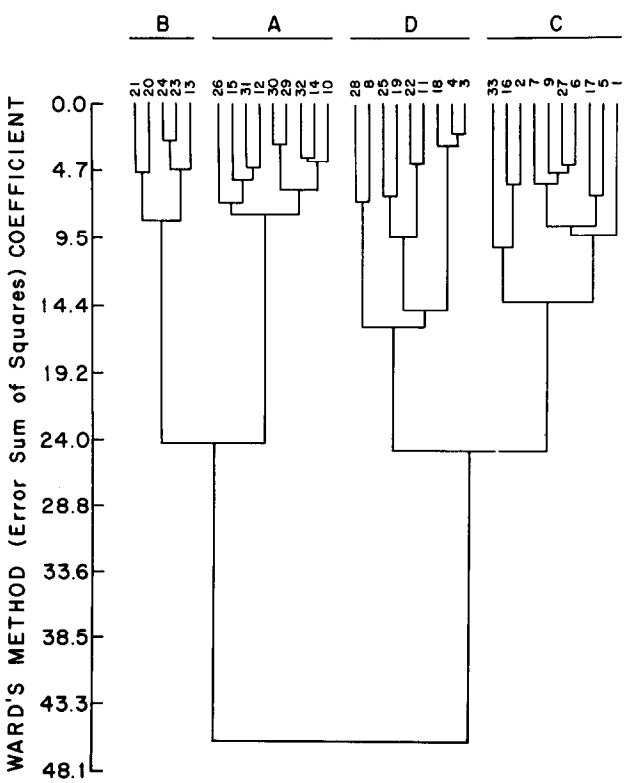

Fig. 10. A dendrogram based on Ward's (error sum of squares) clustering method. The population numbers and group designation letters (see Table 1) are across the top of the dendrogram. The dendrogram is divided into 4 groups for the purpose of comparison with the 4 designated species.

characterization of a *Daphnia* ecotype is a very difficult affair.

The relationship of the populations in all 18 canonical dimensions can be represented by a dendrogram of the populations clustered according to their pair-wise Mahalanobis distances. Figure 10 shows the results of such an analysis. The most important result is that the 4 primary groupings labeled A, B, C, and D do not correspond to the 4 species names used in this study (Table 1). Group A is a homogenous group of *D. pulex*. However, groups B, C, and D also include one or more pulex populations. Two kinds of pulicaria occur: one in C and one in D. The possible existence of 2 (at least) co-occurring species of *D. pulicaria* suggested by the 3 samples from Lake Mendota, Wisconsin (pops. 5 and 9 in group C and pop. 8 in group D) is supported by life history observations by myself in Wisconsin & Cipola (1980) in Michigan. These cryptic species differ mainly in the time of year they have their highest population size, either early June or late summer. Figure 10 also shows 2 kinds of *middendorffiana,* in C and D. *Daphnia catawba* is not in its own cluster, but is combined with 3 populations of small *D. pulex*. The subclonal populations 29, 30, 31 and 32 are joined together by the bottom line of Group A, at about 8.2 error sum of square units (Fig. 10). All but two of the other populations join with other populations, often even with other designated species, at values less than 8.2. That is, the intraclonal ecological amplitude seems to be as large as the variability between clones or even between species of *Daphnia,* which may explain why this study does not reveal morphologically distinct species.

A canonical analysis of the four groups of populations (Fig. 11) is similar in appearance to Fig. 9A. Group B is very slightly separate from A, C and D, which overlap greatly. The overlap of the groups tends to support the impression that the groups are not distinct forms, but are made up of samples (populations) taken from a complex morphological continuum.

There is no evidence based on the 18 characters used in this study that the *D. pulex* group contains two or more morphologically distinct sets of populations. Population means within this group are often statistically distinct but at the same time part of a continuum. Besides the results of the quantitative analyses, a direct visual examination of the

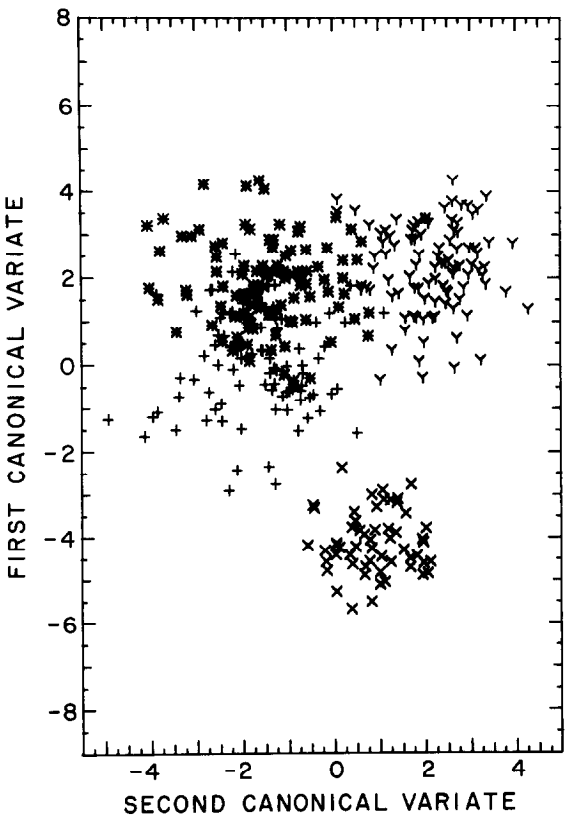

Fig. 11. A canonical analysis of the *Daphnia* grouped in the 4 groups of Fig. 10. The position of each individual is shown relative to the first and second canonical variables. The symbols of the groups are (A(+), B (X), C(★), and D(Y).

morphological variation in the representative individuals of Figs. 1, 2 and 3 suggests a continuum. The nature of the continuum is unclear except that it depends in part on the population's average body size. Although it seems unlikely that there are 4 or so distinguishable species in the *D. pulex* group, the data are compatible with either of two extreme views: that there is only one (possibly two) species in the *D. pulex* group or that there are many species. The lack of distinct population clusters in the canonical analyses suggests the 33 populations of this study may represent ecotypes of one rather variable species. On the other hand, the tendency toward morphological uniqueness of each population and the existence of many asexual populations suggests there may be a large number of biological and asexual species with very similar forms. The latter situation is probably more likely. Careful work with other cladocerans is revealing groups of species where one widely-distributed species was previously recognized (*Bosmina*: Manning *et al.* 1977; *Chydorus*: Frey 1980; *Eurycercus*: Frey 1975). Also, obligatory asexual populations of *D. pulex* (Hebert 1980) might be considered different species with at least the possibility that each species has a subtly different form, just as they differ in their enzyme complement.

A great deal should be learned about the ecological amplitude of different genotypes and about the genetics of *Daphnia* before a taxonomic revision of the group is considered. This study suggests that such a revision is in order, that it will probably be radical, and that the traditional characters and approaches may not be very useful. In the meantime it is probably reasonable to continue using our present keys, but with the understanding that their names are convenient conventions only, not related to morphologically distinct species and probably not related to biological species.

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