

The Evolution of Achene Morphology in *Ceratophyllum* (Ceratophyllaceae), II. Fruit Variation and Systematics of the "Spiny-Margined" Group

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ABSTRACT. Correlations of clustering relationships, phytogeography, and other data justify the continued taxonomic recognition of *Ceratophyllum echinatum*, *C. submersum*, and *C. tanaiticum* as distinct species. Variational patterns indicate a high degree of morphological similarity among *C. australe*, *C. muricatum*, and *C. kossinskyi* although each is separable by specific fruit characters and their geographical distributions are allopatric. They are treated here as vicarious subspecies of *C. muricatum*. Data from variational analyses indicate a close relationship between *C. echinatum* and *C. submersum*. Patterns of morphological variability indicate that spiny-margined species undergo frequent sexual reproduction.

In the first part of this series (Les 1986b) relationships were investigated among three species of *Ceratophyllum* having similar fruit morphologies. In that study, multi- and univariate statistical analyses provided a method for analyzing simultaneously numerous character states of the species, making it possible to distinguish patterns of discrete variational differences from phases of variational continua. The insights into fruit-spine variation provided by that study were useful for making taxonomic decisions. Here, similar investigations are continued with species constituting the "spiny-margined" *Ceratophyllum*. This group comprises those having fruits with lateral appendages in addition to paired basal spines. The spiny-margined *Ceratophyllum* have caused the greatest taxonomic difficulty within the genus for several reasons. They are extremely similar vegetatively; all possess high leaf-orders (for clarification of this term see Les 1985b) and frequently display leaf segment inflation. Their leaf denticles are finely exserted and lack a base of spongy tissue. Accordingly, taxonomic treatments of this group have relied almost entirely on characters of fruit morphology, such as length and number of their lateral appendages (e.g., Fassett 1953). Although these fruit-spine characters display continuous variation, they have been used taxonomically without adequate knowledge of their variational patterns.

The spiny-margined *Ceratophyllum* (fig. 1) are delimited here to include five taxa recognized tentatively at the rank of species: *C. australe* Griseb., *C. echinatum* A. Gray, *C. kossinskyi* Kusen-

Proch., *C. muricatum* Cham., and *C. tanaiticum* Sapi. This scheme generally follows Les (1985a) except for the merger of *C. llerenae* Fassett with *C. australe*. Reasons for this disposition are discussed in more detail below. In a recent revision, Wilmot-Dear (1985) merged the five species of spiny-margined *Ceratophyllum* with *C. submersum*, a species with spineless fruits (see Les 1986b). Wilmot-Dear (1985, p. 255) justified that merger because of a "fairly continuous range of intermediates" between *C. submersum* and members of the spiny-margined group.

Because of the vegetative similarity of this group, this analysis focuses on characters of fruit morphology. The objectives of this study are similar to those outlined in part I (Les 1986b). Specifically, the taxonomic integrity of the spiny-margined group and its putative relationship to *C. submersum* are tested using multivariate clustering analyses. Univariate statistical methods are incorporated to evaluate correlations of fruit-spine characters as done in part I. Comparisons of inter- and intrapopulational variation in fruit-spine characters are made. The results of these analyses should be useful for assessing the relationships and taxonomy of the spiny-margined group, and for elucidating plausible trends in the evolution of their achene morphology.

METHODS

Specimens of *Ceratophyllum* were borrowed from 77 herbaria (see acknowledgments), and identified to species following Les (1986c).

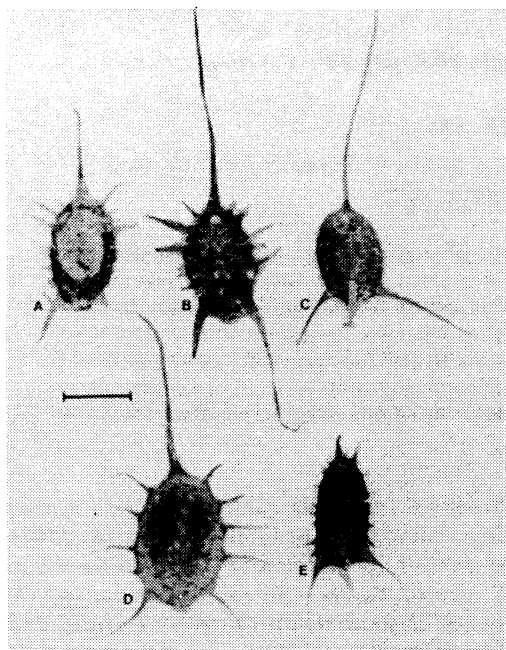


FIG. 1. Achenes of "spiny-margined" *Ceratophyllum*. A. *Ceratophyllum australe* (Lewis 2216, OS). B. *C. muricatum* (Kapoor 1, K). C. *C. kossinskyi* (Ching 3898, UC). D. *C. echinatum* (Skelton & Skelton 1433, TRT). E. *C. tanaiticum* (Sukajev s.n., LE). Bar = 2 mm.

Fruiting specimens of *C. australe*, *C. echinatum*, *C. kossinskyi*, *C. llerenae*, *C. muricatum*, *C. submersum*, and *C. tanaiticum* were selected for use in these analyses. Specimens of *C. llerenae* were merged with and treated as *C. australe* (see discussion). The number of fruits studied for each species was limited by availability; a total of 182 fruits from 18 populations of *C. australe*, 51 fruits from 51 populations of *C. echinatum*, 23 fruits from 4 populations of *C. kossinskyi*, 141 fruits from 14 populations of *C. muricatum*, 50 fruits from 38 populations of *C. submersum*, and 70 fruits from 4 populations of *C. tanaiticum* constituted the operational taxonomic units (OTU's) used in the analyses. Thirteen fruit characters (table 1) were scored for each OTU, but only the eight quantitative characters were used for discriminate analyses (a list of specimens and copy of the original data matrix are available from the author upon request). *Ceratophyllum australe* and *C. muricatum* were compared first. These species are often regarded as conspecific,

TABLE 1. List of characters and character states used in analyses of spiny-margined *Ceratophyllum*.

1. First plumule-leaf morphology (simple/compound). 2. Fruit shape (elliptical/hemispherical). 3. Fruit-body length (mm). 4. Fruit-body width (mm). 5. Fruit-body thickness (mm). 6. Maximum length of basal fruit spines (mm). 7. Styler spine length (mm). 8. Number of lateral fruit appendages. 9. Maximum length of lateral fruit appendages (mm). 10. Fruit surface (smooth/warty/tuberculate/ridged). 11. Fruit wing (present/absent). 12. Styler position (centered/eccentric). 13. Peduncle length (mm).

and both have been merged in recent taxonomic treatments (e.g., Lowden 1978; Wilmot-Dear 1985). Clustering relationships of these species were investigated by analyzing the first two axes of variation from a principal components analysis (PCA). This analysis was made using the NT-SYS numerical taxonomy program (Rohlf et al. 1974). To ascertain the significance of cluster overlap between these two species in the PCA, a stepwise discriminate analysis was performed using the BMDP statistical software package (Dixon 1981).

Clustering relationships of the three Old World species, *C. kossinskyi*, *C. muricatum*, and *C. tanaiticum*, were investigated next. These taxa were merged into one subspecies by Wilmot-Dear (1985). Analysis of the first two axes of variation from a PCA was performed for these three species using NT-SYS. A stepwise discriminate analysis of *C. kossinskyi* and *C. muricatum* was performed with BMDP to test if the clusters of OTU's of these species were significantly different; they overlapped considerably in the PCA. This discriminate analysis also included OTU's of *C. australe* because of the similarity of this species to *C. muricatum* demonstrated by the previous analysis. The *F* values and levels of significance were tabulated for these three species.

All five species of the spiny-margined group were then compared simultaneously. For each species, a dendrogram was generated using the UPGMA-HIGH algorithm of NT-SYS. In each dendrogram, OTU's correlating at the highest levels were removed until a sample size of 50 was obtained (the data set for *C. kossinskyi* was not altered due to the small number of OTU's).

This step provided a more uniform number of OTU's for each species, but retained most of the variation present in the original data set. The OTU's were pooled, and the modified data set was used to perform a PCA analysis using NT-SYS, and a stepwise discriminate analysis with BMDP. Following these analyses, the number of OTU's was reduced to 15 of each species using the same technique (removing those with the highest correlations in the UPGMA-HIGH dendrogram). This modified data set (15 OTU's of each of the five species) was used to generate a UPGMA-HIGH dendrogram with NT-SYS.

Clustering relationships of the spiny-margined group with *C. submersum* were assessed next. The PCA and stepwise discriminate analyses performed in the previous step (50 OTU's of each member of the spiny-margined group) were repeated with inclusion of 50 OTU's of *C. submersum*. The *F* values and level of significance separating each group of species in the discriminate analysis were tabulated.

A test of correlation was made between and among the characters of basal, stylar, and lateral spine length using the original data sets for each species of the spiny-margined group (approximate normal distributions of these variables were first verified). These tests were executed using the SAS package of statistical software (SAS 1982). A correlation matrix was constructed using values of the correlation coefficients (*r*). The values were tested for significance by comparison with two-tailed significance levels of the coefficient (Snedecor and Cochran 1982).

Relationships between intra- and interpopulational fruit-spine variation were investigated in *C. echinatum*, a species for which sufficient fruiting material was available. Frequency distributions of basal, stylar, and lateral spine lengths, and lateral spine numbers, were constructed using values from the 51 different populations in the original data set. Frequency distributions of these same fruit characters were also plotted for values obtained within each of three different populations. The sample number varied according to fruit availability from each population. Data were tabulated for 48 fruits within a population from Illinois, USA (*Patterson s.n.*, F, MO, NY, UC, US, WIS), from 104 fruits within a population from Maine, USA (*Steinmetz & Steinbauer 1118*, B, CAN, DAO, F, KY, MICH, MO, NCSC, NCU, NO, NY, OSC,

PENN, PH, SMU, TENN, UARK, UBC, UC, US, WIS, WTU), and for 40 fruits within a population from Ohio, USA (*Roberts 711*, MICH, MO, OS, PH, UC, US). Frequency distributions of fruit-spine characters were plotted on three-dimensional graphs to illustrate between- and within-population comparisons. Ranges and mean values for each character were tabulated for each comparison; a combined value was obtained by pooling all fruit-spine data obtained for *C. echinatum*. The values for each population were compared to those of the 51 combined populations using the SYSTAT software version of ANOVA (Wilkinson 1986).

RESULTS

By a PCA analysis, OTU's of *C. australe* and *C. muricatum* form separate but overlapping clusters (fig. 2A). In this analysis, factors one and two account for 44.3% of the total variation. The distinctness of these species is statistically significant at the 0.001 level by a stepwise discriminate analysis (fig. 2B) which separates OTU's of *C. australe* and *C. muricatum* along the canonical variable ($F = 144.6; 5$ and 317 degrees of freedom). The distinctness of these groups is supported further by the high percentages of "correct" group classifications calculated in the discriminate analysis (92%). The first four of eight quantitative characters accounting for most of the group separation are: basal spine length, peduncle length, lateral spine length, and lateral spine number. In comparing the two species, the means of these variables are significantly different at *P* less than 0.01 (fig. 2C).

The OTU's of *C. tanaiticum* form a distinct cluster from other Old World representatives of the spiny-margined group by PCA analysis (fig. 3A). In this analysis, factors one and two account for 54.6% of the total variation. The character of peduncle length contributed the highest loading value for the separation of *C. tanaiticum* from *C. muricatum* and *C. kossinskyi*. *Ceratophyllum muricatum* and *C. kossinskyi* do not separate satisfactorily by this PCA, and there is considerable overlap between OTU clusters of these species (fig. 3A). Using a stepwise discriminate analysis, however, *C. kossinskyi* is separable from both *C. muricatum* and *C. australe* (fig. 3C; table 2). From the *F* table (table 2), it is apparent that the degree of separation between *C. kossinskyi* and *C. muricatum* is much less

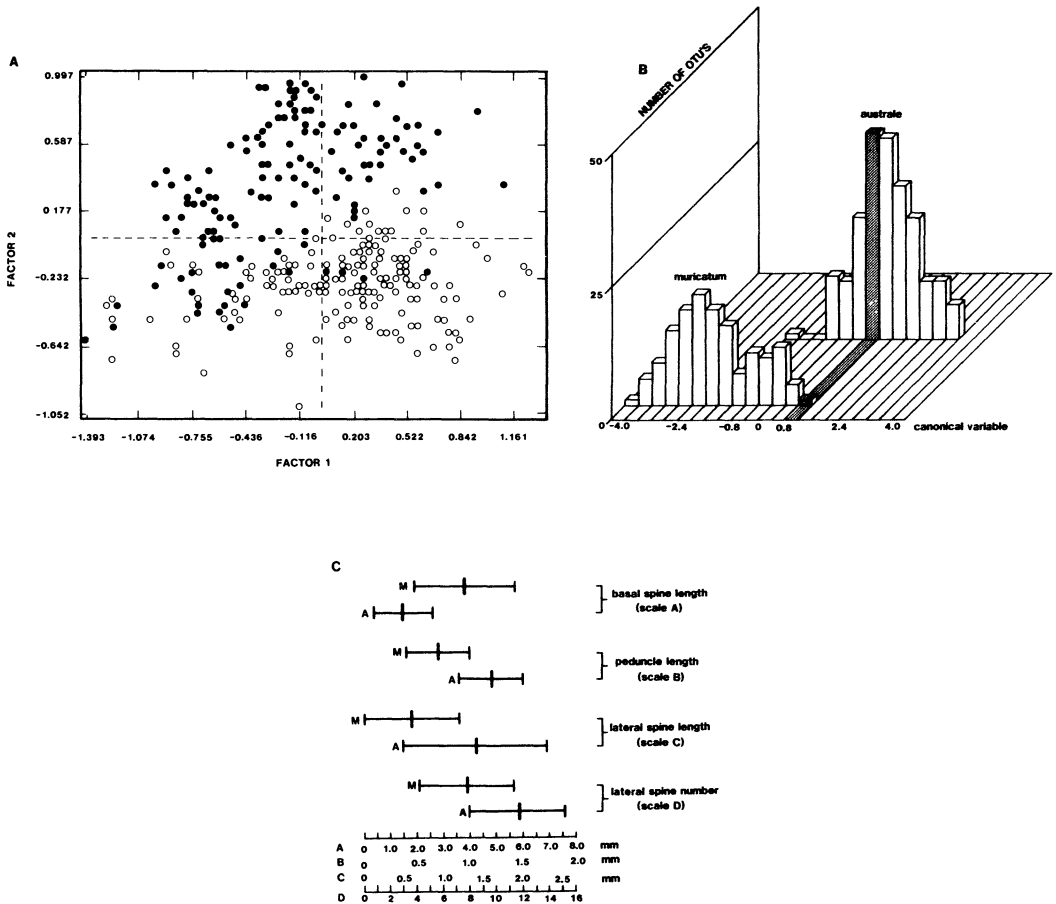


FIG. 2. Numerical analyses of *Ceratophyllum australe* and *C. muricatum*. A. Principal components scatter diagram depicting OTU's of *C. australe* (open circles) and *C. muricatum* (closed circles) generated by plotting the first two factors of variation. B. Histogram plot showing separation of *Ceratophyllum australe* and *C. muricatum* along the canonical variable of a stepwise discriminate analysis of OTU's. C. Means (thick vertical bars) and ranges (thin horizontal bars) compared for four quantitative fruit characters of *Ceratophyllum australe* (A) and *C. muricatum* (M). Means in all paired comparisons are significantly different at P less than 0.01.

than that separating the latter species from *C. australe*. Using the F values to approximate similarities, *C. kossinskyi* and *C. muricatum* have the highest affinity, whereas *C. australe* and *C. muricatum* have the lowest. Although these three species can be separated at a statistically significant level by a stepwise discriminate analysis, clustering relationships demonstrated by PCA indicate their relative similarity.

Three large clusters are produced when all five species of the spiny-margined group are compared simultaneously by PCA (fig. 3B). Factors one and two of this analysis account for 53.7% of the total variation. Characters of fruit

width and fruit thickness have the highest correlation with factor one. In the scatter-diagram plot, *C. echinatum* and *C. tanaiticum* form discrete clusters from the other OTU's, whereas *C. australe*, *C. muricatum*, and *C. kossinskyi* merge into a large central cluster (fig. 3B). A stepwise discriminate analysis of these five species yielded a similar pattern (fig. 3D). The discriminate analysis also identified fruit width and fruit thickness as characters contributing substantially to the separation of these species. On the basis of F values calculated for the discriminate analysis (table 3), *C. kossinskyi* and *C. muricatum* have the highest degree of similarity among

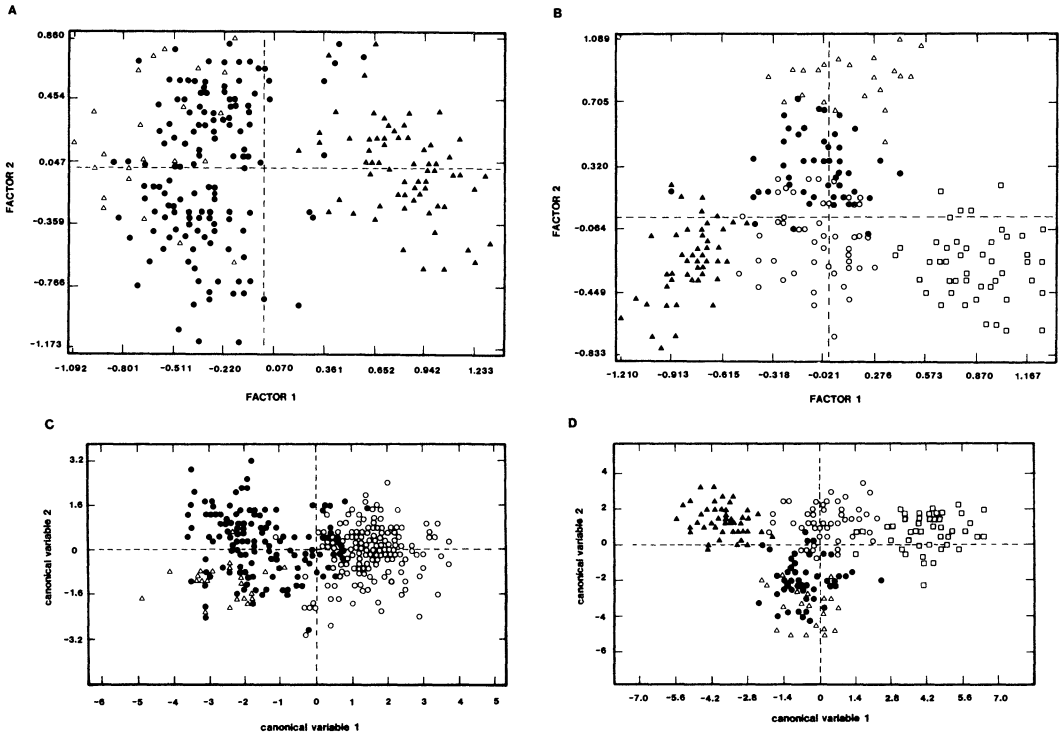


FIG. 3. Numerical analyses of "spiny-margined" *Ceratophyllum* species. A. Principal components scatter-diagram depicting OTU's of *C. kossinskyi* (open triangles), *C. muricatum* (closed circles), and *C. tanaiticum* (closed triangles) generated by plotting the first two factors of variation. B. Principal components scatter-diagram depicting OTU's of *C. australe* (open circles), *C. echinatum* (open squares), *C. kossinskyi* (open triangles), *C. muricatum* (closed circles), and *C. tanaiticum* (closed triangles) generated by plotting the first two factors of variation. C. Scatter-diagram of OTU's of *C. kossinskyi* (open triangles), *C. muricatum* (closed circles), and *C. australe* (open circles) generated by plotting the two canonical variables of a stepwise discriminate analysis of the species. D. Scatter-diagram of OTU's of *C. australe* (open circles), *C. echinatum* (open squares), *C. kossinskyi* (open triangles), *C. muricatum* (closed circles), and *C. tanaiticum* (closed triangles) generated by plotting the two canonical variables of a stepwise discriminate analysis of the species.

any of the spiny-margined group. The same pattern of clustering relationships is maintained in a UPGMA-HIGH dendrogram generated for these species. In this analysis, both *C. echinatum* and *C. tanaiticum* cluster as distinct groups, with *C. muricatum*, *C. australe*, and *C. kossinskyi* grouping in a large central cluster (fig. 4). The co-

phenetic correlation coefficient for this dendrogram is 0.761.

In a PCA analysis including OTU's of *C. submersum* with those of the spiny-margined group, *C. submersum* clusters separately and distinctly

TABLE 2. F-matrix for a stepwise discriminate analysis of *Ceratophyllum australe*, *C. muricatum*, and *C. kossinskyi*. All values are significant at $P < 0.001$; degrees of freedom = 6, 338.

	<i>australe</i>	<i>muricatum</i>
<i>muricatum</i>	121.91	—
<i>kossinskyi</i>	61.33	10.82

TABLE 3. F-matrix for a stepwise discriminate analysis of the spiny-margined *Ceratophyllum*. All values are significant at $P < 0.001$; degrees of freedom = 8, 210.

	<i>echinatum</i>	<i>australe</i>	<i>muricatum</i>	<i>tanaiticum</i>
<i>australe</i>	77.58	—	—	—
<i>muricatum</i>	102.33	39.99	—	—
<i>tanaiticum</i>	192.97	78.00	76.45	—
<i>kossinskyi</i>	76.82	60.47	12.05	66.69

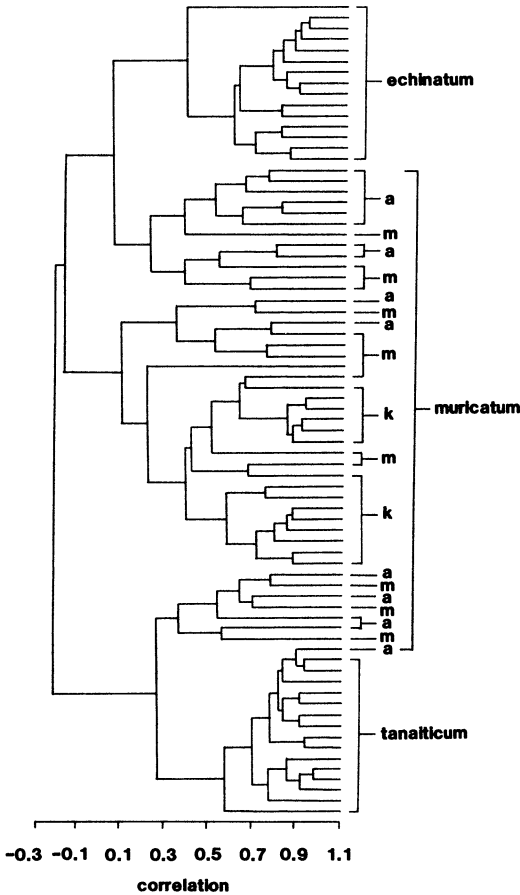


FIG. 4. UPGMA-HIGH dendrogram showing clustering relationships among species of the "spiny-margined" group of *Ceratophyllum*. OTU sub-clusters within *C. muricatum* are identified by: a = *C. australe*, k = *C. kossinskyi*, m = *C. muricatum*.

as a fourth major cluster from all other species (fig. 5A). Factors one and two of this analysis account for 55.6% of the total variation. In a stepwise discriminate analysis of these species (fig. 5B), OTU's of *C. submersum* cluster closest to those of *C. echinatum*, but maintain a marked distinction from this and other groups. All species clusters are significantly different in the discriminate analysis (table 4). For *C. echinatum*, the lowest *F* value from this analysis (table 4) is with *C. submersum*. The highest and lowest *F* values for all pairwise comparisons are between *C. echinatum* and *C. tanaiticum*, and between *C. muricatum* and *C. kossinskyi*, respectively.

The lengths of basal, stylar, and lateral fruit spines are highly correlated ($r = 0.51-0.75$) at a

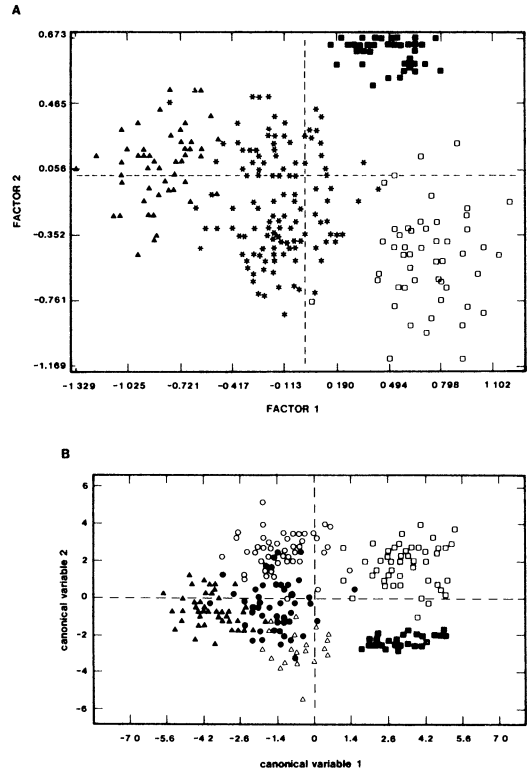


FIG. 5. Numerical analyses comparing "spiny-margined" *Ceratophyllum* species with *C. submersum*. A. Principal components scatter-diagram depicting OTU's of *C. echinatum* (open squares), *C. muricatum* (including *C. australe* and *C. kossinskyi* - asterisks), *C. submersum* (closed squares), and *C. tanaiticum* (closed triangles) generated by plotting the first two factors of variation. B. Scatter-diagram of OTU's of *C. australe* (open circles), *C. echinatum* (open squares), *C. kossinskyi* (open triangles), *C. muricatum* (closed circles), *C. submersum* (closed squares), and *C. tanaiticum* (closed triangles) generated by plotting the two canonical variables of a stepwise discriminate analysis of the species.

statistically significant level (P less than 0.05-0.001) within members of the spiny-margined group (table 5). Only lateral spine lengths in *C. kossinskyi* were not significantly correlated with either basal or stylar spine lengths.

Fruit-spine variation in *C. echinatum* is extensive. The mean and modal values of stylar, basal, and lateral spine length, as well as those of lateral spine number, differ in population comparisons of the species. For basal spines, variability indicated by the range of lengths was

TABLE 4. *F*-matrix for a stepwise discriminate analysis of *Ceratophyllum submersum* with the spiny-margined group of *Ceratophyllum*. All values are significant at $P < 0.001$; degrees of freedom = 8, 258.

	<i>echinatum</i>	<i>australe</i>	<i>muricatum</i>	<i>tanaiticum</i>	<i>kossinskyi</i>
<i>australe</i>	83.02	—	—	—	—
<i>muricatum</i>	110.85	48.64	—	—	—
<i>tanaiticum</i>	195.70	79.04	77.29	—	—
<i>kossinskyi</i>	85.18	73.83	14.48	73.49	—
<i>submersum</i>	67.70	139.24	117.71	168.99	61.88

similar both within and among populations of the species (fig. 6A; table 6). Style length follows a similar pattern (fig. 6B; table 6) although for this variable, within-population variability was somewhat less than the interpopulational variability. Lateral spine lengths (fig. 6C; table 6) followed the pattern of stylar spine lengths, with slightly less intrapopulational variability than interpopulational variation. Lateral spine number showed the most variation between three separate populations. The modal and mean values of this character differed in each of the three populations, however, the range of intrapopulational variation in each case was a fairly good representation of that measured among 51 populations (fig. 6D; table 6). By ANOVA, the Ohio and Maine populations did not differ significantly when compared to the 51 combined populations with the exception of basal spine length (Maine) and lateral spine number (Ohio) (table 6). The population from Illinois, however, differed significantly from the combined populations with respect to all four characters tested by ANOVA (table 6). In summary (fig. 6; table 6), fruit-spine characters in *C. echinatum* differed in three populations studied, but in most cases, their variability was representative of the interpopulational range of variation measured among 51 populations of the species. Values obtained by combining all data (table 6) deviated little from those obtained by sampling only the 51 different populations. These results indicate that spine data collected by sampling fruit characters among populations is as representative of the variability of *C. echinatum* as data including intrapopulational variation. Despite similar ranges of spine character values, several individual population means exhibited different variances when compared to combined population values. Particularly, the population from Illinois is characterized by significantly greater mean values for all variables

(table 6). Overall, these results are consistent with plumule forking characters of *C. echinatum* which exhibit similar patterns of inter- and intrapopulational variability (Les 1985a).

A compilation of fruit-spine data for all members of the spiny-margined group indicates that a wide range of interspecific variability exists in these characters (table 7). Only two of these four characters can be used fairly reliably as a sole means of distinguishing certain taxa. Consistently short stylar-spine length distinguishes *C. tanaiticum* from other members of this group; the maximum stylar length of this species is less

TABLE 5. Values of correlation coefficient (r) for fruit-spine lengths in the spiny-margined *Ceratophyllum* (levels of significance: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$).

	Basal spines	Stylar spines	Lateral spines
<i>C. australe</i>			
Basal spines	1.00	0.51***	0.64***
Stylar spines	0.51***	1.00	0.30***
Lateral spines	0.64***	0.30***	1.00
<i>C. echinatum</i>			
Basal spines	1.00	0.62***	0.64***
Stylar spines	0.62***	1.00	0.61***
Lateral spines	0.64***	0.61***	1.00
<i>C. kossinskyi</i>			
Basal spines	1.00	0.55*	0.29
Stylar spines	0.55*	1.00	-0.28
Lateral spines	0.29	-0.28	1.00
<i>C. muricatum</i>			
Basal spines	1.00	0.73***	0.43***
Stylar spines	0.73***	1.00	0.42***
Lateral spines	0.43***	0.42***	1.00
<i>C. tanaiticum</i>			
Basal spines	1.00	0.68***	0.75***
Stylar spines	0.68***	1.00	0.66***
Lateral spines	0.75***	0.66***	1.00

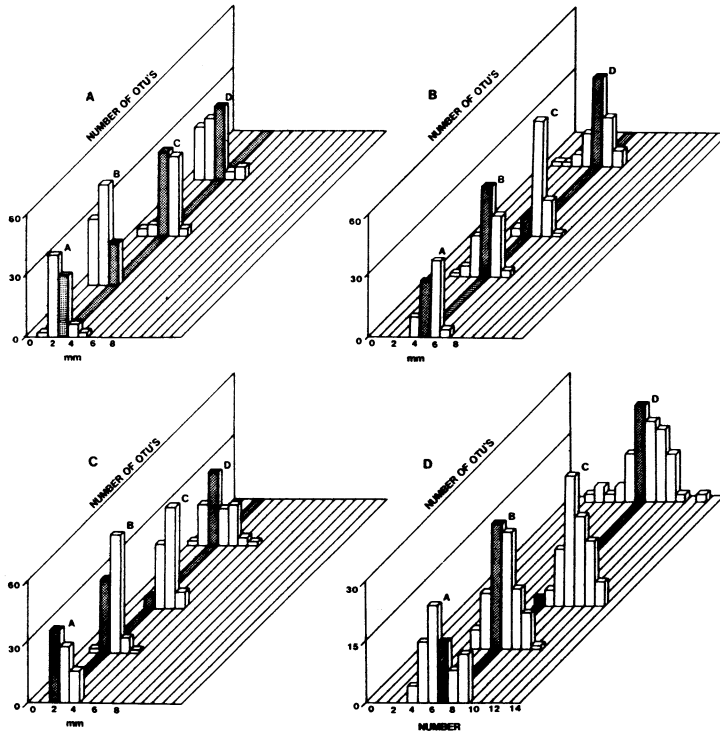


FIG. 6. Histogram plots illustrating variation in selected fruit characters in USA populations of *Ceratophyllum echinatum*. Histogram groupings represent: A. maximum length of basal fruit spines; B. length of stylar fruit spines; C. maximum length of lateral fruit spines; D. number of lateral fruit spines. Histograms within each grouping represent populations from Ohio (A), Maine (B), Illinois (C), and 51 combined populations of the species (D). The modes of "D" are shaded to assist with orientation.

than the mean values of the four other species. Long lateral spines are characteristic of *C. echinatum*, with a mean length 2-8 times greater than that of the four other species.

DISCUSSION

Results of the variational studies presented here indicate that the merger of the spiny-margined *Ceratophyllum* with *C. submersum* by Wilmot-Dear (1985) is not warranted. Simultaneous analyses of fruit characters by multivariate statistical methods have provided evidence to recognize an entirely different pattern of morphological relationships within this complex group of species.

The "range of intermediates" reported between *C. submersum* and taxa of the spiny-margined group (Wilmot-Dear 1985) is an artifact of observations made of one or a few highly variable characters. As illustrated by both PCA

and discriminate analyses (fig. 5), *C. submersum* is actually very distinct morphologically from all representatives of the spiny-margined group. Both *C. echinatum* and *C. tanaiticum* are also relatively isolated in these variational analyses, although Wilmot-Dear (1985) did not recognize either species as distinct. The OTU's of both species group consistently into clusters separate from those of other members of this group (figs. 3, 4, 5). The distinctness of *C. echinatum* indicated by these analyses is consistent with the occurrence of a unique plumule morphology in this species (Les 1985a). These results provide sufficient evidence for continuing to recognize *C. submersum*, *C. echinatum*, and *C. tanaiticum* taxonomically as distinct species.

Ceratophyllum australe, *C. muricatum*, and *C. kossinskyi* cluster as one large group in all multivariate analyses (figs. 3, 4, 5). These results indicate a high degree of morphological similar-

TABLE 6. Variation in fruit-spine characters of *Ceratophyllum echinatum*. Ranges for characters are tabulated; means \pm 1 s.d. are indicated in brackets. Levels of significant deviation from "different populations": * = $P < 0.01$; ** = $P < 0.001$ (ANOVA). "All populations" was excluded from ANOVA.

	Basal spine length (mm)	Stylar spine length (mm)	Lateral spine length (mm)	Lateral spine number
Different populations				
N = 51	1.0-5.0 (2.6 \pm 1.0)	1.5-7.5 (5.2 \pm 1.2)	0.5-6.5 (2.8 \pm 1.3)	2-13 (7.6 \pm 2.1)
Ohio, USA population				
N = 40	1.5-5.0 (2.8 \pm 0.7)	4.0-7.0 (5.6 \pm 0.7)	2.0-4.5 (3.0 \pm 0.8)	4-9 (6.6 \pm 1.5)*
Maine, USA population				
N = 48	1.0-3.5 (2.1 \pm 0.6)*	2.5-7.0 (5.3 \pm 0.9)	1.5-5.0 (2.9 \pm 0.7)	5-11 (7.6 \pm 1.3)
Illinois, USA population				
N = 104	1.5-5.0 (3.6 \pm 0.8)**	4.0-8.5 (6.3 \pm 0.7)**	2.0-5.0 (3.8 \pm 0.7)**	7-13 (10.5 \pm 1.3)**
All populations				
N = 243	1.5-5.0 (2.8)	1.5-8.5 (5.5)	0.5-6.5 (3.1)	2-13 (8)

ity between fruits of these taxa. Despite their similarity, these three taxa are separable morphologically as indicated by a stepwise discriminate analysis of quantitative characters. Wilmot-Dear (1985) recognized *C. kossinskyi* as distinct, but at the rank of variety (*C. submersum* subsp. *muricatum* var. *manschuricum*). Indeed, *C. muricatum* and *C. kossinskyi* are very similar morphologically, and the *F* values obtained in discriminate analyses of these species are relatively low (tables 2, 3). Neither *C. muricatum* nor *C. kossinskyi*, however, show any great degree of similarity to *C. submersum*, with which they were merged by Wilmot-Dear (1985). *Ceratophyllum australe* is also fairly distinct from *C. muricatum*, although this species has not been recognized in recent treatments by either Wilmot-Dear (1985) or Lowden (1978). The OTU's of these species group into two relatively equal halves of a larger cluster (fig. 2A), but are readily separable by a discriminate analysis (fig. 2B) primarily on the basis of four quantitative characters (fig. 2C). The *F* value obtained from the discriminate analysis is relatively low between

C. muricatum and *C. australe* (table 3). On the basis of an overall comparison of both quantitative and qualitative fruit characters, *C. muricatum*, *C. australe*, and *C. kossinskyi* appear to be very closely related, and their geographical distributions are allopatric. *Ceratophyllum australe* has a pan-tropical New World distribution, *C. muricatum* has a pan-tropical Old World distribution, and *C. kossinskyi* has a temperate Old World distribution (Les 1986c). These results lead to the hypothesis that *C. muricatum*, *C. australe*, and *C. kossinskyi* are interrelated, and that their similar fruit morphology is attributable to a common ancestor which diverged into three vicarious entities. These are treated here at the subspecies level with the new combinations made below.

As in *C. demersum* (Les 1986b), basal and stylar spines are significantly correlated in the spiny-margined group of *Ceratophyllum*. In addition, the lateral spine lengths of these species (except for *C. kossinskyi*) correlate with the basal and stylar spine lengths. The correlation of these characters is a major reason that a variational

TABLE 7. Variation in fruit-spine characters of the spiny-margined *Ceratophyllum*. Ranges for characters are tabulated; means are indicated in brackets.

		Stylar spine length (mm)	Basal spine length (mm)	Lateral spine length (mm)	Lateral spine number
<i>C. australe</i>	N = 182	1.5-7.5 [4.3]	0.0-4.5 [1.5]	0.0-4.0 [1.4]	0-20 [11.6]
<i>C. echinatum</i>	N = 243	1.5-8.5 [5.5]	1.0-5.0 [2.8]	0.5-6.5 [3.1]	2-13 [8.0]
<i>C. kossinskyi</i>	N = 23	1.0-8.5 [4.7]	1.5-6.0 [3.8]	0.0-2.5 [0.4]	0-8 [2.6]
<i>C. muricatum</i>	N = 141	0.5-11.0 [5.1]	0.0-8.0 [3.8]	0.0-3.5 [0.6]	0-19 [7.7]
<i>C. tanaiticum</i>	N = 70	0.2-3.5 [0.9]	0.5-4.0 [2.3]	0.1-3.0 [1.0]	4-15 [9.5]

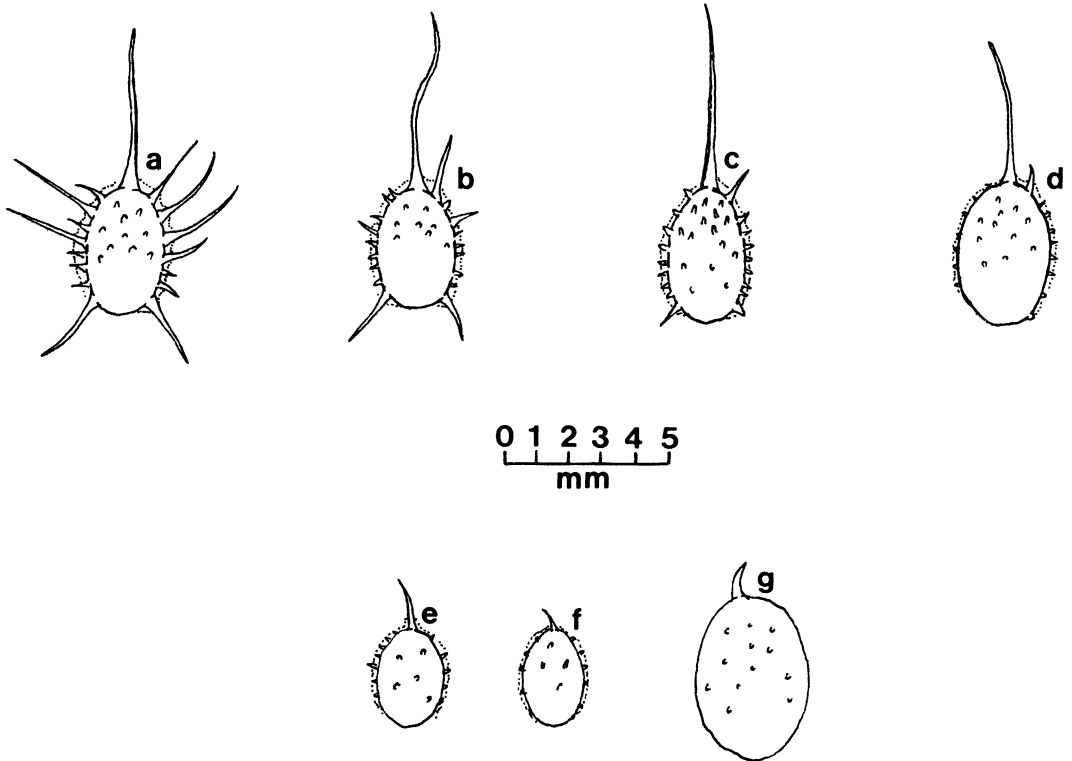


FIG. 7. Fruit variation in *Ceratophyllum muricatum*. a-d. Fruits from a single collection of *C. muricatum* subsp. *australe* showing intrapopulation variability of spine features (drawn from Fassett 28553 (GH), an isotype of *C. llerenae* = *C. australe*). e-f. "Spineless" phenotypes of *C. muricatum* subsp. *muricatum*, drawn from Brass 6458 (A), showing morphological convergence with fruits of *C. submersum*; g, drawn from Les 388 (OS).

continuum in fruit spine characters occurs in all of these species. Like the case of *C. demersum* and *C. submersum* (Les 1986b), individuals at the lower end of the variational continuum can converge morphologically with other species. This convergence has been noted in *C. muricatum*. Short-spined fruit variants of this species (fig. 7) are similar morphologically to *C. submersum*; their margins are essentially spineless, and their styles much reduced. These phenotypes have given rise to reports of *C. submersum* from regions far beyond the usually recognized range of the species, including India and southeast Asia. This has been the case with the reports of "C. submersum" from New Guinea, which actually were based on short-spined variants of *C. muricatum* as proposed earlier by Les (1985b). OTU's from the New Guinea population cluster with *C. muricatum* and not with *C. submersum* in the multivariate analyses.

It is for the same reason that *C. llerenae* and *C. australe* were merged in this study. *Ceratophyllum australe* was the name given to basally spineless variants of the New World species (Grisebach 1879), whereas *C. llerenae* has been applied to New World individuals having fruits with elongate lateral and basal appendages. Both phenotypes occur together (fig. 7) explaining the "mixed population" of both taxa reported by Les (1985a) from Ecuador.

Certain fruit phenotypes of *C. echinatum* also strongly resemble those of *C. submersum* (fig. 8). The size of the fruit body of these species is greater than for other members of the spiny-margined group, and there is other evidence for their close relationship. Both have similar geographical distributions in north temperate regions (Les 1986a, 1986c). Although *C. submersum* lacks the forked first plumule leaves of *C. echinatum* (Les 1985a), there is a recondite sim-

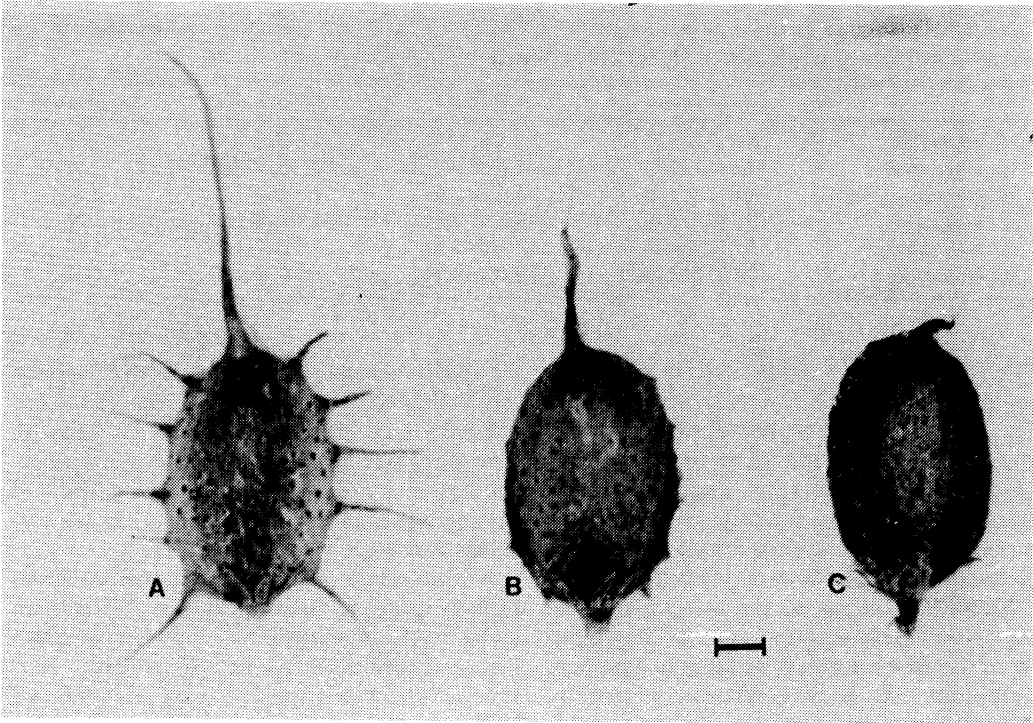


FIG. 8. Convergent fruit morphologies of *Ceratophyllum echinatum* and *C. submersum*. A. "Normal" achene of *C. echinatum* (Skelton & Skelton 1433, TRT). B. "Spineless" achene of *C. echinatum* (Skelton & Skelton 1483, TRT). C. Achene of *C. submersum* (Les 388, OS). Bar = 1 mm.

ilarity in the plumule morphology of these species. In both *C. echinatum* and *C. submersum*, there are four leaves at the second epicotyl node, whereas in other species there are usually five or six (Les 1985a). Both *C. submersum* and *C. echinatum* possess similar flavonoid compounds not found in other species of the genus examined (Les 1986c). These factors point to a close phylogenetic relationship of *C. submersum* and *C. echinatum* which has been obscured because of differences associated with fruit-spine characters. *Ceratophyllum submersum* is probably closely related to *C. echinatum* and the characteristic lateral fruit spines have simply been lost.

The patterns of fruit-spine variation observed within and among populations of *C. echinatum* are useful for estimating the genetic structure of populations of this species. In part I (Les 1986b), the fruit-spine variation within a clonal population of *C. demersum* was found to be considerably less than that measured among populations. In the case of *C. echinatum*, intrapopu-

lational variation in fruit-spine characters is more extensive, and more similar to patterns observed among populations (fig. 6; table 6). These results are consistent with patterns observed in plumule-forking characters (Les 1985a), which within some populations show nearly the same variability as measured among populations. These examples of wide intrapopulational morphological variability indicate that sexual reproduction probably occurs relatively often in *C. echinatum*. This hypothesis is supported by field observations and collection data which show that fruiting is indeed very common in *C. echinatum*, certainly more so than in *C. demersum*. Other members of the spiny-margined group of *Ceratophyllum* are free-fruited, and sexual reproduction probably also occurs frequently in these species as well. This factor could explain why there is extensive variation in fruit features within this group of species, and why the spiny-margined *Ceratophyllum* have been so difficult taxonomically.

TABLE 8. Variation in dimensions and length/width ratios for fruits of *C. submersum* and members of the spiny-margined *Ceratophyllum*. Means are tabulated; ranges are indicated in parentheses.

	Length (mm)	Width (mm)	Thickness (mm)	Length/width
<i>C. echinatum</i>	4.8 (3.5–5.5)	3.5 (2.5–4.5)	2.2 (2.0–3.0)	1.37
<i>C. submersum</i>	4.6 (4.0–5.5)	3.3 (3.0–4.0)	2.3 (1.5–3.0)	1.39
<i>C. australe</i>	4.0 (3.0–4.5)	2.5 (2.0–3.0)	1.9 (1.0–2.0)	1.60
<i>C. kossinskyi</i>	3.8 (3.5–4.0)	2.4 (2.0–2.5)	1.9 (1.5–2.0)	1.58
<i>C. muricatum</i>	3.7 (3.0–4.5)	2.5 (2.0–3.0)	2.0 (1.0–2.5)	1.48
<i>C. tanaiticum</i>	3.9 (3.5–4.5)	1.9 (1.5–2.5)	1.1 (1.0–1.5)	2.05

The wide degree of variation in quantitative fruit-spine characters limits their use taxonomically. In most instances, the overlap of these features between species is too extensive for them to be used reliably in a diagnostic sense (table 7). More reliable quantitative fruit characters are those dealing with dimensions of the fruit body. Fruit width is an important character for separating species of the spiny-margined group in numerical studies, and correlates highly with the first canonical variable in stepwise discriminate analyses. The fruits of *C. submersum* and *C. echinatum* are relatively large and wide, those of *C. muricatum* (including all subspecies) are shorter and narrower, and those of *C. tanaiticum* are very short, narrow, and thin (fig 1; table 8). There are also several qualitative fruit characters which are useful taxonomically in this group. The raised plate-like ridges on the fruit surfaces of *C. kossinskyi* are not found in any other taxa. As mentioned previously, the forking of the first plumule leaves is diagnostic of *C. echinatum* (Les 1985a). Within the spiny-margined *Ceratophyllum*, the best taxonomic differentiation is achieved when both quantitative and qualitative characters are used.

CONCLUSIONS

In summary, this variational study of fruit morphology in the spiny-margined group of *Ceratophyllum* provides evidence to support the recognition of *C. echinatum*, *C. submersum*, and *C. tanaiticum*. Fruits of *C. muricatum*, *C. australe*, and *C. kossinskyi* are morphologically very similar, with overlapping variational patterns. They are allopatric, however, and separable taxonomically by several fruit characters. On the basis of these results, they are treated as three vicarious subspecies of *C. muricatum*. In the variational character space for these taxa, *C. muricatum*

occupies a central position; i.e., both *C. australe* and *C. kossinskyi* overlap with *C. muricatum* but not with each other (e.g., fig. 3B, D). This relationship may indicate that *C. muricatum* subsp. *muricatum* is most similar to the progenitor of the segregates referred to as subsp. *australe* and subsp. *kossinskyi*.

A wide range of intrapopulational variation in fruit-spine characters occurs in *C. echinatum* and is representative of the measured interpopulational variability. Along with frequent fruit production in this species, these observations indicate that *C. echinatum* probably reproduces sexually more typically than *C. demersum*. This conclusion is generalized to pertain to other members of the spiny-margined *Ceratophyllum* which produce abundant fruits. It is possible that populational differences in these fruit characters reflect variation in frequencies of genes regulating spine character expression among populations of *C. echinatum*. Certainly, the genetic structure of populations of putatively sexual and asexual species of *Ceratophyllum* deserves further study.

The correlation of fruit-spine lengths in the spiny-margined group parallels the pattern observed in *C. demersum* (Les 1986b). Achene evolution in the spiny-margined group has apparently proceeded in a fashion similar to that of *C. demersum*, i.e., the divergence of taxa is correlated with reduction in quantitative fruit-spine features. Fruit-body dimensions within this group appear to be more highly conserved and show less intraspecific variability.

As deduced from statistical analyses, *Ceratophyllum* species of the spiny-margined group exhibit relatively discrete variational patterns worldwide, and do not form a "complex net-like or web-like pattern" as Wilmot-Dear (1985, p. 255) concluded. In light of this revised interpretation of morphological variability, a

modified taxonomy of the spiny-margined *Ceratophyllum* is justified.

NEW COMBINATIONS

1. CERATOPHYLLUM MURICATUM Chamisso, *Linnaea* 4:504. 1829.
- C. demersum* γ *filifolium* var. *muricatum* (Cham.) Kuntze, *Revis. gen. pl.* 2:644. 1891. *C. demersum* var. *muricatum* (Cham.) J. D. Hook. ex K. Schum., *Fl. bras.* 3(3):749. 1894.
- C. submersum* subsp. *muricatum* (Cham.) Wilmot-Dear, *Kew bull.* 40:266. 1985.—TYPE: Egypt, Damiatte [Dumyat], 1818, *Sieber s.n.* (holotype: B, destroyed; isotypes: B [acquired after 1829]!, G!, HAL!, K [2 sheets]!, MO [2 sheets]!; lectotype here designated: HAL!).
- 1a. CERATOPHYLLUM MURICATUM subsp. MURICATUM
- C. cristatum* Perrottet & Guillemain, *Fl. seneg. tent.* 296. 1833.—TYPE: Senegal, near Khann and N'Batal, no date, *Perrottet s.n.* (holotype: P [2 sheets]!; isotype: K!).
- C. demersum* β *laxifolium* var. *oxycarpum* Kuntze, *Revis. gen. pl.* 2:644. 1891.—TYPE: Java, Lanata bei Batavia, May 1875, *Kuntze 4212* (holotype: NY!).
- C. demersum* forma *papillosum* Wilmot-Dear, *Kew bull.* 40:261. 1985.—TYPE: Papua New Guinea, Central Dist., Kairuku Subdist., 17 Aug 1962, *Darbyshire 817* (holotype: K!; isotypes: G!, A!).
- 1b. **Ceratophyllum muricatum** subsp. **australe** (Grisebach) Les, *comb. et stat. nov.*—*Ceratophyllum australe* Griseb., *Symb. fl. argent.* 14. 1879.—TYPE: Argentina, Santa Fe Province, uferrand d[er] Laguna del Palmar bei S[a]n Jose, 28 June 1873, *Lorentz & Hieronymus 547* (lectotype, here designated: GOET!; isolectotype: K!); N.O. v. Entrerios, in Tumpeln, Feb 1876, *Lorentz 617* (syntype: GOET!); Province Tucuman, Trancas, Feb. 1873, *Lorentz & Hieronymus 1012* (syntype: GOET!); Gran Chaco, Dragones, 13–18 Aug 1873, *Lorentz & Hieronymus 610* (syntype: GOET!). Bolivia, Bachlein bei [Rio] Yavi[a], Tarija [Bolivia], May 1873, *Lorentz & Hieronymus 704* (syntype: GOET!).
- C. cristatum* Spruce ex K. Schum. [non Perrottet & Guillemain], *Fl. bras.* 3(3):748. 1894, later homonym.
- C. demersum* var. *cristatum* K. Schum., *Fl. bras.* 3(3):748. 1894.—TYPE: Brazil, Solimões River, *Spruce 1583* (holotype: B, destroyed; isotypes: G!, K!).
- C. llerenae* Fassett, *Comun. Inst. Trop. Invest. Ci. Univ. El Salvador.* 2:29. 1953.—TYPE: El Salvador, Laguna Jaguey, 20 km SSW of San Miguel, 4 Jan 1591 [1951], *Fassett 28553* (holotype: F!; isotypes: CU!, F [duplicate acquired after 1953]!, GH!, UC!, MT!, NY!, SMU!, US!, WIS [2 sheets]!); Guatemala, Dept. Amatitlan, 1 July 1921, *Tonduz 594* (paratype: GH!; isoparatype: B!); same locality and date, cited by Fassett as "*Coridux 549*" (paratype: US); Columbia, Dept. Bolivar, 28 Jan 1918, *Pennell 4129* (paratype: NY); Venezuela, Maracay, Edo. Aragua, 26 May 1941, *Salazar 12* (paratype: US); Trinidad, no date, *Crueger s.n.* (paratype: GH!; isoparatype: WIS!); Dutch Guiana, Surinam, no date, *Schweinitz s.n.* (paratype: NY!).
- C. floridanum* Fassett, *Comun. Inst. Trop. Invest. Ci. Univ. El Salvador.* 2:29. 1953.—TYPE: U.S.A., Florida, grassland NW of Watson Hammock, Big Pine Key, Monroe Co., 8 November [November] 1950, *Killip 40723* [40732] (holotype: US!); same locality, 29 Jan–16 Feb 1940, *Killip 32868* (paratype: US; isoparatype: CU!); Florida, no date, *A. W. C.[hapman] s.n.* (paratype: NY!); South Florida, no date, *Blodgett s.n.* (paratype: NY!).
- 1c. **Ceratophyllum muricatum** subsp. **kossinskyi** (Kuseneva-Prochorova) Les, *comb. et stat. nov.*—*Ceratophyllum kossinskyi* Kusen.-Proch., in V. L. Komarov, *Fl. URSS.* 7:721. 1937.—TYPE: Astrachan', Volga delta, near village of Kilinchi, in ponds, 3 Aug 1915, *Kossinsky 799* (holotype: LE!).
- C. submersum* var. *manschuricum* Miki, *Bot. Mag. (Tokyo).* 49:778. 1935.—*C. manschuricum* (Miki) Kitagawa, *Lin. fl. Manshur.* 207. 1939.—TYPE: Manchuria, Nishiheiei in Tetsurei, July 1934, *Suko s.n.* (syntype: OSA); Tsitsihar, Aug 1934, *Ueno s.n.* (syntype: OSA).
- C. inflatum* Jao, in *Fl. Sin.* 27:603. 1979.—TYPE: China, Hupeh, Shashih, in lotus ponds, 20 Oct 1953, *Jao s.n.* (holotype: HIB!).
- C. submersum* subsp. *submersum* var. *squamosum* Wilmot-Dear, *Kew bull.* 40:266. 1985.—TYPE: China, Shandong, Chefoo, [Yentai], 2 Sept 1920, *Cowdry 923* (holotype: K!).

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