

The Evolution of Achene Morphology in *Ceratophyllum* (Ceratophyllaceae), I. Fruit-Spine Variation and Relationships of *C. demersum*, *C. submersum*, and *C. apiculatum*

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ABSTRACT. Variational studies of *Ceratophyllum apiculatum*, *C. demersum*, and *C. submersum* demonstrate that *C. demersum* and *C. submersum* are separable by multivariate statistical analyses, whereas *C. demersum* and *C. apiculatum* are not. In fruits of *C. demersum*, stylar and basal spine lengths are highly correlated and related linearly. A comparison of interspecific spine-length variation with variation observed within a clonal population (identified by enzyme electrophoresis) indicated that a large component of spine-length variation in the species is genetic. In *C. demersum*, the frequency distribution of basal spine lengths is bi-modal as a result of spine abscission. These results provide evidence that *C. apiculatum* fruit phenotypes are simply variational phases of *C. demersum*. The data are useful for inferring possible evolutionary relationships in the genus.

Ceratophyllum is a genus of perennial, fresh-water herbs which has attained an essentially cosmopolitan distribution. Extensive morphological variability has caused considerable taxonomic disarray in the genus (Cook et al. 1974), and systematic relationships are poorly understood due to complicating biological factors. Adaptation to an aquatic existence has been accompanied by conspicuous morphological reduction (Jones 1931), especially in the floral structures (Aboy 1936). Hydrophilous pollination, achenes, and frequent fruit barrenness restrict the application of a comprehensive crossing program to aid in understanding the genetic basis of morphological features in the genus, particularly those used to delimit taxa.

Achene morphology has been accorded considerable evolutionary significance in *Ceratophyllum*, and fruit-spine characters are used most often to delimit taxa within the genus (Les 1980). However, there have not been adequate variational studies made of fruit-spine characters and their use taxonomically has been tenuous. The basic fruit types in *Ceratophyllum* represent character combinations of two kinds, qualitative (presence/absence of apical, basal, facial, and marginal spines) and quantitative (the relative number/length of these spines) (Les 1980). Although somewhat similar, the fruits of *C. demersum* L. (Linnaeus 1753), *C. apiculatum* Cham. (Chamisso 1829), and *C. submersum* L. (Linnaeus 1763) exhibit various differences.

Fruits of *C. demersum* and *C. apiculatum* differ by the length of their basal appendages; i.e., long spines in the former and short tubercles in the latter (fig. 1a, b). Differences in the fruit morphologies of *C. demersum* and *C. submersum* are somewhat more pronounced, with the trispinous achenes of *C. demersum* differing markedly from the essentially spineless fruits of *C. submersum* (fig. 1a, c).

Several studies have dealt with fruit-spine variation in these three species (e.g., McGregor and Sperry 1951), but basic questions remain. Are phenotypic differences between fruits of *C. apiculatum*, *C. demersum*, and *C. submersum* simply phases of a continuous variational gradient? What is the probable genetic basis of the differences between these fruit phenotypes? Does the variable nature of fruit-spine length in *Ceratophyllum* reduce the taxonomic utility of these characters? Do other morphological characters correlate with the "key" characters (i.e., fruit-spine lengths) used to separate these taxa? Should *C. apiculatum*, *C. demersum*, and *C. submersum* be recognized as distinct species?

The purpose of this study is to answer these questions which in turn may provide insights into the evolution of achene morphology in the genus. Such information should aid in understanding the systematic relationships within the genus, and provide a basis for constructing a more meaningful taxonomic treatment of the group.

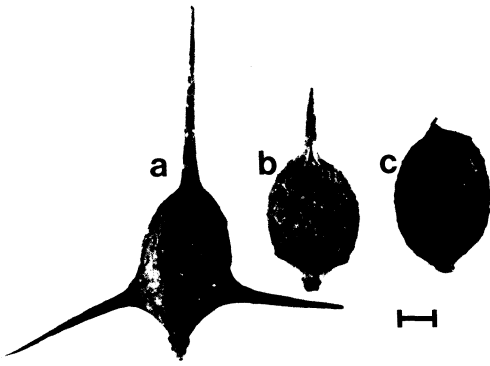


FIG. 1. Achenes of three *Ceratophyllum* species. a. *C. demersum* L. (Les 320, OS). b. *C. apiculatum* Cham. (Stuckey 1951, OS). c. *C. submersum* L. (Les 388, OS). Length of bar is one millimeter.

METHODS

Specimens of *Ceratophyllum* were borrowed for study from 75 herbaria (see acknowledgments), and identified according to Les (1985a). Fruiting specimens of *C. submersum*, *C. demersum*, and *C. apiculatum* were selected for use in these analyses. Although few specimens were identified previously as *C. apiculatum*, it was desirable to treat the taxon as distinct. Following Chamisso (1829), individuals of "demersum-like" plants with fruits bearing basal spines shorter than 1.0 mm in length were treated as *C. apiculatum* (although the type specimen of *C. apiculatum* was destroyed at Berlin, this group included individuals collected near the type locality). A total of 123 fruiting individuals of *C. demersum*, 27 fruiting individuals of *C. apiculatum*, and 32 fruiting individuals of *C. submersum* constituted the operational taxonomic units (OTUs) used in the subsequent analyses. Twenty characters (table 1) were scored for the 182 OTUs (copies of the original data matrix are available from the author). These data were used to perform cluster analysis (UPGMA-HIGH), and principal components analysis (PCA) using the NT-SYS numerical taxonomy program (Rohlf et al. 1974). Data matrices were

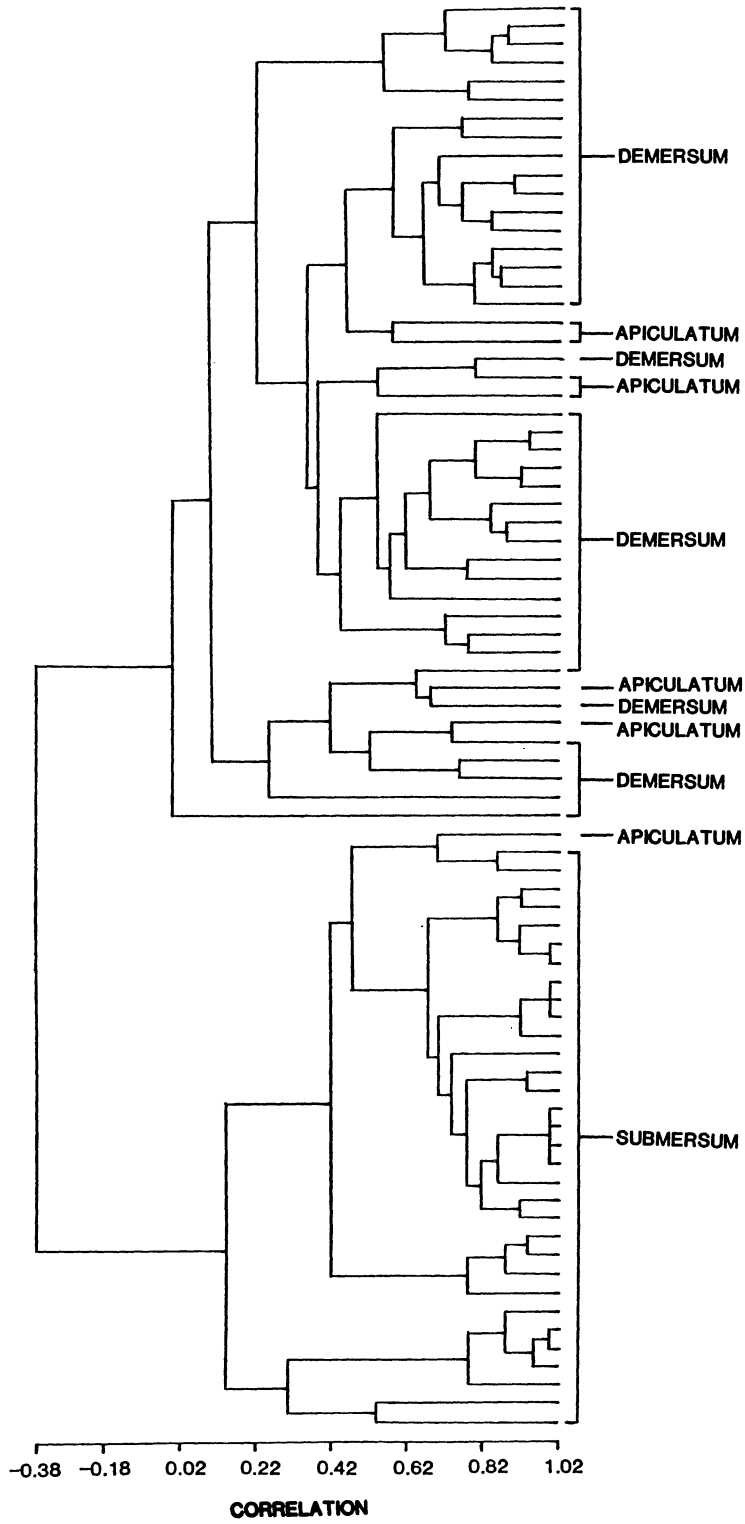
TABLE 1. List of 20 characters and states coded for OTUs of *Ceratophyllum*. Characters 4, 7, 12, 14, and 15 were invariant and were excluded from final analyses.

LEAVES: 1. maximum leaf-order (1-5). 2. segment morphology (inflated/non-inflated). 3. denticle morphology (broad-based/narrow-based). 4. first plumule-leaf morphology (simple/compound). 5. maximum leaf-whorl diameter (mm). 6. petiolar region (present/absent). FRUIT: 7. shape (elliptical/hemi-spherical). 8. fruit-body length (mm). 9. fruit-body width (mm). 10. fruit-body thickness (mm). 11. maximum basal spine length (mm). 12. maximum facial spine length (mm). 13. stylar spine length (mm). 14. number of lateral appendages (#). 15. maximum length of lateral appendages (mm). 16. surface (smooth/warty/tuberculate/ridged). 17. wing (present/absent). 18. phenology (1-12). 19. stylar position (centered/eccentric). 20. peduncle length (mm).

standardized using the STAND subroutine of NT-SYS. The cluster analysis (UPGMA-HIGH) was produced by the TAXON subroutine, and PCA by the FACTOR subroutine. Computations were made by an IBM-4341 computer at the IRCC facility of The Ohio State University.

A preliminary run of the data matrix disclosed five invariable characters (table 1) which were removed to form a 15 column matrix. From this matrix, a UPGMA-HIGH dendrogram was produced and a PCA scatter-diagram was plotted using the first two variational factors as axes. Factors I and II accounted for 43.2% of the total variation; adding a third axis did not change the overall patterns. To produce more evenly-sized groups, the algorithms were repeated using only OTUs of *C. demersum* and *C. apiculatum*, and removing those clustering at a correlation above 0.75. This modified data set (46 OTUs) was more similar in size to that of *C. submersum* (32 OTUs) and depicted most of the original variation (the relative number of *C. apiculatum* OTUs was not critical because individuals of this taxon showed no clustering affinity by either analysis—see results). These 46 OTUs were pooled with the 32 OTUs of *C. sub-*

FIG. 2. UPGMA-HIGH dendrogram for 78 OTUs of *Ceratophyllum* (taxa are labelled by their specific epithets). Cophenetic correlation coefficient is 0.852.



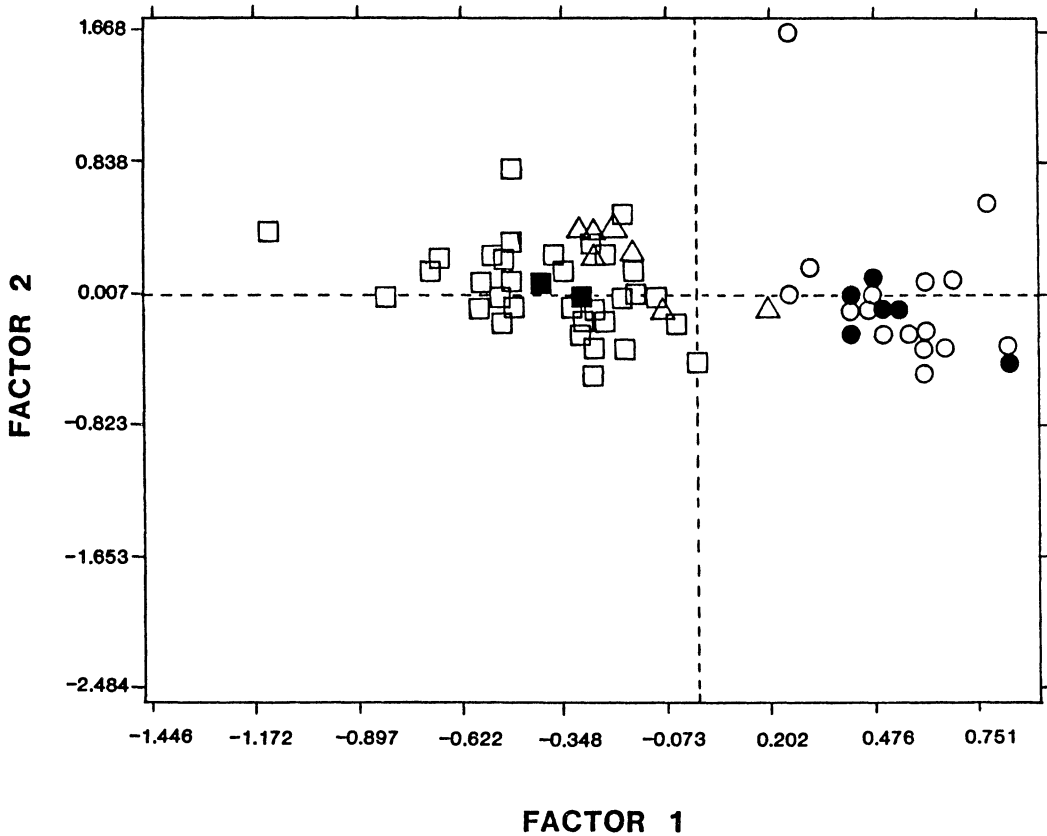


FIG. 3. Principal components scatter-diagram of 78 OTUs of *Ceratophyllum* generated by plotting the first two factors of variation. Squares = *C. demersum* OTUs; triangles = *C. apiculatum* OTUs; circles = *C. submersum* OTUs. Open symbols represent a single OTU at point coordinates; closed symbols represent multiple OTUs at point coordinates.

mersum and the analyses repeated. The newly generated UPGMA-HIGH dendrogram (fig. 2) and PCA scatter-diagram (fig. 3) maintained the same clustering relationships as those generated from the original data set.

Because *C. demersum* and *C. apiculatum* were indistinguishable by multivariate analyses, their data were pooled for subsequent univariate analyses. Measurements of stylar length and maximum basal spine length (from the original data matrix) were used to construct histograms for each character (fig. 4). The OTUs of *C. demersum* with abscised basal spines were removed from the analysis because spine abscission is an external influence upon the relationship between stylar and basal spine lengths (see discussion). The remaining basal and stylar spine lengths were first transformed using the equation $x' = \log(x + 1)$, and then fit-

ted to normal probability plots using the SAS statistical software package (SAS 1982). The log transformation yielded normal distributions for both variables. The transformed values were then used to calculate a correlation coefficient (r and r^2) (SAS 1982) to test for interdependence of these dependent variables (Snedecor and Cochran 1982).

Because standard models of linear regression are inappropriate for analyzing two dependent variables, a "Model II" analysis was performed as a means of assessing their functional relationships (Sokal and Rohlf 1982). Using the original data set, a scatter diagram of style vs. basal spine lengths was constructed (fig. 5) and a regression line (Model II) drawn using the Bartlett 3-group method of analysis (Sokal and Rohlf 1982).

To obtain an estimate of phenotypic plastic-

ity of fruit-spine variation in *C. demersum*, the following procedure was employed. A suspected clonal population of the species was located by its dense, clumplike growth. To estimate the genetic structure of the population, 20 individuals were selected at random for electrophoretic analysis. Plant material was ground in tris-HCl extracting buffer (Gottlieb 1981), the extracts centrifuged, and then electrophoresed on 12% starch gels using two buffer systems, lithium borate and histidine-HCl (Gottlieb 1981). The lithium borate gel (pH 8.3) was run at 60 mA to resolve the following enzyme systems: phosphoglucose isomerase, phosphoglucose mutase, alcohol dehydrogenase, glutamate dehydrogenase, leucine aminopeptidase, and glutamate-oxaloacetate transaminase. The histidine-HCl gel (pH 7.0) was run at 100 volts to resolve the following enzyme systems: 6-phosphogluconate, malate dehydrogenase, isocitrate dehydrogenase, acid phosphatase, and esterase. Gels were stained following Crawford and Smith (1984) and scored. Because no electrophoretic variation was detected (see results), a statistical analysis of these data was unnecessary. Styler and basal spine lengths were measured on 17 fruits collected from the putative clonal population and the data tabulated (table 2).

RESULTS

Cluster and principal components analyses of *C. demersum* and *C. apiculatum* OTUs failed to distinguish these taxa. In both analyses, OTUs of *C. submersum*, *C. demersum*, and *C. apiculatum* produced only two discrete groups (figs. 2, 3). In these analyses, *C. apiculatum* OTUs interspersed among those of *C. demersum* (figs. 2, 3), whereas *C. submersum* OTUs clustered separately. As an exception, an OTU of *C. apiculatum* grouped with *C. submersum* in both instances (figs. 2, 3). The three variables having highest correlations with factor I of the PCA were leaf-order, style-length, and basal spine-length respectively.

Although the transformed values of styler and basal spine lengths in *C. demersum* are normally distributed, the distribution of non-transformed basal spine lengths is bi-modal and includes an inordinately high proportion of short lengths (fig. 4). Basal and styler spine lengths are highly correlated with an r value

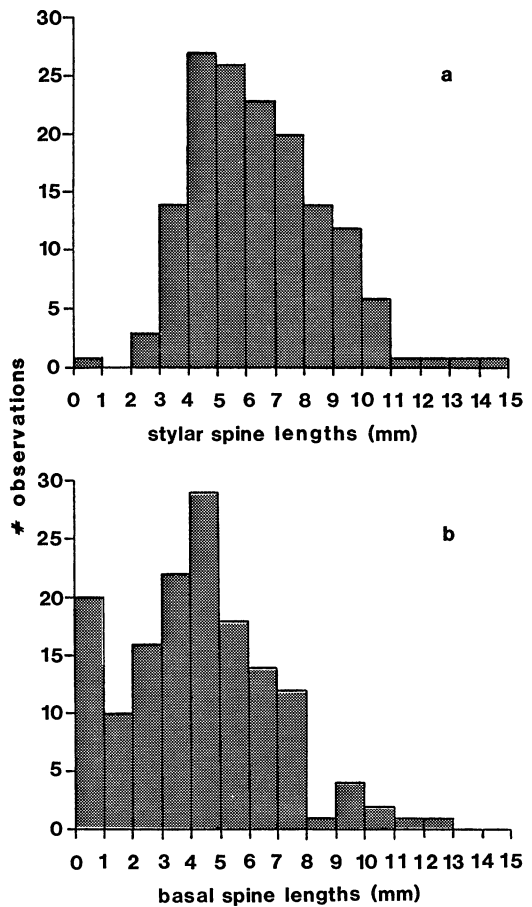


FIG. 4. Frequency distributions of fruit-spine lengths in *Ceratophyllum demersum*.

of 0.77 significant at the 0.001 level ($r^2 = 0.59$). A regression line drawn through the scatter diagram of spine length values illustrates that a linear relationship exists between the variables (fig. 5). This regression line is presented to illustrate the trend of the data rather than as a predictive model. For this reason, the plotted variables were not transformed into normal distributions, and the statistical significance of the line was not evaluated. The interdependence of styler and basal spine lengths is established by the significance of their correlation.

Electrophoretic variation was not detected at any of the 14 loci resolved for individuals of *C. demersum* sampled from a field population. The genetic uniformity indicates either that the population was clonal, or that the level of genetic variation was too small to be detected by

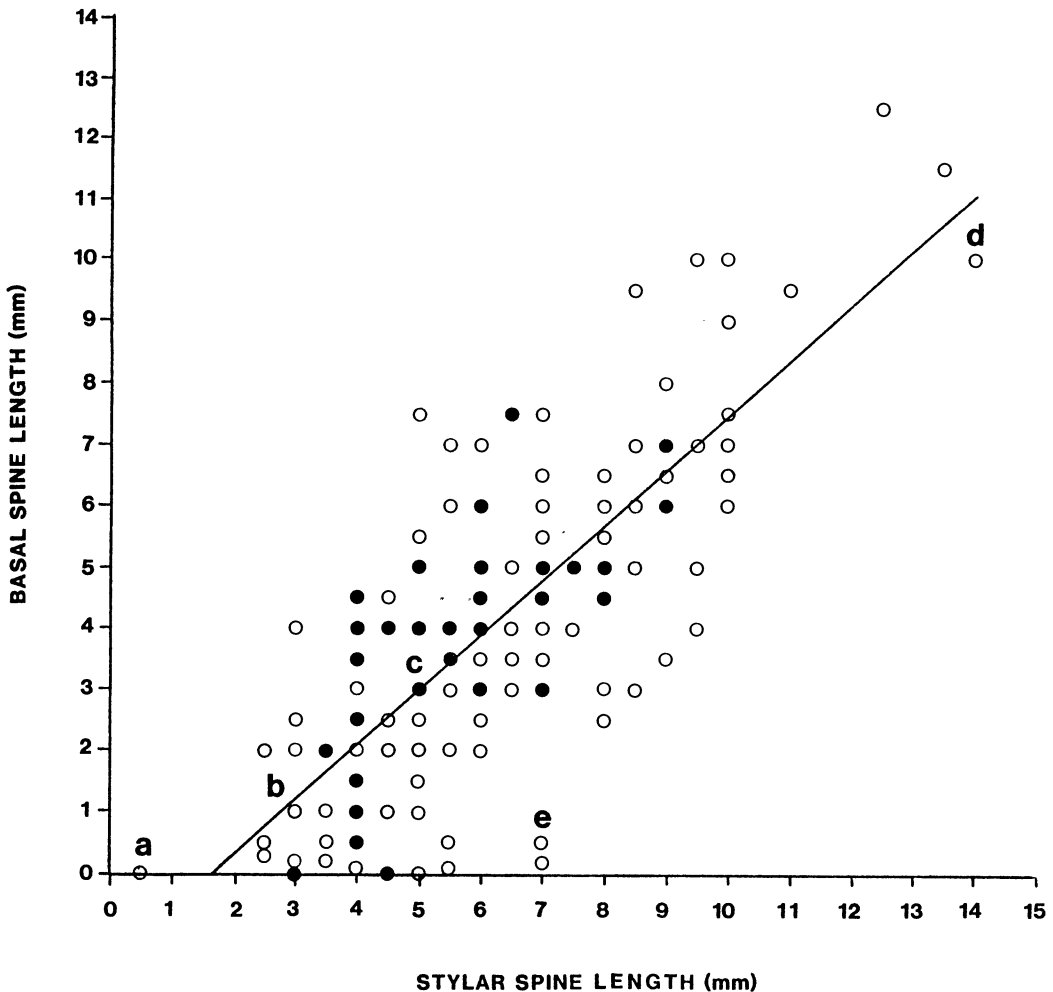


FIG. 5. Scatter-diagram showing linear relationship of fruit-spine lengths in *Ceratophyllum demersum*. Open circles represent a single observation; closed circles indicate multiple observations for given coordinates. Correlation coefficient (r) is 0.77 ($r^2 = 0.59$). Regression line is superimposed. Letters a-e refer to drawings in figure 6.

this method. Fruits from this population displayed considerably less variation in spine lengths than those observed among 150 different populations of the species. Style lengths ranged only from 5.5–9.0 mm within the population vs. 0.5–14.0 mm among populations; basal spine lengths ranged only from 3.5–6.5 mm vs. 0.0–12.5 mm respectively (table 2). Similarly, the "scatter" (range of observed basal spine lengths for a particular style length interval) was much less within the population (1.0–2.0 mm) than among populations (4.5–7.5 mm) in each of the five overlapping intervals compared (table 2).

DISCUSSION

The apparent variational continuum in basal appendage length displayed by the fruit phenotypes of *C. demersum* (with elongate basal spines), *C. apiculatum* (with short basal tubercles), and *C. submersum* (without basal appendages) has complicated an assessment of the relationships of these taxa. Adding to the confusion has been misinterpretation of what actually constitutes a "spineless" fruit. Drawing upon an earlier description and illustration by Vaillant (1719), Linnaeus (1763) described the fruit of *C. submersum* as entirely spineless

TABLE 2. Fruit-spine variation in *Ceratophyllum demersum*. Ranges are given in brackets.

Within clonal population		Among 150 populations	
Style lengths (mm)	Basal spine lengths (mm)	Style lengths (mm)	Basal spine lengths (mm)
5-6	3.5	5-6	0-7.5 [7.5]
6-7	3.5-5.5 [2.0]	6-7	2.0-7.5 [5.5]
7-8	4.5-6.5 [2.0]	7-8	0.5-7.5 [7.0]
8-9	4.5-5.5 [1.0]	8-9	2.5-7.0 [4.5]
9-10	5.5-6.5 [1.0]	9-10	3.5-10.0 [6.5]
5.5-9.0 [3.5]	3.5-6.5 [3.0]	0.5-14.0 [13.5]	0-12.5 [12.5]
(overall)	(overall)	(overall)	(overall)

("fructibus muticis") in his original circumscription of the species. The illustration (Vailant 1719) clearly shows the fruit of the species as lacking elongate appendages. There are no basal appendages, and the terminal style persists only as a short tubercle in contrast to the elongate styles characteristic of other species in the genus. In practice, the short style of *C. submersum* was found to be sufficient for separating this species from *C. demersum* (Sandwith 1924). These observations are supported by the PCA analysis which indicated that style length has the second highest correlation with factor I, the axis which most effectively separates these species. Several investigators, however, have misinterpreted "spineless" fruits as those simply lacking basal appendages and have not considered style length a factor. As a result, fruits of *Ceratophyllum* without discernable basal appendages have been categorized as "spineless" regardless of their style lengths. This interpretation has led to the incorrect application of the name "*C. submersum*" to basally spineless individuals of *C. demersum* (e.g., McGregor and Sperry 1951) and even *C. australe* Griseb. (e.g., Lowden 1978), a species described as lacking basal appendages on the fruit (Grisebach 1879). Similar misconceptions have led to the conclusion that the presence of basal spines is "... clearly not a good character to use in separating *C. demersum* from *C. submersum*" (Norman 1978, p. 51).

Difficulties associated with interpreting the fruit morphology of *C. apiculatum* were reviewed by Sandwith (1924) and Fassett (1953). Nevertheless, occasional discoveries of individuals of *C. demersum* bearing fruits with poorly developed basal appendages continue to create taxonomic difficulties. Should such plants be referred to *C. demersum* or *C. apicula-*

tum? The status of *C. apiculatum* was questioned by Chamisso (1829) when he first named the species. Chamisso expressed uncertainty about the distinctness of *C. apiculatum* from *C. demersum* in a postscript to his account, commenting that the basal fruit spines of *C. demersum* (= *C. oxyacanthum* sensu Chamisso) were scarcely present in younger achenes, but developed into longer spines as the fruits matured. This developmental aspect of basal spine growth has been documented on several occasions (Sandwith 1924; Les 1980). The awareness of this fact by Chamisso indicates that he did not base *C. apiculatum* on an immature specimen of *C. demersum* as some have speculated, but on material with mature fruits, in which the basal spines were not elongated (a conclusive assessment of the status of this species has been complicated by the destruction of the type specimen). Chamisso (1829) also commented on the range of variation in basal fruit-spine length (including fruits with short basal spines) which he observed among specimens of *C. demersum* from different locales. Although these observations apparently concerned Chamisso, he chose to recognize *C. apiculatum* as distinct.

The failure to distinguish *C. demersum* and *C. apiculatum* by cluster and principal components analyses indicates the lack of morphological characters correlating with the species. The key character (basal fruit-spine length) used to separate these species is highly variable. Also, there is no geographic integrity to *C. apiculatum*; its distribution parallels that of *C. demersum*. These observations indicate that the species should not be recognized as distinct. Furthermore, statistical analyses of fruit-spine lengths have revealed the probable relationship of the *C. apiculatum* fruit phenotype to that of *C. demersum*. An important factor is the relationship existing

between stylar and basal spine lengths in *C. demersum*. These lengths are highly correlated and follow a linear relationship (fig. 5). A range of variation in these lengths exists, however, as depicted by the degree of point "scatter" along their regression line (fig. 5). The relationship between style length and basal spine length is not absolute; i.e., fruits of the same style length from various populations have corresponding basal spine lengths of different values (hence the observed scatter). This variational latitude in spine length can be attributed to two components, one genetic, and one of phenotypic plasticity.

The plastic component can be estimated by examining the range of spine-length variation occurring within populations. This estimate is possible because *C. demersum* is highly clonal, as a result of its well-developed means of asexual reproduction by fragmentation (Les 1985b). Sexual reproduction is usually autogamous within clones (Les 1985b) which limits the chances of novel genetic recombination. Unless outcrossing with other clones occurs, the genetic variation within a population is expected to be low. The lack of electrophoretic variation indicated that the field population studied was probably clonal. Therefore, it was assumed that most of the variation in fruit-spine lengths observed within this population reflected environmental rather than genetic effects on the phenotype. The range of variation observed within this population was much smaller than that observed among widely separated populations of the species (table 2). Both the range of spine lengths, and their "scatter" were less within the population, an indication that a large component of interpopulational spine length variation in *C. demersum* is probably genetic.

Generalizations regarding the genetic basis of qualitative and quantitative characters in plants have emerged from results of segregational data amassed from numerous crossing studies. These studies show that many qualitative morphological characters have a relatively simple genetic basis involving only one or two gene loci, whereas quantitative characters are usually governed by multiple gene systems (Gottlieb 1984). These generalizations are useful for interpreting the basis of fruit characters in *Ceratophyllum* where it has not been feasible to carry out crossing studies. As a metric quan-

titative character, spine length is probably regulated polygenically, making possible a wide spectrum of interpopulational variation in the character. As one would expect from the clonal nature of the species, interpopulational variation in spine lengths is extensive. Small genetic differences between populations may affect spine lengths. The high correlation between stylar and basal spine lengths would not be expected if these spine lengths were each regulated independently (e.g., by separate polygenic systems). Therefore, although genetic differences for fruit-spine length may exist among populations of *C. demersum*, the net phenotypic effect is not altered; i.e., fruits with short basal spines concomitantly exhibit short styles and those with long basal spines are long-styled. It is because of this relationship that a variational continuum in fruit spine lengths exists in the species. As a result, fruits at the lower end of the continuum have "*C. apiculatum*" phenotypes, whereas those at the higher end more closely resemble the "typical" *C. demersum* phenotype.

This relationship is well-illustrated by fruits selected at intervals from the lower to upper end of the regression line (figs. 5, 6). Fruits "a" and "d" appear considerably different unless juxtaposed with intermediate types "b" and "c". Also notable is the phenotypic similarity between fruits of *C. demersum* at the lower end of this variational continuum, and those of *C. submersum*. However, based upon overall phenotypic differences, *C. submersum* maintains integrity as a separate group when subjected to cluster and principal components analyses with *C. demersum* (figs. 2, 3). The only instance where an OTU of *C. demersum* clustered with *C. submersum* was a specimen with fruits which were phenotypically identical to those of *C. submersum* (fig. 7). Even though the fruit morphology of this individual converged with *C. submersum*, it is readily separable from that species vegetatively. Leaf-order has the highest correlation with factor I of the PCA analysis.

The spineless fruit phenotype of *C. submersum* could have evolved from a spiny-fruited progenitor by relatively simple genetic changes. As it has been elucidated for many quantitative genetic traits (Johnson 1979), directional selection for reduced spine lengths is one way by which shorter and shorter-spined fruits could have evolved via mutations accumulating at

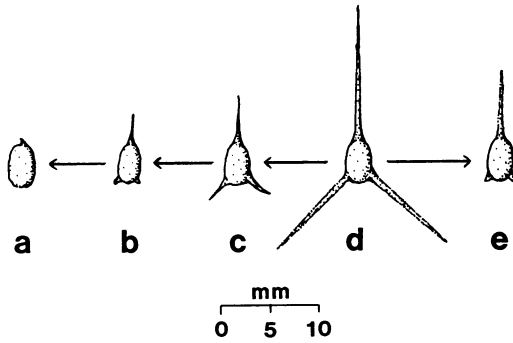


FIG. 6. Phenotypic variation in fruits of *Ceratophyllum demersum*. a. "spineless" phenotype from Nevada (Tiehm & Mozingo 3187, NY). b. "short-spined" phenotype from Pennsylvania (Wahl 6060, PENN). c. "normal" phenotype from Germany (Bischoff s.n., PHIL). d. "long-spined" phenotype from Persia (Bornmüller s.n., B). e. "short basally-spined" phenotype from Oklahoma (Anderson 30, USF). Letters a-e correspond to points on figure 5.

various loci affecting spine lengths. Another possibility is that a major mutation occurred in an ancestral population of *C. submersum* which resulted in a lack of spine production.

In the above discussion, evidence is presented that the name *C. apiculatum* has been associated with specimens of *C. demersum* having fruits with short styles and short basal spines (i.e., tubercles). The original drawing of the fruit of *C. apiculatum* (Chamisso 1829, tab. 5) also supports this argument; in addition to having basal tubercles rather than spines, the fruit illustrated for *C. apiculatum* is also much shorter-styled than those of the other species. Frequency distributions of fruit-spine lengths, however, indicate another possible origin of "*C. apiculatum*" fruit phenotypes. The bi-modal distribution of basal spine lengths (fig. 4b) implicates another factor to account for the high percentage of short basal spines occurring in some fruits with longer style lengths (fig. 6e). This anomaly is explained by abscission of basal spine apices which was observed on several fruits of *C. demersum*. Fruits resulting from basal spine abscission have relatively long styles, but short basal tubercles, which represent the persistent bases of the abscised spines. The resulting phenotype resembles the "*C. apiculatum*" fruit, but are longer-styled (fig. 6e) than their "counterparts" (fig. 6b).

The "*Ceratophyllum apiculatum*" phenotype

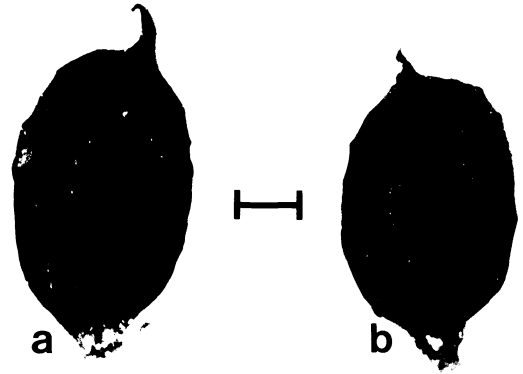


FIG. 7. Convergent fruit morphologies of *Ceratophyllum demersum* and *C. submersum*. a. achene of *C. submersum* from Sweden (Les 388, OS). b. achene of *C. demersum* from Nevada, USA (Tiehm & Mozingo 3187, NY). Length of bar is one millimeter.

actually involves two distinct phases of spine length variation in *C. demersum*. It represents either the lower end of quantitative variation in spine length of the species, or the result of basal spine abscission in longer-styled fruits. Because of these factors, the name *C. apiculatum* is regarded as synonymous with *C. demersum* which has priority of publication. *Ceratophyllum submersum*, however, is separable from *C. demersum* by discrete character states. Fruit-spine length variation in *C. demersum* is probably regulated polygenically, a factor accounting for the wide range of phenotypes observed.

The above observations have enabled some estimation of fruit characters in *Ceratophyllum* from an evolutionary perspective. However, these results also emphasize the need to consider both reproductive and vegetative features when attempting to accurately identify taxa within the genus.

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