

THE ORIGIN AND AFFINITIES OF THE CERATOPHYLLACEAE

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Summary

Historically, the phylogenetic affinities of the Ceratophyllaceae have been vague, however, recent authors have favored a close relationship of the family to the genus *Cabomba* of the Nymphaeales. An evaluation of critical character distributions by cluster analyses confirmed relationships of *Cabomba* with *Brasenia*, *Euryale* with *Victoria*, and *Nymphaea* with *Nuphar*, *Ondinea*, and *Barclaya*, but did not support a close affinity of *Ceratophyllum* to any nymphaealean genus. By phenetic analyses, *Ceratophyllum* is distinct and not recognizable as a natural component of the Nymphaeales. This conclusion is in line with revised interpretations of available data from various sources, which point to an isolated position of the Ceratophyllaceae. Paleobotanical evidence supports a hypothesis that the Ceratophyllaceae are a vestige of ancient angiosperms that diverged early from the line leading to most other modern taxa. The remoteness of Ceratophyllaceae from the Nymphaeales and other extant angiosperms warrants the recognition of a new, distinct order of flowering plants.

Introduction

The Ceratophyllaceae are regarded as monotypic, comprising the single extant genus *Ceratophyllum*. Both the origin and affinities of *Ceratophyllum* have attracted much interest, particularly because this genus possesses many features reminiscent of a relatively ancient lineage of flowering plants. Many discussions of angiosperm diversification, especially those concerning the divergence of the monocots and the dicots, include at least a brief mention of the family (e.g., Dahlgren et al., 1985).

Although it is now commonly believed that the phylogenetic position of *Ceratophyllum* is known with certainty (e.g., Cronquist, 1981), there are two major factors which render this conclusion tenuous. First, the aquatic existence of the genus is correlated with many morphological peculiarities, undoubtedly associated with adaptations to life under water. Consequently, it is difficult to compare character states with other groups which have not manifested similar patterns of morphological divergence. This factor has not been given adequate consideration. Secondly, most discussions have made no mention of the fossil record of the group, and therefore, are based entirely upon comparisons with extant angiosperms. There are, however, several extinct taxa which must be considered in any analysis dealing with the phylogeny of this family.

The ensuing discussion is a synthesis of morphological, anatomical, paleobotanical, and taxometric data relevant to the origin and diversification of this enigmatic group. The purpose of this review is to present a new perspective of the early evolution of Ceratophyllaceae and of their phylogenetic relationship to other angiosperms.

Taxonomic History of Ceratophyllaceae

Few angiosperm families have posed such tenacious defiance to classification as the Ceratophyllaceae. It is understandable that early workers developed some unusual dispositions for the group as a result of the crude state of their scientific knowledge and of their use of simple comparative methods. These early schemes placed the genus among such disparate taxa as *Equisetum* (Plukenet, 1692; Loeselius, 1703), the Najadaceae (Jussieu, 1789), and the Cycadophyta (Richard, 1810). Although most 19th century workers

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eventually recognized the dicotyledonous affinity of *Ceratophyllum*, the classification of the genus was not improved as associations were made with such unlikely families as the Callitrichaceae, Chloranthaceae, Haloragaceae, Lythraceae, Podostemaceae, and Urticaceae (see Gray, 1848; Aboy, 1936; Gibbs, 1974). Because of these varied schemes, Schleiden (1837) referred to the genus as a "vegetable vagabond" whose taxonomic placement was renowned for its interfamilial wanderings.

Asa Gray (1848) took a more objective approach to the classification of the Ceratophyllaceae, which was first recognized as a distinct family by S. F. Gray (1821), and presented convincing arguments for its alliance with various members of the Nymphaeaceae. Using embryological data of Brongniart (1827) as a foundation, he compared *Ceratophyllum* to *Nelumbo*, maintaining that an affinity between these two genera was apparent in their seed characters. He interpreted the ovule to be suspended and orthotropous, the endosperm lacking, the cotyledons large and fleshy, and the plumule unusually well-developed in both. Gray further noted the presence of simple, one-seeded ovaries in both genera. He was aware of discontinuities with his argument as well, but did not consider the lack of petals, sessile anthers, and single ovary of *Ceratophyllum* to weaken the otherwise substantial similarities. Gray attributed the "lesser development" of both vegetative and reproductive structures in *Ceratophyllum* to its entirely submersed habit. Subsequently, he went on to consider the relationship of *Ceratophyllum* to the Cabombaceae (which Gray believed to be closely allied to Nelumbonaceae). Gray pointed out his interpretation of the presence of suspended orthotropous ovules, persistent styles, and stamen number of twice the sepal number in both the Ceratophyllaceae and Cabombaceae. Again, he also pointed out differences between the genera such as the lesser-developed embryo and presence of endosperm in Cabombaceae. Gray concluded that the Ceratophyllaceae were closely allied to Nelumbonaceae and Cabombaceae, and should be placed in a taxonomic position between them.

Many later authors followed Gray's placement of the Ceratophyllaceae near the Cabombaceae with other Nymphaeaceae (e.g., Caspary and Engler, 1891; Li, 1955; Thorne, 1976; Dahlgren, 1980; Takhtajan, 1980; Tamura, 1982; Cronquist, 1981). These authors have devised the major schemes of classification for the family which are summarized in Table 1. The placement of Ceratophyllaceae with the "water-lilies" has become a firmly entrenched taxonomic convention as a result of the above treatments. Both Takhtajan (1980) and Cronquist (1981) support a close relationship of Ceratophyllaceae to Nymphaeales and place the family in that order. Cronquist (1981) in particular asserts (p. 115) that "... there is no doubt about their relationship." Earlier authors, however, have expressed views opposing the alliance. Schaffner (1904) regarded Ceratophyllaceae to be of no direct relationship to Nymphaeales, and doubted a firm affinity of the family to any extant angiosperm group. Aboy (1936) noted that certain features of *Ceratophyllum* are not found among any other known angiosperm, and concluded that the family represented a relictual angiosperm lineage which underwent considerable reduction adapting to its aquatic habitat. With regard to *Ceratophyllum*, Hooker (1849, p. 525) stated, "The careful observations of Schleiden and his elaborate dissertations above quoted, whilst they make us thoroughly acquainted with the real structure of this plant, have shown that no one satisfactory affinity with it has yet been indicated, and it remains an isolated species which cannot be associated with any known Order." However, Hooker (1849, p. 526) added, "The calling it an Order of itself, does not appear to me to throw any additional light on the matter."

Indeed, a close relationship of Ceratophyllaceae to Cabombaceae or other Nymphaeales is not so apparent as to be accepted without skepticism. In Cronquist's (1981) ordinal description of Nymphaeales, for example, the Ceratophyllaceae conflict with no less than eight major characteristics of the group, yet no exceptions are necessary to accommodate either *Cabomba* or *Brasenia* (Cabombaceae). It has become a common practice to "explain" the peculiarities of *Ceratophyllum* as adaptations to an aquatic habitat. What is not clear,

Table 1. Several 19th- and 20th-century classifications showing different placements of the Ceratophyllaceae.

Caspary, 1891	Li, 1955	Thorne, 1976
Order: Ranales	Order: Euryales	Superorder: Annoniflorae
Family: Ceratophyllaceae	Family: Barclayaceae	Order: Nymphaeales
1. <i>Ceratophyllum</i>	1. <i>Barclaya</i>	Family: Nelumbonaceae
Family: Nymphaeaceae	Family: Euryalaceae	1. <i>Nelumbo</i>
Sub-family: Nelumbonoideae	1. <i>Euryale</i>	Family: Cabombaceae
1. <i>Nelumbo</i>	2. <i>Victoria</i>	1. <i>Cabomba</i>
Sub-family: Cabomboideae	Order: Ranales	2. <i>Brasenia</i>
1. <i>Cabomba</i>	Family: Nymphaeaceae	Family: Nymphaeaceae
2. <i>Brasenia</i>	1. <i>Nymphaea</i>	Sub-family: Nymphaeoidae
Sub-family: Nymphaeoidae	2. <i>Nuphar</i>	1. <i>Nymphaea</i>
Tribe: Tetrasepaleae	Family: Cabombaceae	2. <i>Nuphar</i>
Sub-tribe: Euryaleae	1. <i>Cabomba</i>	3. <i>Ondinea</i>
1. <i>Euryale</i>	2. <i>Brasenia</i>	Sub-family: Euryaloideae
2. <i>Victoria</i>	Family: Ceratophyllaceae	1. <i>Euryale</i>
Sub-tribe: Eunymphaeae	1. <i>Ceratophyllum</i>	2. <i>Victoria</i>
1. <i>Nymphaea</i>	Order: Nelumbonales	Sub-family: Barclayoidae
Tribe: Nuphareae	Family: Nelumbonaceae	1. <i>Barclaya</i>
1. <i>Nuphar</i>	1. <i>Nelumbo</i>	Family: Ceratophyllaceae
Tribe: Barclayaeae		1. <i>Ceratophyllum</i>
1. <i>Barclaya</i>		

is why such explanations must be used for *Ceratophyllum*, but not for *Cabomba*, which supposedly, is a closely related submersed aquatic genus. Surely, one can consider divergence between taxa by hypotheses of reduction or specialization to factors such as pollination syndrome. This process is well-known as adaptive radiation and is an integral stage in the transspecific evolution of flowering plants and the formation of groups which we recognize as higher taxonomic categories (Stebbins, 1974). Therefore, assessing relationships of the Ceratophyllaceae involves different problems at various levels. The first is an interpretation of degree. Does the level of divergence displayed by the Ceratophyllaceae jeopardize the integrity of the higher category (i.e., order) within which it is placed? Another difficulty is to assess the likelihood that the Ceratophyllaceae share a relatively close common ancestry with the other families of the group; that is, are the families monophyletic? A third level is to deduce the closest relatives of the family so that some concept of the phylogeny of the group can be ascertained. Only when these questions have been satisfactorily answered can statements of affinity be accepted with a good degree of certainty.

Most classifications strive to depict "natural" groups; i.e., those whose members share a high degree of overall similarity. A high level of homogeneity within a group is often used as evidence of their common ancestry. It is reasonable, therefore, to test relationships of *Ceratophyllum* and other putative Nymphaeales by using a group analysis which compares the similarities and differences among the taxa that have been considered for inclusion within the order. This approach should give a perspective of how the subordinate taxa group within the more inclusive unit. If Nymphaeales is truly a natural assemblage, then the phenetic distance separating component genera should be relatively small. As a corollary, any genus failing to group with the others would not be considered as a group member. A similar approach was taken by Bukowiecki et al. (1972) to evaluate relationships

Table 1. Continued.

Dahlgren, 1980	Takhtajan, 1980	Cronquist, 1981
Superorder: Magnoliiflorae	Order: Nymphaeales	Order: Nymphaeales
Order: Nelumbonales	Family: Nymphaeaceae	Family: Nymphaeaceae
Family: Nelumbonaceae	1. <i>Nymphaea</i>	1. <i>Nymphaea</i>
1. <i>Nelumbo</i>	2. <i>Nuphar</i>	2. <i>Nuphar</i>
Superorder: Nymphaeiflorae	3. <i>Victoria</i>	3. <i>Ondinea</i>
Order: Nymphaeales	4. <i>Euryale</i>	4. <i>Euryale</i>
Family: Nymphaeaceae	5. <i>Barclaya</i>	5. <i>Victoria</i>
1. <i>Nymphaea</i>	6. <i>Ondinea</i>	Family: Barclayaceae
2. <i>Nuphar</i>	Family: Cabombaceae	1. <i>Barclaya</i>
3. <i>Barclaya</i>	1. <i>Cabomba</i>	Family: Cabombaceae
4. <i>Euryale</i>	2. <i>Brasenia</i>	1. <i>Cabomba</i>
5. <i>Victoria</i>	Family: Ceratophyllaceae	2. <i>Brasenia</i>
6. <i>Ondinea</i>	1. <i>Ceratophyllum</i>	Family: Ceratophyllaceae
Family: Cabombaceae	Order: Nelumbonales	1. <i>Ceratophyllum</i>
1. <i>Cabomba</i>	Family: Nelumbonaceae	Family: Nelumbonaceae
2. <i>Brasenia</i>	1. <i>Nelumbo</i>	1. <i>Nelumbo</i>
Family: Ceratophyllaceae		
1. <i>Ceratophyllum</i>		

among the genera included in Nymphaeaceae by Bentham and Hooker. Here, the relationships among the genera of Nymphaeales sensu Cronquist (1981) are assessed using numerical analyses.

Methods

A basic data matrix (Tables 2–3) was constructed from descriptions in Aboy (1936), Batygina et al. (1980), Cook et al. (1974), Cronquist (1981), Davis (1966), Padmanabhan (1970), and Walker (1976). An effort was made to include characters used by Cronquist (1981) in his ordinal description of Nymphaeales. These data were standardized and used to generate a dendrogram incorporating the STAND subroutine and UPGMA-HIGH algorithm of the NT-SYS numerical taxonomy program (Rohlf et al., 1974). A scatter-diagram was generated from the standardized data set using the principal components analysis (PCA) of NT-SYS in which the first three axes of variation were plotted.

Results and Discussion

In both the dendrogram (Fig. 1) and the PCA (Fig. 2), clustering relationships of *Ceratophyllum* and other genera of the Nymphaeales were essentially the same; i.e., *Ceratophyllum* is isolated from the other genera by a relatively large phenetic distance. Genera whose affinity to one another is less debatable, however, grouped closely to each other (e.g., *Cabomba* with *Brasenia*, *Euryale* with *Victoria*, and *Nymphaea* with *Nuphar*). The failure of *Ceratophyllum* to group with other genera of Nymphaeales indicates that its relationship to the order cannot be regarded as natural, at least in terms of the characters included in the analysis. Because these characters represent many of those used to define the order, it is apparent that the relationships of *Ceratophyllum* are seriously in need of re-evaluation. To evaluate the degree of isolation of *Ceratophyllum* from other Nymphaeales, the following technique was used. Alternate runs of the data were made adding characters for four non-nymphaealean submersed aquatic genera (*Hippuris*, *Megalodonta*, *Myriophyllum*, and *Ranunculus*). Although this procedure did not significantly alter the relationships of the nymphaealean genera, the other genera (which resemble *Ceratophyllum* superficially, but are

Table 2. Character codes for basic data matrix (Table 3) used in phenetic analyses of nymphaealean genera.

A.	Habit: rhizomatous (1); rhizoidal (0)
B.	Primary root: developed (1); vestigial or absent (0)
C.	Vascular bundles: numerous (1); single (0)
D.	Tracheids: unmodified (0); modified to store starch (1)
E.	Leaves: cauline (1); from rhizome (0)
F.	Floating leaves: present (1); absent (0)
G.	Dissected leaves: present (1); absent (0)
H.	Peltate leaves: present (1); absent (0)
I.	Laticifers: present (1); absent (0)
J.	Flowers: perfect (1); imperfect (0)
K.	Flowers: hypogynous (1); both hypogynous & perigynous (2); epigynous (3)
L.	Pollination: cleistogamous/autogamous (1); entomophilous (2); hydrophilous (3)
M.	Sepals: present (1); absent (0)
N.	Petals: present (1); absent (0)
O.	Stamens: spiral (1); cyclic (0)
P.	Stamens: free (0), adnate (1)
Q.	Carpels: exposed (0); imbedded in receptacle (1)
R.	Gynoecium: syncarpous (1); apocarpous (0)
S.	Fruit: dry (1); fleshy (0)
T.	Ovules: single (1); 2-6 (2); numerous (3)
U.	Ovule: anatropous (1); orthotropous (0)
V.	Seeds: arillate (1); without arils (0)
W.	Integument: one-layered (1); two-layered (2)
X.	Placentation: laminar (1); apical (0)
Y.	Pollen: monads (0); tetrads (1)
Z.	Tapetum: secretory (0); amoeboid (1)
AA.	Microsporogenesis: simultaneous (1); both simultaneous & successive (2); successive (3)
BB.	Male gametophyte: two-celled (2); three-celled (3)

not believed to be closely related to the genus) grouped no closer to them than did *Ceratophyllum*. Because these four genera represent three different orders, it is difficult to rationalize the inclusion of *Ceratophyllum* with the other genera of Nymphaeales. *Nelumbo*, which is also relatively distant from other nymphaealean genera in these analyses (Figs. 1-2), has been placed in a separate order (Nelumbonales) by Bukowiecki et al. (1972), Dahlgren (1980), Khanna (1965), Li (1955), and Takhtajan (1980). The distinctness of *Nelumbo* is also supported by serological data (Simon, 1970). Although *Ceratophyllum* was not included in the study by Bukowiecki et al. (1972), results for the arrangement of the other nymphaealean genera were similar to those obtained here. In both studies, *Nelumbo* was distinct from all genera comprising Nymphaeales. Groups maintained in both studies were *Victoria* with *Euryale*, and *Cabomba* with *Brasenia*. One discrepancy between the studies regarded the placement of *Nuphar*, which was closest to *Nymphaea* here but was considered distant from *Nymphaea* by Bukowiecki et al. (1972). This discrepancy may have resulted from several factors. The characters used in the two studies were not identical. Bukowiecki et al. (1972) stressed more chemical characters. This study incorporated a larger data set, and the study by Bukowiecki et al. (1972) did not include the genera *Ceratophyllum* or *Ondinea*. Either of these factors could influence the topology of the dendrograms produced in the studies. Regardless, the distance separating *Ceratophyllum* from other Nymphaeales in this study was greater than that between *Nelumbo* and other Nymphaeales in both studies. If these data are viewed as justification for separating *Nelumbo* from Nymphaeales, then it can be argued that a similar disposition is

warranted for *Ceratophyllum*. The isolation of both *Ceratophyllum* and *Nelumbo* from other Nymphaeales is further apparent from studies of their seed anatomy (Kak and Durani, 1986).

The phylogeny of Nymphaeales was considered by Young (1983) who conducted a "cladistic" analysis of the component genera. By the nature of these analyses, Young initially assumed that the group was monophyletic. His study, however, showed that *Ceratophyllum* was quite different from the other nymphaealean genera in terms of the large number of derived character states and reversals required to "join" *Ceratophyllum* to the rest of the cladogram. These results led Young to conclude that the Nymphaeales was not entirely monophyletic, and that a common ancestry of *Ceratophyllum* to other Nymphaeales was unlikely. Young's conclusions are in line with results of the phenetic analyses presented above. In a recent cladistic analysis of Nymphaeales, however, different conclusions were reached (Ito, 1987). Ito (1987) viewed the Nymphaeales as monophyletic and concluded that *Ceratophyllum* and *Cabomba* are closely related phylogenetically. Despite Ito's claim that *Ceratophyllum* and *Cabomba* are defined by two synapomorphies in his analysis (reduced carpel number and lacinate leaves), his table of character distributions indicates only a single synapomorphy (lacinate leaves) between the two genera (reduced carpel number is also shared with *Brasenia*). Therefore, the evidence supporting Ito's interpretation of a close phylogenetic tie between *Ceratophyllum* and *Cabomba* is merely their possession of lacinate leaves. For some reason, Ito did not address the co-occurrence of peltate floating leaves in *Cabomba* and *Brasenia* (and which lack in *Ceratophyllum*) with respect to that conclusion. Ito recognized four apomorphic features which are shared among *Ceratophyllum*, *Cabomba* and *Brasenia*, however, an additional five apomorphies occur in *Ceratophyllum* but not in either *Cabomba* or *Brasenia*. In fact, two of those apomorphies are shared with *Barclaya* yet no phylogenetic tie to that genus is proposed. Several additional unshared apomorphies could be added to Ito's *Ceratophyllum* clade from the list of characters included in the phenetic analysis presented above. Accordingly, I do not accept Ito's conclusion as evidence that *Ceratophyllum* and *Cabomba* are closely related phylogenetically, but view his conclusions as the result of the particular characters used in his analysis and the procedure he used to score and polarize them. Alternatively, Young (1983) arrived at a different cladistic hypothesis using a different data set. Furthermore, I believe that the relatively isolated position of *Ceratophyllum* indicated by Young's analysis is more plausible and is supported by evaluation of other available data.

Proposed Relationships with Cabomba

Another method of testing the relationship of *Ceratophyllum* to other Nymphaeales is by comparing characters of the genus with those of its putatively closest ancestor. Takhtajan (1980) believed that *Cabomba* and *Ceratophyllum* had a common origin. His views were supported by Cronquist (1981) who regarded similarities in the submersed foliage of *Cabomba* to indicate a "link" between *Ceratophyllum* and the rest of Nymphaeales. A close relationship of *Ceratophyllum* to the Cabombaceae has also been proposed in several studies presenting embryological (Batygina et al., 1980), chromosomal (Okada and Tamura, 1981), chemotaxonomic (Sundari et al., 1982), and cladistic (Ito, 1987) evidence. Accordingly, *Cabomba* is the logical genus to use in a comparison with *Ceratophyllum*. It is desirable, however, to compare features of these genera that are not likely to be correlated with the submersed, hydrophilous habit of *Ceratophyllum*. It is fairly obvious that many features of *Ceratophyllum* have evolved as a consequence of adaptation to the aquatic habitat. The unisexual condition of the genus has likely evolved along with the transition to hydrophily, as evidenced by the associated occurrence of imperfect-flowered breeding systems in other hydrophilous taxa such as *Althenia*, *Cymodocea*, *Halodule*, *Lepilaena*, *Najas*, *Phyllospadix*, *Zannichellia*, and *Zostera* (Sculthorpe, 1967). Similarly, the reduction in carpel and ovule number (to one each) is associated with water pollination in many angiosperms (Dahlgren

Table 3. Basic data matrix used in phenetic analyses of nymphaealean genera.

Genus	A	B	C	D	E	F	G	H	I	J	K	L
<i>Barclaya</i>	1	1	1	0	0	1	0	0	1	1	1	1
<i>Brasenia</i>	1	1	1	0	0	1	0	1	1	1	1	2
<i>Cabomba</i>	1	1	1	0	1	1	1	1	1	1	1	2
<i>Ceratophyllum</i>	0	0	0	1	1	0	1	0	0	0	1	3
<i>Euryale</i>	1	1	1	0	0	1	0	1	1	1	3	1
<i>Nelumbo</i>	1	1	1	0	0	1	0	1	1	1	1	2
<i>Nuphar</i>	1	1	1	0	0	1	0	0	1	1	1	2
<i>Nymphaea</i>	1	1	1	0	0	1	0	0	1	1	2	2
<i>Ondinea</i>	1	1	1	0	0	1	0	0	1	1	1	2
<i>Victoria</i>	1	1	1	0	0	1	0	1	1	1	3	2

et al., 1985). The absence of a perianth, the dissected nature of the foliage, drastically reduced xylem, rootlessness, and absence of stomata are all features that can be viewed as adaptations to the hydric condition (Arber, 1920; Sculthorpe, 1967). Such rapid, adaptive divergence greatly obscures phylogenetic relationships with related taxa which have not undergone similar evolutionary changes. Therefore, the best indication of phylogenetic ties to *Ceratophyllum* may be found among characters which are evolutionarily conservative.

Embryological features are often regarded to be highly conserved and good indicators of ancestral relationships. Therefore, a comparison of embryological features of *Ceratophyllum* and *Cabomba* is one means of assessing the degree of their relationship. Batygina et al. (1980) compared the embryological features of genera within Nymphaeales, and concluded that *Ceratophyllum* and *Cabomba* were very similar. Although *Cabomba* and *Ceratophyllum* display a degree of similarity embryologically, there are several noteworthy discrepancies (Table 4). For example, it is clear that the agreement of certain embryological features between *Ceratophyllum* and *Cabomba* is by no means unique to these genera. The *Polygonum*-type embryo-sac, crassinucellar ovule, single archesporial cell, hypostase, and linear arrangement of megaspore tetrads are features common to all other Nymphaeales, and to many other angiosperms as well. All of these states are regarded as relatively unspecialized and primitive in the angiosperms as a whole. The bicellular condition of dispersed pollen is also common to other Nymphaeales and to many other angiosperms. The occurrence of successive cytokinesis in both genera is interesting because of the relative rarity of this condition among the Nymphaeales and other dicots. Whether the co-occurrence of successive cytokinesis in *Ceratophyllum* and *Cabomba* indicates a phyletic tie between the genera is debatable. Actually, both successive and simultaneous types of microsporogenesis are known to occur in different species of *Ceratophyllum*. Also, because cytokinesis is simultaneous in *Brasenia*, the other genus of Cabombaceae, it is obvious that alternate methods of microsporogenesis have been derived independently within the families Ceratophyllaceae and Cabombaceae. What is not certain is which state represents the ancestral condition in these groups. This ambiguity of microsporogenesis offers little support for establishing a close relationship between the families. In essence, the embryological similarity noted between *Ceratophyllum* and *Cabomba* with respect to unspecialized features provides little insight into the question of their relationship. Because these features are of widespread occurrence, they should not be interpreted as good evidence for a close relationship. The embryological differences, however, provide a different perspective of this putative relationship. Specialized features such as unitegmic, orthotropous and exalbuminous ovules, apical placentation, a highly-developed plumule, and amoeboid tapetum are features of *Ceratophyllum* which differ not only from *Cabomba*, but from most other Nymphaeales and dicots in general. If there is a conclusion to be drawn from

Table 3. Continued.

M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	BB
1	1	1	1	0	1	0	3	0	0	2	1	0	0	1	2
1	1	0	0	0	0	1	2	1	0	2	1	0	0	1	2
1	1	0	0	0	0	1	2	1	0	2	1	0	0	3	2
0	0	1	0	0	0	1	1	0	0	1	0	0	1	2	2
1	1	1	0	0	1	0	3	1	1	2	1	0	0	1	3
1	1	1	0	1	0	1	1	1	0	2	0	0	0	1	3
1	1	1	0	0	1	0	3	1	0	2	1	0	1	1	2
1	1	1	0	0	1	0	3	1	1	2	1	0	1	1	2
1	0	1	0	0	1	0	3	1	0	2	1	0	0	1	2
1	1	1	0	0	1	0	3	1	1	2	1	1	0	1	3

these embryological data, it should be that *Ceratophyllaceae* are distinct from other Nymphaeales and possess several derived embryological features.

The conclusion by Okada and Tamura (1981) that both *Ceratophyllum* and *Cabomba* share the same basic chromosome number ($x = 12$) is also subject to dispute. They did not consider an alternate possibility that their count of $2n = 96$ for *Cabomba* could represent a different numerical series even though a count of $2n = 80$ (a number not divisible evenly by 12) was obtained for the closely related genus *Brasenia*. It is likely that $x = 12$ represents a base number for *Ceratophyllaceae*, but not necessarily for *Cabombaceae*. This conclusion is reached by evaluating the chromosome numbers reported for members of both families (Table 5). Reported counts in *Cabombaceae* are not always divisible evenly by 12. In particular, counts reported for *Cabomba* alone are variously divisible by 12 ($2n = 24$; 96) or indivisible by 12 ($2n = 104$). Except for two populations, counts reported for all species of *Ceratophyllum*, however, are divisible by 12 (Table 5). All counts reported for *Cabombaceae* are divisible by eight, but this is not to say that the base number of *Cabombaceae* is necessarily eight (the occurrence of $2n = 24$ and $2n = 104$ species raises some objections to that conclusion). Similarly, it could be argued that counts reported for *Ceratophyllum* are divisible by eight. A base number of eight in this genus, however, would mean that all species exist at odd-ploidy levels. Consequently, the chromosome counts reported for *Ceratophyllaceae* and *Cabombaceae* are not useful for demonstrating that the families share a common basic chromosome number. Such a conclusion is untenable with regard to the discrepancies noted above. What is apparent, however, is that modification of the ancestral chromosomal series has occurred within one or possibly both families. Although it is possible that the families once shared a common basic chromosome number, this conclusion cannot be arrived at by evaluating the available chromosomal data.

Contrary to reports by Sundari et al. (1982), chemotaxonomic relationships between *Ceratophyllum* and *Cabomba* have not been adequately demonstrated, and the significance of chemical data used as evidence of their relationship has been misinterpreted. Sundari et al. (1982) proposed the similarity of *Ceratophyllum* to other Nymphaeales after obtaining negative results from an array of biochemical tests. Many of the tests performed by the authors, however, were for highly specialized compounds (Juglone, Syringin, aucubins, etc.). Because these compounds are restricted to only a few families, similar negative results would be expected from most other angiosperms, and are inconclusive with regard to clarifying relationships of *Ceratophyllum*. Conclusions of relationships were also based on the co-occurrence of groups of compounds rather than specific substances. Accordingly, the positive test cited for phenols is as uninformative phylogenetically as knowing that both groups possess flavonoids. The absence of leucoanthocyanins in *Ceratophyllum* reported by Sundari et al. (1982) may be erroneous because of the documentation of their

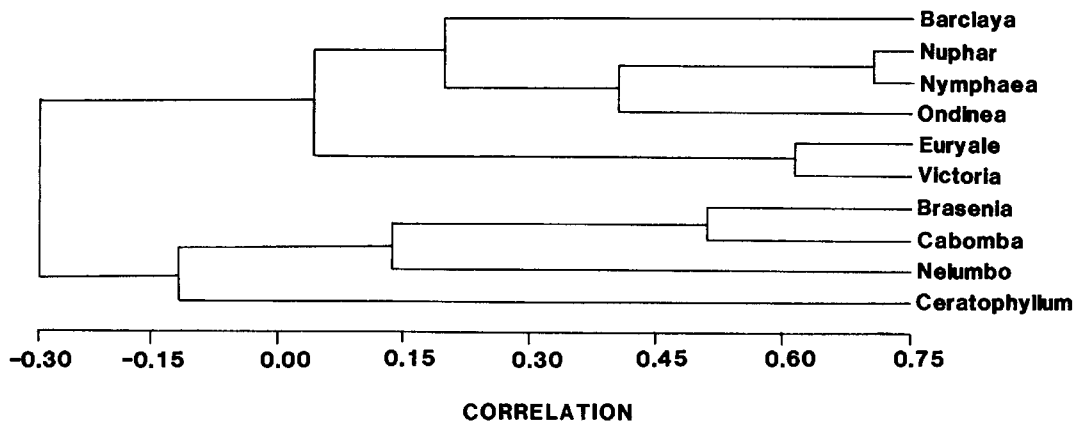


Fig. 1. UPGMA-HIGH dendrogram derived from character matrix (Table 2) of the ten genera placed in the Nymphaeales sensu Cronquist. The cophenetic correlation coefficient is 0.907.

occurrence in the genus by Reznik and Neuhausel (1959). It appears that Sundari et al. (1982) misinterpreted remarks by Dahlgren (1980) regarding the distribution of tannins, benzylisoquinoline alkaloids, and S-type plastids. Dahlgren's comments were not made with reference to *Ceratophyllum* and *Cabomba* as implied by Sundari et al. (1982), but rather to the general nature of the entire Nymphaeiflorae.

Not one of the chemical associations between *Ceratophyllum* and *Cabomba* made by Sundari et al. (1982) is useful for establishing evidence of their close relationship. Ironically, the negative tests for alkaloids and mucilage which they report for *Ceratophyllum* are

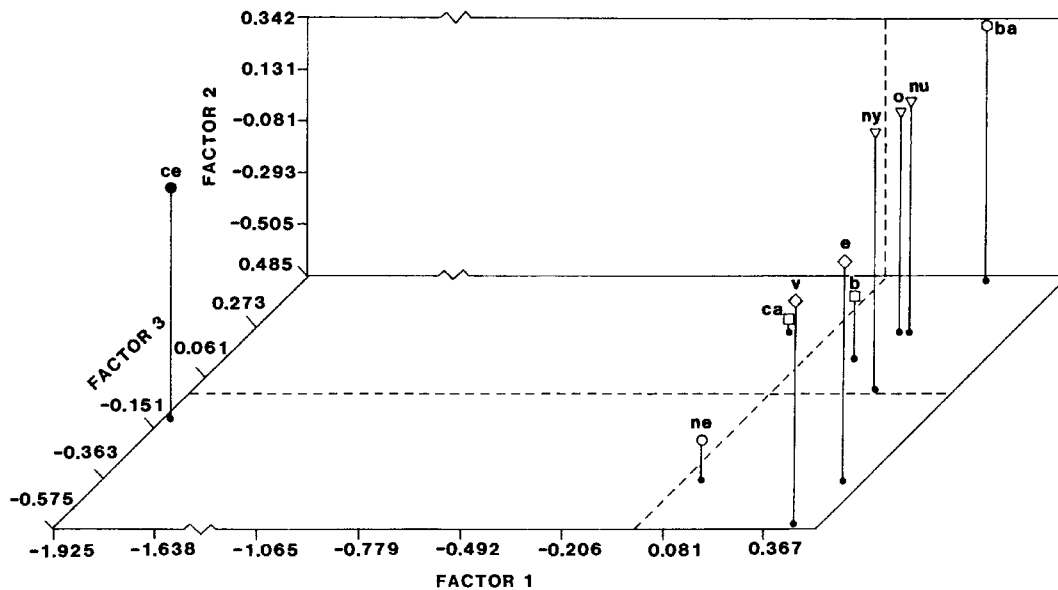


Fig. 2. Principal components plot of the first three axes of variation for the ten genera of Nymphaeales sensu Cronquist. The x-axis (factor 1) is slightly compressed. Letter codes for genera are: ce = *Ceratophyllum*, ne = *Nelumbo*, ca = *Cabomba*, v = *Victoria*, e = *Euryale*, b = *Brasenia*, ny = *Nymphaea*, o = *Ondinea*, nu = *Nuphar*, ba = *Barclaya*; symbol codes are: closed circle = *Ceratophyllaceae*, open circle = *Nelumbonaceae*, open squares = *Cabombaceae*, open diamonds = *Euryalaceae*, open triangles = *Nymphaeaceae*, open hexagon = *Barclayaceae*. Factors 1–3 account for 77% of the total variation in the data set.

Table 4. Embryological features of *Ceratophyllum* and *Cabomba* (from Batygina et al., 1980; Bhojwani and Bhatnager, 1979; Davis, 1966; Shamrov, 1981).

Character	<i>Ceratophyllum</i>	<i>Cabomba</i>	Remarks
Embryo sac	polygonum-type	polygonum-type	same for most dicots; regarded as primitive state
Ovule	crassinucellar	crassinucellar	same for most dicots; regarded as primitive state
Integument	unitegmic	bitegmic	most angiosperms are bitegmic
Archeporial cell	single	single	single archeporial cell is usual condition
Ovule position	orthotropous	anatropous	most angiosperms have anatropous ovules
Hypostase	present	present	hypostase present in many angiosperm families
Megaspores	linear tetrads	linear tetrads	most common configuration
Placentation	apical	laminar	laminar placentation is regarded as primitive state
Tapetum	amoeboid/secretory	secretory	secretory type is most common; amoeboid type also reported in Nymphaeaceae
Dispersed pollen	bicellular	bicellular	most common condition
Plumule	highly developed	minute	most dicots possess minute plumule
Microsporogenesis	both successive and simultaneous	successive	successive type common in monocots; simultaneous in dicots; <i>Bra-senia</i> is simultaneous
Perisperm	absent	copious	present in most Nymphaeales
Tapetal cells	uninucleate	uni- or binucleate	polyploidy is common in tapetal cells

indicative of divergence from Cabombaceae which possess both classes of compounds. Cronquist (1981) lists the presence of alkaloids as a familial character of Cabombaceae.

The study of pollen fine structure has proven to be a useful means for assessing relationships among taxa of various ranks. Therefore, a comparison of the pollen structure of *Ceratophyllum* and *Cabomba* is particularly appropriate. Although the pollination modes in these genera are quite different (hydrophily in *Ceratophyllum*; entomophily in *Cabomba*), it is conceivable that similarities, at least at the ultrastructural level, may be detectable if these genera are closely related.

The pollen of *Cabomba* has been studied both at the light-microscopic (Snigiryevskaya, 1955) and the electron-microscopic (Walker, 1974a, b, 1976) levels. *Cabomba* pollen is shed as monads, and is anasulcate (occasionally anatrachotomosulcate), heteropolar, bilaterally symmetrical, boat-shaped, oblong, and tectate with a striate suprategal surface (Snigiryevskaya, 1955; Walker, 1974b, 1976). The striate sculpturing of the grains is unique among all genera of primitive dicotyledons (Walker, 1976).

Pollen of *Ceratophyllum* has also been examined at the light-microscopic (Mouraviev, 1945) and ultrastructural (Pettitt and Jermy, 1975) levels. Some scanning electron microscopy has also been carried out on the genus (Walker, 1974a; Kurmann, unpublished). Certain details of the pollen structure in *Ceratophyllum* are still controversial; it has been described variously as inaperturate (Walker, 1976) or with several germination pores

Table 5. Synopsis of chromosome counts reported for Ceratophyllaceae and Cabombaceae.

Taxon	2n	Reference
Ceratophyllaceae		
<i>Ceratophyllum demersum</i>	24	Langlet and Soderberg, 1927
<i>C. demersum</i>	24	Tischler, 1934
<i>C. demersum</i>	24	Vachova and Ferakova, 1978
<i>C. demersum</i>	24	Les (Appendix A)
<i>C. demersum</i>	24	Skalinska et al., 1978
<i>C. demersum</i>	38	Bir and Sindhu, 1978
<i>C. demersum</i>	40	Wulf, 1938
<i>C. demersum</i>	48	Skalinska et al., 1978
<i>Ceratophyllum pentacanthum</i>	72	Okada and Tamura, 1981 (= <i>C. oryzetorum</i>)
<i>Ceratophyllum echinatum</i>	24	Les (Appendix A)
<i>Ceratophyllum submersum</i>	24	Strasburger, 1902
<i>C. submersum</i>	24	Tischler, 1934
<i>C. submersum</i>	24	Delay, 1947
<i>C. submersum</i>	72	Jedrychowska and Sroczyńska, 1934
Cabombaceae		
<i>Cabomba aquatilis</i>	104	Sobha and Ramachandran, 1980
<i>Cabomba caroliniana</i>	24	Nitzschke, 1914
<i>C. caroliniana</i>	104	Langlet and Soderberg, 1927
<i>C. caroliniana</i>	104	Langlet, 1936
<i>C. caroliniana</i>	24	Delay, 1947
<i>C. caroliniana</i>	96	Okada and Tamura, 1981
<i>Brassenia purpurea</i>	80	Langlet and Soderberg, 1927
<i>B. purpurea</i>	80	Langlet, 1936
<i>B. purpurea</i>	80	Ohga et al., 1962
<i>Brasenia schreberi</i>	80	Löve and Löve, 1964
<i>B. schreberi</i>	80	Gurzenkov, 1973
<i>B. schreberi</i>	80	Okada and Tamura, 1981

(Mouraviev, 1945); tectate (Walker, 1976) or with an extremely reduced exine (Snigiryevskaya, 1955).

The grains are apolar, radiosymmetric, globose-spherical, "psilate", and are shed as monads (Walker, 1976). Pollen of *Ceratophyllum* contains numerous starch grains (Jedrychowska and Sroczyńska, 1934; Les, 1980) presumably accounting for their specific gravity greater than water (Les, 1980). Occasionally the pollen may germinate before being released from the anther (Sehgal and Ram, 1981). The pollen tubes are long, coiled (Sehgal and Ram, 1981) and can develop into branched sac-like structures (Schleiden, 1837; Les, 1986). Pettitt and Jermy (1975) described some of the ultrastructural features of *Ceratophyllum* pollen. The exine of *Ceratophyllum* is greatly reduced, unsculptured, and covered by a fine fibrillar network when examined by transmission electron microscopy (TEM). Schroter (1917) and Mouraviev (1945) have speculated that this layer is made up of cutin or a "cutin-like" substance; however, the exact chemical nature of the layer has not yet been ascertained. The intine layer is fairly thick. TEM's and SEM's (Kurmman, unpublished) show the outer wall to possess curious processes in some populations (Fig. 3).

The pollen morphologies of *Ceratophyllum* and *Cabomba* (Table 6), are quite dissimilar. Although this divergence is probably a result of adaptation to different pollination syndromes, the pollen structure of these genera appears to be of little use in demonstrating a close phyletic tie between them. Furthermore, consideration must also be given to the probable origin of hydrophily in *Ceratophyllum*. It is likely that hydrophily in *Cerato-*

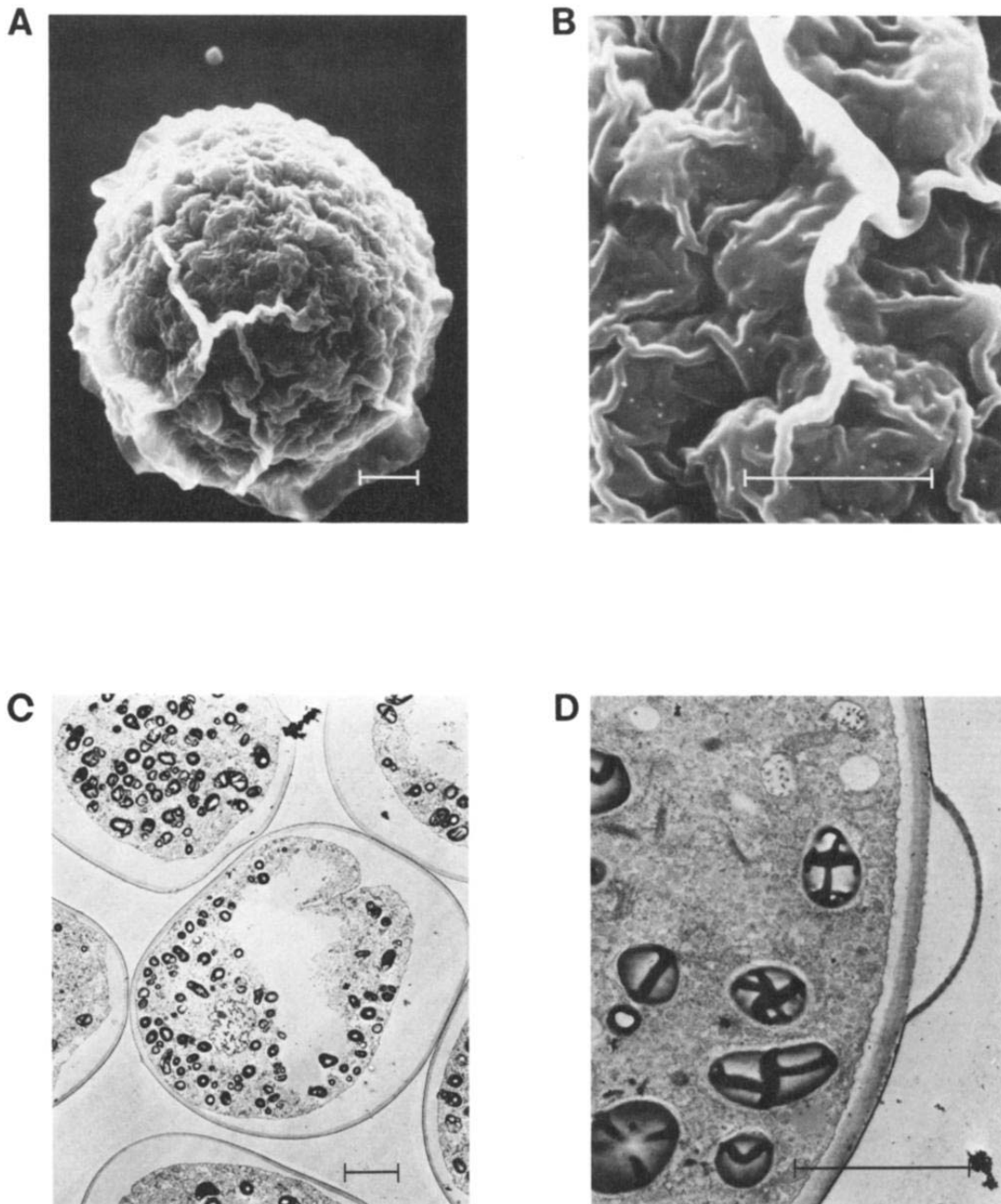


Fig. 3. Pollen structure in *Ceratophyllum demersum*. A: SEM of grain showing unusual exine processes. B: magnified surface of same grain. C: TEM of "normal" pollen grain. D: TEM showing expanded exine ridge. Length of bar is 5 μm for all photos. Voucher: *Les 401* (OS). Micrographs provided by M. Kurmann.

phyllum evolved from an anemophilous syndrome. In other hydrophilous angiosperms, there is a strong correlation between these two abiotic systems (Table 7). The biological features of monoecy, self-compatibility, and clonal growth in *Ceratophyllum* (Les, 1986) also characterize many wind-pollinated angiosperms (Niklas, 1985). The branching of pollen tubes in *Ceratophyllum* is apparently unique among angiosperms. Interestingly, branched pollen-tubes are also known in several primitive wind-pollinated groups such as

Table 6. Summary of pollen features in *Ceratophyllum* and *Cabomba*.

Feature	<i>Ceratophyllum</i>	<i>Cabomba</i>
Aperture type	inaperturate	anasulcate-anatrichotomosulcate
Polarity	apolar	heteropolar
Symmetry	radial	bilateral
Shape	globose	boat-shaped, oblong
Exine structure	highly reduced	tectate
Exine sculpturing	smooth	striate
Intine thickness	massive	thin
Pollen units	monads	monads
Pollen size	17–45 μm	50–200 μm

Cycadophyta, Pinophyta, and Pteridospermophyta (Schleiden, 1837; Taylor, 1981). Pollen-tube branching in these archaic groups is usually haustorial (Taylor, 1981), thus the occurrence of the trait in *Ceratophyllum* may be relictual and inherited from similarly primitive ancestors. Other correlations such as high pollen/ovule ratios, naked flowers, and unusual pollen surfaces (Les, 1986) link hydrophily in *Ceratophyllum* to anemophily in other plants. Although reduced in extant species, *Ceratophyllum* also once possessed a highly branched inflorescence (Aboy, 1936). To propose a relationship of *Ceratophyllum* to any genus of Nymphaeales (all of which are entomophilous), one must disregard these factors, and assume that hydrophily in *Ceratophyllum* evolved instead from entomophily, a much less parsimonious interpretation.

From the available data, I conclude that a close relationship of Ceratophyllaceae to the Nymphaeales is unlikely. The presence in both groups of numerous character states regarded as primitive in angiosperms indicates, however, that their ancestry is with a primitive angiosperm group. In particular, the characters of dichotomously forking leaves and foliaceous appendages on the stamens of *Ceratophyllum* may be truly archaic. It is likely that the group of plants that gave rise to Ceratophyllaceae and the Nymphaeales were not along the "main line" of angiosperm ancestors. The unusual feature of extra-axillary branching found in both groups (Aboy, 1936; Cronquist, 1981) is not known to persist in any other line of extant angiosperms. This branching pattern may be an indication that Ceratophyllaceae and Nymphaeales diverged from a common ancestor, although it has been proposed that the configuration represents an adaptation to maximize leaf/flower position in aquatic plants having large, peltate, floating leaves (Moseley et al., 1984). If this is then case, the extra-axillary branching may represent a highly derived condition which evolved inde-

Table 7. Distribution of pollination modes in eight hydrophilous angiosperm families and the probable condition ancestral to hydrophily. HYD = hydrophily, ANEM = anemophily, ENTO = entomophily. Compiled from Sculthorpe (1967).

Family	Pollination modes	Condition ancestral to hydrophily
Ceratophyllaceae	HYD	?
Hydrocharitaceae	HYD, ANEM, ENTO	ANEM
Najadaceae	HYD	ANEM
Posidoniaceae	HYD	ANEM
Potamogetonaceae	HYD, ANEM	ANEM
Ruppiceae	HYD	ANEM
Zannichelliaceae	HYD	ANEM
Zosteraceae	HYD	ANEM

Table 8. Macrofossil taxa with affinity to *Ceratophyllum*.

Epoch (period)	Approximate age (MYBP)	Taxon	References
Holocene/Pleistocene	0-2	<i>Ceratophyllum demersum</i> L.	Gruger, 1973
Holocene/Pleistocene	0-2	<i>Ceratophyllum demersum</i> L.	Ohlhorst et al., 1982
Holocene/Pleistocene	0-2	<i>Ceratophyllum demersum</i> L.	Pals et al., 1980
Holocene/Pleistocene	0-2	<i>Ceratophyllum demersum</i> L.	Ritchie and DeVries, 1964
Holocene/Pleistocene	0-2	<i>Ceratophyllum demersum</i> L.	Terasmae and Craig, 1958
Holocene/Pleistocene	0-2	<i>Ceratophyllum demersum</i> L.	Van Zant, 1979
Holocene/Pleistocene	0-2	<i>Ceratophyllum demersum</i> L.	Watts and Bright, 1968
Holocene/Pleistocene	0-2	<i>Ceratophyllum submersum</i> L.	Backman, 1943
Holocene/Pleistocene	0-2	<i>Ceratophyllum submersum</i> L.	Griffin, 1980
Holocene/Pleistocene	0-2	<i>Ceratophyllum submersum</i> L.	Hessland, 1946
Upper Pliocene	2	<i>Ceratophyllum demersum</i> L.	Kirchheimer, 1957
Lower Pliocene	4	<i>Ceratophyllum dubium</i> (Ludwig) Kirchheimer	Kirchheimer, 1957
Upper Miocene (Pannonian)	9	<i>Ceratophyllum dubium</i> (Ludwig) Kirchheimer	Knobloch, 1977
Middle Miocene	14	<i>Ceratophyllum sinjanum</i> Kerner	Kerner, 1905
Lower Miocene	20	<i>Ceratostratiotes sinjanus</i> (Kerner) Buzek	Buzek, 1982
Lower Miocene	20	<i>Trapa zapfei</i> Berger	Berger, 1957
Lower Oligocene	35	<i>Ceratophyllum zaisanicum</i> Avakov	Avakov, 1962
Eocene	45	<i>Ceratophyllites faujasii</i> (Brong.) Unger	Unger, 1850 Brongniart, 1822

pendently in these aquatic genera. In the case of *Ceratophyllum* which lacks floating leaves, the feature may be relictual. Despite this possible association, the morphological divergence undergone by *Ceratophyllum* is of such magnitude that a close relationship to any of the extant nymphaealean genera cannot be demonstrated. It is unlikely that *Ceratophyllum* and *Cabomba* share a relatively close relationship because of discrepancies in embryological features and floral appendage arrangements (cyclic in Cabombaceae; spiral in Ceratophyllaceae). The floral anatomy of Cabombaceae is, in fact, regarded as distinct and derived with respect to all Nymphaeales (Moseley, 1958; Schneider and Jeter, 1982). There has been no convincing evidence from chromosomal or chemosystematic studies to support a relationship between *Ceratophyllum* and *Cabomba*. Preliminary molecular data also support a relatively isolated position for the Ceratophyllaceae. Oganezova and Nalbandyan (1976) reported that the amino acid sequences of plastocyanin and ferredoxin proteins in *Ceratophyllum* have diverged considerably from those of terrestrial plants. Such molecular divergence suggests a long period of genetic isolation between Ceratophyllaceae and other angiosperm lineages.

The available evidence supports the removal of Ceratophyllaceae from the order Nymphaeales, as has already been proposed for Nelumbonaceae by several authors. This conclusion, however, leaves the question of affinities unresolved. Comparisons with extant taxa have failed to provide indications of relationship with Ceratophyllaceae, and the investigation of this question must turn to the paleontological record.

Table 9. Achene features compared between *Ceratophyllum* and *Ceratostratiotes* (compiled from Buzek, 1982).

<i>Ceratophyllum</i>	<i>Ceratostratiotes</i>
Fruit stalked	fruit sessile
Two planes of symmetry	one plane of symmetry
Surface punctate or papillate	surface polygonally pitted
Spines in two planes	spines radiate in a circular plane
Vascularized apical style	non-vascularized apical spines
Two-valved germination	two-valved germination
Unilocular	unilocular
One-seeded	one-seeded
Apical/ventral placentation	basal/ventral placentation
Endocarp two-layered	endocarp two-layered
"Micropylar" canal present	"micropylar" canal present

Paleobotanical Evidence for Relationships of Ceratophyllaceae

Paleobotanical data have not been used previously to assess the relationships of the Ceratophyllaceae, although the family is fairly well represented in the fossil record. This record consists mostly of macrofossils, presumably because the pollen of *Ceratophyllum* does not fossilize well (Pals et al., 1980).

Macrofossils of Ceratophyllaceae (Table 8) are usually fruits and have resulted in the establishment of several presumably extinct taxa. Such remains have been found in strata dating from the Eocene and document the early diversification of the group. One interesting aspect of the fossil record is that most of the extinct taxa can be associated with the extant genus *Ceratophyllum*, and often even with extant species (e.g., *Ceratophyllum dubium* with *C. submersum*; *C. zaisanicum* with *C. tanaiticum*). This phenotypic similarity between extinct and extant taxa indicates the conservative nature of fruit features which apparently has resulted in a long period of morphological "stasis" in the group.

Of considerable interest is work by Buzek (1982) on a peculiar group of Miocene macrofossils. Buzek (1982) deduced the relationship of an extinct taxon belonging to the form-genus *Ceratostratiotes* (Gregor, 1980) to the extant genus *Ceratophyllum*. Previously, the affinities of this fossil taxon were dubious due to various misinterpretations. The failure of several workers to interpret properly the fruit structure led them to associate *Ceratostratiotes* with the genera *Trapa* and *Stratiotes*. The more critical analysis by Buzek revealed that *Ceratostratiotes* showed a great deal of similarity to *Ceratophyllum* and that its placement with the other groups was unfounded. Several fundamental differences, however, led him to conclude that the fossil taxon should not be included within the genus *Ceratophyllum*, but rather should be regarded as a separate, related genus. A comparison of fruit characters between *Ceratostratiotes* and *Ceratophyllum* is made in Table 9. Fruits of these genera are quite similar by this comparison, and differ mainly by their spinature, surface features and placentation. The common occurrence of a "micropylar pore" ("stigmatic pocket" of Jones, 1931) is particularly convincing evidence for their relationship because such a feature is not known to exist in any other extant angiosperm group and is an important functional component of the hydrophilous mechanism in *Ceratophyllum*. Buzek's arguments warrant his conclusions that *Ceratostratiotes* and *Ceratophyllum* are closely related, and that *Ceratostratiotes* is distinct enough to be treated as a separate genus.

Coupled with evidence that presently extinct species of *Ceratophyllum* once flourished several million years ago (Avakov, 1962), these factors shed new light on the early evolution of Ceratophyllaceae. It is inappropriate to view the genus simply as an unusual offshoot from the Nymphaeales as authors have done in the past. There is better reason to believe that *Ceratophyllum* represents the remains of a more ancient lineage which once was more diverse, comprising at least two genera, and possibly several others. The diversity of the

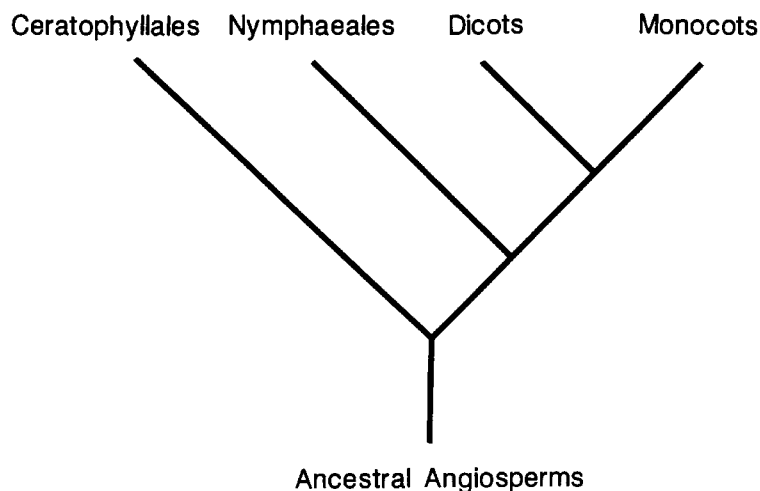


Fig. 4. Hypothetical phylogenetic relationship of Ceratophyllales, Nymphaeales, and modern monocots and dicots.

group was reduced by extinctions occurring over millennia, resulting in the absence of related groups being represented in our extant flora. It is for this reason that the affinities of the family have been so difficult to determine.

Conclusions

Ceratophyllaceae show no close affinity to any extant angiosperm group. The family is viewed as a vestige of ancient angiosperms that diverged quite early from the line giving rise to most other modern taxa. The occurrence of typically monocotyledonous features in *Ceratophyllum* and Nymphaeales (Dahlgren et al., 1985; Haines and Lye, 1975) may indicate a common gene pool somewhere in the remote ancestry of all three groups. The mosaic pattern of these features, however, prevents more exact relationships to be ascertained from the evidence presently at hand. The above discussion has provided sufficient grounds for proposing a new, albeit hypothetical relationship of Ceratophyllaceae and other primitive angiosperms. The family is viewed here as occupying a relatively isolated position along the major evolutionary line leading to modern angiosperm groups, a conclusion reached previously by both Schaffner (1904) and Abov (1936). In this regard, the Ceratophyllaceae are truly "living fossils" and represent plants which probably diverged from some of the earliest angiosperm progenitors. To better reflect the isolated phylogenetic position of Ceratophyllaceae in classification schemes, a new order, Ceratophyllales, is proposed and named below (Fig. 4).

More precise details of the origin of this unusual family must remain speculative. Further interpretations of relationships of Ceratophyllaceae using morphological data have reached an impasse. It is possible that a better idea of the time of divergence and exact relationships of the Ceratophyllaceae could be obtained by studying the degree of molecular divergence among *Ceratophyllum* and the genera of Nymphaeales. This technique has proven to be a useful tool in the phylogenetic analysis of other problematic angiosperms and would be an appropriate method for testing these hypotheses.

Taxonomy

CERATOPHYLLALES Les, ord. nov. Type genus: *Ceratophyllum* L., Sp. pl. 992. 1753.

Herbae submersae, perennes. Radices absentes. Folia verticillata, denticulata, divisa dichotome. Monoeciae. Inflorescentiae reductae ad floribus solitaris, subtentae a verticillo bractearum connatarum et foliacearum. Flores nudi. Stamina subsessilia, numerosa, spiralia, centripeta. Carpellum unus. Stylus persistens, spinescens vel apiculatus. Stigma mar-

supiiiformis. Ovulum singulare, cum tegmen unum. Embryo magnus; cotyledona 2, carnosae; plumula evoluta multum.

Submersed, rootless, perennial herbs. Leaves whorled, dichotomously divided, denticulate. Plants monoecious. Inflorescences reduced to solitary flowers, subtended by a whorl of fused, foliaceous bracts. Flowers naked. Stamens sessile, numerous, arranged spirally, maturing centripetally. Pollination hydrophilous. Carpel one. Style persistent, spinescent or apiculate. Stigma pocket-like. Ovule single, unitegmic; placentation apical/ventral or basal/ventral. Embryo large; cotyledons two, fleshy; plumule highly developed. Fruit an achene, usually spiny.

A monotypic order comprising the single family Ceratophyllaceae.

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APPENDIX A

Chromosome Number Reports for *Ceratophyllum demersum* and *C. echinatum*

Methods

Staminate inflorescences were fixed in the field using “Farmer’s Fluid” preservative (1:3 acetic acid: alcohol). After 24 hours, buds were transferred into 70% alcohol and kept under refrigeration until examined. Prior to staining, buds were soaked for 24 hours in 45% acetic acid. Buds were transferred onto a glass slide, stained with aceto-carmine, gently heated, covered with a coverslip, and squashed.

Results

Ceratophyllum demersum L. $n = 12$. U.S.A. MICHIGAN. Washtenaw Co., pond on W side of Earhart Rd., ca. ½ mi S of Plymouth Rd., but E of US-23, *Les 335* (OS). CANADA. BRITISH COLUMBIA. Vancouver Island, Thetis Lake, outside Victoria, *Les 430* (OS).

Ceratophyllum echinatum A. Gray. $n = 12$. U.S.A. MICHIGAN. Washtenaw Co., Loessel Field Lab of the Eastern Michigan University, E of Golfside Dr., ca. ½ mi S of Huron River Dr., in pond, *Les 337* (OS). CANADA. BRITISH COLUMBIA. Nanaimo, Brannen Lake, *Les 434* (OS); Devil’s Lake, near Hope, small pond about 2 km W of junction of HWY 7 with HWY 1, just N of Hope, directly across from truck scales on S side of HWY 7, bordered by RR tracks, *Les 436* (OS).