

Comparative pollen structure and pollination biology in the *Callitrichaceae*

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Summary.- The *Callitrichaceae* is a widespread, monotypic (*Callitriche*) family, consisting of approximately 50 terrestrial, amphibious, and obligately submersed species. Several pollination syndromes have been suggested to occur within the genus, including anemophily, epihydrophily (pollen transfer at the water surface), and hypohydrophily (entirely submersed pollination). However, cross-pollination has only recently been unequivocally documented in the family. Terrestrial species appear to be geitonogamous, and produce distinctly tricolpate pollen, with a well-defined, intectate exine. In the various amphibious species, fertilization can occur in two different ways: "typical geitonogamy", as in terrestrial species, or by "internal geitonogamy", whereby pollen grains precociously germinate within indehiscent anthers and pollen tubes subsequently grow through nodal and pistillate tissues directly to the ovules. Anemophily is also likely in these amphibious species. Pollen grains have weakly differentiated apertures, but well-developed, intectate exines. Molecular data confirm that the obligately submersed *C. hermaphroditica* is hypohydrophilous. The actual mechanism of pollen transfer has not yet been observed, but several features suggest that pollen grains are effectively dispersed by the water. Pollen of *C. hermaphroditica* is exineless in perennial forms of the species, and has only a rudimentary exine in annual forms. *Callitriche* represents the only infrageneric system in which both aerial and underwater pollination co-occur, and provides an unprecedented opportunity to investigate the evolution of hypohydrophily from aerial systems.

Résumé.- La famille des *Callitrichaceae* est une famille monotypique (*Callitriche*), cosmopolite, comprenant environ 50 espèces terrestres, amphibies ou totalement submergées. Plusieurs syndromes de pollinisation ont été évoqués dans le genre : l'anémophilie, l'épiphyllophilie (transport du pollen à la surface de l'eau) et l'hypohydrophilie (pollinisation entièrement dans l'eau). Cependant, c'est seulement récemment que l'allogamie a été mise en évidence. Les espèces terrestres semblent être génotogames et possèdent un pollen distinctement tricolpé, avec une exine intectée, bien définie. Dans différentes espèces amphibies, la pollinisation peut se réaliser de deux manières :

soit par "geitonogamie typique", comme dans les espèces terrestres, soit par "geitonogamie interne", processus selon lequel les grains de pollen germent précocement à l'intérieur des anthères indéhiscentes et les tubes polliniques atteignent directement les ovules à travers les tissus du gynécée. L'anémophilie est également remarquable chez certaines espèces amphibies. Les données moléculaires confirment que l'espèce totalement submergée, *C. hermaphroditica*, est hypohydrophile. Le véritable mécanisme du transport de pollen n'a pas encore été observé, mais plusieurs faits suggèrent qu'il est effectivement dispersé par l'eau. Le pollen de *C. hermaphroditica* est dépourvu d'exine dans les formes pérennes de l'espèce, et a seulement une exine rudimentaire dans les formes annuelles. *Callitriche* est le seul genre dans lequel les modes de pollinisation aérienne et sous l'eau coexistent à l'échelle infragénérique et fournit ainsi une occasion sans précédent permettant d'étudier l'évolution de la pollinisation aérienne vers l'hypohydrophilie.

Key words : *Callitrichaceae* - *Callitriche* - pollen - ultrastructure - pollination - hydrophily - evolution.

INTRODUCTION

The *Callitrichaceae*, or water starworts, is a relatively small, monotypic family. *Callitriche* is widespread in its distribution and consists of approximately 50 terrestrial, amphibious, and obligately submersed species (Philbrick and Jansen, 1991; Philbrick and Anderson, 1992). Plants are diminutive and monoecious; flowers are also greatly reduced in size. Pistillate flowers are composed of a single, bicarpellate ovary with one ovule in each of four locules, and two filiform, papillate styles which may be entirely stigmatic (*i.e.*, receptive) in their length. Staminate flowers consist of a single stamen, with a relatively robust filament supporting a single apical anther. The 4-locular anther dehisces longitudinally by two slits; however, these become apically confluent. Although all flowers lack perianth parts, those of several species are subtended by a pair of delicate, gas-inflated bracts. Staminate and pistillate flowers occur in various combinations in the axils of opposite leaves (*i.e.*, sometimes singly and other times together). Moreover, the number of each flower type per node may also vary, both within and among plants. For

most species, plants are partially protogynous; in instances when both staminate and pistillate flowers develop at a node, pistillate flowers become receptive prior to anther dehiscence.

As *Callitriche* is so diverse in terms of its growth habit, several pollination syndromes have been suggested to occur within the genus, including anemophily, in both terrestrial and amphibious species, and two forms of water-mediated pollination (*i.e.*, hydrophily). Epihydrophily, pollen transfer at the water surface, has been reported exclusively in several amphibious taxa, while hypohydrophily, entirely submersed pollination, has been attributed to both amphibious and obligately submersed species (see Philbrick and Anderson, 1992, and references therein).

However, the evidence supporting most reports of pollination is, for the most part, anecdotal, with terminological problems regarding pollination systems in the genus contributing to the confusion. For example, Schotsman (1982) has used the terms "epihydrophily" and "hypohydrophily" to describe pollen transfer in *Callitriche* regardless of whether or not self-pollination (*i.e.*, geitonogamy) or cross-pollination (*i.e.*, xenogamy) has taken place. However,

such a broad usage fails to reflect the important distinction between cross- and self-pollination. Thus a more conservative approach to the use of these two terms has been recommended (Philbrick, 1988 ; Philbrick and Anderson, 1992). Moreover, attempts to unquestionably determine the specific mechanisms of pollination in *Callitriche* have been problematic. For instance, due to the extremely reduced sizes of flowers the appropriate crossing experiments have proved difficult to carry out ; most controlled pollinations fail as a result of floral manipulations (*i.e.*, pistillate flower abortion following emasculation of the staminate flower). Molecular approaches, however, have recently proven to be more useful in addressing the problem, and cross-pollination has clearly been demonstrated in the obligately submersed species *C. hermaphroditica*, thereby unequivocally documenting hypohydrophily in the *Callitricheaceae* for the first time (Philbrick, unpublished).

These new data indicate that *Callitriche* represents the only angiosperm genus in which both aerial pollination systems and hypohydrophily co-occur. The genus therefore provides an unprecedented opportunity to investigate queries concerning the evolution of hypohydrophily. It is clear that underwater pollination in general is derived from aerial floral systems (*e.g.*, Faegri and Van der Pijl, 1979), and that the evolution of this pollination system results in a suite of morphological adaptations in flowers (*e.g.*, Sculthorpe, 1967 ; Philbrick, 1988). We would also predict that palynological characters would be acted upon by strong selective pressures, as pollen is in direct contact with the water. If true, marked differences in pollen structure would be expected between aerial versus hypohydrophilous species. However, relatively few studies

have critically examined pollen within the *Callitricheaceae*. The majority of these have been based on observations in transmitted light (*e.g.*, Erdtman, 1952 ; Moar, 1960), while several others have employed scanning electron microscopy (SEM ; *e.g.*, Diez, Talavera, and Garcia-Murillo, 1988). Transmission electron microscopy (TEM), on the other hand, has only recently been utilized in the investigation of *Callitriche* pollen (Philbrick and Osborn, unpublished ; Martinsson, 1993).

The intent of the present paper is to review current information about the pollination systems exploited by the three principal growth forms of *Callitriche*, and to overlay new data on pollen morphology and ultrastructure of the genus. We will also consider the degree to which palynological evidence can be utilized to trace the origins of hypohydrophily in *Callitriche*. It is beyond the scope of this paper to address all of the species for which data are now available ; therefore we will focus on representative terrestrial, amphibious, and obligately submersed species. The taxa considered here are principally new world species, but reflect the ecological and pollination diversity for the genus overall.

TERRESTRIAL SPECIES

Terrestrial species of *Callitriche* typically grow on seasonally muddy to moist soils that often become dry. Individual plants produce small, prostrate growth mats ranging from 2-7 cm in diameter. Three species have been reproductively studied, including *C. peploides* Nutt., *C. nuttallii* Torrey, and *C. terrestris* Raf. (Philbrick and Anderson, 1992). All three produce only aerial flowers, and these appear to be geitonogamous. Geitonogamy apparently takes place most readily when both staminate and

pistillate flowers co-occur at a node, either by pollen falling from a dehiscent anther to the style or by the elongate style coming into direct contact with the dehiscent anther. Cross-pollination (anemophily?) is also possible in terrestrial species, but has yet to be confirmed.

Pollen of *Callitriche peploides*

Pollen of *C. peploides* is tricolpate, subtriangular in shape, and averages 18.7 μm in diameter (Pl. I, fig. 1). Ornamentation ranges from irregularly verrucate to echinate, with sculptural elements forming polygonal clusters (Pl. I, fig. 1). Pollen is intectate and has a distinct exine, ranging from 0.70-0.95 μm in thickness (Pl. I, fig. 2). Sculptural elements may be laterally fused and are borne on a relatively thick, homogeneous nexine. A dark-staining commissural line is present near the bottom of the nexine, and is underlaid by several minute granules (Pl. I, fig. 2).

AMPHIBIOUS SPECIES

The growth habit exhibited by amphibious *Callitriche* species is variable, although most individuals grow in clumps and are multibranched. Plants may inhabit moist soil thereby growing terrestrially, or they may grow aquatically as one of two forms. Aquatic individuals may either be entirely submersed, or mostly submersed with the principal stem(s) extending to the water surface supporting a rosette of floating leaves. Depending on the environmental growth form, amphibious species may produce only aerial flowers, both aerial and submersed flowers on the same individual, or only submersed flowers. Two amphibious species studied by Philbrick and Anderson (1992), *C. stagnalis* Scop. and *C. marginata* Tor-

rey, produce only aerial flowers. *Callitriche heterophylla* [var. *heterophylla* Pursh and var. *bolanderi* (Hegelm.) Fassett], *C. verna* L., and *C. trochlearis* Fassett, on the other hand, are known to produce both aerial and submersed flowers.

Seed production in amphibious species may be facilitated by two strikingly different events. Fertilization can occur by "typical geitonogamy" as described above for terrestrial species, or by an anomalous reproductive mechanism termed "internal geitonogamy" (Philbrick, 1984) which is known from seven species (Philbrick and Bernardello, 1992). In the latter, flowers exhibit precocious pollen germination within indehiscent anthers and subsequent pollen tube growth through vegetative (nodal) and pistillate tissues directly to the ovules. Interestingly, the species that exhibit internal geitonogamy also show some variation in flower structure. In the "floating-rosette" forms, anthers reach full development and produce a large number of pollen grains (ranging from 1,214 - 2,939) per anther. These flowers also develop full-size ovaries and styles. By comparison, in the "submersed" and "terrestrial" amphibious forms, the anthers are typically only poorly developed and produce a small number of pollen grains (as low as four) per anther. Furthermore, although these flowers form a full-size ovary they lack well-developed styles, and if these rudimentary structures are present, characteristic papillae are typically absent.

Pollen of all amphibious species examined to date, both those with and without internal geitonogamy, have a well-developed exine.

Pollen of *Callitriche marginata*

Typical geitonogamy, and perhaps anemophily, seems to occur *C. marginata*. Pollen grains are spherical, ave-

rage 20.9 μm in diameter, and typically have three ill-defined apertures (colpi) (Pl. I, fig. 9). Grains are intectate and have gemmate to faintly echinate ornamentation, with sculptural elements slightly fused laterally forming weakly defined, circular clusters (Pl. I, fig. 9). The exine ranges in thickness from 0.55–1.17 μm and is two-layered, consisting of the sexinous sculptural elements and a relatively thin nexine (Pl. I, fig. 10). The nexine is irregularly homogeneous to granular in ultrastructure, with larger granules penetrating the underlying intine (Pl. I, fig. 10).

Pollen of *Callitriche heterophylla* var. *bolanderi*

Callitriche heterophylla var. *bolanderi* is an internally geitonogamous species. Pollen grains are spherical, also with weakly differentiated colpi, and have an average diameter of 21.2 μm . Pollen of this species is more gemmate in surface ornamentation in comparison with *C. marginata*, with the sculptural elements forming poorly defined clusters (Pl. I, fig. 3). The exine is intectate and two-layered, averaging 0.75–1.00 μm in thickness (Pl. I, fig. 4). The sculptural elements may also be laterally fused, especially at their bases (Pl. I, fig. 4). The nexine is more granular than *C. marginata*, and has a dark commissure present near the base, which is underlaid by smaller granules (Pl. I, fig. 4).

OBLIGATELY SUBMERSED SPECIES

The reproductive aspects of one obligately submersed species have been investigated thus far, *C. hermaphroditica* L., and two genetically based growth forms of this species have been encountered in the field (Philbrick and Anderson, 1992, and references therein). Annuals are relatively small plants consisting of only one to a few upright stems. By comparison, the pe-

rennial growth form is larger and thought to be clonal. Plants consist of multiple stems that branch profusely, and form roots at their nodes.

In *C. hermaphroditica*, staminate and pistillate flowers occur at alternate nodes, with individual staminate flowers producing, on average, over 2,400 grains per anther. Pollen germination is also precocious in this species. Although here it occurs subsequent to anther dehiscence, but still within the locule of the dehisced anther, and results in a tangled mass of pollen tubes (Pl. II, fig. 11). In many individuals, the elongate styles of pistillate flowers become reflexed and bend to the contiguous node where they become closely positioned to staminate flowers (Pl. II, fig. 12). As *C. hermaphroditica* is protogynous, and based on observations of pollen grain/pollen tube masses on receptive stylar surfaces, geitonogamous pollination was thought to be the exclusive mode of pollination (Philbrick and Anderson, 1992). However, Philbrick (unpublished) has recently completed genetic analyses of maternal plants and their progeny, and has confirmed that outcrossing (hypohydrophily) occurs in this species. Nonetheless, the details concerning the mechanism of pollen transfer remain unclear.

Pollen of *Callitriche hermaphroditica*

Pollen grains of *C. hermaphroditica* are spherical, average 20.1 μm in diameter, and are inaperturate (Pl. I, fig. 5). Grains have a distinct two-layered intine, ranging from 0.25–0.45 μm in thickness, but lack a well-developed exine (Pl. I, figs. 6–8). Due to the absence of a distinct exine, pollen often appears smooth-walled and irregularly shaped when viewed by SEM (Pl. I, fig. 5). The irregular shape of grains may, in part, be due to intine compression during preparation for SEM. Plants of the perennial growth form lack an exine altogether (Pl. I,

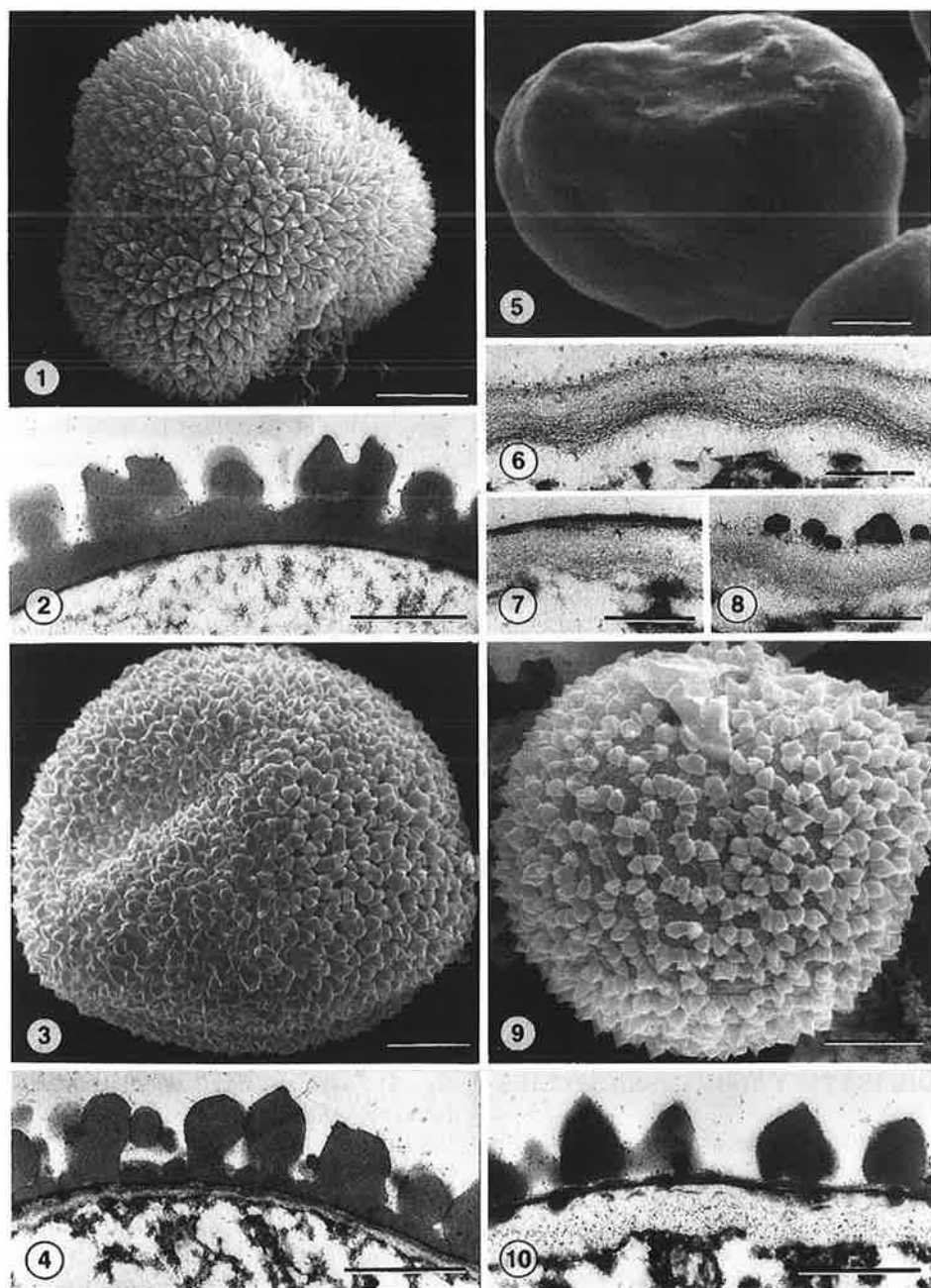


Plate I

fig. 6); whereas plants of the annual growth form have a reduced exine. In annuals, this rudimentary exine may be manifested either as a thin, homogeneous to slightly granular band not more than 0.1 μm in thickness (Pl. I, fig. 7), or as small, discontinuous and irregularly shaped granules ranging from 0.05-0.16 μm in size (Pl. I, fig. 8).

DISCUSSION

Although the functional association of pollen morphology and ultrastructure to particular pollination syndromes is not always related (e.g., Taylor and Levin, 1975) nor well-understood, a number of angiosperm families have in

fact been shown to demonstrate positive correlations between these palynological characters and their pollination systems (e.g., Osborn, Taylor, and Schneider, 1991). This is especially true for hydrophilous species in which reduction of exine thickness or lack of an exine altogether characterizes the pollen (e.g., Cox, 1988; Philbrick, 1988). For example, exine reduction or complete loss has been documented in *Najas* (*Najadaceae*; e.g., Pettitt and Jermy, 1975), *Enhalus*, *Thalassia* (*Hydrocharitaceae*; e.g., Pettitt, 1980, 1981), *Amphibolis*, *Thalassodendron* (*Cymodoceaceae*; e.g., Pettitt, Ducker, and Knox, 1978; Pettitt, 1981), *Lepilaena* (*Zannichelliaceae*; e.g., McConchie,

Plate I.- *Callitriche* pollen

Fig. 1-2.- *C. peploides* (terrestrial). Fig. 1.- Polar view of grain showing polygonal clusters of sculptural elements; note also the three equatorial colpi (Scale bar = 3 μm). Fig. 2.- Transverse section of the nonapertural exine showing laterally fused sexine elements and relatively thick nexine (Scale bar = 1 μm). Fig. 3-4.- *C. heterophylla* var. *bolanderi* (amphibious). Fig. 3.- Equatorial view of a pollen grain showing poorly defined, circular clusters of gemmate sculptural elements and one weakly differentiated aperture (Scale bar = 3 μm). Fig. 4.- Transverse section of nonapertural exine showing gemmate sexine elements and granular nexine; note the dark-staining commissural line in the base of the nexine which is underlaid by relatively small granules (Scale bar = 1 μm). Fig. 5-8.- *C. hermaphroditica* (obligately submersed). Fig. 5.- Surface view of a pollen grain from the perennial form; note the irregular shape and smooth wall (Scale bar = 3 μm). Fig. 6.- Transverse section of the sporoderm from the perennial form; note the two-layered intine and absence of an exine component (Scale bar = 0.5 μm). Fig. 7.- Transverse section of the sporoderm from the annual form showing a rudimentary, homogeneous exine; note also the well-defined, thicker intine (Scale bar = 0.5 μm). Fig. 8.- Sporoderm from the annual form in transverse section showing a rudimentary exine; note the discontinuous, irregularly shaped exine granules and thicker, underlying intine (Scale bar = 0.5 μm). Fig. 9-10.- *C. marginata* (amphibious). Fig. 9.- Polar view of a pollen grain showing slightly echinate to gemmate sculptural elements (Scale bar = 3 μm). Fig. 10.- Transverse section of nonapertural exine showing sexine elements and relatively thin, slightly granular nexine; note that the nexine is also underlaid by small granules (Scale bar = 1 μm).

Planche I.- Pollen de *Callitriche*

Fig. 1-2.- *C. peploides* (terrestre). Fig. 1.- Grain de pollen en vue polaire montrant les amas polygonaux des éléments ornementaux; noter aussi les trois colpi équatoriaux (Echelle = 3 μm). Fig. 2.- Section transversale de l'exine non aperturale montrant les éléments sexiniques soudés latéralement et la nexine relativement épaisse (Echelle = 1 μm). Fig. 3-4.- *C. heterophylla* var. *bolanderi* (amphibie). Fig. 3.- Grain de pollen en vue équatoriale montrant les amas circulaires, mal délimités, d'éléments ornementaux gemmés et une aperture faiblement différenciée (Echelle = 3 μm). Fig. 4.- Section transversale de la région non aperturale montrant les éléments gemmés de la sexine et la nexine granulaire; noter, dans la partie interne de la nexine, la ligne de coloration sombre au-dessus des granules relativement petits (Echelle = 1 μm). Fig. 5-8.- *C. hermaphroditica* (strictement submergée). Fig. 5.- Surface d'un grain de pollen provenant de la forme pérenne; noter la forme irrégulière et la paroi lisse (Echelle = 3 μm). Fig. 6.- Section transversale du sporoderme de la forme pérenne; noter l'intine bistratifiée et l'absence de constituant exinique (Echelle = 0,5 μm). Fig. 7.- Section transversale de la forme annuelle montrant une exine homogène rudimentaire; noter aussi l'intine épaisse, bien délimitée (Echelle = 0,5 μm). Fig. 8.- Sporoderme de la forme annuelle en section transversale, montrant une exine rudimentaire; noter les granules discontinus, de forme irrégulière et l'intine sous-jacente plus épaisse (Echelle = 0,5 μm). Fig. 9-10.- *C. marginata* (amphibie). Fig. 9.- Vue polaire d'un grain de pollen montrant les éléments ornementaux légèrement échinulés à gemmés (Echelle = 3 μm). Fig. 10.- Section transversale de l'exine non aperturale montrant les éléments sexiniques et la nexine mince, légèrement granulaire; noter que la couche interne de la nexine couvre aussi de petits grains (Echelle = 1 μm).

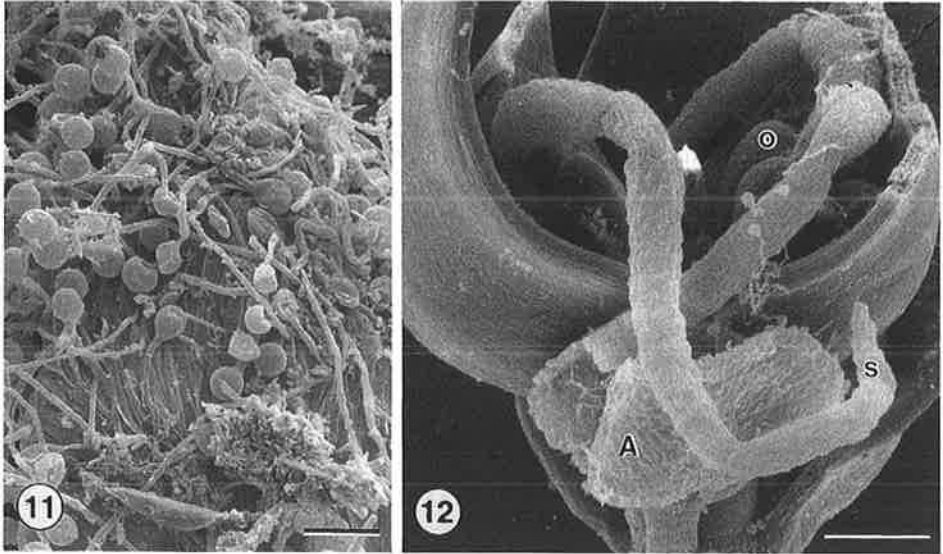


Plate II.- *Callitriche hermaphroditica*

Fig. 11.- Open locule of a dehiscent anther showing precociously germinated pollen grains; note the numerous pollen tubes (Scale bar = 50 μ m). Fig. 12.- Portion of a stem showing two contiguous nodes and three flowers. Two pistillate flowers are present in the axils of opposite leaves at the upper node ; each flower consists of a 4-locular ovary (O) and two elongate styles (S). Note how the styles are reflexed and thereby come into contact with the open theca of the dehiscent anther (A) at the lower node ; several germinated pollen grains are present on one style of the pistillate flower at right (Scale bar = 250 μ m).

Planche II.- *Callitriche hermaphroditica*

Fig. 11.- Loge ouverte d'une anthère montrant les grains de pollen germés précocement ; noter les nombreux tubes polliniques (Echelle = 50 μ m). Fig. 12.- Portion de tige montrant deux nœuds contigus et trois fleurs. Deux fleurs pistillées sont présentes à l'aisselle des feuilles opposées au nœud supérieur ; chaque fleur est constituée d'un ovaire 4-loculaire (O) et de deux styles allongés (S). Noter la manière dont les styles sont réfléchis, ce qui les amène en contact avec la thèque ouverte de l'anthère (A) au nœud inférieur ; plusieurs grains de pollen germés sont présents sur un style de la fleur pistillée, à droite (Echelle = 250 μ m).

Knox, and Ducker, 1982), and *Ceratophyllum* (*Ceratophyllaceae* ; e.g., Pettitt and Jermy, 1975 ; Les, 1988).

Confirmation of hypohydrophily in *C. hermaphroditica*, in combination with the virtual lack of an exine in this species, supports the idea that loss of exine is a fundamental component of the evolution of hypohydrophilous pollination in angiosperms overall. A corollary to this is that the presence of a well-developed exine in aerial flowering callitriches reflects the aerial floral biology of these species. However, there are also apparent differences in pollen

structure between terrestrial and amphibious species that may reflect the nature of the floral biology ; this variation in structure may relate to a floral system that functions in close proximity to the water surface (increased humidity levels ?) in amphibious species. For instance, the terrestrial *C. peploides*, *C. nuttallii*, and *C. terrestris* have distinct colpi compared with amphibious species such as *C. heterophylla*, *C. verna*, *C. trochlearis*, and *C. marginata* where the aperture margins are poorly differentiated. Preliminary comparisons also indicate differences in nexine thick-

ness ; terrestrial species (e.g., *C. peploides*) have a thicker nexine than amphibious species (e.g., *C. marginata*, *C. heterophylla*, *C. stagnalis* ; Philbrick and Osborn, unpublished). Notably, phylogenetic analysis (Philbrick and Jansen, 1991) placed *C. marginata* and the aforementioned terrestrial species in the same clade, suggesting that the palynological differences are not simply phylogenetic constraint. Expanded analyses of both exine structure and phylogenetic relationships among species are clearly needed to test this apparent pattern.

The evolution of underwater cross-pollination involves significant modification in floral biology ; in particular, transition from an aerial pollination mode into one characterized by the release and capture of wet, water-borne pollen by underwater flowers. As noted above, an important feature in this transition has been the reduction or complete loss of an exine. Related to this is the observation that pollen grains of the majority of hypohydrophilous taxa are filiform to ellipsoidal in shape (e.g., Pettitt, 1981). Cox and coworkers (Cox, 1988 ; Cox, Cromar, and Jarvis, 1992) have attributed this elongate shape to pollination efficiency. The pollen of *C. hermaphroditica* is spherical in shape but germinates precociously soon after anther dehiscence. Although hypohydrophily in *C. hermaphroditica* seems to occur in a three-dimensional medium, the precocious germination, and resultant tangled masses of pollen grains/tubes, may indicate parallelisms with the ideas presented by Cox for "search vehicle" efficiency in epihydrophilous pollination systems.

Pollen of hypohydrophilous species is often coated with some type of a mucilaginous tapetal product (e.g., Pettitt, 1981). However, the type of tapetum and nature of its derivatives remain to

be confirmed in *C. hermaphroditica*. Detailed developmental studies may provide new insights in this regard, as well as provide information concerning patterns of pollen wall ontogeny. In a broader context, there appear to be differences between the developmental patterns of exineless pollen of aerial flowering species compared with hypohydrophilous species (see Blackmore and Crane, 1988 and references therein), although these distinctions are only based on studies of a few taxa and need to be clarified within others. Lastly, the lack, or extreme reduction, of endothelial thickenings is well known in hypohydrophilous angiosperm anthers (e.g., Pettitt and Jermy, 1975 ; Philbrick, 1988). Such is also evident in *C. hermaphroditica* as well as *C. truncata*, another obligately submersed species (Schotsman, 1982 ; Philbrick, unpublished).

Another wide-ranging character of hypohydrophilous angiosperms is a high degree of structural divergence of the flower (i.e., reduction ; see Philbrick, 1988) in comparison with their aerially flowering relatives. However, floral morphology within all species of *Callitriche* is generally uniform. This floral similarity directly contrasts with the differences in exine structure observed among species of the genus (i.e., degree of reduction). Based on these structural data taken in concert with the fact that *Callitriche* represents the only infrageneric system in which both aerial and hypohydrophilous pollination occurs, the evolution of hypohydrophily from aerial systems may involve the initial selection for pollen structure rather than other components of the floral structure (Philbrick and Osborn, unpublished). Moreover, variation in exine structure observed between perennial (exine absent) and annual (exine rudimentary) forms of *C. hermaphroditica*

may further indicate that hypohydrophily has a recent origin in the species (Philbrick and Osborn, unpublished). Additional observations of other obligately submersed species within the genus (e.g., *C. truncata*) may help clarify these hypotheses.

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