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VARIATION, ADAPTATION AND REPRODUCTIVE BIOLOGY IN *RUPPIA MARITIMA* L. POPULATIONS FROM NEW HAMPSHIRE COASTAL AND ESTUARINE TIDAL MARSHES

BY

Frank David Richardson
B.A., Boston University, 1968
M.S., University of New Hampshire, 1976

DISSERTATION

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Doctor of Philosophy
in
Botany

December, 1983

This dissertation has been examined and approved.

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Arthur C. Mathieson

Arthur C. Mathieson, Professor of Botany

30 November, 1983
Date

This Dissertation
is dedicated to the memory of
Professor Albion Reed Hodgdon
who first introduced me to *Ruppia*.
His intellect, intuition and keen insights
on the complexities of variation and adaptation
in the botanical world were inspirational to me.
His appreciation of art, science
and love of life in all forms
has been a pattern in my own development.
If ever I had a mentor,
'Doc' Hodgdon is that man.

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ABSTRACT

VARIATION, ADAPTATION AND REPRODUCTIVE BIOLOGY IN *RUPPIA MARITIMA* L. POPULATIONS FROM NEW HAMPSHIRE COASTAL AND ESTUARINE TIDAL MARSHES

by

FRANK DAVID RICHARDSON

University of New Hampshire, December, 1983

Ruppia maritima L. populations in coastal and estuarine tidal marshes in New Hampshire exhibit considerable variation in morphology, reproductive strategy and pollination ecology in response to a range of environmental conditions. Variation among the populations correlates with seasonal changes in environmental conditions recorded at various sites. A comparison of coastal and estuarine habitats of *Ruppia* such as pannes, pondholes, ditches and creeks has shown them to differ significantly with respect to such factors as: origin and development, substrate, epibiota, floristic and faunistic components and seasonal patterns in water depth, temperature and salinity. The three varieties of *Ruppia maritima* L. occurring in New Hampshire are phenotypically plastic in response to the seasonal environment in ecologically distinct habitats. Phenotypic plasticity associated with responses to environmental parameters, however, accounts for only a part of the variation exhibited by Ruppia populations. Genetic and ecological differentiation has occurred and populations characterized by a particular reproductive strategy and morphology may be genetically discrete. The adaptive significance of phenotypic plasticity and genetic variation relative to seasonal periodicity and phenological phenomena in Ruppia populations is discussed. A reciprocal transplant field study and a controlled environment greenhouse experiment contribute to an assessment of autecological data, environmental conditions and phenological studies. Morphological variation in vegetative organs, reproductive parts and in the pollen was observed in populations along an ecocline. Coastal populations have consistently larger anther sacs and larger pollen grains with heavier exine deposition than estuarine plants.

Coastal and estuarine populations were examined and compared for several ecological, phenological and reproductive criteria. Coastal populations are biennials or short-lived perennials producing relatively little seed; pollination occurs at the surface of the water facilitating an outcrossing strategy. Estuarine populations are for the most part annuals producing abundant seed; an underwater pollination mechanism promotes selfing. The physiology of the underwater pollination mechanism is documented by anatomical evidence and time-lapse cinemicrography. The sediments in most of the study areas contain a seed bank although the seed concentration is higher in the estuarine habitats. A seed bank provided for the reestablishment of large populations of *Ruppia* at several locations which had been devoid of plants for one or two years. Electrophoretic variation was investigated but the results were inconclusive. Chromosome numbers, however, indicate polyploidy in the coastal populations where $2n = 28$ while the estuarine populations were found to be $2n = 14$. Field and laboratory techniques developed and utilized in this study may be applied to investigations in reproductive biology and variation in related submersed aquatics (e.g., section *Pusilli* of *Potamogeton*), seagrasses and other groups needing further research. Implications of variation and reproductive biology in *Ruppia* relative to its taxonomy are discussed.

INTRODUCTION

Ruppia populations in New Hampshire tidal marshes are distributed along an environmental gradient of coastal and estuarine habitats. Seasonal fluctuations in water temperature, depth, salinity and other environmental conditions affect the growth, development and reproductive biology of the plants at different sites (Richardson, 1980). Considerable variation in morphology, phenology and pollination systems has been observed within and between the populations. The present research seeks to determine the possible exogenous and endogenous factors which contribute to this variation. An assessment is made on the adaptive significance of variation in morphology and reproductive biology. It has been a major consideration to gain an understanding of the level to which the variation expressed in these populations represents a phenotypic response to the environment or a function of the genotype. These findings will contribute to the development of a functional taxonomy for _Ruppia_ representatives based on an integration of morphological, genotypic and ecological information.

The cosmopolitan genus _Ruppia_ has been widely studied from ecological, anatomical, morphological and taxonomic perspectives. Considerable attention has been paid to the diverse habitats it occupies and the broad range of environmental conditions it has adapted to or will tolerate. Setchell (1924, 1946), Bourn (1935), McKay (1935), Conover

(1958, 1964, 1966), Conover and Gough (1966), Mayer (1969), Anderson (1970), Sullivan (1977) and Richardson (1976a, 1980) have reported on the ecology of Ruppia in North America. Verhoeven (1975, 1978, 1979, 1980a, 1980b) and Verhoeven and van Vierssen (1978) have made substantial contributions to the understanding of Ruppia-dominated communities in Western Europe. Reese (1963) reported on the distribution of Ruppia and Zannichellia species and varieties bordering the North Sea in Schleswig-Holstein. Kiorboe (1980) studied the production of Ruppia in Denmark. Congdon and McComb (1979) and Brock (1981, 1982a, 1982b) have studied the ecology of Ruppia in Australia. Various aspects of the biology of the genus have been investigated in Mexico (Gutierrez, 1977), Argentina (Gamerro, 1968) and Brazil (Oliveira F., et al., 1983).

The anatomy and morphology, and their ecological significance in this submersed vascular hydrophyte, was examined in detail in the profusely illustrated monograph of Graves (1908). Floral development in Ruppia maritima var. maritima has been well documented by Posluszny and Sattler (1974). Roze (1894) was the first to provide a treatment of the reproductive biology and ecological anatomy/ morphology of various species of Ruppia, and cites many obscure earlier works. McCann (1945) discussed the morphology and floral biology, including pollination ecology, in his treatment of the genus as a new addition to the flora of India. Mason (1967) examined the morphology and taxonomy of Ruppia species in New Zealand. The floral biology of R.

cirrhosa (Petag.) Grande (= *R. spiralis* L. ex Dum.) has been investigated by Gamerro (1968). Singh (1964, 1965) studied the vegetative anatomy and vascular anatomy of the flower of *Ruppia* in his work on the Potamogetonaceae. Miki (1937) and Uhl (1947) studied the floral anatomy and morphology of certain members of the Helobiae including *Ruppia*. Chrysler (1907) briefly explored the structure and relationship of *Ruppia* to the Potamogetonaceae and allied families. Tomlinson (1982) provides the most recent treatment of the anatomy of *Ruppia*, considered as the subfamily Ruppioideae of the Potamogetonaceae, as well as compiling an extensive listing of significant literature. Observations on the floral anatomy of *Ruppia*, with particular attention to the gynoecium, was published by Serbanescu-Jitariu (1974).

Pollination in the genus *Ruppia* has been widely discussed in the literature. Most authors have described a method of surface pollination in which the inflorescence axis is elevated above the surface of the water on an elongated peduncle. Pollen is shed, floats on the water, and is intercepted by stigmas of flowers with dehiscent anthers on peduncles which have reclined and are lying on the surface (Arber, 1920; Graves, 1908). Gamerro (1968) described a method of surface pollination involving a gas bubble which helps transport an abscised anther sac to the surface. The bubble gives the sac buoyancy on the surface until dehiscence is complete and the pollen is dispersed.

Another method of pollination may occur when the inflorescence is completely submersed. McCann (1945) described pollen grains drifting upward to be intercepted by a "stigmatic-canopy", however, he made no mention of a pollen release or transport mechanism. In considering morphological criteria such as length of peduncle and podogyne, which may exhibit ecophenic variability (Setchell, 1946), Mason (1967) differentiated *Ruppia spiralis* L. from *R. maritima* L. on the basis of fertilization behavior; the former being fertilized at the surface, the latter underwater, but gave no description of pollination in either case.

Schwanitz (1967), utilizing transmission electron microscopy (TEM), presented a very detailed analysis of microsporogenesis, pollen morphology and pollination in *Ruppia*, with particular emphasis on physiology and cytology. Pettitt and Jermy (1975) investigated the structure of the pollen wall in a number of hydrophilous angiosperms, including *Ruppia*, with TEM. Richardson (1976b) published the first detailed work on the anatomy and physiology of an underwater pollination mechanism in *Ruppia*. This provided the basis for Verhoeven's (1979) treatment of the subject and is further developed in the present study.

The first detailed study of the embryology of *Ruppia* is that of Murbeck (1902). The embryology of *Ruppia maritima* and its affinities to other genera was examined by Yamashita (1972). Ly Thi Ba and Guignard (1979) examined *Ruppia* in their work on phylogeny of the Helobiae and embryogenic criteria.

The taxonomy of the genus *Ruppia* in Eastern North America was treated by Fernald and Wiegand (1914). They established a series of varieties based on morphological characters of the peduncle, podogyne and mature carpel. Based on their treatment, the varieties of *Ruppia maritima* L. found in New Hampshire correspond to var. *subcapitata* (peduncles 0.5-1.5 cm. long; podogynes 1-6 mm. long), var. *rostrata* (peduncles 1.5-3.0 cm. long; podogynes 1-3.5 cm. long) at estuarine sites, and var. *longipes* (peduncles 3-30 cm. long; podogynes 1-3.5 cm. long) at coastal sites. The size and shape of the mature carpel was found to be highly variable at all sites in New Hampshire coastal and estuarine tidal marshes. Jacobs and Brock (1982) recently revised the genus *Ruppia* in Australia. Davis and Tomlinson (1974) described a new species of *Ruppia* (*R. tuberosa*) occupying hypersaline habitats in Western Australia. Ostenfeld (1915) and St. John and Fosberg (1939) described a new species (*R. anomala*) and variety (*R. maritima* var. *pacifica*), respectively, from tropical regions.

The taxonomic position of *Ruppia* has been a debated issue with no concensus among authors until recently. *Ruppia* has been placed in the monogeneric family, Ruppiaceae, by Hutchinson (1934, 1959). It has also been included in the Potamogetonaceae, Zosteraceae, and Najadaceae. Davis and Tomlinson (1974) reviewed the systematic position of *Ruppia*. Similarities between *Ruppia* and *Potamogeton* in vegetative morphology, anatomy, and especially floral morphology (Uhl, 1947; Posluszny and Sattler, 1974) appear to outweigh dissimilarities, thereby substantiating its inclusion in the Potamogetonaceae. Published accounts of Gamerro (1968), Davis and Tomlinson (1974), Richardson (1980), Tomlinson (1982) and Jacobs and Brock (1982) concurred on its position in the Potamogetonaceae as a subfamily or genus.

Chromosome numbers for Ruppia and other Potamogetonaceae have been reported for European, Asian and South American representatives, but are poorly known in North America. Although Graves (1908) reported the haploid *n* = 8 in pollen daughter nuclei he found it impossible to get an exact count. Reese (1962, 1963) studied *Ruppia* populations in Schleswig-Holstein. He found *R*. *maritima* s.l. to be a diploid (2 *n* = 20) and *R*. *spiralis* s.l. a tetraploid (2 *n* = 40), with a hexaploid variety of *R*. *spiralis* (2 *n* = 60) also being reported. Van Vierssen *et al*. (1981) found similar counts for *Ruppia* taxa in Western Europe as did Harada (1956) in Japan, and Gamerro (1968) in Argentina. Snoeijs and van der Ster (1983) reported a diploid (2 *n* = 20) for both *R*. *megacarpa* Mason and *R*. *tuberosa* Davis and Tomlinson with an apparent triploid reported as 3 *n* = 30 for *R*. *tuberosa* found at one location in South Australia. The chromosome numbers for *Ruppia* populations given in the present account are the first well documented records for New England and the east coast to the best of my knowledge.

The purpose of the present investigation is to demonstrate the nature of the relationships between clinal variation and reproductive biology in *Ruppia* and its ecology.

Habitats for *Ruppia* in the coastal marshes are creeks, drainage ditches and, most frequently, vertically sided pools or pond-holes 20 - 60 cm in depth. All of these habitats are aquatic throughout the year. The estuarine marshes also contain creeks, ditches and pools which may be inhabited by *Ruppia*, but most of the larger populations are found in extensive systems of shallow pannes on the high marsh. Pannes develop as secondary features in the marsh topography (see Redfield, 1972) and are subject to a highly variable seasonal environment, with some of them losing most or all of their water to evaporation by mid to late summer. The ecology of *Ruppia* populations in New Hampshire tidal marshes has been detailed in an earlier publication (Richardson, 1980). Table 1 summarizes characteristics and environmental parameters of the study areas with which this report is concerned.

My investigations in the reproductive biology of coastal and estuarine populations of *Ruppia* in New Hampshire reveal a number of reproductive strategies and physiological and morphological adaptations corresponding to a range of environmental conditions. Field studies, as well as laboratory and greenhouse experiments, all contribute to the information on growth and reproduction phenomena reported here. Tables 2 and 3 provide an outline for comparison of environmental parameters, growth and reproduction between coastal and estuarine sites.

MATERIALS AND METHODS

Field Studies - Reciprocal Transplants

Nine sites were selected as being representative of the diversity of habitats and variation in growth forms noted for Ruppia populations in New Hampshire coastal and estuarine tidal marshes (see Richardson, 1980). Where possible, each site was a donor and receptor of transplants to and from the other sites. However, habitats having strong currents (e.g., Cain's Creek) or seasonal desiccation (e.g., shallow pannes at Vols Island) were only donors. See Fig. 2 for distribution of transplants.

Collections were made at the beginning of the growing season after seedlings had become rooted in the substratum or after perennating rhizomes had initiated renewal growth. Sections of substrata containing the plants were removed with as little disruption to roots or rhizomes as possible and placed in 5 cm deep by 30 cm diameter plastic containers with a drain hole in the bottom. The rim of each container was encoded by punched holes.

Herbarium collections were made at all donor and receptor sites at the time of initial collections and at the completion of the study.

The transplants were left relatively undisturbed at most locations over a 2 - 2½ month period. During this time, environmental parameters were measured and phenological occurrences recorded. The transplants were collected at reproductive maturity or when the local population began to show signs of senesence (e.g., uprooting from the substratum). Of 38 transplants, 26 were recovered, representing a 68% return.

Peduncle and podogyne lengths were measured and compared within and between donor and receptor populations. These variables were then compared with those measured on the transplant collections and subjected to multiple range tests (LSD: Scheffe). Further, correlations were made with environmental variables (water temperature, depth and salinity) for all sites in an attempt to determine the predictability of a particular growth form or variety of Ruppia occurring in a habitat having a certain range of these variables. Although the logistics of a field transplant study with Ruppia precluded the use of a large enough set of replicates to yield data for powerful statistical analysis, the information generated in this phase of the study provides insight on Ruppia population dynamics which is substantiated by other experimental work.

Greenhouse Experiment

Thirty 5 cm thick x 15 cm diameter discs of firm peat substratum, each containing 8-10 recently established seedlings of Ruppia, were removed from the panne at Lubberland Creek to a controlled environment room in the UNH research greenhouse. Installed here is a temperature regulation bench with 9 water baths in sets of three arranged on a latin square design (see Figs. 7 and 8). Each set of three baths is connected to a heat exchanger calibrated to maintain the desired temperature range and through which the water is constantly circulated.

Each peat disc with seedlings was rinsed to remove debris and algae and planted in washed sand in a four gallon white plastic container. Three discs of seedlings were grown in water collected at the site as controls while the rest were grown in artificial sea water at selected salinity levels. Salinities were monitored with an optical refractometer/salinometer and adjusted with distilled water to compensate for evaporation.

The 30 containers were placed in the water baths with the surrounding surface area of water being covered with a 10 cm thick layer of styrofoam insulation to minimize water temperature fluctuations due to changes in the ambient air temperature. Copper vs. constantan thermocouple wires located in each water bath and above the table were connected to a strip chart recorder to determine diel air and water temperature patterns and detect fluctuations. Fans were run on a time clock in the greenhouse to help control ambient temperature. An airstone was placed in each container to keep the water aerated and in circulation to avoid physicochemical stratification in the water column. Two "Silent Giant" (Aquarium Pump Supply, Inc.) aquarium pumps provided the air supply through conventional plastic tubing regulated by gang valves.

The assignment of treatments in the experimental design assured representation of every possible combination of the three environmental parameters: water temperature, salinity and depth. However, due to spatial limitations, simultaneous replication of experimental units could not be done with only one trial being performed. After the three month duration of the experiment, 22 of the 30 units yielded plants for analysis of variation in peduncle and podogyne length and comparative phenological data, representing a 73% return. Of the 22 survivors, 3 were the control units, leaving 19 of the 27 test units to provide information on the effects of the three variables on growth and development; representing a 70% return. The 8 units in which plants died had become contaminated with blue-green algae after about 2 months growth.

Seed Recovery from Sediments and Germination Studies

The seed bank was investigated through sediment cores taken either by a frozen core technique designed by H. E. Wright, Jr., Limnological Research Center, University of Minnesota (A. L. Baker, pers. comm.) at Johnson Creek, where deep, unconsolidated sediments could not be otherwise collected while maintaining their structural continuity, or with a McCauley peat sampler at locations where the substrate was more cohesive. The cores often contained seeds deposited over a number of years. Seeds retrieved from a depth of 0 - 15 cm below the soil-water interface were germinated after being removed to the lab and washed with fresh water.

The frozen core technique yields a relatively undisturbed "core" of watery sediments within which the horizons containing seed can be examined and an estimate of age and composition of the seed bank determined. A 7 cm square by 2 m long hollow aluminum shaft tipped with a 15 lb. tapered lead head is filled with crushed dry ice over which butanol is poured. The shaft is then pushed into the soft sediments where the super-cooling effect freezes the sediments to the outside of the casing. With the help of field assistants, the shaft is withdrawn after 3-5 min. The CO_2-butanol mixture is washed out with water and as the casing warms slightly the "core" can be slipped off like a sleeve. The core is then wrapped in plastic or foil and packed in a cooler with dry ice for transport (see Fig. 9). The hollow cores are then split open, laid out and seeds extracted as the material thaws. The cores can be stored indefinitely in the frozen state, but for germination studies the seeds should be removed as soon as possible.

Ecological Anatomy and Morphology

A. Field collections were made from all study sites at various stages of growth and development from 1974-1982. Voucher specimens for all sites treated in Richardson (1980) are deposited in the Hodgdon Herbarium, University of New Hampshire (NHA).

B. Optical Microscopy. Whole plants and inflorescence axes were killed and fixed in formalin-acetic-alcohol (50%) (FAA), stored in 70% ethyl alcohol (ETOH), dehydrated and cleared through an ETOH-xylene-tertiary butyl alcohol (TBA) series, infiltrated in vacuo, and embedded in Paraplast using standard techniques (Johnasen, 1940). Transverse, longitudinal and oblique serial sections of inflorescence axes were cut on a rotary microtome at 10-12 microns for gross anatomy and 6 microns to trace the lacunar system through the inflorescence axis and for examination of pistils for the presence of pollen tubes. The sections were stained with safranin and fast green and the slides sealed with Harleco synthetic resin. Appropriate sections were photographed with a Nikon Microflex Model AFM on a Wild M20 compound microscope using Polaroid type 105 film. The morphology of vegetative and reproductive organs was examined with a Wild M5 dissecting microscope equipped as above.

C. Scanning Electron Microscopy. Whole and subdivided inflorescence axes and flowers were killed and fixed in phosphate buffered Karnovsky's glutaraldehyde-paraformaldehyde, and post-fixed in 1-3% osmium tetroxide, following the technique for electron microscopy preparation of *Lemna minor* L. by Melaragno and Walsh (1976). The specimens were dehydrated in a very gradual ethanol series and stored in 70% ETOH at 1-3°C. Dehydration to 100% ETOH then preceded CO_2 critical point drying. All stages of fixation and dehydration were carried out in vacuo. Floral parts and pollen were mounted with carbon paint or double stick tape on aluminum stubs. The stubs were then coated with paladium-gold on a Technics Hummer 2 sputter coater and examined and photographed with an AMR 1000 scanning electron microscope

(SEM) at the Museum of Comparative Zoology at Harvard University.

Investigation and Documentation of Surface and Underwater Pollination Mechanisms

A. Comparative anatomy and morphology of the inflorescence axes and reproductive organs of plants from coastal and estuarine populations was examined by optical and scanning electron microscopy as noted previously. Collections were made from natural populations and from plants grown from seedlings in aquaria. Plants from Lubberland Creek (LC), an estuarine population where underwater pollination was observed, and Awcomin Marsh (AWC), a coastal site having surface pollination, were grown in large, deep glass chromatography cylinders in filtered and aerated water collected at the sites. Individual inflorescences were tagged and a record kept of their phenology. Specimens were collected just prior to anthesis.

B. Pollen Viability. Pollen from each population was tested for viability by cytoplasmic staining with 1% aniline blue in 45% propionic acid. Pollen was tested for starch and oil content by staining with iodine in aqueous potassium iodide (IKI) and Sudan IV, respectively, following the method of Baker and Baker (1979). Pollen grains were germinated on glass slides in a 5% sucrose solution and examined for pollen tube development.

C. Pollen Studies - Clinal Variation. Pollen grain length and anther sac width measurements were recorded for

7 populations (3 estuarine, 4 coastal). Pollen was taken from 4 anthers from each of 4 different plants, with 90-120 grains being measured for each population. The lengths of 10 anther sacs from 10 different inflorescences (4 plants) were measured. These data were then correlated with seasonal means of water temperature, depth and salinity for each site. Variation in pollen morphology was examined with SEM. An estimate of the number of pollen grains per anther sac was made using two representative populations: Lubberland Creek (LC) and Awcomin Marsh (AWC). In this procedure, the pollen was shaken out of the anther sac, stained with aceto-carmine and transferred to a settling chamber on a Leitz inverted microscope. Pollen grains were counted by scanning the chamber twice, the second scan perpendicular to the first. The total number of grains per anther sac was then estimated by multiplying the sample count by a factor of 7.854, derived from a ratio of the area of the chamber to the area covered by the scan.

D. Evidence of Underwater Pollination - Pollen Tubes in the Style. Fifty carpels from each of the 3 populations where underwater pollination was observed - Johnson Creek (JC), Lubberland Creek (LC) and Vols Island (VI) - were collected after anthesis. These were sectioned by hand with microdissecting tools and stained according to the method described by Bucholz (1931). Because of the extremely small size of the carpels of *Ruppia*, this proved to be a very tedious method yielding only fair results. The following year, plants from LC were grown from seedlings in aquaria to provide material for paraffin sectioning. The developing inflorescences were observed daily and were killed and fixed in a 70% ETOH and 6.5% formalin solution immediately following anthesis when the anthers had emptied and abscised from the floral axis. This material was then carefully prepared for sectioning to minimize disturbance to the tissues and pollen tubes. The paraffin blocks were trimmed and aligned on the microtome to produce near-median longitudinal sections of the carpels at 6 microns.

E. Time-Lapse Cinemicrography. To document underwater pollination, Ruppia was grown in aquaria and the process of anther dehiscence, bubble formation and transference of pollen to the stigma recorded on cine film. Plants from an estuarine site (LC) and a coastal site (AWC) were used for comparison. The coastal surface pollinating plants, which send their flowers to the surface on extended peduncles, were periodically weighted down in the aquarium so that even with considerable peduncle elongation (20-30 cm) the flowers were forced to reach anthesis while still underwater.

An apparatus was constructed in which the body of a Wild M5 dissecting microscope was held horizontally and attached to a Bell & Howell 16 mm Filmo cine camera. A solenoid intervalometer controlled room lights and photo floods, and tripped the camera shutter release at intervals of one frame per 10 seconds. Stem sections terminated by an inflorescence on which anther dehiscence appeared to be imminent were placed in a photo chamber constructed of lantern slide glass 7.5 cm deep x 8 cm wide with an inside dimension of 0.6 cm from front to back. The chamber was sealed to a large test tube to contain the rest of the stem section. The tube and chamber were filled with water cooled by an ice bath and fan to offset the heat from the lights. The 400 feet of film exposed during filming was edited to a sequence of approximately 6 minutes.

Electrophoresis

Five populations of Ruppia (3 estuarine and 2 coastal) were analyzed for 7 enzyme systems (peroxidase, alpha-esterase, fluorescent esterase, phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), glutamate dehydrogenase (GDH), malate dehydrogenase (MDH) using starch gel electrophoretic techniques. Freshly collected material was transported to the laboratory on ice in a cooler and stored at 1°C or frozen. Plants from each site were kept in aquaria in a cold growth chamber at the Jackson Estuarine Laboratory (J.E.L.). Only newly formed leaves free of epibiota or other contaminants were used. The analyses were run under the direction of Dr. Donald P. Cheney at J.E.L., employing techniques developed for the marine alga Chondrus crispus, and Dr. Jang-Bal Ryu at the U.N.H. Forest Genetics Laboratory using methods developed for Pinus strobus L.

Chromosome Numbers

Five populations of Ruppia (3 estuarine, 2 coastal) were analyzed to determine chromosome counts and ploidy levels. Freshly collected plants were transported to the laboratory on ice in a cooler and processed immediately. Although tissues for both meiotic (young anthers and carpels) and mitotic (root tips) chromosomes were collected and examined, root tips gave superior results and were easier to handle. Mucilaginous secretions in the young floral buds created problems in maceration and staining. Root tissues were prepared for chromosome squashes by methods described in Löve and Löve (1975). The following pretreatments were used: a) cold treatment - root tips excised in distilled water and kept at 1-3°C in a refrigerator for 6-24 hrs.; b) 0.002 mol. 8-hydroxyquinoline at 10-18°C (cooled by running tap water) for 2-4 hrs.; c) saturated aqueous solution of paradichlorobenzene at room temperature for 2-4 hrs. After pretreatment the root tips were washed and fixed either in Farmer's fluid (1:3 acetic-alcohol), Carnoy's fluid (1:3:6 acetic-chloroform-alcohol) or Newcomer's fluid (6:3:1:1:1 Isopropyl alcohol-propionic acid-petroleum ether-acetone-dioxane). In all cases the fixative was mixed daily as needed. Farmer's fluid gave consistently good results and was used most frequently. After 6-24 hrs. fixation at room temperature the tissue was transferred to 70% ETOH for storage at 1-3°C. Material stored in ETOH was transferred to 45% acetic acid overnight prior to hydrolysis and maceration. Hydrolysis with 10% HCL required as much as 3 hrs. at 60°C to achieve a maceration that resulted in a monolayer of cells when the root tips were squashed under a coverslip. Several stains were used, including aceto-carmine, aceto-orcein, lacto-propiono-orcein, and the Feulgen technique with leuco-basic fuchsin. Ultimately, best results were obtained with a modified Feulgen technique utilizing 3% pararosaniline hydrochloride, staining for 1-3 hrs. until an intense purple stain developed. The root tips were squashed in 45% acetic acid. Chromosome counts were obtained with an American Optical phase-contrast microscope equipped with camera lucida. Drawings of late prophase figures were made at 1000 x. Selected figures were photographed by focusing at 3 planes through the cell to account for all parts of all chromosomes. Three transparencies were then sandwiched together and brought into register to project the complete chromosome complement.

RESULTS

Reciprocal Transplants

Coastal and estuarine populations of _Ruppia_ exhibit a range of variation in vegetative, floral, pollen and fruit-seed morphology. Annual and perennial reproductive strategies and underwater versus surface pollination mechanisms are employed by different populations in relation to their environmental parameters. A reciprocal transplant study was designed and executed to demonstrate the effects of recorded levels of water temperature, salinity and depth at different sites on the growth and development of _Ruppia_ from the various populations. Other characteristics of the habitats such as oxygen content, turbidity, epibiota and algal mats (see Richardson, 1980) were also considered, but their influence on morphological variation is not quantified in the present study. The locations of the sites are shown in Fig. 1, while the distribution pattern of the transplants is illustrated in Fig. 2, where two way arrows indicate reciprocal exchange between donor and receptor sites, while one way arrows indicate sites which were only donors, such as the shallow pannes at Vols Island (VIs), where extreme evaporation and desiccation precluded recovery, and Cains Creek (CC), where strong currents washed away the transplants. Seasonal means for the environmental parameters for all sites are presented in Table 1.

Peduncle and podogyne length and fruit-seed ('mature carpel') size and shape, the morphological criteria upon which Fernald and Wiegand (1914) based the separation of Ruppia maritima L. into a series of varieties in Eastern North America, are shown to be very plastic. Recorded lengths for peduncles and podogynes from six populations (3 coastal; 3 estuarine) are plotted against water temperature, salinity and depth in Figs. 3 and 4. In Fig. 3, the relationship between peduncle length and the mode of pollination is apparent, with the coastal, surface-pollinating plants having considerably longer peduncles than the estuarine, underwater-pollinating plants in all cases. An increase in peduncle length between the coastal sites shows a direct relationship to mean water depth. In Fig. 4, podogyne length is shown to exhibit variation, however, the pattern of variation is less well associated with the mode of pollination or an environmental condition. Variation in podogyne length may be a function of post-fertilization behavior, at least partially under endogenous control of the plants. Fruit-seed morphology within a population and even on an individual plant, as shown in Fig. 10C, is so highly variable that this character was eliminated as having little value as a morphological criterion for taxonomic distinctions. Least significant difference (LSD) and Scheffe multiple range tests were used to assess the difference or similarity of donor and receptor populations and the transplants. These results, summarized in Table 4, indicate that the estuarine plants were more plastic than the coastal plants, showing a significant variation in both peduncle and podogyne length in a number of cases. Those transplants which showed a significant difference in both peduncle and podogyne lengths, indicated as $^{+}/+$, were in all but one case from estuarine donor populations. A significant difference in peduncle length in coastal donor population reflects the effect of water depth and pollination behavior, i.e., the extension of the flowers to the surface on the peduncle. In some cases, coastal plants from deep pond-holes still grew elongated peduncles when transplanted to shallower receptor sites.

In several cases, the peduncle and podogyne lengths and the growth habit of a transplant more closely resembled the receptor population than the donor population from which it came. Figure 5 shows specimens of the donor population from a shallow panne at the Vols Island site on Great Bay and the receptor population at Johnson Creek, a deep pond-hole in the marsh on the upper reaches of a small estuary, together with the transplant. All three specimens were collected on the same day at the termination of the field study. A graphic representation of this example is given in Fig. 6, where the mean and range values for peduncle and podogyne lengths in var. subcapitata Fern. & Wieg., which best describes the Vols Island plants, and var. rostrata Agardh, which best describes the Johnson Creek plants, are plotted on the ordinate, while points arbitrarily equidistant on the abscissa allow for the construction of a polygon to visualize the relationship. The polygon representing the transplant (S. 2 n 4) is shown to conform more closely to that of the receptor (JC) than that of the donor (VIs).

Transplants from estuarine populations exhibited more plasticity as a result of response to receptor habitat conditions than did those from coastal populations. Although Figs. 3 and 4 indicate greater variation in peduncle and podogyne lengths for coastal populations, the reproductive biology and associated morphology of these plants account for more of the variation than do the environmental parameters. Variation in peduncle length in the coastal plants is a function of the surface pollination mechanism in which the flowers are elevated from the point of initiation on the sympodium to just above the water surface where pollination occurs. First formed flowers rise up from deeper water and as the plants grow toward the surface, the later formed flowers rise to the surface on progressively shorter peduncles. Peduncle length in the coastal populations is not, however, entirely associated with water depth and the point of origin of the inflorescence; occasionally long flexuous peduncles were observed lying horizontally just under the water surface even when water depth was at a maximum.

The peduncles of the underwater pollinating estuarine plants varied less and are more uniform in length on an individual plant regardless of time of origin or position on the sympodium.

Podogyne lengths varied considerably less than peduncle length in a given population. However, the shortpodogyned var. subcapitata transplants developed longer podogynes in var. rostrata or var. longipes Hagstr. receptor sites. The adaptive significance and function of the podogyne is discussed later. Clinal variation in floral and pollen morphology was not measured in the transplants, but was examined for all populations and the results are reported in the section on pollination.

Greenhouse Experiment

The purpose of this phase of the study was to determine the effect of three ranges of water temperature, salinity and depth on Ruppia plants from the same natural population - Lubberland Creek (LC). The plants were grown under controlled conditions over a 3 month period, after which all were collected, phenological data recorded and peduncle and podogyne lengths were measured for comparison. The experimental design and the set-up in the UNH research greenhouse are shown in Figs. 7 and 8. Occasional extremes in the greenhouse ambient air temperature and malfunctions of compressors in the heat exchangers caused temperature fluctuations beyond the prescribed ranges and must be considered as confounding factors in the experiment. Summary data for the experiment are presented in Table 5. Spatial limitations precluded the use of a large enough set of replicates of the experimental units to generate a substantial data base for regression analysis, however, examination of the effects of the independent variables on growth and development of the plants yielded further information on the adaptive capacity of Ruppia to tolerate various levels of these variables.

In the reciprocal transplant study, estuarine plants exhibited a broader range of morphological variation than the coastal representatives. The Lubberland Creek plants used in this experiment appear to substantiate this observation in that there was growth and reproduction for almost every treatment, with the most vigorous growth in the deeper water (30 cm) containers.

The maximum reproductive effort (number of fruits produced) corresponded to the following levels of the 3 variables: $T^{o}C = 16.5$, S o/oo = 30, Dcm = 30 in the experimental units. The maximum reproductive effort for the control units was $T^{o}C = 22.5$, S o/oo = 15, Dcm = 20 with 54 mature fruits being produced, while at 12.5°C and 16.5°C, there were 8 and 13 mature fruits produced, respectively. The salinity and depth in the control units were constants, using the natural water collected at the time the experiment was initiated.

All treatments which reached reproductive maturity had underwater pollination. A range in variation in peduncle and podogyne length was recorded; 0.5 - 1.8 cm for the former and 0.6 - 1.3 cm for the latter, indicating that both var. subcapitata and var. rostrata could be represented on the basis of these characters. However, results of the statistical analysis showed no significant correlation between peduncle and podogyne length and levels of any of the three independent variables. Table 6 gives the total reproductive effort and mean peduncle and podogyne lengths for each level of water temperature, salinity and depth.

Growth habit was influenced by depth, with the shallow water (Dcm = 10) treatments yielding procumbent plants with short internodes while the deeper containers yielded more erect plants with longer internodes. Variation in growth habit corresponding to depth was observed *in situ* at LC where the population grows over an area of expansive pannes with shallow and deeper regions.

Seed Recovery from Sediments and Germination Studies

All sites were sampled to determine the presence and nature of a seed bank in the sediments. Cores taken either by the frozen core technique (Fig. 9) or with the McCauley peat sampler were examined for structure and content. Stratification of sediments and plant remains in the 50-100 cm core samples obtained contributed information on the ontogeny of the habitat, the length of time *Ruppia* may have occupied a site and the viability of seeds in the seed bank. Table 7 summarizes this information.

The dispersal unit in *Ruppia* is a fruit-seed; the fleshy pericarp wall rapidly decomposes at or soon after the time of shedding, leaving a persistent bony endocarp surrounding a membranous testa. The structure and function of the fruit-seed-podogyne unit is discussed later.

Sediments containing the most abundant seeds were found at the 0-5 cm level in estuarine populations. However, seeds recovered from levels down to 15 cm germinated when placed in fresh (tap) water and exposed to moderate daylight at ca. 20°C in the laboratory. It is problematic as to how in situ conditions at that depth, in a reducing anaerobic environment, would allow for germination unless the sediments are turned over by burrowing organisms or otherwise disturbed.

Seeds collected by sieving the upper layers of sediments in late fall or during the winter of 1974-1975 from JC, LC, VI, TR and AWC were stored in the dark at 3°C in filtered natural water for 8 years. Each spring 10-15 seeds from each collection were removed, placed in petri dishes in fresh water as mentioned above and germinated. During the first 5 years after the collections were made, germination was nearly 100% in all cases and 40-60% of the seedlings could be grown to reproductive maturity when transferred to containers of filtered and aerated natural water. After 6-8 years of cold storage, the percent of germination lessened and most seedlings lost vigor and died.

The estuarine annual populations (JC, LC, VI) produce their seed crop over a 4-6 week period in July-August, shedding abundant mature fruit just prior to senescence and/or desiccation of the habitats (see Richardson, 1980). The coastal populations (AWC, TR) develop seed over a longer period due in part to the permanence of the aquatic habitat and to the slower maturing perennial plants, however, considerably less seed per plant was produced.

In the coastal populations there was usually limited seasonal recruitment from seeds. However, in areas where there had been extensive die-back, the seed reserve was essential to reestablish a population. In 1974 the plants at AWC produced very few fruits and almost no seeds, even though flowering was abundant and the water surface was nearly covered with yellow masses of pollen. Over the following 2 years this site was devoid of Ruppia, but a sediment sample revealed seeds in the upper 5 cm layer. In 1977 growth of Ruppia was reestablished from the seed bank and fruit-seeds were produced on vigorous plants. Perennating rhizomes then provided renewal growth until 1979, when the population again died back. From 1980-1983, however, vigorous growth was again observed. The seed bank contributed substantially to the reproductive success of this population, providing a back-up system when regular seasonal growth was interrupted.

TR, a deep pond-hole which contained abundant Ruppia in 1974, was also nearly devoid of the plant in 1975. The water in the pond-hole became increasingly milky-cloudy during spring and summer of 1975. Ruppia has yet to reestablish in this pond-hole even though a panne adjacent to it has supported vigorous growth every season. The disappearance of Ruppia has been noted at other coastal sites as well, and almost always in isolated, deep, vertically-sided pond-holes.

Following scenescence of the estuarine populations in late summer, the habitats appeared to contain no Ruppia, since the plants detach from the bottom and are washed out or become tangled in algal mats and float up into the surrounding grasses in flood tides. Each spring germination and seedling establishment occurred at most of the estuarine sites examined. Although some areas of extensive pannes at LC and a pond-hole at VI had very scant growth during one or two seasons, vigorous growth subsequently returned while other areas suffered seasonal population depletion.

Biomass and percent cover of Ruppia in panne areas may vary from year to year (Richardson, 1980). Relatively sparse growth observed following a season of dense growth and high fecundity at LC and VI may be attributed to adverse environmental conditions in either late summer (e.g., extended drought) or the following spring, when conditions were not conducive to a high rate of germination (e.g., high soil salinity resulting from evaporation during the previous season, followed by insufficient dilution by snowmelt or rainfall). Sediment samples collected during the season of sparse growth contained many ungerminated seeds. The following season, when a dense growth of Ruppia again flourished, the sediments contained fewer ungerminated seeds. The assumption is that reestablishment of these populations is contingent on favorable environmental conditions and on seed production from the previous season, as well as on the seed reserve from two and possibly more years past.

Ecological Morphology and Anatomy

Numerous voucher specimens of Ruppia, collected from populations throughout its distribution in ecologically distinct habitats in coastal and estuarine tidal marshes in New Hampshire, show variation in growth habit corresponding to differences in environmental conditions. Photographs of specimens of mature Ruppia plants from 6 sites (3 coastal, 3 estuarine) together with a detailed description of their ecology are given in an earlier paper (Richardson, 1980). In the present study, morphological variation in growth habit (Figs. 5, 12), reproductive organs (Figs. 13, 14), pollen grains (Figs. 12, 25-28) and fruit-seeds (Fig. 10) was observed within and between populations.

Annual populations of Ruppia found in the extensive shallow pannes at estuarine sites such as LC and VI are characterized by a procumbent growth habit with distinctly forking stems and short internodes. Collections revealed persistent fruit-seed coats attached to the rhizomes throughout most of the growing season (Fig. 11). The first inflorescence develops early, at the third to the fifth node of the distichous monopodium. Subsequent inflorescence development occurs with progressively shortened internodes as the plant reaches the surface. The growth habit becomes sympodial distally in association with flowering, and is otherwise modified in response to environmental factors. The number of inflorescences formed per plant in a given population is approximately the same, but the arrangement of inflorescences relative to internodal length and branching is largely a function of water depth.

Differences in water depth in the same panne or pond-hole, or between sites, is reflected in the internodal lengths and erectness or procumbency of the plants. Plants in shallow areas of a panne exhibit a very dense, compact, procumbent growth habit with short internodes and a fan-like display of inflorescences, while in adjacent deeper water they are loose and flexuous, with a more diffuse appearance to the display of inflorescences.

Stems lying closely over the bottom sediments become stoloniferous, usually with a single adventitious root developing at each node. These roots grow rapidly, and when they become attached to the substratum, root hairs anchor them. The stem is drawn closer to the bottom as other roots likewise secure the bifurcating stems. As sediments are deposited over the spreading stolon system, additional adventitious roots develop at the nodes. Although the roots, once attached, apparently pull the stem down from near vertical to a horizontal plane along the bottom, there is no anatomical evidence of contractile tissue. However, some roots become very elongated (10-15 cm) and then coiled following contact with the substratum. Many of the nodes on the vertical monopodium, as well as on the reproductive sympodium, also have an adventitious root which is often quite short (2-6 mm) and surrounded by a sheath.

Anatomically, the coastal plants showed somewhat more abundant vascular tissue than the estuarine plants, although still highly reduced, as well as a slightly thicker surrounding mantel of cortical and epidermal tissues in the rhizomes, stolons and vertical stems. The coastal plants exhibited a more robust and vigorous appearance, no doubt the result of adaptation to an environment in which tidal flooding, currents and higher salinities affect their gross morphology.

Water temperature differentials at a site, due to differences in depth or surface cover by algal mats and the canopy of Ruppia, were found to cause the flowering period to continue throughout the time required for all areas to reach the optimal temperature for reproduction (18-22°C). Sites characterized by isothermal conditions had shorter flowering periods once the water temperature reached the thermal optima for reproduction. Temperature stratification in the water column influenced flowering and fruit-seed production by lengthening the duration of the reproductive phase (Richardson, 1980).

The effect of salinity levels on growth and development of Ruppia along an ecocline of estuarine sites with a seasonal range of 2-20 o/oo, to coastal sites having a range of 20-32 o/oo for most populations, corresponds to the reproductive strategy of the population. In estuarine populations, low spring season salinities facilitate rapid germination and seedling establishment, and a phenology in which vegetative growth, flowering and fruit-seed production occur prior to the seasonal salinity maximum. Ruppia populations at these sites employ an annual reproductive strategy. In the more saline coastal habitats, the populations employ a short-lived perennial or biennial reproductive strategy, in which renewal growth is initiated in the spring on overwintering rhizomes. Following a period of winter quiescence, renewal growth in early spring occurs in buds on the rhizomes and on fragments of detached vertical stems which persist through fall and winter on the bottom or along the edges of deeper pools. Occasionally, renewal growth is observed in late fall at nodes on otherwise senescent stems. The new growth persists throughout the winter as bright green vigorous shoots developing under several inches of ice. After iceout, these precocious shoots develop very rapidly and are the first to form inflorescences. However, they account for only a small portion of the total biomass of seasonal growth from rhizomes for the population.

Sexual reproduction in the estuarine populations is almost exclusively associated with an underwater pollination mechanism (Fig. 19) resulting in a large seasonal seed crop. The coastal populations were found in all cases to have surface pollination (Fig. 18) which results in fruit-seed production, but often with considerable fluctuations in fecundity. On occasion, I noticed bubbles emerging from dehisced anther sacs on the flowers of coastal, surface-pollinating plants while still submersed. However, no fruits were produced on flowers which did not reach the water surface. Similar results were obtained when plants from these sites (AWC, TR) were forced to reach anther dehiscence underwater in aquaria.

Fruit development in Ruppia is very rapid, with the carpel being extended on a podgyne which reaches maximum length only a few days after fertilization. Initially, the podogyne is somewhat curved and flexible but soon becomes straight and rigid due to sclerification of tissues. The fruit color changes from green to reddish-brown and finally black. As the endocarp becomes sclerified and darkened, the outer layers of the pericarp become increasingly translucent. The peltate stigma drops off very soon after fertilization and the rostrum becomes more pronounced. The mature fruit has a greater specific gravity than water and when shed, with the podogyne still attached, sinks rapidly to the bottom. As mentioned earlier, the dispersal unit in Ruppia is actually a fruit with only the bony endocarp remaining; the testa is membranous. The term fruit-seed, as used by McMillan (1981) in his discussion of seed reserves and seed germination in Halodule and Syringodium, most accurately describes this structure in Ruppia, although the terms achene, druplet, nutlet and mature carpel have also been applied.

The morphology of the fruit-seed with podogyne intact is well suited for implantation of the seed in the substratum. The seed becomes oriented in a position that ensures the attachment of the first adventitious root to emerge during germination. The primary root in Ruppia is vestigal or abortive (see Graves, 1908; Gamerro, 1968). The fruit-seed-podogyne structure functions like an arrowhead on its shaft; the pointed tip (rostrum) of the fruit-seed is directed into the bottom material by the podogyne.

The fruit-seed, when mature, has a distinct dehiscence mechanism for germination. There are specialized perforated areas in the fruit-seed coat through which water is apparently imbibed prior to germination (see Fig. 10A,B). As the endocarp becomes sclerified, these areas of loose parenchyma do not become as hard or consolidated as the rest of the wall (cf. Mason, 1967; Gamerro, 1968). These areas are located on either side of the fruit-seed near the rostrum, close to the point of dehiscence where the cotyledon and adventitious root first emerge at germination (Fig. 10, B). Mason (1967) suggested that the function of the perforated areas may be mechanical, allowing the endocarp to change shape during germination. The fruit-seed coats I examined did not appear to change shape at germination, and mechanical or biochemical scarification of the endocarp was not detected, except for a gradual softening of the perforated areas. During the process of dehiscence a triangular operculum opens, with its pointed end toward the rostrum, hinging proximally near the podogyne (Fig. 10,B). It is through this opening that the germinated seedling emerges. A similar structure opens at germination in Potomogeton.

In early spring, prior to germination and when the water is clear, myriad podogynes have been observed bristling from the bottom, with the fruit-seeds covered by sediments. Seedling collections reveal that the fruit-seed-podogyne remains attached to the new plant for a considerable period (Fig. 11), and possibly throughout the season in annual populations, although retrieving them after the reproductive period is difficult due to senescence of the root-rhizome system. In late season, the vertical axis of the plant often becomes detached from the rhizome, although flowering and fruit production may continue for a short while thereafter.

Investigation and Documentation of Surface and Underwater Pollination Mechanisms

Comparative anatomy and morphology of the inflorescence axis and reproductive organs of Ruppia from coastal and estuarine sites revealed significant differences in the size and relative proportions of organs, cells and, to some degree, tissues. The size and shape of anther sacs (Fig. 13), the size and conformation of stigmatic surface epidermal cells (Fig. 14) and the size and exine architecture of pollen grains (Figs. 12, 25-28) all exhibit considerable variation. These differences were consistent between coastal and estuarine populatons, and provide a more reliable measure of variation than does vegetative morphology.

The upper flower of an inflorescence of a coastal (AWC) and an estuarine (LC) population are shown for comparison at the same magnification in Fig. 13. Although the flower from AWC appears considerably larger than the one from LC, it is the difference in anther size and shape which accounts for this. The reniform anthers of coastal surface-pollinating plants were consistently larger than the ellipsoidal ones of estuarine underwater-pollinating plants.

Based on earlier work (Richardson, 1980) and more recent observations, the seasonal salinity regime was the factor shown to be most closely associated with variation in floral morphology. Figure 15 shows the relationship between salinity and anther size for 5 sites distributed along an ecological gradient from estuarine to coastal tidal marshes. It can be seen that a similar relationship also exists for pollen grain size (Fig. 16). A plot of pollen grain size against anther size (Fig. 17) shows a very close relationship between these two characters for the 3 estuarine populations, as distinct from the 2 coastal populations. A pronounced cluster of the data points separates the coastal sites (OP, AWC) from the estuarine sites (JC, LC, VI) in each of these plots. The smaller anther sac and pollen grain size for the 3 estuarine populations all fall within a considerably lower salinity regime than those from the coastal populations. Collectively, the coastal and estuarine populations are significantly different from each other. Mean pollen grain length and anther sac length for 7 Ruppia populations (3 estuarine, 4 coastal) is given in Table 8. It is interesting that TIB and CC fall about midway in the range of variation. Both are coastal sites, but have unique habitat conditions. TIB is an impounded marsh subject to freshwater runoff and, with almost no tidal influence over the past 10 years, has a very low salinity level (see Table 1). CC is a creek on the landward edge of an expansive coastal marsh with a broad range of salinity (2 - 32 o/oo) accounted for by readings taken at low (freshwater runoff) and high (salt water intrusion) tides. The habitat conditions at CC also explain the lack of similarity between this and other coastal populations for peduncle and podogyne length relative to levels of water temperature, salinity and depth (Figs. 3 & 4).

The number of pollen grains per anther sac for a coastal (AWC) and an estuarine (LC) population was computed, and estimated per flower and per inflorescence (Table 9). The coastal population was shown to have more than twice the pollen grain complement. The random scattering of pollen by wind and water in a coastal, surface-pollinating population such as AWC would surely be more effective with a higher number of dispersal units.

Aspects of surface pollination are illustrated in Fig. 18. The flowers are extended above the surface on peduncles (Fig. 18A) where, during anther dehiscence (Fig. 18B), pollen is shed and collects on the surface film in chains (Fig. 18C). The pollen grains in the chain are held together by the surface tension of the meniscuses formed between the reticulate exines of adjacent grains. The pollen chains are blown across the water surface where they may be intercepted by the stigmas of flowers on inflorescences which have reclined to lie in the surface film following anthesis. The exine architecture of the pollen of surface-pollinating plants is thicker and more pronounced than that of the pollen of underwater-pollinating plants (see Figs. 25-28). The more elongate curved shape of the pollen grains of surface-pollinating plants with the almost bulbous, dumbbell-like protruberances of the modified colpus areas, which are devoid of reticulate exine, are structural features which aid in pollen-chain formation. The epidermal cells on the stigmatic surface of surface-pollinating plants also showed variation from those of underwater-pollinating plants (Fig. 14). The stigmas of the surface-pollinating plants (Fig. 14A) have deeper furrows between the epidermal cells, giving them a more papillate surface than the smoother, more undulate stigmatic surface seen in the underwater-pollinating plants (Fig. 14B). This may represent an adaptation in the surface-pollinating plants to prevent desiccation (although a thicker cuticle layer was not detected) and may possibly be designed in such a way as to overcome the physical forces between the surface film and the pollen chains in order for floating pollen grains to adhere to the stigma upon contact.

Underwater pollination was studied in great detail, since it is of major adaptive significance to the reproductive biology and ecology of Ruppia populations which inhabit estuarine sites subject to large fluctuations in seasonal levels of water temperature, salinity and depth. The mechanism by which underwater pollination occurs has never been thoroughly documented in the literature. To verify underwater fertilization it was necessary to establish that the pollen was viable and, secondly, to show evidence of pollen tubes in the styles of underwater-pollinating plants. Pollen samples from the estuarine, underwater-pollinating populations (JC, LC and VI) all showed nearly 100% viability when examined by cytoplasmic staining, and were packed with starch grains. Pollen from the coasta, surface-pollinating populations (OP, AWC, TIB and CC) likewise had nearly 100% viability, but with somewhat less densely packed starch grains. The round starch grains of a sectioned pollen grain can be seen in Fig. 24C. Numerous fixations and serial sections of carpels from underwater-pollinated plants (LC) resulted in direct evidence of underwater pollination. Figure 20A shows a median longitudinal section through a carpel with stigma pollinated underwater. A germinated pollen grain on the stigmatic surface with its pollen tube penetrating the stylar canal is shown in Fig. 20B.

In underwater pollination in Ruppia, gas bubbles emerge from within the anther sac as the anther dehisces, carrying pollen grains out of the theca into proximity with receptive stigmas of the same flower. Since the source of this gas was unknown, the lacunar system of the inflorescence axis was investigated anatomically to see if it serves as a conduit for photosynthetic gases evolved elsewhere in the plant to the anther sac.

An open lacunar system develops above the node at which the inflorescence axis originates. The term inflorescence axis, as used here, refers to the upper portion of the peduncle upon which the two sessile, subopposite flowers are located. My observations concur with those of Gamerro (1968) and Graves (1908) that the lacunae of the inflorescence axis are extensive, traverse its entire length and are larger and more numerous per unit area than in the vegetative axis. The lacunae of the floral axis, however, do not pass through the anther connective, but terminate among fairly loosely packed parenchyma cells at the base of the connective (Figs. 21, 22, 23). The parenchyma of the connective is more closely packed, but has distinct intercellular spaces (Figs. 21F, 22D) through which gases may diffuse. In none of the sections examined was a lacuna observed which actually entered the base of an anther sac, thereby supplying a direct source of the internal gas to function in the pollination mechanism.

Each stamen in Ruppia is composed of two large bilocular anther sacs separated by a broad connective (Figs. 13, 23). The stamen was examined in detail in transverse, longitudinal and oblique sections of the inflorescence axis (Figs. 21, 22). Deeply staining tanniniferous cells were found scattered as idioblasts throughout the inflorescence axis and on stigmas, but were especially concentrated in the epidermis of the anther connective in contiguous bands, often entirely surrounding the connective (Figs. 21A,B). The tanniniferous cells surrounding the anther connective may serve to direct the diffusion flow to the anther sac by creating a boundary through which the gas cannot escape. In a study of the floral morphology and anatomy of the seagrass Thalassia testudinum, Tomlinson (1969) found that tannin sacs /sic/ were conspicuous, especially in the anther connective. Esau (1965) stated that the function of tanniniferous cells may be to protect the plant against desiccation, decay or injury.

When the internal lacunar system is charged with gases produced during photosynthesis, the pressure is sufficiently high to force gas through intercellular spaces which may be partially occluded with mucilaginous material. This substance, which is produced in the parenchyma of the anther connective and elsewhere in the inflorescence, becomes more abundant and increases in viscosity as the plant matures. Young specimens showed only slight traces of mucilage whereas transverse sections of older floral structures were more deeply staining due to increased amounts of mucilage. The mucilaginous secretions may function to plug injured areas of inflorescence axes. When a portion of an axis was nicked so as to cut through the epidermis, bubbles emerged from the incision, but were later blocked by these secretions. When an anther was removed from the connective prior to dehiscence, profuse bubbling was evidenced at the point of separation in the exposed tissue. However, when an anther which has gradually released its pollen through the bubbling mechanism abscises from the axis, the abscission zone is effectively sealed off by mucilaginous substances so that no further loss of gases from the internal lacunar system occurs. The development and function of mucilage on the external surface of organs of a number of submersed aquatic plants (e.g., Potamogetonaceae, Nymphaeaceae) has been documented (see Arber, 1920; Sculthorpe, 1967), but the significance of internal mucilage production is less well known. The trichome-diaphrams formed across the intercellular spaces in _Nuphar lutea_ are coated with mucilage even though they are only in contact with the internal atmosphere (Arber, 1920). Also noted in sections of the anther connective were apparently suberized cell walls which may indicate the formation of an abscission zone just prior to or during anther dehiscence. These cells appear as a band of darkened, deeply-staining cell walls where the anther sac/connective is attached to the inflorescence axis in Fig. 21E. Shortly after the anther sac is emptied of most of its pollen by the bubbling mechanism, it is abscised from the connective.

The internal atmosphere of submersed vascular hydrophytes has been dealt with in detail by Williams and Barber (1961), Hartman and Brown (1967), Sculthorpe (1967), Dacey (1980) and Weaver and Wetzel (1980). The components of the gaseous atmosphere within the lacunar system are carbon dioxide, oxygen and nitrogen in order of increasing percent by volume under most conditions. Sculthorpe (1967) stated that during periods of active photosynthesis the gas pressure in the lacunar system steadily increases, with leaves, stems and petioles becoming distended until the internal pressure overcomes the surface tension of the water at small openings (e.g., vestigial stomata) or tears in the epidermis through which bubbles are forced out. At high rates of photosynthesis evolution of oxygen proceeds at a faster rate than that of the other gases, and oxygen therefore accounts for the largest percent by volume of the gases in the lacunae. The rate of bubbling increases with light intensity. Due to the gas pressure generated in the lacunar system, diffusion of gases evolved in photosynthesis occurs between the termination of the lacunae in the parenchyma of the anther connective and the interior of the anther sac.

Time-lapse cinemicrographic sequences of underwater pollination in plants from an estuarine population (LC) unequivocally revealed the mechanism by which pollen grains are carried out of the dehiscent anther sac on the outer surface of a gas bubble (Figs. 19C,D). It was further substantiated that the formation of the gas bubbles is not attributable to gases produced within the anther sac during lysis of the tapetum, but is caused by gases produced in the lacunar system of the plant during photosynthesis. This was evidenced by the formation of numerous bubbles from a single anther sac during the period of maximum photosynthesis. Few or no bubbles were noted both in situ and in the laboratory on cloudy days or under low light conditions. Gases produced by the breakdown of tapetal cells or other tissues in the anther sac may function in and be released at the onset of anther dehiscence. However, a functional endothecium (see Fig. 24B) probably contributes the actual force resulting in dehiscence. The photosynthetically produced gas bubbles appear to open the sac further as they expand. In most cases dehiscence is gradual rather than explosive.

As the pollen-bearing bubble enlarges, often into a teardrop or pear-shaped form, it comes in contact with an adjacent receptive stigma. A portion of the pollen grains borne on the surface of the bubble adhere to the stigmatic surface. Some of the pollen grains may also be carried to the water surface when the bubble separates from the stomium of the anther sac. Upon reaching the water surface, the bubble may burst, dispersing the pollen grains, or the bubble may be trapped for some time within the surface film of the water. This is especially true if, as under natural conditions, there are strands of filamentous algae present in the surface film. The chances of a pollen-coated bubble, once released from the anther sac, being intercepted by the stigma of another flower in the three-dimensional sub-surface realm is remote indeed. The bubbles, once released, do not drift underwater but rise very rapidly and directly to the surface.

Occasionally, cleistogamy was observed where anther dehiscence occurred in flowers that were still enclosed in their sub-floral leaf sheaths (Figs. 19A,B). Successful fertilizations were recorded in all cleistogamous pollinations observed in aquaria. In very shallow pannes at LC, and in shallow areas at other estuarine sites, cleistogamous pollination was observed to occur on approximately 20-35% of the inflorescences. Cleistogamy may be of considerable adaptive significance in the estuarine Ruppia populations inhabiting shallow pannes subject to evaporation, resulting in a rapid loss of water depth, and temperature and salinity extremes. The sub-floral leaf sheaths serve to protect the flowers from desiccation by maintaining an aqueous environment around the flowers inside their envelope.

Inflorescences of plants from a coastal population (AWC) which were raised in aquaria and forced to reach anthesis underwater were filmed to determine whether underwater pollination and fertilization occur. The results show that although bubbles emanate from dehiscent anther sacs, the bubbles do not function in the transference of pollen to the stigma. Bubbles from a single anther would form, enlarge and break away to be followed by another one, often over a period of 2-4 hrs., but no pollen was carried out of the sac on the surface of the bubble. The pollen in these surface-pollinating plants may be held in mass in the anther sacs by a thecal slime, as noted in certain seagrasses by Pettitt

(1980), until the flowers reach the water surface. Eventually the anther sac would abscise from the inflorescence axis and float to the surface where the pollen was ultimately dispersed in a fashion similar to that described for Ruppia cirrhosa by Gamerro (1968). Flowers of this species which did not reach the water surface to intercept pollen delivered to or shed on the surface produced no fruit.

The reports by Schwanitz (1967), Gamerro (1968) and Verhoeven (1979) concerning the possibility that both Ruppia maritima s.l. and R. cirrhosa (Petag.) Grande (= R. spiralis L. ex Dum.) may employ either a surface or underwater pollination mechanism depending on changes in water level were not confirmed by my studies.

The mode of pollination and the associated mechanisms were found to be the most markedly different characteristics in the reproductive biology of coastal versus estuarine Ruppia populations in New Hampshire tidal marshes.

Electrophoresis

Electrophoretic techniques were used in an attempt to assess the degree of genetic differentiation between coastal and estuarine Ruppia populations. The results were largely inconclusive. Although bands of activity were obtained on the gels, no apparent variation between populations was detected. Had discrete banding patterns revealed electrophoretic variation in the enzyme systems tested, the degree of polymorphism observed between populations and its genetic basis might have been quantified using the phenotypic identity statistic described in Cheney and Babbel (1978). The grinding/extracting procedures used were designed for Chondrus and Pinus and not developed specifically for Ruppia. At my request, Drs. Cheney and Ryu allowed Ruppia samples to be run simultaneously with their analyses. However, limitations of time and space precluded a more in depth experiment under the direction of a qualified researcher. The nature of phenotypic plasticity and life history differences expressed by the populations, therefore, could not be determined at the molecular level. The recently developed techniques for microelectrophoresis of single pollen grains (see Mulcahy et al., 1979; Ruchel, 1976) involving polyacrylamide gradient gels and isoelectric focusing might be a more appropriate diagnostic test (D. L. Mulcahy, pers. comm.).

Chromosome Numbers

After considerable trial and error and modifications of techniques, a relaible method of effectively staining chromosomes from root-tip squashes of Ruppia was obtained. The pararosaniline hydrochloride technique consistently provided well defined, intensely staining chromosomes from which accurate counts could be made. Even in a well stained, monolayer squash preparation, however, the blocky root-tip cells in which late prophase or early metaphase figures were found required focusing through several planes to draw or photograph the chromosomes. Figure 29 shows Camera Lucida drawings of root-tip chromosomes from 5 populations (3 estuarine, 2 coastal; LC, JC, VI and AWC, TR, respectively). Chromosome numbers for the estuarine underwater-pollinating populations are diploid (2 _n_ = 14) while those for the coastal, surface-pollinating populations represent a tetraploid (2 _n_ = 28). The chromosome complement for the diploid estuarine plants consists of 6 larger somewhat curved chromosomosomes and 8 smaller dot-shaped ones. There are 4 large curved chromosomes, 8 linear or slightly curved ones and 16 dot-shaped chromosomes in the tetraploid coastal plants. Figure 30 shows optical micrographs made from 3 transparencies taken at different planes through a cell and then sandwiched together and brought into register along with a Camera Lucida drawing of the same chromosomes for a diploid and a tetraploid population. The root-tip cells showed variation, with those from the tetraploid population being consistently larger, although comparative measurements were not taken. Morphological features such as larger anther sacs and larger and more numerous pollen grains in the coastal as opposed to the estuarine plants are also associated with polyploidy. The chromosome numbers reported here for _Ruppia_ populations in New Hampshire coastal and estuarine tidal marshes differ from any reported in the literature for _Ruppia_ taxa elsewhere.

DISCUSSION

This study in conjunction with my previously published work (Richardson, 1976b, 1980) contributes further to the understanding of autecological and genecological phenomena in _Ruppia_ populations in New England, a region in the cosmopolitan distribution of the genus which has received little attention. _Ruppia_ taxa have been studied world-wide from nearly as many perspectives as there are disciplines in natural science. The data resulting from this investigation of variation, adaptation and reproductive biology in _Ruppia_ populations from New Hampshire coastal and estuarine tidal marshes reveal both similarities between these populations and those studied by other researchers, and significant differences having ecological, physiological and taxonomic implications.

Reciprocal transplants: transplant studies designed to test the taxonomic integrity of the morphological criteria which were used to describe the series of varieties of _Ruppia maritima_ L., s.s., in Eastern North America (Fernald and Wiegand, 1914) revealed that the characters of length and degree of spiraling (coiling) of peduncles, length of podogynes and size and shape of mature carpels or fruits, upon which the present intraspecific designations are based, are, in fact, mutable. Manipulation of environmental conditions in a controlled greenhouse experiment produced a range of variation in plants from one population source which corresponded to morphologies not represented in the natural population. So plastic are these vegetative features, that even under natural conditions a single plant may possess characteristics ascribable to two or more named varieties based on measurements given in the taxonomic keys currently in use.

At this writing, there are no published reports on reciprocal transplants of Ruppia. However, transplant experiments have been undertaken with seagrasses and other aquatic vascular plants. Ranwell et al. (1974) worked on feasibility studies for large scale transplantation of Zostera spp. to reclaim steadily declining losses in intertidal feeding grounds for wild-fowl. These field trials unfortunately were not directed towards a genecological assessment. However, the transplantation techniques might be effectively applied to future transplantation studies of Ruppia or other submersed aquatic plants in lieu of the container system used in the present study. An attempt to develop a phenological index for seagrasses utilizing reciprocal transplants across a tidal zone and integrating information on the effects of changes in water temperature, salinity and substrate was undertaken by Phillips (1976). He sought to distinguish whether the responses represented phenotypic plasticity or ecotypic differentiation. Although his results addressed some of the spatial and logistics problems of seagrass transplantation experiments, they offer nothing more conclusive than a suggested phenological index of limited consequence. Dawson (1980) investigated flowering times for Rannunculus penicillatus (Dum.) Bab. using transplants in an estuary and suggested that an ecocline of genetic material exists. He concluded, however, that the initiation of flowering was controlled by an endogenous rhythm rather than by the effects of photoperiod and temperature. My results on variation of peduncle and podogyne length in reciprocal transplants showed significant differences between the donor population and the transplants retrieved from the receptor site. In several cases, the transplants showed a closer affinity to the receptor population than with the donor population from which they were taken. The estuarine plants exhibited a greater response to the treatments than did the coastal plants for variation in both peduncle and podogyne length.

Greenhouse experiment: controlled experiments on growing Ruppia and other submersed aquatics in the laboratory or in outdoor tanks have yielded more substantial data than field studies due largely to better monitoring and collection techniques but, nevertheless, the resulting information regarding genecological phenomena is quite limited. Most experiments of this sort have simply recorded the effects of environmental conditions (e.g., water temperature and salinity) on growth and development without making any determinations relative to phenological or genotypic responses (cf. Bourn, 1935; Mayer, 1969). Verhoeven (1979) raised Ruppia cirrhosa (Petag.) Grande (= R. spiralis L. ex Dum.) and Ruppia maritima L. (var. maritima and var. brevirostris) in nine outdoor culture tanks under 3 salinity regimes (ca. 6.5 o/oo S; 33.5 o/oo S; 50.5 o/oo S), but water depth was adjusted during the experiment (15 cm to 40 cm after 10 wks.) to compensate for growth of the plants, and temperature was not regulated. Therefore, these two variables (water temperature and depth) were eliminated from a valid assessment of the combined effect of the three environmental factors (water temperature, depth and salinity) discussed by Richardson (1980) and in the present report. Verhoeven (1979) did, however, substantiate the fact that, as in its natural habitat, R. maritima s.l. flowered and fruited more abundantly than R. cirrhosa. He also reported from field studies that R. maritima var. brevirostris was well adapted to temporary environments and was capable of completing its entire life cycle in a few months. This is a very close parallel to the phenology for the estuarine plants under consideration in my studies. His data also show similarities in the ecology of R. cirrhosa with the New Hampshire coastal plants, in that it inhabits more permanent water bodies with a higher mean seasonal salinity than R. maritima.

McMillan (1978) found morphological variation in five seagrass species grown under controlled conditions. His results report an ecotypic status for leaf width suggesting a selective role of habitat conditions. Further, McMillan stated that variation in leaf width is dependent not only on environmental factors, but that the limits of its variation (ecoplasticity) varies geographically depending on the genotype. In McMillan's study the selective influence of the habitat on the genotype is not clear but, nevertheless, he rejects the ecad or habitat form status (environmental modification of a single genotype) in favor of an ecotype.

However, he qualifies this conclusion as needing further investigation.

Although the data presented here on the reciprocal transplants and the greenhouse experiment are not in themselves sufficient to generate predictive patterns, interpretation of the results does indicate that the value of morphological criteria for taxonomic differentiation in Ruppia is critically limited.

My contention regarding the assignment of a taxonomic category to morphological variants at an infraspecific level in the genus Ruppia is that such recognition must be based on a functional taxonomy involving not only morphological criteria, but also an assessment of the effects of environmental factors and reproductive biology.

Seed bank: this study contains the first report of a seed bank in Ruppia populations. The ecological significance of the seed bank is to ensure re-establishment (without immigration) of the population at the same site, even if no individuals survive to produce a seed crop in a given year, or even in several consecutive years. The seed bank may also increase genetic variability and stability in the population (Harper, 1977; Baskin and Baskin, 1978). McMillan (1981) supported this conclusion in his observations on the seed reserves and seed germination in the seagrasses Halodule and Syringodium. The ability of Ruppia seeds to remain dormant and stored in the soil during conditions not conducive to germination, such as high salinity, allows a population of seeds to survive extreme conditions which developing or mature plants could not survive (cf. Ungar and Riehl, 1980). Seed dimorphism (Ungar, 1979) and variation in seed coat thickness in a population (Mayer, 1969) may ensure an irregular germination period which would allow for seedling establishment under a range of conditions. This is an especially important consideration with regard to the annual populations where prolonged germination periods may be necessary for the perpetuation of growth and development in a constantly changing environment. Templeton and Levin (1979) hypothesized that the seed bank acts as an evolutionary filter determining the survival of certain genotypes. The significance of the seed bank in providing assurance for recovery and growth in heterogenous environments typical of the various habitats of Ruppia, should not be underestimated (see, Leck and Graveline, 1979; McMahon and Ungar, 1978; Milton, 1939; Thompson and Grime, 1979; Ungar, 1978; Williams and Ungar, 1972).

Pollination biology: careful observations as well as some rather bizarre descriptions of pollination in Ruppia have been reported in the literature (see, Roze, 1894; Graves, 1908; McCann, 1945; Setchell, 1946; Reese, 1962; Mason, 1967; Schwanitz, 1967; Gamerro, 1968, Richardson, 1976b; Verhoeven, 1979). While some authors have described surface and underwater pollination in the genus Ruppia there is considerable latitude for interpretation with respect to the anatomy and physiology of the mechanisms and the adaptive significance each may have. Verhoeven's (1979) description of a surface and an underwater pollination mechanism in R.

cirrhosa and *R. maritima*, respectively, was based on a thorough review of the literature, but hardly more than observational data on the anatomy and physiology of the pollination systems were reported. His observations on pollination in *R. cirrhosa*, which was well documented by Gamerro (1968), described a surface pollination system in which the flowers are either raised above the surface on extended peduncles or where entire anther sacs, at the time of dehiscence, would form a gas bubble, abscise from the inflorescence and be transported to the water surface where they burst open on arrival, dispersing pollen on the surface film. Verhoeven's (1979) treatment of an underwater pollination mechanism in *R. maritima* was based almost entirely on my earlier work (Richardson, 1976b). From my report, Verhoeven published a description of the physiology of the underwater pollination process which, albeit well thoughtout, presented little or no experimental work to support his assumptions. My original hypothesis on the anatomy and physiology of the underwater pollination mechanism has been refined and is thoroughly documented in the present study.

By means of a thorough anatomical examination of the inflorescence of *Ruppia*, and time-lapse cinemicrography of the underwater pollination mechanism, unequivocal evidence is presented here on the passage of photosynthetically produced gases through the internal lacunar system of the plant and delivery of the gas to the anther sac by diffusion through intercellular spaces in the anther connective. The gas bubbles. formed during the process of anther dehiscence carry pollen grains out of the anther sac on their outer surface and into contact with adjacent receptive stigmas.

Underwater pollination and surface pollination are almost certainly mutually exclusive adaptations for estuarine, annual populations of _Ruppia_ inhabiting temporary water bodies subject to desiccation (e.g., shallow pannes), and coastal, perennial populations inhabiting permanent water bodies (e.g., deep pond-holes), respectively. Although a decrease in the water level due to evaporation in shallow water habitats often exposed underwater-pollinating flowers on the surface, where pollen was shed, it was not possible to determine whether surface cross-pollination occurred in these plants. An attempt to duplicate these conditions in the laboratory was unsuccessful because I could not be sure if partial anther dehiscence had already taken place underwater, and when immature inflorescences were exposed above the surface they failed to reach anthesis. Similarly, no successful underwater pollination was recorded for coastal, surface-pollinating plants either in nature or in the laboratory. Therefore, my earlier report (1976b) on the possibility that both mechanisms may function in the same population under different environmental conditions remains unverified. Verhoeven (1979) and Schwanitz (1967), however, reported that in rare situations, when _Ruppia cirrhosa_ flowers were prevented from reaching the surface, sporadic underwater pollination can take place, but gave no supporting evidence. This is contrary to the conclusions of Luther (1951), with whom I concur, who found no fruits produced where peduncles of Ruppia spiralis (= R. cirrhosa) did not raise the inflorescence to the water surface.

In considering the reproductive strategies of coastal and estuarine Ruppia populations, the adaptive significance of the two pollination systems becomes evident. The coastal plants are dependent on vegetative reproduction to ensure continued growth at sites having higher seasonal salinities which are not conducive to seed germination and seedling establishment. The seed that is produced as a result of surface cross-pollination remains viable through extended periods of dormancy as a seed bank until environmental conditions promote germination. The estuarine plants, for the most part, are found in seasonal environments subject to late summer drying conditions which may result in the complete evaporation of water from shallow pannes (Richardson, 1976a, 1980). Prior to desiccation, however, the plants in these populations flower and set fruit over a short period of time by an efficient underwater self-pollinating mechanism. The annual estuarine plants have a very high fecundity, with nearly every carpel ultimately producing a viable seed. These seeds are stored over the summer in the drying algal mats on the mud surface where some moisture remains, or until the pannes are recharged with water by flood tides or rainfall. If exposed to sun and air, Ruppia seeds will not survive prolonged periods of drying.

Pollen morphology: Ruppia pollen showed considerable variation in size and exine architecture. The coastal plants had larger pollen grains with heavier reticulate exine deposition. This condition is consistent with the mode of pollen dispersal which is epihydrophilly. The adaptive significance of the size of the pollen grains and their exine morphology relates to exposure to air, transference across the water surface film and subsequent attachment to receptive stigmas. The stigmas of the coastal plants have deeper furrows between the surface epidermal cells than do those of underwater-pollinating plants. This condition is apparently a morphological adaptation which functions to attract and secure the pollen grains from the surface film by creating a stronger physical force across the surface of the meniscus between the stigmatic surface and the reticulate exine of the pollen grain than between the pollen and the water surface film.

Comparative morphology of pollen grains from coastal and estuarine Ruppia populations showed that, for the most part, the pollen from estuarine underwater-pollinating plants had a less well-developed reticulate exine. Details revealed in SEM examination of sections of the pollen grain walls suggest a thinner intine in the underwater-pollinating plants. However, accurate measurements of pollen grain wall thickness were not obtained. The reduction or absence of exine in the pollen of hypohydrophilous marine angiosperms has been well documented (cf. Cranwell, 1952; Sculthorpe, 1967; Ducker and Knox, 1976; Pettitt et al., 1978; Pettitt et al., 1980). Extreme cases of reduction of the pollen wall have been reported for Zostera and other seagrasses in which the pollen is filamentous and may coil about the stigma on contact (cf. Ducker and Knox, 1976; DeCock, 1980; Cox, 1983). In some cases, precocious germination occurs in which the pollen is dispersed with pollen tubes already developed (Wodehouse, 1935; Cox, 1983). In this event, the pollen grain/pollen tube becomes the search vehicle (Cox, 1983). There was no precocious germination of pollen noted in the *Ruppia* I studied. However, the formation of pollen chains, both on the water surface in the coastal, surface-pollinating populations and on the surface of the gas bubble in the estuarine, underwater-pollinating plants, results in a filament-like function during dispersal to the stigma. The pollen chains deliver a number of gametes to the stigma, in proximity with the stylar canal, thereby increasing the chances of successful pollination/fertilization. The mature pollen of *Ruppia* I examined at shedding was trinucleate. The trinucleate condition was reported for *Ruppia* in Western Europe by Schwanitz (1967) and in various seagrass genera (Ducker *et al*., 1978).

Cox (1983) presented a rather unconventional but very illuminating treatment on search theory, random motion and convergent evolution of pollen morphology in aquatic plants. He generated several predictions concerning the evolution of aquatic plants with pollen moving at random. Among these were the predictions that 1) adaptations which place pollen and stigmas in the same plane will be favored, and 2) because of the increase in pollination efficiency, plants with two-dimensional hydrophilous pollination will probably have lower pollen/ovule ratios than aquatic plants with three-dimensional wind or underwater pollination. These concepts are valid in many cases. However, the underwater-pollinating Ruppia representatives, although being hypohydrophilous (i.e., completely submersed throughout the entire pollination/fertilization phase) actually employ a two-dimensional system of transference of pollen to the stigma. The pollen is carried out of the anther sac on the outer surface film of a gas bubble, rather than being released underwater to drift into contact with or be intercepted by receptive stigmas, as is the case in Zannichellia (Sculthorpe, 1967; Proctor and Yeo, 1973), Zostera (DeCock, 1980) and in the seagrasses of the Helobiae (see Pettitt and Jermy, 1975; Ducker and Knox, 1976; Ducker *et al.*, 1978; Pettitt, 1980; Pettitt *et al.*, 1980; Pettitt *et al.*, 1981; Cox, 1983).

Electrophoresis: the relationship between life history characteristics and electrophoretically detectable genetic variation in plants, including investigations of polyploidy, have been studied with regard to analysis of taxonomic status, geographic range and distribution, perennial versus annual reproductive strategies, mating systems, pollination mechanisms, chromosome number and other aspects (see Hamrick *et al.*, 1979; Carr and Johnson, 1980). As with the results of the present study, electrophoretic variation in submersed aquatics at the infraspecific level has not been demonstrated. McMillan (1980) compared isozymes of tropical seagrasses from the Indo-Pacific region and the Gulf of Mexico, McMillan and Williams (1980) investigated the systematic implications of isozymes in the seagrass *Halophila* grown under laboratory conditions, and Gagnon *et al*. (1980) attempted to determine the genetic identity of annual and perennial forms of *Zostera marina* L. from an estuary in Penobscot County, Maine, electrophoretically by using sixteen enzyme and general protein systems. In each of these studies the results were inconclusive due to lack of significant electrophoretic variation. Electrophoresis does, however, promise to be a powerful research tool in the study of variation in submerged aquatic plants. Techniques such as microelectrophoresis of single pollen grains (Mulcahy *et al*., 1979) encourage further research.

Cytological evidence: chromosome numbers have provided a basis for taxonomic distinctions and have also revealed polyploidy in *Ruppia* populations in Western Europe (Reese, 1962; Van Vierssen *et al*., 1981), South Australia (Brock, 1982a; Jacobs and Brock, 1982; Snoeijs and van der Ster, 1983), Argentina (Gamerro, 1968), New Zealand (Mason, 1967) and other less well documented reports summarized by Harada (1956). In almost all cases, a diploid chromosome number of $2\ \underline{n} = 20$ was reported for representatives of *Ruppia maritima* L. s.l., while plants identified as *R. cirrhosa* (Petag.) Grande (= *R. spiralis* L. ex Dum.) were tetraploids ($2\ \underline{n} = 40$). The general descriptions of morphology, reproductive biology, ecology, and other life history characteristics, show many similarities between the diploid plants (*R. maritima* s.l.) described in the literature and the estuarine underwater-pollinating plants examined in the present study, as well as between the tetraploid R. cirrhosa and the coastal surface-pollinating plants in New Hampshire tidal marshes. My chromosome counts of 2 n = 14 for the estuarine plants and 2 n = 28 for the coastal plants, however, provide a strong genetic basis for evaluating Ruppia maritima L. taxa described in previous studies and the representatives in New Hampshire.

Following the treatment of Fernald and Wiegand (1914), the diploid (2 n = 14) estuarine plants examined in this study would correspond to R. maritima L. var. rostrata Agardh and var. subcapitata Fern. and Wieg., and their intermediates. The tetraploid (2 n = 28) coastal plants would best fit the description for R. maritima L. var. longipes Hagstr. These cytological data have obvious taxonomic implications, but chromosome numbers for other Ruppia representatives recognized by Fernald and Wiegand (1914), should be obtained from a broader geographical range before a revision is made. Chromosome numbers together with data on reproductive strategies, pollination, morphological variation and environmental factors will provide a substantial basis for a thorough revision of the genus in Eastern North America, and will create a more useful and functional taxonomic system (cf. Mosquin, 1966; Heywood, 1973; Snaydon, 1973).

Taxonomic evaluation: considering the evidence presented here on ecological, reproductive and genetic differences between coastal and estuarine Ruppia populations in New Hampshire, a strong argument could be made for recognition of two distinct taxonomic entities. The different chromosome numbers between the two populations could be sufficient to form reproductive barriers, and the different pollination systems could enforce such an isolating mechanism. Based on a biological species concept, the differing populations may deserve serious consideration for recognition as distinct species. However, a formal designation of taxonomic rank for these genetically distinct coastal and estuarine *Ruppia* populations in New Hampshire should wait until more comprehensive data are available for a wider geographic range of populations. The designation of a higher rank than variety is called for (see, Fernald, 1940; Clausen, 1941; Fosberg, 1942) and further experimental work may justify the recognition of two separate species in parallel with the European *R. maritima* L., s.l., and *R. cirrhosa* (Petag.) Grande. The wider range of phenotypic plasticity exhibited in the estuarine plants may represent a series of ecads or ecophenes within this entity (Mooring *et al.*, 1971; Shea *et al.*, 1975). In the estuarine populations, where significant changes in environmental conditions may occur over a short span, both spatially and temporally, the adaptive significance of phenotypic plasticity may be considerable (cf. Bradshaw, 1965).

In a closely related group, the genus *Potamogeton*, Haynes (1974, 1978) observed considerable vegetative plasticity of undetermined adaptive significance and noted the possible occurrence of underwater pollination in subsection *Pusilli* (cf. Voss, 1972). However, Haynes was unable to obtain chromosome counts to determine the genetic status of the taxa in this subsection. Les (1983) compiled chromosome number reports published for Potamogeton and recognized the diploid level as $2\underline{n} = 14$ with two polyploid series.

The adaptive significance of the phenotypic plasticity and reproductive strategies observed in coastal and estuarine Ruppia populations is difficult to quantify. However, the variation which promotes successful growth, development and reproduction of Ruppia populations in heterogeneous environments is surely adaptive (see Bradshaw, 1965; Jain, 1979). Phillips et al. (1983) in a study of reproductive strategies in Zostera, applied the classification systems of MacArthur (1962) and Grime (1977) to categorize the life histories of populations having phenotypic and ecological differences. Coastal and estuarine Ruppia populations correspond well to this scheme. The life history strategy categories of Grime (1977) are a modification of earlier work by MacArthur (1962) in that they recognize life history adaptations to stress tolerance as a distinct plant strategy. The coastal populations of Ruppia, characterized by more stable habitat conditions and longer lived plants with predominantly asexual reproduction, correspond to the K-selected strategy of MacArthur or the C-selected (competitive) life history of Grime. The estuarine populations, on the other hand, which are short lived, have high seed production and are adapted to habitats having considerable fluctuation in environmental factors, correspond to the R-selected strategy of MacArthur and may be characterized by the R-selected (ruderal) life history strategy of Grime.

CONCLUSIONS

It has been the intent of this investigation to elucidate the salient features of Ruppia populations distributed along an environmental gradient in coastal and estuarine tidal marshes in New Hampshire. The variation found within and between these populations is an expression of the adaptive capacity of Ruppia for growth and reproduction in heterogeneous environments. Phenotypic plasticity and genetic differentiation may be alternative strategies in adaptation, but in a given population both strategies may operate simultaneously in relation to environmental variables. The genotypes of the coastal and estuarine plants have a range of phenotypic plasticity which is genetically determined. The coastal populations have a relatively narrow (stenoplastic) range of expression, while the estuarine populations have a broader (euryplastic) range of expression, especially with regard to morphological variation.

Local variation in levels of water temperature, depth and salinity all affect growth, development and reproduction. The temperature regime of the water column is largely determined by the amount of surface cover of algal mats and the canopy of Ruppia. Water temperature has been shown to be a controlling factor in the onset and duration of the flowering and fruiting period in Ruppia populations. Differences in water depth, at the same or different sites, result in a variation in growth habit. In shallow areas, at both the coastal and estuarine sites, the plants had a more procumbent growth form, shorter internodes, and initiated inflorescences earlier in the season due to higher light intensity and warmer water temperature. In deeper areas, the plants had longer internodes and grew up through the water column to develop inflorescences at a level having the necessary light and temperature requirements. Although water temperature and depth had a spatial-temporal effect on growth and development, neither showed a net effect on the reproductive strategies and associated mechanisms in the populations studied.

Variation in Ruppia populations was most closely associated with the salinity gradient between coastal and estuarine sites. The relationship between salinity and reproductive biology was even more distinct than with morphological variation and phenological differences. It is along the salinity gradient that a distributional pattern for phenotypic and genotypic variation can be best described. The lower salinity estuarine populations are characterized by diploid ($2\underline{n} = 14$) plants having a broad range of phenotypic expression in morphology and an annual reproductive strategy employing an underwater, self-pollinating mechanism. The heretofore poorly understood underwater pollination mechanism in Ruppia, utilizing photosynthetically produced gas bubbles to transfer pollen grains from the anther sac to adjacent stigmas, has been thoroughly documented in the present study. Reproduction in the estuarine populations is almost exclusively by sexually produced seeds. The higher salinity coastal populations are represented by tetraploid ($2\underline{n} = 28$) plants having a narrower range of phenotypic expression and a biennial or short-lived perennial reproductive strategy utilizing a surface, cross-pollinating mechanism. Only a small percentage of each season's growth in these populations develops from sexually produced seed. Renewal growth in the coastal populations is mainly from buds on overwintering rhizomes and stolons. The exception to this is when depleted populations are reestablished from the seed bank.

The origin of the two genotypes of Ruppia is problematical. The question remains as to what evolutionary processes may have contributed to the origin of the two genotypes of Ruppia. The effect of environmental conditions on the genotype has resulted in phenotypic plasticity, the expression of which has heretofore been interpreted as taxonomic varieties. Further evidence is required to determine whether a diploid Ruppia population, with a highly specialized underwater pollination mechanism, could have given rise to tetraploid plants having a less specialized pollination system, but being better adapted to the environmental conditions at coastal sites. The differences in base chromosome numbers between Ruppia populations in New Hampshire coastal and estuarine tidal marshes and those of other populations world-wide leaves this open to speculation. There are similarities in the life-history patterns of the cosmopolitan species R. cirrhosa (Petag.) Grande (= R. spiralis L. ex Dum.) with the New Hampshire coastal populations, and of R. maritima s.l. with the estuarine populations. However, the ploidy levels of the New Hampshire populations make them genetically distinct from both *R. cirrhosa* and *R. maritima* s.l. as reported in the literature.

The results of this investigation suggest the basis for an eventual, thorough revision of the genus *Ruppia* in Eastern North America. However, it is my opinion that such revision should not be attempted until more comprehensive data are available for populations representing the range of the species, especially with regard to chromosome numbers, and including reproductive biology.

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Table 1. RUPPIA POPULATIONS IN NEW HAMPSHIRE COASTAL AND ESTUARINE MARSHES: STUDY AREAS *

			Mean Seasonal Environmental Parameters 1974 - 1980				
Site	Location	Habitat Type	Water Temp. °C	Salinity o/oo	Depth cm	Reproductive Strategy	Pollination Mechanism
Johnson Creek (JC)	Durham	Upper Estuary. Pond Hole in Creek Meander	23.3	15.5	29.0	Annual	Underwater
Lubberland Creek (LC)	Newmarket	Estuarine - Extensive Shallow Pannes Adjacent to Great Bay	27.0	22.6	14.6	Annual	Underwater
Vols Island (VI)	Newmarket	Estuarine - Pond Hole (VId) and Panne (VIs) Adjacent to Great Bay	23.9	21.0	24.7	Annual	Underwater
Odiornes Point (OP)	Rye	Coastal - Pond Hole Adjacent to Little Harbor	23.8	30.3	38.5	Short Lived Perennial/Biennial	Surface
Awcomin Marsh (AWC)	Rye	Coastal - Pond Hole (AWCd) and Scattered Pannes (AWCs)	24.5	31.5	49.7	Perennial	Surface
Little River Saltmarsh (TIB)	Hampton	Coastal - Impounded Tidal Creek. Fresh Water Runoff and Drainage has Lowered Salinity	24.9	4.4	36.6	Perennial	Surface
Taylor River (TR)	Hampton	Coastal - Pond Hole (TRd) and Panne (TRs) Adjacent to Taylor River	21.6	26.8	24.4	Perennial	Surface
Cain's Creek (CC)	Seabrook	Coastal Estuary (Fresh Water Pond Upstream)	20.2	18.4	65.5	Vigorous Perennial	Surface at Low Tide

*See Richardson 1976a; 1980, for detailed descriptions of habitats and environmental parameters

Table 2. COMPARATIVE ENVIRONMENTAL PARAMETERS FOR *RUPPIA* POPULATIONS

ESTUARINE SITES	COASTAL SITES
HABITATS TEND TO BE SECONDARY FORMATIONS ON THE MARSH	HABITATS TEND TO BE PRIMARY FORMATIONS ON THE MARSH
SEASONAL SALINITY LOW (2 - 20 o/oo)	SEASONAL SALINITY HIGHER (20 - 32 o/oo)
THERMAL & HALINE STRATIFICATION IN WATER COLUMN	SYSTEM TENDS TO BE ISOTHERMAL AND ISOHALINE
CONSIDERABLE FLUCTUATION IN SEASONAL OXYGEN PATTERNS	LITTLE FLUCTUATION IN SEASONAL OXYGEN PATTERNS
SURFACE ALGAL MATS DENSE	SURFACE ALGAL MATS THIN TO NONE
EPIBIOTA SCANT	EPIBIOTA DENSE
LOW TURBIDITY	HIGHER TURBIDITY
DESICCATION MAY OCCUR IN LATE SUMMER	PERMANENTLY AQUATIC ENVIRONMENT

Table 3. COMPARATIVE GROWTH AND REPRODUCTION IN *RUPPIA* POPULATIONS

ESTUARINE SITES	COASTAL SITES
PLANTS LOOSE AND FLEXUOUS	PLANTS MORE ROBUST
ANNUAL REPRODUCTIVE STRATEGY	PERENNIAL REPRODUCTIVE STRATEGY
FLOWER, FRUIT AND SENESCENCE BY MID - SUMMER	PLANTS PERSISTING WELL INTO AUTUMN
LARGE SEED CROP	SMALL SEED CROP
SEEDS GENERALLY SMALL (1.5 - 2 mm) OBLIQUE / ACUTE	SEEDS MOSTLY LARGER (2 - 3 mm) OVOID / OBTUSE
FLOWERS DIMINUTIVE w/SMALL ANTHER SACS	FLOWERS LARGER w/ LARGE ANTHER SACS
UNDERWATER POLLINATION AND PSEUDOCLEISTOGAMY	SURFACE POLLINATION
SELF - POLLINATING: POLLEN CARRIED TO STIGMA via GAS BUBBLE	CROSS - POLLINATING: POLLEN DISPERSED ON SURFACE FILM
POLLEN GRAINS ca. 60 MICRONS LONG	POLLEN GRAINS ca. 75 MICRONS LONG
RETICULATE EXINE THINNER AND COVERING LESS SURFACE AREA	RETICULATE EXINE WELL DEVELOPED w/ PELTATE MURI
INTINE VERY THIN	INTINE THIN
DIPLOID 2N = 14	TETRAPLOID 2N = 28

Table 4. RECIPROCAL TRANSPLANTS. COMBINED RESULTS OF LEAST SIGNIFICANT DIFFERENCE (LSD) AND SCHEFFE'S MULTIPLE RANGE TESTS ON PEDUNCLE AND PODOGYNE LENGTHS FOR DONOR AND RECEPTOR POPULATIONS AND TRANSPLANTS FROM 6 SITES.

		RECEPTORS					
		JC	LC	VIs	VId	AWCs	AWCd
DONORS	JC		0	D	+/+	-/-	0
	LC	-/+		D	-/+	0	-/-
	VIs	+/+*	NTP		+/+	+/+	0
	VId	0	-/+	D		+/+	0
	AWCs	-/-	+/-	D	-/+		+/-
	AWd	+/-	+/+	D	+/-	+/-	

Note: Read slashes as: Peduncle/Podogyne

Note: - indicates no significant difference between transplant and donor site at the 0.05 level

+ indicates a significant difference between transplant and donor site at the 0.05 level. In these cases the donor and receptor sites are significantly different from each other at the 0.05 level. The difference recorded for the transplant is attributed to phenotypic plasticity.

D = donor only; NTP = no transplant; 0 = no recovery, transplant lost or died

s = shallow panne; d = deep pond-hole

*See Figs 5 and 6

Table 5. SUMMARY DATA FOR GREENHOUSE EXPERIMENT

TREATMENTS RECOVERED			RESULTS					
T°C	S°/oo	Dcm	Hcm	#II	#MI	#Frts.	$\bar{x}$Ped.	$\bar{x}$Pod.
12.5	2	10	12	2	2	1	0.8	0.6
"	"	20	20	3	2	8	1.4	0.9
"	"	30	20	-	8	-	1.0	-
"	15	10	15	1	3	4	1.1	1.1
"	"	20	30	10	12	20	0.8	0.6
"	"	20 (c)	20	-	4	8	0.7	0.6
"	"	30	25	-	10	4	0.5	0.6
"	30	20	20	10	18	6	0.8	0.7
16.5	2	10	14	2	14	16	0.9	0.6
"	"	20	20	-	6	6	1.4	1.0
"	"	30	31	2	18	12	1.8	0.9
"	15	20	20	2	1	3	0.8	0.8
"	"	20 (c)	22	-	3	13	1.1	0.7
"	"	30	34	5	6	47	1.2	1.2
"	30	10	18	10	8	9	1.6	0.8
"	"	20	22	3	12	5	0.8	0.7
"	"	30	35	12	10	42	1.8	1.2
22.5	15	20	20	-	1	2	0.6	0.6
"	"	20 (c)	25	4	9	54	1.3	0.8
"	"	30	25	5	5	12	1.7	1.1
"	30	20	18	6	10	20	1.0	0.9
"	"	30	30	8	16	52	1.3	1.3

Note: (c) = control unit; Hcm = ave. plant height; #II = number of immature inflorescences still within sub-floral leaf sheath; #MI = number of mature inflorescences extended on peduncle at or near anthesis with or without mature fruit; #Frts. = number of mature fruits on podogynes found attached to plants or in container; $\bar{x}$Ped = mean peduncle length in cm; $\bar{x}$Pod = mean podogyne length in cm.

Table 6. GREENHOUSE EXPERIMENT. REPRODUCTIVE EFFORT AND MEAN PEDUNCLE AND PODOGYNE LENGTHS (cm) AT THREE LEVELS OF WATER TEMPERATURE (T^oC), SALINITY (So/oo) AND DEPTH (Dcm).

		TOTAL NUMBER EXPERIMENTAL UNITS RECOVERED FOR EACH LEVEL		TOTAL NUMBER MATURE FRUITS PRODUCED		$\bar{x}$ PEDUNCLE LENGTH (cm)		$\bar{x}$ PODOGYNE LENGTH (cm)	
T^oC	12.5	8	(1)*	51	(8)	0.89	(0.70)**	0.73	(0.60)
	16.5	9	(1)	153	(13)	1.27	(1.10)	0.88	(0.70)
	22.5	5	(1)	140	(54)	1.18	(1.30)	1.08	(0.80)
S^o/oo	2	6		43		1.22		0.80	
	15	10	(3)	167	(75)	0.98	(1.03)	0.81	(0.70)
	30	6		134		1.20		1.22	
Dcm	10	4		30		1.10		0.85	
	20	11	(3)	145	(75)	0.97	(1.03)	0.75	(0.70)
	30	7		169		1.33		1.05	

* Numbers in ( ) represent that portion of the total accounted for by 3 control units

** Mean peduncle and podogyne lengths per control unit

Table 7. SEDIMENTARY AND SEED BANK CHARACTERISTICS FOR 8 SITES

		Origin of habitat as per Redfield, 1972*	Substrate type	Depth in sediments from which seeds were recovered	Lowest level at which seeds germinated in fresh water	Approximate number of seeds per upper 5 cm^3 layer
JC	estuarine	primary lentic	very soft deep organic muck	0-45 cm	15 cm	25 ± 5
LC	estuarine	secondary lentic	firm partially decomposed peat	0-10 cm	5 cm	30 ± 5
VI	estuarine	secondary lentic	soft highly decomposed peat	0-25 cm	10 cm	30 ± 5
OP	coastal	primary lentic	firm sandy	0-10 cm	5 cm	10 ± 5
AWC	coastal	primary lentic	partially decomposed peat over sand	0-10 cm	5 cm	8 ± 2
TIB	coastal	secondary lentic/semi-lotic	very soft deep organic muck	0-30 cm	5 cm	10 ± 5
TR	coastal	primary lentic	partially decomposed to highly decomposed peat over sand	0-20 cm	5 cm	10 ± 5
CC	coastal	primary lotic	firm sandy-peaty creek bottom	0-10 cm	5 cm	5 ± 2

* Primary habitats develop from original features in the ontogeny of the tidal marsh secondary habitats develop by subsidence, impounded water or changes in the topography of the marsh surface.

Table 8. MEAN POLLEN GRAIN LENGTH AND ANTHER SAC LENGTH FOR SEVEN *RUPPIA* POPULATIONS

		Pollen (microns)		Anther sac (mm)	
		$\bar{x}$	95% Conf. Int. for $\bar{x}$	$\bar{x}$	95% Conf. Int. for $\bar{x}$
ESTUARINE	VI	57.50	± 0.94	.464	± 0.02
	JC	58.89	± 0.60	.432	± 0.04
	LC	59.58	± 1.06	.586	± 0.04
COASTAL	TIB	61.00	± 0.95	.774	± 0.06
	CC	67.94	± 1.03	.864	± 0.06
	OP	75.28	± 0.90	.932	± 0.09
	AWC	77.97	± 1.10	.904	± 0.05

Table 9. APPROXIMATE NUMBER OF POLLEN GRAINS PER ANTHER SAC, PER FLOWER AND PER INFLORESCENCE FOR A COASTAL (AWC) AND AN ESTUARINE (LC) RUPPIA POPULATION

	Anther Sac	Flower	Inflorescence
AWC	4665	18,660	37,320
LC	2105	8420	16,840

Fig. 1. Location map of study areas for *Ruppia* in New Hampshire coastal and estuarine tidal marshes.

JC = Johnson Creek, Durham
LC = Lubberland Creek, Newmarket
VI = Vols Island, Newmarket
OP = Odiornes Point, Rye
AWC = Awcomin Marsh, Rye
TIB = Little River Swamp, Hampton
TR = Taylor River, Hampton
CC = Cain's Creek, Seabrook

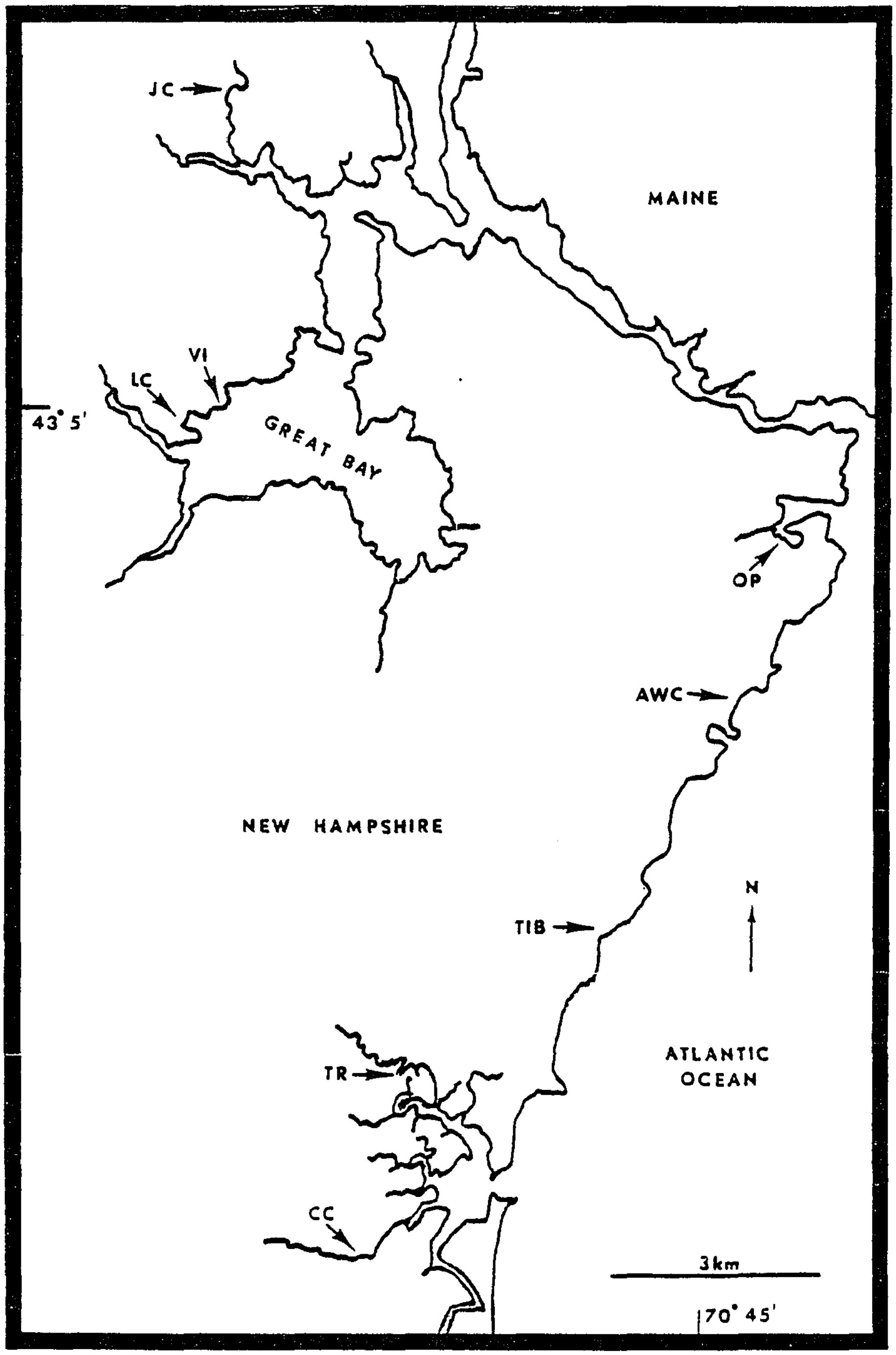
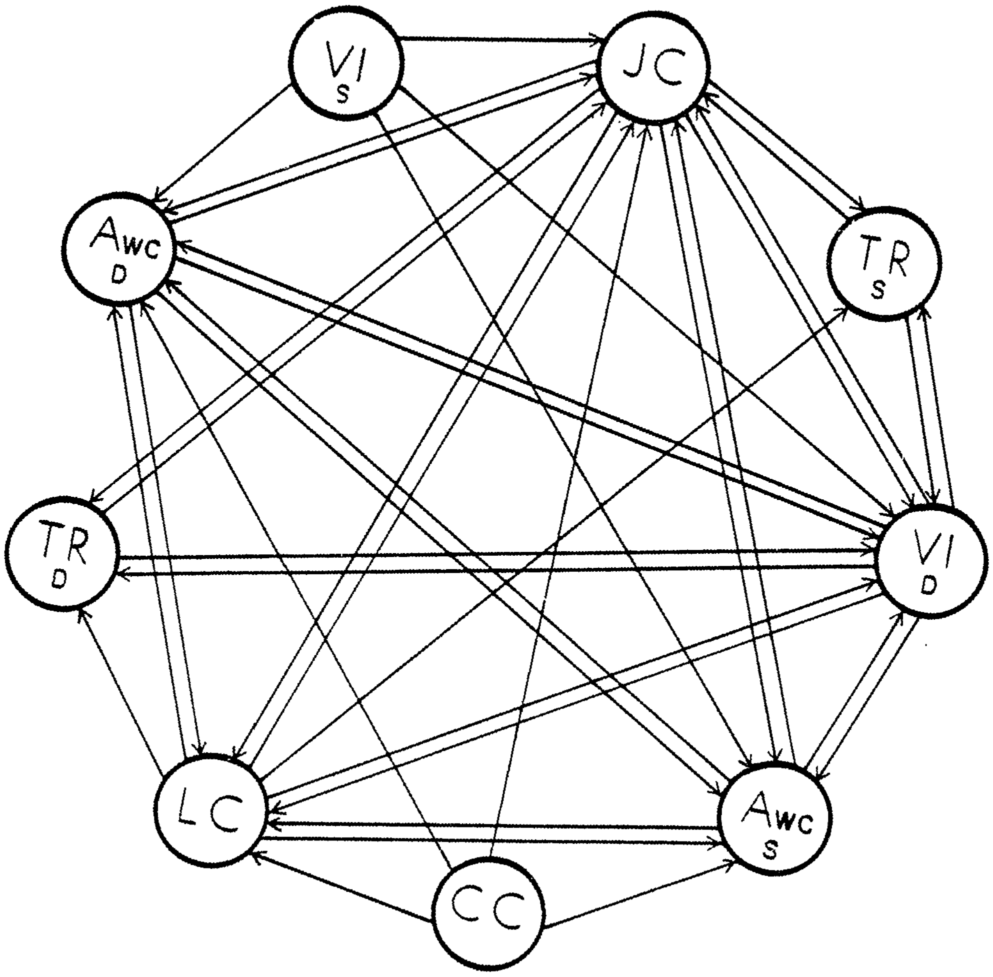

JC
MAINE
VI
LC
43° 5'
GREAT BAY
OP
AWC
NEW HAMPSHIRE
N
TIB
ATLANTIC OCEAN
TR
CC
3km
70° 45'

Fig. 2. Schematic for donor and receptor reciprocal transplants. Two way arrows indicate reciprocal exchange between two populations while one way arrows indicate certain sites were only donors (see text for explanation).

s = shallow pannes

d = deep pond-hole



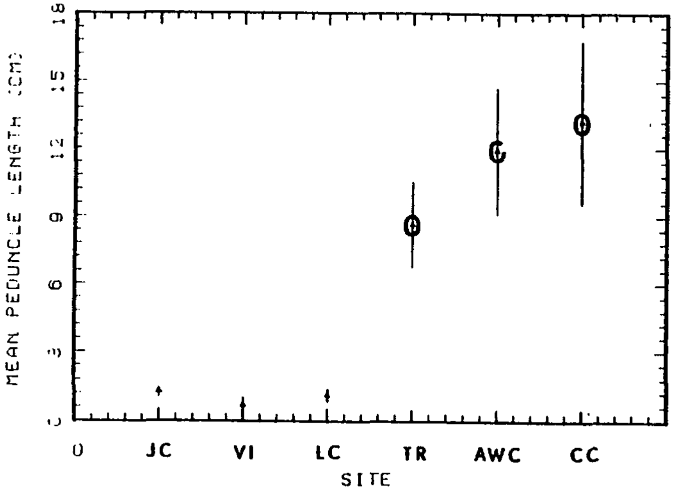
$V I_S$
JC
A_{WC_D}
TR_S
TR_D
VI_D
LC
A_{WC_S}
CC

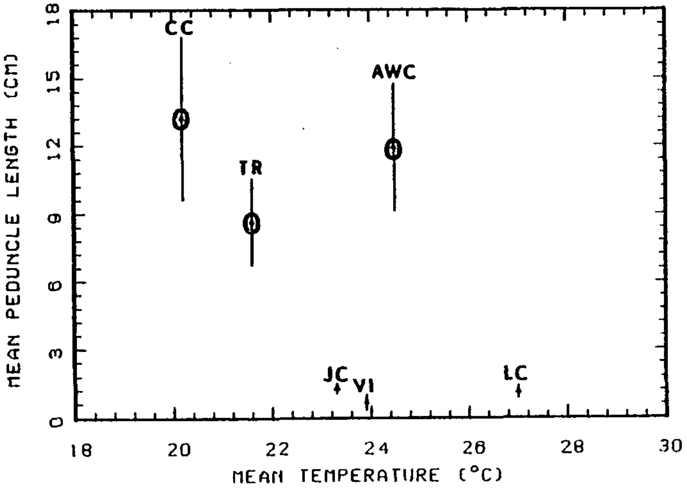
Fig. 3. Mean peduncle lengths for six *Ruppia* populations plotted against water temperature (°C), salinity (o/oo) and depth (cm). Error bars represent the 95% confidence interval for the mean.

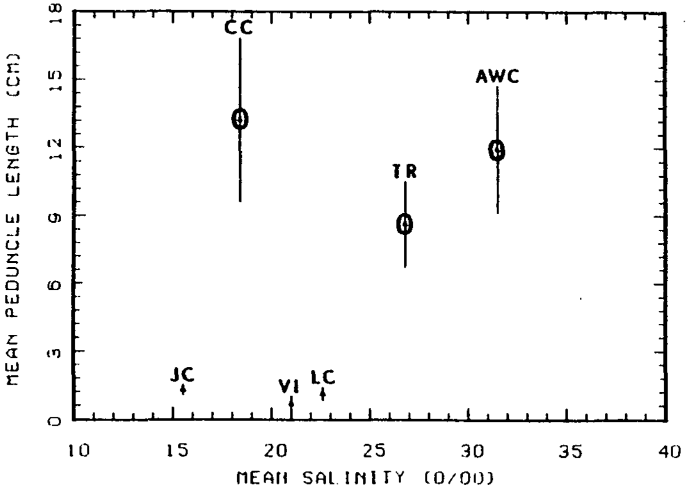
JC = Johnson Creek - upper estuarine pond-hole
VI = Vols Island - estuarine pond-hole
LC = Lubberland Creek - estuarine panne
TR = Taylor River - coastal deep panne
AWC = Awcomin Marsh - coastal pond-hole
CC = Cain's Creek - coastal marsh creek.

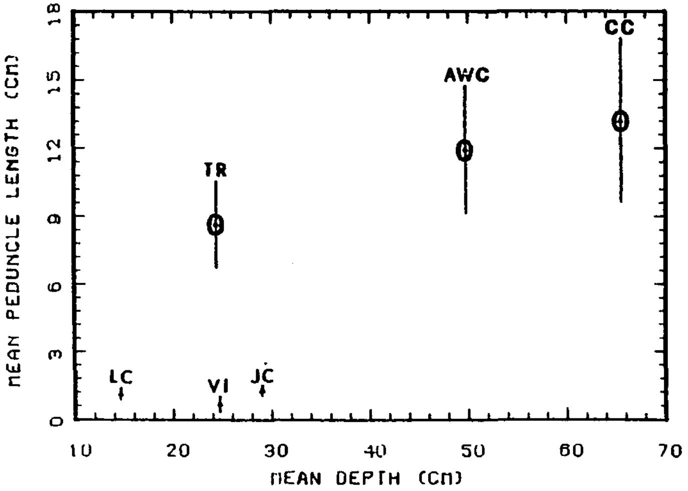
Recognition pattern: Coastal ○

Estuarine


MEAN PEDUNCLE LENGTH (CM)
0
3
6
9
12
15
18
0
JC
VI
LC
TR
AWC
CC
SITE


MEAN PEDUNCLE LENGTH (CM)
0
3
6
9
12
15
18
CC
TR
AWC
JC
VI
LC
18
20
22
24
26
28
30
MEAN TEMPERATURE (°C)


MEAN PEDUNCLE LENGTH (CM)
0
3
6
9
12
15
18
CC
AWC
TR
JC
VI
LC
10
15
20
25
30
35
40
MEAN SALINITY (0/00)


MEAN PEDUNCLE LENGTH (CM)
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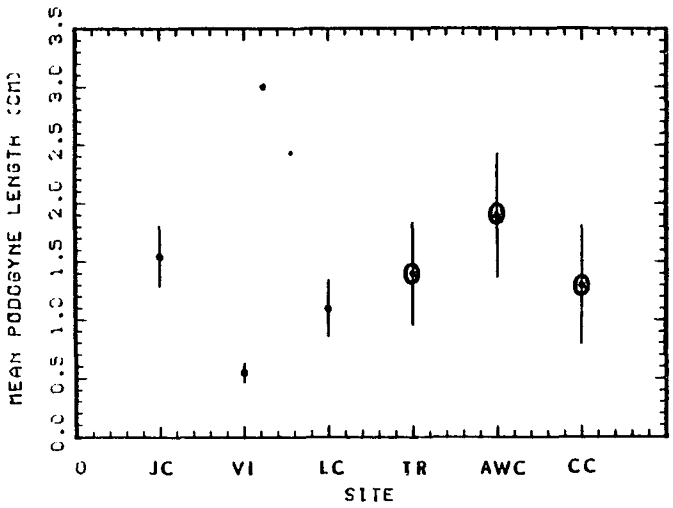
3
6
9
12
15
18
CC
AWC
TR
LC
VI
JC
10
20
30
40
50
60
70
MEAN DEPTH (CM)

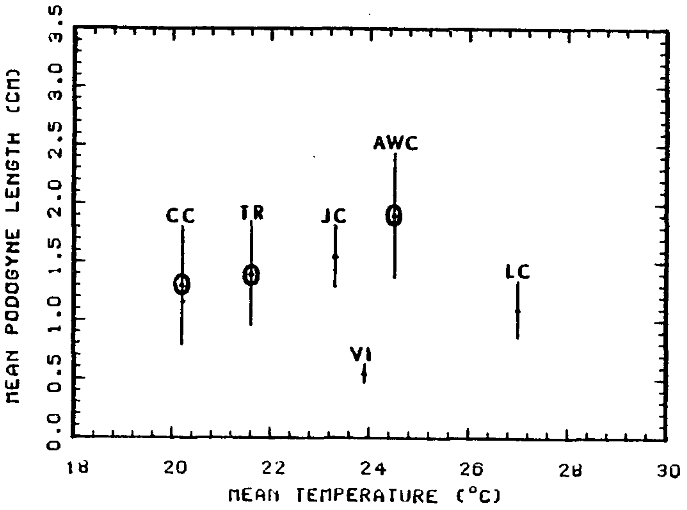
Fig. 4. Mean podogyne lengths for six *Ruppia* populations plotted against water temperature (°C), salinity (o/oo) and depth (cm). Error bars represent the 95% confidence interval for the mean.

JC = Johnson Creek - upper estuarine pond-hole
VI = Vols Island - estuarine pond-hole
LC = Lubberland Creek - estuarine panne
TR = Taylor River - coastal deep panne
AWC = Awcomin Marsh - coastal pond-hole
CC = Cain's Creek - coastal marsh creek

Recognition pattern: Coastal ○

Estuarine


MEAN PODOGYNE LENGTH (CM)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
0
JC
VI
LC
TR
AWC
CC
SITE


MEAN PODOGYNE LENGTH (CM)
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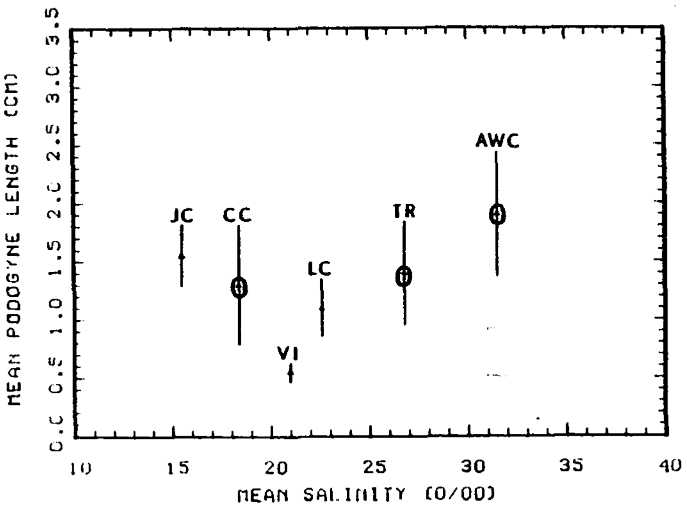
0.5
1.0
1.5
2.0
2.5
3.0
3.5
CC
TR
JC
AWC
VI
LC
18
20
22
24
26
28
30
MEAN TEMPERATURE (°C)


MEAN PODOGYNE LENGTH (CM)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
JC
CC
VI
LC
TR
AWC
10
15
20
25
30
35
40
MEAN SALINITY (0/00)

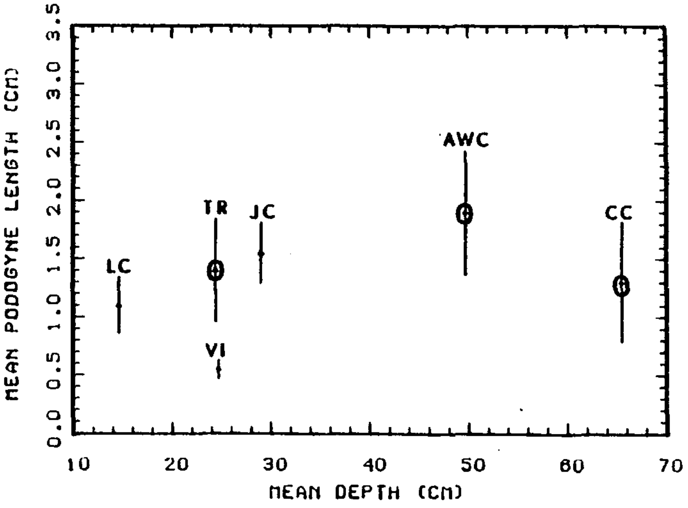

MEAN PODOGYNE LENGTH (CM)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
LC
TR
VI
JC
AWC
CC
10
20
30
40
50
60
70
MEAN DEPTH (CM)

Fig. 5. Herbarium specimens of:

A. donor population (VI-D)

B. transplant (series 2 n 4) (VI-T)

C. receptor population (JC-R)

collected on same date at the end of the reciprocal transplant experiment

Compare with fig. 6.

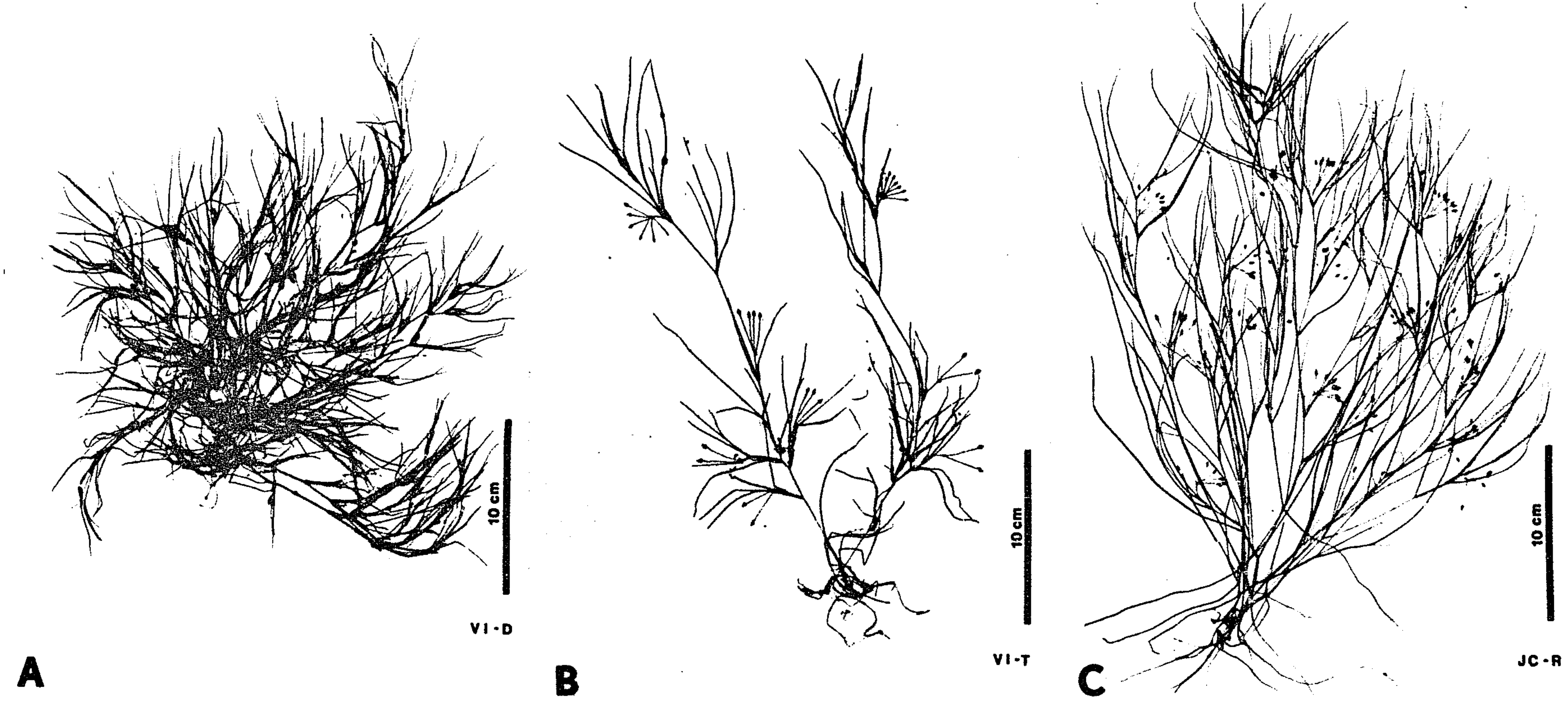
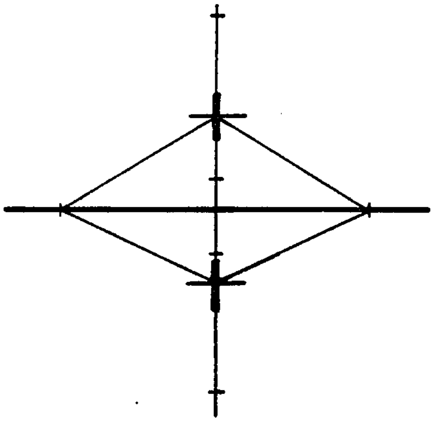
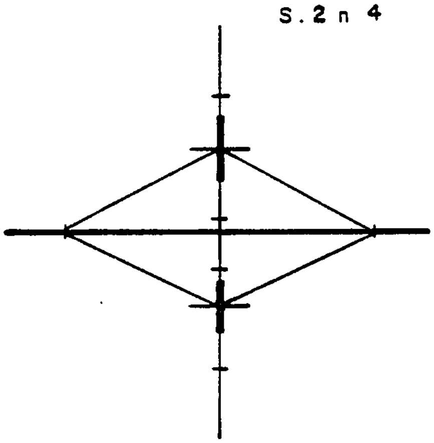

10 cm
VI-D
A
10 cm
VI-T
B
C
10 cm
JC-R

Fig. 6. Graphic representation of the results of a reciprocal transplant (S.2 n4) from Vols Island marsh (VIs, an estuarine shallow panne) to Johnson Creek marsh (JC, an upper estuarine pond-hole). Mean standard error and range of podogyne and peduncle length are plotted on the upper and lower ordinate, respectively. Arbitrarily set points, equidistant from center, on the left and right abscissa allow for the construction of a polygon to visualize the relationship between the donor (VIs) and receptor (JC) populations and the transplant (S.2 n4). The figures on the right are similarly constructed for *Ruppia maritima* varieties *rostrata* and *subcapitata*, based on measurements for podogyne and peduncle length given in Fernald and Wiegand (1914) and in the 8th edition of Gray's Manual of Botany (1970), for comparison. Specimens of the plants represented here are shown in Fig. 5.

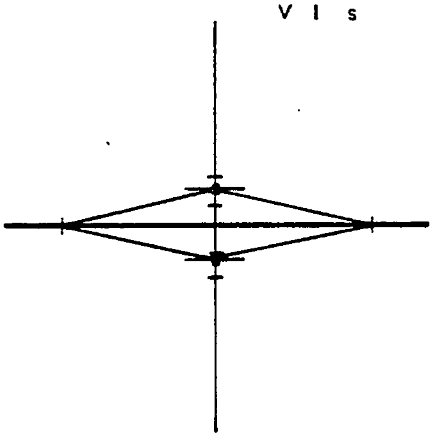
JC



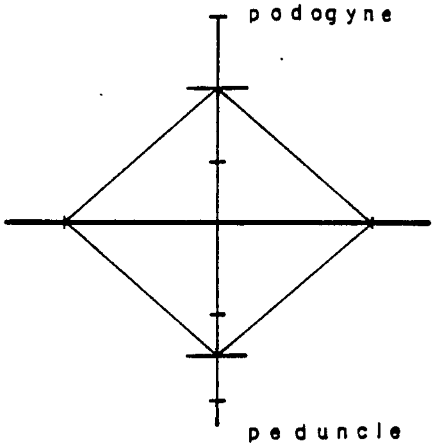
S.2n4



VIs



var. ROSTRATA


podogyne
peduncle

var. SUBCAPITATA

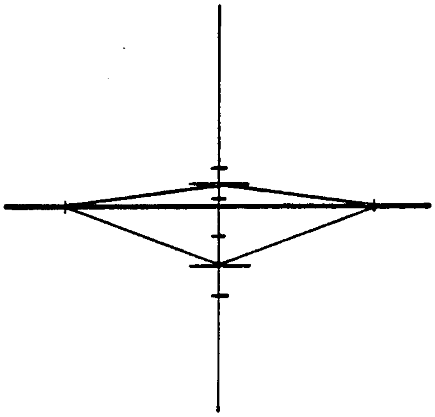


Fig. 7. Experimental design for Ruppia growth study at U.N.H. greenhouse.

Treatments:		1	2	3
Water Tempreature	(T)	20 - 25°C	15 - 18°C	11 - 14°C
" Salinity	(S)	2 o/oo	15 o/oo	30 o/oo
" Depth	(D)	10 cm	20 cm	30 cm

Control (C): Seedlings grown in natural water from collection site in the three temperature regimes

Duration: May 20, 1976 - August 20, 1976

Transplant Material: Lubberland Creek Site (annuals). Plants collected immediately after germination and seedling establishment (rooting) in firm peat bottom material

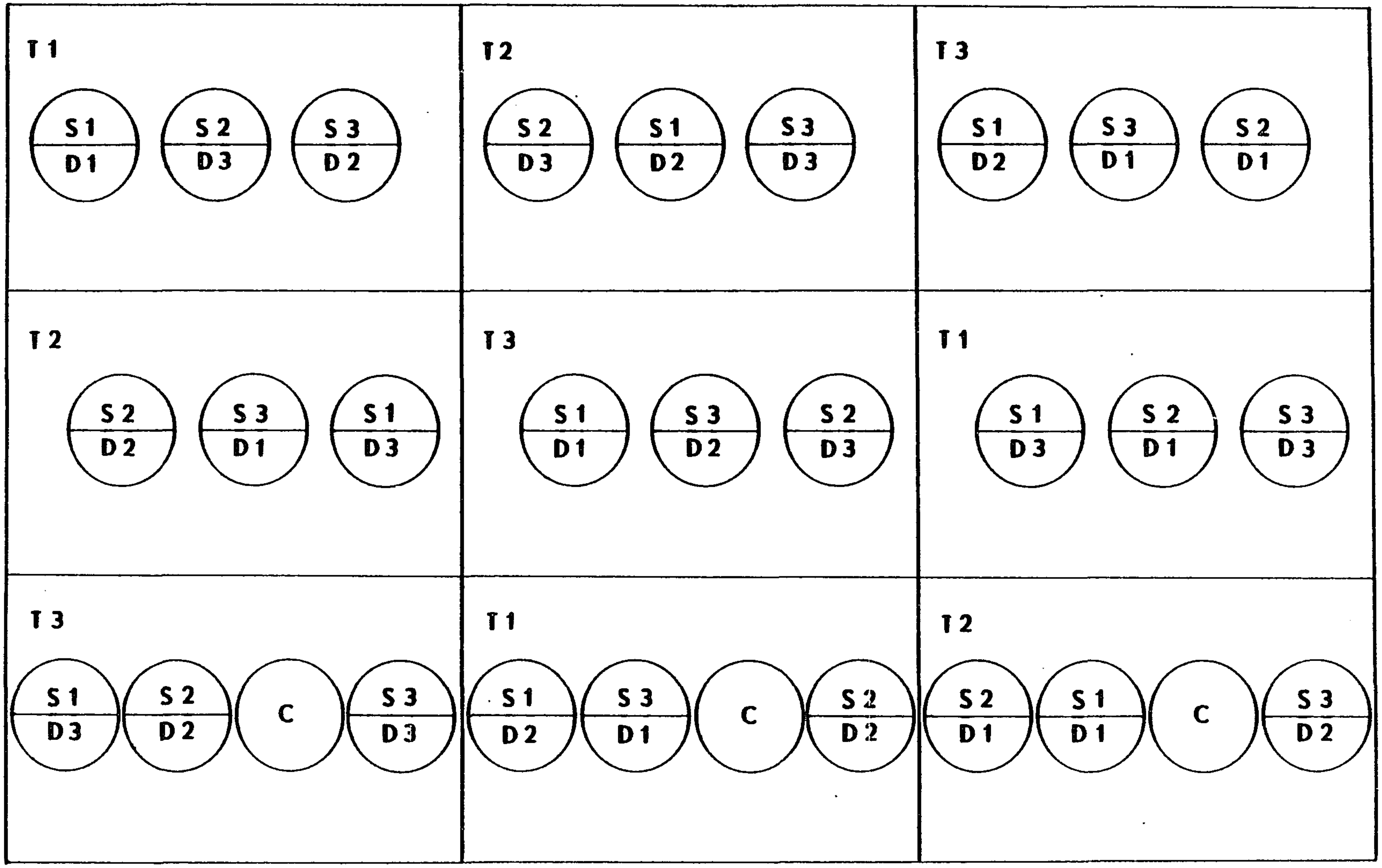
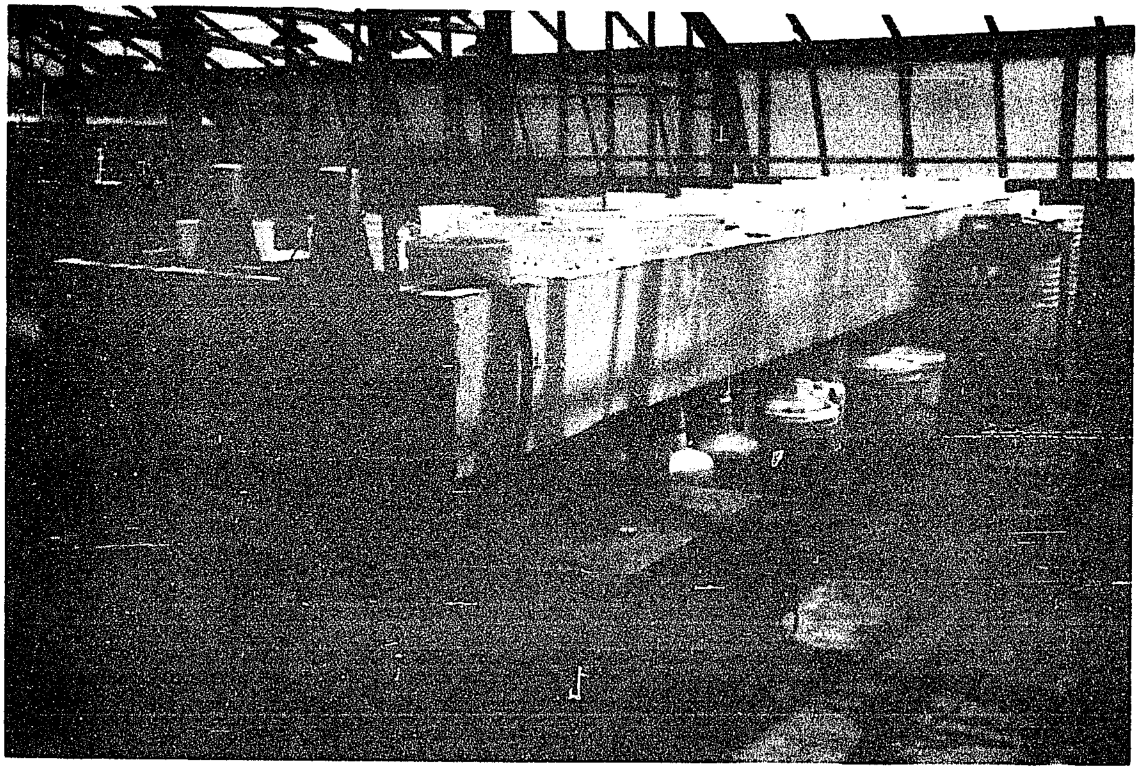

T 1
S 1 / D 1
S 2 / D 3
S 3 / D 2
T 2
S 2 / D 3
S 1 / D 2
S 3 / D 3
T 3
S 1 / D 2
S 3 / D 1
S 2 / D 1
T 2
S 2 / D 2
S 3 / D 1
S 1 / D 3
T 3
S 1 / D 1
S 3 / D 2
S 2 / D 3
T 1
S 1 / D 3
S 2 / D 1
S 3 / D 3
T 3
S 1 / D 3
S 2 / D 2
C
S 3 / D 3
T 1
S 1 / D 2
S 3 / D 1
C
S 2 / D 2
T 2
S 2 / D 1
S 1 / D 1
C
S 3 / D 2

Fig. 8. A. & B. Two views of temperature regulation bench in the U.N.H. research greenhouse with containers of Ruppia installed. The aeration pumps and water heat exchangers can be seen in the foreground. See fig. 7 for experimental design and arrangement of treatments.



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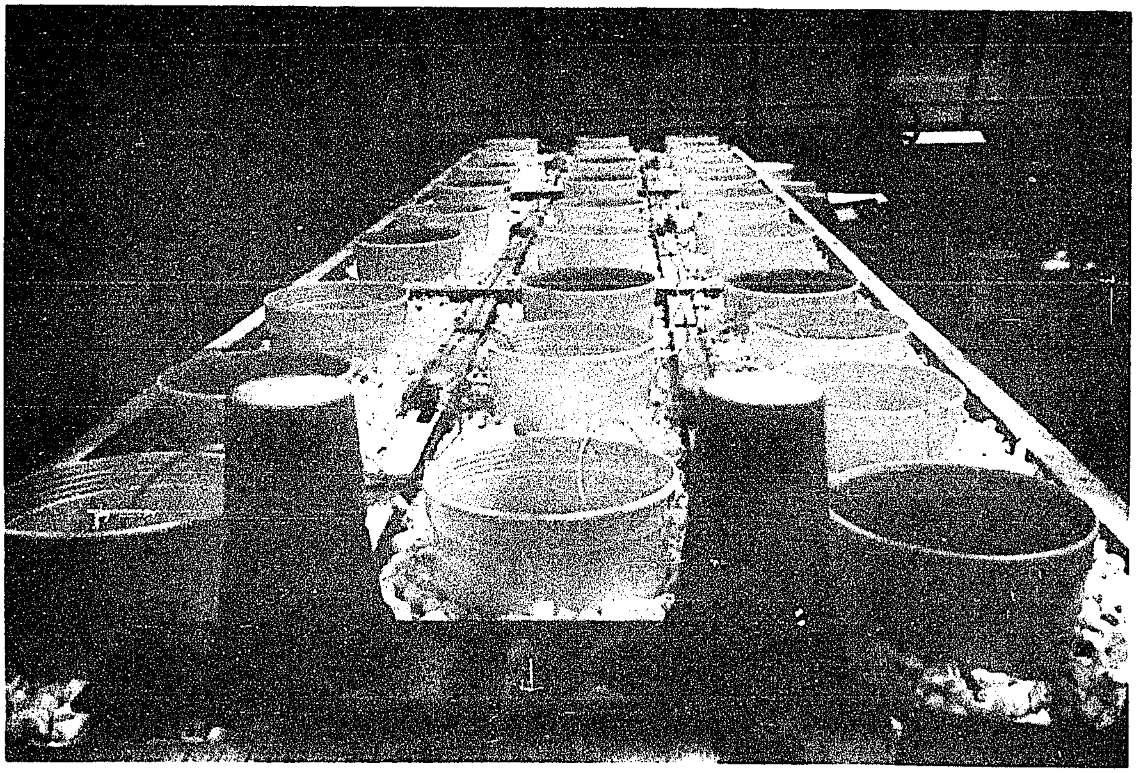


B

Fig. 9. Frozen core seed recovery technique.

A. Dry-ice and butanol are loaded into sampler

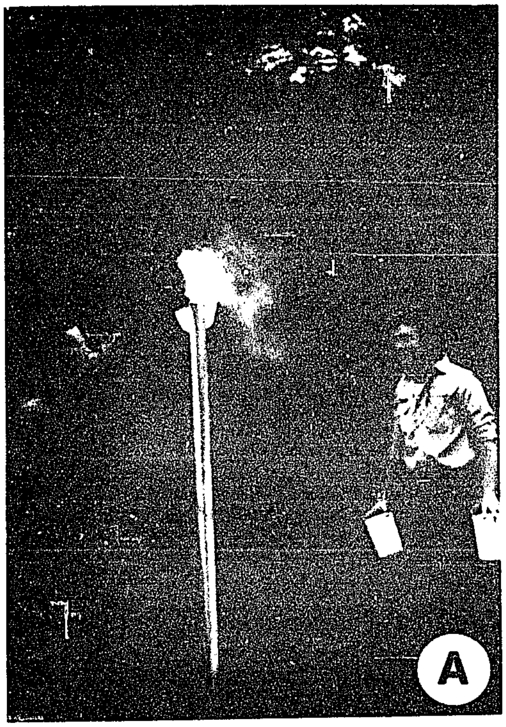
B. Sampler is pushed into soft bottom sediments

C. Teamwork is required to remove sampler from sediments after ca. 5 minutes with frozen material attached

D. Sampler is rinsed out with water to slightly thaw sleeve 'core' of material to be slid off

E. 'Core' is carefully marked and packaged for transport in a cooler of dry ice to the laboratory

Photos by Garrett E. Crow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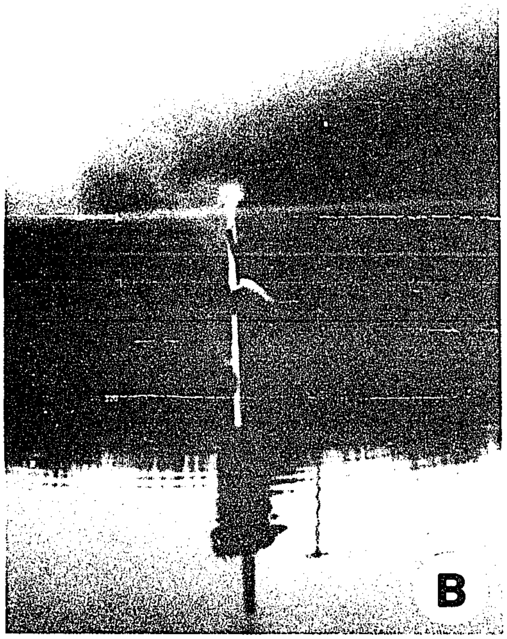
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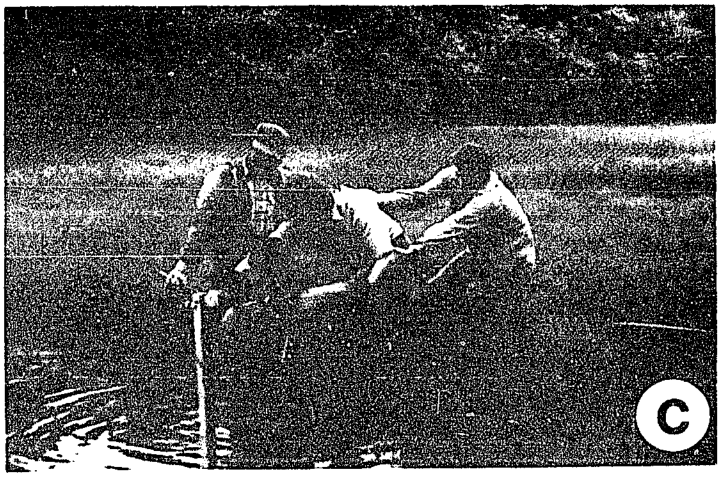

C

D

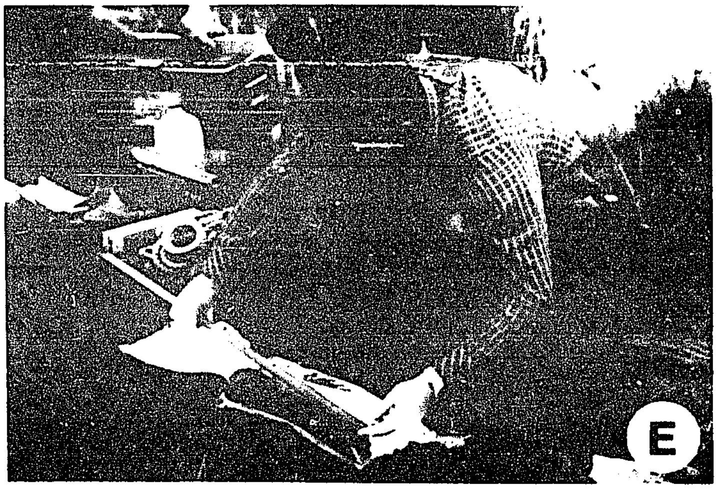
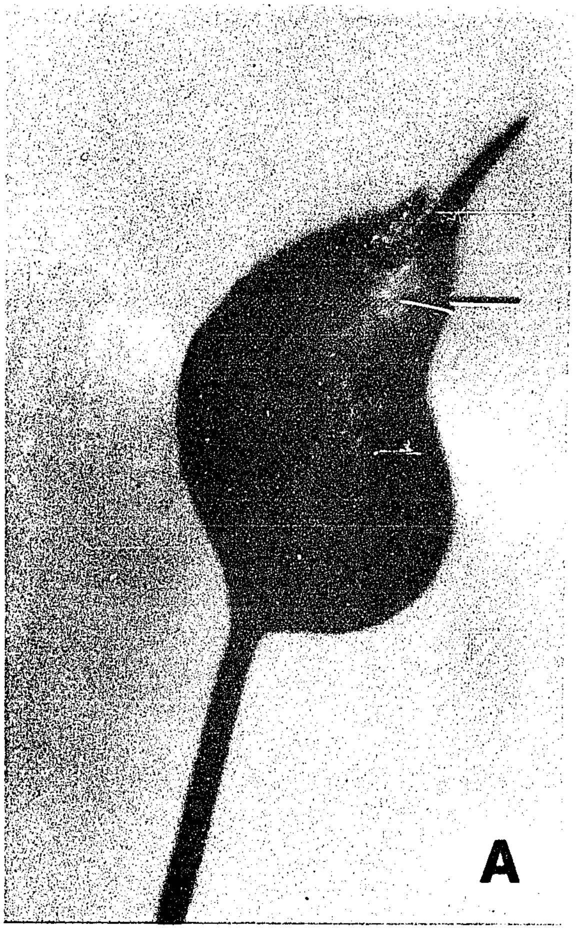

E

Fig. 10. Fruit-seeds of Ruppia.

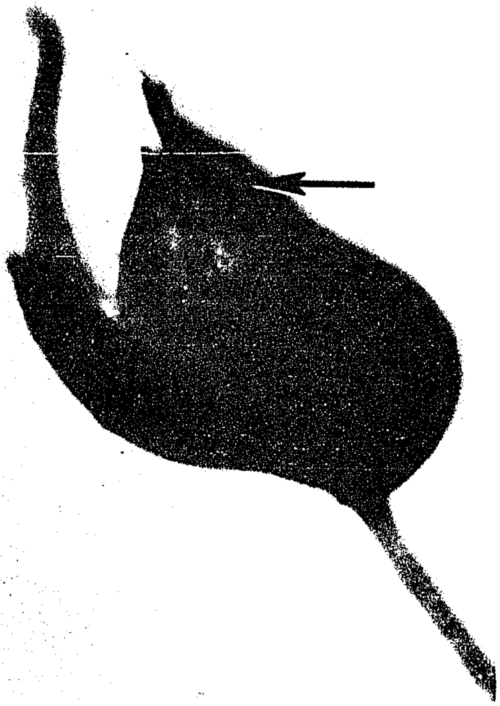
A. arrow indicates specialized perforated area in the fruit-seed coat through which water is apparently imbibed prior to germination. x 25.

B. Germinated fruit-seed. Arrow indicates perforated area. x 25.

C. Variation in fruit-seed morphology found on a single plant at the Vols Island site. x 16.



A



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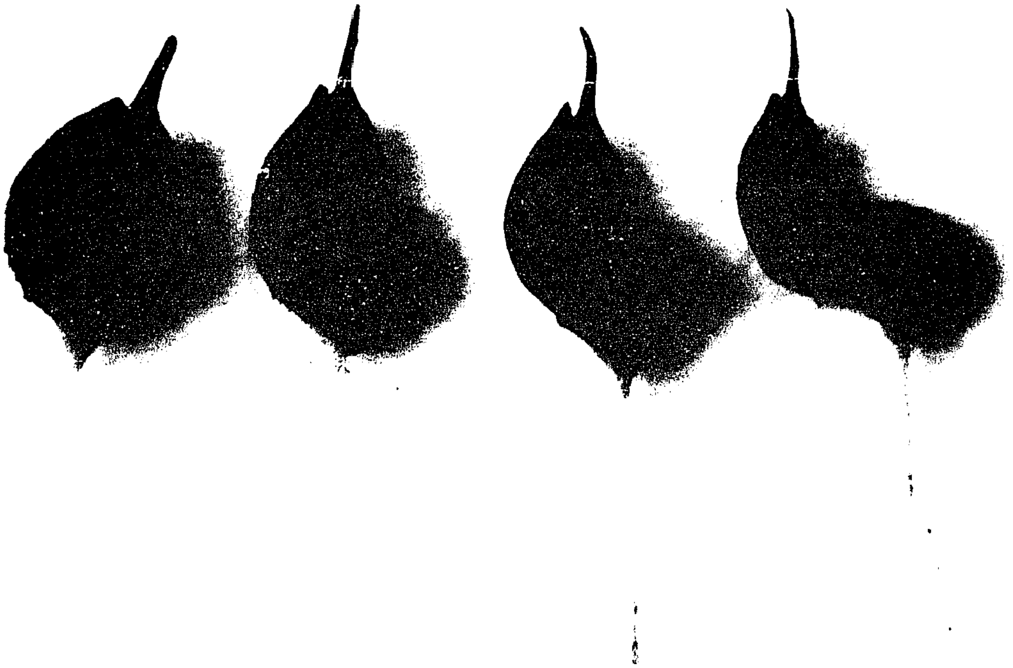


C

Fig. 11. Specimens representing different stages in the growth and development of annual plants collected at Lubberland Creek showing the persistent fruit-seed attachment (arrows)

A. Seedlings collected 28 May, 1975; approximately 3 weeks after germination. Darkened areas at base of leaf clusters are developing inflorescences.

B. Plants collected 10 June, 1975; period of underwater pollination and development of young fruits.

C. Plants collected 30 June, 1975; abundantly fruiting with some mature fruits shedding.

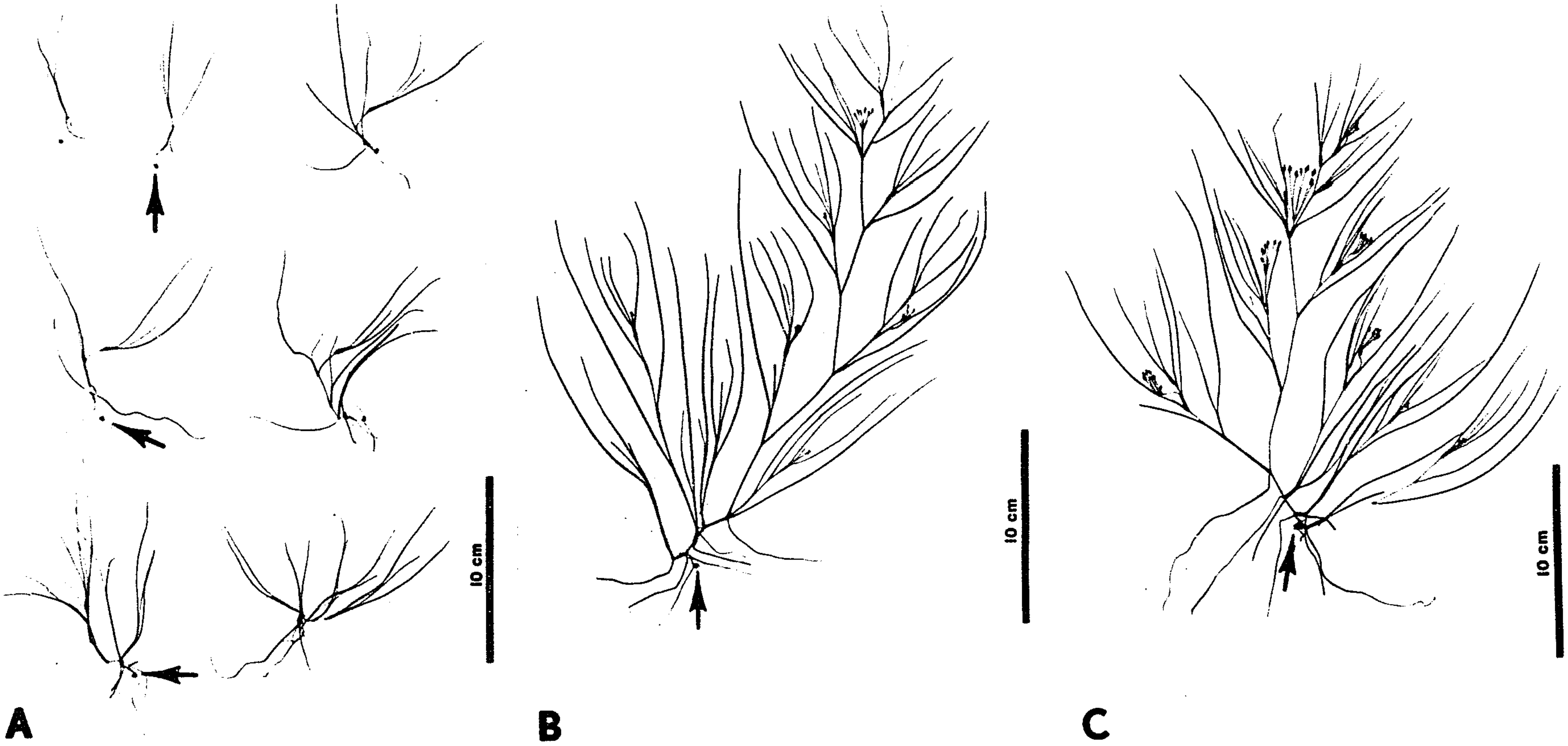

10 cm
A
10 cm
B
10 cm
C

Fig. 12. Comparative growth habit, pollen grain size and exine architecture for three populations of Ruppia.

A. Awcomin Marsh - coastal pond-hole

B. Vols Island - estuarine pond-hole

C. Lubberland Creek - estuarine shallow panne

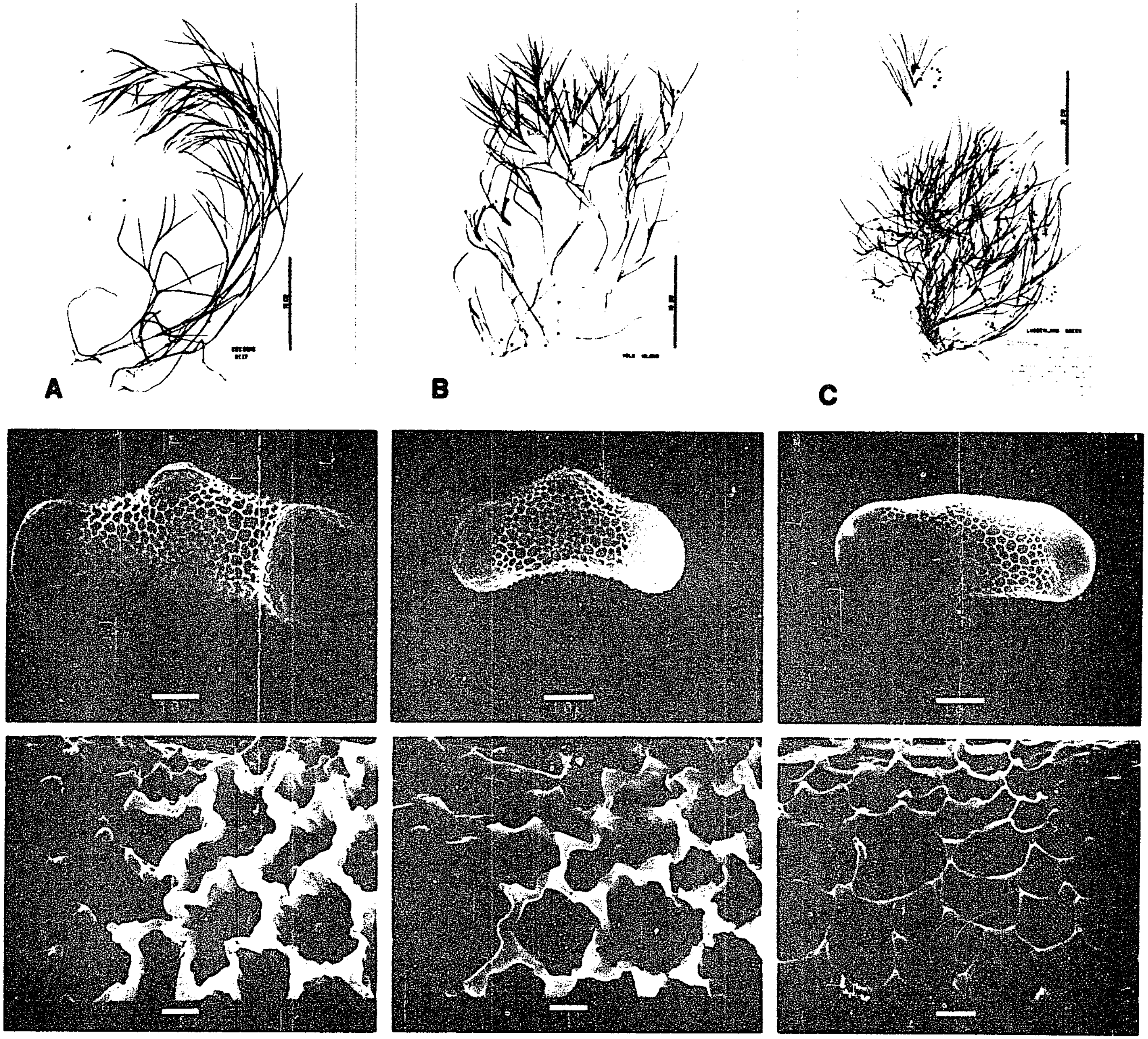
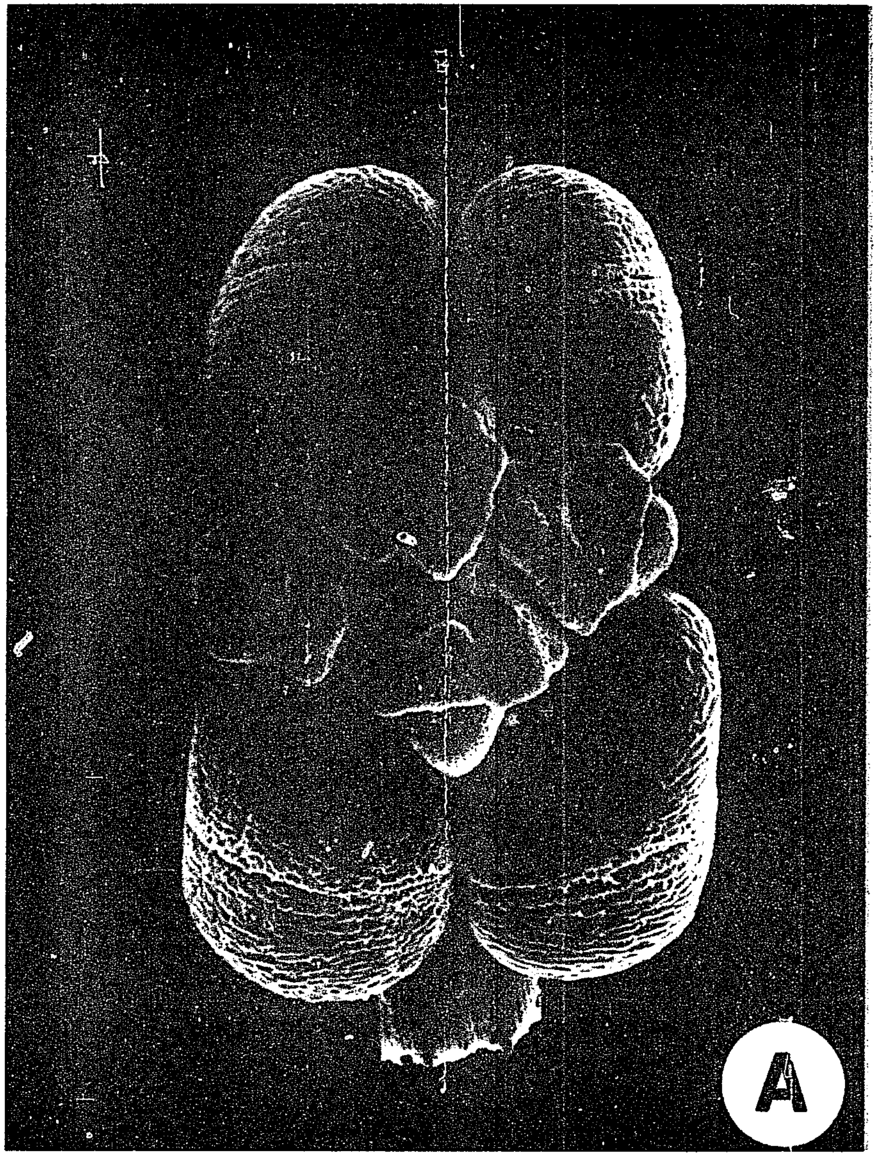

A
B
C

Fig. 13. Comparison of anther sacs and floral morphology of an estuarine (Vols Island) and a coastal (Awcomin Marsh) population.

Upper flower of inflorescence. both x65.


A

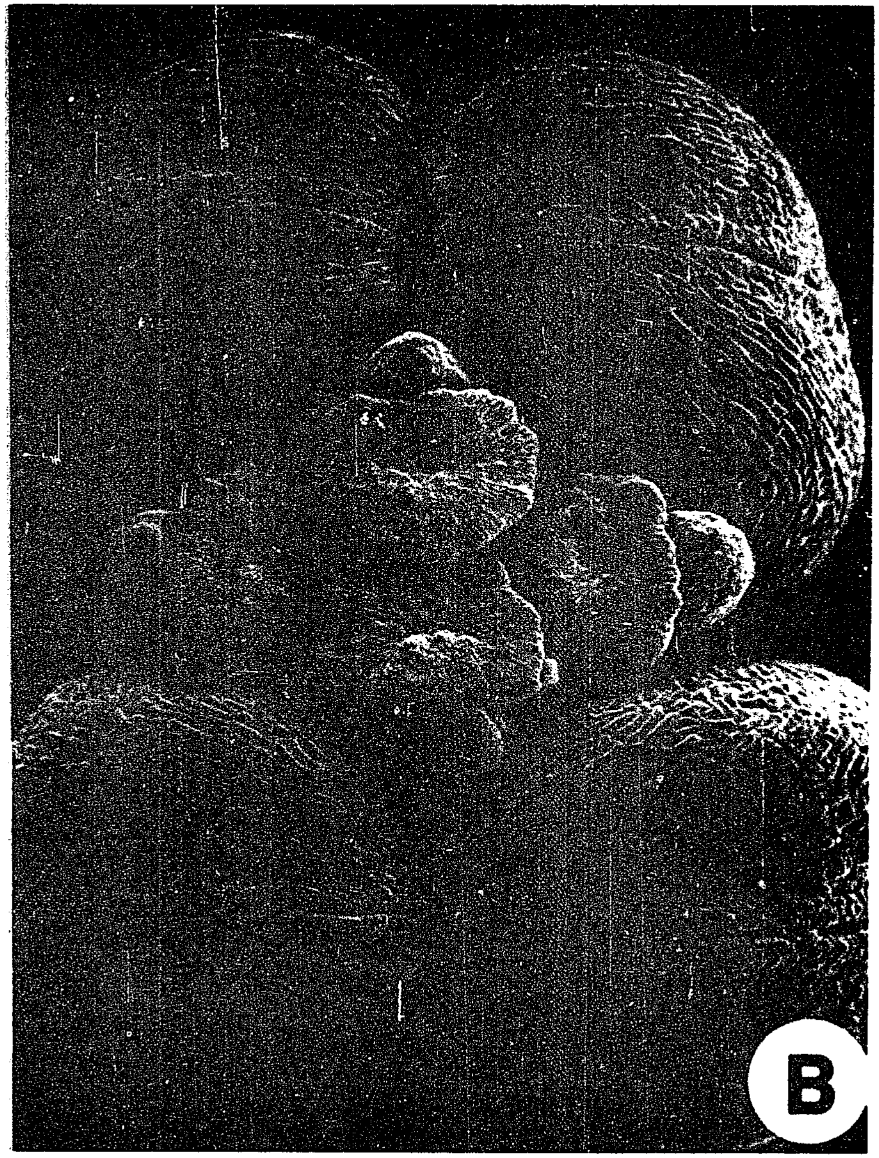
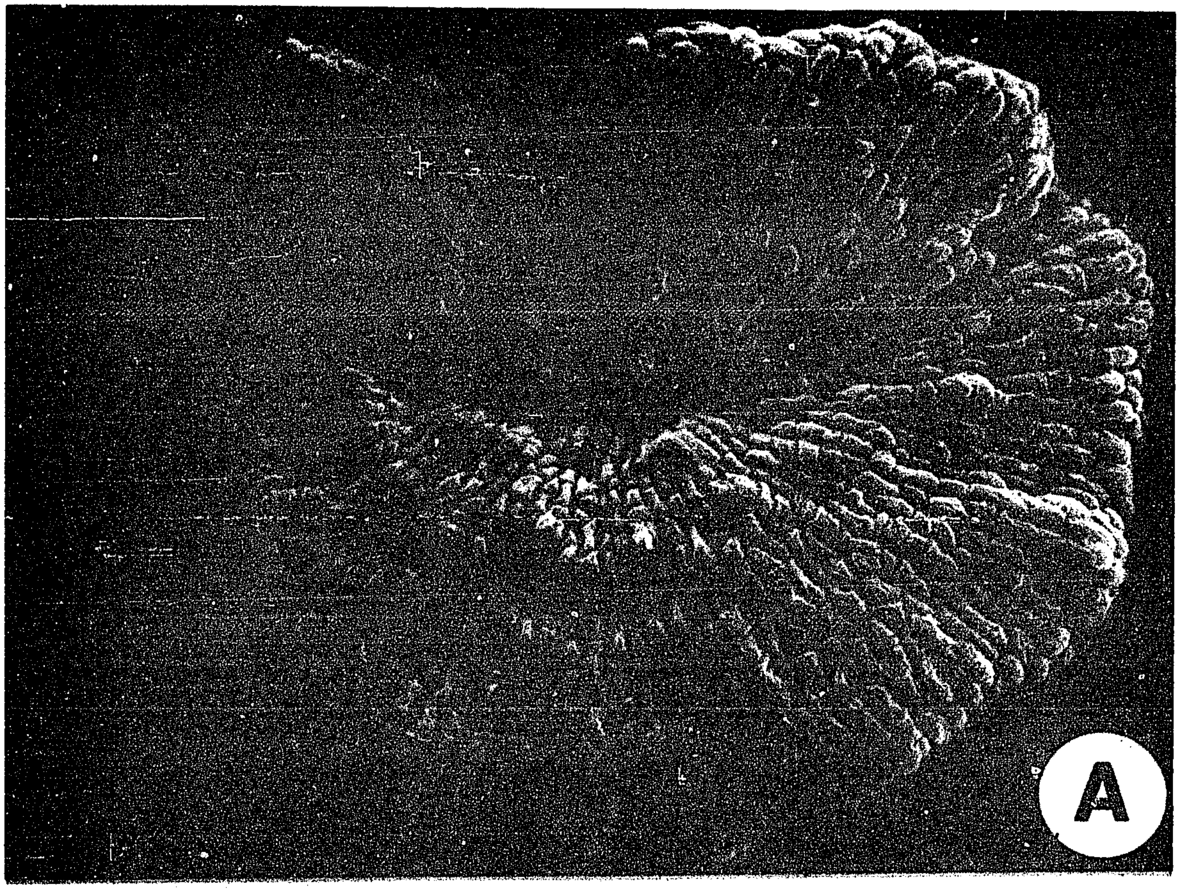

B

Fig. 14. Comparison of stigmatic surfaces of Ruppia from:

A. a coastal population (Awcomin Marsh). x 312.

B. an estuarine population (Johnson Creek). x 260.


A

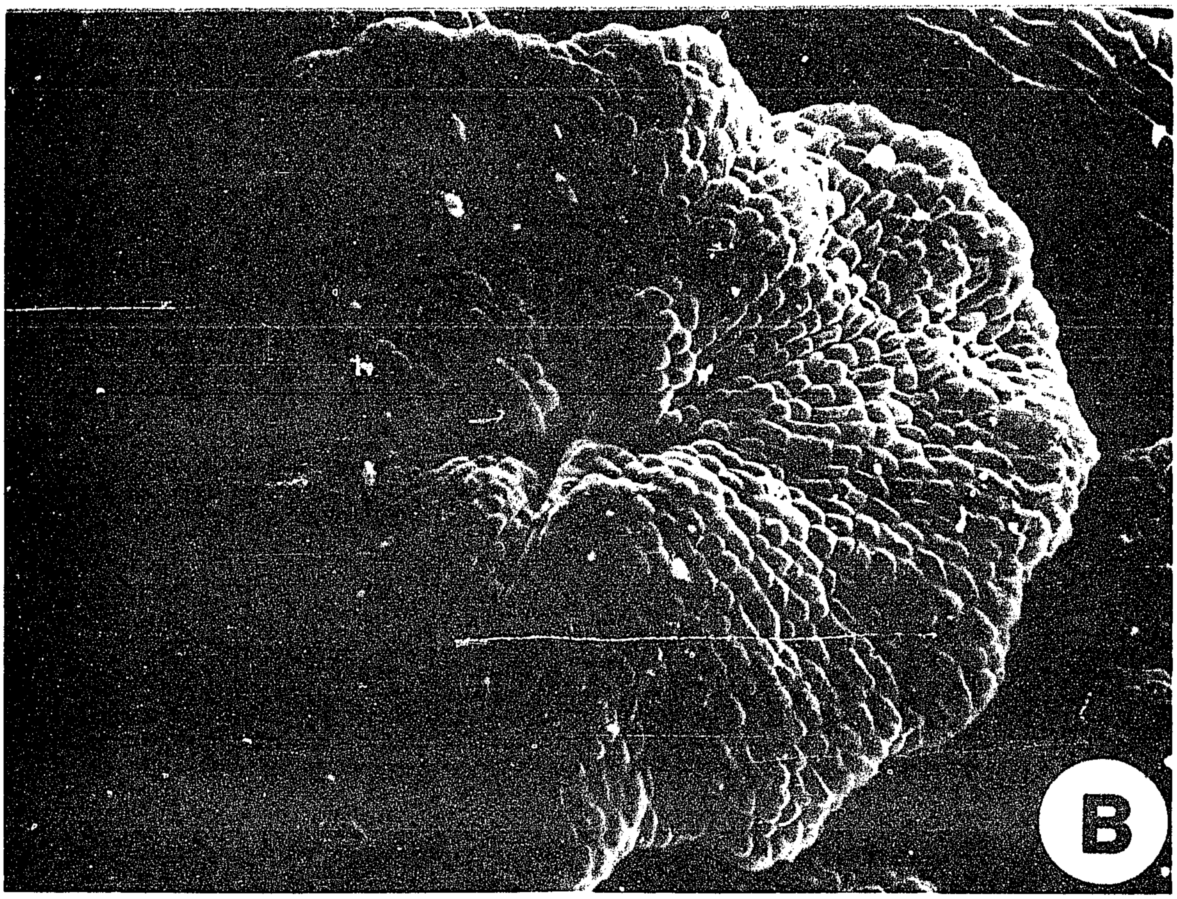

B

Fig. 15. The relationship between anther sac size (mm) and salinity (o/oo) for five sites.

JC = Johnson Creek
LC = Lubberland Creek
VI = Vols Island
AWC = Awcomin Marsh
OP = Ordiones Point

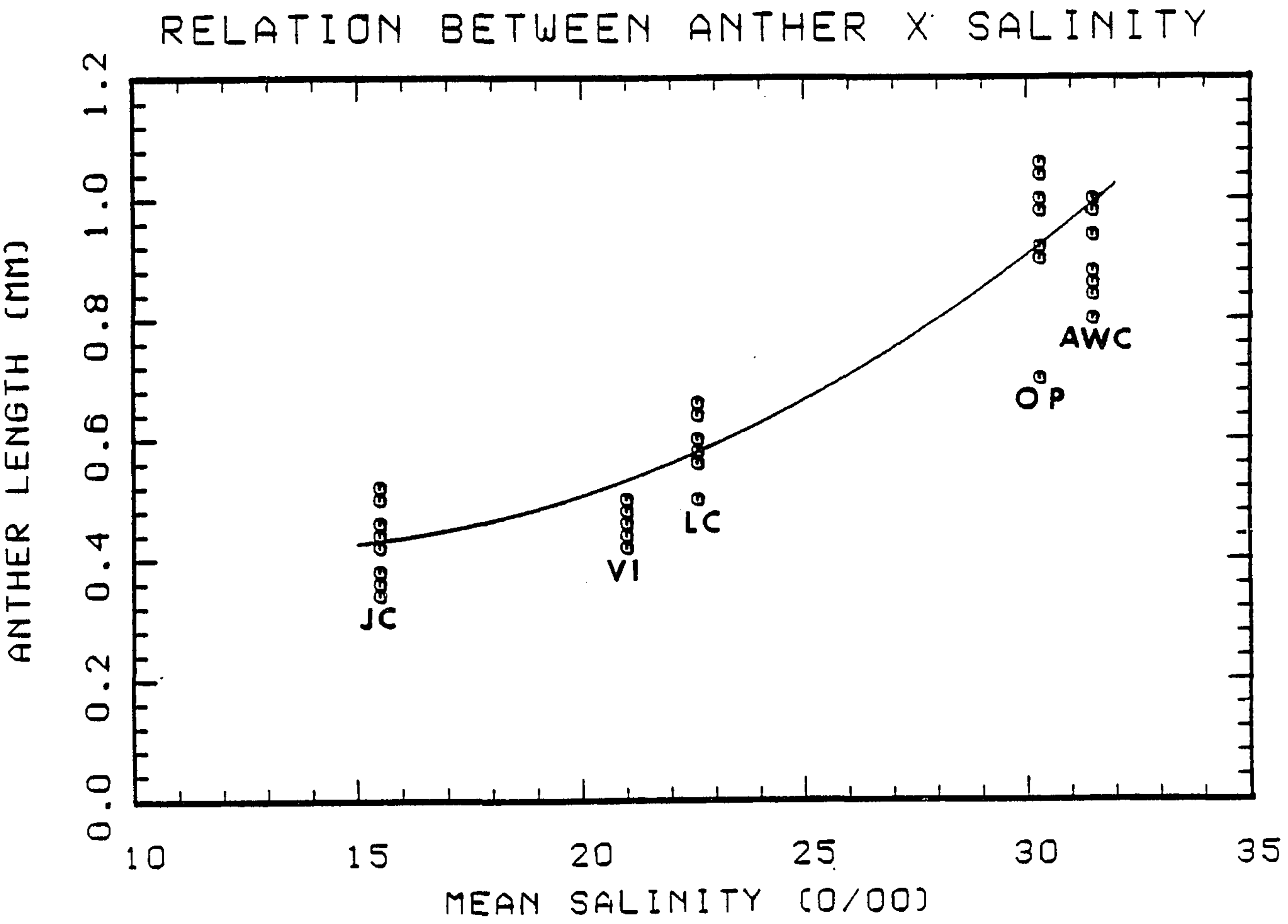

RELATION BETWEEN ANTHER X SALINITY
ANTHER LENGTH (MM)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
10
15
20
25
30
35
MEAN SALINITY (0/00)
JC
VI
LC
OP
AWC

Fig. 16. The relationship between pollen size (microns) and salinity for five sites.

JC = Johnson Creek
LC = Lubberland Creek
VI = Vols Island
AWC = Awcomin Marsh
OP = Ordiones Point

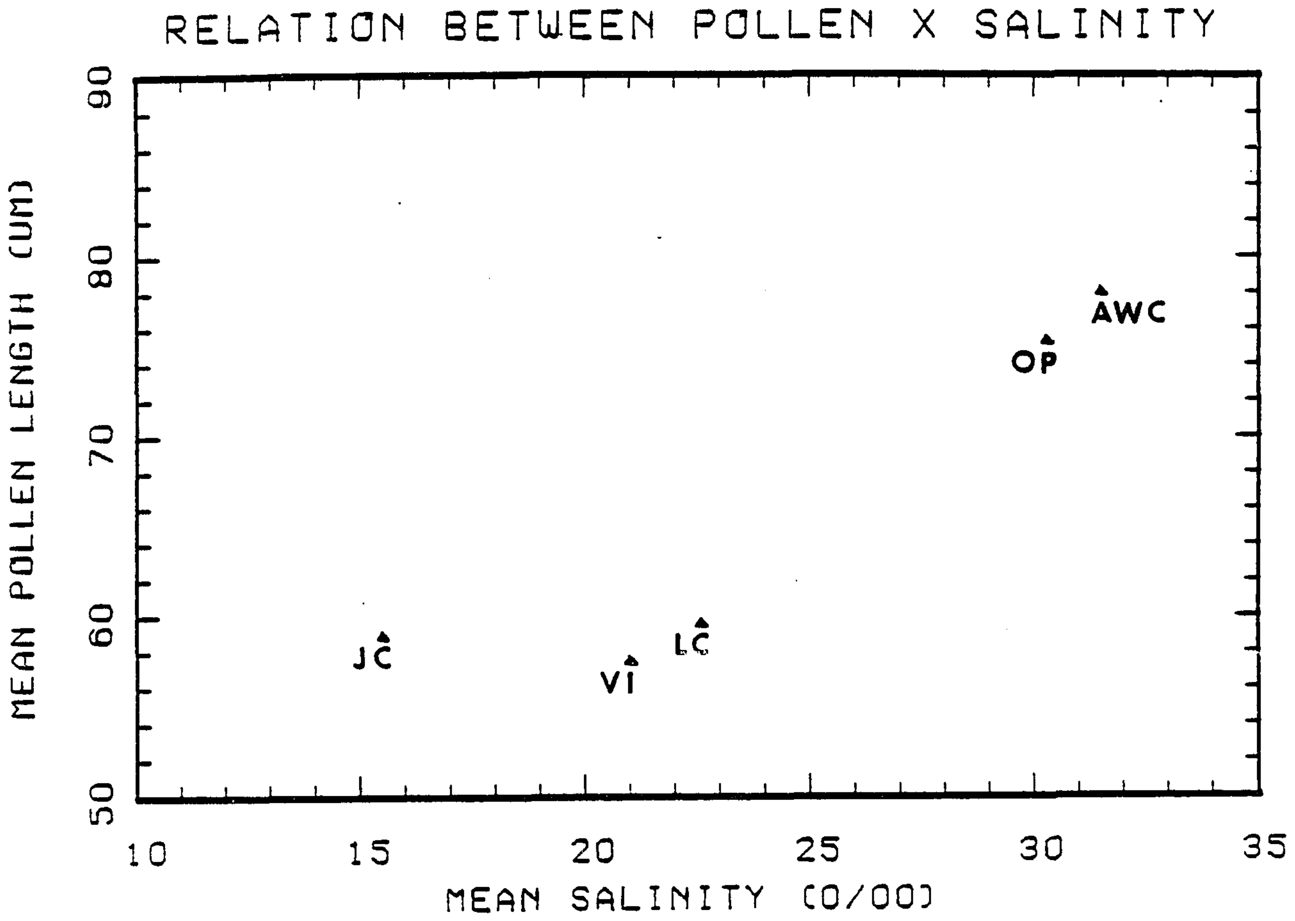

RELATION BETWEEN POLLEN X SALINITY
MEAN POLLEN LENGTH (UM)
90
80
70
60
50
10
15
20
25
30
35
MEAN SALINITY (O/OO)
JC
VI
LC
OP
AWC

Fig. 17. The relationship between anther sac size (mm) and pollen size (microns) for five sites.

JC = Johnson Creek
LC = Lubberland Creek
VI = Vols Island
AWC = Awcomin Marsh
OP = Ordiones Point

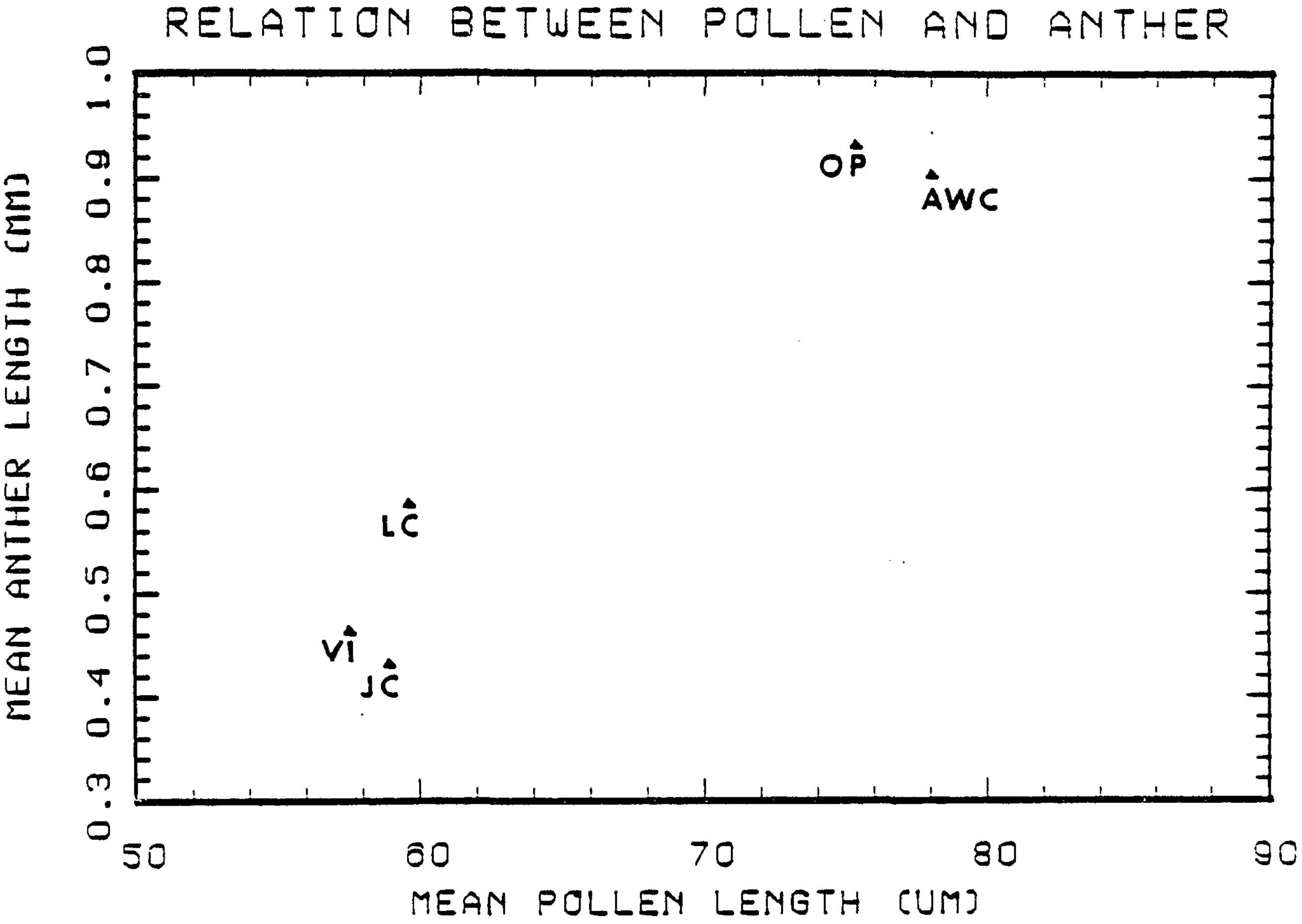

RELATION BETWEEN POLLEN AND ANTHER
MEAN ANTHER LENGTH (MM)
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
50
60
70
80
90
MEAN POLLEN LENGTH (UM)
OP
AWC
LC
VI
JC

Fig. 18. Surface pollination.

A. Inflorescences protruding above the water surface and shedding pollen. x 0.5.

B. Surface pollinating flowers with dehiscent anther sacs. Upper and lower flower on inflorescence axis terminating peduncle. x 20.

C. Chains of pollen grains after shedding on the surface film of water. The chains are dispersed across the surface by wind or wave action. x 80.

B

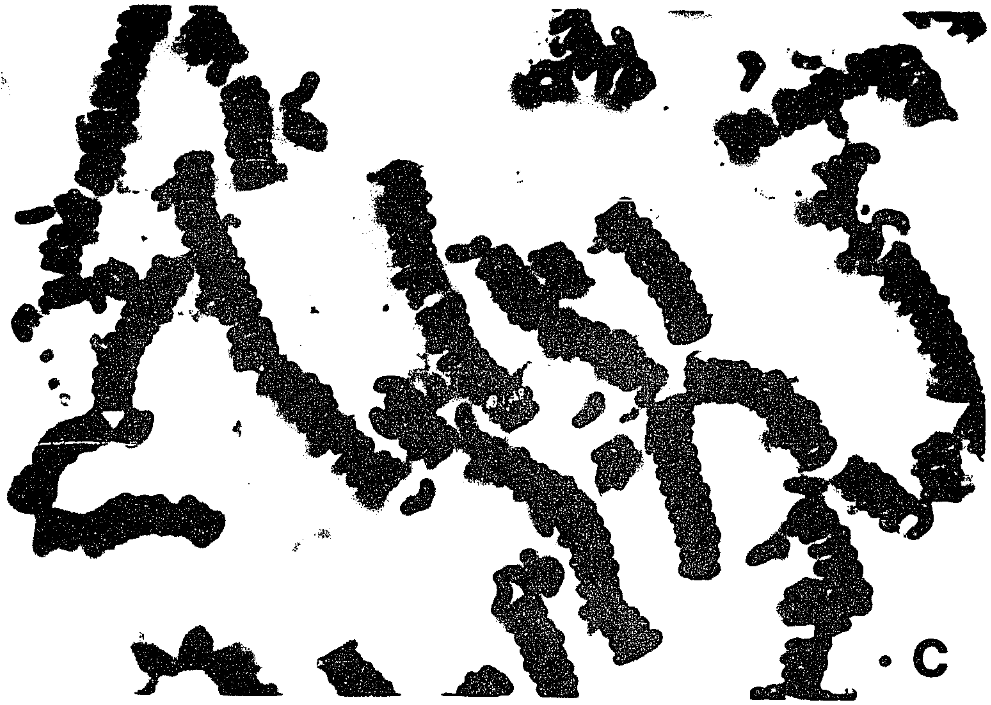


Fig. 19. Underwater pollination.

A&B. Pseudocleistogamy. Anther dehiscence and pollination occurring while inflorescence is still within subfloral leaf sheaths. A, x 15. B, x 15.

C. Inflorescence extended on peduncle with many anther sacs releasing gas bubbles and pollen. x 25.

D. Pollen grains visible (arrows) on outer surface of gas bubbles emanating from dehisced anther sacs. x 25.

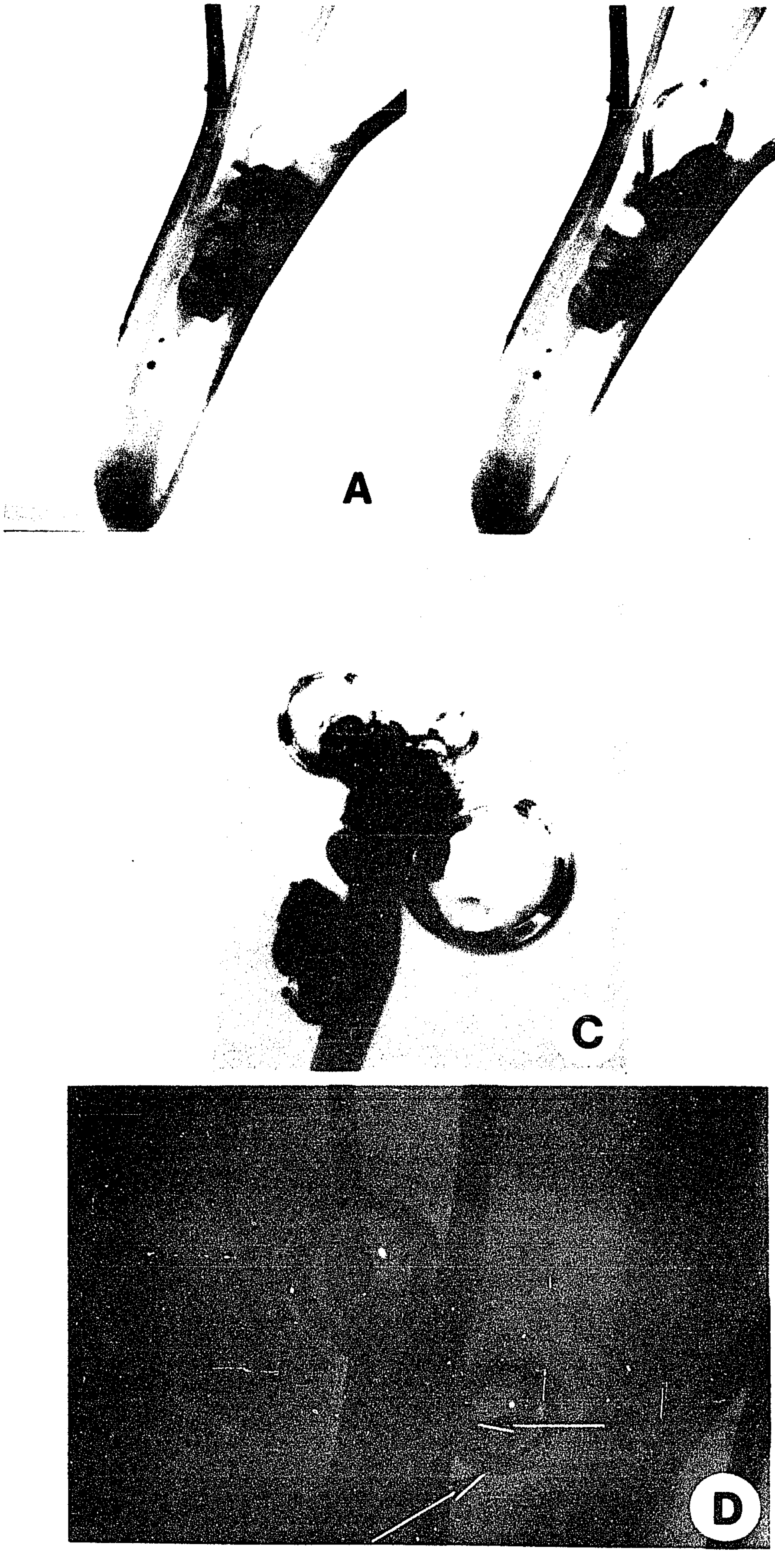
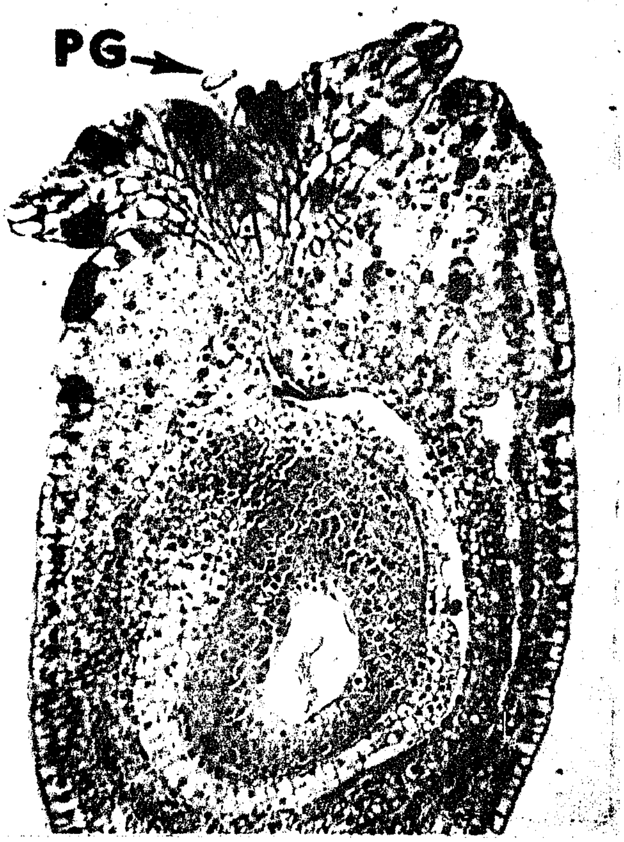

A
B
C
D

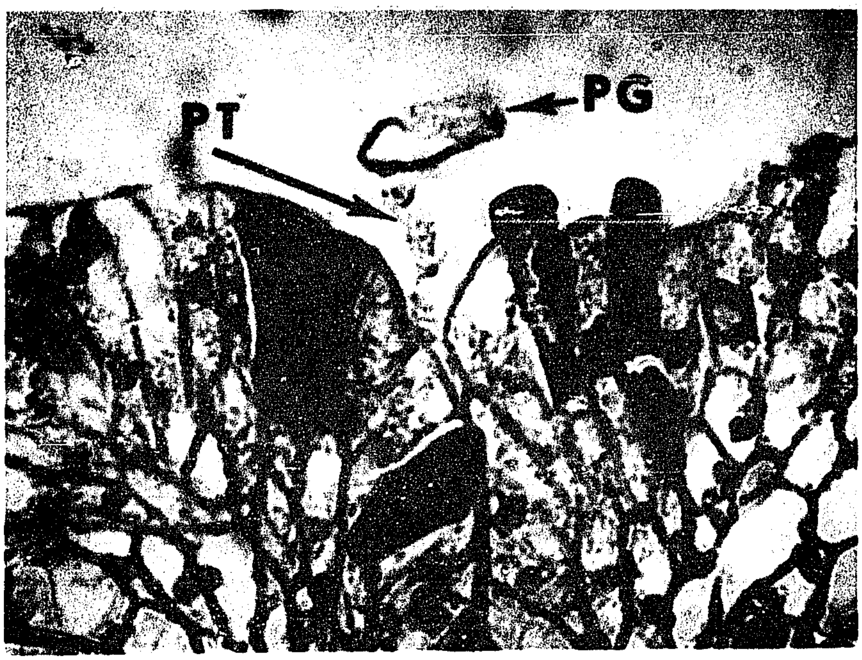
Fig. 20. Median longitudinal sections through the carpel of an underwater pollinated plant.

A. Pollen grain (PG) on stigma with pollen tube (PT) growing down through stylar canal. x 120.

B. Enlargement of same showing reticulate exine of germinating pollen grain and the pollen tube. Dark staining cells on stigma and carpel wall are tanniniferous cells (see text description). x 456.



A



B

Fig. 21. The anatomy of underwater pollination.

A. & B. Arrow indicates terminus of lacunae in base of anther connective. Darkly staining cells along outermost cell layer of connective are tanniniferous cells (see text explanation). Longitudinal section. A, x 105; b, x 75.

C. Loosely packed parenchyma with intercellular spaces (arrows) in longitudinal section of anther connective. x 330.

D. & E. longitudinal and transverse sections of inflorescence axis showing position of lacunae near terminus to anther connective. D, x 85; E, x 60.

F. Transverse section of internal anther connective tissue with intercellular spaces (arrows) through which photosynthetic gases diffuse into anther sac. x 330.

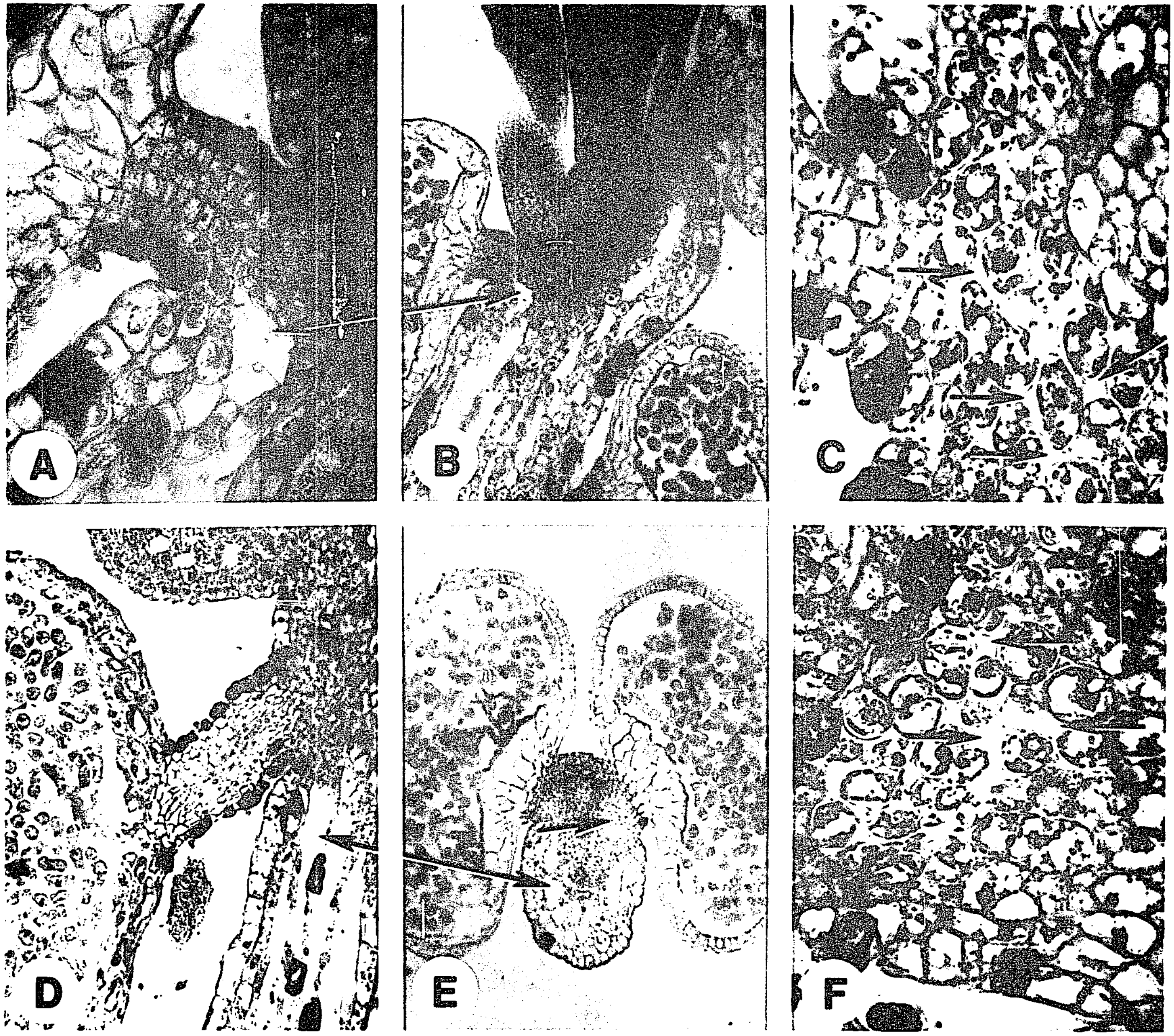

A
B
C
D
E
F

Fig. 22. The anatomy of underwater pollination.

A. & B. arrows 1, 2, 3, show diminishing size of lacunae through which gases produced in photosynthesis diffuse from the inflorescence axis into the anther connective and ultimately to the interior of the anther sac (AS). A, x 85; B, x 325.

PG = pollen grain

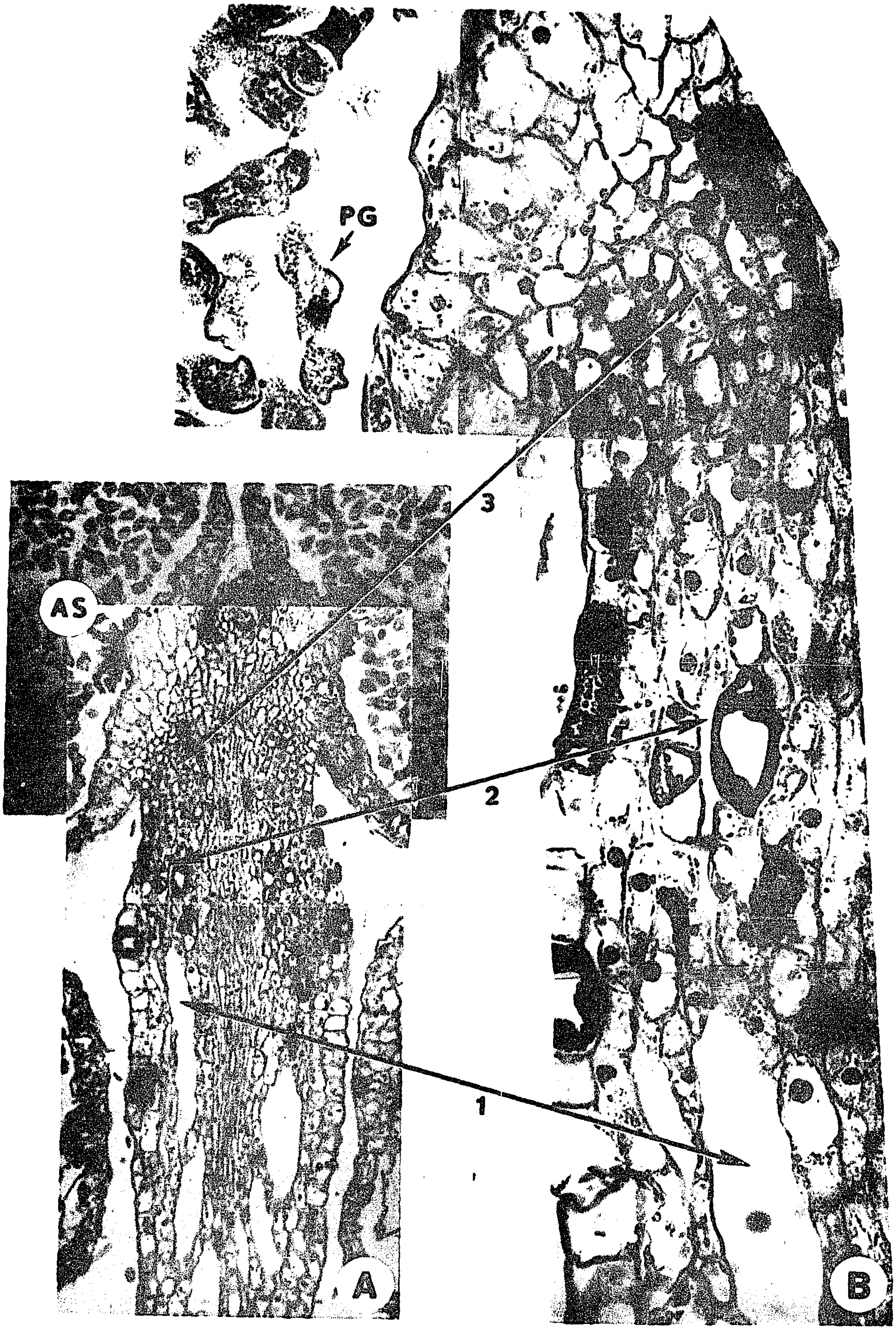

PG
AS
3
2
1
A
B

Fig. 23. Floral morphology and anatomy of underwater pollination.

A. Cluster of four carpels. Anther sacs have been broken off to show broad connectives at base of carpels. x 70.

B. Perianth segment attached to anther connective x 165.

C. Anther (composed of two bilocular anther sacs) broken off inflorescence axis to show base of connective. x 65.

D. Tissue of anther connective reveals intercellular spaces (arrows) through which gases produced during photosynthesis in the internal lacunar system of the plant are diffused to the anther sac. x 550.

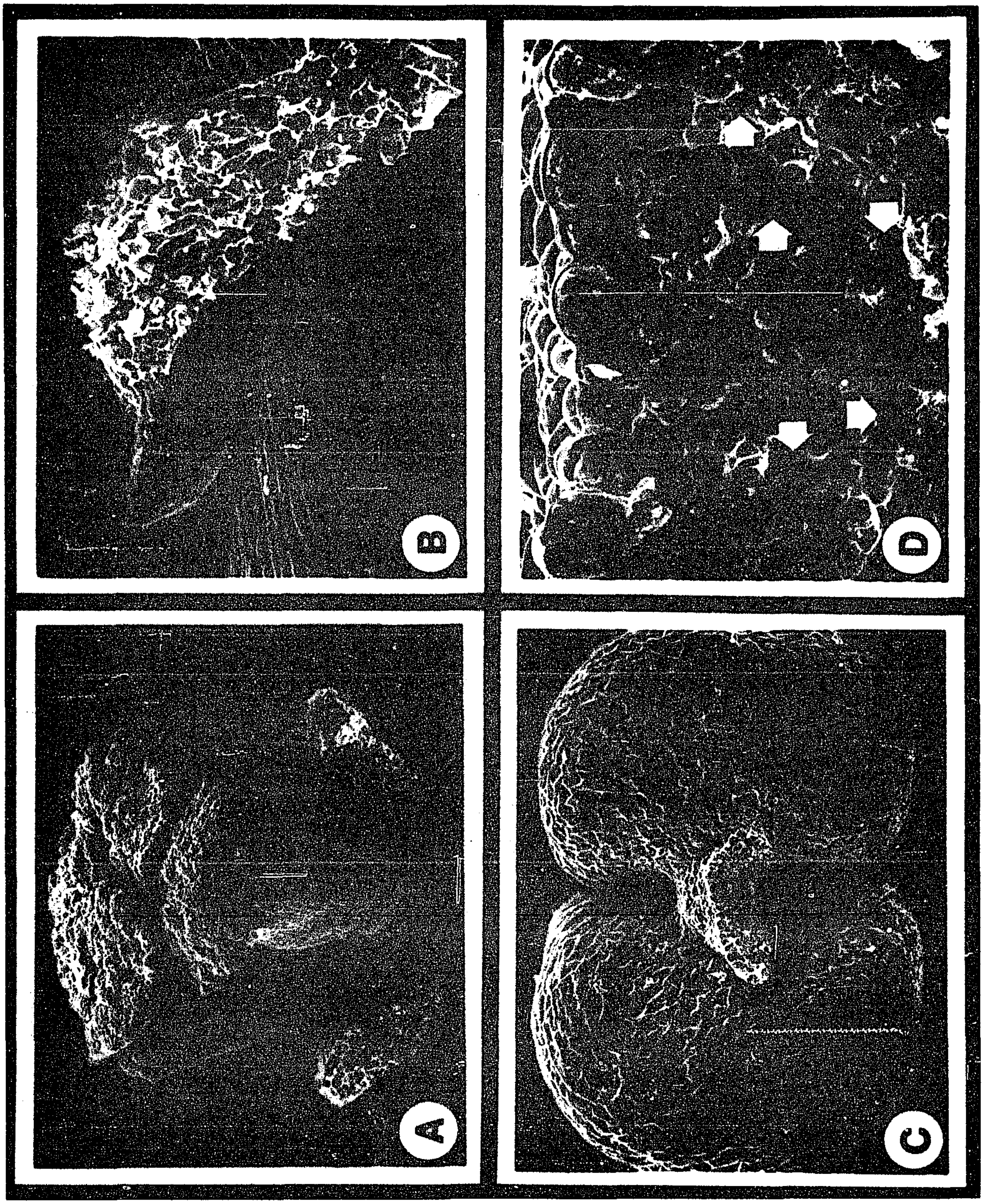

A
B
C
D

Fig. 24. A. An anther sac split open perpendicular to septum containing nearly mature pollen grains. x 96.

B. Pollen grains with traces of mucilaginous material attached in another sac. Secondary cell wall thickenings of endothecium are visible in sectioned anther sac wall. x 680.

C. Sectioned pollen grain (non-acetolyzed) reveals remains of cytoplasm and starch grains (round objects). x 3400.

D. Section of pollen grain wall showing sculptured exine layer and very thin intine. x 17, 140.

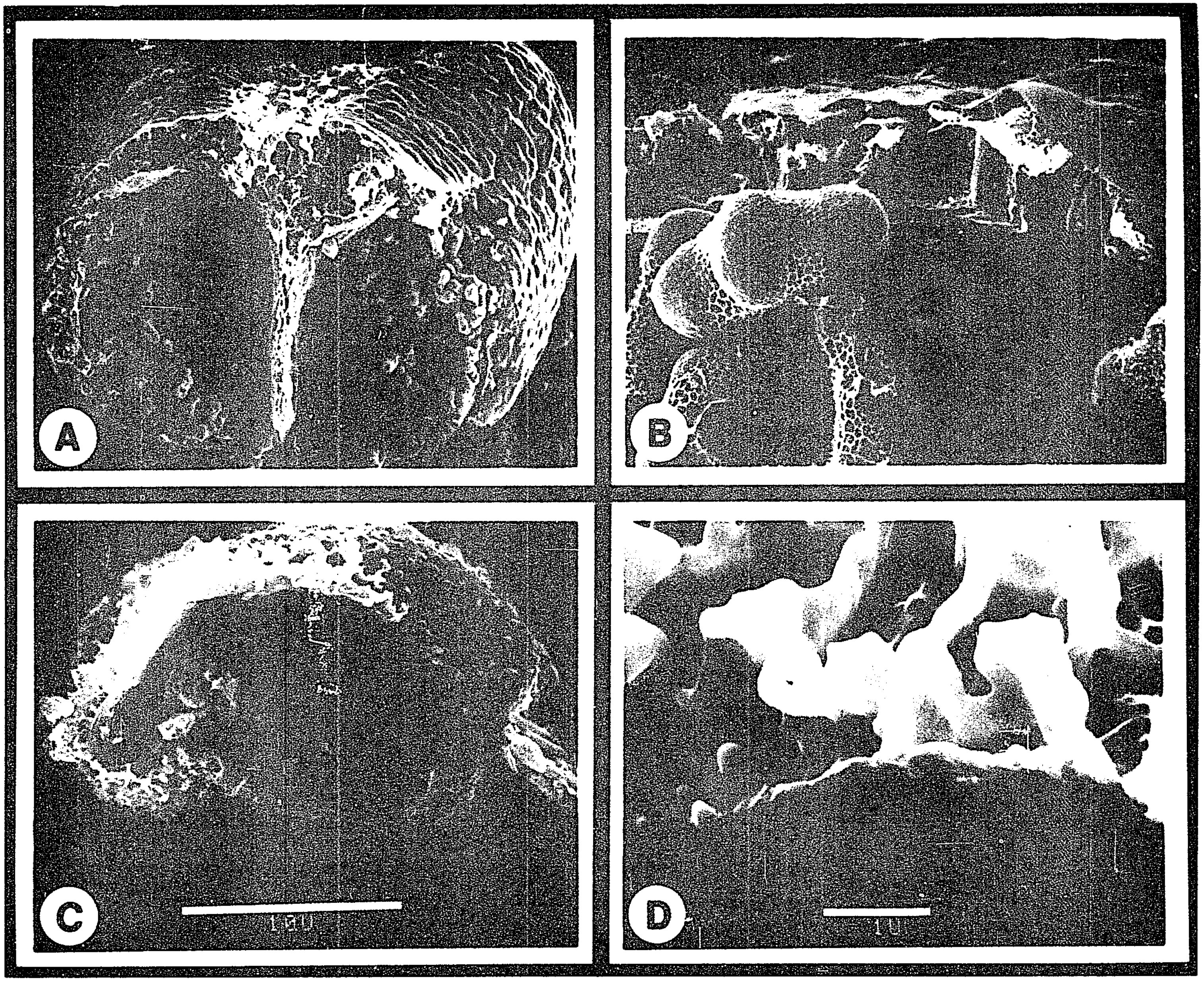

A
B
C
10U
D
1U

Fig. 25. Comparative pollen grain morphology for eight populations of Ruppia

A. Lubberland Creek (LC) - estuarine extensive shallow pannes
B. Vols Island (VI) - estuarine panne
C. Johnson Creek (JC) - upper estuarine pond-hole
D. Little River Swamp (TIB) - coastal low salinity impounded creek
E. Cain's Creek (CC) - coastal marsh creek
F. Taylor River (TR) - coastal deep panne
G. Awcomin Marsh (AWC) - coastal pond-hole
H. Odiornes Point (OP) - coastal pond-hole

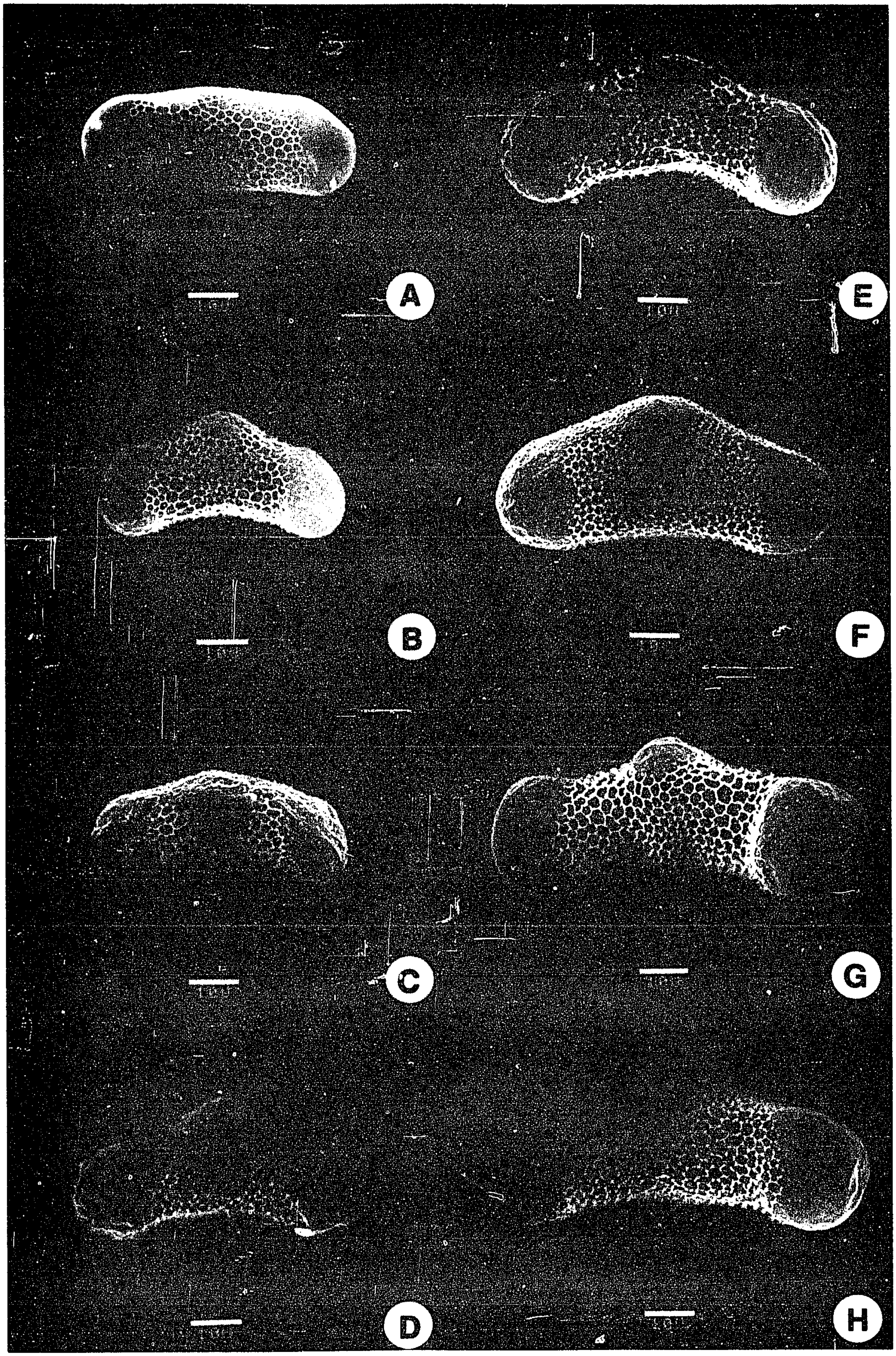

A
E
B
F
C
G
D
H

Fig. 26. Comparative pollen grain exine architecture for eight populations of Ruppia. Reticulate exine surface view.

A. Lubberland Creek (LC) - estuarine extensive shallow pannes
B. Vols Island (VI) - estuarine panne
C. Johnson Creek (JC) - upper estuarine pond-hole
D. Little River Swamp (TIB) - coastal low salinity impounded creek
E. Cain's Creek (CC) - coastal marsh creek
F. Taylor River (TR) - coastal deep panne
G. Awcomin Marsh (AWC) - coastal pond-hole
H. Odiornes Point (OP) - coastal pond-hole

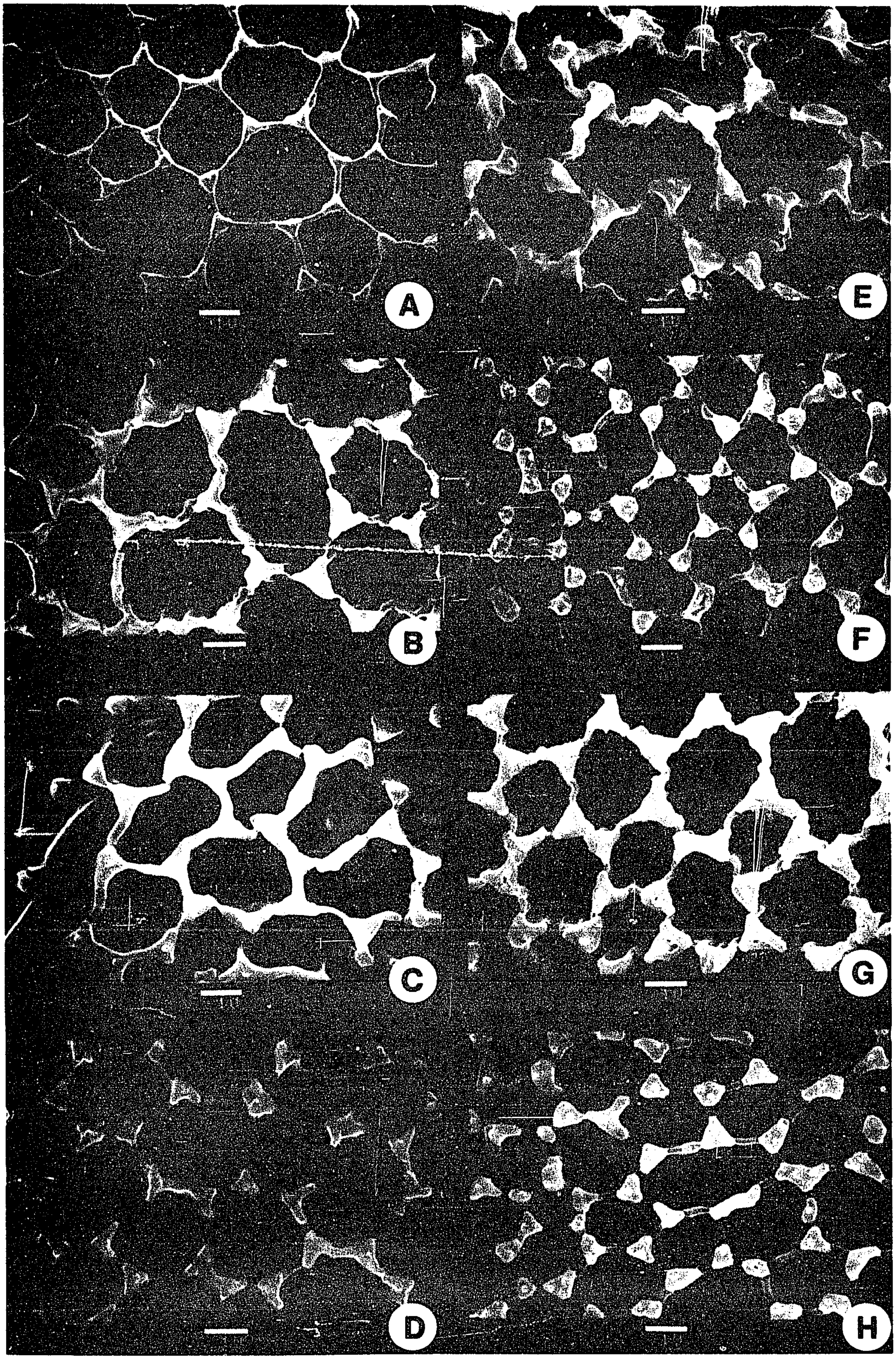

A
E
B
F
C
G
D
H

Fig. 27. Comparative pollen grain exine architecture for eight populations of Ruppia. Oblique views showing reticulate exine and thinner modified colpus area.

A. Lubberland Creek (LC) - estuarine extensive shallow pannes
B. Vols Island (VI) - estuarine panne
C. Johnson Creek (JC) - upper estuarine pond-hole
D. Little River Swamp (TIB) - coastal low salinity impounded creek
E. Cain's Creek (CC) - coastal marsh creek
F. Taylor River (TR) - coastal deep panne
G. Awcomin Marsh (AWC) - coastal pond-hole
H. Odiornes Point (OP) - coastal pond-hole

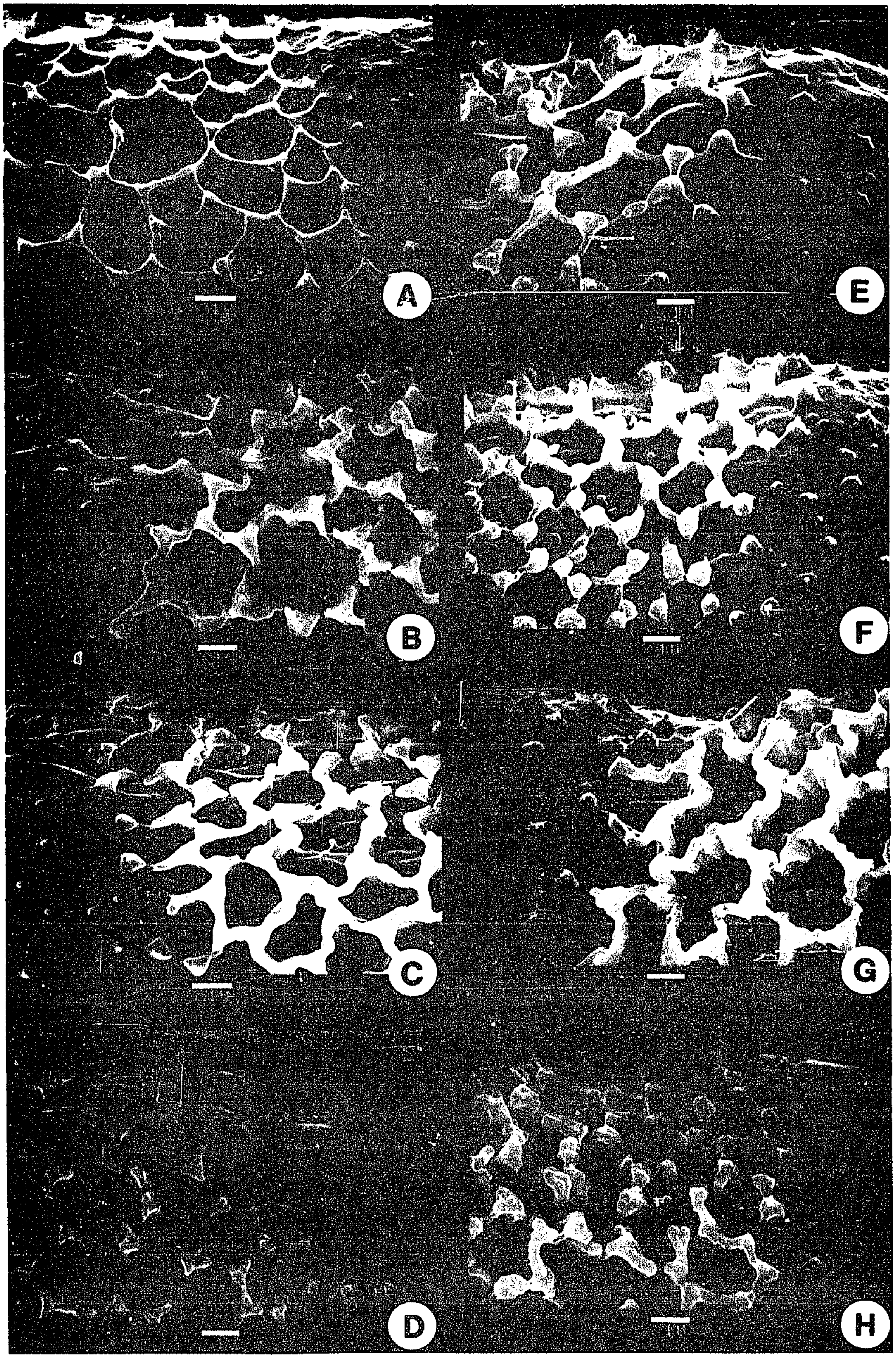

A
E
B
F
C
G
D
H

Fig. 28. Comparative pollen grain wall morphology for eight populations of Ruppia. Sectional views from 'chopped' grains.

A. Lubberland Creek (LC) - estuarine extensive shallow pannes
B. Vols Island (VI) - estuarine panne
C. Johnson Creek (JC) - upper estuarine pond-hole
D. Little River Swamp (TIB) - coastal low salinity impounded creek
E. Cain's Creek (CC) - coastal marsh creek
F. Taylor River (TR) - coastal deep panne
G. Awcomin Marsh (AWC) - coastal pond-hole
H. Odiornes Point (OP) - coastal pond-hole

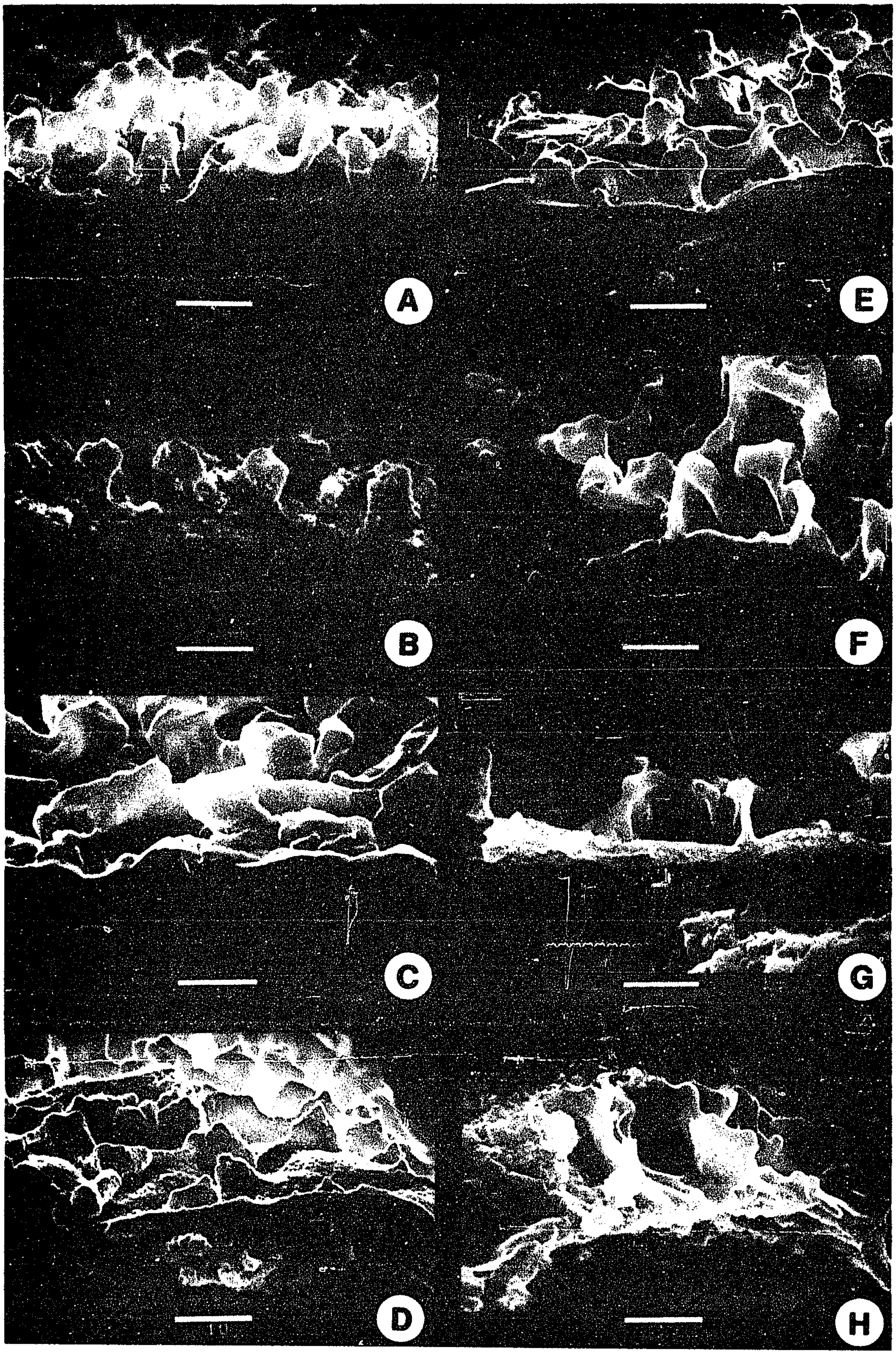
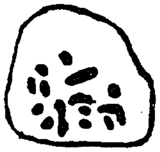

A
E
B
F
C
G
D
H

Fig. 29. Camera Lucida drawings of somatic chromosomes (root-tips) from five populations of *Ruppia*.

LC = Lubberland Creek 2n = 14
JC = Johnson Creek 2n = 14
VI - Vols Island 2n = 14
AWC = Awcomin Marsh 2n = 28
TR = Taylor River 2n = 28

All at x 1000.

LC



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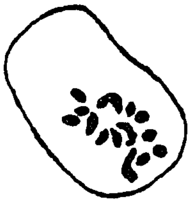
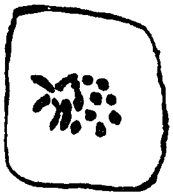
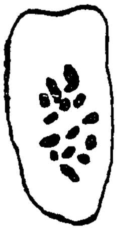


2



3

JC



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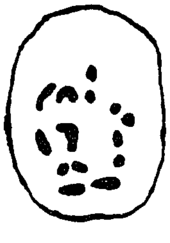

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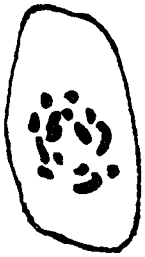
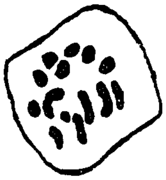


3

VI



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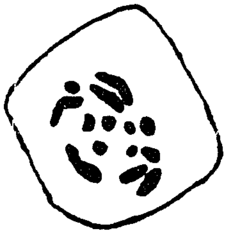

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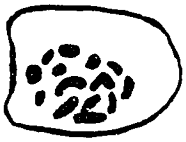
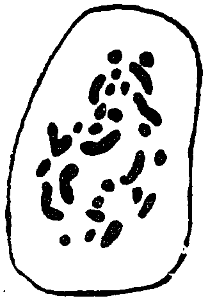


3

AWC



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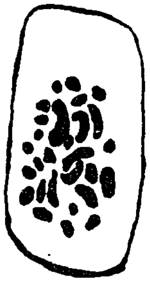
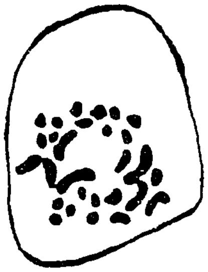
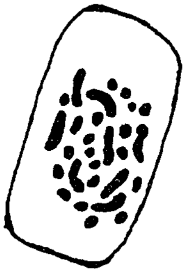


2

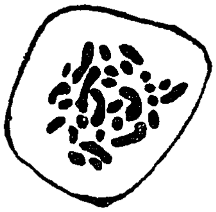


3

TR



1



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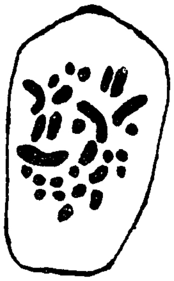


3

Fig. 30. Optical micrographs and camera lucida drawings of somatic chromosomes (root-tips) from an estuarine population and a coastal population.

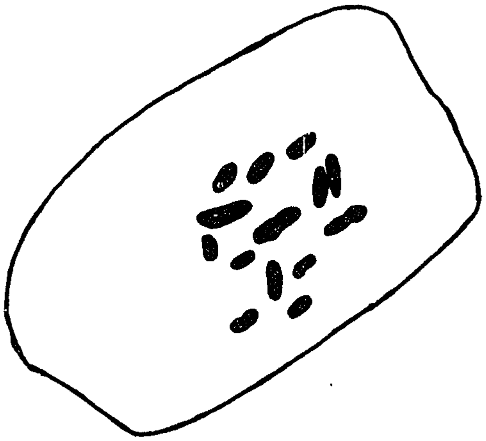
A, Al. Johnson Creek - estuarine pond-hole.

2n = 14. A, x 3370. Al, x 2400.

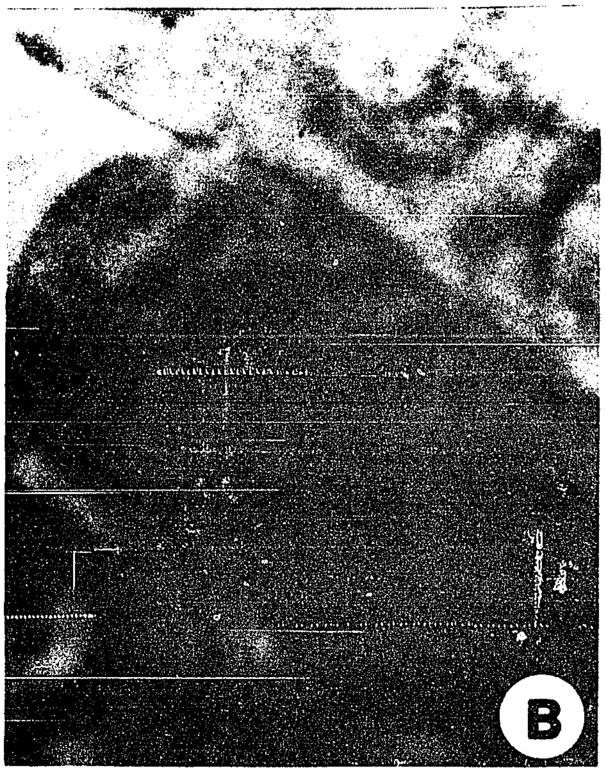
B, Bl. Awcomin Marsh - coastal pond-hole.

2n = 28. B, x 2455. Bl, x 1830.

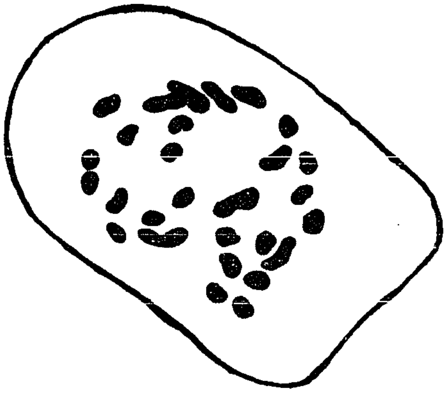
A



A1



B



B1