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Role of Morphological Characteristics of Leaves and the Sporangial Region in the Taxonomy of *Isoetes* in Northeastern North America

L. S. Kott[1] and D. M. Britton
Department of Botany and Genetics, University of Guelph, Guelph, Ontario

Vegetative features have often been used as key characters in distinguishing between species of *Isoetes* as well as for supportive evidence in taxonomic treatments of the genus (Pfeiffer, 1922; Fernald, 1950). As part of a larger taxonomic study of a group of species within this genus from northeastern North America, morphological characteristics of the vegetative body were studied in order to assess their taxonomic significance. Taxonomically valuable character states were considered to be those that correlated with distinctive spore ornamentation (Kott & Britton, 1983), cytological (Kott & Britton, 1980), chemical (Kott & Britton, 1982a) and biological groupings (Kott & Britton, 1982b). Those characters that varied within taxa and overlapped greatly among taxa were considered of no taxonomic value. Characters of the leaf, corm, roots, velum, ligule, leaf margins, and sporangium were investigated.

Materials and Methods

Morphometric studies were made from specimens borrowed from herbaria (CU, DAO, GH, LKHD, MICH, MIN, MO, NEBC, NY, OAC, PFES, QFA, QUE, SFS, TRT, UVIC, VT, WAT, and WIS). Additional specimens were received from Algonquin Park Museum (APM), and the personal collection of B. Gauthier. The authors' collections have been deposited at the University of Guelph (OAC). The species of *Isoetes* considered in this study were *I. macrospora* Dur., *I. hieroglyphica* A. A. Eaton, *I. riparia* Engelm. in A. Braun, *I. tuckermanii* A. Braun, *I. acadiensis* Kott, *I. echinospora* Dur., *I. eatonii* Dodge, and *I. engelmannii* A. Braun, all from northeastern North America.

Leaf measurements were made to the nearest half centimeter with a plastic millimeter rule, which could be bent to conform to the curvature of the leaves. Measurements were made from the upper surface of the corm at the base of the leaf to leaf tip; only the longest leaf per plant was measured. On most plants, leaves of one age class were generally similar in height, the younger and shorter ones being nearer the center of the corm at the apex. Leaf margin measurements were made on the oldest complete leaf.

Leaf number was determined by counting all leaves per plant measuring over 1 cm including old, outer leaves, often represented only by persistent leaf bases.

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[1] Present address: Department of Crop Science, University of Guelph, Guelph, Ontario.

Leaf number and length were determined on more than 3000 plants in about 600 populations.

Variation in leaf number and length was calculated for plants within a few large but typical populations and between all populations available of each species. The statistic used for comparisons of the variability of leaf features was the coefficient of variability:

$$CV = \frac{\text{standard deviation} \times 100}{\text{mean}}$$

Leaf habit (relating to the presence or absence of curvature of individual leaves) was scored as follows: 1) erect, leaves upright and stiff; 2) slightly recurved, leaves essentially erect but slightly reflexed at the tips; and 3) recurved, leaves decidedly recurved or reflexed in a fixed position.

Leaf texture (relating to thickness of individual leaves) was scored only when the leaves were 1) of unusual thinness or appeared fine and soft, or 2) unusually thick or stout, almost brittle. Leaves that had neither of these characters were considered of "typical" texture and were not scored.

Leaves were scored for color when they were unusually dark green, yellow-green, or red-green, but the color of most dried specimens had deteriorated or faded and accurate data were impossible to accumulate for all specimens. The more recently collected specimens gave better color data. Stomata were observed by the acetate peel method. Data for leaf habit, texture, and color were scored for 466 populations.

Height and width of mature sporangia were measured in mm at the longest dimension. Velum length was measured from the base of the ligule down to the free edge of the velum towards the center of the sporangium. Ligules of mature leaves were observed, measured, and drawn from live specimens only. Sporangium coloration was observed and photographed from slides of dry sporangial walls mounted in Euparol, or observed directly with a stereoscope. Sporangium coloration was scored as unspotted (clear), spotted, patchy, or tan.

Results and Discussion

General Morphology.—The most obvious feature of an *Isoetes* plant is the tuft of green, linear leaves, congested at their bases, borne on a solid corm in a centrifugal, spiral pattern. The corm is a short stem-like organ that may be variously lobed. Some species such as *I. japonica* A. Braun of Japan, *I. durieui* Bory of southern Europe, and *I. coromandelina* L.f. of India (Pfeiffer, 1922) have three-lobed corms. Plants of northeastern North America generally have a two-lobed corm, and therefore this feature has no discriminatory value.

Lobes of the corm are separated by shallow fossae. Young roots develop from the basal meristem in the lower part of the corm along this fossa (Paolillo, 1963). The flattish shoot apex sits in a central concavity on top of the corm. Older leaves are displaced from the shoot apex by development of younger leaves. Therefore, the oldest leaves and roots are found at the periphery of the corm where old leaves, roots, and deteriorated sections of old corm tissues are sloughed. The age

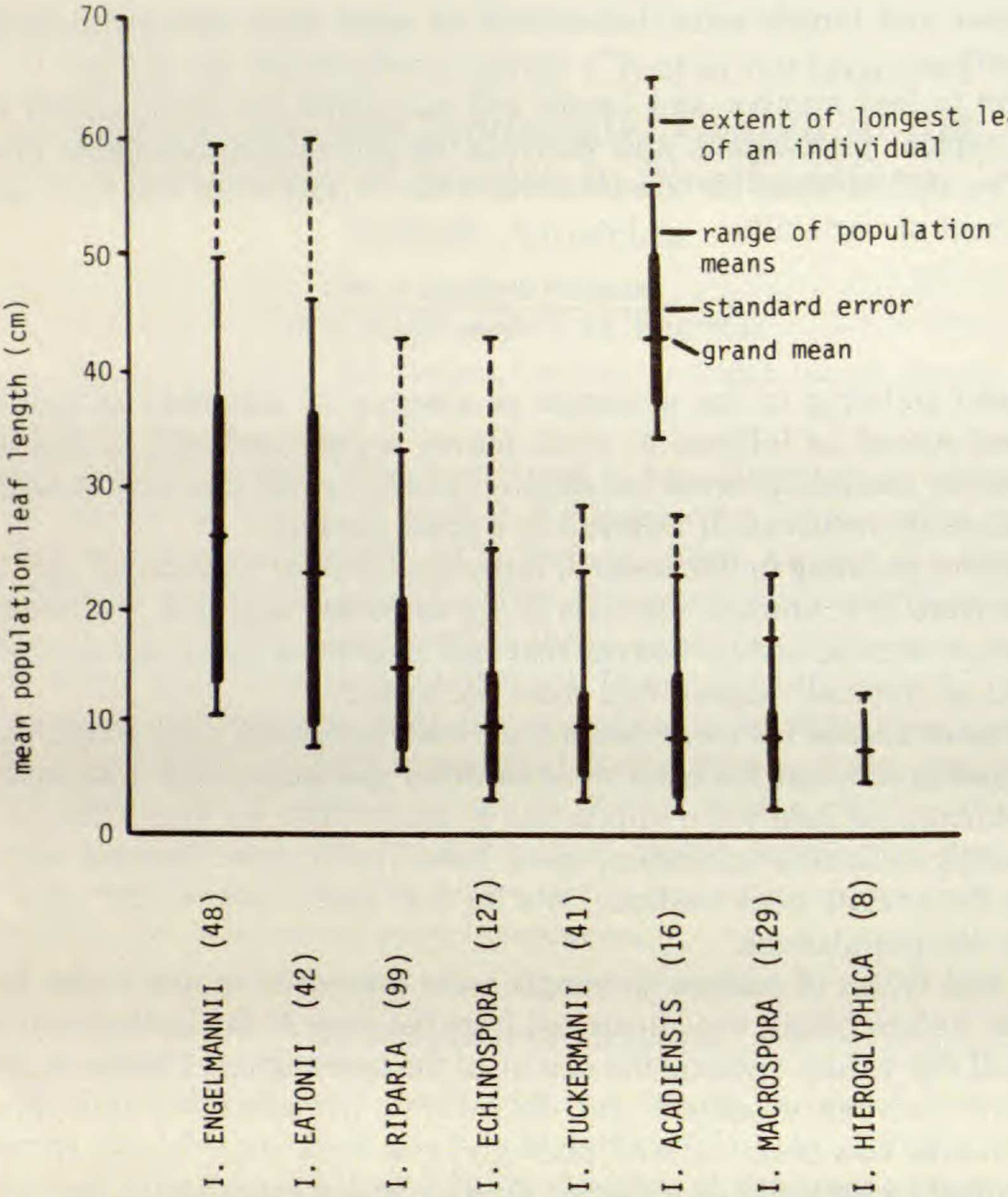

FIG. 1. Comparison of leaf length of *Isoetes* based on population means. Number of populations sampled indicated parenthetically for each taxon.

of a corm is therefore virtually impossible to determine with accuracy. Karrfalt (1977) speculated on the age of *Isoetes* corms; however, his studies did not consider tissue previously shed, and therefore his results cannot be conclusive.

Roots emerging from the base of the corm have a large root cap (Peterson et al., 1979). The roots branch dichotomously and have a stigmarian type of anatomy, possessing a large central air cavity surrounded by cortex, with the vascular tissue eccentrically placed. While roots are young and actively growing, they appear white. Later, only the root tip remains white, while the body of the root becomes a uniform brown. The gross morphology of roots does not provide characteristics of taxonomic value because the roots are morphologically very constant and generally behave in the same manner in all species considered in this study.

The leaves provide all of the taxonomically important vegetative information

TABLE 1. Variability of Leaf Length and Number within Sample Populations of *Isoetes* spp.[1]

	Leaf length			Leaf number		
	Mean (cm)	SD	CV	Mean	SD	CV
I. macrospora	5.6	1.3	22.2	11.6	3.1	26.4
	6.8	2.0	28.7	21.7	4.0	18.6
I. tuckermanii	8.9	2.6	28.7	11.1	3.0	26.7
	9.1	1.7	18.5	13.4	3.1	22.7
I. riparia	14.8	2.8	19.1	11.0	3.7	34.0
	10.5	1.3	12.6	11.3	4.2	37.5
I. acadiensis	22.2	3.0	13.5	17.8	8.8	49.8
	9.1	2.7	29.7	28.0	12.6	45.0
I. echinospora	9.6	3.1	32.0	15.5	4.3	29.9
	7.6	1.5	20.0	14.2	2.9	20.2
I. eatonii	14.5	2.7	18.9	34.0	7.2	21.1
	47.4	7.5	15.8	46.0	49.8	108.3
I. engelmannii	11.4	1.8	15.6	21.4	2.7	12.6
	18.8	1.3	6.9	16.0	9.2	57.6

[1] *Isoetes hieroglyphica* plants were too few to analyze statistically.

about the species. Leaves are awl-shaped, but vary from very fine and thread-like throughout to thick and stout, flaring at the base and with an abrupt point at the tip. Leaf color is typically bright green, but varies from yellow-green, to dark green, and occasionally to reddish-green. Those parts of the leaves that are buried in the substrate are opaque white or brown. Leaf bases of some species that occur outside the study area are chestnut-brown and persistent as in *I. melanopoda* Gay & Dur.

Internally the leaves have four longitudinal air chambers that are traversed by thin diaphragms at regular intervals. The vascular tissue is located in the central tissue of the leaf.

Leaf Characteristics.—Leaf measurements have been used as supporting diagnostic evidence in several keys (Eaton, 1900, 1908; Pfeiffer, 1922) but data presented here indicate that leaf length, at least in part, is variable and perhaps strongly dependent on ecological conditions and the vigor of the plant. Measurements of leaf length yielded results seen in Figure 1. The standard error is large for taxa that have large leaf length ranges reflecting the great sensitivity of the leaf length to environmental conditions.

Isoetes engelmannii and *I. eatonii* had the longest leaves, with means of 26 and 23 cm respectively, but both also had considerable range of variability as shown by the high standard errors. *Isoetes riparia* had a grand mean of 15 cm with population means of 33 cm at the upper end of its range. The remaining species had leaf length grand means between 8 and 10 cm. Even the leaf length ranges are similar for these last species. This observation indicates that leaf length is not a good taxonomic character for most of these species.

The variability of leaf length within some sample populations is shown in Table 1 as coefficients of variability (CV). A high CV value indicates a high

TABLE 2. Variability of Leaf Length and Number between Populations within Species of *Isoetes*.

	Leaf length				Leaf number			
	Grand mean (cm)	Range of $\bar{x}$	SE	CV	Grand mean (cm)	Range of $\bar{x}$	SE	CV
I. macrospora	7.6	3–17	2.7	34.4	19.1	4–53	11.6	60.6
I. hieroglyphica[1]	8.0	5–11	—	—	13.0	7–16	—	—
I. tuckermanii	9.2	4–23	3.6	39.1	22.4	10–45	8.5	37.9
I. riparia	14.6	6–33	6.2	42.5	14.8	5–36	7.0	47.3
I. acadiensis	9.3	2–21	5.0	53.9	25.6	9–35	15.2	59.6
I. echinospora	9.6	4–25	4.4	46.2	14.5	7–26	4.4	30.4
I. eatonii	22.7	8–46	13.5	59.3	46.6	12–98	31.2	67.1
I. engelmannii	26.0	10–50	11.8	45.6	27.2	9–61	17.1	63.1

[1] *Isoetes hieroglyphica* populations too few to analyze statistically.

degree of variability, whereas a low CV value indicates a low degree of variability. All CV values for leaf length were above 6.9, generally most were over 18, and the highest being 32 (Table 1).

Between populations, leaf length CV values were even higher than within populations. These high values, which ranged from 34 to 59 (Table 2), reflect the effect of environment on individual plants during the time of leaf development.

Leaf number per plant was recorded because this number has been used as diagnostic or supportive taxonomic evidence in the past. Results of the means and the ranges of leaf number per plant are shown in Figure 2. *Isoetes eatonii* had the highest grand mean at 47 leaves per plant, as well as the largest range of population means (12–98). *Isoetes acadiensis, I. tuckermanii* and *I. engelmannii* had grand means in the 20s, while the remaining species had grand means below 20 leaves per plant. The extremely large variation in leaf number (=range) within species strongly suggests that leaf number is a function of factors such as age and vigor of plants. Habitat perhaps may play an important role since plants inhabiting shallow waters may have a reduced leaf number due to wave or ice action, while those in deep, undisturbed waters may exhibit higher leaf numbers since older leaves can accumulate on the corm.

Variability in leaf number was compared among plants of a few sample populations and among populations within a species. The CV values of leaf number within a population are again very high and are generally considerably higher than comparable CV values of leaf lengths (Table 1). They range from 12 to 108, half being 30 or higher. Older plants tend to have more leaves because they have a larger corm, while it takes new plants several years to build up to an average number of leaves. Because a population consists of both young and old individuals, leaf number is not reliable diagnostic feature.

The CV values also tend to be higher within species (i.e., between populations) than within populations (Table 2). They range from 30 to 67. Such values show great variability of leaf number from plant to plant and from population to population.

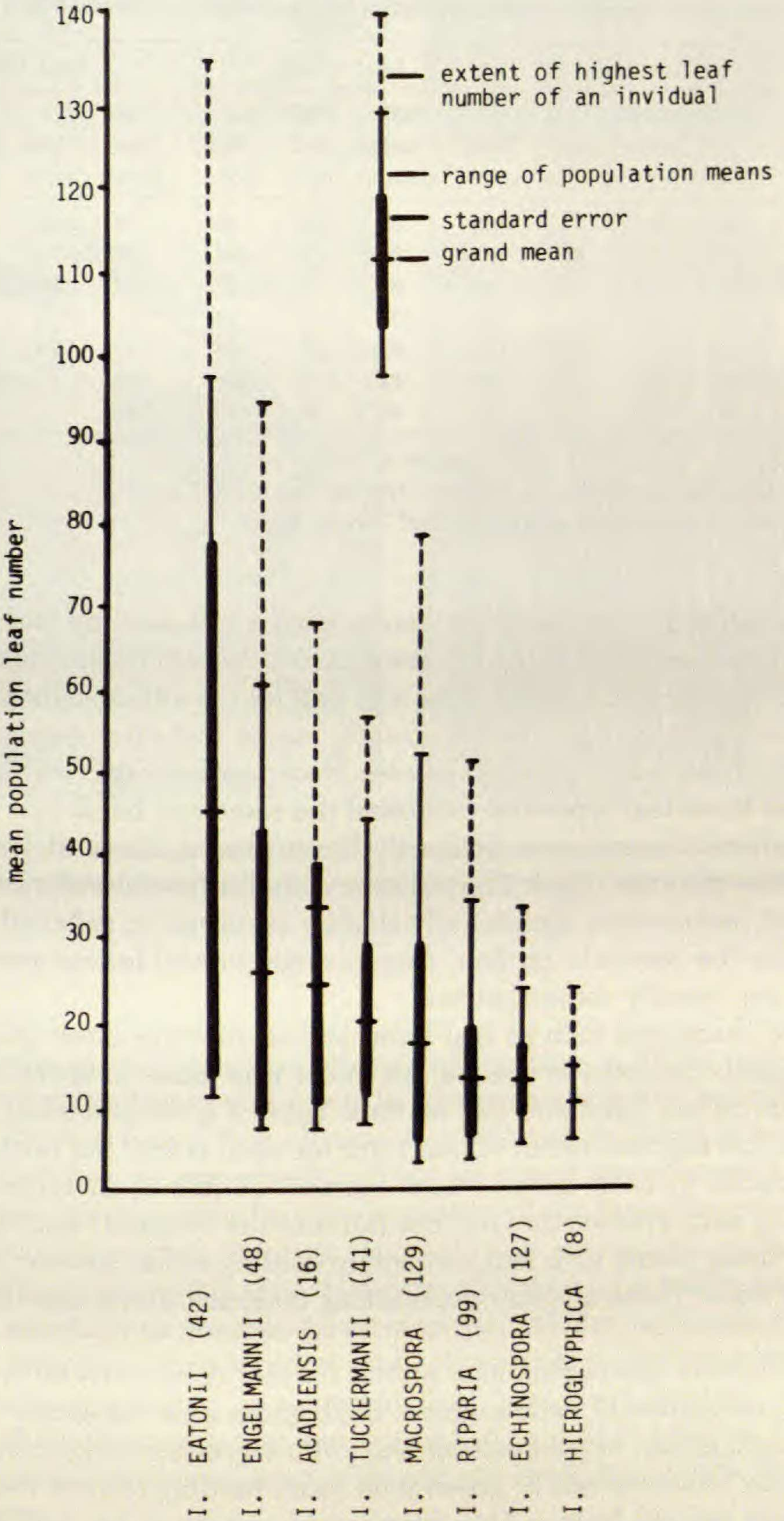

FIG. 2. Comparison of leaf number of *Isoetes* based on population means. Number of populations sampled indicated parenthetically for each taxon.

TABLE 3. Tendencies in Gross Leaf Characteristics in *Isoetes* spp.[1] (Expressed as a Percentage).

	Leaf habit %				Leaf texture %[2]			Leaf color %[3]			
	Erect	Slightly recurved	Recurved	Total pops.	Soft and slender	Stiff and stout	No. pops.	Yellow-green	Dark green	Red-green	No. pops.
I. macrospora	39	27	34	106	35	65	26	6	91	3	33
I. tuckermanii	63	19	18	77	91	9	56	71	14	14	14
I. riparia	100			87	95	5	42	79	21		14
I. acadiensis	25	25	50	12	100		12		75	25	4
I. echinospora	73	14	14	103	83	17	30	43	29	29	8
I. eatonii	100			40	92	9	26	100			10
I. engelmannii	98	2		41	80	20	15	100			7

[1] *Isoetes hieroglyphica* had too few specimens for reliable data.
[2] Includes only those plants that do not fall into "typical" texture for genus.
[3] Includes only those plants that were not "typical" bright green.

Isoetes eatonii generally had erect leaves with a soft and lax leaf texture, yet a few specimens were erect with very stout leaves. *Isoetes tuckermanii* exhibited erect, fine leaves, but many populations also had leaves either slightly or severely recurved. *Isoetes riparia* had erect or straight leaves that often appeared to have been trained by the water current. *Isoetes macrospora* and *I. acadiensis* had a mixture of all three leaf types but exhibited the recurved habit half of the time. While *I. acadiensis* leaves were primarily fine-textured, those of *I. macrospora* were most often stout and rigid. The common state for *Isoetes echinospora* leaves was erect, but leaves were occasionally slightly recurved or reflexed. There was a tendency for the leaves to be fine. *Isoetes engelmannii* leaves were also generally erect and usually somewhat lax.

Qualitative characters such as leaf habit and texture are often quite arbitrary and do not easily conform to classes. An effort was made to score leaf form as a combination of leaf habit and leaf texture. Table 3 is not intended to show the actual numerical representation of leaf form for each taxon, but instead, to show prevailing trends in each taxon. "Leaf texture" (Table 3) includes only those populations in each species that did not fall into the "typical" texture state, and is a score of those plants with leaf characters tending either toward fine and soft or thick and stout. These atypical populations included about one third of those scored.

Of approximately 600 populations scored for leaf color, only 90 were atypical and fell into categories of yellow-green, dark green and red-green. The typical color was bright green. *Isoetes eatonii* was yellow-green or bright green, *I. tuckermanii* mostly yellow-green or green with some tending toward reddish-green. *Isoetes riparia* ranged from a high number of yellow-green and bright green populations to some that were dark green. *Isoetes acadiensis* and *I. macrospora* both tended towards the dark green leaf color, occasionally being reddish-green. The color of *I. echinospora* and *I. engelmannii* leaves were typically bright green, but both tended towards yellow-green (Table 3).

FIG. 3. Coloration of sporangial walls. Bar = 50 μm. a, Pale brown scattered cells of *Isoetes riparia* sporangium. b, Groups of brown cells give a patchy appearance to *I. riparia* sporangium.

Pfeiffer (1922) and earlier workers refer to the presence or absence of bast bundles (fibers) in the peripheral tissues of the leaves. These tissues probably act as strengthening tissue and occur in plants as a result of specific ecological conditions. Pfeiffer found that all taxa in northeastern North America usually lacked bast bundles, except occasionally in emergent specimens of *I. eatonii* and *I. engelmannii*. All the other taxa are aquatic and the formation of bast bundles in the leaves never occurs. Because this feature is uncommon in the taxa under study, it has no taxonomic value. However this may be a useful feature in terrestrial and amphibious species. Of 62 taxa that Pfeiffer reviewed, all terrestrial species had peripheral bast bundles while those truly aquatic species mostly had none. Amphibious species, as expected, had few, weak bundles. Eaton (1900) and Clute (1905) also agree that the amount of bast depended on the exposure of the plant and that this character should not have very much importance in aquatic species.

Stomata are probably formed in developing leaves that are exposed to air. In most species studied here this does not occur regularly, because during early leaf development in spring, water is at a high level and plants are well covered with water, although they may become emergent later. This feature, like the bast

TABLE 4. Characters of the Sporangial Region in *Isoetes* spp.

	Sporangium			Velum	Ligule
	Length (mm)	Width (mm)	Color of mature sporangia	Length (mm)	Length (mm)
I. macrospora	3–5	3–4	clear to brown-spotted	1–1.5	to 2
I. hieroglyphica	5	3	usually clear	1	—
I. tuckermanii	3–5	3	clear to tan	1	to 2
I. riparia	3–7	2–4	clear to very pale tan groups or cells in streaks	to 2	to 3 (gen. less)
I. acadiensis	3–5	2.5–3	clear to single dark or pale tan cells scattered over sporangium	1–1.5	to 3
I. echinospora	4–10	3	clear, or patches of tan cells, or evenly tan	1–2.5	to 2.5
I. eatonii	6–12	3.4–5	clear or tan	1–2	to 3.5
I. engelmannii	3–10	2–4	clear	1–3	to 3.5

bundles, appears to be correlated with growing conditions. Species such as *I. macrospora* that grow in deeper water never have stomata. Those growing nearer to the shoreline occasionally show the presence of some stomata on leaves, as in *I. riparia* and *I. tuckermannii*, but in these it is not a constant feature (Eaton, 1900; Pfeiffer, 1922).

The length of membranous leaf margins that extend some distance up from the base of the leaf tends to be related to the length of the leaves and therefore indirectly to the vigor of the plants. This feature showed a great degree of variability from plant to plant within a given taxon. The maximum distance that this tissue reached on the leaves of most taxa was between 18 and 23 cm from the leaf base; however in most cases this distance was much shorter.

Characteristics of the Sporangial Region.—Sporangia vary in size and color depending on maturity or growing conditions. Young sporangia are unpigmented and appear transparent, while mature sporangia may or may not have some cells that have acquired a brown pigment. The number of cells affected by the pigment, their distribution, and intensity of the pigmentation determined whether the sporangium was scored as clear (no pigment), spotted (individual cells that are brown) (Fig. 3a), patchy (groups of brown cells in patches) (Fig. 3b), or tan (all cells slightly colored). Table 4 shows the sporangium coloration for all species, but data for *I. hieroglyphica* are based on relatively few specimens.

Matthews and Murdy (1969) found sporangial color patterns extremely variable in two southeastern species. This character tends to be constant for most specimens within species of the northeast, although occasionally there are striking exceptions to the otherwise typical patterns. *Isoetes eatonii*, *I. engelmannii*, *I. tuckermanii*, and *I. macrospora* had sporangia that were usually unspotted but a few specimens of *I. tuckermanii* had light brown or tan sporangia, and otherwise typical specimens of *I. macrospora* sometimes had sporangia that were

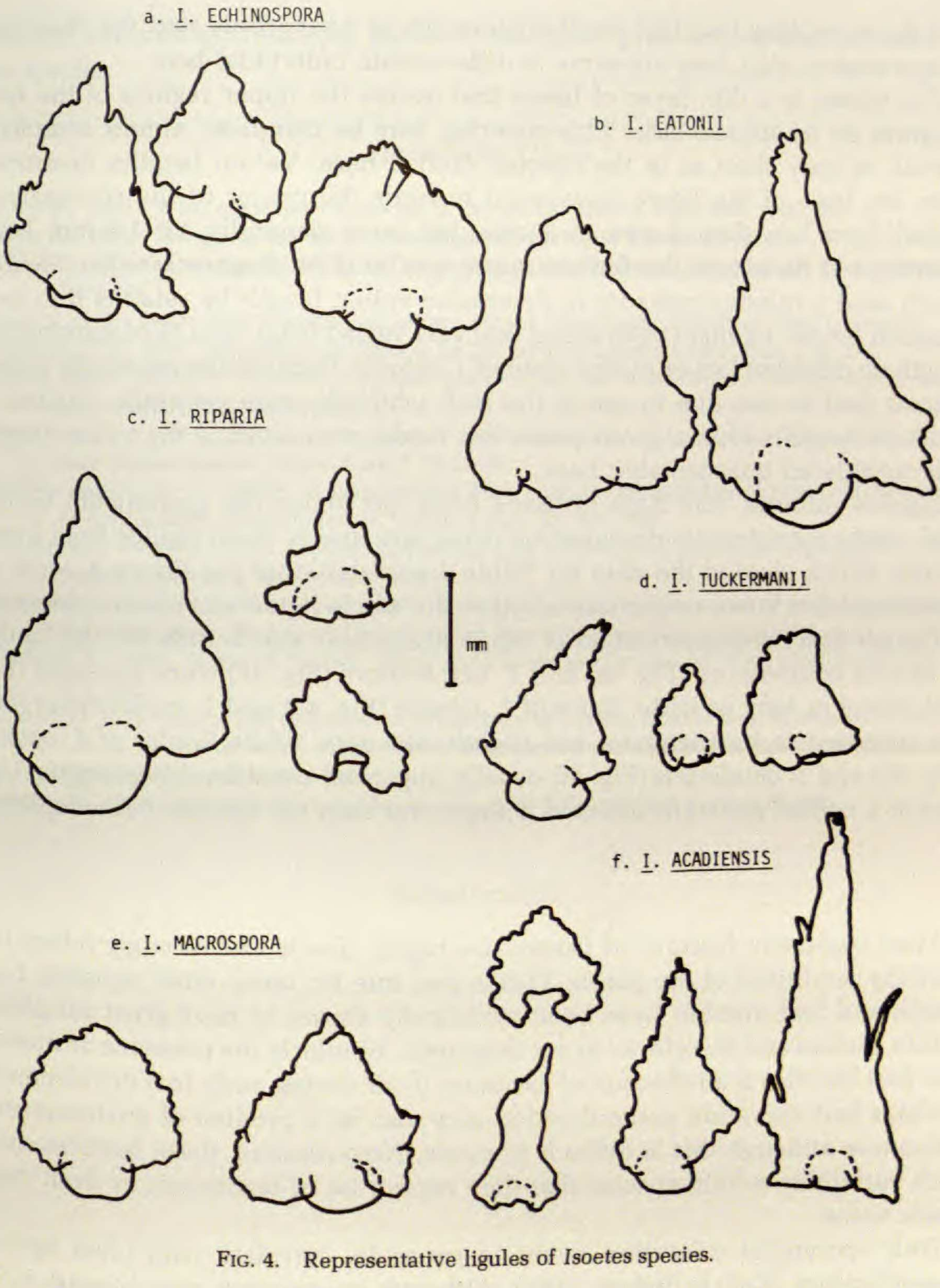

FIG. 4. Representative ligules of *Isoetes* species.

brown-spotted. *Isoetes echinospora* and *I. riparia* typically had colored cells in groups either sparsely or closely placed, which gave the sporangia a coarsely mottled aspect. *Isoetes acadiensis* usually had a tan or spotted sporangium wall.

Table 4 shows data collected on sporangium size for each taxon. Although *I. eatonii*, *I. engelmannii*, and *I. echinospora* appear to have the largest sporangia, the last generally had sporangia much smaller than 10 mm in length. Because *Isoetes eatonii* and *I. engelmanni* had large sporangia of generally the same size, and the remaining taxa had smaller sporangia of comparable size, the character of sporangium size does not serve to differentiate individual taxa.

The velum is a thin layer of tissue that covers the upper regions of the sporangium on its adaxial side. This covering may be complete, almost complete, partial, or very short as in the species studied here. Velum lengths, measured from the base of the ligule downward towards the center of the sporangium, varied from less than 1 mm to 3 mm, but were generally 1.0–1.5 mm long. Therefore in these taxa this feature appears to be of no diagnostic value. Pfeiffer (1922) used a relative measure to determine velum length by relating it to sporangium length. Palmer (1896) found that vela varied from ¼ to ¾ of sporangium length on different leaves of one plant of *I. riparia*. Because the sporangia varied a great deal in size due to age of the leaf, while the vela generally retained a constant length within a given plant, this mode of measuring the velum length was considered unacceptable here.

Ligules—minute, fine flaps of tissue lying just above the sporangium on the leaf—were considerably damaged on dried specimens. Even ligules from living plants, which yielded the data for Table 4 and drawings for Figure 4, were so ephemeral that it was not certain whether the whole ligule was always observed. Although exact characterization of ligule shape and size is difficult, the ligules of *Isoetes echinospora* (Fig. 4a) and *I. macrospora* (Fig. 4e) were generally deltoid, being as long as wide; those of *I. riparia* (Fig. 4c) and *I. tuckermanii* (Fig. 4d) appeared to be triangular but slightly elongate, while ligules of *I. eatonii* (Fig. 4b) and *I. acadiensis* (Fig. 4f) usually appeared considerably elongate. Ligules of *I. eatonii* generally attained a larger size than the ligules of other species.

CONCLUSIONS

Most vegetative features of *Isoetes* are highly plastic and strongly reflect the growing conditions of the plants. This is also true for many other aquatics. Leaf length and leaf number have been statistically shown to have great variability within species and therefore are not diagnostic. Similarly the presence of stomata and bast bundles is a reflection of exposure to air during early leaf development. Perhaps leaf curvature and coloration may also be a product of environmental influences although this is difficult to prove. Nevertheless, these features show such variability within species that they cannot be of taxonomic or even diagnostic value.

Only sporangial coloration seems to generally correlate with other species characteristics (Kott & Britton, 1983). Although sporangium size appears to be somewhat constant within species, the size is often so similar among the taxa that it is not a valuable diagnostic aid. Both the velum and ligule do not yield further distinguishing information about these species.

It therefore must be concluded that vegetative features in *Isoetes* species of northeastern North America are either so constant (such as corm lobing and nature of roots) or so variable as to be of no taxonomic value. Species identification must rely primarily on spore characteristics (ornamentation and size), which agree well with other species specific characteristics such as chromosome number, chromatographic spot patterns, ecological preferences, and behavior of the species.

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