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APPALACHIAN QUILLWORT (*ISOETES APPALACHIANA*, SP. NOV.; ISOETACEAE), A NEW PTERIDOPHYTE FROM THE EASTERN UNITED STATES

DANIEL F. BRUNTON

216 Lincoln Heights Road, Ottawa, Ontario K2B 8A8

DONALD M. BRITTON

Department of Molecular Biology and Genetics,
University of Guelph, Guelph, Ontario N1G 2W1

ABSTRACT. *Isoetes appalachiana,* sp. nov., is an element of the *I. engelmannii* complex of North America. Its distinct cytology, spore morphology, and distribution are illustrated and described. Although widely distributed in the eastern United States, it appears to be most common in the Appalachian Mountains. *Isoetes appalachiana* is tetraploid; it is suspected to be an allopolyploid which evolved from the chromosome doubling of *I.* × *altonharvillii,* the sterile hybrid between *I. engelmannii* (*s. str.*) and *I. valida.*

Key Words: *Isoetes appalachiana,* Isoetaceae, pteridophyte, Appalachian Mountains

Isoetes engelmannii A. Br. (*s. lat.*) is widely distributed in the eastern United States, rarely extending into southern Canada. It is considered to be a basic diploid ($2n = 22$), although a single tetraploid population ($2n = 44$) has been reported from northern Florida (Taylor et al. 1993). Although several varieties of *I. engelmannii* were recognized in the 19th and early 20th centuries (var. *fontana* A. A. Eaton, var. *georgiana* Engelm., var. *valida* Engelm., var. *gracilis* Engelm., var. *caroliniana* A. A. Eaton; Engelmann 1867; Eaton 1900, 1905), these were largely ignored by subsequent workers. Morphological and distributional studies combined with cytological data and the application of Scanning Electron Microscopy (SEM) of spores, however, have led to a reconsideration of some of these subspecific taxa, resulting in the description of new species. *Isoetes engelmannii* var. *caroliniana,* for example, was recently recognized as the diploid species *I. valida* (Engelm.) Clute (Luebke 1992; Brunton and Britton 1996), a decision supported by the electrophoretic studies of Duff and Evans (1992). Luebke (1992) also described *I. georgiana* N. Luebke and *I. boomii* N. Luebke from south central Georgia pop-

ulations previously attributed to *I. engelmannii.* Both are hexaploid ($2n = 66$). *Isoetes engelmannii* var. *gracilis* is widely considered to represent only a larger-than-normal form of *I. engelmannii* (*s. str.*; Taylor et al. 1993).

The discovery of a number of additional tetraploid *Isoetes engelmannii* populations encouraged us to investigate the ecological, morphological, and distributional characteristics of these polyploids and to consider their relationships to previously named taxa in the *I. engelmannii* complex. These investigations indicate that tetraploid populations closely resemble *I. engelmannii* var. *georgiana.* This large-spored taxon was described by Engelmann (1882) from material collected in the 1870s from Floyd County, northwestern Georgia. *Isoetes engelmannii* var. *fontana* described from southeastern Pennsylvania by Eaton (1905) appears to be identical to Engelmann's var. *georgiana* in gross morphology and spore characteristics. Eaton apparently did not consider that the Pennsylvania material could represent the same taxon that was known only from the deep south. We consider these taxa to be synonymous.

In this paper we present evidence for considering tetraploid *Isoetes appalachiana* to represent a previously undetected allopolyploid species.

MATERIALS AND METHODS

Extensive field investigations and collecting throughout the southeastern United States by Brunton since 1990 as part of ongoing systematic studies of *Isoetes* in North America have included efforts to re-locate the locations of type populations of taxa in the *I. engelmannii* complex. Cytological investigation of a selection of southeastern *Isoetes* populations has been conducted by Britton during this period. Over 700 herbarium specimens of the *I. engelmannii* complex from DUKE, GA, NCSC, NCU, FLAS, USF, UNA, FSU, NYS, PH, PSU, UNCC, VDB, VPI and DFB (D.F. Brunton personal herbarium), as well as selected specimens from GH, MO, NY and US, have been studied. Scanning electron micrographs of selected samples were taken using the standard methods of Britton and Brunton (1989, 1992).

Microspores were measured in Euparol, as described by Britton (1991). Megaspore widths (to the outer edges of spore ornamentation) were measured on SEM stubs or in sporewells (Brunton

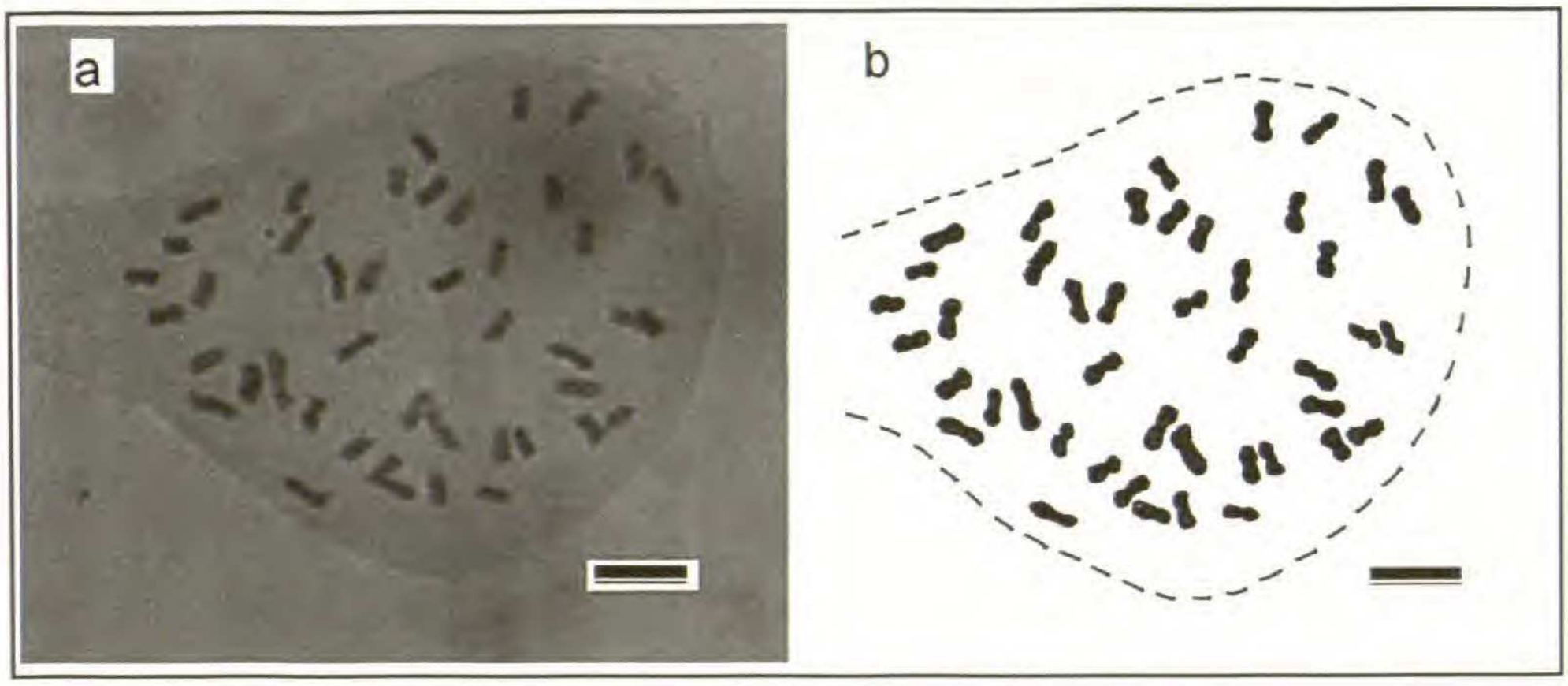


Figure 1. Photomicrograph (a) and interpretive drawing (b) of mitotic root tip plates of *Isoetes appalachiana* (*D.F. Brunton and K.L. McIntosh 11,171,* Huntingdon Co., PA). Scale bar = 5 μm.

1990) by using a binocular stereo microscope at a magnification of 40X or 50X equipped with an ocular micrometer.

Chromosome counts were obtained from *Isoetes engelmannii* (*s. lat.*) populations in Ontario, Pennsylvania, North Carolina, Florida, and Virginia. Plants from each population were grown in distilled water in a growth cabinet. The developing root tips were excised and pretreated in aqueous paradichlorobenzene (PDB) at room temperature for four hours. They then were washed in distilled water, fixed in acetic alcohol (3:1 absolute ethyl alcohol to glacial acetic acid) for 30 minutes or more, hydrolyzed in Warmke's solution (1:1 concentrated HCL to absolute ethyl alcohol) for 7–10 minutes at room temperature, and stained in leucobasic fuchsin (Feulgen) for two hours. The meristems were squashed under a cover glass in 45% acetocarmine stain and examined.

RESULTS

Cytology. Living material from large-spored *Isoetes engelmannii* (*s. lat.*) populations in Pennsylvania, Florida, North Carolina, and Virginia (see listing of type material, below) were confirmed to be tetraploid ($2n = 44$; Figure 1). Specimens from these populations form the sample that was used in the morphological analysis of the tetraploid populations.

Morphology. Tetraploid populations consist of relatively

Figure 2. *Isoetes appalachiana* on shore of Juniata River 8 km S of Huntingdon, Huntingdon Co., PA (6 July 1992).

large quillworts with broad, usually somewhat reflexed leaves (Figure 2) which are curved on their abaxial side and flattened on their adaxial side. Four large lacunal air canals occupy most of the leaf interior. In cross-section, the two abaxial air canals of tetraploid plants from Gadsden Co., Florida, are distinctly smaller than the two adaxial air canals of *Isoetes engelmannii* (*s. str.*) plants from Dinwiddie Co., Virginia. Tetraploid plants are typically more robust than *I. engelmannii* (*s. str.*).

The sporangia of mature individuals usually have a larger proportion of their surface covered by the opaque velum than do plants of *Isoetes engelmannii* (*s. str.*; Table 1). The surface of the sporangium is brown streaked—often heavily so—as opposed to the unmarked or only sparsely marked sporangia of *I. engelmannii* (*s. str.*).

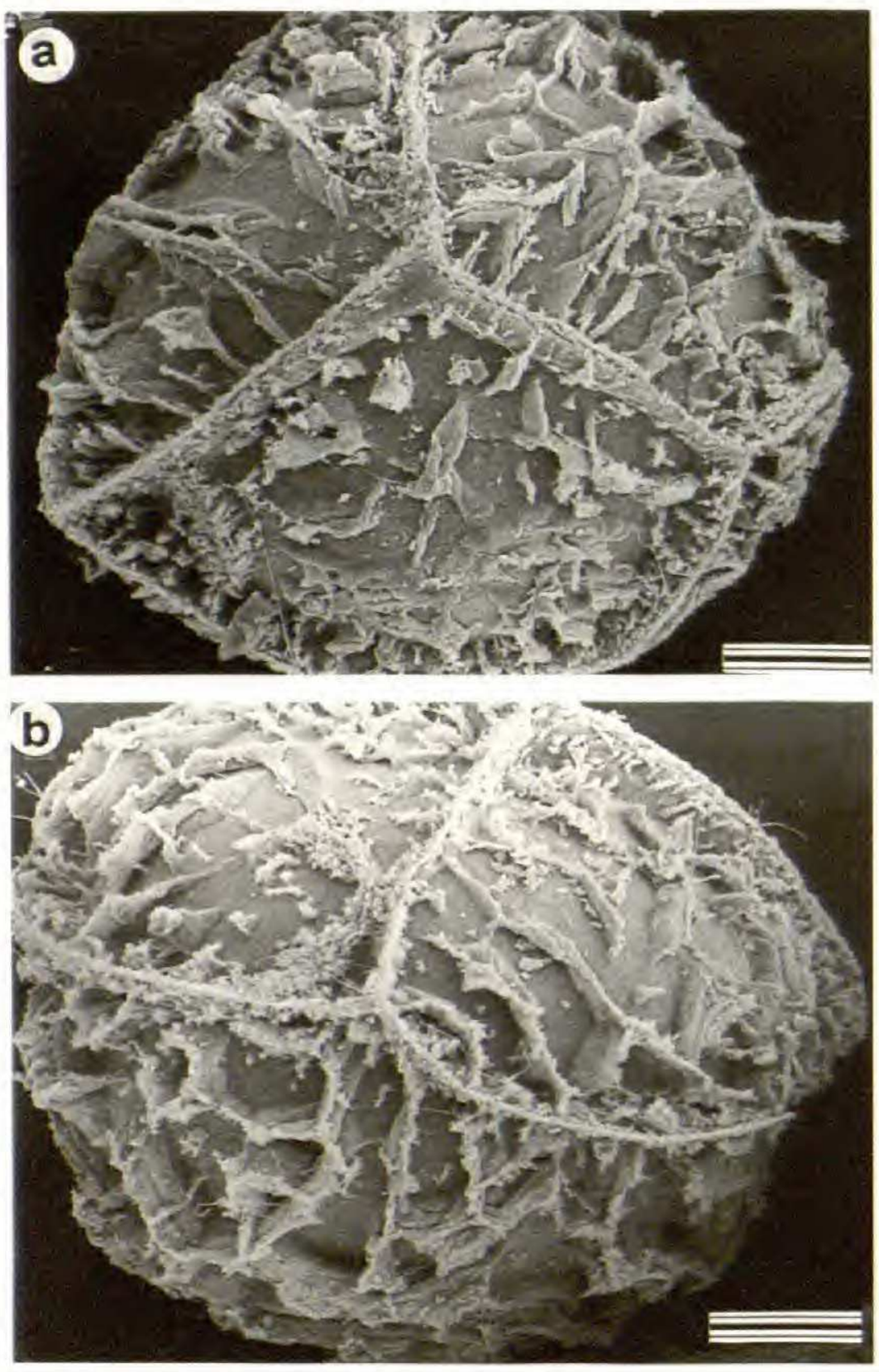
As with virtually all *Isoetes* taxa, spore morphology is critical in the discrimination of these tetraploid plants. Figure 3 illustrates typical tetraploid and diploid *I. engelmannii* spores from various perspectives. Kott and Britton (1983), Britton et al. (1991), Brunton and Taylor (1990), and Musselman et al. (1995) illustrate megaspores and microspores of diploid *I. engelmannii* from throughout the species' range. The megaspores of most tetraploid

Table 1. Comparison of *Isoetes appalachiana* with *I. engelmannii* (Standard deviations and sample size in square brackets).

	I. appalachiana	*I. engelmannii*
Corm	Two-lobed; typically 1.5–2.5 cm wide.	Two-lobed; typically 1.0–1.5 cm wide.
Leaves	Erect to loosely reflexed; narrow to broad, dull green with white basal portion.	Weak, loosely reflexed to sprawling; narrow, dull dark green with paler or white basal portion.
Sporangia		
—pigmentation	Hyaline to white, usually moderately to heavily brown-streaked.	White or pale tan, rarely with scattered brown dots.
—velum coverage	20–25% (rarely to 45%)	10–15%
Megaspore		
—size	(450) **534.1 μm** (611) [SD = 43.41; n = 130]	(380) **461.1 μm** (508) [SD = 29.74; n = 176]
—tri-radial face	Irregular pattern of high, ragged-crested muri.	Regularly reticulate pattern of smooth-crested muri.
—lateral face	Pseudo-reticulate pattern of ragged-crested ridges; short spines distributed individually or as a narrow band along distal side of equatorial ridge.	Continuation of distal face ornamentation to equatorial ridge.
—distal face	Broken-reticulate pattern of ragged-crested, ± thickened muri of variable height.	Reticulate pattern of ± smooth-crested, thin muri of even height.
Microspore		
—size	(28.6) **30.4 μm** (32.0) [n = 100]	(22.0) **25.5 μm** (28.7) [n = 40]
—ornamentation	Sparsely to densely low tuberculate.	Smooth (rarely obscurely tuberculate).
—color	White or pale tan in mass.	White or pale gray in mass.

Table 1. Continued.

	I. appalachiana	*I. engelmannii*
Chromosome Number	$2n = 44$	$2n = 22$
Site Ecology	Submerged to emergent along creek banks, boggy woodland pools and lakes (reservoirs) in acidic sand, clay, or gravel substrate.	Shallow lake and river shores in subacid to calcareous substrate.
Phenology	Leaves well developed by late April (south) or late June (north); spores maturing May (south) to mid-July (north).	Leaves well developed by May (south) or July (north); spores maturing June (south) to August (north).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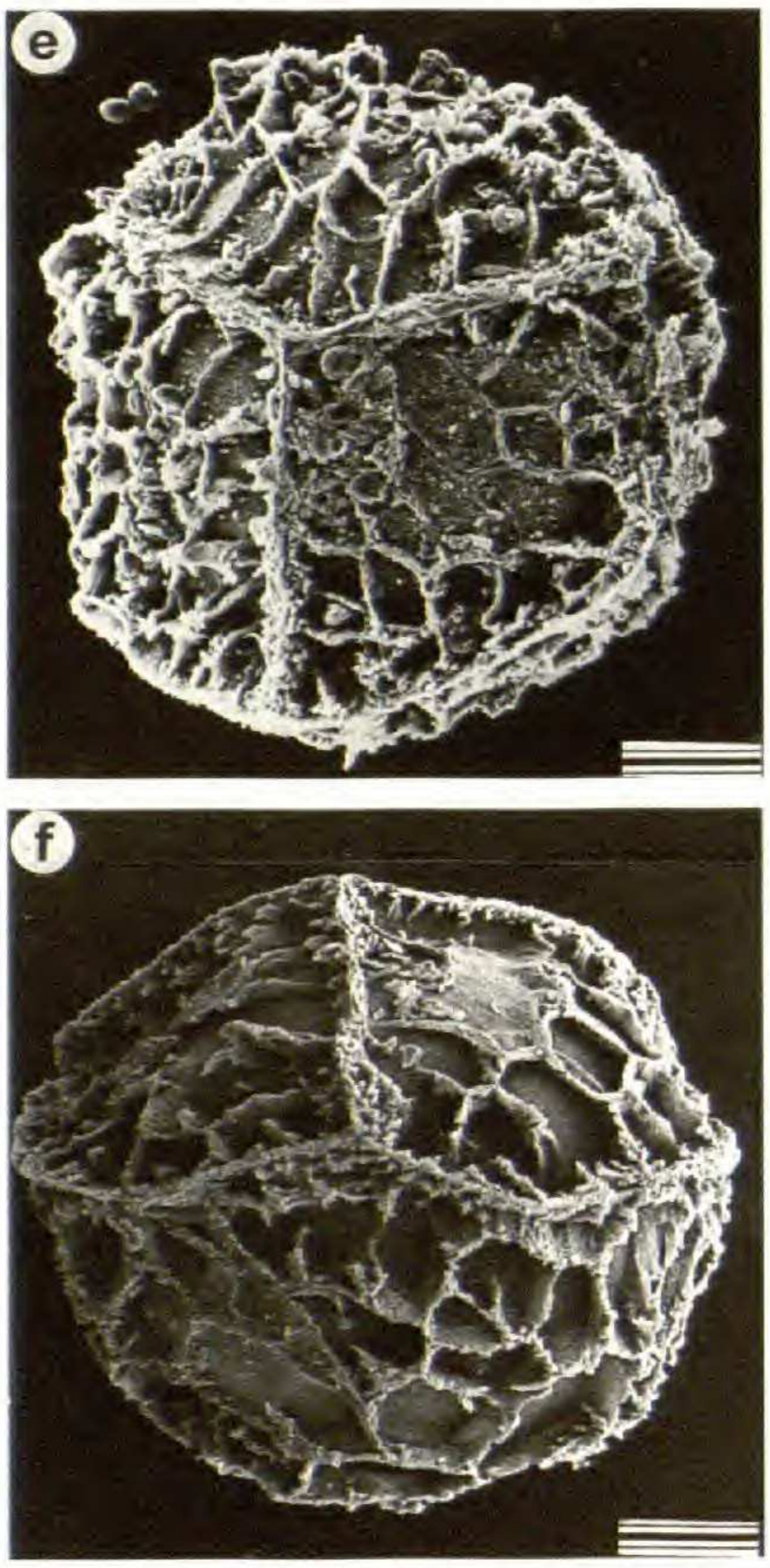
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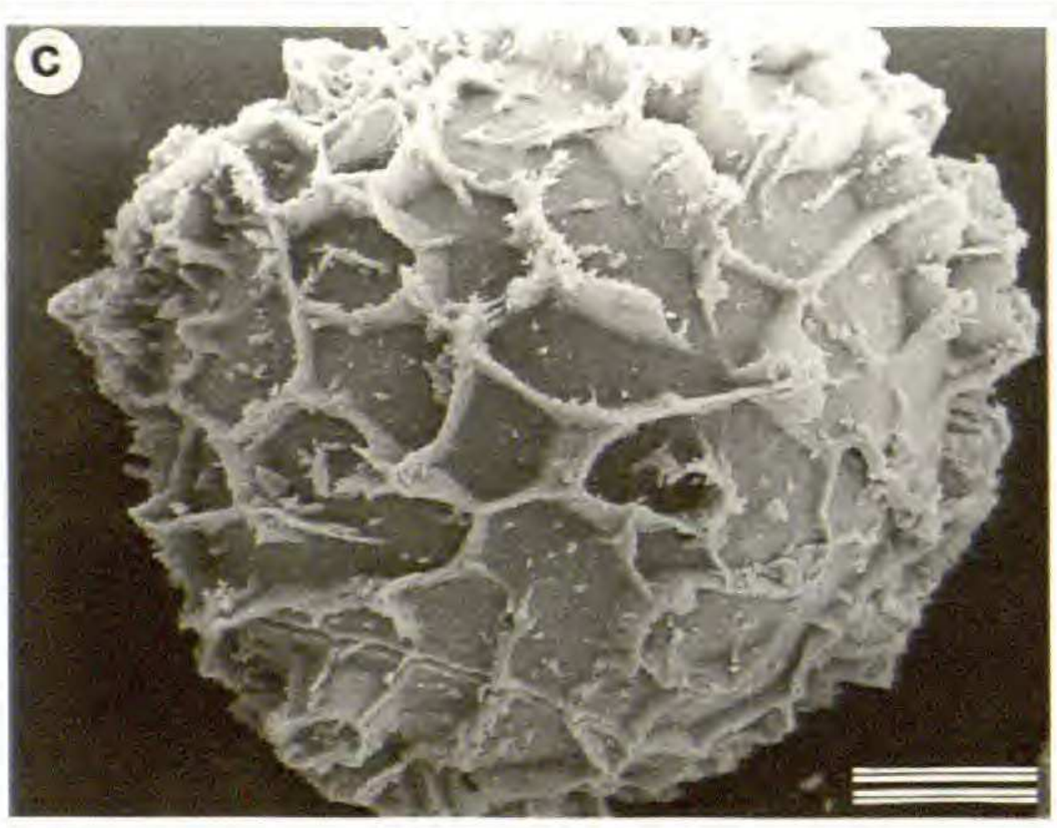
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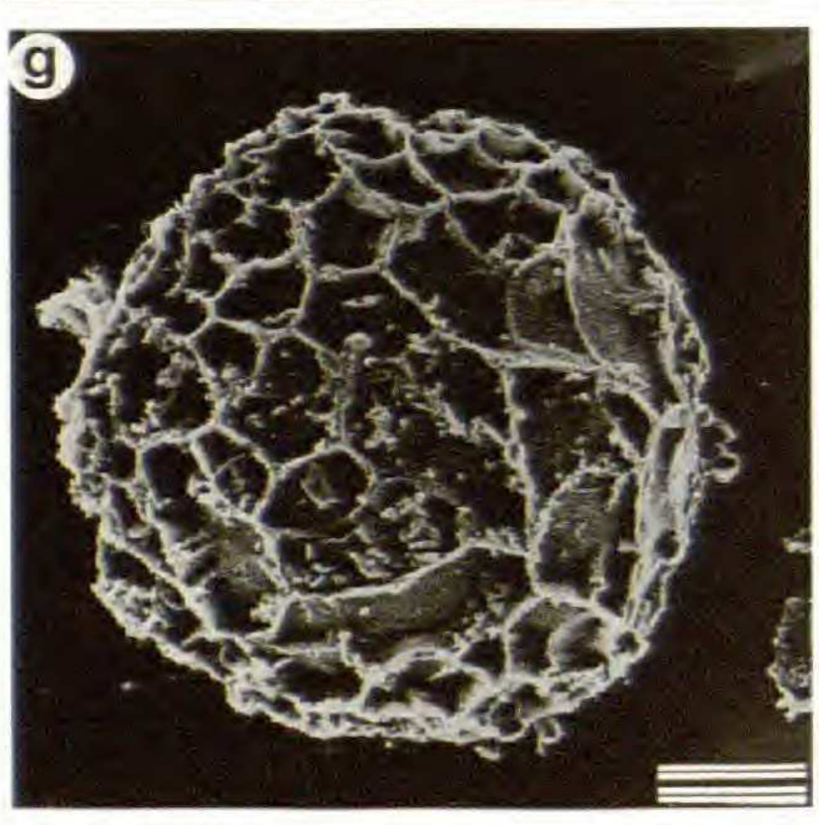
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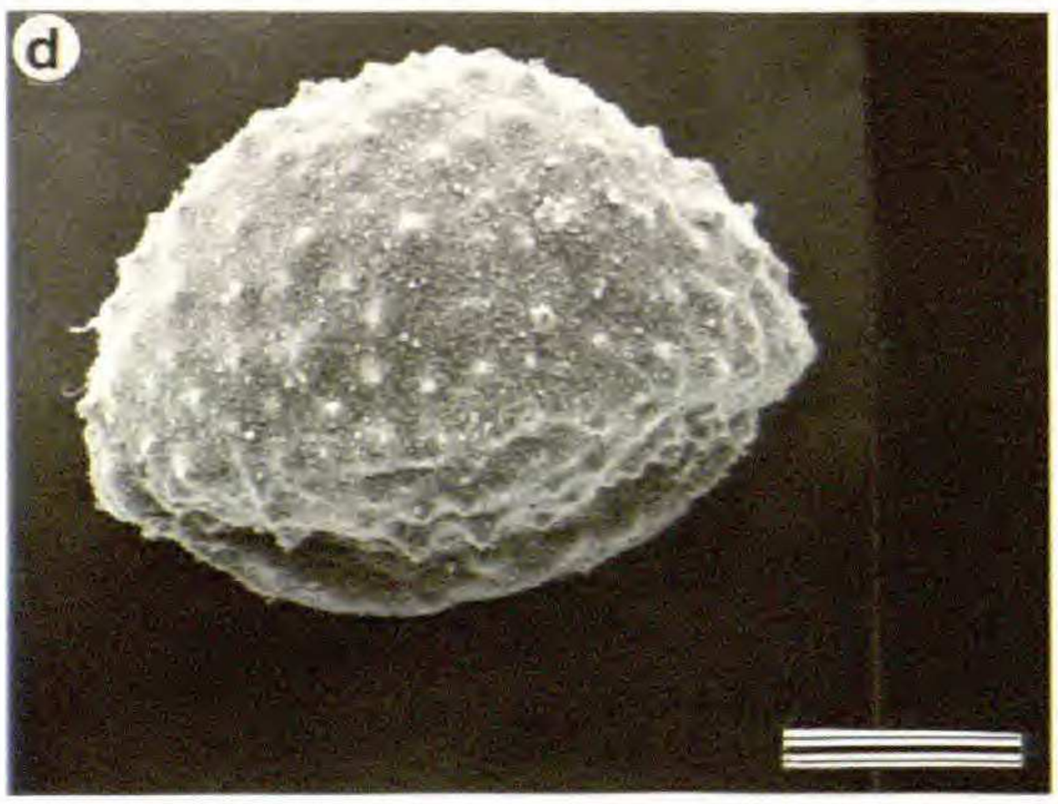
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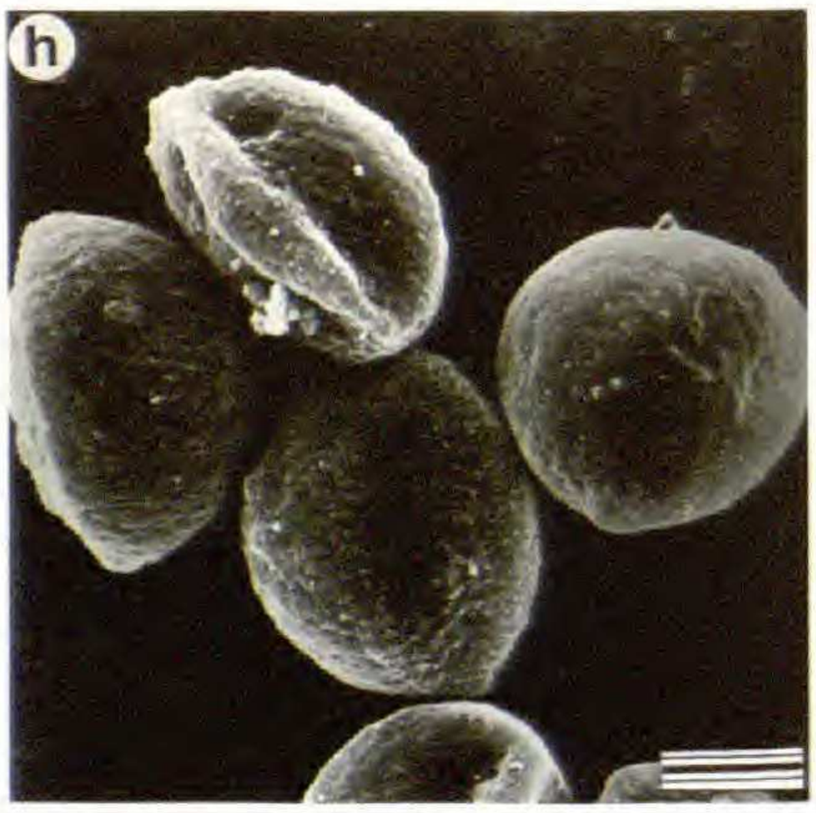
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populations have a ragged-reticulate appearance (Figures 3a–3c), in contrast to the regularly reticulate ornamentation of *I. engelmannii* (*s. str.*) megaspores (Figures 3e–3g). Some cytologically determined tetraploids, however (e.g., the Gadsden Co., Florida, population), have a regular megaspore ornamentation pattern very similar to *I. engelmannii* (*s. str.*). The substantial difference in spore size and microspore ornamentation (Table 1) can be used to separate mature specimens of most problematic populations.

Megaspore muri (ornamentation ridges) of the tetraploid are typically thicker than those of *Isoetes engelmannii* (*s. str.*). They also spread outward at their point of attachment to the megaspore and are more irregular in form than the thin, straight-sided and smooth-topped muri of *I. engelmannii* (*s. str.*; Figures 3c and 3g). A narrow, though frequently obscure, band of short spines distal to the equatorial ridge is often present on tetraploid megaspores (Figure 3b) but has not been observed with *I. engelmannii* (*s. str.*; Figure 3f).

The ploidy level of the tetraploid is reflected in a larger megaspore size. While it averages about 535 μm, individual spores over 600 μm have been observed. *Isoetes engelmannii* (*s. str.*) megaspores average only about 460 μm with individuals larger than 500 μm being rarely observed.

Microspore size is significantly different between the tetraploid and *Isoetes engelmannii* (*s. str.*) as well. Microspore length from a sample of Pennsylvania tetraploid plants averages about 30 μm compared with about 25 μm for diploids (Table 1). Ornamentation on mature tetraploid microspores is strikingly different, exhibiting a low tuberculate pattern (Figure 3d) reminiscent of *I. riparia* Dur. (*s. str.*; Britton and Brunton 1989). In contrast, the surface of *I. engelmannii* (*s. str.*) microspores is smooth (Figure

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Figure 3. SEM composite of *Isoetes engelmannii* (*s. str.*) and *I. appalachiana* spores. a–d: *I. appalachiana.* a–c: holotype, (*D.F. Brunton and K.L. McIntosh 12,274* [OAC]); a: Proximal view of megaspore; b: Lateral view of megaspore; c: Distal view of megaspore; d: microspore (*D.F. Brunton and K.L. McIntosh 11,559* [OAC]). e–h: *I. engelmannii* (*s. str.*) e, g, h: holotype, (*G. Engelmann s.n.*, 1843 [MO]); e: Proximal view of megaspore; f: Lateral view of megaspore (*D.F. Brunton and K.L. McIntosh 11,170* [OAC]); g: Distal view of megaspore; h: Microspore. Scale bar = 100 μm (megaspores), 10 μm (microspores).

3h). Table 1 summarizes the morphological characteristics of the tetraploid and indicates its morphological differences from *I. engelmannii* (*s. str.*).

Some tetraploid populations (in the northern Appalachian Mountains in particular) are strikingly similar to *Isoetes valida.* The much smaller megaspores (450 μm) with more congested, more raggedly reticulate ornamentation, larger velum coverage (50% or more) and unmarked sporangium distinguish mature *I. valida* specimens (Brunton and Britton 1996).

Site ecology. We have observed the tetraploid in a variety of aquatic and emergent habitats. It is found in seeps in mature swamp forest and on emergent clay flats at swamp forest edges (Florida), on emergent clay, silty-sand and/or cobble river shores (Pennsylvania, Virginia), in back-eddies along tributary streams (Georgia, Pennsylvania) and submerged in shallow water in a manmade reservoir (a former mountain stream in Pennsylvania). The flora at these sites appears to be dominated by species preferring acidic substrates. It was the only *Isoetes* taxon present at all but the Jersey Shores, Lycoming County, Pennsylvania, site where it grows with *I. riparia* (*s. str.*). Typically it is found growing with few other vascular plants in the immediate vicinity (Figure 2).

Distribution. The tetraploid is most frequently—almost commonly—found at lower to middle elevation areas of the Appalachian Mountains in Pennsylvania. It also occurs in a number of counties in the southern Appalachians in Georgia, North Carolina, and South Carolina. Inexplicably, it seems to be absent from northern and western Virginia, Maryland, and West Virginia. A similar gap in range was noted initially for *Isoetes valida* (Brunton and Britton 1996). Both may, in part, represent limitations of the floristic record rather than actual distributional gaps. The tetraploid is also known from a number of Coastal Plain counties in the Carolinas, Virginia, and northern Florida. The Florida Coastal Plain stations are found in areas known for their concentrations of regionally uncommon Appalachian disjuncts (Mitchell 1963).

In addition to on-site examination of the populations at which cytological material was collected, between 90 and 100 specimens displaying the morphological features of the tetraploid were

noted (and so annotated) in herbaria. Figure 4 illustrates (one per county) the distribution of these specimen records. They include specimens from Florida, Georgia, New Jersey, North Carolina, South Carolina, and Virginia. The material includes those specimens upon which the Florida reports of *Isoetes engelmannii* (*s. str.*) and *I. riparia* are based.

Despite the segregation of these tetraploid populations as *Isoetes appalachiana, I. engelmannii* (*s. str.*) continues to display a widespread distribution across eastern North America. It appears to be particularly concentrated along the Atlantic seaboard from Maine to South Carolina and along the Mississippi River valley into Missouri and Illinois. Stations become increasingly widely scattered inland from the Atlantic, with populations in Ontario and Michigan being components of a disjunct Atlantic Coastal Plain flora (Britton et al. 1991; Reznicek 1994). This revised distribution for *I. engelmannii* (*s. str.*) is similar to that of other riparian deciduous swamp species which are rare in the northern United States, such as *Populus heterophylla* L. and *Fraxinus tomentosa* Michx. f. (McCormac 1993).

The distribution of *Isoetes appalachiana* (Figure 4) overlaps with other swamp and emergent shore quillworts including *I. riparia* (*s. str.*), *I. valida,* and *I. engelmannii* in the north and *I. engelmannii, I. valida,* and *I. hyemalis* Brunton in the south (Taylor et al. 1993; Brunton et al. 1994). It is found in higher, more inland sites in the north and lower, more coastal areas in the south. Additional field studies will be required to clarify this distribution and to evaluate the apparent rarity of stations in the central portion of its range.

DISCUSSION

Taxonomy. We believe that the distinctive cytology, distribution, and morphology of the tetraploid indicate that it is a distinct species and that it probably represents the taxon first recognized as *Isoetes engelmannii* var. *georgiana* by George Engelmann over a century ago. Cytological determination of Engelmann's material is presently impossible, however, and we were unable to re-locate *I. engelmannii* var. *georgiana* at its Floyd County, Georgia, type location. The connection between that taxon and the tetraploid described here, therefore, cannot be established with certainty. Recombination of this name at the species

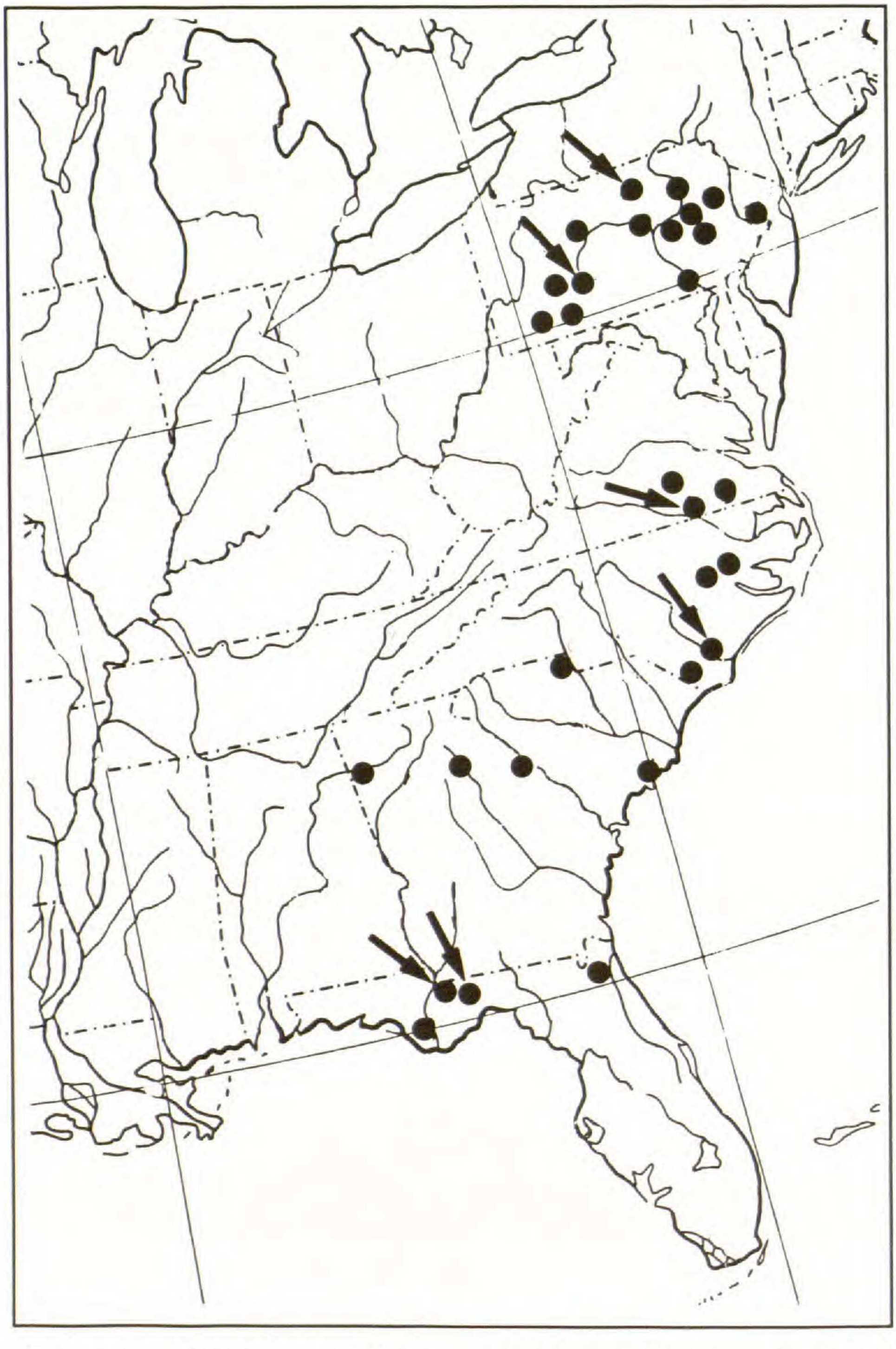

Figure 4. Distribution of *Isoetes appalachiana* (by county) in North America. Arrows point to cytologically confirmed populations.

level, in any event, would be inappropriate due to its previous application to another species (Luebke 1992). Similarly, Eaton's *I. engelmannii* var. *fontana* cannot be cytologically linked to the tetraploid since the type population along the Susquehanna River of southern Pennsylvania has been destroyed by power dam construction (pers. obs.).

We propose the following name and description based entirely on cytologically confirmed type material:

Isoetes appalachiana D. F. Brunton & D. M. Britton, *sp. nov.* (Figures 1, 2, and 3a–3d). *I. engelmannii* A. Braun var. *georgiana* Engelm. (Engelmann 1882); *I. engelmannii* A. Braun var. *fontana* A. A. Eaton (Eaton 1905); TYPE: U.S.A. Pennsylvania: Blair Co., Tipton Reservoir, 6 Aug 1995, *D. F. Brunton and K. L. McIntosh 12,274* [HOLOTYPE: OAC (Plant A); ISOTYPES: MIL, MICH, DAO, GH, VPI, PH, DFB]. The specific epithet reflects the concentration of populations along the Appalachian Mountains.

Isoetes magna et aquatica, emergens foliis colore oleae rigidis vel reflexis, e cormo bilobato exoriens; megasporae similes *I. engelmannii* (*s. str.*), sed majores (plerumque 535 μm), ornatione inique reticulata signatae, quae consistit in muris cum lateribus crassis et fundamentis attenuatis et cristis irregularibus; zona spinarum brevium, densa, angusta vel obscura, secundum latus distale iugi medialis, microsporae ovales, tuberculis humilibus ornatae (plerumque 30 μm); chromosomata $2n = 44$.

FORM: Robust (25–30 cm tall), semi-terrestrial herb arising from a rounded, two-lobed corm 1.5–2.5 cm wide; LEAVES: erect when submerged, reflexed when emergent, dull olive-green with whitish-green to pale brownish-green bases, 1–2 mm wide at mid-length, abaxial side curved, flattened adaxially, four large lacunal air canals conspicuous in cross-section; SPORANGIA: oblong to oval, to 10 mm long, white or hyaline surface moderately to heavily brown-streaked; VELUM: narrow, descending across ca. 20%–25% (rarely to 45%) of the sporangium; LIGULE: delicate, narrowly triangular; MEGASPORES: 535 μm, white, with ragged-reticulate, relatively thick, irregularly crested muri (ornamentation walls) which spread outwards at their point of attachment to the megaspore surface; short spines distributed along distal side of equatorial ridge, often forming a narrow, dense band; MICROSPORES: white to pale tan in mass when

mature, 30 μm long, with low tuberculate ornamentation; CYTOLOGY: $2n = 44$.

PARATYPES (all cytologically determined $2n = 44$): FLORIDA: Gadsden Co., Ochlockonee River at Highway 27, 18 Mar 1994, *D.F. Brunton and K.L. McIntosh 11,816* (MICH, FSU, DFB, OAC); Leon Co., Geddies Road, Tallahassee, 15 Mar 1995, *D.F. Brunton and K.L. McIntosh 12,147* (OAC, DFB, MICH); NORTH CAROLINA: Jones Co., north side of Hunters Creek at Highway 58, 2 Jul 1996, *D.F. Brunton and K.L. McIntosh 12,586* (OAC, MIL, MICH, DFB, ODU); PENNSYLVANIA: Huntingdon Co., Juniata River 8 km S of Huntingdon, 6 Jul 1992, *D.F. Brunton and K.L. McIntosh 11,171* (OAC, DFB, MIL, MICH, DAO, WIS, NCSC); Lycoming Co., W channel of West Branch, Susquehanna River, Jersey Shore, 6 Jul 1992, *D.F. Brunton and K.L. McIntosh 11,176* (OAC, DFB); VIRGINIA: Greenville Co., Highway 301, Fountains Creek, Emporia, 9 Jul 1993, *D.F. Brunton and K.L. McIntosh 11,559* (OAC, DFB, DAO, VPI).

Because many specimens of large-leaved quillworts have been collected before megaspores have matured, precise discrimination of herbarium material in the *Isoetes engelmannii* complex is not always possible. The close morphological similarity between *I. appalachiana* and *I. engelmannii* has a parallel with two quillworts in western North America, the diploid *I. echinospora* Dur. and the tetraploid *I. maritima* Underw. Although some specimens of these latter species can be separated confidently only by spore size, the recent description of their sterile triploid hybrid, *I.* × *pseudotruncata* Britton & Brunton, has confirmed that they are cytologically distinct species (Britton and Brunton 1996). Coincidentally, *I. echinospora* and *I. maritima* also can be distinguished by the same microspore characteristic seen with *I. appalachiana* and *I. engelmannii* (*s. str.*). In both cases the diploid has smooth microspores while the tetraploid is conspicuously marked with spines or tubercles.

Origins. *Isoetes appalachiana* likely represents an allopolyploid resulting from the chromosome doubling of a sterile hybrid between two diploids. The involvement of *I. engelmannii* (*s. str.*) is strongly suggested by the morphological similarity of the two taxa and by range considerations. Based on present and fossil distribution (e.g., Taylor et al. 1993; Craig 1969) as well as morphology, the other likely diploid parental candidates might be *I. echinospora* or *I. valida.* Assuming that *I. appalachiana* should reflect the morphological traits of both progenitor species, the absence of elements of the strongly echinate megaspore mor-

phology of *I. echinospora* should eliminate this northern taxon from serious consideration. The morphology of *I. valida,* however, fits well, as this member of the *I. engelmannii* complex displays an even more congested, ragged-reticulate ornamentation and a larger velum coverage than *I. appalachiana* (Brunton and Britton 1996). The distribution of these two species also is similar. *Isoetes* × *altonharvillii* Musselman & Bray, the sterile hybrid between *I. engelmannii* and *I. valida,* has been described recently from Virginia (Musselman et al. 1995) and has also been collected in Delaware and North Carolina. It seems reasonable, therefore, to speculate that *I. appalachiana* evolved from the doubling of diploid *I.* × *altonharvillii* just as it is believed that *I. riparia* has evolved from a comparable doubling of the sterile diploid hybrid *I.* × *eatonii* Dodge (Taylor et al. 1993).

Possible *Isoetes appalachiana* × *engelmannii* (*s. str.*) hybrid specimens have been seen in herbarium collections from Lancaster, Northampton, and Berks Counties, Pennsylvania, Cecil Co., Maryland, and Passaic Co., New Jersey. Cytological confirmation of such a triploid taxon would provide important support for the specific status of *I. appalachiana.* The relationship of this species with other elements of the *I. engelmannii* complex may be clarified further by molecular genetic investigations.

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