TAXONOMY AND IDENTIFICATION OF *ISOETES* (ISOETACEAE) IN TEXAS BASED ON MEGASPORE FEATURES

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Abstract: Scanning electron microscopy examination of Texas specimens of *Isoetes* revealed differences in megaspore ornamentation patterns of the proximal and distal surfaces that support the recognition of four species of the genus in Texas. These include *I. butleri* and *I. melanopoda,* both of widespread occurrence in the central United States, *I. lithophila,* a central Texas endemic, and *I. piedmontana,* which we report as new to the state. A key to species based upon megaspore characteristics, distributions, a limited list of exsiccate, and descriptions and micrographs of megaspores are included.

Keywords: Isoetaceae, *Isoetes,* Texas.

The Isoetaceae consists of several genera known from fossils and one living genus, *Isoetes,* which is recognized to include 60–75 species (Lellinger, 1985) to perhaps 150 species (Taylor et al., 1993) of nearly worldwide distribution. Lellinger (1985) recognized 19 species in the United States and Canada, whereas Taylor et al. (1993) recognized 24 species within the same area. Presently, three species, *I. melanopoda, I. butleri,* and *I. lithophila* have been reported in Texas (Turner et al., 2003). The first two species are of widespread distribution in the central United States, while the latter is endemic to granite outcrops in south central Texas.

Features typically used to distinguish *Isoetes* taxa are geography, habitat, megaspore texture, spore size and velum, with texture and size of mature, dry megaspores usually required for identification (Taylor et al., 1993). Several of the features (including leaf morphology) produce unreliable results or, particularly megaspore texture, are not visible using conventional methods. Geography (distribution) is of limited usefulness in Texas because all species recorded within the state occur in an area centered on Llano County in the Edward's Plateau vegetation region of central Texas. Based upon morphological and ecological characteristics, all currently known Texas species, including the one reported here for the first time, are linked into the *melanopoda-melanospora-butleri-lithophila* complex (Reed, 1965), which further complicates determinations.

Lellinger (1985) states that advances in taxonomy of the genus should come about as a result of examining megaspores with the scanning electron microscope. For example, Taylor et al. (1975) found clear differences in megaspore "micro-ornamentation" between *Isoetes melanopoda* and *I. butleri,* species previously confused that could not be adequately distinguished with light microscopy. During 2002 and 2003, several specimens from the Edward's Plateau and Pineywoods vegetation regions of the state were discovered that were not identifiable by use of the conventional methodology mentioned above and the available literature concerning the Texas species. As a result, scanning electron microscopy of megaspores was used as a method of distinguishing between the Texas species of the genus and little reference is made of other morphological characteristics that have traditionally been used to distinguish members of the genus. Correll and Johnston (1970), Lellinger (1985), Reed (1965), Taylor et al.

(1993) or similar works provide morphological information.

The study is largely based upon examination of herbarium specimens from BAYLU, BRIT, IBE, and TEX/LL and limited field studies to obtain additional specimens from glauconite deposits in extreme east Texas. Mature spores of *Isoetes* were obtained from dried herbarium specimens from BAYLU and TEX/LL. Megaspores were mounted on aluminum stubs using double-sided tape, coated with gold, and viewed using a JEOL JSM 5410 scanning electron microscope operated at 15kV. Images were acquired digitally.

Generic descriptions are in Correll and Johnston (1970), Lellinger (1985), Taylor et al. (1993), and Diggs et al. (1999) and need not be repeated here. Complete citations of exsiccatae are provided only for *Isoetes piedmontana,* which is reported as new to the state, and for the east Texas records of *I. butleri.* County distributional data of *I. lithophila* and *I. melanopoda* are in Turner et al. (2003). Additional county records for east Texas are given for the latter species because of its rarity in that part of the state.

Based upon our observations, the species of *Isoetes* in the Texas flora can be readily distinguished by the megaspore features of size, color and ornamentation patterns as determined by scanning electron microscope. The following key, based upon these characteristics, may be used to distinguish the four species of the genus now known to occur in the state.

KEY TO THE ISOETACEAE OF TEXAS BASED UPON MEGASPORE CHARACTERISTICS

1. Megaspores light gray to gray-brown, surfaces glabrous, smooth to rugulate to obscurely tuberculate ..... 2. *I. lithophila*
1. Megaspores white, surfaces cobwebby to variously pubescent, rugulate to tuberculate to echinate.
   2. Megaspores usually > 450 μm, surfaces densely cobwebby ..... 1. *I. butleri*
   2. Megaspores usually < 450 μm (200–450 μm), variously pubescent but not cobwebby.
      3. Surfaces of megaspores sparsely rugulate with low, fused ridges ..... 3. *I. melanopoda*
      3. Surfaces of megaspores tuberculate to echinate ..... 4. *I. piedmontana*

1. ISOETES BUTLERI Engelm., Bot. Gaz. 3: 1.1878. (Figs. 1, 2).

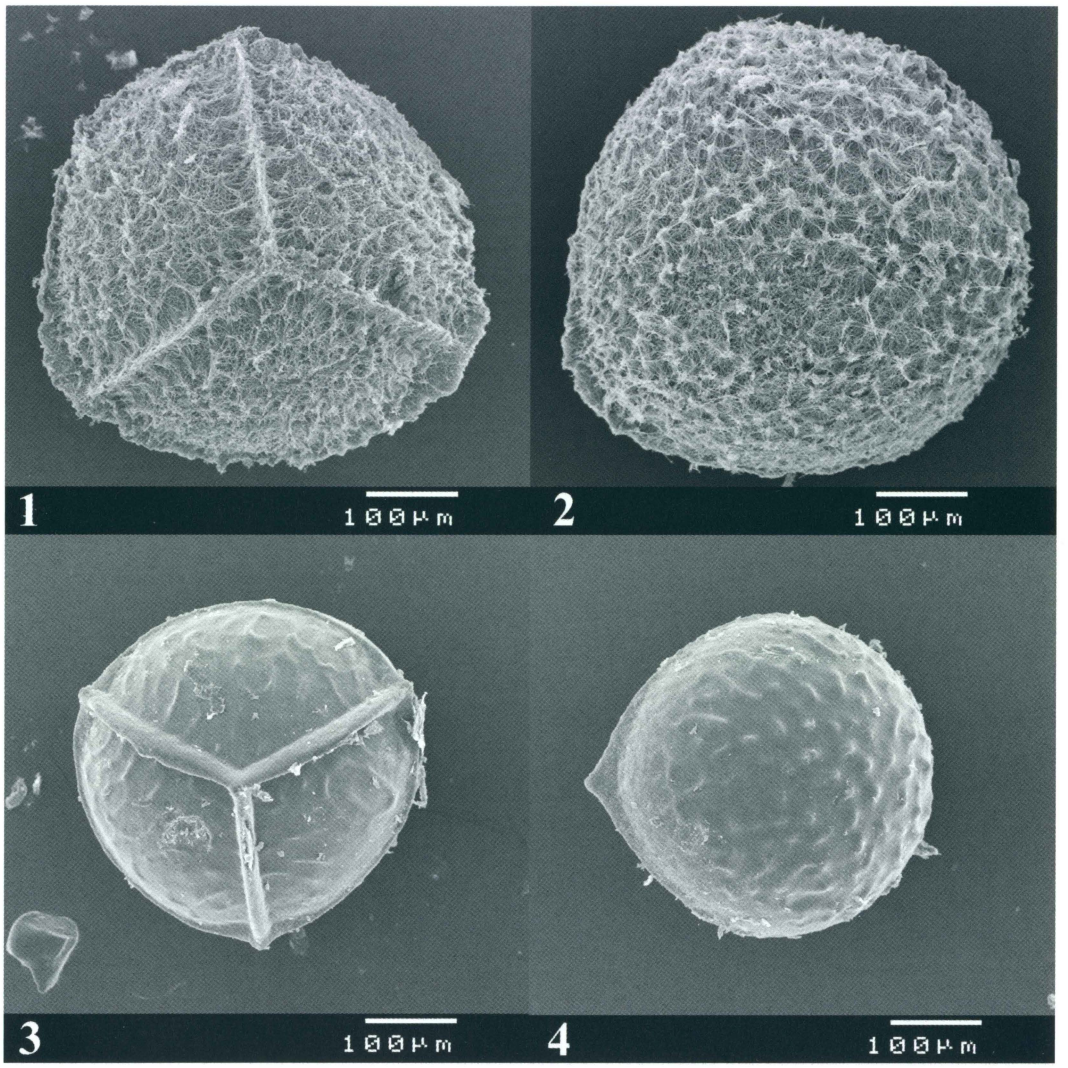
Megaspores white, large for the genus, 450 μm or more in diameter; proximal and distal spore surfaces obscurely tuberculate; surface stands of perine material extending from the tuberculae resulting in arachnoid or cobwebby appearance; trilete ridges, equatorial ridge, and girdle are obscured by the surface strands.

DISTRIBUTION AND HABITAT. NW Georgia, N Alabama, C Tennessee, SC Kentucky, S Missouri, Arkansas, SE Kansas, E and SE Oklahoma, disjunct in Illinois and Texas; terrestrial on alkaline soils saturated by early spring rains; generally on limestone or limestone cemented sandstone (Lellinger, 1985).

TEXAS DISTRIBUTION. Rare: Comal (Turner et al., 2003), Llano, Sabine, San Augustine counties; in east Texas, on glauconite deposits.

SPECIMENS EXAMINED. **Sabine Co.**: 1.5 mi. S. of the jct. of Hwy 21 and St Rt 330 on Hwy 21, W side of Hwy 21, *Singhurst 11749* (BAYLU); **San Augustine Co.**: 1.0 mi. S of Hwy 21 and the jct. of Chapel Hill Rd. on Chapel Hill Rd., near Fords Corner Community, *Singhurst 11750* (BAYLU).

Isoetes butleri was first reported in Texas by Lott (1982). The San Augustine and Sabine county records are restricted to glauconite glade pools of the Weches Formation. Associated plants included *Crassula aquatica, Potamogeton nodosum, Sedum pulchellum, Arenaria drummondii, Allium drummondii, Leavenworthia aurea,* and *Calamintha arkansana.* These records are about



FIGS. 1–4. Scanning electron micrographs of megaspores. *Isoetes butleri* proximal (1) and distal (2) faces are obscurely tuberculate. Surface strands of perine material extend from the tuberculae and result in cobwebby or arachnoid appearance. *Isoetes lithophila,* proximal (3) and distal (2) faces are nearly smooth to obscurely rugulate with low ridges. The trilete markings and equatorial ridge are prominent.

265 km south of the nearest Arkansas station and about 440 km east northeast of the nearest Texas records in Llano and Comal counties. The habitat requirements for the species in Comal and Llano counties are similar to that described for *I. lithophila.*

2. ISOETES LITHOPHILA N.Pfeiff., Ann. Missouri Bot. Gard. 9: 135. 1922. (Figs. 3, 4).

Megaspores gray to gray brown, ranging in size from 250–400 µm, proximal and distal spore surfaces are nearly smooth, obscurely regulate with low ridges to occasionally obscurely tuberculate; trilete markings and equatorial ridge are prominent but the girdle is not well-defined.

DISTRIBUTION AND HABITAT. Endemic to Texas. Known only from Burnet, Gillespie,

Llano, and Mason counties (Turner et al., 2003); shallow depressions and temporary pools on granite outcrops.

Isoetes lithophila is restricted to the larger granite domes of the Llano Uplift. The plants occur in pools that form in late winter and dry up in late spring-summer. Common associated flora included *Marsilea vestita* subsp. *vestita, Pilularia americana, Crassula aquatica, Heteranthera limosa, Eleocharis microcarpa, Fimbristylis autumnalis, F. puberula, Fuirena simplex, Rhynchospora nivea, Schoenoplectus pungens, Potamogeton nodosus, Rorippa sessiliflora, Callitriche heterophylla, Hypericum mutilum, Utricularia gibba, Ammannia coccinea, Rotala ramosior, Anagallis minima, Samolus ebracteatus, Bacopa rotundifolia, Buchnera americana, Lindernia dubia* var. *anagallidea, Mecardonia procumbens,* and *Mimulus glabratus* var. *jamesii.*

The species is considered rare (Lellinger, 1985) and of conservation concern (Taylor et al., 1993). It is currently classified as G2S2 by the Wildlife Diversity Program of Texas Parks and Wildlife Department, Austin.

3. ISOETES MELANOPODA J.Gay & Durieu, Bull. Soc. Bot. France 11: 102. 1864. (Figs. 5, 6).

Megaspores white, 250–450 μm in diameter; proximal and distal spore surfaces sparsely rugulate with low fused ridges, rarely reticulate; trilete markings and equatorial ridge are prominent but covered by perine projections; girdle not well-defined.

DISTRIBUTION AND HABITAT. Widespread thought most of central and southeast United States, also Utah and Montana (USDA, NRCS 2004); terrestrial or amphibious in temporary pools, intermittent streams, prairies, meadows, and wallows in granite outcrops and clay soils underlain by sandstone.

TEXAS DISTRIBUTION: Widely distributed [but uncommon] on spring runs and pools in non-calcareous soils in central and east Texas. The species is noticeably absent from most of [deep] east Texas (see Turner et al. 2003). This seems to be a result of being overlooked or represents inadequate collecting for the region. The following counties are here added to the known distribution: Hardin, Sabine, and Shelby.

SPECIMENS EXAMINED. **Hardin Co.**: 1 mi. N of FM 418 N of confluence of Village Creek and Dry Creek, 2 Jun 2004, *Keith 713* (BAYLU); **Sabine Co.**: without location and date, *Singhurst 11450* (BAYLU); **Shelby Co.**: FM 2694, adjacent to sandy site of pine with some longleaf, 6 May 2004, *Walker 3003* (BAYLU).

This is the most widespread species of the genus in the state. The bases of the plants are commonly blackish.

4. ISOETES PIEDMONTANA (N.Pfeiff.) C.F.Reed. Phytologia 12: 392. 1965. (Figs. 7, 8).

I. virginica N.Pfeiff. var. *piedmontana* N.Pfeiff., Bull. Torr. Bot. Club 66: 411.1939.

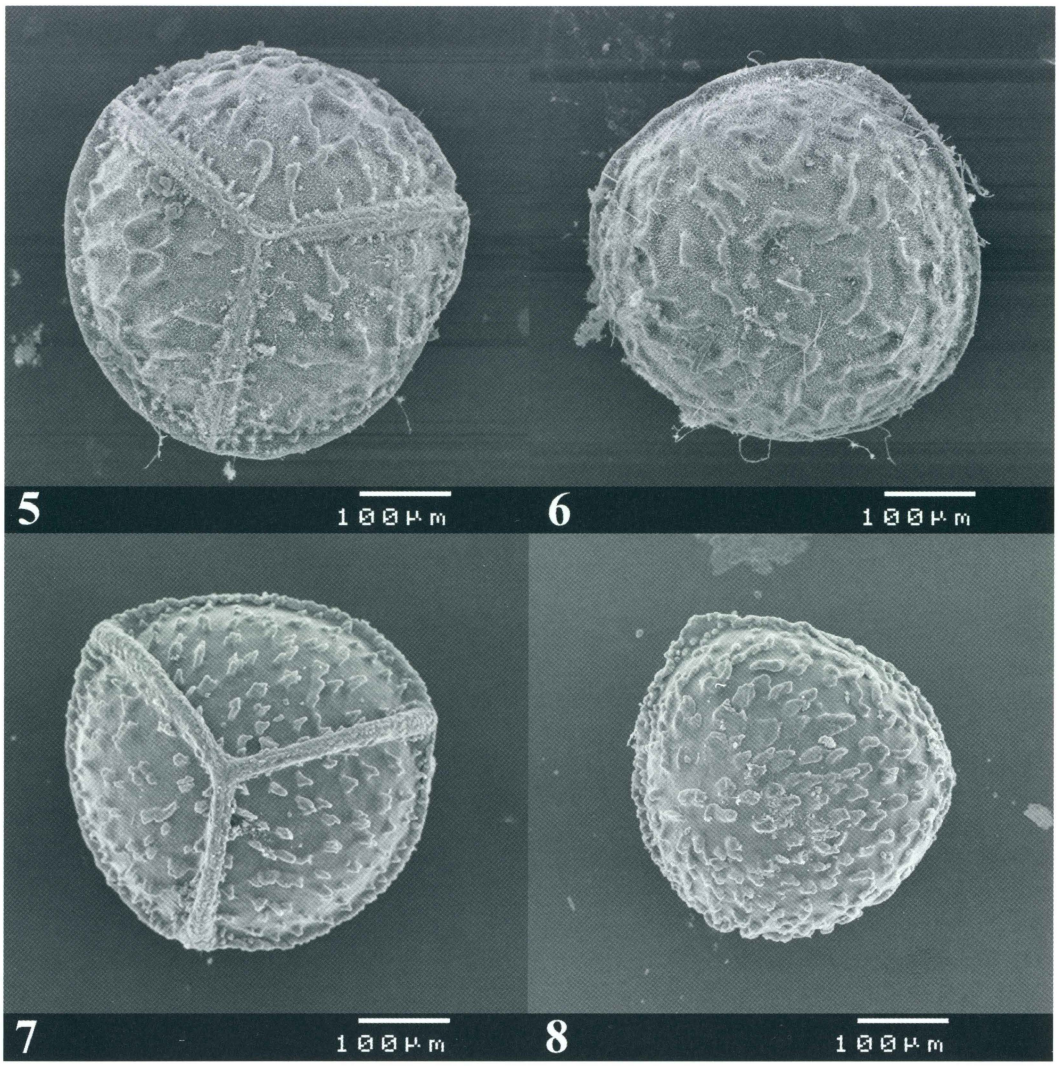
Megaspores white, 300–400 μm in diameter; proximal and distal spore surfaces tuberculate to echinate, rarely cristate; trilete ridges are proximate; equatorial ridge and girdle are sparsely covered by short tuberculae.

DISTRIBUTION AND HABITAT. Virginia to Alabama, Texas; shallow depressions and temporary pools on granite outcrops.

TEXAS DISTRIBUTION. Mason and Llano counties; granitic outcrops,

SPECIMENS EXAMINED. **Llano Co.**: Granite Mountain, 30 June 1957, *Correll & Johnston 17332* (TEX); Enchanted Rock, Enchanted Rock State Natural Area, 8 May 1990, *Carr 10569* (TEX); Town Mountain granite outcrop, LCRA tracts at Ferguson Power Plant, 20 March 1992, *Carr 11625* (TEX); **Mason Co.**: Mason Mountain Wildlife Management Area, 0.4 mi. NE of Mile-O-More Lake, 23 April 2001, *Sanchez 2364 & Stanford* (BAYLU).

This is the only species currently recognized in the Texas flora with a tuberculate to echinate ornamentation pattern not obscured by perine projections. In the treatment of *Isoetes* in the Flora of North America (Taylor et al. 1993), this species keys to



FIGS. 5–8. Scanning electron micrographs of megaspores. *Isoetes melanopoda,* proximal (5) and distal (6) faces are sparsely rugulate with low, fused ridges. Both faces, the trilete markings, and the equatorial ridge are covered by short perine projections. *Isoetes piedmontana,* proximal (7) and distal (8) faces are sparsely tuberculate to echinate. The trilete markings and equatorial ridge are prominent and sparsely covered by short tuberculae.

and is submerged into *I. virginica* N.E. Pfeiffer. Reed (1965), however, includes *I. virginica* as part of the *engelmannii-flaccida* complex, thus does not consider the two species (*I. piedmontana* and *I. virginica*) particularly closely allied. Because the species occurs in near proximity to both *I. lithophila* and *I. melanopoda,* the possibly exists that the Texas expressions of *I. piedmontana* could be a hybrid between those two species. However, our megaspore studies do not support this. We are following USDA, NRCS (2004), where it is treated as a valid species.

Habitat requirements are similar to those of *I. lithophila* discussed above.

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