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Interesting, Provocative, and Enigmatic: Morphological Observations on Southeastern Quillworts (*Isoetes* Isoetaceae, Lycopodiophyta)

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ABSTRACT The unique morphology of quillworts has received limited attention, and such studies have been limited to only a few species. Our extensive field work during the past two decades has revealed variation in the structures of the plant, including the rhizomorph, scales, phyllopodia, and abscission caps. Polarity of the axes of southeastern quillwort rhizomorphs varies from discoid (most species), to elongate, to upright with axis branching in several species. In species of intermittent streams, these branched rhizomorphs produce plantlets that break off in running water. Scales are tiny brown or black structures in alternating whorls with sporophylls (leaves). They are often overlooked and easily removed when rinsing specimens. Found in most southeastern terrestrial/amphibious species, scales are absent from aquatic species. Scales are distinct from phyllopodia, the sclerified bases of sporophylls, but intermediates between scales and phyllopodia occur. Not all species with scales have phyllopodia. Although scales cannot be used for determination of species, their presence or absence is of taxonomic value. Research on southeastern *Isoetes* phylogeny could help determine which of the rhizomorph and scale characters are plesiomorphic.

Key words: Abscission cap, *Isoetes*, phyllopodia, rhizomorph.

INTRODUCTION Quillworts, species of the genus *Isoetes* (Isoetaceae), have long fascinated plant morphologists because of their unique suite of structures. These characters have changed little over evolutionary time. Quillworts are successful, occurring in a wide range of habitats on all continents except Antarctica.

The literature on quillwort morphology is not reviewed here. In his challenge to plant morphologists to use modern techniques, Kaplan (2001) included Lycopsida, paying special attention to leaf development in *Isoetes*. But morphological studies with modern technology have been few. One recent example is Freund (2016), who used elliptical Fourier analysis to elucidate differences in the glossopodia of three *Isoetes* species.

For the classically trained plant morphologist—an endangered species—quillworts remain, in the words of the pre-eminent plant morphologists Adriance Foster and Ernest Gifford (Foster and Gifford 1974)—"interesting, provocative, and enigmatic." They have a distinct suite of characters, including the ligule, glossopodium, velum, and attendant structures. Likewise, the rhizomorph (also known as the stem, corm, or rootstock) is also unique and has no corresponding structure in plants with its cambial activity, unique tissues ("prismatic cells"), and abscission caps. Scant attention has been paid to these and other vegetative features as possible taxonomic characters.

†Rebecca D. Bray died 21 January while this paper was in review. She was the beloved Curator of the Herbarium at Old Dominion University for many years where she generously volunteered her time curating the collection and caring for innumerable students through her widely sought sage advice and patient, listening ear. Rebecca was part of the *Isoetes* research group and her fastidious cytological techniques provided chromosome counts for all known southeastern quillworts. Dr. Bray is sorely missed. This paper memorializes her contributions to mentoring students and understanding the enigmatic quillworts. LJM

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Figure 1. Rhizomorphs of *Isoetes valida* showing the discoid shape. Sporophylls and roots have been removed. Giles County, Virginia.

Recent research on the genus *Isoetes* in the southeastern United States, the North American center of diversity of the group, has intensified during the past two decades, resulting in a better understanding of the biology of this remarkable group of Lycophytes and stimulating critical insights into the structural biology of these neglected plants. Numerous new species and hybrids have been described (P. W. Schafran, E. A. Zimmer, W. C. Taylor, and L. J. Musselman, unpubl.) revealing unexpected diversity and widespread reticulate evolution. Before the advent of molecular systematics, determination of species depended almost exclusively upon the features of the megaspores and, to a far lesser extent, on vegetative morphology.

That is because quillwort species are notoriously difficult to identify, sometimes requiring chromosome counts or DNA sequencing—hardly techniques useful for field botanists and ecologists. This is because the quillwort plant is remarkably morphologically uniform across taxa. It is easy to recognize a quillwort as a species of *Isoetes* with its whorled leaves (more accurately sporophylls; the term leaf is used interchangeably), four air chambers with diaphragms, and slightly channeled adaxial sporophyll surfaces. Difficulty in species determination often results in reports listing them simply as *Isoetes* sp.

MATERIALS AND METHODS

This is a report of our field observations during the past 20 yr, chiefly in the southeastern United States. Specimens are deposited in the Old Dominion University Herbarium (ODU), with type specimens deposited at the Missouri Botanical Garden (MO) and the US National Herbarium (US). This work is ancillary to a broad phylogenetic study in the genus.

The taxonomy of *Isoetes* in the southeastern United States is in a state of flux due to several taxa being polyphyletic, unresolved nomenclatural tangles, under-collection of these grass-like wetland plants, and a lack of experimental work on the role of environmental factors in vegetative features. Thus, we have used quotation marks for species we have not yet named or which have been named but shown to be polyphyletic.

OBSERVATIONS

Rhizomorph Axis

We use the term rhizomorph for the corm-like perennial portion of the quillwort plant because that term is morphologically neutral. Gómez (1980) suggested rhizomorph as an alternate to the more widely used "corm" but the term rhizomorph has not been widely used. As noted above, the rhizomorph is unique among vascular plants, producing roots only in the crevices between the lobes, and exhibiting cambial activity (unique in the Lycopodiophyta) (Foster and Gifford 1974). Polarity of the axis varies from discoid (Figure 1) as in most southeastern species, to elongate (Figure 2; *I. tegetiformans* Rury), to upright (Figure 3) as in some *I. valida* (Engelm.) Clute populations), although most collections of this species have discoid rhizomorphs.

The rhizomorph can branch and produce buds on the branches. Branching has been known for over 125 yr (Motelay and Vendryes 1882, pl. XVI) but is seldom recorded. As they elongate, these branches can break from the rhizomorph as a means of asexual reproduction. This form of rhizomorph branching was detailed by Gómez (1980) in *I. storkii* Palmer in Costa Rica. He suggested that the "cracking" of the rhizomorph was caused by desiccation and splitting. From these splits, new growth points arose, producing "bulbils." The term "plantlet" seems more appropriate and less freighted by other morphological applications than bulbil (i.e., the

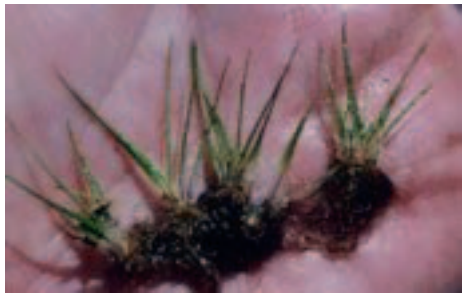

Figure 2. *Isoetes tegetiformans* rhizome-like rhizomorph. Greene County, Georgia. This is the only North American quillwort with this habit.

development of bulbils from true bulbs). We report here branched rhizomorphs in *I. flaccida* Shuttlw. ex A. Braun, *I. "hyemalis,"* and *I. "snowii,"* and have found them on quillworts in Turkey, Syria, and Lebanon (Bolin et al. 2008, 2011; Figure 4) as well. All the species that exhibit this phenomenon are amphibious, contra submersed.

These plantlets should not be confused with the gemmae reported in several species including *I. lacustris* L., *I. andicola* (Amstutz) L.D. Gómez, and others where buds are produced in sporangia (Amstutz 1957, Jermy 1990).

Scales and Phyllopodia

There are of two types of modified sporophylls—phyllopodia and scales produced by the apical meristem. Phyllopodia are sporophylls that have become indurate. Scales are much smaller than the surrounding sporophylls and are usually thin, papyraceous, and brown. Both scales and phyllopodia were apparently first described by Braun (1868).

Phyllopodia, in contrast to scales, are easily seen and easy to collect. The level of sclerification can vary depending on the age of the sporophyll and, to a lesser extent, on the taxon. Like other researchers (e.g., Hickey 1986) we have found intermediates between diminutive scales and indurated fertile sporophylls (Figure 5).

Phyllopodia are of two types. The first is exemplified by the widespread *I. melanopoda* J. Gay and Durieu where the base of the leaf, including the alae (the wings present on both sides of the leaf base) and the subula (the tip of the scale) sclerifies. The green portion of the leaf disintegrates, leaving the usually jet-black sclerified tissue remaining at the base of the plant until the next growing season. The second type includes only the basal portion of the leaf. In other words, little of the subula remains—only the basal portion of the leaf, including the alae, is sclerified and remains on the plant. Examples of this second type of phyllopodium are found in *I. capensis* Duthie (L. J. Musselman, unpubl.) and *I. hystrix* Bory (Bolin et al. 2008). There are no representatives of this second type of scale in our flora.

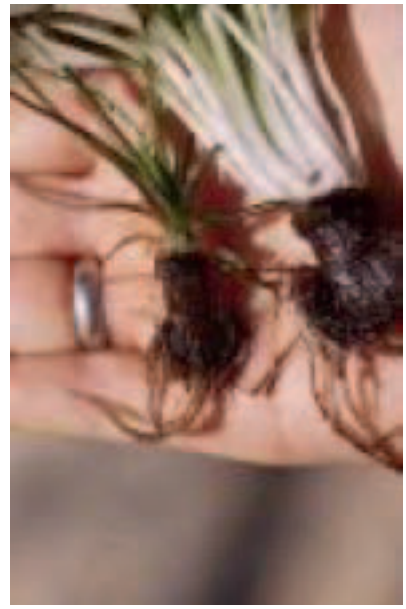

Figure 3. Upright rhizomorph of *Isoetes valida* (right), Butler County, Alabama.

The structure of scales varies considerably among taxa. Some are sclerified, but in other taxa scales are papyraceous, thin, caducous structures (Figure 6). None of the scale-bearing quillworts we have examined have the highly modified spine-tipped scales characterized by the Mediterranean species *I. duriei* Bory (Bolin et al. 2008).

We have documented scales in the following southeastern taxa: *Isoetes "Forty Acre Rock," I. appalachiana* Brunton and Britton, *I. boomii* N. Luebke, *I. butleri* Engelm., *I. "chesterfield"* (an unnamed tetraploid), *I. engelmannii* Braun, *I. georgiana* Luebke, *I. "hyemalis"* (a polyphyletic tetraploid), *I. louisianensis* Thieret, *I. melanopoda*, *I. piedmontana* (Pfeiffer) Reed, *I. riparia* Engelm. ex Braun, *I. "snowii"* (an unnamed

Figure 4. Left: Branched rhizomorph of *Isoetes "hyemalis,"* Chesterfield County, Virginia. The development of the prismatic center of the rhizomorphs is evident in the mother rhizomorph (upper right) and the daughter (center left). Right: *I. "silvatica"* Wayne County, Mississippi.

diploid), *I. virginica* Pfeiffer, and *I. "york county"* (an unnamed tetraploid). Scale size, length of the subula, and degree of sclerification all vary even on the same plant, and therefore scales are of no obvious taxonomic value except for their presence or absence. In all of these taxa, scales occur only on the lobes of the corm, not above the root-producing region. All of these have the delicate, small, papyraceous scales that are easily overlooked and readily shed even with cautious collecting. None of these scale-bearing species are truly aquatic. Further careful collecting is necessary to determine how widespread the presence of scales is. Our list of approximately 15 taxa is a large proportion of the estimated 30 taxa in the Southeast, and contradicts Hickey's statement (1986) that most North American species lack scales.

As noted, we have never found scales on aquatic species, consonant with the findings of Hickey (1986). In his review of scales, Hickey (1986) states "Virtually all of the species which produce phyllopodia or scales in any abundance are seasonally inundated and subject to periods of drought-associated dormancy" (p. 315).

Scales have been neglected and inconsistently reported. For example, they are not mentioned

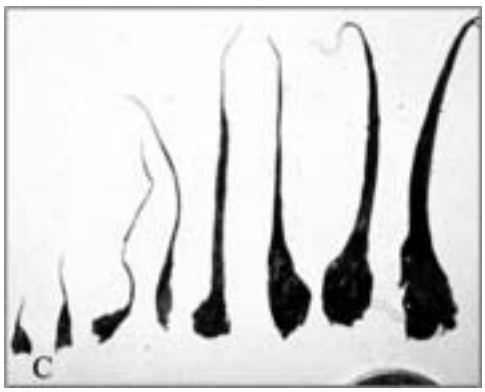

Figure 5. Scales and phyllopodia from a single plant of *Isoetes piedmontana*, Franklin County, North Carolina. The small scales (first and second from left) are only found in the outermost whorl of sporophylls. Like all the scales of southeastern quillworts, scales are longer than broad. Intermediate phyllopodia are third and fourth from the left.

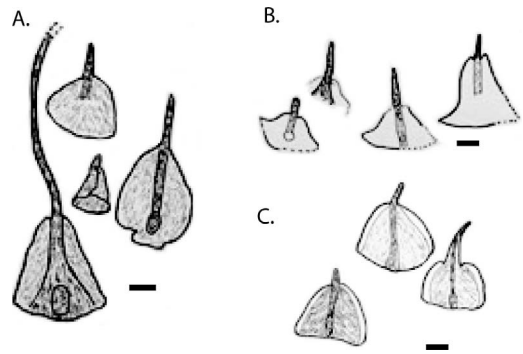

Figure 6. Scales from southeastern quillworts. (A) *Isoetes "snowii,"* diploid; Coffee County, Georgia. Scale on the left is intermediate between a small scale and a phyllopodium. (B) *I. engelmannii*, diploid, Chesterfield County, Virginia. This species lacks phyllopodia. (C) *I. virginica*, diploid, Person County, North Carolina. Scale = 1 mm.

Figure 7. Abscission caps, *Isoetes louisianensis* Wayne County, Mississippi. Left shows the lobe of the rhizomorph after removal of the abscission cap. Right, two abscission caps with remnant roots and scales.

in the treatment of the genus in Virginia (Musselman and Knepper 1994), Flora of the Southern and Mid-Atlantic States (Weakley 2015) or Flora of North America (Taylor et al. 1993). Likewise, in her monograph of the genus, Pfeiffer (1922) mentions scales only for *I. nuttallii* A. Braun ex Engelm. and *I. melanopoda*. However, the Flora of Virginia (Weakley et al. 2012) explicitly mentions scales and phyllopodia.

Some recent descriptions of new species of *Isoetes* include scales (Bolin et al. 2008, Troia and Raimondo 2009, Bolin et al. 2011, Ernandes and Marchiori 2012). Roux et al. (2009) specifically mention a lack of scales in *I. eludens* Roux, Hopper, and Smith—unusual for a granite outcrop species. Other authors include no reference to scale presence or absence (Rosenthal et al. 2014, Zare et al. 2016). Scales presence or absence is a trait usable by field biologists. For example, Ernandes and Marchiori (2012) show that one of the characters separating two species in Sicily is the presence or absence of scales.

The role of scales in the biology of quillworts remains elusive. Protection of the rhizomorph apex during periods of desiccation has been suggested (e.g., Hickey 1986), but many of the plants we have studied have scales only in the outer whorls of sporophylls where protection would be unnecessary. Could such scales be arrested phyllopodia? This is suggested by the presence of vestiges of the fovea and alae in some scales (Figure 6).

Hickey (1986) suggests scales are plesiomorphic in the genus and that scales gave rise to phyllopodia. Considering the presence of clearly transitional forms between tiny, thin, unsclerified scales and phyllopodia, could the reverse be true: could phyllopodia have given rise to scales? The ontogeny of scales can only be elucidated through developmental studies.

Abscission Caps

The abscission cap is the outer layer of the rhizomorph that is shed when growth is resumed at the end of the dormant season (Figure 7). The origin of the term abscission caps is unknown. They are common in southern quillworts and we have found them on *I. boomii*, *I. butleri*, *I. "hyemalis," I. georgiana*, *I. "chickahominy," I. "leary," I. louisianensis*, *I. melanopoda*, *I. piedmontana*, and *I "snowii."* Scales, if present, are frequently shed with the abscission cap.

Future Work

Southeastern field botanists are becoming more aware of how widespread quillworts are in our region and of the great diversity in species. When collecting *Isoetes*, the presence or absence of scales should be noted. They are evanescent structures that are hard to find on most herbarium specimens. The role of rhizomorph branching and the associated production of young plants (plantlets) in the population ecology of the species needs attention. Quillworts are usually thought of as plants with no asexual reproduction, yet plantlets are regularly produced on branched rhizomorphs. Lastly, do abscission caps leave scars? If so, could this be a way to age a plant? These features should be mapped on phylogenetic trees to shed light on their evolution.

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LITERATURE CITED

Amstutz, E. 1957. *Stylites*, a new genus of Isoetaceae. Ann. Missouri Bot. Gard. 44:121–123.

Bolin, J.F., R.D. Bray, M. Keskin, and L.J. Musselman. 2008. The genus *Isoetes* L. (Isoetaceae, Lycophyta) in South-Western Asia. Turk. J. Bot. 32:447–457.

Bolin, J.F., R.D. Bray, and L.J. Musselman. 2011. A new species of diploid quillwort (Isoetes, Isoetaceae, Lycophyta) from Lebanon. Novon 21:295–298.

Braun, A. 1868. Über die australischen Arten der Gattung *Isoëtes*. Monatsber. Königl. Preuss. Akad. Wiss. zu Berlin 1868:523–545.

Ernandes, P. and S. Marchiori. 2012. A comparative study of two endemic *Isoëtes* species from south Italy. International Scholarly Research Network. 1272501-7.

Foster, A.S. and E.M Gifford. 1974. Comparative morphology of vascular plants, 2nd ed. W. H. Freeman & Co., New York, New York.

Freund, F.D. 2016. Characterizing quantitative variation in the glossopodia of three western North American *Isoëtes* species. Amer. Fern J. 106:87–115.

Gómez, L.D. 1980. Vegetative reproduction in a Central American *Isoetes* (Isoetaceae), its morphological, systematic, and taxonomical significance. Brenesia 18:1–14.

Hickey, R.J. 1986. The early evolutionary and morphological diversity of *Isoetes*, with descriptions of two new Neotropical species. Syst. Bot. 11:304–321.

Jermy, A.C. 1990. Isoetaceae. p. 26–30. *In:* Kramer, K.U. and P.S. Green (eds.). The families and genera of vascular plants. Volume l. Springer, Berlin, Germany.

Kaplan, D.R. 2001. The science of plant morphology: definition, history, and role in modern biology. Amer. J. Bot. 88:1711–1741.

Motelay, L. and A. Vendryes. 1882. Monographie des Isoetaceae. Actes de la Société linnéenne de Bordeaux, Bordeaux, France.

Musselman, L.J. and D.A. Knepper. 1994. Quillworts of Virginia. Amer. Fern J. 84:48–68.

Pfeiffer, N.E. 1922. Monograph of the Isoëtaceae. Ann. Missouri Bot. Gard. 9:79–232.

Rosenthal, M.A., S.R. Rosenthal, G. Johnson, W.C. Taylor, and E.A. Zimmer. 2014. *Isoëtes viridimontana:* a previously unrecognized quillwort from Vermont, USA. Amer. Fern J. 104:7–15.

Roux, J.P., S.D. Hopper, and R.J. Smith. 2009. *Isoetes eludens* (Isoetaceae), a new endemic species from the Kamiesberg, Northern Cape, South Africa. Kew Bull. 64:123–128.

Taylor, W.C., N.T. Luebke, D.M. Britton, R.J. Hickey, and D.F. Brunton. 1993. Isoetaceae. p. 64–75. *In*: Flora of North America Editorial Committee, eds. Volume 2. Pteridophytes and Gymnosperms. Oxford University Press, New York, New York.

Troia, A. and F.M. Raimondo. 2009. *Isoetes todaroana* (Isoetaceae, Lycopodiophyta), a new species from Sicily (Italy). Amer. Fern J. 99:238–243.

Weakley, A.S. 2015. Flora of the Southern and Mid-Atlantic States. (http://www.herbarium.unc.edu/flora.htm, 15 May 2015). University of North Carolina, Herbarium, Chapel Hill, North Carolina.

Weakley, A.S., J.C. Ludwig, and J.F. Townsend. 2012. Flora of Virginia. *In* Bland, C. (ed.). Foundation of the Virginia Flora Project, Inc., Richmond. Botanical Research Institute of Texas Press, Fort Worth, Texas.

Zare, G., M. Keskin, A. Doğru-Koca, and M. Armağan. 2016. *Isoëtes vanensis* (Isoëtaceae) sp. nov. from Turkey. Phytotaxa 209:294–300.