ABSTRACT

COMPARATIVE MORPHOLOGY: THREE DIMENSIONAL RENDERINGS OF THE GLOSSOPODIA OF NORTH AMERICAN *ISOETES* LIGULES

by Shane William Shaw

One of the most distinctive features of the heterosporous lycopsids is the presence of a ligule. This enigmatic structure is currently found only in the genera *Isoetes* and *Selaginella*. It is comprised of a basal embedded glossopodium and a free distal tongue. Previous studies have demonstrated small variations in glossopodium shape in Indian species, leading to the possibility that this structure could have taxonomic use. Serial cross, paradermal, and sagittal sections of glossopodia from three different North American species, representing three different ploidy levels, were made. 3-D digital rendering of this complex organ provided comparative data for the three North America species and allowed comparisons with previous published descriptions. In general, the overall shape of the glossopodium is similar in all three North American species. There are several structural differences among them, such as the shape of the cornuae, the length of the medimoles, and the angle of the glossopodium in comparison to the leaf axis.

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GLOSSOPODIA OF NORTH AMERICAN *ISOETES* LIGULES

A Thesis

Submitted to the
faculty of Miami University
in partial fulfillment of
the requirements for the degree of
Master of Science
Department of Botany

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2004

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*In loving memory of
Nana*

ACKNOWLEDGMENTS.

I thank:

The MU herbarium for financial support for a collecting trip to Puerto Rico. Although the main focus of the trip was shelved, it did provide material for a wonderful side project. I am also appreciative of the herbarium for hiring me during the summer of 2004; without the job, finishing my thesis would have been a lot more difficult.

All the people associated with the science department at Lyndon State College. They all had a hand in fueling my interest in science. I especially want to thank Dr. Conant for his generosity and for passing his enthusiasm for botany, especially ferns, to me. Thank you, Dave!

All the botany students that I met and befriended at MU, I enjoyed talking shop, learning how to become a better TA, and spending time with you outside of work. I will never forget the time we spent together, good luck in your future endeavors!

My family for all their support, encouragement, and love over the years. Without you, none of this would have been possible.

My lab mates at Miami University, Kerry and Jessica, for the use of their plants and their help in getting me up to speed on *Isoetes*. Lara for sharing techniques and music, and especially Mike for the work we did together and time we spent away from the lab. It was nice to work with people who have such a passion for botany, their work, and the work of each person in the lab. I'll always remember the fun we had working together.

Most of all Dr. Hickey. I enjoyed working with you. You gave me the freedom to explore my work the way I thought would be best, but with enough guidance to keep me pointed in the right direction. You always made time to talk and you were always there when I needed reassurance. You believed in me and gave me confidence in myself. Thank you, Jim!

INTRODUCTION

The ligule is an enigmatic structure found only in the extant genera *Isoetes* and *Selaginella*. It is located on the adaxial surface of the leaf and is comprised of two major regions: a basal embedded glossopodium and a free visible portion, the tongue. The *Isoetes* glossopodium has a complex shape consisting of a transverse cellular band with globose lobes at each end, giving the overall appearance of a dumbbell. The tongue is parallel to the adaxial leaf surface and is deltate to triangular with auriculate bases. The tongue also consists of two sections, a multicellular central cushion and a thin peripheral margin.

The ligule is thought to have originated at least 408 million years ago during the Devonian (Goswami, 1976; Pigg, 1992, 2001) in lycopsids such as *Leclercqia complexa* Banks, Bonamo and Grierson (Grierson and Bonamo, 1979; Pigg, 2001). *Leclercqia complexa* is the earliest known ligulate lycopod and, as in all lycopsids, its ligule is positioned distal to the sporangium, but unlike any other ligulate lycopod, it is located 2/3 of the way up the leaf. *Leclercqia complexa*'s tongue tapers quickly to a rounded tip and averages 1.95 mm long by 1.80 mm wide (Grierson and Bonamo, 1979). Due to the type of fossil preservation, it is impossible to determine if *L. complexa*'s ligule possessed a glossopodium. Morphologists had long held that heterospory and ligules were correlated features in the lycopod line. However, with the discovery of *L. complexa*, this idea was proven untrue. *Leclercqia complexa* illustrates that the "origin of heterospory and the ligulate condition were not linked" because *L. complexa* is homosporous (Grierson and Bonamo, 1979; Pigg, 2001).

Isoetaleans of the Carboniferous are represented by the lycopsids *Chaloneria cormosa* Pigg and Rothwell and *Chaloneria periodica* Pigg and Rothwell (= *Polysporia mirabilis* Newberry *sensu* DiMichele; Pigg and Rothwell, 1983). Anatomical characters are known for both species because they were both well preserved and it is evident that *C. cormosa* and *C. periodica* both had corm-like axes and were anatomically similar to each another. Also, their leaves possessed small ligules that lacked glossopodia (Pigg and Rothwell, 1983; Pigg, 1992; Retallack, 1997; Pigg, 2001).

By the Triassic, several morphological features characteristic of modern day *Isoetes* had evolved. These include: monolete microspores, sunken sporangia, velum, labium, and elaborate ligules with glossopodia (Pigg, 2001). However, no single Triassic plant possessed all of these features (Pigg, 2001). Complex glossopodia were well developed in the mid-Triassic, as seen in *Takhtajanodoxa mirabilis* Snigirevskaya and *Isoetes beestonii* Retallack (Pigg, 1992; Retallack, 1997; Pigg, 2001).

Takhtajanodoxa mirabilis had transfusion tissue between the ligule and the vascular bundle and possessed an intricate ligule with an anchor-like glossopodium that is similar to those of modern day *Isoetes* (Pigg, 1992). *Isoetes beestonii* is considered to be the oldest geological species of the genus *Isoetes* (Retallack, 1997). *Isoetes beestoni* leaves had sporangia at their base and contained ligules with sunken glossopodia (Retallack, 1997). Pigg suggested that plants similar to modern day *Isoetes*, such as *Isoetites rolandii* Ash and Pigg, emerged during the Jurassic (Pigg, 2001). Not only did *I. rolandii* have the general appearance and growth form of extant *Isoetes*, it was also completely fertile, producing only sporophylls at maturity as in modern *Isoetes* (Pigg, 2001).

Pigg (1992) stated that such fossils document an evolutionary trend of increasing complexity in the glossopodial region of the *Isoetalen* ligule. By this, she was alluding to how the glossopodium has changed from the Carboniferous ligules that lacked glossopodia, through ligules with simple glossopodia, to extant ligules comprised of large glossopodia with intricate cornuae.

Ligules of *Isoetes* develop precociously on young leaves, being initiated on primordia just peripheral to the stem apex (Smith, 1900a; Sporne, 1966). The ligule arises from a large, single epidermal cell located on the adaxial surface of a leaf primordium when it is approximately five to seven cells tall (Smith, 1900a; Bhambie, 1963; Sporne 1966; Sharma and Singh, 1984). The ligule initial undergoes a paradermal division to form two daughter cells (Smith, 1900a; Bhambie, 1963). The inner cell develops into the glossopodium (Bhambie, 1963) and the outer, daughter cell continues to divide, giving rise to a vertical file of three cells. These then undergo repeated transverse and longitudinal divisions, forming a plate of cells parallel to the epidermis (Bhambie, 1963; Sharma and Singh, 1984). This plate of cells develops into

the laminate tongue of the ligule (Smith, 1900a; La Motte, 1933; Bhambie, 1963). The tongue grows quickly and soon overtops the young leaf primordium (Smith, 1900a; Bhambie, 1963; Sporne 1966; Sharma and Singh, 1984). Up to this point, most of the growth of the ligule has been two dimensional, but the central region eventually divides paradermally to become multiseriate (Smith, 1900a; Bhambie, 1963; Sharma and Bohra 2002). This thickening begins in the basal region of the tongue and proceeds acropetally, but never reaches the margins or apex of the tongue (Smith, 1900a; Bhambie, 1963). As a result, a central cushion and peripheral margins are differentiated.

As the ligule matures, the original interior cell divides vertically to form two cells. These cells divide irregularly to form a small horizontal band-like mass of cells. On either side of the band, rapid cell divisions occur, resulting in acropetal and basipetal growth leading to the development of the two lobes known as the cornuae (Fig. 1.A; Smith, 1900a; Bhambie, 1963).

Thus, at maturity the ligule consists of two major regions: the tongue, commonly referred to simply as the ligule, and the glossopodium (Bhambie, 1963). The tongue is usually triangular in shape and, after reaching maturity, is partly or wholly deciduous. It consists of a central cushion (Fig. 1.B) whose cells are similar in size and shape to those of the glossopodium and which contain large quantities of protein and highly developed Golgi bodies. Lateral to the cushion is the margin (Fig. 1.C), a region of the tongue that is only 1-3 cells thick. Margin cells usually have well developed endoplasmic reticulum (ER), but the cellular components and the cells themselves degrade quickly once the ligule reaches full size (Smith, 1900a, 1900b; Bhambie, 1963; Goswami, 1976; Kristen *et al.*, 1982; Sharma and Singh, 1984).

The glossopodium remains embedded and in most species is surrounded by a layer of sheath cells, which may be uniseriate or multiseriate (Fig. 1.D; Bhambie, 1963; Goswami, 1976). This sheath is composed of small isodiametric gland-like cells and is the contact/boundary layer between the ligule and the leaf. The rest of the glossopodium is composed of isodiametric, parenchymous cells that are arranged in an irregular pattern and are larger than the sheath cells (Fig. 1A). The free portion of the tongue is connected to the glossopodium by an embedded region of the tongue that we

FIG. 1. Sagittal sections through *Isoetes melanopoda* (A, D) and *I. virginica* (B, C) to show the four sections of the ligule and glossopodium. A. Glossopodium with cornuae and embedded section of the tongue, the medimoles. B. Multicellular central cushion of the tongue. C. Thin peripheral margin of the tongue. D. Surrounding sheath cells and medimoles. C= cushion, Co= cornua, G= glossopodium, M= margin, Me= medimoles, S= sheath.

call the medimoles (L., *media* = middle, *moles* = shapeless mass; Figs. 1.A, 1.D).

The physiological significance of *Isoetes* ligules and glossopodia are unknown (Kristen *et al.*, 1982), but there are numerous hypotheses that include:

1. In *Isoetes*, the large size, overarching form, and early development of the ligule suggests that it may function to protect the leaf from mechanical harm (Sharma and Bohra, 2002).

2. Due to the frequent development of transfusion tissue around the glossopodium (i.e. between the sheath and vascular trace), the presence of glandular hairs on some ligule margins, and the fact that some ligules are known to exude mucilage and/or water Bierhorst (1971), Goswami (1976), Sharma and Singh (1984), and Gifford and Foster (1989) have suggested that the ligule might prevent the sporangia and leaves from desiccation.

3. Given that there is considerable amounts of accumulated proteins found within the ligule, the resemblance between *I. pantii* basal glossopodial cells to the palisade structure of the foot region of *Anthocerus* sporophyte, the similarity between sheath cells and endodermal cells, and again, the presences of transfusion tissues, Goswami (1976), Kristen and Biedermann (1980), and Sharma and Bohra (2002) suggested that the ligule may serve a nutritive role.

4. Based on the presences of mucilage and transfusion tissue, Bierhorst (1971) and Sharma and Singh (1984) suggest that the ligule may act as a water reservoir during xeric conditions.

5. Based on F.C. Steward's work on *Cucurbita*, Bierhorst (1971) suggested (although admittedly lacking real evidence) the ligule may be involved with the upward movement of solutes as they are passed among leaf primordia.

6. Kristen *et al.* (1982) speculated that at one time the ligule may have been a digestive organ, but now may be vestigial.

7. Since no bacteria are found within the mucilage, but can be found growing in the remains of cells located at the ligule margins that are devoid of mucilage Kristen *et al.* (1982) suggested that the ligule may have antibacterial properties.

8. Finally, it is possible that the ligule performs multiple functions.

In light of the fact that there are multiple opinions about ligule function, most of which are based on the same data, further studies are obviously needed to resolve this issue.

Selaginella and *Isoetes* are both ligulate heterosporous pteridophytes (Sharma and Singh, 1984; Gifford and Foster, 1989) sharing numerous developmental and vegetative characters. For example, both have endosporic gametophytes (Bierhorst, 1971; Gifford and Foster, 1989), similar embryo orientation (La Motte, 1933), and leaves that initiate from an assemblage of superficial cells (Smith, 1900b; Bhambie, 1963; Gifford and Foster, 1989). Although they share a lot of characters, there are many differences as well. For instance, *Selaginella* embryos have suspensors, whereas *Isoetes* embryos lack them (Gifford and Foster, 1989); *Selaginella* sperm is biflagellate, whereas *Isoetes* sperm is multiflagellate (Bierhorst, 1971; Gifford and Foster, 1989); *Selaginella* can have dimorphic leaves and lack air chambers, whereas *Isoetes* leaves are all monomorphic and contain four lacunae (Webster, 1992; Moran, 1995; Sharma and Bohra, 2002).

Ligule ontogeny is also dissimilar between *Selaginella* and *Isoetes*. In *Selaginella* the ligule develops from two rows of superficial cells (Smith, 1900a; Horner *et al.*, 1975; Gifford and Foster, 1989), whereas the *Isoetes* ligule originates from a single epidermal cell (Smith, 1900a; Bhambie, 1963; Sporne, 1966; Gifford and Foster, 1989). In both, the ligules are attached to the adaxial leaf surface just distal to the sporangium, produce callose, achieve maturity before their corresponding leaves, and both lack chlorophyll, starch, and intercellular spaces (Smith, 1900a; Bierhorst, 1971; Horner *et al.*, 1975; Jagels and Garner, 1979; Kristen and Biedermann, 1981; Webster, 1992). At maturity, the ligules of each consist of four sections. In *Selaginella* these are the sheath, glossopodium, bulbous base, and tip or neck (Fig. 2A). These are comparable to the four sections of the *Isoetes* ligule (Fig 2B; Smith, 1900a, 1900b; Bhambie, 1963; Sigee, 1974; Horner *et al.*, 1975; Kristen *et al.*, 1982; Bilderback, 1987; Bilderback and Slone, 1987) but the individual parts differ in size and extent of development. *Selaginella* ligules are rarely if ever triangular and instead are shaped like a slightly curved, cupped hand. They also have a much simpler glossopodium

FIG. 2. Comparable sections of the mature ligules of *Selaginella* and *Isoetes*. A. *Selaginella* ligule is comprised of a sheath, glossopodium, bulbous base, and tip/neck. B. *Isoetes* ligule consists of a sheath, glossopodium, cushion and margin. Drawings are NOT to scale. [Fig 2.A redrawn from Gifford and Foster. 1989. *Morphology and Evolution of Vascular Plants*. W.H. Freeman and Company, New York.]

(Smith, 1900; Horner *et al.*, 1975; Gifford and Foster, 1989), lacking the broad cornuae typical of *Isoetes*.

The ligules of *Selaginella* and *Isoetes* are quite similar at the ultrastructure level. Both contain dense cytoplasm, protein bodies, Golgi, ER, and mitochondria, although *Isoetes* appears to contain more Golgi and ER than *Selaginella* (Paolillo, 1962; Sigee, 1974; Kristen and Biedermann, 1981; Kristen *et al.*, 1982; Bilderback and Slone, 1987). The presence of ER suggests significant amounts of protein synthesis (Kristen and Biedermann, 1981; Kristen *et al.*, 1982) as evidenced also by temporary protein bodies within the ligule (Kristen *et al.*, 1982). These ligular protein bodies are identical to those found in the external mucilage (Kristen and Biedermann, 1981; Kristen *et al.*, 1982). Finally, the secreted mucilage from *Selaginella* and *Isoetes* ligules (Bhambie, 1963; Kristen *et al.*, 1982; Bilderback, 1987; Bilderback and Slone, 1987; Webster, 1992) consists of two major components: proteins and polysaccharides (Paolillo, 1962; Kristen *et al.*, 1982; Webster, 1992).

Despite these similarities, there are ultrastructural differences. Kristen *et al.* (1982) showed that the ligule cushion of *Isoetes lacustris* L. possess numerous protein bodies, has connections between Golgi and ER, and lacks cell wall ingrowths within the cushion. The ligule margins also showed well developed ER, Golgi, and mucilage. Kristen *et al.* (1982) argued these as evidence that the ligule is to be “considered a secretional organ”.

In contrast, the ligule base of *Selaginella kraussiana* (Kunze) A. Braun. lacks protein bodies, has no known Golgi-ER connections, and in the ligule bases of *Selaginella pilifera* A. Braun and *Selaginella uncinata* (Desv. ex Poir.) contain cell wall ingrowths (Kristen *et al.* 1982). Furthermore, *Selaginella* ligule margins lack well developed ER, Golgi, and mucilage (Kristen *et al.* 1982). The lack of these structures, suggest that *Selaginella* ligules do not secrete mucilage. That hypothesis was supported by the studies of Sigee (1974), Bilderback (1987), and Webster (1992) who showed that some *Selaginella* species do not secrete mucilage.

Previous studies on the ligule of *Isoetes* have demonstrated variation in glossopodium shape in the Indian species *I. reticulata* Gena and Bhardwaja, *I. coromandelina* L.f., and *I. rajasthanensis* Gena and Bhardwaja. The cornuae of the

glossopodia have been shown to be triangular, anchor-shaped, or globular (Figs. 3A-C; Sharma and Singh, 1984), suggesting that the glossopodium may have some taxonomic value. Sharma and Singh's work inspired the current research on glossopodium morphology of several North American *Isoetes*. Specifically, the current work investigates three questions:

1. Is the glossopodium shape consistent among North American *Isoetes*?
2. Are the cornuae of North American species similar in shape to any of those described for the Indian *Isoetes* species?
3. Can 3-D images derived from different sectioning planes be used to faithfully reflect glossopodium morphology?

METHODS AND MATERIALS

Three specimens of *I. melanopoda* Gay and Durieu (2n), four of *I. virginica* N. Pfeiff. (4n), and five of *I. tennesseensis* Luebke and Budke (8n) were collected (Table 1) and fixed in FAA. After fixation, the plants were moved into 70% ethanol for long-term storage. Twelve basal portions of mature megasporophylls were removed from four different plants, dehydrated in a TBA series, and embedded in Paraplast (Johansen, 1940). Serial cross, paradermal, and sagittal sections were prepared using a rotary microtome set at 10 μ m. Ribbons were mounted onto glass slides using egg albumen and stained with 0.2% Toluidine Blue (Johansen, 1940). Each section was magnified 37X with a Rayoscope slide projector and the glossopodium was traced onto paper. Angular orientation of the glossopodium and medimoles relative to the leaf axis were measured from these tracings. Nine glossopodium tracings, three from each species, were scanned into a computer and aligned by hand using Adobe Photoshop (version 6.0) and Image Pro Plus (version 4.5). 3-D images of each were created using Voxblast (version 3.0) and were saved in Quick Time and AVI format. In the 3-D images, the tongues were removed, except in the three sagittal sections, to conserve computer memory and because they were not an integral part of this study. Photographs of thin sections were taken with a Nikon Coolpix 4500 digital camera mounted on an

FIG. 3. Reconstructions of ligules and glossopodia of three Indian *Isoetes*. A. *Isoetes coromandelina*. B. *I. rajasthanensis*. C. *I. reticulata*. L= ligule (tongue), G= glossopodium. [Redrawn from Sharma and Singh. 1984. The ligule in *Isoetes*. Amer. Fern J. 74:22-28.]

Table 1. Collection information for study material.

Species	Collection	N
<i>I. melanopoda</i> Gay and Durieu	New Salem Union County, North Carolina. <i>Kerry Heafner 99011</i> (MU) 16-April-2002.	3
<i>I. virginica</i> N.E.Pfeiffer	Person County, North Carolina. <i>Kerry Heafner</i> <i>99015</i> (MU) 18-April-2002.	4
<i>I. tennesseensis</i> Luebke and Budke	Polk County, Tennessee <i>Jessica Budke et al.</i> <i>3-04TN</i> (MU) 15-July-2001.	5

Olympus BH-2 microscope; all measurements were made directly from slides with the aid of an ocular micrometer. Glossopodia and cornuae shapes were determined by comparing with a standard for symmetric plane figure shapes (Radford, 1986).

RESULTS

Isoetes tennesseensis.—In the most distal cross section of *I. tennesseensis* (Fig. 4.A), a labium is visible in front of the ligule tongue. At this point, the cornuae are transversely elliptical and are surrounded by a noticeable sheath (Fig. 4.A). The vascular trace is positioned between the cornuae and two lacunae are visible near the abaxial leaf surface (Fig. 4.A). In Fig. 4.B, the tongue (arrow) is encroaching the foveola (ligular pit), adaxial to one of the cornuae. Protuberances are noticeable on either side of the concave adaxial surface of the cornuae. Slightly lower, the tongue is connected to the adaxial surface of the cornuae by two lateral portions of the medimoles (Fig. 4.C). At this point, both cornuae curve adaxially on their outer edges (Fig. 4.C). In Fig. 4.D, only the basal auricles of the ligule are visible and the glossopodium is connected to the broad medimoles. A thin transverse band linking the curved cornuae is evident (Fig. 4.D). In successively lower sections, the labium has merged with the leaf and a simple elliptical pad of tissue represents the glossopodium (Figs. 4.E, 4.F). The fovea is noticeable in front of the glossopodium (Fig. 4.F).

In peripheral sagittal section of *I. tennesseensis* (Fig. 5.A) the tongue is seen intruding into the foveola. At this point, the glossopodium is somewhat ovate and is enveloped by a noticeable sheath. Two lacunae are evident above the glossopodium

FIG. 4. Serial cross sections of *Isoetes tennesseensis*, proceeding from the top down. A. Section through the elliptical cornuae lobes. B. The tongue encroaching into the foveola (see arrow). C. The tongue is connected to the cornuae by two lateral portions of the medimoles. D. The glossopodium is attached to the broad medimoles. E. The labium has merged with the leaf and the glossopodium is reduced to a simple pad. F. The fovea is noticeable in front of the simple glossopodium. Scale bars = 500 μm. A= lacuna, Co= cornua, F= fovea, G= glossopodium, L= labium, L= tongue, Me= medimoles, S= sheath, V= vascular trace.

FIG. 5. Serial sagittal sections of *Isoetes tennesseensis*. A. The tongue is intruding into the foveola in front of the ovate glossopodium. B,C. The tongue is attached to the glossopodium by a long, horizontal medimoles. D. In the medial section, the glossopodium is reduced. E. The glossopodium is again at its larger, more complex form (as in sections B and C). F. The tongue is not connected to the glossopodium, but segments of it and the medimoles remain in the foveola. Scale bars = 500 μ m. A= lacuna, Co= cornua, G= glossopodium, L= labium, L= tongue, Me= medimoles, S= sheath, V= vascular trace.

(Fig. 5.A). More medially, the tongue is attached to the adaxial face of the glossopodium by a long medimoles (Fig. 5.B). At these points (Figs. 5.B, 5.C), the glossopodium is at its thickest and most intricate with the upper cornuae tilting adaxially at an acute angle. In medial section, the labium is visible in front of the ligule (Fig. 5.D). The ovate glossopodium is diminutive and the vascular trace is visible abaxial to it (Fig. 5.D). Fig. 5.E is a section through the proximal complex segment of the glossopodium and has features similar to those of Fig. 5.B. In Fig. 5.F, the tongue is not attached to the glossopodium, but it is still contained within the foveola. Also, a short peripheral portion of the medimoles can be seen projecting toward the ligule from the upper portion of the cornuae.

Fig. 6 shows sections in near paradermal section beginning from the adaxial face and proceeding abaxially. Because of the curvature of the leaf, the sections cut obliquely through the upper portions of the tissue. In Fig. 6A, the broad medimoles is evident within the foveola. More abaxially, two transversely-elliptical cornuae are visible just above the medimoles, along with four lacunae and the vascular trace (Fig. 6.B). In Figs. 6.C and 6.D, the glossopodium is at its most complex form and is surrounded by a noticeable sheath. At these points, protuberances on the cornuae and invaginations in the transverse band (Figs 6.C, 6.D) are evident. Also, the right cornua (Fig. 6.C) enclose a small circular patch of leaf tissue (arrow in Fig. 6.C). Abaxially, the glossopodium is bilobed (Fig. 6.E) and the transverse band is lost, leaving two isolated cornuae lobes (Fig. 6.F).

Isoetes virginica.—In distal serial cross section the ligule cushion is noticeably thick and the cornuae are depressed-ovate with flattened adaxial faces (Fig. 7.A). At this point, the vascular trace is located between the cornuae, and the two abaxial lacunae are evident (Fig. 7.A). Slightly lower, a small portion of the labium can be seen in front of the tongue (Fig. 7.B). At this point, the cushion is attached to the cornuae by means of two broad but radially short portions of the medimoles (arrow in Fig 7.B) and small protuberances on the adaxial corners of the cornuae are visible (Fig. 7.B). In lower sections, the transverse band connecting the cornuae thickens and the cornuae lose their distinctness (Fig. 7.C, 7.D). In Figs. 7.E and 7.F the glossopodium is relatively simple.

FIG. 6. Near paradermal long sections of *Isoetes tennesseensis*, starting from the adaxial face and proceeding abaxially. A. The medimoles is seen within the foveola. B. The transversely-elliptical cornuae lobes are distal to the medimoles. C,D. The glossopodium is at its most complex form. Note the small circular patch of tissue enclosed within the glossopodium (arrow in section C). E. The glossopodium is simpler the before (C,D). F. The transverse band is lost, leaving only two isolated cornuae lobes. Scale bars = 500 μm . A= lacuna, Co= cornua, G= glossopodium, Me= medimoles, S= sheath, V= vascular trace.

FIG. 7. Serial cross sections of *Isoetes virginica*, proceeding basipetally. A. The cornuae are depressed-ovate in shape. B. The cushion is attached to the cornuae by broad, radially short medimoles sections (arrow). C,D. The transverse band connects the cornuae forming the glossopodium. E,F. The glossopodium is relatively simple and has reduced cornuae. Scale bars = 500 μ m. A= lacuna, Co= cornua, G= glossopodium, I= labium, L= tongue, S= sheath, V= vascular trace.

In the first peripheral sagittal section, the glossopodium is elongate and bean shaped; its orientation is parallel to the leaf axis. The ligule cushion is seen as detached from the rest of the leaf, but the extreme edge of the foveola is noticeable between the base of the tongue and the center of the cornua (Fig 8.A). More medially, (Fig. 8.B) the tongue is connected to the glossopodium by a short medimoles that is angled slightly upward away from the cornua. In medial sagittal section (Fig. 8.C), the cushion, medimoles, and glossopodium are similar in thickness forming a continuum that cannot be differentiated. At this point, the entire glossopodium lies quite close to the adaxial leaf surface. In Fig. 8.D, two portions of the cornua lobe are evident distal to the glossopodium. In sequentially peripheral sections (Figs. 8.E, 8.F) the proximal cornua exhibits a form similar to that of the distal one (Fig. 8.B). Lacunae are evident above the glossopodium in Fig. 8.E. In the last section (Fig. 8.F), the tongue margin is isolated and the peripheral portion of the glossopodium is visible.

In proximal paradermal longitudinal sections of *I. virginica* the curved medimoles is shown within the foveola with two unattached cornuae lobes distal to it (Figs. 9.A, 9.B). Moving abaxially, the thin, broadly curved transverse band attaches to the cornuae between small protuberances (Fig. 9.C). Abaxially, the transverse band flattens and thickens as the cornuae become more extensive (Fig 9.D). At this point, two lacunae are visible distal to the glossopodium and a noticeable sheath surrounds the glossopodium (Fig. 9.D). In the next two figures, the glossopodium is reduced and the vascular trace extends between the cornuae (Figs. 9.E, 9.F). In the last view (Fig 9.F), the transverse band is gone and the glossopodium appears as two cornuae lobes.

Isoetes melanopoda.—In the most distal cross section of *I. melanopoda* (Fig. 10.A) the cornuae of the glossopodium are depressed-ovate with a somewhat flattened adaxial face. A multiseriate sheath surrounds each cornuae. The vascular trace is located between the cornuae and two lacunae are evident. Moving basipetally, the tongue connects to the adaxial face of the cornua by means of the two (one shown in Fig. 10.B) lateral edges of the medimoles. In successively lower sections the medimoles transitions into the transverse band, which connects the two cornuae (Figs. 10.C, 10.D). Small cellular protuberances are located on the inside (Fig. 10.C) and

FIG. 8. Serial sagittal sections of *Isoetes virginica*. A. The glossopodium is bean shaped and not connected to the tongue. B. The tongue is connected to the glossopodium by a short, slightly upwardly-angled medimoles. C. In medial section, the tongue, medimoles, and glossopodium are impossible to differentiate. D. There are two portions of the cornua lobe distal to the glossopodium. E. The glossopodium is at it more complex form, as in section B. F. The detached margin and a simple conua lobe are evident. Scale bars = 500 μ m. A= lacuna, Co= cornua, G= glossopodium, L= tongue, M= margin, Me= medimoles, S= sheath, V= vascular trace.

FIG. 9. Near paradermal longitudinal sections of *Isoetes virginica*, starting from the adaxial face and proceeding abaxially. A,B. The curved medimoles is in the foveola with two cornuae lobes distal to it. C. Abaxially, the transverse band connects the cornuae lobes between small protuberances. D. The cornuae lobes are more extensive and the transverse band is flatter and thicker. E. The glossopodium is reduced to a simpler form. F. The transverse band is gone, leaving only two cornuae lobes. Scale bars = 500 μm . A= lacuna, Co= cornua, G= glossopodium, Me= medimoles, S= sheath, V= vascular trace.

FIG. 10. Serial cross sections of *Isoetes melanopoda*, proceeding basipetally. A. The cornuae are depressed ovate and the tongue is at the edge of the foveola. B. The tongue is connected to the cornuae by two (one shown) edges of the short medimoles. C,D. The transverse band connects the cornuae forming a glossopodium that resembles a dumb-bell. E,F. The cornuae are less distinct, giving the glossopodium a relatively simpler form. Scale bars = 500 μm . A= lacuna, Co= cornua, G= glossopodium, I= labium, L= tongue, Me= medimoles, S= sheath, V= vascular trace.

outside corners of the cornuae (Fig. 10.D) giving them a bulbous angular appearance. At this level the glossopodium resembles a curved dumb-bell (Figs. 10.D, 10.E). In lower sections (Figs. 10.E, 10.F), the transverse band thickens and the cornuae are less distinct.

In the peripheral sagittal sections of *I. melanopoda* (Fig. 11.A), the detached ligule cushion intrudes into the foveola. The small obovate patch of glossopodium is adjacent to the intruding tongue and two lacunae are located above the glossopodium (Fig. 11.A). Closer to the center, a pronounced labium and extensive medimoles is evident. The medimoles connects centrally to the near vertically oriented glossopodium (Fig. 11.B). Also at this point, the vascular trace can be seen above the glossopodium and a noticeable sheath is evident (Fig. 11.B). In medial sections, cut through the transverse band, the glossopodium is small and terete, with the vascular trace curving around and abaxial to it (Fig. 11.C). Fig. 11.D is a section through a more proximal segment of the glossopodium just at the edge of the cornuae. It has features similar to those of Fig. 11.B. More laterally, the ligule margin is visible intruding into the foveola, but at this point, is not attached to the medimoles (Fig. 11.E). Fig. 11.F is a peripheral section through the cornuae. Only the ligule margin is visible as is a small, elliptical patch of tissue representing the glossopodium.

Starting from an early adaxial paradermal longitudinal section and proceeding abaxially, the first figure (12.A) shows the medimoles within the foveola. Due to the oblique sectioning, one lobe of the glossopodium is seen as a small, detached patch of tissue (see arrow) and as a swelling on one end of the medimoles as it merges with the cornua (Fig. 12.A). More abaxially (Figs. 12.B, 12.C, 12.D), the cornuae of the glossopodium are at their most complex form and a noticeable sheath surrounds them. Throughout this region, the vascular trace is located centrally, above the glossopodium (Figs. 12.B, 12.C, 12.D). Abaxially, the cornuae reduce (Figs. 12.E, 12.F) and only one cornua lobe is visible in Fig. 12.F.

Using the cross sectional and sagittal sectional views, each North American species was measured to determine its maximum glossopodium width and cornua height. *Isoetes tennesseensis* is the largest with a maximum width of 1900 μ m and a cornua height of 1120 μ m. *Isoetes virginica* has a maximum glossopodium width of

FIG. 11. Serial sagittal sections of *Isoetes melanopoda*. A. The tongue is intruding into the foveola. Adjacent to the tongue is a small obovate glossopodium. B. The tongue is connected to the glossopodium by an ascending medimoles. C. In the medial section, the glossopodium is small and terete. D. The more proximal segment has a complex glossopodium similar to that of section B. E. The tongue is no longer attached to the glossopodium, but it and a segment of the medimoles is seen in the foveola. F. The ligule margin and a small elliptical patch of glossopodium are evident. Scale bars = 500 μ m. A= lacuna, G= glossopodium, I= labium, L= tongue, Me= medimoles, S= sheath, V= vascular trace.

FIG. 12. Near paradermal longitudinal sections of *Isoetes melanopoda*, starting from the adaxial face and proceeding abaxially. A. The medimoles is in the foveola; due to the oblique sectioning, the glossopodium is evident as a small detached patch of tissue (arrow) and as a swelling at one end of the medimoles. B,C, and D. The glossopodium is at its most complex form. E. The glossopodium is reduced to a simpler form. F. Only one cornua lobe is evident. Scale bars = 500 μ m. A= lacuna, G= glossopodium, Me= medimoles, S= sheath, V= vascular trace.

1200 μ m and a cornua height of 860 μ m. *Isoetes melanopoda* is the smallest of the three, with a glossopodium width of 820 μ m and a cornuae height of 540 μ m.

DISCUSSION

The nine images in Fig. 13 are the starting images of the nine movie reconstructions (included CD). Each movie rotates through 360°, first horizontally and then vertically so that all sides can be viewed and compared.

In overall shape, the ligule is similar in all three North American species. All the mature ligules have a well developed sheath, glossopodium, cushion, and margin. In each the tongue is attached to the glossopodium adaxially via the medimoles. Each glossopodium is symmetrical and bilobed, has complex cornuae, is proximal to the lacunae, and has the vascular trace passing between the cornuae lobes. Despite these similarities, there are several structural variations among the North American species. In face view, the cornuae of *I. tennesseensis* are stout-triangular and somewhat flattened ventrally (Figs. 6.C, 13.B, and corresponding movies; CM). Viewed from the side the cornuae appear reniform in shape (Figs. 5.B, 13.C, CM) and the ligule is attached slightly below the center of the glossopodium (Figs. 5.E, 13.C, CM). A well developed medimoles connecting the tongue and glossopodium (Figs. 5.B, 13.C, CM) is evident in cross sectional and sagittal views (Fig. 13.A, CM). In *I. tennesseensis* the glossopodium leans towards the ligule at an acute angle (Figs. 5.B, 13.C, CM).

In abaxial face view, the cornuae of *I. virginica* are elliptic (Figs. 9.D, 13.E, CM). In sagittal view, the glossopodium appears thin and narrowly elliptic, and the tongue attaches high on the adaxial face (Figs. 8.E, 13.F, CM). In side and cross sectional views, it is evident that the medimoles is stout but not extensive (Figs. 7.B, 8.B, 13.D, 13.F, CM). The glossopodium axis is nearly parallel to the leaf axis (Figs. 8.B, 13.F, CM).

The cornuae of *I. melanopoda* are ovate in face view (Figs. 12.D, 13.H, CM) and elliptic in side view (Figs. 11.B, 13.I, CM). The ligule is attached centrally to the adaxial face of the glossopodium by means of a short medimoles (Figs. 10.B, 11.B, 13.G, 13.I,

FIG. 13. Reconstructions of three North American *Isoetes* glossopodia. The first column is *I. tennesseensis* (A-C), the second column is *I. virginica* (D-F), and the last column is *I. melanopoda* (G-H). The first row of images is derived from transverse cross sections and this view is from the top (A, D, and G). The second row of images is derived from paradermal long sections. These views are from an adaxial position (B, E, and H). The third row of images are from sagittal sections. These views are lateral (C, F, and I). The asterisk references the adaxial sides of each image (no asterisks are seen in B, E, and H because they are outside the plane of the paper).

CM). In *I. melanopoda*, the upper section of the glossopodium leans slightly away from the ligule (Figs. 11.D, 13.I, CM) and is angled toward the abaxial side of the leaf.

In summary, the glossopodia of *Isoetes tennesseensis*, *I. virginica*, and *I. melanopoda* are generally similar in shape. Regardless of this resemblance, there are numerous structural variations among them. They differ in cornua shape, ligule attachment position, size of the medimoles, its angle of departure from the glossopodium, the glossopodium angle, and the maximum height and width of the cornua and glossopodium (Table 2). The glossopodia of the North American *Isoetes* are structurally different from the Indian taxa (Fig. 14. A-F). Sharma and Singh (1984) demonstrated variation in size, shape, and complexity in the glossopodia of three Indian species. *Isoetes coromandelina* has the largest, most complex glossopodium with distinctly anchor-shaped cornuae and an extended transverse band (Fig. 14.D). *Isoetes rajasthanensis* has a medium sized glossopodium with globular cornuae (Fig 14.E) and *Isoetes reticulata* has the least complex glossopodium of the three, with fusiform cornuae (Fig. 14.F). From the descriptions provided by Sharma and Singh (1984), *I. rajasthanensis* (Fig. 14.E) appears to be the most similar to any of the North American species. The glossopodia of *I. coromandelina* and *I. reticulata* are either too complex or too simple. Due to the small number of images available and their diagrammatic nature, a more comprehensive study of the Indian species is necessary to fully compare them with ours and other species.

The reconstructions of the glossopodium for a single species created from serial cross, paradermal, and sagittal sections are generally congruent (CM). However, due to the thickness of each section, there is loss of minor detail between reconstructions. For example, the small protuberances and invaginations are often best seen in only one or two sectional planes. It is important for future investigators to include all three sectional planes to insure fine-detail fidelity in reconstructions and illustrations of the glossopodia so that these small differences are not overlooked or interpreted incorrectly. However, these minor variations do not affect the overall appearance of the reconstructions.

Sharma and Bohra (2002) propose that *Isoetes* glossopodia became complex because of the presence of the lacunae. They state that the glossopodium “sends

Table 2. Summary of structural variations in the glossopodia of *Isoetes tennesseensis*, *I. virginica*, and *I. melanopoda*.

	<i>I. tennesseensis</i>	<i>I. virginica</i>	<i>I. melanopoda</i>
Paradermal view of cornuae	Stout triangular	Elliptic	Ovate
Saggital view of cornuae	Reniform	Thin, narrowly elliptic	Elliptic
Ligule attachment position on the glossopodium	Slightly below the center	High	Centrally
Medimoles size	Large: well developed	Stout: not extensive	Short: not extensive
Medimoles angular departure from the glossopodium	Parallel	Parallel	Ascending
Angle of the glossopodium compared to vertical leaf axis	Upper sections leans acutly adaxially	Near parallel to the leaf axis	Upper section leans slightly abaxially
Maximum cornua height and glossopodium width (μm)	Height = 1120 Width = 1900	Height = 860 Width = 1200	Height = 540 Width = 820

branches toward the four cavities” and the cornuae are “arranged in a regular manner into the (air) cavities”. Thus, they hypothesize that the lacunae develop prior to the glossopodium. However, many studies suggest that the ligule develops faster than its associated leaf (Smith, 1900a; Bhambie, 1963; Sporne 1966; Sharma and Singh, 1984; Gifford and Foster, 1989) and photographs of longitudinal sections of *Isoetes* corms depict young leaves with well developed glossopodia that lack lacunae (Bierhorst, 1971; Gifford and Foster, 1989). Even though these studies are inconclusive, they warrant future investigations to determine if Sharma and Bohra’s ontogenetic hypothesis is correct.

FIG. 14. Reconstructions of the three North American (A-C) and three Indian (D-F) *Isoetes* glossopodia. A. *Isoetes tennesseensis*. B. *I. virginica*. C. *I. melanopoda*. D. *I. coromandelina*. E. *I. rajasthanensis*. F. *I. reticulata*. All views are from an abaxial vantage point looking toward the ligules; the glossopodia are in front of the ligules. L= ligule (tongue), G= glossopodium. [Indian taxa redrawn from Sharma and Singh. 1984. The ligule in *Isoetes*. Amer. Fern J. 74:22-28.]

This work clearly shows that there is interspecific variation in glossopodium shape among the three North American taxa. These differences may be due to habit, environmental influences, different ploidy levels or phylogenetic history. Each North American species in this study is from a different habitat. *Isoetes tennesseensis* is an obligate aquatic and like other species of this type of environment are not generally surrounded by a compact, encroaching substrate. The lack of substrate pressure on the leaves allows for a looser packed assemblage of leaf bases at the apex of the corm. *Isoetes virginica* is an amphibious and its leaf bases are somewhat compressed by the surrounding substrate resulting in a shorter, radial leaf base dimension. *Isoetes melanopoda* is terrestrial and as such the entire base of the plant is generally surrounded by a tightly packed substrate. This substrate exerts pressure on the young leaves, compresses the leaf bases radially, and results in a very tight, compact plant base. Thus habitat differences could affect the growth patterns of the ligule, effecting changes in the size and shape of the glossopodium. Alternatively, glossopodium variation maybe a reflection of chromosome number: each of the North American species examined has a different ploidy level (2n, 4n, and 8n for *Isoetes melanopoda*, *I. virginica*, and *I. tennesseensis* respectively). It is commonly known that plants with higher ploidy levels often have larger cells and organs than plants of lower ploidy (Smith, 1946; Sinnott, 1960). Thus ploidy level variation alone could explain the differences in glossopodia size and complexity within these three North American plants. It was not possible to establish a correlation between glossopodia morphology of the Indian species to habit, environmental influences, or different ploidy levels due to the limited plant information provided by Sharma and Singh (1984). Additional studies on plants of similar chromosome number and varying habitats or different chromosome numbers in a common habitat are required to establish correlations of this type.

If no correlation can be established between form and either chromosome number or habitat preference, then the observed variations may be a function of phylogenetic history. This would be significant because hypotheses of *Isoetes* relationships are complicated due to the simplicity of the plant body, owing to morphological convergence and reticulate evolution (Taylor and Hickey, 1992). Therefore, any new area of insight is valuable. Characters commonly used to identify

Isoetes are habitat, various vegetative features, megaspore ornamentation, and chromosome numbers. Unfortunately, vegetative characters are usually viewed as either too conservative or too variable. For example, the corms, roots, and velum lengths are very similar throughout *Isoetes*, whereas leaf length, ala length, number of leaves per plant, and sporangium size are thought to be dependent on environmental conditions, plant vigor, and age (Kott and Britton, 1985). Leaf texture and color are deemed arbitrary, and non-quantitative; Kott and Britton (1985) argued that they should not be used. For an alternative viewpoint on the systematic use of vegetative characters however see Hickey (1986a) and Budke *et al.* (in press). Furthermore, megaspore ornamentation is more variable than previously thought (Hickey, 1986b, 1986c). Since many of the characters used to identify *Isoetes* are not conclusive, they should be re-examined and additional morphological characters should be analyzed for taxonomic utility. Some of the character variations noted in this study provide not only phylogenetic data but also a identification aid to different *Isoetes* species in the field. This could be accomplished by performing a simple hand dissection of the sporangial region; total reconstructs are not necessary to analyze characters such as the medimoles and cornua development and orientation.

The glossopodium has been present in lycopsids at least from the Triassic and is presently found only in the extant genera *Isoetes* and *Selaginella*. This relictual organ must be under some type of selective pressure in order to maintain such a complex form for such a long duration of time. Despite a long history of scientific investigations, there is a great deal we do not understand about *Isoetes* and it continues to be a profitable source of scientific inquires.

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