

**GLOBAL REVIEW OF RECENT TAXONOMIC RESEARCH INTO
ISOETES (ISOETACEAE), WITH IMPLICATIONS FOR
BIOGEOGRAPHY AND CONSERVATION¹**

D.F. BRUNTON* & A. TROIA**

*216 Lincoln Heights Road, Ottawa, Ontario Canada K2B A8A,
bruntonconsulting@rogers.com

**Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche,
Sezione di Botanica ed Ecologia Vegetale, Università degli Studi di Palermo, Via
Archirafi 38, I-90123, Palermo Italy, angelo.troia@unipa.it

Key Words: *Isoetes*, lycophytes, taxonomy, global research, biogeography,
conservation

ABSTRACT

With the advent of cytological analysis, Scanning Electron Microscopy, the recognition of sterile hybrids, improved access to wild populations and increasingly comprehensive molecular investigations, there has been a revolution in *Isoetes* taxonomy in recent times. The last 40 years have seen an almost 100% increase in the diversity documented during the previous two centuries. Four geographic areas account for over 90% of this diversity: northern-central South America, eastern North America, western Eurasia (including the northern Mediterranean) and South to Tropical Africa. There is a significant global conservation concern for *Isoetes*, with many taxa being rare (known from one to a handful of populations) and some known only from herbarium specimens. The taxa of the extraordinary rock-outcrop pools and the ephemeral wetlands found in disjunct areas around the world appear to be particularly vulnerable to habitat destruction. We expect 100 or more new taxa to be described in coming years. Such taxonomic productivity will require more systematic cytological and molecular investigations to be undertaken. It also requires the assembly of a larger global inventory of high-quality voucher specimens to provide the material for such investigations.

INTRODUCTION

Speaking of quillworts (*Isoetes*, Isoetaceae), the late geneticist and pteridologist, Donald Britton, said only half-jokingly "... *this is a crazy genus! Just add water and stir*" (D. M. Britton in lit, 1989). In the following, we undertake a review of the dramatic changes that have occurred in our global understanding of the taxonomy of this 'crazy genus' in recent decades as a result of the work of Britton and many others.

Isoetes (Isoetaceae) is an ancient heterosporous lycophyte dating back to the Jurassic Period (Pigg, 2001). With few exceptions, species exhibit a similar simple form and structure (Figure 1). A spirally-arranged, typically small (3 - 30 cm tall), set of

¹ The authors dedicate this paper to the memory of Donald M. Britton (1923-2012) and Enrico Bellini (1940-2002) who in addition to being fine gentlemen and scholars, inspired and developed our interest in *Isoetes*.

unbranched quill-like leaves with usually partially velum-covered sporangia embedded into their basal abaxial faces, is developed from a shallowly buried two or three-lobed corm. At a gross scale there is not much to separate one taxon from another. Just the same, a high degree of taxonomic diversity as well as a high degree of endemism (Jermy, 1990) exists with approximately 200 species, subspecies and varieties currently recognized (Troia et al., 2016).

Spore morphology is the primary character employed for distinguishing individual *Isoetes* taxa. The megaspores demonstrate a remarkable diversity of perispore (surface) ornamentation expressions (Figure 2), ranging from laevigate (virtually featureless) to tuberculate (covered in broad, low, symmetrical projections) to echinate (spiny) or cristate-reticulate (intricate patterns of muri (walls), tubercles and/ or spines) (Hickey, 1986b; Punt et al., 2007). These ornamentation patterns are typically expressed consistently throughout the range of each taxon.

The notable exception to consistent morphological representation is with sterile hybrids. The megaspores of such taxa have been the source of much confusion for over a century (Engelmann, 1882; Motelay, 1892; Eaton, 1900), exhibiting a range of sizes and morphological features intermediate between their putative parents. Deformed (often flattened) individual megaspores with congested ornamentation patterns likened to brain coral and/ or with irregular, even ‘dumb-bell’ shapes (Figure 3) are frequent and are strongly indicative of sterile hybrid status.

Microspores are typically less boldly ornamented, varying from plain to tuberculate to echinate (Figure 4). While consistent in size in relation to the cytology of the particular taxon (Kott & Britton, 1983; Taylor et al., 1993; Musselman, 2002; Macluf et al., 2006), they often vary widely in appearance, even within individual populations. Microspores of hybrids are variable in size. Typically they are amphibious and misshaped and, as with megaspores, exhibit ornamentation patterns intermediate between those of the putative parents.

All *Isoetes* occupy sites which are at least seasonally wet and grow in fresh (rarely brackish) water, typically being amphibious or purely aquatic, most growing in sterile or at least nutrient poor, typically acidic to circumneutral substrates. These habitats vary widely from deep-water oligotrophic lakes (growing as deeply as 4-5 metres), permanently flowing rivers and high elevation bog pools, to open woodland seepages, shallow ephemeral swales and briefly inundated pools in open bedrock outcrops.

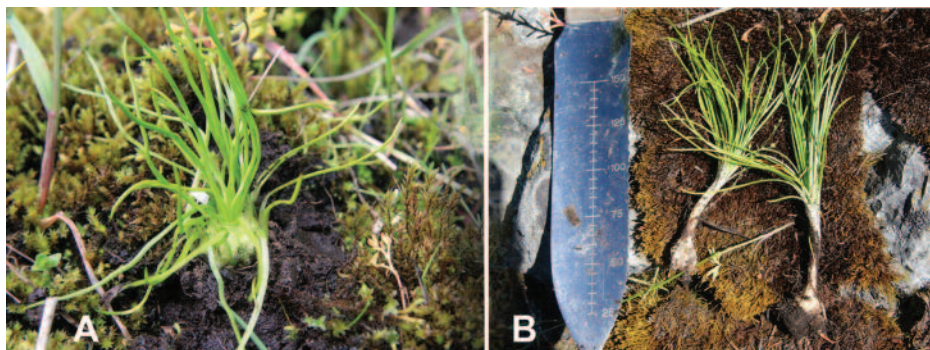


Figure 1: A typical *Isoetes* plant: A) in situ, and B) excavated, also showing corm and roots (*Isoetes minima* A.A.Eaton, Salmo, British Columbia, Canada, 11 June 2017 and 28 June 2008, respectively) (Photos: D. F. Brunton).

Through all of this, be they like the more than half metre long, flexulose aquatic species *Isoetes japonica* A.Braun of eastern Asia, the more typically less than 30 cm long tall, reflected, emergent *I. drummondii* A.Braun of southern Australia, or the stiffly-erect, 5-15 cm tall rock pool ephemeral *I. piedmontana* (N.Pfeiffer) C.Reed of the southeastern United States (USA), virtually all *Isoetes* retain this simple gross-scale

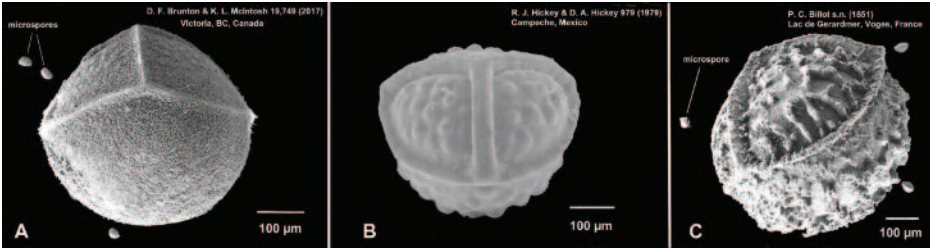


Figure 2: Variations in *Isoetes* megaspore ornamentation: A) diploid *I. nuttallii* A.Braun, B) diploid *I. cubana* Engelm., C) decaploid *I. lacustris* L.(s. str.). (SEM images A & C - P. Sokoloff; B - D. M. Britton).

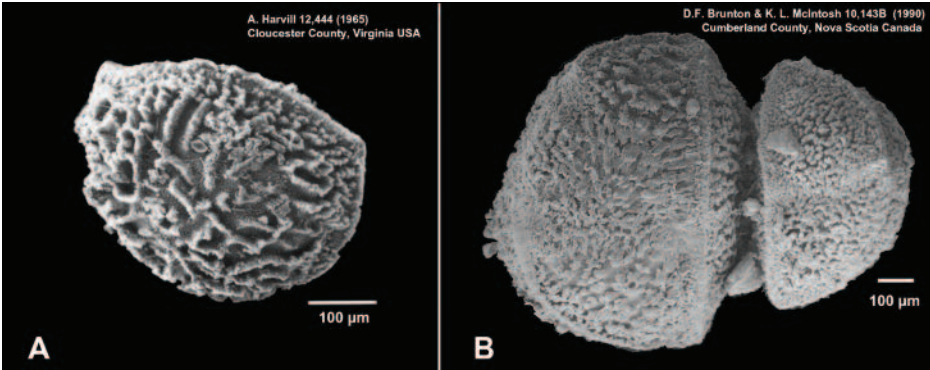


Figure 3: Aborted, sterile *Isoetes* hybrid megaspores: A) triploid *I. engelmannii* A.Braun x *hyemalis* D.F.Brunton, b) heptaploid *I. x harveyi* A.A.Eaton, pro sp. (*I. echinospora* ssp. *muricata* (Durieu) Löve & Löve x *lacustris*) (SEM image A - P. Sokoloff; B - D. M. Britton).

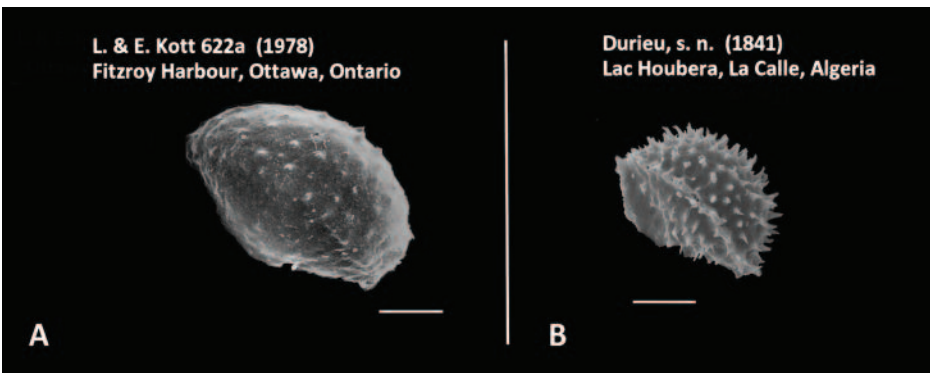


Figure 4: Variation in *Isoetes* microspore ornamentation: A) tetraploid *I. septentrionalis* D.F.Brunton., B) *I. longissima* Bory (s. str.) (SEM image A - P. Sokoloff; B - Carmela Di Liberto, University of Palermo, Italy (with Angelo Troia). (Scale bar = 10 µm)

appearance.

There has been a substantial change in our global understanding of the taxonomic diversity of *Isoetes*, however, since it was last enumerated almost 100 years ago (Pfeiffer, 1922). Change has been particularly dramatic in the latter half of that period (Troia et al., 2016), which is the focus of this review. For our purpose, ‘recent’ commences ca. 1980 when Scanning Electron Microscope (SEM) and cytology were first applied in comprehensive reviews of *Isoetes* in North America (Boom, 1979; Kott, 1980; Kott & Britton, 1983), Europe (Berthet & Lecoq, 1977) and Australia (Marsden, 1979).

METHODS

The following assessments are based upon a comprehensive reading of the *Isoetes* literature as well as the authors’ experience from investigations of the genus since the 1980s. This includes on-site ecological and population investigations in southern Europe (A.T.) and across North America (D.F.B.). In concert with various research associates and supplemented by extensive investigations of herbarium material, SEM imagery and the application of cytological data, these studies have clarified or described a substantial number of taxonomic issues (see *References*, below). We pay special attention to the literature concerning systematics, phylogeny, karyology and biogeography. We also considered the literature in other areas of investigation such as ecology, vegetation science, physiology and conservation biology. For the nomenclatural purposes of the present study we follow Troia et al. (2016).

DISCUSSION

Historical Context

Pfeiffer (1922) conducted the most comprehensive global review of the genus, producing a mostly herbarium-based enumeration of the traditional 19th Century - early 20th Century understanding of *Isoetes* diversity. Her study advanced the more modest reviews of Baker (1880) and Motelay (1883) and relied especially upon the work of Braun (1846), Durieu (1864), Engelmann (1867; 1888) and Eaton (1900). Working independently, she described 77 species and varieties globally. The publication of her remarkably insightful review coincided with an independent description of 15 new South American species (Weber, 1922), resulting in a total of 92 taxa being accepted at a species, subspecies or varietal level by the end of that year. That fewer than 25 species were described worldwide in the subsequent 50 years indicates how the technology and raw data available to researchers in that earlier era had largely been exploited by Pfeiffer’s time.

After a long period of relative nomenclatural inactivity following Pfeiffer (1922), *Isoetes* has experienced a surge in taxonomic innovations. In effect, there has been a late 20th Century - early 21st Century *Isoetes* taxonomic ‘renaissance’. This results in part from geographically expanded investigations of previously poorly explored regions and habitats. More substantially, however, it reflects the application of new investigative tools for the reconsideration of both long-standing interpretations and new data.

Cytology and SEM

Following the pioneering work of Manton (1950) on various pteridophyte genera, the connection between chromosome number and *Isoetes* spore size was investigated in detail in northern North America (Kott, 1980; Taylor et al., 1985; Taylor & Hickey, 1992). These break-through investigations provided confident determination of both the consistency and taxonomic significance of physical distinctions that had previously been

thought to represent only insignificant morphological variations within broadly circumscribed species. Cytological data quickly became a major line of evidence in subsequent global taxonomic investigations.

Cytological investigations revealed that genetically distinct polyploid taxa are common in *Isoetes*, derived from basic diploid species ($2x=2n=22$) through hybridization and subsequent chromosome doubling (Taylor et al., 1985; Taylor & Hickey, 1992; Taylor et al., 1993). Significant progress was made in documenting *Isoetes* cytology in this period, with chromosome counts for over 80 taxa reported in Takamiya (1999) alone. The chromosome numbers for over 46% of all named taxa are presently known (Troia et al., 2016).

The production of detailed, clear illustrations of seemingly subtle variations of various *Isoetes* morphological features through the systematic application of SEM became common in the 1980s and 1990s (Kott & Britton, 1983; Prada, 1983; Ferrarini et al., 1986; Schelpe & Anthony, 1986; Srivastava et al., 1993; Musselman & Knepper, 1994). For the first time this permitted commonly occurring but minute features to be confidently and reliably related to larger-scale characteristics such as velum size, sporangium shape and colouring, sporangium-topping ligule characteristics and site ecology.

In the late 1980s the application of cytological data and SEM imagery in concert with comprehensive reviews of preserved herbarium material and significantly expanded field investigations, lead to the detection and description of sterile hybrids in North America. The existence of natural *Isoetes* hybrids had been identified in Canada 50 years earlier (Jeffrey, 1937) in a publication that was overlooked by researchers for some decades thereafter (Britton & Brunton, 1992). Independently, Matthews and Murdy (1969) reported probable naturally occurring hybrids in the southeastern USA. Other possible hybrids were subsequently reported in the eastern USA (Boom, 1979, Taylor et al., 1985). The first sterile hybrid *Isoetes* to be formally named was *I. ×hickeyi* W.C. Taylor & N. Luebke (Taylor & Luebke, 1988).

The certain identification of sterile hybrids is taxonomically important. Their existence confirms that genetic barriers are in place between previously described and morphologically similar species. They also indicate the existence of previously unsuspected species. Although research has not been undertaken evenly throughout the globe, over a dozen hybrids have now been formally described in North America (Taylor et al., 1985; Taylor & Luebke, 1988; Britton & Brunton, 1989; Musselman & Knepper, 1994; Brunton, 2015), with others named in Japan (Takamiya et al., 1997), Russia (Mochalova et al., 2015) and India (Singh et al., 2018). Undescribed sterile hybrids have been detected in western Europe (A. C. Jermy, pers. comm., Prada & Rolleri, 2003; D.F.B., pers. obs.) and northern South America (J. R. Hickey, pers. comm.) and are suspected in South Africa (Musselman & Roux, 2002), Australia (Tasmania) (pers. obs.) and New Zealand (Hofstra & de Winton, 2016).

A Case Study of Recent Diversity

The combination of cytological data, SEM imagery and hybrid detection has confirmed the existence of distinct taxa previously thought only to represent clusters of populations demonstrating insignificant morphological variation within broadly circumscribed species. The widespread North American *Isoetes engelmannii* s.l. provides an excellent example of how dramatic (and complex) this recent clarification has been. The specific distinction of diploid *I. engelmannii* s.str. (Figure 5A and C) and *I. valida* (Engelm.)

W.Clute (Luebke, 1992, Brunton & Britton, 1996a) was confirmed by the recognition of their sterile diploid hybrid *I. × haltonharvillii* Musselman & R.Bray (Musselman et al., 1995). A widespread tetraploid ($4x=2n=44$) derivative, *I. appalachiana* D.F.Brunton & D.M.Britton (Brunton & Britton, 1999) was subsequently described (Figure 5B and D), apparently the fertile product of doubled *I. × haltonharvillii* (Brunton & Britton, 1997).

The publication of *Isoetes appalachiana* was followed in turn by the determination of hexaploid ($6x=2n=66$) *I. microvela* D.F.Brunton, suspected to be the fertile doubling of triploid ($3x=2n=33$) *I. appalachiana × engelmannii* (Brunton & Britton, 1998). Tetraploids *I. hyemalis*, *I. riparia* Engelm. s.str. and *I. septentrionalis* (Brunton et al., 1994, Caplen & Werth, 2002; Brunton & McNeill, 2015), as well as hexaploids *I. boomii* N.Luebke and *I. georgiana* N.Luebke (Luebke, 1992; Brunton & Britton, 1996b), all represent recently described or clarified taxa within or connected to *I. engelmannii* s.l.

Three additional sterile hybrids were identified in this period from within the *Isoetes engelmannii* s.l. species complex: tetraploids *I. × brittonii* D.F.Brunton & W.C.Taylor (*I. appalachiana × septentrionalis*) (Brunton & Taylor, 1990; Brunton, 2015), *I. × bruntonii* D.Knepper & Musselman (*I. appalachiana × hyemalis*) (Musselman et al., 1996; Brunton, 2015) and hexaploid *I. × fairbrothersii* J.D.Montgomery & W.C.Taylor (*I. engelmannii × lacustris*) (Montgomery & Taylor, 1994). In addition, 19th Century taxa *I. × foveolata* A.A.Eaton, pro. sp. (*I. engelmannii* Dur. $×$ *tuckermanii* A.Braun) and *I. × eatonii* R.Dodge, pro. sp. (*I. echinospora × engelmannii* and its synonym *I. × gravesii* A.A.Eaton, pro sp.) were recognized to represent sterile *I. engelmannii* hybrids (Taylor et al., 1985; Jermy, 1990). Thus since the mid 1980s, over a dozen taxa in eastern North America were recognized to have been derived in whole or in part from *I. engelmannii* s.l.!

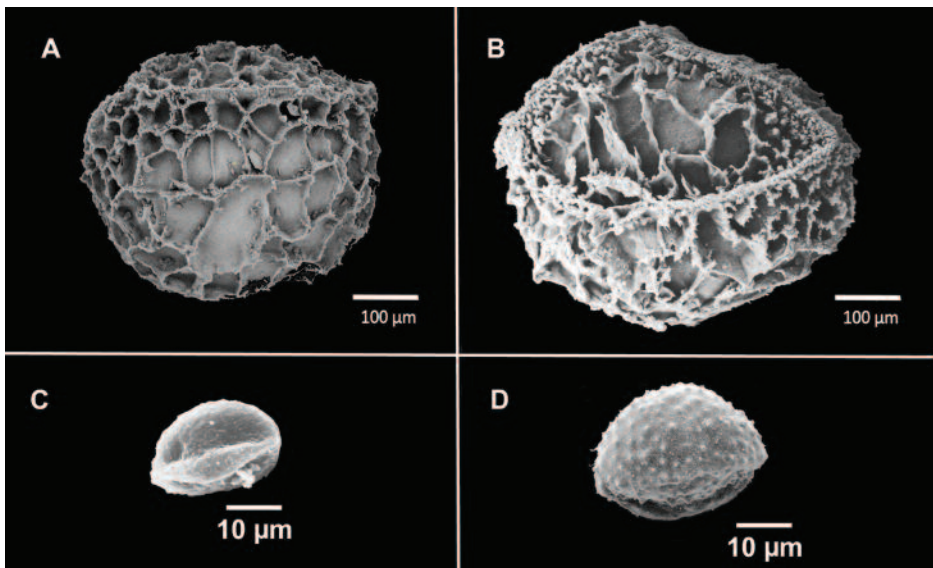


Figure 5: Megaspore (A) and microspore (C) of diploid *Isoetes engelmannii* (St Louis, Missouri, USA, G. Engelmann s. n., 1842 (MO 100901 - Syntype) compared with megaspore (B) and microspore (D) of tetraploid *I. appalachiana* (Huntington County, Pennsylvania, D. F. Brunton & K. L. McIntosh 11,171, 6 July 1992) (SEM images - D. M. Britton).

Recent Diversity Trends

Approximately 80 new taxa have been described in the recent era (Troia et al., 2016). The early to mid 1980s was a particularly productive time with over 20 species or subspecies being described globally, followed by eight new taxa in 1986 and four in each of 1991 and 1994 (Figure 6). A second even larger surge in taxonomic activity occurred early in the 21st Century (almost 25 taxa), with five new taxa recognised in each of 2005 and 2010.

Figure 7 illustrates our contemporary understanding of *Isoetes* diversity, with four global ‘hot spots’ centred on 1. northern-central South America (64 taxa), 2. eastern North America (45 taxa), 3. western Eurasia (including the northern Mediterranean) (39 taxa), and 4. South to Tropical Africa (35 taxa) (adapted from Troia et al., 2016). Together these account for over 180 taxa, or more than 90% of global diversity. That is a radically different picture from the traditional global understanding of *Isoetes* diversity. Based on

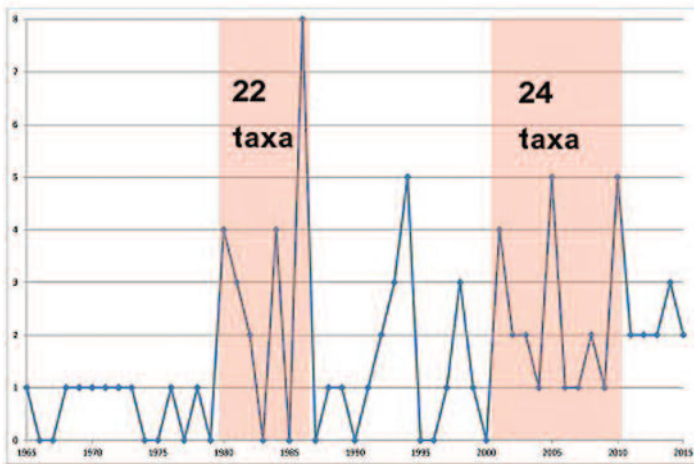


Figure 6: taxonomic activity (number of taxa described) since 1960s (adapted from Troia et al. 2016); particularly active periods shaded.

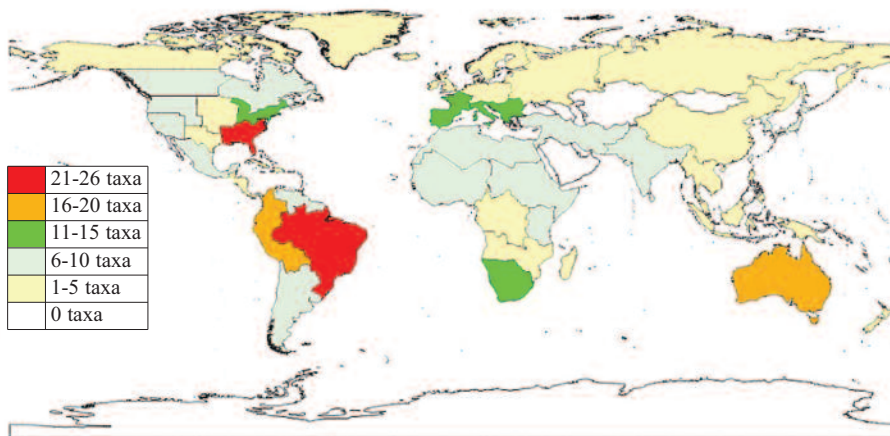


Figure 7: Global *Isoetes* distribution centres of diversity (Troia et al., 2016).

Pfeiffer (1922) and Weber (1922), the traditional five primary centres of diversity would have been identified as western Eurasia (including the northern Mediterranean) (19 taxa - 21% of global diversity), western North America (16 taxa - 17% of global diversity), eastern North America (12 taxa - 13% of global diversity), northern South America (12 taxa - 13% of global diversity), and Australia-New Zealand (9 taxa - 10% of global diversity).

While the prominence of these contemporary ‘hot spots’ is undoubtedly biased towards regions where ‘Isoetologists’ have lived and/ or could most easily access, we believe the overall pattern at least broadly represents global reality. Recent developments within the major *Isoetes* diversity areas identified in Figure 7 are summarized below.

Northern-central South America (31% of global diversity)

The expansion of known documented *Isoetes* diversity in northern South America in recent years is remarkable, with more than a doubling of the number of taxa that were known to exist prior to 1930. Most of these are from Brazil, with a particular focus in the Atlantic Rainforest region where almost 40% of pteridophytes are considered endemic (Prado, Sylvestre & Labiak et al., 2015; Prado, Sylvestre & Pereira, 2015). This total includes at least seven *Isoetes* taxa (Pereira, Mittelbach & Labiak, 2015).

A number of new species represent clarifications of taxa first described (though frequently not legitimately so) in the works of Fuchs-Ekert (1982; 1992). Most new taxa are rare, with several being known from only a single (or handful of) site(s) (Pereira et al., 2016). At least one is known only from 19th Century herbarium specimens (Pereira & Stutzel et al., 2017).

This recent neotropical work was substantially built upon the foundation of taxonomic research established by James Hickey and his associates. Their work focused particularly on species diversity in the northwest of the continent (Hickey, 1984; 1985; 1986a; 1989). The taxonomy of *Isoetes* in the northwestern portion of the continent remains substantially unresolved (Pinto-Zarata et al., 2017), being hampered by the limited availability of herbarium material and a confused nomenclatural history. A number of new species have been described in this region (e.g. five of nine species reported from Peru (Hickey, 1994) have been described since 1979 as well as others in the high elevation *I. karstenii* A. Braun complex (Small & Hickey, 2001)). Undescribed species and hybrids remain to be discriminated and documented, however (R. J. Hickey, pers. comm.).

Eastern North America (22% of global diversity)

The recent expansion of our knowledge of *Isoetes* diversity on the eastern side of the North American continent commencing with the investigation of species diversity in the southeastern USA by Boom (1979; 1982) has been simply explosive. The systematic review of herbarium collections in the 1980s and 1990s and subsequent field investigations supported by cytological analysis of morphologically, ecologically and/ or distributionally distinctive elements detected within long-established species, has led to the recognition of numerous new taxa. While 32 taxa (24 species or subspecies and eight named hybrids) were enumerated in the definitive *Flora of North America* (Taylor et al., 1993), the total now stands at 51 taxa (35 species and subspecies and 16 described hybrids). A number of additional candidate species, subspecies and hybrids are under active investigation. This growth represents an almost 60% increase in total North American *Isoetes* diversity over the last 25 years.

Somewhat akin to the geographic concentration witnessed in the Atlantic Coastal Rainforest region of South America (see *Northern-central South America*, above), the Atlantic Coastal Plain of the southeastern USA is particularly species rich and supports a number of rare and endemic taxa (Reed, 1965; Musselman et al., 2001; Brunton & Britton, 2006; Brunton, 2016).

Considerable attention has been focussed in recent years on species of shallow, ephemeral pools occurring on open, acidic, granitic or sandstone bedrock outcrops (flat-rocks) in the southeastern USA (Matthews & Murdy, 1969; Heafner & Bray, 2005; Brunton, 2016). Comparable species have been described from central and southern Africa (Hall, 1971; Schlepe & Anthony, 1986; Roux et al., 2009), the Mediterranean region (Troia & Raimondo, 2009; Troia & Greuter, 2015b), southern and central Australia (Johnson, 1984; Chinnock, 1993; 1998) and Mexico (Mora-Oivo, 2013) from similar outcrop pools and ephemeral swales. Such pools are otherwise referred to as erosion pits, !gua, gnammas and vleis. These outcrop taxa include the only known distichous-leaved *Isoetes*, the diminutive and remarkably similar *Isoetes tegetiformans* Rury of Georgia, USA (Rury, 1985; Allison, 1993) and *I. australis* Williams of western Australia (Williams, 1943).

Outcrop taxa are frequently rare and are confined to widely disjunct sites. Many species, such as the aforementioned *Isoetes tegetiformans* and *I. australis* or *I. melanospora* Engelm. of the southeastern USA and *I. pusilla* C.R.Marsden & R.J.Chinnock of eastern Australia (Entwhistle, 1994), bear remarkable ecological and morphological similarities (Figure 8) despite being hugely separated geographically.

Western Eurasia (including northern Mediterranean) (19% of global diversity)

Pfeiffer (1922) initially enumerated 13 taxa in this region. Although Berthet & Lecocq (1977) made an important contribution to European *Isoetes* research with their SEM-based review of French taxa, taxonomic reconsiderations in the period began with the work of Carmen Prada (Prada, 1979; 1980; 1983) focussed on the Iberian peninsula. Beyond that, only occasional taxonomic investigations (including karyology) were undertaken (Schneller, 1982; Britton & Brunton, 1996). With the start of the new Millennium, however, dedicated *Isoetes* research was initiated in Italy (Troia & Bellini, 2001; Cesca & Peruzzi, 2001; Peruzzi et al., 2003), including the review and reconsideration of known diversity and the description of new species (Troia & Raimondo, 2009; Troia & Azzella, 2013).

In this period Prada and associates expanded their studies beyond Spain (Prada & Rolleri, 2005; Rolleri & Prada, 2007). Romero and associates focused on critical Spanish taxa (Romero et al., 2004; Romero & Real, 2005) while Musselman and associates (Musselman, 2002; Bolin et al., 2008; 2011) studied *Isoetes* in the eastern part of the Mediterranean basin. Troia and associates concentrated their studies on expanding and clarifying previously documented *Isoetes* taxonomy in the central and eastern Mediterranean region. This included national syntheses for Italy (Troia & Greuter, 2014, 2015a) and Greece (Troia & Greuter, 2015b). In addition, a nomenclatural clarification of the taxonomically important but perplexing *I. longissima-velata* complex of the Mediterranean has been prepared (Troia & Rouhan, 2018).

The treatment of *Isoetes* in *Flora Europaea* (Jermy & Akeroyd, 1993) lists 14 taxa. Jermy also studied aquatic taxa in northwestern Europe, providing the first confirmation of a European sterile hybrid (*I. echinospora* s.str. x *lacustris* - A. C. Jermy, pers. comm.). Documentation was also provided at that time for triploid plants of *I. echinospora* (s.

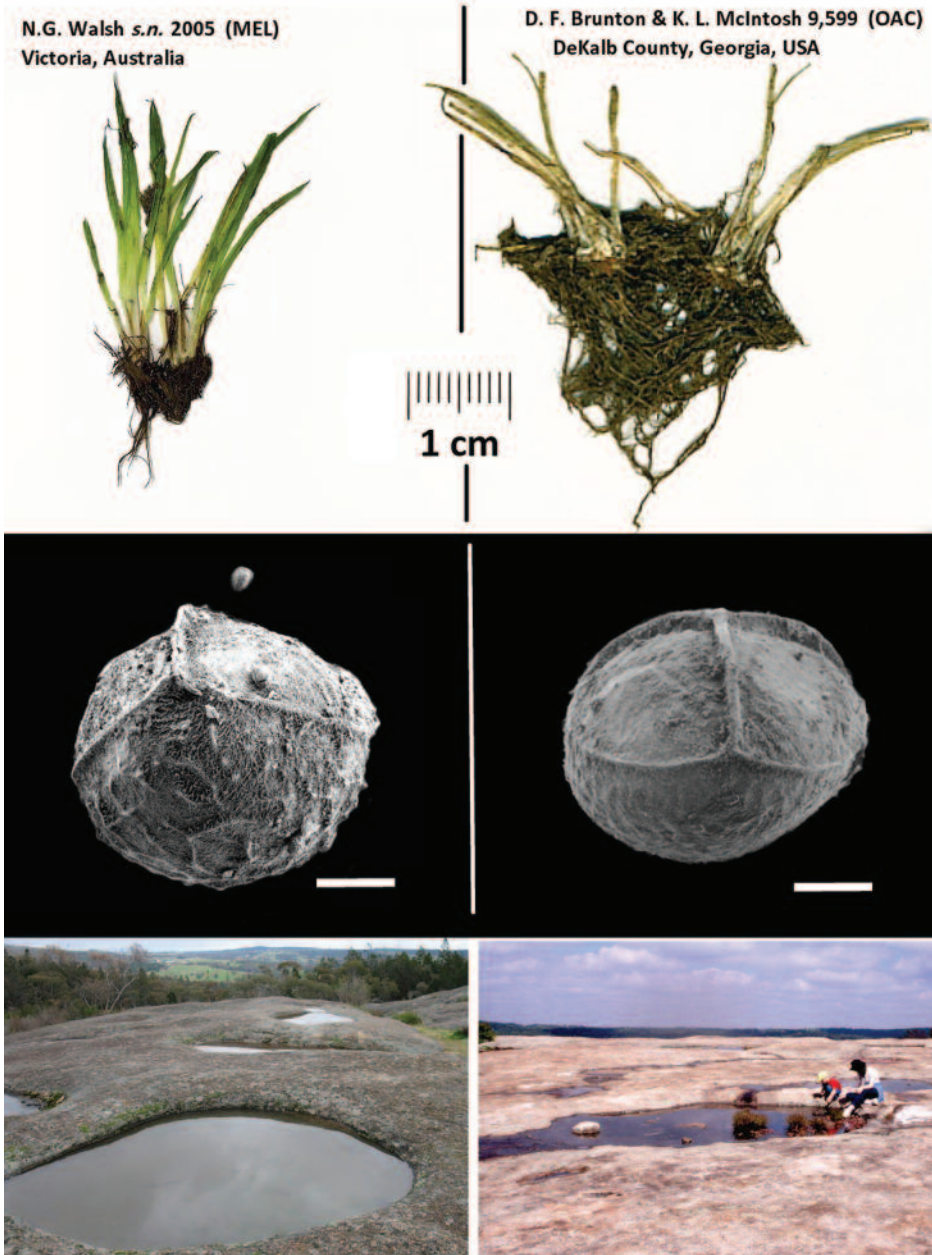


Figure 8: Morphologically similar bedrock outcrop pool species *Isoetes pusilla* of eastern Australia, LEFT (top to bottom): full plant; megaspore and microspore from this specimen (SEM image P. Sokoloff); habitat at Ingram's Rock, Victoria (Photo: N. Walsh), and *I. melanospora* of the southeastern USA, RIGHT (top to bottom): full plant; megaspore from this specimen (SEM image D. M. Britton); habitat at Mount Arabia, DeKalb County, Georgia (Photo: D. F. Brunton). Scale bar = 100 μ m.

str.) in England that were morphologically indistinguishable from the otherwise diploid plants of this species but showed no evidence of hybridization (Rumsey et al., 1993); this unique circumstance for at least a Northern Hemisphere *Isoetes* species remains unresolved (F.J Rumsey, pers. comm.).

Vegetation inventories of subalpine *Isoetes* communities in the Pyrenees Mountains of France, Spain and Andorra have led to the unexpected discovery and description of *I. creussensis* J.J.Lazare & S.Riba (Lazare & Riba, 2010; Aymerich & Saez, 2013), probably the duodecaploid ($12x=2n=132$) detected earlier in this region by Taylor & Hickey (1992). A hexaploid ($6x=2n=66$) or heptaploid ($7x=2n=77$) *I. ×hickeyi*-like sterile hybrid has also been reported (Taylor & Hickey, 1992) here. A reconsideration of the rare and ambiguous (possibly hybrid) taxon *I. brochonii* M.L.Motelay, and its relationship to other Pyrenean taxa, is on-going (D.F.B., pers. obs.).

South to Tropical Africa (17% of global diversity)

Pfeiffer (1922) enumerated only four taxa in this region, probably because she was limited by the availability of voucher material and the restricted nature of botanical explorations in the region up to that time. That number of documented taxa has increased steadily over the last century, with at least 10 taxa now known. The increase to date is entirely from southern Africa (Wanntorp, 1970; Schelpe & Anthony, 1980; Musselman & Roux, 2002). Most new taxa are found in briefly-flooded ephemeral ponds (vleis) or bedrock outcrop solution pits (Roux et al., 2009).

Tropical African species remain less well known, presumably due to continuing limited field exploration opportunities and relatively limited herbarium collections. A variety of amphibious species in ephemeral grassy wetlands and perhaps also in flat rock-like habitats over sandstone bedrock, are known here (Halls, 1971).

Areas of Lesser Diversity

A number of areas of lesser diversity have also received particular attention in recent years. These are described below in descending order of the documented diversity that is apparent.

Australia - New Zealand (16 - 23 species; 8 to 11% of global diversity): *Isoetes* of that region, along with those in a major area of eastern Asia, were enumerated in a comprehensive investigation by Marsden (1979). In that foundational study a number of new taxa from Australia were described, several of which are confined to ephemeral open bedrock pools (Chinnock, 1993). These share remarkably close morphological and ecological affinities with flat rock species in the southeastern USA (see *Eastern North America*, above). Approximately double the traditional number of Australian *Isoetes* known to Pfeiffer (1922) are now recognized (Chinnock, 1998) - a total of 16 species, with at least one Tasmanian species remaining undescribed (Garrett, 1996). Much remains to be resolved here, however, with possible hybrids and cryptic species suggested by abnormal morphological characteristics in several species (referred to by Marsden (1979) as Type I, Type II and Type III megaspores) and multiple ploidy levels reported from other taxa such as apparently apomictic *I. muelleri* A.Braun s.l. (Chinnock, 1998).

The taxonomy of New Zealand taxa was unresolved in Marsden's (1979) study and remains so today. There appear to be up to five aquatic taxa represented here, probably including hybrids (Hofstra & de Winton, 2016).

Eastern Asia (Japan/ China/ Korea) (12 taxa; 6% of global diversity): Research over the last three decades has doubled the known diversity of *Isoetes* taxa in this region.

All the newly described taxa are aquatics and most are very rare (Liu et al., 2005), being known from one to a handful of sites. Two (including *I. ×michinokuana* M.Takamiya, M.Watanabe & K.Ono, the first Asian hybrid to be described) result from the especially comprehensive morphological and cytological investigations of Japanese *Isoetes* by M. Takamiya and associates (Takamiya et al., 1997; Takamiya, 2001). Another two new species are found on a single island off the coast of Korea (Choi et al., 2008).

Central America - Caribbean (10 taxa; 5% of global diversity): Known diversity in this floristically transitional area between North and South America is moderate in extent but demonstrates wide ecological variation and significant endemism (e.g. four species confined exclusively to Mexico). Known diversity has increased in recent years with the description of three new endemic species, including *I. jamaicensis* J.R.Hickey from its namesake island (Hickey, 1981), the lowland *I. pallida* J.R.Hickey of the South American *I. triangula* Weber s.l. complex found in southern Mexico (Hickey, 1988), and a bedrock outcrop pool taxon *I. tamaulipana* A.Mora-Olivio, A.Menoza-Ruiz & J.G.Martinez-Ávalos, known from a single site in north-eastern Mexico (Mora-Olivio et al., 2013).

India (6-23 taxa; 3 to 11% of global diversity): Ironically, while the endemic *Isoetes coromandalina* L.f. s.str. was one of the first *Isoetes* to be described (Linnaeus 1781), it was the only known member of the genus from India known to Pfeiffer (1922). The 18 species now reported from the subcontinent (Shukla et al., 2002, Pantil & Rajput, 2017) were almost all described after 1960 (Troia et al., 2016).

The disposition of a number of recently described taxa and the various subspecies and varieties within several species complexes (Pant & Shrivastava, 1962; Gena & Bhardwaja, 1984; Shrivastava et al., 1993; Srivastava, 1998; Shukla et al., 2002) is controversial and remains unresolved. This is due to uncertainties from the reports of multiple and irregular chromosome counts and possibly apomictic reproduction amongst widely scattered, rarely occurring taxa with distinctions dependent on variable morphological characteristics. At least six of the 23 described taxa (Pantil & Rajput, 2017), however, seem solidly established at a species level (Fraser-Jenkins et al., 2016; Troia et al., 2016). The first Indian sterile hybrid, *I. xgopalkrishnae* S.K.Singh, P.K.Shukla & N.K.Dubey, has also recently been described (Singh et al. 2018).

North Pacific Beringia (ca. 7 taxa; 3% of global diversity) - The *Isoetes* taxa of the area between Far East Asia (Siberia, Russia) and northwest North America (Alaska, USA and Yukon, Canada) have received attention in recent years due to their location within the phytogeographically significant Beringian region (Brunton et al., 2015), and one sterile hybrid, *I. ×gopalkrishnae* S.K.Singh, P.K.Shukla & N.K.Dubey (Singh et al., 2018) has recently been described. Britton and Brunton (1999), Mochalova (2006) and Mochalova et al. (2015) document at least seven taxa (three hybrids) intermixing across this North Pacific transition zone. One of these, *Isoetes ×paratunica* D.F.Brunton, O.Mochalova & A.A.Bobrov (*I. asiatica* (Makino) Makino x *I. maritima* Underw.) (Mochalova et al., 2015), represents the first hybrid reported between putative parents, that occur predominantly on separate continents (Asia and North America, respectively). Research continues on the intercontinental relationships of these taxa and their phytogeographic implications (A. Bobrov, pers. comm.). Kim et al. (2009) and Kim and Choi (2016) have conducted DNA analyses that further refine the origins and taxonomic distinctions of this Beringian complex.

Molecular Investigations

Molecular investigations of *Isoetes* commenced with the Hoot & Taylor (2001) assessment of the value of these data for species determination and the clarification of *Isoetes* phylogeny. Although employed in support of some morphologically and cytologically determined specific taxonomic decisions (e.g. Bolin et al., 2011) and to examine the genetic diversity of particular taxa (Chen et al., 2005), molecular research to date has largely been applied at a higher level. Molecular data have also been employed in many areas of the world over the last 15 years to investigate genetic diversity within and between populations of rare and endangered *Isoetes*. This research has particular importance for the development of conservation management strategies aimed at establishing sustainable populations with such species as *I. hypsophila* H.vonHandel-Mazzetti (Chen et al., 2005; Li et al., 2012), *I. sinensis* T.C.Parker (Kang et al., 2005; Li et al., 2012) and *I. yunguiensis* Q.F.Wang & W.C.Taylor (Chen et al., 2007; Dong et al., 2018) in China, *I. coreana* Y.H.Chung & H.K.Choi (Kim et al., 2008) in Korea and *I. malinverniana* V.deCesati & G.DeNotaris (Gentili et al., 2010; Abeli et al., 2017) in Italy. It has been similarly applied for clarifying diversity in undetermined rare taxa in New Zealand (Hofstra & de Winton, 2016) and with both *I. butleri* G.Engelmann in the United States (Vander Stelt et al., 2017) and *I. durieui* J.Bory in Italy (Troia, 2003). Carrying on from fossil and morphological interpretation-based studies such as Retallack (1997) and Taylor and Hickey (1992), molecular research has focussed on broader questions of phylogeny and the evidence for extinct lineages (Rydin & Wikstrom, 2002; Hoot et al., 2004; Taylor et al., 2004; Larsen & Rydin, 2016; Pereira et al., 2017). This permitted Hoot et al. (2006), for example, to identify six major well-supported *Isoetes* clades globally. In recent years Xiang Lui and associates have applied a significant focus of *Isoetes* molecular research in China on regional origins and prehistoric dispersal patterns (X. Liu, pers. comm.).

Bolin et al. (2018) employed DNA flow cytometry to consider the utility of C Values for the identification of particular species and Freund et al. (2018) combined molecular phylogeny data with morphological documentation to clarify the evolution of particular character states (e.g. the number of corm lobes).

Molecular investigations, both at a high level and in regards to particular taxa represent a new and powerful tool that will be fundamental to future taxonomic studies of *Isoetes*, as they now are to virtually all groups of vascular plants.

Other Areas of Investigation

While the limited scope of our review directs focus onto recent taxonomic accomplishments, it is important to note that significant strides in our understanding of other aspects of *Isoetes* were made during this period. The following briefly notes some of the major achievements of other areas of biological investigation.

Vegetation classification involving *Isoetes* communities was conducted extensively in Eurasia, with considerable work being undertaken recently in the Mediterranean region. A preliminary synthesis of the *Isoetes* - related vegetation classification in this region was made by Quézel (1998). Studies on the ecology and vegetation science of *Isoetes* communities are now available for most (though not all) species around the Mediterranean: northwest Africa (Boutin et al., 1982), Azores (Pietsch, 1994), Spain (Romero & Amigo, 1995; Molina, 2005; Molina et al., 2011), France (Médail, 2003; M. Rhazi et al., 2004; L. Rhazi et al., 2009), Italy (Abeli et al., 2014), Sardinia (Bagella & Caria, 2013) and Turkey (Kürschner & Parolly, 1999). These studies are clearly

conservation-oriented in some cases (e.g. Médail, 2003; M. Rhazi et al., 2004; L. Rhazi et al., 2009; Gentili et al., 2010).

Troia (2016) investigated the dispersal and possible migration routes of some of the current Mediterranean taxa, especially those with African affinities and particularly considering faunal and climatic vectors. Amongst climatic issues, severe wind storms were speculated to be a distributional vehicle for bedrock outcrop *Isoetes* taxa in the southern USA (Brunton, 2001) as they are for other types of terrestrial and wetland vascular plants (Matthews et al., 1991; Brunton et al., 2018).

Presumably discouraged by long-standing taxonomic uncertainty in regards to many species, other contemporary investigations of *Isoetes* biogeography have been relatively few. Studies in East Asia employing geological, cytological and molecular data, however, have examined the origins and original dispersal patterns of the genus in that region (Liu et al., 2004; Taylor et al., 2004). Other investigations on a more local scale considered possible recent Trans-Pacific dispersal between North America and Asia (Brunton et al., 2015).

Ecological and physiological investigations of *Isoetes* have been relatively few, despite being initiated early in the recent era in the pioneering studies of Matthews & Murdy (1969) regarding rock outcrop communities in the southern USA. Indeed, the benefit of considering ecological characteristics as an aid to taxonomic clarification was demonstrated earlier with recognition of the importance of substrate chemistry for distinguishing the morphologically similar ephemerals *I. melanopoda* Gay & Durieu s.str. and *I. butleri* Engelm. in the southern USA (Taylor et al., 1975). Substrate considerations have subsequently been applied to investigations of many aquatic and amphibious species in North America and elsewhere.

Karrfalt and Hunter (1980) examined how the uniquely mucilage-covered sporophylls of South American *Isoetes andicola* (Amstutz) L.D.Gomez (*Stylites andicola* Amstutz) were literally squeezed out of the alpine vegetation in which they developed by the expansion of new plant growth, thus aiding the dispersal of sporangia. Other physiological and morphometric investigations in this period relate to leaf structure (ligule) functions (Kristen et al., 1982; Sharma & Singh, 1984; Singh, 1984), the early development of sporophytes (Hilger et al., 2002) leaf (intrastelar canal) anatomy (Romeo et al., 2000), and the early development of sporophytes (Hilger et al., 2002).

The only extensive *in situ* ecological investigations of purely aquatic *Isoetes* to date were undertaken in central and northern Europe. Margrit Voge conducted underwater studies of numerous deep-water populations of *I. lacustris* at this time (Voge, 1997; 2003). She focussed especially on the importance of water quality parameters for controlling the health and size of particular populations. The importance of water quality to population sustainability was also investigated in China by Wen et al. (2003). More recently, Chappuis et al. (2015) examined the relative importance of light and organic elements in the stability (or instability) of southern European *I. lacustris* populations. Sharma (1998) and Sudova et al. (2011) examined the apparently symbiotic role of mycorrhizal fungi connected with the roots of *Isoetes* taxa in India and central Europe, respectively.

The most dramatic physiological *Isoetes* investigations in recent decades resulted from the remarkable work of Jon Keeley and associates who determined that the CAM photosynthetic process typically employed by desert plants was also widely employed by *Isoetes* (Keeley, 2014). Their field and laboratory studies determined that CO₂ uptake of *Isoetes* plants was at least substantially through their roots, enabling these plants to

prosper in sterile, otherwise prohibitively challenging habitats (Keeley & Busch, 1984; Keeley & Sandquist, 1991). Those investigations focussed mostly on ephemeral pool taxa in semi-arid habitats in southern California, USA but also included subalpine American taxa and South American tropical alpine species (Keeley et al., 1994).

Conservation Biology and Management

Conservation concerns are increasingly significant motivators of *Isoetes* investigations, with many global conservation initiatives focussing on rare species of precarious status that are in danger of extinction. Taxa known from only a single population are known around the world, including *I. pallida* in southern Mexico (Hickey, 1988), *I. viridimontana* M.A.Rosenthal & W.C.Taylor in the northeastern USA (Rosenthal et al., 2014), *I. sabatina* A.Troia & M.M.Azzella (Troia & Azzella, 2013) in central Italy, *I. hallasanensis* H-K.Choi, Ch.Kim & J.Jung in Korea (Choi et al., 2008), *I. libanotica* Musselman, J.F.Bolin & R.D.Bray in Lebanon (Bolin et al., 2011) and *I. junciformis* D.F.Brunton & D.M.Britton in the southeastern USA (Brunton & Britton, 1999). Liu et al. (2005) note that all *Isoetes* taxa in China are in danger of extinction and several eastern Mediterranean taxa are similarly at risk (Bolin et al., 2011; Troia & Greuter, 2015a). Amphibious bedrock outcrop and ephemeral pool taxa may be the most threatened class of *Isoetes* globally, these being considered to be of even greater conservation value by sharing habitat with other specialized (often endemic) flora and fauna (Allison, 1993; Bauder & McMillian, 1998; Bagella & Caria, 2013; Ernandes & Marchiori, 2013; Bagella et al., 2016; Brunton, 2016).

FUTURE POSSIBILITIES

Based on the rate of nomenclatural innovation in recent years (Figure 6) and the incentive provided by new research techniques supplementing traditional databases, we speculate that 100 or more additional *Isoetes* taxa will be identified in future years. The richest, perhaps globally most important areas for investigation would appear to be the bedrock outcrop and ephemeral pool habitats of the USA, Australia and South Africa and the less well investigated areas of Tropical Africa and interior South America. In addition to potentially providing taxonomic insights, the remarkably strong ecological and morphological similarities of geographically widely dispersed bedrock outcrop taxa (Figure 8) suggest an exceptional opportunity for the study of convergent evolution.

Despite no *Isoetes* being known from the area, a strong case can be made for including the Caucasus region of central Asia amongst those having a high potential for future taxonomic contributions. The extraordinarily rich biodiversity of this region is known to support the highest level of Temperate Zone endemism in the Northern Hemisphere (Batsatsachvii et al., 2013).

Molecular clarification of the subtle and even cryptic relationships within *Isoetes* species complexes could have major conservation management implications in many parts of the world. Again, this is especially true in regards to bedrock outcrop and ephemeral wetland taxa.

Particular regions such as India, central and northern South America, South to Tropical Africa, central Asia and the eastern Mediterranean appear to be particularly in need of the initiation or continuation of systematic cytological and molecular taxonomic investigations and clarification. That applies as well to a number of unusually challenging species complexes including *Isoetes muelleri* s.l. in Australia, *I. coromandelina* s.l. in India, *I. longissima* s.l. in the Mediterranean region of Europe, *I. karstenii* s.l. in South

America, and both *I. riparia* s.l. and *I. melanopoda* s.l. in North America.

Although considerable improvements have been seen in recent decades in the growth of *Isoetes* herbarium collections globally, more systematic gathering of high quality material is needed to facilitate future physiological and molecular investigations, particularly with morphologically challenging species groups. This is especially important in geographic regions (identified above) which traditionally have been deficient in preserved plant material. Similarly, cytological determinations remain outstanding for over half of the described taxa (Troia et al., 2017). These determinations are fundamental to understanding polyploid taxa and interspecific hybrid relationships. And of course, the application of rapidly evolving and increasingly sophisticated molecular investigative tools is required in a systematic basis, not just in regards to individual taxa or species complexes.

Despite the laudable achievements of pioneering 19th and 20th Century investigators and the remarkable advances of recent decades, the potential for the identification of new taxa remains high. Indeed, one could accurately state that such potential exists *because* of those pioneering efforts. Regardless, it is clear that previously undetected *Isoetes* taxa can be expected just about anywhere in the world where these curious - and ‘crazy’ - plants are to be found.

ACKNOWLEDGMENTS

Our thanks to W. Carl Taylor, Botany, Smithsonian Institution, Washington, DC, USA for providing background information and encouragement for this project. We appreciate permission for use of the SEM images produced by Paul Sokoloff, Botany, Canadian Museum of Nature, Ottawa, Ontario, Canada (with Daniel Brunton), the late Donald M. Britton, University of Guelph, Guelph, Ontario, Canada and Carmela Di Liberto, University of Palermo, Italy (with Angelo Troia). We are grateful as well to Alexander Bobrov, I. D. Papanin Institute for Biology of Inland Waters, Borok, Russia, R. James Hickey, University of Miami, Ohio, USA and Xiang Lui, Laboratory of Plant Systematics and Evolutionary Biology, Wuhan University, Wuhan, China for their contributions of regional information. Neville Walsh, Royal Botanic Gardens, Melbourne, Australia also provided regional information and photographic material and offered helpful comments on the treatment of Australian taxa. We are particularly appreciative of the invaluable observations that James R. Hickey, Miami University, Oxford Ohio USA. Jim possibly has the longest continuous record of and perspective on *Isoetes* research in the world. And finally our sincere thanks to Editor Bridget Laue, without whose patience and persistence this paper would never have seen the light of day.

REFERENCES

- ABELI, T., ORSENIGO, S., ARDENGHI, N.M.G., LUCASSEN, E.C.H.E.T. & SMOLDERS, A.J.P. 2014. Hydrochemical characterization of a stand of the threatened endemic *Isoetes malinverniana*. *Amer. Fern J.* 103: 241-244.
- ALLISON, J.R. 1993. *Recovery plan for three granite outcrop plant species: black-spored quillwort (Isoetes melanospora), mat-forming quillwort (Isoetes tegetiformans), and little amphianthus (Amphianthus pusillus)*. United States Fish and Wildlife Service, Jackson, Mississippi.
- AYMERICH, P. & SAEZ, L. 2013. Noves dades pteridològiques dels Pirineus catalans. *Orsis* 27: 175-194.
- BAGELLA, S. & CARIA, M.C. 2013. Sensitivity of ephemeral wetland swards with *Isoetes histrix* Bory to environmental variables: implications for the conservation of

- Mediterranean temporary ponds. *Aquatic Conservation* 23: 277-290.
- BAGELLA, S., GASCON, S., FILIGHEDDU, R., COGONI, A. & BOIX, D. 2016. Mediterranean temporary pools: new challenges from a neglected habitat. *Hydrobiologia* 782: 1-10.
- BAKER, J.G. 1880. A Synopsis of the species of *Isoetes*. *J. Bot. Br. Foreign* 9 65-70, 105-110.
- BATSATSACHVIL, K., SCHATZ, G.E. & SCHULKINA, T. (Eds) 2013. *Caucasus Plant Initiative: A Regional Plant Conservation Strategy*. https://www.bgci.org/files/Worldwide/News/2013/caucasus_strategy.pdf
- BAUDER, E.T. & MCMILLAN, S. 1998. Current distribution and historical extent of vernal pools in southern California and northern Baja California, Mexico. In: WITHAM, C.W., BAUDER, E.T., BELK, D., FERREN, W.R. & ORNOFF, R. (Eds) *Ecology, Conservation and Management of Vernal Pools*, pp. 56-70. Proceedings from a 1996 Conference. California Native Plant Society, Sacramento.
- BERTHET, P. & LECOCQ, M. 1977. Morphologie sporale des espèces françaises du genre *Isoetes* L. *Pollen & Spores* 19: 329-359.
- BOLIN, J.F., BRAY, R.D., KESKIN, K. & MUSSELMAN, L.J. 2008. The genus *Isoetes* L. (Isoetaceae, Lycopphyta) in South-Western Asia. *Turk. J. Bot.* 32: 447-457.
- BOLIN, J.F., BRAY, R.D. & MUSSELMAN, L.J. 2011. A new species of diploid quillwort (*Isoetes*, Isoetaceae, Lycopphyta) from Lebanon. *Novon* 21: 295-298.
- BOLIN, J.F., HARTWIG, C.L., SCHAFFRAN, P. & KOMARNYTSKY, S. 2018. Application of DNA flow cytometry to aid species delimitation in *Isoetes*. *Castanea* 83: 38- 47.
- BOOM, B.M. 1979. *Systematic Studies of the Genus Isoetes in the Southeastern United States*. Master's Thesis, University of Tennessee, Knoxville.
- BOOM, B.M. 1982. Synopsis of *Isoetes* in the southeastern United States. *Castanea* 47: 38-59.
- BOUTIN, C., LESNE, L. & THIÉRY, A. 1982. Ecologie et typologie de quelques mares temporaires à isoètes d'une région aride du Maroc occidental. *Ecol. Medit.* 8: 31-56.
- BRAUN, A. 1846. Über die nordamericanischen *Isoetes* Arten. *Flora oder Bot. Zeit.* (Regensburg) 12: 177-180.
- BRITTON, D.M. & BRUNTON, D.F. 1989. A new *Isoetes* hybrid (*Isoetes echinospora* x *riparia*) for Canada. *Canad. J. Bot.* 67: 2995-3002.
- BRITTON, D.M. & BRUNTON, D.F. 1992. *Isoetes* x *jeffreyi*, *hyb. nov.*: a new *Isoetes* (*I. macrospora* x *riparia*) from Quebec, Canada. *Canad. J. Bot.* 70: 447-452.
- BRITTON, D.M. & BRUNTON, D.F. 1996. *Isoetes* x *pseudotruncata*, a new triploid hybrid from western Canada and Alaska. *Canad. J. Bot.* 74: 51-59.
- BRITTON, D.M. & BRUNTON, D.F. 1999. *Isoetes* in Alaska and the Aleutians. *Amer. Fern J.* 79: 133-141.
- BRUNTON, D.F. 2001. Quillwort dispersal: which way is the wind blowing? *Chinquapin* 9: 20; 24-26.
- BRUNTON, D.F. 2015. Key to the quillworts (*Isoetes*: Isoetaceae) of the southeastern United States. *Amer. Fern J.* 105: 86-100.
- BRUNTON, D.F. 2016. Flat Rock Quillwort, *Isoetes graniticola* sp. nov. (Isoetaceae), a new lycophyte from the southeastern United States. *Rhodora* 118: 261-275.
- BRUNTON, D.F. & BRITTON, D.M. 1996a. The status, distribution and identification of Georgia Quillwort (*Isoetes georgiana*; Isoetaceae). *Amer. Fern J.* 86: 105-113.

- BRUNTON, D.F. & BRITTON, D.M. 1996b. Taxonomy and distribution of *Isoetes valida* (Isoetaceae). Amer. Fern J. 86: 16-25.
- BRUNTON, D.F. & BRITTON, D.M. 1997. Appalachian Quillwort, (*Isoetes appalachiana*, sp. nov.; Isoetaceae), a new pteridophyte from the eastern United States. Rhodora 99: 118-133.
- BRUNTON, D.F. & BRITTON, D.M. 1998. *Isoetes microvela* (Isoetaceae), a new quillwort from the southeastern coastal plain of the United States. Rhodora 100: 261-275.
- BRUNTON, D.F. & BRITTON, D.M. 1999. Rush Quillwort (*Isoetes junciformis*, sp. nov.), a new pteridophyte from southern Georgia. Amer. Fern J. 79: 187-197.
- BRUNTON, D.F. & BRITTON, D.M. 2006. *Isoetes melanopoda* spp. *silvatica* (subsp. nov.), a new quillwort (Isoetaceae) from eastern North America. *Castanea* 71: 15-30.
- BRUNTON, D.F., BRITTON, D.M. & TAYLOR, W.C. 1994. *Isoetes hyemalis*, sp. nov. (Isoetaceae); a new quillwort from the southeastern United States. *Castanea* 59 (1): 12-21.
- BRUNTON, D.F. & MCNEILL, J. 2015. Status, distribution and nomenclature of Northern Quillwort, *Isoetes septentrionalis* (Isoetaceae), in Canada. *Canad. Field-Nat.* 129: 174-180.
- BRUNTON, D.F., MOCHALOVA, O.A. & BOBROV, A.A. 2015. *Isoëtes* (Isoëtaceae) in the northern Pacific: diversity, taxonomy and distribution. In: *Problems of taxonomy and geography of aquatic plants*, pp. 90-91. Proceedings of the International Conference (Borok, Russia, 21-24 October, 2015), Yaroslavl, Filigran.
- BRUNTON, D.F. & TAYLOR, W.C. 1990. *Isoëtes* × *brittonii* hyb. nov. (Isoëtaceae): A naturally occurring hybrid (*I. engelmannii* ' *I. riparia*) in the eastern United States. Amer. Fern J. 80: 82-89.
- CAPLEN, C.A. & WERTH, C.R. 2000. Isozymes of the *Isoetes riparia* complex, II. Ancestry and relationships of polyploids. *Syst. Bot.* 25: 260-280.
- CESCA, G. & PERUZZI, L. 2001. *Isoetes* (Lycophytina, Isoetaceae) with terrestrial habitat in Calabria (Italy). New karyological and taxonomical data. *Flora Medit.* 11: 303-309
- CHAPPUIS, E., LUMBRERAS, L., BALLESTEROS, E. & GACIA, E. 2015. Deleterious interaction of light impairment and organic matter enrichment on *Isoetes lacustris* (Lycopodiophyta, Isoetales). *Hydrobiologia* 760: 145-158.
- CHEN, J.-M., LUI, X., WANG, J.-Y., GITURU, W.R. & WANG, Q.F. 2005. Genetic variation within the endangered quillwort *Isoetes hypsophila* (Isoetaceae) in China as evidenced by ISSR analysis. *Aquatic Bot.* 82: 89-98.
- CHINNOCK, R.J. 1993. Notes on *Isoetes* and *Tmesipteris* in Victoria. *Muelleria* 8: 57-60.
- CHINNOCK, R.J. 1998. *Isoetaceae*. In: *Flora of Australia, Volume 4*. Australian Biological Resources Study, Department of the Environment and Energy, Canberra.
- CHOI, H, JUNG, J. & KIM, C. 2008. Two new species of *Isoetes* (Isoetaceae) from Jeju Island, South Korea. *J. Pl. Biol.* 51: 354-358.
- DURIEU DE MAISONNEUVE. 1864. Notes sur quelques espèces nouvelles d'Isoetes de l'Amérique du Nord et de l'Australie. *Bull. Soc. Bot. France* 11(4): 100-105.
- EATON, A.A. 1900. The genus *Isoetes* in New England, In: *Fernwort Papers*, pp. 1-16. W. N. Clute & Co., Binghamton.
- ENGELMANN, G. 1867. *Isoetaceae*. In: GRAY, A. *Manual of the Botany of the*

- Northern United States, 5th Edition*, pp. 698-700. Iverson, Blakeman & Company, New York.
- ENGELMANN, G. 1882. The Genus *Isoetes* in North America. *Trans. Acad. Sci. St. Louis* 4: 358-390.
- ENTWISLE, T.J. 1994. Ferns and allied plants (Psilophyta, Lycopodiophyta, Polypodiophyta). In: WALSH, N.G. & ENTWISLE, T.J. (Eds), *Flora of Victoria Vol. 2, Ferns and Allied Plants, Conifers and Monocotyledons*. Inkata Press, Melbourne.
- ERNANDES, P. & MARCHIORI, S. 2013. Mediterranean temporary ponds in Puglia (South Italy): a “joyau floristique” to protect. *Acta Bot. Gallica* 160: 53-64.
- FERRARINI, E., CIAMPOLINI, F., PICHI SERMOLLI, R.E.G. & MARCHETTI, D. 1986. *Iconographia Palynologica Pteridophytorum Italiae*. *Webbia* 40: 1-202.
- FRASER-JENKINS, C.R., GANDHI, K.N., KHOLIA, B.S. & BENNIAMIN, A. 2016. Isoetaceae, In: *An annotated checklist of Indian pteridophytes Part I (Lycopodiaceae to Thelypteridaceae)*, pp. 54–60. Bishen Singh Mahendra Pal Singh, Dehradun, India.
- FREUND, F.D., FREYMAN, W. & ROTHFELS, C. 2018. Inferring the evolutionary reduction of corm lobation in *Isoetes* using Bayesian model-averaged ancestral state reconstruction. *Amer. J. Bot.* 105: 275-286.
- FUCHS-ECKERT, H.P. 1982. Zur heutigen Kenntnis von Vorkommen und Verbreitung der sudamerikanischen *Isoetes*-Arten. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Series C* 85: 2015-260.
- FUCHS-ECKERT, H.P. 1992. Supplementum ad indicem Isoetalium. *Bionature* 12: 99-159.
- GARRETT, M. 1996. *The Ferns of Tasmania, their ecology and distribution*. Tasmanian Forest Research Council, Hobart.
- GENA, C.B. & BHARDWAJA, T.N. 1984. Three new species of genus *Isoetes* from Rajasthan. *J. Bombay Nat. Hist. Soc.* 81: 165-168.
- GENTILI, R., ABELI, T., ROSSI, G., LI, M., VAROTTO, C. & SGORBATI, S. 2010. Population structure and genetic diversity of the threatened quillwort *Isoetes malinverniana* and implication for conservation. *Aquatic Bot.* 93: 147-152.
- HALLS, J.B. 1971. Observations on *Isoetes* in Ghana. *Bot. J. Linn. Soc.* 64: 117-139.
- HEAFNER, K.D. & BRAY, R.D. 2005. Taxonomic reassessment of North American granite outcrop *Isoetes* species with emphasis on vegetative morphology and *I. piedmontana* (Pfeiffer) Reed sensu lato. *Castanea* 70: 204-221.
- HICKEY, R.J. 1981. A new *Isoetes* from Jamaica. *Amer. Fern J.* 71: 69 - 74.
- HICKEY, R.J. 1984. Chromosome numbers of Neotropical *Isoetes*. *Amer. Fern J.* 74: 9-13.
- HICKEY, R.J. 1985. *Revisionary Studies of Neotropical Isoetes*. University of Connecticut, Storrs University of Adelaide [Phd Thesis].
- HICKEY, R.J. 1986a. The early evolutionary and morphological diversity of *Isoetes*, with descriptions of two new Neotropical species. *Syst. Bot.* 11: 304-321.
- HICKEY, R.J. 1986b. *Isoetes* megaspore surface morphology: nomenclature, variation and systematic importance. *Amer. Fern J.* 76: 1-16.
- HICKEY, R.J. 1988. *Isoetes pallida*, a new species from Mexico. *Amer. Fern J.* 78: 35-36.
- HICKEY, R.J. 1989. A new species of *Isoetes* from Territorio Federal Amazonas, Venezuela. *Ann. Missouri Bot. Gard.* 76: 1160-1162.
- HICKEY, R.J. 1994. Pteridophyta of Peru, Part VI - Family 28. Isoetaceae. *Fieldiana* 34: 88-97.

- HILGER, H.H., WEIGEND, W. & FREY, W. 2002. The gametophyte-sporophyte junction in *Isoetes boliviensis* Weber (Isoetaceae, Lycopodiophyta). *Phyton* 42: 149-157.
- HOFSTRA, D. & DE WINTON, M. 2016. Geographic scales of genetic variation amongst *Isoetes* in New Zealand. *Aquatic Bot.* 131: 28-37.
- HOOT, S.B., NAPIER, N.S. & TAYLOR, W.C. 2004. Revealing unknown or extinct lineages with *Isoetes* (Isoetaceae) using DNA sequences from hybrids. *Amer. J. Bot.* 91: 899-904.
- HOOT, S.B. & TAYLOR, W.C. 2001. The Utility of nuclear ITS, a LEAFY homolog intron, and chloroplast atpB-rncL spacer region data in phylogenetic analyses and species delimitation in *Isoetes*. *Amer. Fern J.* 91: 166-177.
- HOOT, S.B., TAYLOR, W.C. & NAPIER, N.S. 2006. Phylogeny and biogeography of *Isoetes* (Isoetaceae) based on nuclear and chloroplast DNA sequence data. *Syst. Bot.* 31: 449-460.
- JEFFREY, E.C. 1937. The cytology of a heterosporous *Isoetes*. *Bot. Mag. (Tokyo)* 521: 203-209.
- JERMY, A.C. 1990. Isoetaceae, In: KRAMER, K.U. & GREEN, P.S. (Eds) *The Families and Genera of Vascular Plants Volume 1: Pteridophytes and Gymnosperms*, pp. 24-31. Springer-Verlag, Berlin.
- JERMY, A. C. & AKEROYD, J. R. 1993. *Isoetes* L. In: TUTIN, T.G., BURGESS, N.A., CHATER, O., EDMONDSON, J.R., HEYWOOD, V. MOOR, D., VALENTINE, D.H., WALTERS, S.M. & WEBB, D.A. *Flora Europaea, Volume 1 Psilotaceae to Platanaceae*, 2nd ed., pp. 6-7. Cambridge University Press, Cambridge.
- JOHNSON, E.R.L. 1984. Taxonomic revision of *Isoetes* L. in Western Australia. *J. Roy. Soc. Western Australia* 66, Part 4: 28-43.
- KARRFALT, E.E. & HUNTER, D.M. 1980. Notes on the natural history of *Stylites gemmifera*. *Amer. Fern J.* 70: 69-72.
- KEELEY, J.E. 2014. Aquatic CAM Photosynthesis: a brief history of its discovery. *Aquatic Bot.* 118: 38-44.
- KEELEY, J.E. & BUSCH, G. 1984. Carbon assimilation characteristics of the aquatic CAM plant *Isoetes howellii*. *Pl. Physiol.* 76: 525-530.
- KEELEY, J.E. & SANDQUIST, D.R. 1991. Diurnal photosynthesis Cycle in CAM and non-CAM seasonal-pool aquatic macrophytes. *Ecology* 72: 716-727.
- KEELEY, J.E., DEMASON, D.A., GONZALEZ, R. & MARKHAM, K.R. 1994. Sediment-based nutrition in tropical alpine *Isoetes*. In: RUNDEL, P.W., SMITH, A.P. & MIENZER, F.C. (Eds) *Tropical Alpine Environments*, pp.167-194. Cambridge University Press, Cambridge.
- KIM, C. & CHOI, H.-K. 2016. Biogeography of North Pacific *Isoetes* (Isoetaceae) inferred from nuclear and chloroplast DNA sequence data. *J. Pl. Biol.* 59: 386-396.
- KIM, C., NA, N.R., SHIN, H. & CHOI, H.-K. 2009. Systematic evaluation of *Isoetes asiatica* Makino (Isoetaceae) based on FLAP, nrITS and chloroplast DNA sequences. *J. Pl. Biol.* 52: 501-510.
- KOTT, L. 1980. Chromosome numbers for *Isoetes* in northeastern North America. *Canad. J. Bot.* 58: 980-984.
- KOTT, L. & BRITTON, D.M. 1983. Spore morphology and taxonomy of *Isoetes* in northeastern North America. *Canad. J. Bot.* 61: 3140-3164.
- KRISTEN, U., LIEBEZEIT, G. & BIEDERMANN, M. 1982. The ligule of *Isoetes lacustris*: ultrastructure, mucilage composition. and a possible pathway of secretion.

- Ann. Bot. 49: 569-584.
- KÜRSCHNER, H. & PAROLLY, G. 1999. On the occurrence of *Isoëtes hystrix* in the Menderes Massif of western Turkey - a synecological study and the first record of an Isoëtion community for Turkey. Bot. Jahrb. Syst. 121: 423-451.
- LARSÉN, E. & C. RYDIN. 2016. Disentangling the phylogeny of *Isoetes* (Isoetales), using Nuclear and Plastid Data. Int. J. Pl. Sci. 177: 157-174.
- LAZARE, J.J. & RIBA, S. 2010. Les Isoètes de l'Estany de Creussans (Andorre). J. Bot. Soc. France 30: 19-26.
- LINNAEUS, C. 1781. *Supplementum Plantarum Systematis Vegetabilium Editionis Decimae Tertiae, Generum Plantarum Editiones Sextae, et Specierum Plantarum Editionis Secundae*. Brunsvigne.
- LIU, X., GITURU, W.R. & WANG, Q.-F. 2004. Distribution of basic diploid and polyploid species of *Isoetes* in East Asia. J. Biogeogr. 31: 1239-1250.
- LIU, X., WANG, J.-Y. & WANG, Q.-F. 2005. Current status and conservation strategies for *Isoetes* in China: a case study for the conservation of threatened aquatic plants. Oryx 39: 335-338.
- LUEBKE, N.T. 1992. Three new species of *Isöetes* for the southeastern United States. Amer. Fern J. 82: 23-26.
- MACLUF, C.C., MORBILLI, M.A. & GIUDICE, G.E. 2006. Microspore morphology of *Isoetes* Species (Lycophyta) from southern South America. Bot. Review 72: 121-124.
- MANTON, I. 1950. *Problems of cytology and evolution in the Pteridophyta*. Cambridge University Press, New York.
- MARSDEN, C.R. 1979. *Morphology and Taxonomy of Isoetes in Australia, India, North-East and South-East Asia, China and Japan*. Department of Botany, University of Adelaide [Phd Thesis].
- MATTHEWS, J.F., FAIRCLOTH, W.R. & ALLISON, J.R. 1991. *Portulaca biloba* Urban (Portulacaceae), a species new to the United States. Syst. Bot. 130: 53-61.
- MATTHEWS, J.F. & MURDY, W.M. 1969. A study of *Isoetes* common to the granite outcrops of the southeastern Piedmont, United States. Bot. Gaz. 130: 53-61.
- MÉDAIL, F. 2003. La colonisation des mares temporaires méditerranéennes par les ligneux menace la survie à court terme d'une ptéridophyte très rare et menacée, l'isoète sétacé (*Isoetes setacea* Lam.). Ecol. Medit. 29: 251-252.
- MOCHALOVA, O.A. 2006. The genus *Isoetes* (Isoetaceae) in the northern Far East of Asia. Bot. Zhurn. (Moscow & Leningrad) 91: 94-98 [in Russian].
- MOCHALOVA, O.A., BOBROV, A.A. & BRUNTON, D.F. 2015. *Isoetes* in Kamchatka (northern Russian Far East) with description of a new hybrid *I. ×paratunica* (*I. asiatica* x *I. maritima*). Amer. Fern J. 105: 101-112.
- MOLINA, J.A. 2005. The vegetation of temporary ponds with *Isoetes* in the Iberian Peninsula. Phytocoenologia 35: 219-230.
- MOLINA, J.A., LUMBRERAS, A., GALLARDO, T., AGOSTINELLI, E., CASERMEIRO, M.A. & PRADA, C. 2011. *Isoetes* distribution pattern in a Mediterranean vernal pool system. Acta Bot. Gallica 58: 27-36.
- MONTGOMERY, J.D. & TAYLOR, W.C. 1994. Confirmation of a hybrid *Isoetes* from New Jersey. Amer. Fern J. 84: 115-120.
- MORA-OLIVO, A., MENDOZA-RUIZ, A. & MARTÍNEZ-ÁVALOS, J.-G. 2013. *Isoetes tamaulipana* (Isoetaceae), a new species from Mexico. Phytotaxa 267: 113-120.

- MOTELAY, M.L. 1883. Monographie des Isoëteae. Actes Soc. Linn. Bordeaux 36: 309-404.
- MOTELAY, M.L. 1892. *Isoetes brochoni* Motelay, spec. nov. Actes Soc. Linn. Bordeaux 45: 45-48 + Plate II.
- MUSSELMAN, L.J. 2002. Ornamentation of *Isoetes* (Isoetaceae, Lycophyta) microspores. Bot. Review 68: 474-487.
- MUSSELMAN, L.J., BRAY, R.D. & KNEPPER, D.A. 1996. *Isoetes* × *bruntonii* (*Isoetes engelmannii* ' *I. hyemalis*), A new hybrid quillwort from Virginia. Amer. Fern J. 86: 8-15.
- MUSSELMAN, L.J. & KNEPPER, D.A. 1994. Quillworts of Virginia. Amer. Fern J. 84: 48-68.
- MUSSELMAN, L.J., KNEPPER, D.A., BRAY, R.D., CAPLEN, C.A. & BALLOU, C. 1995. A new *Isoetes* hybrid from Virginia. Castanea 60: 245-254.
- MUSSELMAN, L.J. & ROUX, J.P. 2002. *Isoetes toximontana* (Isoetaceae), a new quillwort with green megaspores from Northern Cape of South Africa. Novon 12: 504-507.
- MUSSELMAN, L.J., TAYLOR, W.C. & BRAY, R.D. 2001. *Isoetes mattaponica* (Isoetaceae), a new diploid quillwort from freshwater tidal marshes of Virginia. Novon 11: 200-2004.
- PANT, D.D. & SRIVASTAVA, G.K. 1962. The genus *Isoetes* in India. Proc. Natl. Inst. Sci. India 28 (Part B): 242-280.
- PATIL, S.M. & RAJPUT, K.S. 2017. The genus *Isoetes* from India: An Overview. Plant Science Today 4: 213-226.
- PEREIRA, J.B.S., LABIAK, P.H., STUTZEL, T. & SHULTZ, C. 2017. Origin and biogeography of the ancient genus *Isoetes* with focus on the neotropics. Bot. J. Linn. Soc. 185: 253-271.
- PEREIRA, J.B.S., MITTELBAACH, M. & LABIAK, P. 2015. Studies on chromosome numbers and spore size in Brazilian *Isoetes*. Amer. Fern J. 105: 226-237.
- PEREIRA, J.B.S., SALINO, A., ARRUDA, A. & STUTZEL, T. 2016. Two new species of *Isoetes* (Isoetaceae) from northern Brazil. Phytotaxa 272: 141-148.
- PEREIRA, J.B.S., STUTZEL, T. & SCHULZ, C. 2017. *Isoetes nana*, a new species from the coastal mountains of southeastern Brazil. PhytoKeys 89: 91-107.
- PERUZZI, L., CESCO, G. & PUNTILLO, D. 2003. *Isoetes* (Isoetaceae), *Ophioglossum* and *Botrychium* (Ophioglossaceae) in Calabria (S. Italy): more karyological and taxonomical data. Caryologia 56: 359-363.
- PFEIFFER, N.E. 1922. Monograph on the Isoetaceae. Trans. Acad. Sci. St. Louis 9: 79-233.
- PIETSCH, W. 1994. *Isöetes azorica* Durieu ex Milde - ein Endemit der Azoren - Vegetations-und Standortsverhältnisse, Gefährdung und Schutzmaßnahmen. Phytocoenologia 24: 649-665.
- PIGG, K.B. 2001. Isoetalean lycopsid evolution: from the Devonian to the present. Amer. Fern J. 91: 99-114.
- PINTO-ZÁRATE, J.H., OROZCO-RINCÓN, E. & MARQUÍNEZ-CASAS, X. 2017. Panorama de la investigación sobre los Isoëtes de Colombia (Isoëtaceae, Lycopodiopsida). In: MORENO-GAONA, D.A. (Ed) *Memorias IX Congreso Colombiano de Botánica, 30 de julio al 3 agosto de 2017, Tunja-Boyacá*. Ciencia en Desarrollo 8 (1, Supl. Esp.): 304-305, Asociación Colombiana de Botánica y Universidad Pedagógica y Tecnológica de Colombia.

- PRADA, C. 1979. Estudio de la anatomía foliar de las especies Españolas del género *Isoetes* L. *Lagascalia* 9: 107-113.
- PRADA, C. 1980. Estudios palinológicos y cromosómicos en las especies Españolas del género *Isoetes* (Isoetaceae). *Palinología* 1: 211-225.
- PRADA, C. 1983. El género *Isoetes* L. en la Península Ibérica. *Acta Bot. Malac.* 8: 73-100.
- PRADA, C. & ROLLERI, C.H. 2003. Caracteres diagnósticos foliares en taxones Ibéricos de *Isoetes* L. (Isoetaceae, Pteridophyta). *Anales Jard. Bot. Madrid* 60: 371-386.
- PRADA, C. & ROLLERI, C.H. 2005. A new species of *Isoetes* (Isoetaceae) from Turkey, with a study of microphyll intercellular pectic protuberances and their potential taxonomic value. *Bot. J. Linn. Soc.* 147: 213-228.
- PRADO, J., SYLVESTRE, L., LABIAK, S. H., WINDISCH, P.G., SALINO, A., BARROS, I.C.L., HIRAI, R.Y., ALMERIA, T.E., SANTIAGO, A.C.P., KLIELING-RUBIO, M.A., N. PEREIRA, A., OLLGAARD, B., RAMOS, C.G.V., MICKEL, J.T., DIETRICH, V.A.O., MYNSEN, C.M., SCHWARTSBURD, P., CONDACK, J.P.S., PEREIRA, J.B.S. & MATOS, F.B. 2015. Diversity of ferns and lycophytes in Brazil. *Rodriguesia* 66: 1073-1083.
- PRADO, J., SYLVESTRE, L. & PEREIRA, J.B.S. 2015. Isoetaceae, In: *Lista de Espécies da Flora do Brasil*. Jardim Botânico do Rio de Janeiro. <<http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB119852> [accessed 22 March 2018]
- PUNT, W., HOEN, P.P, BLACKMORE, S., NILSSON, S. & LE THOMAS, A. 2007. Glossary of pollen and spore terminology. *Review of Palaeobotany and Palynology* 143: 1-81.
- QUEZEL, P. 1998. La végétation des mares transitoires à *Isoetes* en région méditerranéenne, intérêt patrimonial et conservation. *Ecol. Medit.* 24: 111-117.
- REED, C.F. 1965. *Isoetes* in the southeastern United States. *Phytologia* 12: 369-400.
- RESTALLACK, G.J. 1997. Earliest Triassic origin of *Isoetes* and quillwort evolutionary radiation. *J. Paleontology* 71: 500-521.
- RHAZI, L., FRILLAS, P., RHAZI, M. & AZNAR, J.-C. 2009. Ten-year dynamics of vegetation in a Mediterranean temporary pool in western Morocco. *Hydrobiologia* 634: 185-194. <https://doi.org/10.1007/s10750-009-9893>
- RHAZI, M., GRILLAS, P., CHARPENTIER, A. & MEDAIL, F. 2004. Experimental management of Mediterranean temporary pools for conservation of the rare quillwort *Isoetes setacea*. *Biol. Conservation* 118: 675-684.
- ROLLERI, C. & PRADA, C. 2007. Caracteres diagnósticos foliares en *Isoetes* (Pteridophyta, Isoetaceae). *Ann. Missouri Bot. Gard.* 94: 202-235.
- ROMEO, D., TROIA, A., BURGARELLA, C. & BELLINI, E. 2000. Casparian stripes in the leaf intrastelar canals of *Isoetes durieui* Bory, a Mediterranean terrestrial species. *Ann. Bot. (Oxford)* 86: 1051-1054.
- ROMERO, M.I. & AMIGO, J. 1995. Autecology and distribution of *Isoetes longissimum* in Europe. *Nordic J. Bot.* 15: 563-566.
- ROMERO, M.I., AMIGO, J. & RAMIL, P. 2004. *Isoetes fluitans* sp. nov.: the identity of Spanish plants of '*I. longissimum*'. *Bot. J. Linn. Soc.* 146: 231-236.
- ROMERO, M.I. & REAL, C. 2005. A morphometric study of three closely related taxa in the European *Isoetes velata* complex. *Bot. J. Linn. Soc.* 148: 459-464.
- ROSENTHAL, M.A., ROSENTHAL, S.E., JOHNSON, G., TAYLOR, W.C. &

- ZIMMER, E. 2014. *Isoetes viridimontana*: a previously unrecognized quillwort from Vermont, USA. Amer. Fern J. 104: 7-15.
- ROUX, J.P., HOPPER, S.D. & SMITH, R.J. 2009. *Isoetes eludens* (Isoetaceae), a new endemic species from Kamiesburg, Northern Cape, South Africa. Kew Bull. 64: 123-126.
- RUMSEY, F.J., THOMPSON, P. & SHEFFIELD, E. 1993. Triploid *Isoetes echinospora* (Isoetaceae: Pteridophyta) in northern England. Fern Gaz. (UK) 14: 215-221.
- RURY, P.M. 1985. A new and unique, mat-forming Merlin's-grass (*Isoetes*) from Georgia. Amer. Fern J. 68: 102-104.
- RYDIN, C. & WIKSTROM, N. 2002. Phylogeny of *Isoetes* (Lycopsidea): resolving basal relationships using rbcL sequences. Taxon 51: 83-89.
- SCHELPE, E.A.C.L.E. & ANTHONY, N.C. 1986. *Pteridophyta*. In: LEISTER, O.A. (Ed) *Flora of Southern Africa*. Botanical Research Institute, Pretoria.
- SCHNELLER, J.J. 1982. Cytological investigations on *Isoetes malinverniana*. Webbia 35: 307-309.
- SHARMA, B.D. 1998. Fungal associations in the roots of three species of *Isoetes* L. Aquatic Bot. 61: 33-37.
- SHARMA, B.D. & SINGH, R. 1984. The ligule In *Isoetes*. Amer. Fern J. 74: 22-28.
- SHRIVASTVA, G.K. 1998. Isoetaceae in India: morphology and taxonomy. Indian Fern J. 15: 165-177.
- SHRIVASTVA, G.K., PANT, D.D. & SHUKLA, P.D. 1993. The genus *Isoetes* L. in India. Amer. Fern J. 83: 105-119.
- SHUKLA, P.K., SHRIVASTAVA, G.K. & SHUKLA, S.K. 2002. The quillworts (*Isoetes*) of India: distribution, endemism and species radiation. Biodivers. & Conservation 11: 959-973.
- SINGH, S.K., SHUKLA, S.K., DUBY, N.K. & SHUKLA, P.K. 2018. *Isoetes* × *gopalkrishnae*, a new interspecific hybrid from central India. Phytotaxa 362: 68-76.
- SMALL, R.L. & HICKEY, R.J. 2001. Systematics of the North Andean *Isoetes karstenii* Complex. Amer. Fern J. 91: 41-69.
- SUDOVA, R., RYDLOVA, J., CTVRILKOVA, M., HAVRANEK, P. & ADAMEC, L. 2011. The incidence of arbuscular mycorrhiza in two submerged *Isoetes* species. Aquatic Bot. 94: 183-187.
- TAKAMIYA, M. 1999. Natural history and taxonomy of *Isoetes* (Isoetaceae). Acta Phytotax. Geobot. 50: 101-138.
- TAKAMIYA, M. 2001. *Isoetes sinensis* var. *sinensis* in Korea (Isoetes: Pteridophyta). Fern. Gaz. (UK) 16: 169-176.
- TAKAMIYA, M., WATANABE, M. & ONO, K. 1997. Biosystematic studies on the genus *Isoetes* in Japan IV. Morphology and anatomy of sporophytes, phytogeography and taxonomy. Acta Phytotax. Geobot. 48: 89-122.
- TAYLOR, W.C. & HICKEY, R.J. 1992. Habitat, evolution and speciation in *Isoetes*. Ann. Missouri Bot. Gard. 79: 613-622.
- TAYLOR, W.C., LEKSCHAS A.R., WANG, Q.-F., LIU, X., NAPIER, N.S. & HOOT, S.R. 2004. Phylogenetic relationships of *Isoetes* (Isoetaceae) in China as revealed by nucleotide sequences of nuclear ribosomal ITS region and second intron of a *LEAFY* homolog. Amer. Fern J. 94: 196-205.
- TAYLOR, W.C. & LUEBKE, N.T. 1988. *Isoetes* × *hickeyi*: A naturally occurring hybrid between *I. echinospora* and *I. macrospora*. Amer. Fern J. 78: 6-13.

- TAYLOR, W.C., LUEBKE, N.T., BRITTON, D.M., HICKEY, R.J. & BRUNTON, D.F. 1993 *Isoetaceae* - Quillwort family. In: FLORA OF NORTH AMERICA EDITORIAL COMMITTEE (Eds) *Flora of North America, volume 2: Pteridophytes and Gymnosperms*, pp. 64-75. Oxford University Press, New York.
- TAYLOR, W.C., LUEBKE, N.T. & SMITH, M.B. 1985. Speciation and hybridization in North American Quillworts. *Proc. Royal Soc. Edinburgh* 86B: 259-263.
- TAYLOR, W.C., MOHLENBROCK, R.H. & MURPHY, J.A. 1975. The spores and taxonomy of *Isoetes butleri* and *I. melanopoda*. *Amer. Fern J.* 65: 33-38.
- TROIA, A. 2016. Dispersal and colonization in heterosporous lycophytes: palynological and biogeographical notes on the genus *Isoetes* in the Mediterranean region. *Webbia* 71: 277-281.
- TROIA, A. & AZZELLA, M.M. 2013. *Isoetes sabatina* (Isoetaceae, Lycopodiophyta), a new aquatic species from central Italy. *Pl. Biosyst.* 147: 1052-1058.
- TROIA, A. & BELLINI, E. 2001. Karyological observations on *Isoetes durieui* Bory (Lycophyta, Isoetaceae) in Sicily. *Bocconea* 13: 397-400.
- TROIA, A. & GREUTER, W. 2014. A critical conspectus of Italian *Isoetes* (Isoetaceae). *Pl. Biosyst.* 148: 13-20.
- TROIA, A. & GREUTER, W. 2015a. A conspectus of and key to Greek *Isoetes* (Isoetaceae) based on a reassessment of Haussknecht's gatherings of 1885. *Willdenowia* 45: 391-403.
- TROIA, A. & GREUTER, W. 2015b. Flora Critica d'Italia: *Isoetaceae*. Fondazione per la Flora Italiana, Firenze. <http://www.floraditalia.it/pdf/Isoetaceae.pdf> [accessed 29 April 2018].
- TROIA, A., PEREIRA, J.B.S., KIM, C. & TAYLOR, W.C. 2016. The genus *Isoetes* (Isoetaceae): a provisional checklist of the accepted and unresolved taxa. *Phytotaxa* 277: 101-145.
- TROIA, A. & RAIMONDO, F.M. 2009. [published 2010]. *Isoetes todaroana* (*Isoetes*, Isoetaceae), a new species from Sicily (Italy). *Amer. Fern J.* 99: 238-243.
- TROIA, A. & ROUHAN, G. 2018. Clarifying the nomenclature of some Euro-Mediterranean quillworts (*Isoetes*, Isoetaceae): Indicator species and species of conservation concern. *Taxon*, <https://doi.org/10.12705/675.10>.
- VOGE, M. 1997. Plant size and fertility of *Isoetes lacustris* L. in 20 lakes of Scandinavia. *Arch. Hydrobiologia* 139: 171-185.
- VOGE, M. 2003. Environmentally related demography: field studies on *Isoetes lacustris* L. (Lycophyta, Isoetaceae) in Europe. In: CHANDRA, S. & SHRIVASTAVA, M. (Eds) *Pteridology in the New Millennium*, pp. 233-260, Kluwer Academic Publishers, Dordrecht.
- WAGNER, W.H., WAGNER, F.S. & TAYLOR, W.C. 1986. Detecting abortive spores in herbarium specimens of sterile hybrids. *Amer. Fern J.* 76: 129-140.
- WANNTORP, H-E. 1970. The genus *Isoetes* in South West Africa. *Svenska Botanisk Tidskrift* 64: 141-157.
- WEBER, U. 1922. Zur anatomie und systematik der gattung *Isoetes* L. *Hedwigia* 63: 219-262.
- WEN, M.C., PANG, X.-A., WANG, Q.-F. & TAYLOR, W.C. 2003. Relationship between water chemistry and the distribution of the endangered aquatic quillwort *Isoetes sinensis* Palmer in China. *J. Freshw. Ecol.* 18: 361-367.
- WILLIAMS, S. 1943. On *Isoetes australis* S. Williams, a new species from Western Australia. *Proc. Roy. Soc. Edinburgh, Sec. B*, 62: 1-11.

SHORT BIOGRAPHIES OF THE AUTHORS

Daniel Brunton was born in Ottawa, Canada on 17 May 1948. He grew up in that area and graduated with a Geography degree from Carleton University, Ottawa, in 1973. A keen naturalist from an early age, he translated that interest into summer employment and then permanent work in provincial parks in Ontario and Alberta (1967-1979), involved in the areas of environmental interpretation, ecological inventories and systems planning.

The lessons learned in parks prepared him for a career as an independent ecological consultant from which he retired (mostly) in 2017. Encouragement from federal government scientists in Ottawa and insights gained from expert associates in those field endeavors encouraged his interest in natural history generally and botany in particular.



Isoetologists (left to right) Carl Taylor, Angelo Troia and Daniel Brunton visiting bedrock outcrop *Isoetes* sites (*I. piedmontana* is abundant in the flooded erosion pits visible behind Taylor) at Clover, South Carolina, USA, 2 April 2016 (Photo: Gerry Taylor).

An extensive series of field based studies across Canada resulted in numerous publications on rare flora and fauna, especially pteridophytes, from the 1970s through the 1990s and the development of a very large (20,000 specimen) private herbarium.

Association with the late Donald M. Britton, foremost Canadian pteridologist, drew Brunton into the challenging - perplexing! - world of 'Isoetology' in the late 1980s. Beginning with their discovery of an undescribed hybrid, the Britton and Brunton team explored *Isoetes* throughout North American and beyond. They generated numerous papers between 1989 and 2006, describing over a dozen new species, hybrids and subspecies. To date, Brunton has produced 49 publications dealing with the taxonomy, diversity, distribution, ecology and conservation of *Isoetes* in North America, Asia and Europe.

Angelo Troia was born in Palermo, Italy on 3 September 1967 and grew up in Sicily. After obtaining a degree in Natural Sciences at the University of Palermo in 1991 and doing his (obligatory) service in the Italian Navy, he undertook PhD studies in Plant Biosystematics and Ecology between Florence and Bologna (Italy). His doctoral thesis was on the systematics and biogeography of the rare insular endemic *Cytisus aeolicus* Guss. (Fabaceae). Returning to Sicily in 1997, he was introduced to the fantastic world of Mediterranean quillworts by the late Prof. Enrico Bellini, his studies focussing on caryological, taxonomic and ecological aspects of various species.

At the IX OPTIMA Meeting in Paris, in May 1998, Troia and Bellini presented a poster about the (previously unknown) chromosome number of the Mediterranean *Isoetes durieui*. After a period as Director of the Trapani Saltworks Nature Reserve in western Sicily, he returned to academic research in 2007, working as a botanical researcher at the University of Palermo. There he prepared the treatments for the three lycophyte families for the project "Flora Critica d'Italia".

Troia's ongoing research is focused on the diversity, ecology and biogeography of plants, with a particular focus on the genus *Isoetes* (and other lycophytes and ferns), freshwater plants and their habitats, the role of islands in the ecology of the Mediterranean, and a wide variety of conservation issues. In the course of his studies, he has produced 27 publications involving the taxonomy, biogeography, cytology, ecology and conservation of *Isoetes*. His most recent area of research is the study of Characeae (green algae).