

## The Genus *Isoetes* L. (Isoetaceae, Lycophyta) in South-Western Asia

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**Abstract:** Quillworts or Çim Eğreltisi, species of the genus *Isoetes* L. in Turkey and the Levant, are poorly studied despite extensive floristic studies in the region. The Flora of Turkey lists 4 taxa: *Isoetes olympica* A.Braun ( $2n = 22$ ), *I. histrix* Bory var. *histrix* ( $2n = 20$ ), *I. subinermis* (Bory) Cesca & Peruzzi [= *I. histrix* Bory var. *subinermis* Durieu ( $2n = 22$ )] and *I. duriei* Bory ( $2n = ca. 121$ ). An additional species, *I. anatolica* Prada & Rolleri, was described in 2005 from Bolu province, and we report the first count for this species,  $2n = 22$ . In Syria and Lebanon 2 species are known: *I. olympica* from a site in Syria and a known but undescribed plant from Akkar province (Lebanon) and adjacent Syria ( $2n = 22$ , first count). A preliminary molecular phylogeny for these species is presented; their distribution and cytology are discussed. The taxonomy and nomenclature of the *I. histrix* and *I. duriei* groups need considerable further study. The first hybrids for the region are documented.

**Key Words:** Quillworts, Çim Eğreltisi, Lebanon, Syria, Turkey, megaspores, chromosome counts, molecular phylogeny

### Güneybatı Asya'daki *Isoetes* L. (Isoetaceae, Lycophyta) Cinsi

**Özet:** Türkiye ve Levant olarak adlandırılan coğrafik alanda İngilizce 'Quillworts', Türkçe 'Çim Eğreltisi' olarak adlandırılan *Isoetes* cinsi, bu alandaki büyük floristik çalışmalara karşın, çok az incelenmiştir. Türkiye Florasında 4 takson bulunmaktadır: *Isoetes olympica* A.Braun ( $2n = 22$ ), *I. histrix* Bory var. *histrix* ( $2n = 20$ ), *I. subinermis* (Bory) Cesca & Peruzzi [= *I. histrix* Bory var. *subinermis* Durieu ( $2n = 22$ )] ve *I. duriei* Bory ( $2n = ca. 121$ ). Daha sonra 2005 yılında Bolu'dan *I. anatolica* Prada & Rolleri türü tanımlanmış ve bu türün kromozom sayısı  $2n = 22$  tespit edilip burada ilk kez rapor edilmektedir. Suriye ve Lübnan'da iki tür bilinmektedir: Suriye'den tek alandan bilinen *I. olympica* ve Akkar ilinden, Lübnan ve komşu Suriye'den henüz adlandırılmamış bir *Isoetes* türü ( $2n = 22$ , ilk sayım). Bu türlere ait başlangıç seviyesindeki moleküler filogenetik çalışma sunulmakta; onların yayılışları ve sitolojik özellikleri tartışılmaktadır. *I. histrix* ve *I. duriei* gruplarının taksonomisi ve sınıflandırılması çok daha detaylı büyük çalışmalara ihtiyaç duymaktadır. Çalışma alanı için ilk hibrit rapor edilmektedir.

**Anahtar Sözcükler:** Quillworts, Çim Eğreltisi, Lübnan, Suriye, Türkiye, megaspor, kromozom sayıları, moleküler filogeni

### Introduction

Regions of the Mediterranean include some of the best researched floras in the world. Yet, species in the genus *Isoetes* L. (known as quillworts in English) remain remarkably understudied and represent one of the most poorly researched of all vascular plant genera in western Asia. This is even more surprising since Mediterranean quillworts have been studied since the middle of the 1800s (e.g., Braun, 1863; Motelay & Vendreyes, 1884).

The monograph by Motelay & Vendryes remains a source of helpful information and description with beautifully executed colour plates.

Unfortunately, the nomenclature of the taxa clustered around *I. histrix* Bory is confused and needs further work. The magisterial *Flora of Turkey* (Jermy & Akeroyd, 1965) lists 4 taxa: *Isoetes olympica* A.Braun, *I. histrix* Bory var. *histrix*, *I. histrix* Bory var. *subinermis* Durieu, and *I. duriei* Bory. Our work as well as that of

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others (cf. Cesca & Peruzzi, 2001) clearly shows that the 2 varieties of *I. histrix* are best treated as separate, distinct species that we are tentatively referring to as *I. histrix* Bory and *I. subinermis* (Bory) Cesca & Peruzzi. Another apparent synonym for this species appears to be *I. gymnocarpa* (Genn.) Braun (Arrigoni, 2006). The first South-West Asian quillwort described in more than a century is *I. anatolica*, published in 2005 (Prada & Rolleri, 2005).

In the Levant, Mouterde (1966) recorded 2 species of quillworts, *I. olympica* and what he calls *I. histrix* forma *subinermis* Durieu, a plant of intermittent pools. He suggests it could also occur in adjacent Syria. We have recently discovered populations on the Syrian side in the vicinity of Lake Homs, a large intermittent lake formed by spring rains. This is a new species that will soon be described (Musselman, in prep.), in this paper referred to as the Lebanon quillwort. However, because of morphological convergence coupled with nomenclatural confusion, the presence of *I. subinermis* in the Levant is possible, even likely. For accurate distribution data, a careful examination of all collections from the region is needed. It is also possible that a third species, *I. histrix*, may be found in Syria and Lebanon.

This paper is a review of the species in the region with an objective of making botanists more aware of the genus and of the possibility of discovering new taxa. In addition, we present cytological data, including the first count for *I. anatolica*, and a preliminary phylogeny of the species in Western Asia.

All quillworts are remarkably similar in appearance because of extreme reduction of the plant body to a rootstock bearing sporophylls (Figures 1 and 2). A good search image for field botanists is the whorled leaves with a slight groove on the adaxial surface, features found in no other wetland plant in the region.

We include representative images of megaspores as these are traditionally the most reliable criterion for species determination. However, megaspore ornamentation exhibits considerable parallel and convergent evolution and is therefore unreliable for some species' determination. We have not included information on microspores. Our work is based on extensive field work in Lebanon, Syria, and Turkey.

## Materials and Methods

### Molecular Methods

The primary goal of the molecular study was to determine the phylogenetic position of western Asian quillworts in the context of the major world clades of

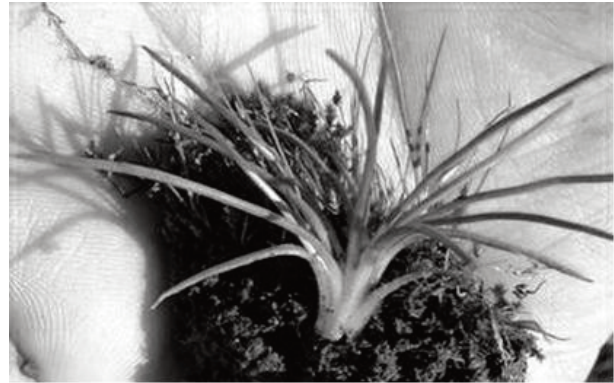


Figure 1. *Isoetes olympica* showing the diagnostic whorled sporophylls characteristic of the genus. Most quillworts have a similar appearance. Near Suweida, Syria. May 2002.



Figure 2. *Isoetes anatolica* from the type locality above Abant Lake, Bolu province, Turkey, July 2007. A species of intermittent pools, this is the most vigorous of the western Asian quillworts.

*Isoetes* delineated by Rydin and Wikström (2002) and Hoot et al. (2006). A secondary goal was to test the species boundaries particularly the recently elevated *I. subinermis* (= *I. hystrix* var. *subinermis*) and a new quillwort from Lebanon and Syria.

**Plant Material.** Specimens of *I. anatolica*, *I. duriei*, and *I. subinermis* were collected in Western Anatolia. The Lebanon quillwort was obtained from Akkar province in Lebanon, and contiguous Syria. *I. olympica* was collected from the Suweida region of Syria. All quillworts were cultured at Old Dominion University in Norfolk, Virginia, USA.

ITS sequence data for 22 *Isoetes* spp. representing Mediterranean *Isoetes* and placeholders for the major world clades were sequenced by Taylor et al. (2004) and Schuettpehl and Hoot (2006) and thus were available on GenBank. These data were included in all analyses to provide a backbone phylogeny. All accessions used in the analyses and sequenced for this study are available on GenBank. Collection information and GenBank accession numbers are in Appendix A.

**DNA Isolation.** Silica dried leaf material was macerated for extraction using a Mini-Beadbeater (BioSpec Products, Bartlesville, OK, USA). DNA was extracted using a Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) using the recommended protocol.

**DNA Amplification.** The ITS nuclear region was selected for amplification based on its utility in the genus *Isoetes* (Hoot & Taylor, 2001). The entire ITS region, including ITS1, 5.8S rDNA, and ITS2 was amplified using polymerase chain reaction (PCR) on an ABI 2720 thermal cycler. PCR reactions were prepared in 25 µl reactions using Promega GoTaq DNA Polymerase. ITS primer pairs and thermocycler programs are as in Hoot and Taylor (2001). Amplified PCR products were gel purified with the Qiagen QIAquick Gel Extraction Kit.

**DNA Sequencing.** Both complementary DNA strands were sequenced using forward and reverse amplification primers with the ABI Prism BigDye terminator Cycle Sequencing Ready Reaction Kit. Sequences were generated using an ABI 3130 XL genetic analyser. Sequences were assembled using Vector NTI Suite 7.0 and aligned in ClustalX 1.83. Gaps were binary coded using the conservative gap coding method of Simmons and Ochoterena (2000).

**Phylogenetic Analyses.** Maximum parsimony (MP) analysis of the ITS data was implemented in PAUP\* 4.0b10 (Swofford, 1998). For the MP analysis a heuristic

approach was implemented, with the following settings: random addition of sequences (100 replicates), TBR branch swapping, maximum trees set to 10,000, and MULTREES on. Each character was weighted equally. Gaps were included in a separate data partition as binary data. Bootstrap support values were estimated from 1000 replicates, using TBR branch swapping, one random addition of taxa, and no steepest descent. For the Bayesian analysis (BA) the data were first examined using Modeltest 3.7 (Posada & Crandall, 1998) and the best fit model of nucleotide evolution selected was HKY (Hasegawa et al., 1985). MrBayes 3.1.2 was implemented for Bayesian analysis (Ronquist & Huelsenbeck, 2003). The data were again partitioned, and nucleotide data were analysed under the HKY model of substitution and indel data under a binary model of substitution. A total of  $2 \times 10^6$  generations were run in 2 independent analyses with 4 Markov chains each, with trees sampled every 1000 generations until the standard deviation of the split frequencies fell to below 0.01. We used unrooted phylogenetic trees. Unrooted trees allow for assessment of relationships among species without implying the direction of evolution.

#### Cytological Methods

Plants were floated in tap water at room temperature until new roots emerged.

In late morning, excised root tips were treated in a 1:1 mixture of saturated PDB and 0.1% colchicine solution for 3 h. They were then fixed in Newcomer's solution (3:1, absolute ethanol:glacial acetic acid). After 1 h at room temperature, the roots were stored at -20 °C.

The root tips were removed from the fixative and hydrolysed in 1 N HCl for 10 min, neutralised for 15 min in 95% ethanol, and then stained with Wittman's haematoxylin for 1 h. After destaining in glacial acetic acid for 2-3 min, the root tips were placed in a drop of Hoyer's medium and squashed. The slides were made semi-permanent by sealing the cover slip edges with clear nail polish and photographed with an AV 70 Olympus microscope with a DP70 camera system.

#### Scanning Electron Microscopy

Materials for scanning electron microscopy were fixed and critical point dried, coated with a gold palladium mixture, and examined with a LEO scanning electron microscope.

### Results

The analyses of the ITS data included 808 total characters of which 340 were parsimony informative including 49 indels. Overall, 52 characters in the dataset were parsimony uninformative and scored as missing and 416 characters were constant. *Isoetes subinermis* had a run of 9 nucleotides scored as ambiguous at the interface of ITS1 and 5.8S. MP analysis yielded 2 equally parsimonious trees. Tree 1 and tree 2 differed in the arrangement of the 3 taxa in the fully resolved clade containing *I. longissima* Bory, *I. olympica*, and *I. velata* Braun. Bootstrap analysis and strict consensus yielded a

MP tree with the following parameters: 762 steps, consistency index of 0.70 (excluding uninformative characters), and a retention index of 0.88. The strict consensus tree topology from BA and MP analyses were identical with one exception, the BA fully resolved the clade with *I. longissima*, *I. olympica*, and *I. velata* while the MP strict consensus produced a well-supported trichotomy. Thus, only the BA topology with Bayesian posterior probability (PP) values is given; MP bootstrap support (BS) values are displayed on the common branches (Figure 3). BS and PP for terminal and backbone nodes were strong, above 90 BS and 0.98 PP

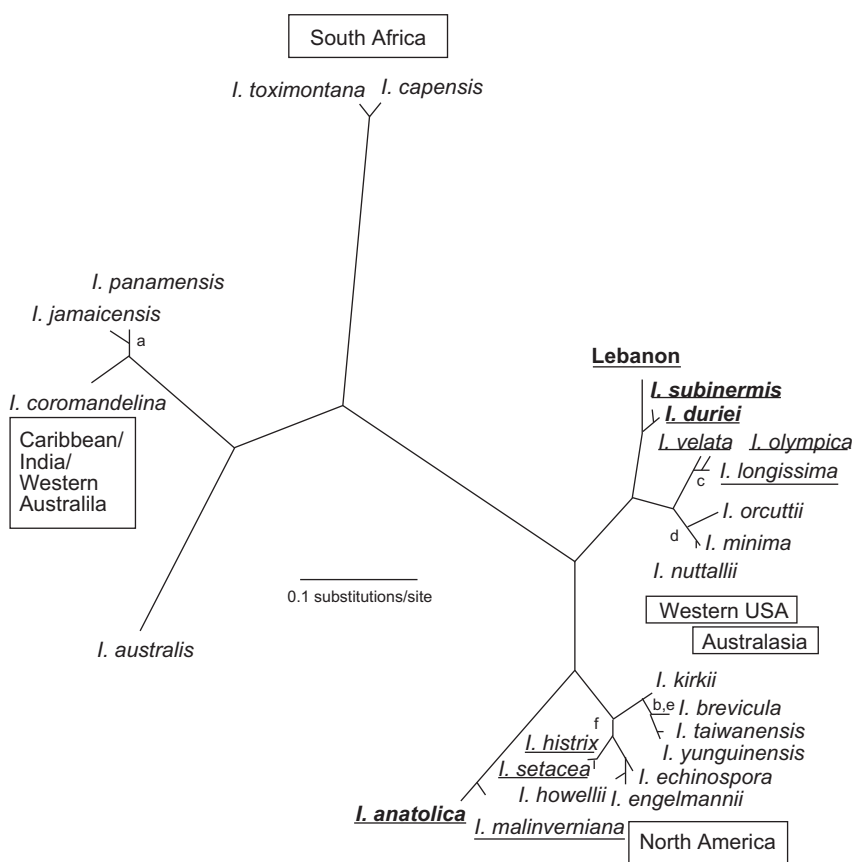


Figure 3. Unrooted Bayesian phylogram of *Isoetes* representing the major world clades and the phylogenetic positions of the western Asian *Isoetes* sequenced for this study (in bold). Other Mediterranean *Isoetes* are underlined. Bayesian posterior probability values for all nodes were greater than 0.98 with the exception of 3 branches indicated with lowercase letters “a” (= 0.85), “b” (0.86), and “c” (0.63). MP topology was identical with the exception of a trichotomy at the terminal *I. olympica*, *I. longissima*, *I. velata* clade. Maximum parsimony bootstrap support values for congruent branches were greater than 90% with the exception of 3 branches indicated with lowercase letters “d” (84), “e” (58), and “f” (82).

for all branches with 5 exceptions (still, all are greater than 55 BS and 0.60 PP). *Isoetes anatolica* is in a well-supported clade (BS = 100; PP = 1.0) with *I. malinveriana* Cesca & De Notaris. The other western Asian *Isoetes*: *I. duriei*, *I. subinermis* and the Lebanon *Isoetes* are located on a well supported clade (BS = 100; PP = 1.0) sister to a clade composed of other Mediterranean and western North American taxa.

The overall tree phylogeny recovered after the inclusion of the 4 western Asian *Isoetes* ITS sequences was similar to the topology of the 2 gene (ITS and *atpB-rbcL*) world *Isoetes* phylogeny published by Hoot et al. (2006). This is not surprising considering the variability and utility of ITS for higher level relationships within the genus (Hoot & Taylor, 2001; Hoot et al., 2006). Still, the absence of an independent source of molecular characters is admittedly absent from this study and can explain some differences in support values and general topology of the phylogram generated here and the best current hypothesis of world *Isoetes* phylogeny (Hoot et al., 2006).

“The morphologically distinct taxa, *I. duriei*, *I. subinermis*, and the Lebanon quillwort, form a well supported clade. However, the level of ITS sequence divergence among these western Asian species is low.” When considering only nucleotide transversions and transitions and without counting indels the Lebanon quillwort has ITS sequence divergence from *I. anatolica*, *I. subinermis*, and *I. duriei* of 13.5%, 2.8%, and 2.8%, respectively. Using the same criteria, the closely related *I. subinermis* and *I. duriei* have ITS sequence divergence of 0.84%, with only 6 base pair transversions and transitions. To put these values in context, 2 morphologically distinct and basic diploid Asian taxa *I. taiwanensis* DeVol and *I. yunguiensis* Q.F.Wang & W.C.Taylor differ by only 1 base pair (0.1%) in the ITS region. ITS data are most useful for delimiting higher level relationships in *Isoetes* (Hoot & Taylor, 2001). Polyploidy in *I. duriei* may obscure interpretation of ITS data due to potential concerted evolution or recombination events. More intensive molecular work is required to resolve relationships better within closely related taxa.

#### Species Notes

The following are notes on each of the species that occur in Lebanon, Syria, and Turkey. These are not

exhaustive descriptions but rather present differences among species and emphasise features previously unreported as well as areas for further study.

The presence of phyllopodia and scales can be diagnostic features important in determination of several species that we are considering. Some literature confuses these 2 structures—phyllopodia are the hardened sporophyll bases (sometimes the entire sporophyll) typically dark brown or black and often produce spores (Figures 4 and 5). These structures persist on the rootstock. Scales, on the other hand, are modified



Figure 4. Phyllopodia of *I. subinermis*. Each phyllopodium has 2 horns formed from the margins of the sporophyll. Plants collected at Maltepe, İstanbul, Turkey, and grown at the Old Dominion University greenhouse.



Figure 5. Phyllopodia (A) and scales (B) of *I. subinermis*. Phyllopodia (C) and scales (D) of *I. duriei*. Scale bar = 3 mm.

sporophylls that develop from sporophyll primordia and never produce spores (Figure 5). In other words, phyllopodia and scales differ ontogenetically—phyllopodia arise from typical primordia, scales from specialized primordia. All *Isoetes* taxa that we have examined from different parts of the world contain scales, fewer have phyllopodia. However, scales are seasonal and easily removed from the plants by handling and so uniformly under-reported.

#### *Isoetes olympica*

Originally described from “Mount Olympus”, now known as Uludağ near the Turkish city of Bursa in western Anatolia, *I. olympica* is of great conservation concern. It is known only from the type locality and one locality in south-eastern Syria near the city of Suweida (Musselman, 2002). A recent search at the type locality failed to turn up any plants, which we attribute to the extensive building of ski resorts and resultant environmental destruction. There are only a few plants remaining in Syria (Musselman, 2002). Megaspores are tuberculate with distinctive flanges on the equatorial ridge (Figure 6). It would be informative to compare the Syrian and Turkish populations of this species to determine any divergence.

Comparison of plants from the Homs region of Syria with *I. olympica* specimens from the type locality in Turkey as well as plants from the Suweida, Syria site strongly suggests that *I. olympica* occurs near Homs in

Syria and adjacent Lebanon based on similarity in megaspore characters and chromosome counts ( $2n = 22$ ). Mouterde (1966) applied the name *I. hystrix* var. *subinermis* to these plants which, as we note below, also includes the Lebanon quillwort, a new species. Both the Suweida site and the Homs site are basaltic and the habitats are similar. *Isoetes olympica* should be looked for at similar sites in eastern Turkey.

#### *Isoetes anatolica*

This was first described from an intermittent pool above Abant Lake in Bolu province, Turkey (Prada & Roller, 2005). A search of similar habitats around Abant Lake located no additional populations. This is the largest quillwort in western Asia and the only species in that region completely lacking a velum (Figures 2 and 7). It is a diploid with  $2n = 22$ , a first count for this species (Figure 8).

Another population of *I. anatolica* has been found in Eskişehir province; these are the only 2 known populations. Megaspores of *I. anatolica* have distinctive large tubercles that are as tall as broad (Figure 9).

The relationship between *I. anatolica* and *I. malinverniana* bears further study because of their possible relationship. Jermy and Akeroyd (1993) note that the rootstock of *I. malinverniana* is 3-lobed and the sporophylls lack a velum, features shared with *I. anatolica*.

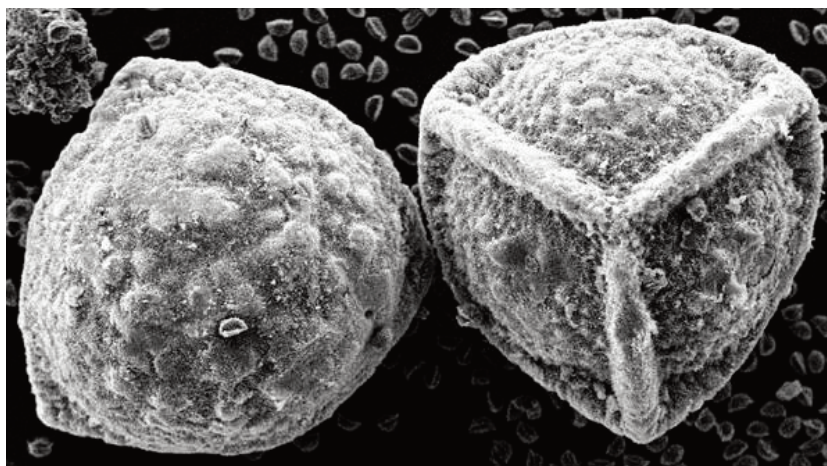


Figure 6. Megaspores of *Isoetes olympica*, microspores are evident above centre. Note the pronounced flange on the equatorial ridge evident on the left megaspore. Near Suweida, Syria. Scale: microspores are ca. 10  $\mu$ m long.

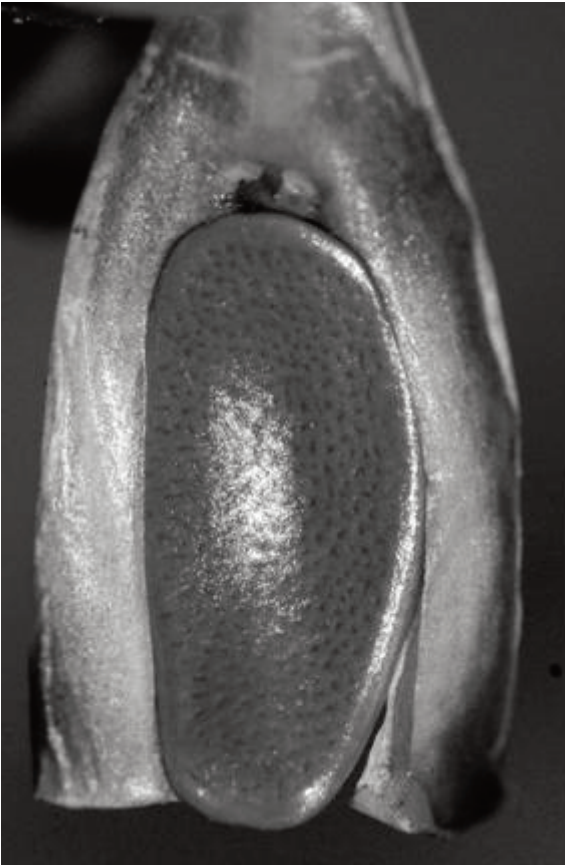


Figure 7. Microsporophyll of *I. anatolica* showing the complete lack of a velum (the membranous covering of the sporangium). This is the only species of *Isoetes* in the west Asian flora without a velum.

Molecular studies support this relationship. *Isoetes anatolica* forms a well supported clade with *I. malinverniana*, a plant of north-western Italy unknown in South-Western Asia. This *I. anatolica* - *I. malinverniana* clade is basal to a large and diverse clade that includes the American species complex, a number of eastern Asian and eastern Australian taxa, and notably 2 other Mediterranean quillworts (Figure 3). *Isoetes malinverniana* was not included in the biogeographic discussion by Hoot et al. (2006) because it lacked a closest relative. The close association of *I. malinverniana* ( $2n = 44$ ) and *I. anatolica* ( $2n = 22$ ) supports Schneller's (1982) hypothesis of an Asian origin for *I. malinverniana*. Our molecular and chromosome data suggest that *I. anatolica* may have contributed to the genome of *I. malinverniana* via polyploidy.

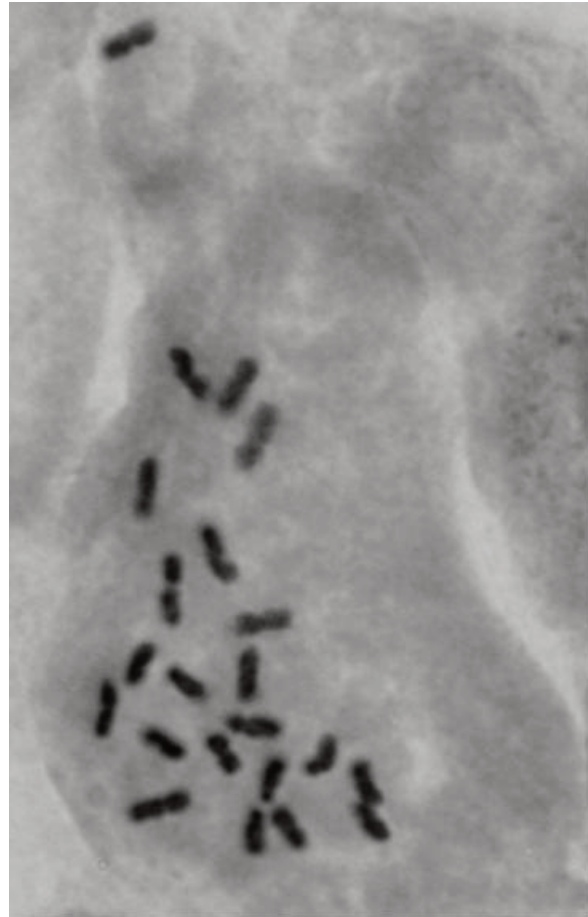


Figure 8. Mitotic metaphase figure from root tip of *I. anatolica* showing 22 chromosomes.

#### *Isoetes duriei*

Chromosome counts for this widespread Mediterranean species range from 55 to 121 (Troia & Bellini, 2000; Cesca & Peruzzi, 2001; Giovannini et al., 2001). Our count is ca. 121 (Figure 10). These varying numbers might be attributed to the presence of hybrids (see below). *Isoetes duriei* is abundant in Mediterranean woodlands, where it occurs with *I. subinermis*. Both species have phyllopodia. The phyllopodia of *I. duriei* are less than 3 mm in length (Figure 5). The spores of *I. duriei* are distinctive with alveolate ornamentation (Figure 11).

#### *Isoetes subinermis*

While further work is necessary to determine the correct application of this name, there is little doubt that

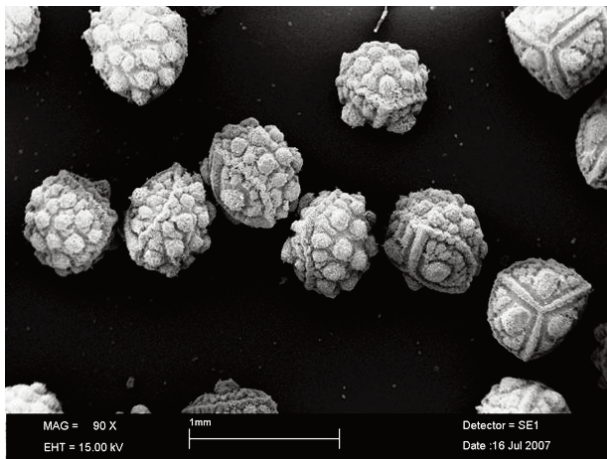


Figure 9. Megaspores of *I. anatolica*. These are the most distinctive megaspores of any of the diploid species.

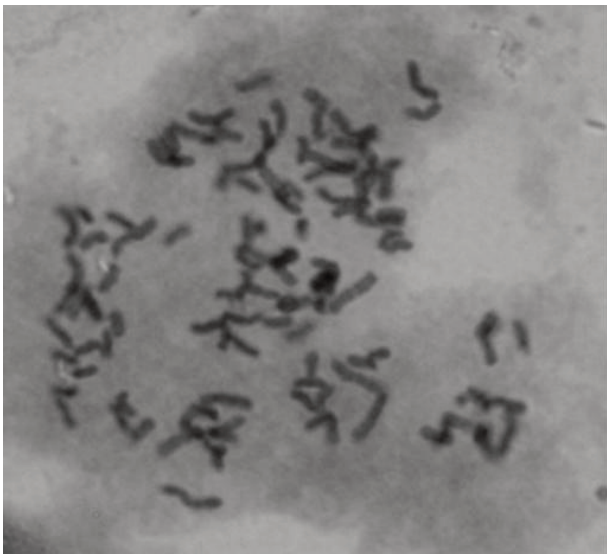


Figure 10. Chromosomes of *I. duriei* from plants collected at Maltepe in İstanbul, Turkey. In this preparation, ca. 121 chromosomes are evident (not all present in this plane).

this taxon is distinct from *I. hystrix* based on both chromosome count and molecular evidence despite the fact that they are morphologically similar. Because of confusion in applying this name, references to *I. hystrix* must be questioned as the plants may actually be *I. subinermis*. The chromosome number of *I. hystrix* var. *hystrix* has consistently been reported as  $2n = 20$  (Jermy & Akeroyd, 1965, 1993), while the taxon we call *I. subinermis* is  $2n = 22$ .

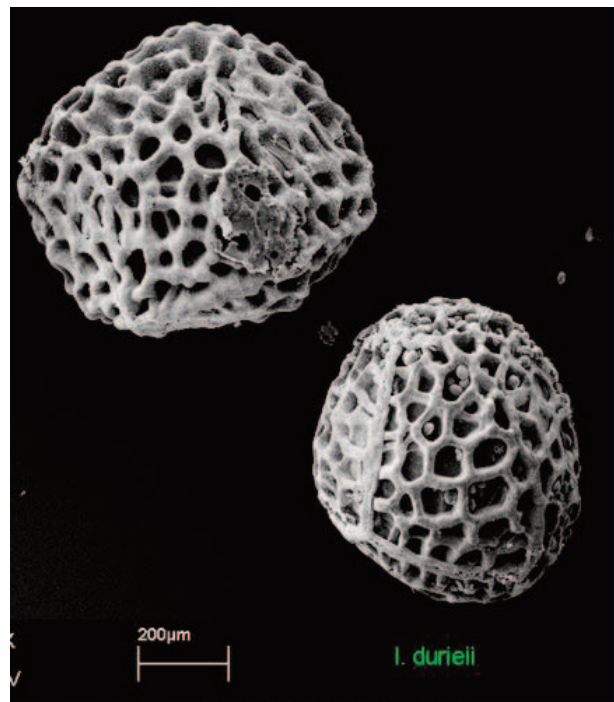


Figure 11. Megaspores of *I. duriei* collected at Maltepe, İstanbul, Turkey.

Phyllopodia and scales were present on our *I. subinermis* collections from Turkey. However, the constancy of this character remains to be determined. Mouterde (1966) considers phyllopodia sometimes present but reduced, while Jermy (1965) states that the “leaf bases [are] rarely black and horny, lacking the spine like edges”. Cesca and Peruzzi (2001) unequivocally state that phyllopodia are present but irregular, “often trunked at the base”.

The most striking finding of the molecular analyses is the molecular divergence of *I. hystrix* and *I. subinermis*. The *I. hystrix* sampled by Hoot et al. (2006) and included here is a  $2n = 20$  plant from Crete. Based on their cytology and similar morphology it was presumed that *I. hystrix* is derived from *I. subinermis* via aneuploidy (Cesca & Peruzzi, 2004).

The megaspores of *I. subinermis* have low tubercles (Figure 12) and resemble those of *I. hystrix* though both cytological and molecular data clearly separate them as distinct species.

#### *Isoetes hystrix*

Because of nomenclatural confusion involving the subspecific taxonomy of this group, distribution of this



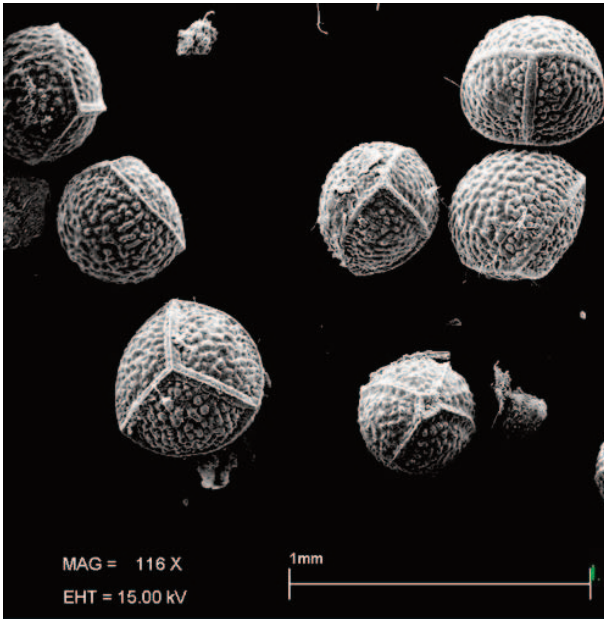


Figure 12. Megaspores of *I. subinermis* collected at Maltepe, İstanbul, Turkey.

species remains unclear. It inhabits intermittently wet sites around the Mediterranean like both *I. duriei* and *I. subinermis*. Records from England and areas out of its Mediterranean range (Jermy & Akeroyd, 1993) need to be investigated cytologically. At least one treatment

suggests that *I. hystrix* and *I. subinermis* are a single wide ranging and variable species (Kurschner & Parolly, 1999). Because we now know that at least 2 distinct taxa are included, future studies must consider these taxonomic differences. *Isoetes hystrix* is characterised by having distinct phyllopodia that are often bi- or trifurcate (see illustrations in Motelay & Vendreyes, 1884). Cytologically, it is a unique species because of its reduced chromosome number, which has been attributed to aneuploidy, an unusual phenomenon in quillworts (Manton, 1950). The megaspores are low tuberculate (Figure 13). Clearly, further study is required to clarify the circumscription of *I. hystrix*.

Megaspore features are the most frequent character for *Isoetes* identification. We have found limited utility in the megaspore and microspore characters presented by Cesca and Peruzzi (2004). Yet, as noted, both cytology and molecular studies clearly separate *I. hystrix* and *I. subinermis*. This is another example in this genus of strong convergent evolution of characters.

**Lebanon quillwort.** The description of this new species is in preparation. For the purpose of this paper it is important to note that it possesses scales (Figure 14), lacks phyllopodia, and is genetically distinct from other South-West Asian quillworts (Figure 3).

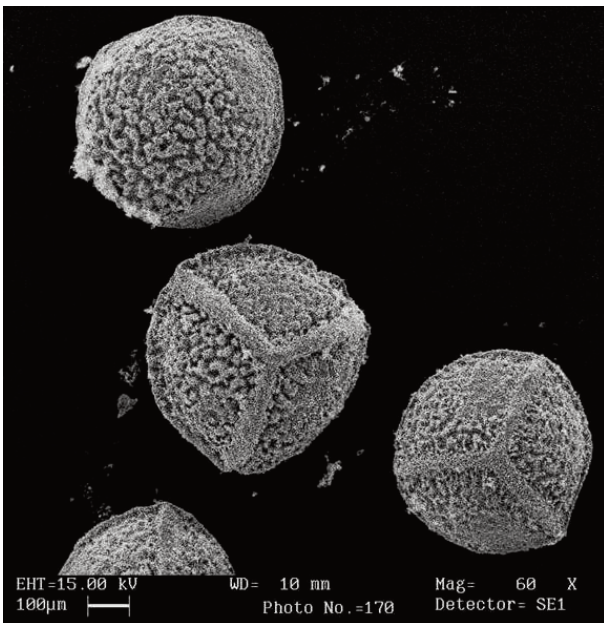


Figure 13. *Isoetes hystrix* megaspores. These are from material collected by Bory in Algeria (BM).

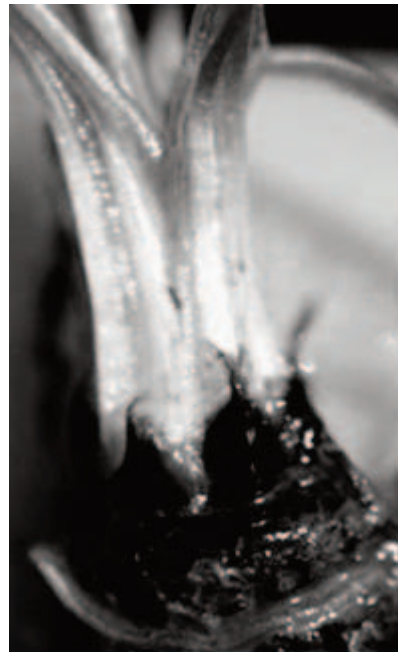


Figure 14. Scales at the base of the Lebanon quillwort, Akkar province, Lebanon.

In his flora of the Levant, Mouterde (1966) includes this quillwort as *I. histrix* forma *subinermis*. However, it differs in chromosome number from *I. histrix* and, more strikingly, is in an entirely different clade. The megaspores are distinct from *I. histrix* and *I. subinermis*, by having only few prominent tubercles on the proximal face (Musselman in prep.).

This quillwort inhabits intermittent pools and adjacent saturated areas in the vicinity of Lake Homs, a natural temporary lake in a basaltic region on the border of Lebanon and Syria west of Homs, Syria.

### Hybrids

We have found hybrid plants in the Istanbul region; they possess the characteristic polymorphic megaspores and hybrid vigour. Further work is necessary to ascertain parentage and ploidy level. This is the first report of *Isoetes* hybrids in western Asia, though our work (Musselman et al., unpublished) shows that quillwort hybrids are to be expected anywhere.

### Key to the Species in Western Asia

To summarise differences among these species, we present this simple key to the species in Western Asia. Considerably more data on morphology are needed to circumscribe clearly *I. histrix*, *I. subinermis*, and the Lebanon quillwort.

1. Megaspores with tuberculate ornamentation, tubercles various.....2
1. Megaspores with alveolate (honeycombed) ornamentation.....*I. duriei*
2. Tubercles, at least on the proximal surface, as tall as wide..... *I. anatolica*
2. Tubercles never as tall as wide.....3
3. Phyllopodia with well developed horns.....5
3. Phyllopodia without well developed horns.....4
4. Velum complete..... Lebanon quillwort
4. Velum incomplete.....*I. olympica*
5.  $2n = 20$ .....*I. histrix*
5.  $2n = 22$ .....*I. subinermis*

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### Future Work

Our work is preliminary to understanding the presence, distribution, species delineations, and relationships among quillworts of South-West Asia. There is a great opportunity for field botanists to locate and collect material without which an understanding of these furtive plants is impossible. It is likely that new species will be found and that these overlooked plants are more frequent and abundant than previously thought.

Collectors should be careful to obtain spores. Even if the plants are not spore bearing at the time of collection, it is usually possible to find spores in the soil around the rootstock. For this reason it is ill advised to wash the plants rigorously before looking for spores or scales. Even gentle washing could remove scales (see discussion above). Phyllopodia are persistent.

Spirit collections are desirable as features of the sporophylls in cross section can be useful in determining species. Features of the ligule and associated structures might also be useful in taxonomy.

It is likely that several species grow together in a small area. This is certainly the situation in western Anatolia where we have found *I. duriei* and *I. subinermis* growing together. Therefore, special care is needed to determine each plant to avoid mixed collections.

*Isoetes anatolica* and *I. olympica* can be submersed. In these cases, the sporophylls will look dramatically different than the leaves of the same species growing in a terrestrial situation. For this reason, sporophyll length is often of little diagnostic value.

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## APPENDIX A.

*Isoetes* spp. sequenced for this study, ploidy level, collection site and date, voucher information, and GenBank accession number. *I. anatolica*,  $2n = 44$ , Vicinity of Abant Lake, Bolu province, Turkey, 3 July 2007, *Musselman and Keskin TR-2007-01* (ODU), EU444000; *I. duriei*,  $2n = ca\ 121-132$ , Mediterranean forest near hospital complex in Maltepe, İstanbul, Turkey, 4 July 2007, *Musselman and Keskin TR-2007-02* (ODU), EU444001; *I. subinermis*,  $2n = 22$ , Mediterranean forest near hospital complex in Maltepe, İstanbul, Turkey, 4 July 2007, *Musselman and Keskin TR-2007-03* (ODU), EU444003; *I. "Lebanon"*,  $2n = 22$ , Menjez (Akkar), Lebanon, 16 May 2006, *Musselman and Al-Zein MSA 2006-11* (ODU), EU444002.

*Isoetes* spp. included in this study from sequences published by Taylor et al. (2004) and Hoot et al. (2006), ploidy level, collection site, GenBank accession number. *I. australis* MR,  $2n = 44$ , Mt. Ridley, Australia, DQ284990; *I. brevicula*,  $2n = ?$ , Lily McCarthy Rock, Australia, AY641098; *I. capensis*,  $2n = 22$ , S. Africa, Stellenbosch Univ. Campus, South Africa, DQ284991; *I. coromandelina*,  $2n = ?$ , Matkuli, India, DQ284992; *I. echinospora*,  $2n = 22$ , Sauk Co, WI, USA DQ479977; *I. engelmannii*,  $2n = 22$ , Greenville Co., VA, USA, DQ479978; *I. hystrix*,  $2n = 20$ , Greece, Crete, DQ284994; *I. howellii*,  $2n = 22$ , Butte Co., CA, USA, DQ479983; *I. jamaicensis*,  $2n = 22$ , Clarendon Parish, Jamaica, DQ479984; *I. kirkii*,  $2n = 22$ , Lake Brunner, South Island New Zealand, AY641100; *I. longissima*,  $2n =$

44, Galica, Spain, DQ479987; *I. malinverniana*,  $2n = 44$ , Cultivated at Zürich Botanic Gardens, from Prov. di Vercelli, Italy, DQ284995; *I. minima*,  $2n = 22$ , Kititas Co., WA, USA, DQ479989; *I. nuttallii*,  $2n = 22$ , San Diego Co., CA, USA DQ284997; *I. orcuttii*,  $2n = 22$ , Riverside Co., CA, USA, DQ284998; *I. olympica*,  $2n = 22$ , Jebel Druze region, Syria, DQ479991; *I. panamensis*,  $2n = 44$ , Santa Elena, Guanacaste, Costa Rica, DQ284999; *I. setacea*,  $2n = 22$ , Madrid, Spain, DQ285000; *I. taiwanensis*,  $2n = 22$ , Menghuan Lake, Taiwan, AY641101; *I. toximontana*,  $2n = 22?$ , N. Cape Province, South Africa, DQ479997; *I. velata*,  $2n = 22$ , Madrid, Spain, DQ285001; *I. yunguiensis*,  $2n = 22$ , Guizhou Prov., China, AY641102.