REVIEW

THE ETHNOBOTANY OF FERNS AND LYCOPHYTES

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ABSTRACT

A summary is presented of the most important ways in which ferns have been important to humanity. Many of these categories are positive such as the use of ferns for subsistence. On the negative side is their role as weeds and as bearers of substances harmful to human health. Many of the traditional uses such as for medicines have been transferred to modern life as societies have modernized. Some uses have even become important in industrial society, for example in the assay of new medicines.

INTRODUCTION

Ferns are distributed in all climate zones of the planet, but have a greater diversity in the tropics (Smith et al., 2006; Strasburger et al., 2003). The discipline that studies the relationship between the uses of ferns and humans has been termed ethnopteridology, and it was well explained and amplified by Boom (1985). These reciprocal interactions may or may not be related to a particular use category in family or regional cultures, but the concept of ethnobotany goes beyond the utilitarian spheres of economics and uses to include symbolic values, nomenclature, religion and also the place that particular plants occupy in the cosmology of peoples. Strictly economic aspects of uses are addressed by the discipline of economic botany. It is not easy to separate economic botany and ethnobotany and indeed economic botany implies a type of relationship between human groups and plants.

The object of this work is to present a global panorama of the state of the art in ethnobotany of pteridophytes. We will show the great diversity of relationships between these plants and humans. Pteridophytes feature in some way in many papers about ethnobotany and economic botany and here we will concentrate mainly on papers whose object was specifically about the study of ferns or lycophytes. A few previous papers have addressed similar topics at least locally. For example, May (1978) made a summary of the economic and folkloric uses of ferns. Díaz de León et al. (2007) reviewed the many uses of ferns and lycophytes in Mexico and to some extent the rest of the world. Murillo (1983) produced a major work on the uses of ferns in South America with a special emphasis on the ferns of Colombia. Mannar Mannan et al. (2008) gave a short review of the potential uses of ferns. We have used the names as cited in the various papers rather than trying to update the nomenclature in any way. The literature about the reciprocal relationships between humans and pteridophytes is so extensive that here we cannot possibly cover it all, but we hope to show the variety of possible relationships through presenting a number of wide-ranging examples.
ARCHAEOBOTANY
Archaeobotany is the study of ancient plant remains found in archaeological contexts. The name is obviously a parallel to, and a derivative from, ethnobotany. In both archaeobotany and ethnobotany the focus of attention is upon the uses of plants by, and their association with people (Wiley, 1995). Since remote times human populations in many parts of the world knew and made use of ferns and lycophytes. The archaeological literature offers much evidence of the close relationship between humans and these plants over many generations. Anderson & White (2001) suggested that *Cyathea* was used for consumption by humans at least 888 years ago in Norfolk Island. Dental evidence from human remains in New Zealand show ancient use of the rhizomes of *Pteridium esculentum* (G.Forst.) Cockayne (Houghton, 1978). In China there was use of edible ferns at least 3000 years ago (Zhang, 2007). There is evidence of the prehistoric use of the leaves of *Marattia fraxinea* Sm. in ritual involving the incipient use of iron in Africa (Schmidt & Avery, 1983).

FERNS IN PRE-LINNEAN LITERATURE
Our study has shown that fern ethnoobotany is nothing new and that there is much about ferns and local cultures in ancient literature. We have mainly concentrated here on the more recent literature, but a good example of early studies of fern ethnoobotany is that of Georgius Everhardus Rumphius (1627-1702) in Ambon, now in Indonesia (Rumphius, 2011). Rumphius devoted 44 pages to descriptions of ferns, lycophytes and their uses. The pre-Linnean names are often hard to identify to species, but the good drawings and the interpretation by E. D. Merrill (1917) make it possible to relate to current species names. These pages are full of ethno botanical information about fern uses by the Ambonese and natives of other islands in the seventeenth century. Many of these uses come under the subheadings we have used below. For example one chapter is entitled “The Edible Fern” and is about *Athyrium esculentum* (Retz.) Copel. Rumphius said that this fern and related species “are a renowned potherb of all of these islanders. One can make a good salad from its leaves and shoots….it cools moderately and loosens the bowels, especially if one drinks some tree-wine after it.” Rumphius describes the culinary use of several different species of ferns together with great details about the effects of eating them. The Balinese stick the tops of *Tectaria crenata* Cav. behind their ears when they go to war, because this plant will keep them from getting hurt by dart poison, rendering it powerless. They also rub the dry leaves over their bodies when bathing to get rid of sweat and odours. The lower stems of *Lygodium circinnatum* (Burm. f.) Sw. are split into four strips and used for seams around the edges of baskets by the Ambonese. An interesting use of the leaves of *Drynaria sparsisora* (Desv.) Moore is to tie them to a baited fishhook and use them as sails to carry the hooks out to sea until fish bites. The leaves of the same species are suspended over little children to keep them safe from evil spirits. The pages of Rumphius contain much interesting fern ethnoobotany and many similar uses are reported in the more recent literature on the subject.

ETHNOPTERIDOLOGY
In the literature surveyed we found a number of articles that were specifically about ethnopteridology of human groups and also ethnobotany about individual species of pteridophytes, and we highlight examples of recent works from different continents. From the Americas, Navarrete *et al.* (2006) presented information on the uses attributed to more than 200 species of pteridophytes of Ecuador, Peru and Bolivia. Boom (1985)
treated the use of ferns by the Chácobo tribe of Amazonian Bolivia and Macía (2004) compared the ethnopteridology of the Tacana of Bolivia with that of the Huaorani of Ecuador. The only species in common was *Cyathea pungens* (Willd.) Domin. Hernández Cibrián & Sutherland (2007) carried out an ethnobotanical study of the ferns of a national park in Honduras and found only eight species that were used by the local population. For Argentina Keller *et al.* (2011) treated the various uses of 50 species of ferns and lycophytes by the Guaranies of Misiones Province (Figures 1-3) and Hurrell & De La Sota (1996) did the same for the villagers of Santa Victoria in Salta Province. In Asia Christensen (1997) studied the ethnopteridology of ethnic groups in Malaysia. A study of the ethnobotanical uses of ferns in the Indian States of Jammu and Kashmir (Kirn & Kapahi, 2001) listed 17 species of which 11 were medicinal, four used for thatching roofs and three as foods. Joshi (1997) listed ethnobotanical uses of 44 species of ferns in Uttar Pradesh State of India.

For Africa the ethnopteridological study of ethnic groups by Nwosu (2002) mentioned 36 species in 23 families. In addition to fern uses for food and medicines, many of these studies mention uses in rituals of love, for magic ceremonies, as indicators of cardinal points and the presence of animals and also as material to make crafts and weapons. The demands of today’s markets have led various indigenous groups to commercialize ornamental ferns and flowerpots made out of erect rhizomes and the trunks of tree ferns.

**FERNS IN TRADITIONAL MEDICINE**

Studies of uses of ferns in ethnomedicine are abundant on all inhabited continents. In Córdoba (Argentina) the study of ferns used in traditional medicine has developed to

*Figure 1. Microgramma squamulosa* (Kaulf.) de la Sota, a multipurpose species for the Guarani, used for slimming, menstrual analgesic, post partum washing, and treatment of lumbago.
such an extent that anatomical and morphological evidence is used to detect adulterants of the products (Luján et al., 2007; 2011). However, the adulteration of medicinal ferns is nothing new. Hipólito Ruiz (1805) described the species *Polypodium calguala* Ruiz with the intention of clearly differentiating this medicinal ethnospecies of the indigenous peoples of Peru from other fern species that were being imported into Spain from the New World as adulterants of the legitimate “calaguala.”

In a comparison between the ethnopteridology of the Tacana of Bolivia and the Huaorani of Peru, Macía (2004) found that 76% of the recorded uses for ferns were medicinal either for people or for animals to heal wounds or expel parasites. Most of the uses by the Tacana are external, whereas the Huaorani uses are mainly internal. This study cites uses for 24 species of ferns and lycophytes. There are many medicinal uses of ferns in India. For example, Sharma and Vyas (1985) described the use of six species in Rajastan. Srivastava (2007) emphasised the importance of ferns in tribal medicine from a study made in various places throughout India. Benniamin (2011) reported on the use of 51 species of ferns in the east of India and Kumari et al. (2011) gave information about the use of 66 species in ethnomedicine in India. Dixit (1982) is an example of the ethnobotanical use of a single species, *Selaginella bryopteris* (L.) Bak. This species is much revered in local medicine and commands a high market price. The medicinal use of *Helminthostachys zeylanica* (L.) Hook. has had serious effects on its state of conservation in Himalaya (Joshi, 2011). *Osmunda regalis* L. is used as a medicine in the north of Spain (Molina et al., 2009).

Of the 36 species of ferns reported in the ethnobotany study of ferns of Southern Nigeria by Nwosu (2002), 34 have medicinal uses. The paper reads like a complete

![Figure 2. Alsophylla setosa Kaulf. The base of the rhizomes are used as stands for ornamental plants. The Guaranis and local farmers agree that the presence of this fern is an indicator that the soil is not suitable for agriculture. The Guarani use a soup of the petioles for the treatment of herpes.](image-url)
pharmacy to treat many different ailments and all from pteridophytes. Ferns supply
treatment for external injuries and wounds and many are taken internally to treat such
diseases as malaria, ulcers, intestinal worms, liver disease etc. Many of the species listed
have multiple uses, for example, a decoction of the rhizome and leaves of *Polypodium
microrhizoma* Clarke ex Bak. is used for the relief of gastrointestinal disorders, backache
and jaundice; a paste from the dried leaves (dried over an open fire) is applied externally
for fissures on hands and wound healing; and a paste mixed with palm-kernel oil is
applied externally to domestic animals such as sheep and cattle. The whole plant of
*Osmunda regalis* is taken internally for psychosis as it is believed that the tonic can chase
away evil spirits, and an infusion of the roots is used to treat malaria and jaundice.

The importance of medicinal ferns is evidenced by the growing interest in methods
for their reproduction. An example is the achievement of *in vitro* propagation of the
Asiatic fern *Drynaria quercifolia* (L.) J.Sm. that is much used in traditional phytotherapy
(Mazumder *et al*., 2011).

**VETERINARY**
The literature has many examples of the use of ferns for treating animals. According to
Nwosu (1922), the leaves of *Tectaria macrodonta* (Fée) C.Chr. are powdered and mixed
with castor oil and given to goats and sheep to stop a running stomach; young fronds are
chewed by cows after delivery of a calf to accelerate the expulsion of the afterbirth.

**EDIBLE FERNS**
Fern rhizomes were an important source of food for Native Americans in western North

![Figure 3](image-url). The rhizome of *Dicksonia sellowiana* Hook. in a Guarani basket offered for
sale at a roadside stand.
America. Turner et al. (1992) produced a summary of this in a detailed paper that listed at least 15 species of ferns together with their native nomenclature. Ferns are much eaten in India. For example, Pandy and Pangtey (1987) list seven different species of ferns consumed in Western Himalaya and Joshi (1997) lists 10 edible species used in Uttar Pradesh State. For China, Liu et al. (2012) listed a total of 42 edible pteridophytes, but they estimated that the potential total could be as high as 144 species. Some ferns are eaten as though they were sweets, as in the case of Pectinatula pectinatiformis (Lindm.) M.G.Price, where the sweet leaves are commonly chewed by Guaraní children in Misiones, Argentina (Keller et al., 2011).

Some species of Polypodium are known for their property of sweetness. A variety of Polypodium vulgare L. was used to flavour tobacco for its liquorice taste and it contains small amounts of ostadin, a steroid saponin 3000 times as sweet as sucrose. In former times the fronds of this species were used in Ireland to treat coughs, colds and asthma. Polypodium glycyrrhiza D.Eaton also has a liquorice flavour and was eaten by Native American peoples (Mabberley, 2008).

FERNS AS BUILDING MATERIALS
The Guaranies of southeastern Brazil use the stems of Dicksonia sellowiana Hook. to support the walls of their traditional houses (Prudente, 2007).

Joshi (1997) lists Cyathea spinulosa Wall. ex Hook. and Dicranopteris linearis (Burm. f.) Underw. as used for thatching roofs in India. Kirn and Kapahi (2001) mention Pteridium aquilinum (L.) Kuhn. var. wightianum (Ag.) Tryon, Pteris vittata L., Thelypteris erubescens (Wall. ex Hook.) Ching and Woodwardia unigemmata (Makino) Nakai for the same purpose.

FERNS IN ORNAMENTATION AND ART
Ferns are often used for body ornamentation. The stipes of Cheilanthes farinosa (L.) Brogn. and Adiantum lunulatum Burm. are used as nose and ear studs by children and poor women in Uttar Pradesh (Pande & Pangey, 1987) and Adiantum venustum D. Don is used as ear studs by girls in Kashmir (Kirn & Kapahi, 2001). The tree ferns Cyathea divergens var. tuerckheimii R.M.Tryon and C. fulva (Martens & Galeotti) Fée are harvested to produce handicrafts for garden ornamentation by artisans of the mountains of Cuetzalan, Mexico (Elutério, 2006). In the Philippines the petiole and leaf rachis of Lygodium japonicum (Thunb.) Sw. are used to decorate baskets (Novellino, 2006). In Argentina the Guaranies use the petioles of various ferns to make necklaces (Keller et al., 2011).

USES OF FERN SPORES
Frye (1934) reported that the spores of Lycopodium clavatum L. were used for dusting on open raw wounds and chafed infants by natives of northwestern North America. The spores are fine and light and so repel water and prevent stickiness. Lycopodium powder has also been used as a lubricating dust on latex gloves and condoms, though the latter use is not recommended (see Balick & Beitel, 1989), because these spores have been known to cause allergic reactions, ranging from hay-fever to more serious giant cell granulomases. May (1978) reported that the easily flammable spores of species of Lycopodium have been used in theatre as a flash powder.
FERNS AS PLACE NAMES

The importance of ferns to local communities has often led to the names of places, topographic formations, watercourses and political divisions. In Misiones Province (Argentina), the most tropical and wettest province of Argentina, there are various places based on plant names. The “Diccionario geográfico toponímico” of Stefañuk (2009) gives several examples. “Los helechos” is the name of a stream and a municipality in this province. In the town of Oberá there is a place and a stream named “Samambaya” the common name for ferns derived from the Guarani word for them “amambái” which translated into English is a generic name of ferns. Locally amambái refers to the large populations of Pteridium arachnoideum (Kaulf.) Maxon. In the Department of L.N. Alem there is a stream called “Chachi” which is the Guarani name for the tree fern Cyathea atrovirens (Langsd. & Fisch.) Domin (Cyatheaceae). In the Department of San Martín there is stream called “Culandrillo”, a term that refers to species of the genus Adiantum (Pteridaceae). In Paraguay the derivation of the name of the political division Amambay Department is derived from the Guarani word for fern. There are many place names in the United Kingdom associated with ferns, for example, Ferndown in Dorset, Fernilee in Derbyshire and Ferness in the Highlands. Fern is a town in Tayside and Fernie a stream and a castle in Fife. Bracken (Pteridium aquilinum) features in Brackenfield in Derbyshire and Brackenthwaite in Cumbria.

FERNS IN PHARMACOLOGY

Substances with antioxidant activity are now used in medicine to reduce the effects of oxidation stress. Antioxidant activity has been reported in Adiantum capillus-veneris L., a widely distributed fern (Kumar, 2009). A recent study of the lateral branches of Equisetum giganteum L. of South and Central America showed that they can be used as a source of antioxidant compounds (Ricco et al., 2011). In Malaysia studies have shown similar properties in various ferns: Blechnum orientale L., Cibotium barometz (L.) J.Sm., Cyathea latebrosa (Wall. ex Hook.) Copel., Dicranopteris linearis Burm., Drynaria quercifolia (L.) J.Sm. and Stenochlaena palustris (Burm. f.) Bedd. (Chai et al., 2012; Lai & Lim, 2011, Lai et al., 2010). In China several fern rhizomes have been shown to have antioxidant properties: Drynaria fortunei (Kze.) J.Sm., Pseudodrynaria coronans (Wall. ex Mett.) Ching, Davallia divaricata Bl., D. mariesii Moore ex Bak., D. solida (Forst.) Sw., and Humata griffithiana (Hook.) C.Chr. (Chang et al., 2007).

Ferns also contain substances with antibacterial activity as was shown in the study by Thomas (2011) of Osmunda regalis. India has made important advances in this area and Kumarpal (2013) showed that there was good antimicrobial activity in three species of ferns from three different families used in traditional medicines in Darjeeling, Athyrium filix-femina (L.) Roth (Woodsiaceae), Dicranopteris linearis (Burm. f.) Underw. (Gleicheniaceae) and Ploepeltis macromarpa (Bory ex Willd.) Kauff. (Polypodiaceae). Patric Raja et al. (2012) found antibacterial and antifungal activity in Cyathea nilgiriensis Holttum, C. crinita (Hook.) Copel., Leptochilus lanceolatus Fée and in Osmunda hugeliana Presl. Studies made in Romania by Soare et al. (2012) showed that the bladder fern Cystopteris fragilis (L.) Bernh. and Polypodium vulgare L. strongly inhibited various bacteria, especially Escherichia coli. Most of the species that have been studied for their pharmaceutical properties have notable antecedents in traditional medicine. The development of pharmaceuticals from plants used in the pharmacopeia of local peoples ought to ensure that the benefits are shared with the traditional communities from where the original information came (Prance, 1991).
FERNS IN TOXICOLOGY

The relationship between plants and people is not always a positive one. This includes those species that have strong substances that are damaging either to human health or to that of domesticated animals. *Pteridium aquilinum* has been studied for the relationship between the various uses and its toxicity (Alonso Amelot, 1999; Franca *et al*., 2002; Ortega, 1993; Vetter, 2011). Recent studies show that *Blechnum orientale* is able to absorb heavy metals from the environment and it is therefore a potentially toxic source of food and medicine. The accumulation is mostly in the rhizomes and leaves, the very parts of the plant that are eaten (Zhu *et al*., 2013). A positive side for the environment of this absorptive capacity of ferns is the accumulation of arsenic by *Pteris vittata* L., which could be used as a method of decontaminating toxic sites. Phytoremediation of arsenic contaminated environments will involve growing the arsenic hyperaccumulator ferns in the contaminated environment, harvesting the arsenic-rich biomass and the safe disposal of the biomass (Gumaelius, 2004; Rathinasabapathi *et al*., 2006).

FERNS AS ORNAMENTAL PLANTS

A large number of fern species are used as ornamentals in the gardens around the world, and this dates back to ancient times and often to domestication for other uses. Studies of the ornamental potential of pteridophytes of regional floras has led to an increase of their use as cultivated plants. Abraham *et al.* (2012) listed a total of 153 ferns and 18 lycophytes from Nilgiris, India with ornamental potential. Macaya (2004) mentioned 20 species of ferns native to Chile that are cultivated as ornamentals and in Macaya (2008) he expanded this to a list of 75 species. An example of the relevance of ethnomotony for making strategies for use of populations of native vegetation is that of Baldauf *et al.* (2007), who made an ethnomotanical study of the management systems of *Rumohra adiantiformis* (G. Forst.) Ching. This species is used as an ornamental in southern Brazil. Another fern of the southern cone region of South America with high ornamental potential is *Blechnum tabulare* (Thunb.) Kuhn, a species that resembles the Cycadaceae in appearance. A population study of this species was made in southern Brazil (Rechenmacher *et al*., 2007). The stems of some tree ferns are used extensively as a substrate for the cultivation of epiphytes. However, the indiscriminate use of this resource is threatening populations of these plants in some places. An example of this is the use of *Dicksonia sellowiana* Hook., the conservation of which has led to studies of growth, phenology and germination of spores (Filippini *et al*., 1999; Schmitt *et al*., 2009).

FERNS AND SOIL QUALITY

*Azolla* Lam. has long been used as a fertilizer in rice paddies (Jones, 1987). In Misiones, Argentina farmers identify compacted and degraded soils by means of observations on the presence of *Pteridium aquilinum*. In the central Andean region of Peru indigenous peoples of the Quechua language group use the fronds of *Dennstaedtia glauca* (Cav.) Looser to fertilize the soils where they cultivate potatoes (Camino & Jhons, 1988), which is similar to the use of bracken peat as a fertilizer in some places in the United Kingdom.

CONCLUSIONS

This brief summary has shown the diversity of important uses to which humans have put ferns and lycophytes, and some of these are illustrated in Figures 1-3. Many of these use categories originated in traditional societies (the white area of Figure 4) such as use in rituals, the construction of houses and to make weapons. Other uses are typical of
contemporary society (the dark area of Figure 4) such as the large scale commercialization of ornamental plants and for drug prospecting. However, the division between traditional and modern uses is not precise because many uses fall into both social categories (the grey area of Figure 4). For example many medicinal uses arose in traditional societies that have gradually modernized, still using ferns in their medicine but also leading to prospecting and use in pharmacology. This much more intensive use of ferns in modern societies has led to the destruction of the habitats where they grow. This has increased the importance of habitat conservation of ferns and the development of commercial production rather than harvesting from the wild.

Figure 4. Examples of the importance of ferns in traditional and modern contexts.
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A SHORT BIOGRAPHY OF THE AUTHORS

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The authors in a Guarani village in Misiones, Héctor Keller at left, Ghillean Prance second from left.
NOTE ON THE REDISCOVERED TYPE SPECIMEN OF
ANGIOPTERIS INDICA DESV. (MARATTIACEAE)

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ABSTRACT
The type of the tree fern Angiopteris indica Desv. (Marattiaceae) was rediscovered in Herb. Desvaux at P and its status is discussed.

INTRODUCTION
Three species of the marattioid fern genus Angiopteris Hoffm. (Marattiaceae) are generally accepted to occur in India (Fraser-Jenkins, 2008; Fraser-Jenkins & Benniamin 2010), namely Angiopteris indica Desv., A. helferiana C.Presl, and A. palmiformis (Cav.) C.Chr. Fraser-Jenkins (2008) accepted A. indica as the oldest available name for plants characterized by the combination of the following characters: the soral lines are located close or at the margin, the lamina segments possess prominent teeth near their tips, the lamina colour is darker than in other Indian species, and with the false (recurrent) veins reaching the soral line or just passing beyond it. In contrast, A. helferiana is distinguishable from A. indica by its inframarginal sori, whereas A. palmiformis has long false veins extended up to the pinnule-midrib.

Angiopteris indica was described by Desvaux in 1813 (Desvaux 1813: 267) and not in 1811 (Desvaux 1811: 207), as misquoted by Moore (1857: 75) and Christensen (1906: 57), but see Hooker & Greville (1831) for the correct citation of the name. In the protologue, Desvaux (1813: 267) described the plants as “frondibus pinnatis, pinnis lanceolatis utrinque attenuates” and mentioned the area of origin as “Habitat in India orientali”. He further considered the non-cordiform base on the segments as the main feature distinguishing this species from previously described taxa. Desvaux in his protologue mentioned that he saw a specimen of the species at Herb. de Jussieu. Later authors (Vriese & Harting, 1853; Fraser-Jenkins, 2008) concluded that no specimens of this species were found in the Herb. de Jussieu, which is located in the herbarium at the “Museum national d’Histoire naturelle” at Paris (P).

Subsequently, Fraser-Jenkins (2008) designated a neotype: Herb. Wight e Nilghiry [“S. India”], alt. 5000 ped. Wallich List No. 187.8. (K-W). This designation is based on the crucial assumption that no specimen seen by Desvaux can be found.

RESULTS
Here, I present the discovery of a single specimen located at “Herbier de A.N. Desvaux Donné par Mme Vve Lavallée en 1896” in the herbarium P (Fig. 1A) with the barcode P01646217 (http://sonneratphoto.mnhn.fr/2010/08/09/P01646217.jpg). The specimen carries two labels on the left side that were interpreted as written by Desvaux showing the name “Angiopteris indica Desv., journ. bot. appl, p. 267, Habitat in India orientali” (Fig. 1D). The second label mentions (in French) “was not described in my memoir given at Berlin” (Fig. 1D). By this annotation Desvaux indicated that the name A. indica was
not published in “Berlin Magazine” in 1811 along with many new taxa he described there. A third label below the pinna fragment shows the name “Angiopteris glauca Desv.” (Fig. 1B). This name does not correspond to Angiopteris glauca Alderw. Mal. Ferns, Suppl. I, Corr. 61 (1917) and it is interpreted here as an unpublished name considered by Desvaux.

Figure 1. Specimens of *Angiopteris indica* Desv. A. Herbarium sheet (P01646217) with two pinnule-segments, B. fragmented pinnule (Type), C. second pinnule with long false veins, D. labels written by Desvaux. Images © Muséum national d’Histoire naturelle, Paris Herbarium (P), reproduced with permission.
The putative type is a fragment of pinnule showing prominent marginal teeth, inconspicuous false veins and sori inframarginal (Fig. 1B). A morphologically distinct second pinnule pasted above it (see Fig. 1A) has long false veins extended up to pinnule-midrib, rounded base and nearly median sori (Fig. 1C).

**DISCUSSION**

One of these pinnules should be considered as type of *A. indica* as it was definitely studied by Desvaux, supported by the evidence of his own handwritten labels, and which can be assumed to be the lost type of *A. indica* from Herb. de Jussieu. The second pinnule (Fig. 1C) is not a separate portion of the same plant but was pasted from a different specimen (pinna): Java, *Leschenault 642* (barcode P01646218, [http://sonneratphoto.mnhn.fr/2010/08/09/4/P01646218.jpg](http://sonneratphoto.mnhn.fr/2010/08/09/4/P01646218.jpg)); Jean-Baptiste Leschenault was a French botanist and his plant collections from Java were subsequently used by A.L. de Jussieu and others. But there is no evidence to support the view that Desvaux selected this second pinnule (Fig. 1C) as type and pasted it himself. Actually the second pinnule was originally cut and pasted in an inverted position in P01646218. As a result it was easily detached and later erroneously pasted in P01646217.

Desvaux coined the name *A. glauca* Desv. probably due to glaucous lamina of the pinnule (Fig. 1B), but later revised it as *A. indica* considering the distribution “India Orientali,” which includes modern India, Sri Lanka, Indonesia, Philippines and Marianas (Cook 2009).

**CONCLUSION**

The fragment of pinnule (Fig. 1B) is chosen here as the type of *A. indica* from the two pinnules in Figure 1A. This type, actually lectotype (designated here), refutes the application of the name *A. indica* to plants from India with marginal sori by Fraser-Jenkins (2008) in displaying inframarginal sori (Fig. 1B), similar to *A. helferiana* (having sori about 1 mm distant from margin). But due to wide range of morphological variability of species of *Angiopteris* further additional data are required to confirm the identity and correct number of species of *Angiopteris* in India.

**ACKNOWLEDGEMENTS**

I thank Mr C.R. Fraser-Jenkins, Nepal for discussing the present topic and translation of Desvaux’s French annotation; Muséum national d’Histoire naturelle (MNHN), Paris Herbarium (P) and Dr G. Rouhan, Curator of Ferns & Lycophytes, MNHN for granting permission for publication of the image; and an anonymous reviewer for improving the manuscript.

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FIRST CHROMOSOME NUMBER REPORT FOR *CYSTOPTERIS FRAGILIS* (CYSTOPTERIDACEAE: PTERIDOPHYTA) IN IRAN

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Keywords: fern, polyploidy, tetraploid.

ABSTRACT

The chromosome number and ploidy level of *Cystopteris fragilis* (L.) Bernh. are documented for the first time from Iran. The count was tetraploid, with 2n= 4x= 168. This agrees with previous reports of tetraploid *C. fragilis* from Europe, Asia and the United States.

INTRODUCTION

*Cystopteris* Bernh. (Cystopteridaceae) comprises about 27 species distributed throughout the temperate regions and tropical mountains (Rothfels et al., 2013). Three species occur in Iran: *C. alpina* (Lamark) Desvaux, *C. dickieana* Sim and *C. fragilis* (L.) Bernh. (Roux 2001; Khoshravesh et al., 2009). The two latter species have also been reported from high elevations in the Hindu Kush Mountains of neighboring Afghanistan (Breckle, 1987). In general, these species of *Cystopteris* are found in shady, rocky, wet habits (Bhellum and Razdan, 2012). The taxonomy and distribution of its species in Iran have been reported by Khoshravesh et al. (2009). *Cystopteris fragilis* differs from *C. dickieana* in having spiny spores (vs. verrucate in *C. dickieana*).

The chromosome numbers of n=84 and n=126 (tetra- and hexaploid, respectively) of *C. fragilis* were first reported from Europe (Manton, 1950). Vida (1976) later reported octaploids in Central Europe. In North America, tetraploids and hexaploids of *C. fragilis* have been found (Wagner, 1955; Haufler and Windham, 1991; Haufler et al.; 1995, Paler and Barrington, 1995). In Asia, tetraploids and hexaploids have been reported from India and Japan (Bhellum and Razdan, 2012; Mitui, 1970). In 2010, a pentaploid with 2n=210 was also recorded from Mongolia Altai by Kawakami et al. (2010). For Iran, however, chromosome numbers of *C. fragilis* have not been reported previously. This paper represents the first such report for the country.

MATERIAL AND METHODS

Fixed cytological material and voucher specimens were collected by the first author from three populations in natural habitats in Akhlamad waterfall valley, ca. 65 km NW of Mashhad, NE Iran at an elevation 1500 m in May and June 2013. The populations occurred on stony or rocky slopes exposed to waterfall, within shaded habitats, and occasionally in the cement of the roadside. Vouchers of examined specimens are deposited in IAUM and NY. Our initial identification was checked by comparison with descriptions in the floristic literature (Khoshravesh et al., 2009) and confirmed by Robbin C. Moran at NY.

The chromosome squash method used followed that of Windham and Yatskievych (2003). Pinnae with whitish sporangia were collected every half hour from 9 am to 1 pm. The pinnae were fixed in Farmer’s Solution (1:3 glacial acetic acid-ethanol alcohol)
and Carnoy’s Solution (ethanol, glacial acetic acid and chloroform in the proportion of 6: 1: 3). Large numbers of sporangia were squashed in acetocarmine. Photography were taken with a digital camera Dino-Lite connected to Labomed LX 400 microscope.

RESULTS AND DISCUSSION
We found n=84 pairs (bivalents) at meiotic metaphase I (Figure 1). This is the first chromosome count of C. fragilis from Iran. This number agrees with previous counts from Jammu and Kashmir ((Bhellum and Razdan, 2012), Mongolian Altai (Kawakami et al., 2010) in Asia; Finland, (Sorsa, 1961), and Iceland (Löve & Löve, 1943) in Europe; Ontario, British Columbia, and Greenland in Canada (Britton, 1953; Taylor and Mulligan, 1968; Dalgaard, 1989), and Michigan, USA (Wagner, 1955).

Each sorus contained 6–19 sporangia. The most important factor in obtaining meiotic figures at the proper stage was the time of fixation. Descriptions and the figures in the present paper refer to fixation at 11am. Tetrad spores were produced by the second meiotic division and many well filled, spiny spores were observed (Figure 2). The size of 25 spores from one leaf was measured in distilled water as followed: 38.48–48.60 × 23.40–28.65 mm. The size straddles that reported for tetraploids and hexaploids within Cystopteris by Blasdell (1963). He found that tetraploids had spore lengths from 32–42 mm and hexaploids from 38–48mm. It is not known what mounting media Blasdell used to measure his spores—whether distilled water, Hoyer’s solution, or Canada balsam. Use of different mounting media might explain why our results of spore length measurements do not fall exactly within the tetraploid range reported by Blasdell (1963).

ACKNOWLEDGMENTS
We thank Dr. R. C. Moran (NY) for protocol advice, comments on the manuscript, and confirmation of the species identification. We also thank Mr. M. Joharchi for the original identification the species and M. Basiri for his assistance in the field.

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Figure 1. A. Chromosomes of *C. fragilis* at meiosis, n= 168; B. Explanatory diagram for a, showing 84 bivalents. Scale bars= 20 μm.

Figure 2. Surface (A) and internal (B) features of spiny spores of tetraploid *C. fragilis*. Scale bar= 20 μm.


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THE DUTCH RUSH:
HISTORY AND MYTH OF THE EQUISETUM TRADE

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Key words: Equisetum hyemale, economic botany, ethnobotany, trade history

ABSTRACT
In England in the early 19th century at least two products went by the commercial name Dutch Rush, viz. the Rough Horsetail Equisetum hyemale L. used in cabinet making and similar crafts, and the Common Club-rush/Bulrush Schoenoplectus lacustris (L.) Palla used in matting and chair manufacturing. Some authors did not heed the scientific names and confused the properties and geo-cultural backgrounds of both products. Thus the myth took hold that E. hyemale was in culture in the Netherlands and that is was deliberately planted and cared for to protect that country from the sea. Scarce but widespread evidence of trade reveals that this species was economically insignificant. The idea that it owes its common name to imports from Holland could be correct; however, other parts of North and Central Europe, especially the upper Rhine Valley, are more likely to be the original sources from where the Dutch obtained the plants. North America can be reasoned to be an alternative origin, but evidence for this hypothesis is still lacking.

INTRODUCTION
Ever since the sixteenth century, many authors in a number of Western European countries have reported the use of Equisetum hyemale L. by various trades (e.g. Fuchs, 1543; Gerarde, 1597; Bock & Sebisch, 1630; Pexenfelder, 1670; Ruppe & Haller, 1745; Anon., 1749; Pernety, 1771; Krüniitz, 1785; J. E. Smith, 1802; Headrick, 1813; Stewart, 1815; Phillips, 1818; Hooker, 1821; Gill, 1822). Material evidence of its use is found in inventories of workshops (e.g. Giskes, 1979) and characteristic scratches from its siliceous skin on antique woodcraft (Esterly, 1998). In addition, several authors during the past two centuries showed that the vernacular name “Dutch Rush” used in England relates to large-scale imports from Holland (Pratt, 1846; Francis, 1851; Johnson & Sowerby, 1856; Moore, 1861; Pratt, 1866). Up to the present day the statement is repeated frequently (Weeda et al., 1985; Øllgaard & Tind, 1993; Page, 1997). The apparent source to which this can be traced back is Edward Newman (Newman, 1842, 1844), who also noted that the species was cultured in Holland and played an important role in the defence of the coast against the eroding action of the sea:

“… for this purpose it is imported, under the name of “Dutch Rush” in large quantities, from Holland, where it is grown on the banks of canals and on the sea ramparts, which are often bound together and consolidated by its strong and matted roots.”

1Wherever the toponym Holland is used, literal citations excepted, it is to be understood in the strict sense, i.e. the western part of the Netherlands, or the present provinces Noord-Holland and Zuid-Holland.
This is at odds with the ecological preferences of the species, as well as with both its historical and present day distribution (de Winter, 2007). These problems can be summarised under three main points:

1. the alleged abundance of *E. hyemale* in Holland finds no evidence in its current distribution in that country, nor is it supported in historical Dutch reports;

2. the reported occurrence and even cultivation of *E. hyemale* along canals and the practice of using it for the solidification of dykes, dunes, or any kind of coastal protection constructions finds no reference in Dutch literature, nor in the present distribution and habitat preferences of European *E. hyemale*;


If these doubts are well-founded, then the question must be addressed as to whether *E. hyemale* really was imported from Holland to England, and where the origin should be sought of the plants sold on London markets.

### HISTORY OF THE NAME “DUTCH RUSH”

A number of vernacular names have been used for *E. hyemale* in English, but most of them never became popular and have disappeared into oblivion (Table 1). The oldest historic one is “shave-grass”, which was first recorded in the 14th century: “Cauda equina, cauda Caballina idem est. angl, schaugres” (Anon., 1350-1400; Lancaster, 1887; Murray, 1971).

To *shave* is derived from the Anglo-Saxon *scaftan* through the Old English *shaven*, and Middle English *schaven/schafen* (Flexner & Hauck, 1993), whence there is a direct relation with the Old High German *scafti(h)awi* (Graff, 1838) and the Dutch stem *schaaf* - as in *schaafstro* (de Winter, 2012). Although the verb “to shave” at present is predominantly used to refer to the process of removing the beard, the general meaning is to make a surface smooth (Webster, 1913). The name is continuous through Turner (1538) and Gerard (1597). “Shave-grass” has been largely replaced now by the more official “rough horse-tail” (Bolton, 1790; J. E. Smith, 1802), and “Dutch Rush”.

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**Table 1. Vernacular names used in British English**

<table>
<thead>
<tr>
<th>Name</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shave-grass</td>
<td>(discussion in this paper)</td>
</tr>
<tr>
<td>Dutch Rush</td>
<td>(discussion in this paper)</td>
</tr>
<tr>
<td>Rough horsetail</td>
<td>From the French/Latin <em>asprella</em> (de Winter, 2012), introduction unknown</td>
</tr>
<tr>
<td>Dutch shave-grass</td>
<td>species list of Scarborough (Hinderwell, 1811)</td>
</tr>
<tr>
<td>Holland rushes</td>
<td>In advertisements (Glover, 1843; Measom, 1861)</td>
</tr>
<tr>
<td>Dutch reed</td>
<td>rarely (Bailey, 1756; Martin, 1813; Henslow, 1839; Trimmer, 1842)</td>
</tr>
<tr>
<td>polishing rushes</td>
<td>customs’ duties lists (Burn <em>et al.</em>, 1831; Parnell, 1831; Ellis, 1837)</td>
</tr>
<tr>
<td>shave weed</td>
<td>(Aubrey, 1848)</td>
</tr>
<tr>
<td>shauynge gyrs</td>
<td>(Turner, 1538)</td>
</tr>
<tr>
<td>dyßhewaßhynges</td>
<td>(Turner, 1538)</td>
</tr>
<tr>
<td>Pewterwort</td>
<td>e.g. (Withering, 1776; Targioni Tozzetti, 1813; Wilkinson, 1858)</td>
</tr>
<tr>
<td>scouring[-]rush</td>
<td>American (Law Olmstedt <em>et al.</em>, 1924)</td>
</tr>
</tbody>
</table>
The latter name has been in use since around 1700 and first appears in manuals of practical trades (C.K., 1701; Artlove, 1730; Barrow, 1735; Anon., 1754; Dossie, 1758). Eighteenth century dictionaries show a more conservative attitude towards this emerging name and strictly print “shave-grass”. The name “Dutch Rush” therefore remains dormant in written language until the second edition of the cryptogamic volume of William Withering’s *Botanical Arrangement* was published (Withering, 1792), in which the name “Dutch Rush” is used equivalent to “Shave-grass”. The *Botanical Arrangement* was an immediate and sustained success that would see many reprints (Lee, 2001). Inclusion in this work apparently made the name “official” and acceptable for use in subsequent publications (Figure 1). This pattern strongly suggests that since the onset of the eighteenth century something has changed that made people name the product by its origin rather than its purpose. Just assuming that the increasing trade with the low countries flooded the market with Dutch horsetails seems unsatisfactory as an explanation. A size and/or quality difference as suggested by Newman might have changed the perception and initiated the desire to distinguish the new product by name.

**IMPORTS OF HORSETAILS INTO BRITAIN**

Even though *E. hyemale* is not especially rare in Britain (Wardlaw & Leonard, 2005), it was certainly felt so (Camden, 1722; James, 1745). Since urbanisation and industrialization in England started long before the invention and acceptance of sand paper in the mid 19th century, it is conceivable that here, more than elsewhere in Europe, the discrepancy was felt between urban demand and rural supply. Especially the reconstruction of London after the fire (1666) may have elevated the demand.

The earliest account of exports from the Netherlands are found in a manuscript of the late 17th century by John Aubrey (Aubrey, 1847): “Watchmakers and fine workers in brasse use it after smooth filing. They have it from Holland”. This can be dated more exactly, for in a letter to John Ray of August 5, 1691 he writes: “Shave-weed used by artists (which they have from Holland)” (Aubrey, 1848).

![Figure 1](image-url)

**Figure 1.** Relative usage of the names “Dutch Rush” (darker bars) and “Shave-grass” (lighter bars) in English literature from 1720-1860. Counts made per 20 years periods, based on 178 printed publications, not including advertising.
The demand declined when glass paper came into use, but it never completely disappeared. In the USA it was still advertised in 1938 (Kaliban’s Grocery and Market, 1938). Nowadays some still prefer it to scrape their clarinet reeds (Intravaia & Resnick, 1965; Taillard & Dalmont, 2012).

Testimony of imports into England, which lasted more than one and a half centuries, exists in lists of import duties of the English customs (Burn et al., 1831; Parnell, 1831; Ellis, 1837), but is absent in earlier legislation (e.g. Steel, 1796). Often the horsetails could be imported, transported, and exported tax-free (de Martens & Murhard, 1836; Anon., 1837; MacGregor, 1843; Anon., 1849a, 1853, 1858) or they were not considered important enough to justify their own category (Koninkrijk der Nederlanden, 1816, 1822). As a consequence, no accounts of their trade were kept and they do not show in yearly statistical surveys (Departement van Financiën, 1848). England and Holland were the two countries with the worst kept statistics on agriculture and trade (MacGregor, 1843). Yet, although far from complete, a few statistics of the early period remain, covering the imports into the port of London and allowing sampling of the existing data of imports of *E. hyemale*. However, in all of them the horsetail trade is conspicuous by its absence, *viz.* in January 1683 (Houghton, 1728), from May to October 1735 (Anon., 1735), and from January to June, 1776 (Whitworth, 1777b, 1777a).

In the nineteenth century Dutch newspapers meticulously reported freight loaded and unloaded per port, frequently even per ship. Any product of trade of any importance would be expected to be listed. Nonetheless horsetails are not mentioned by any of their known names. With one exception, however: in the second half of 1855, 142 inland navigation barges with in total 6050 bundles of shave-grass arrived in Brussels (Anon., 1856). Estimating the total volume of *Equisetum* transported requires acquaintance with the size of such a bundle, which we do not know. A rough estimate for a bundle of 80 cm perimeter (as is used at present for bulrushes: de Vries, 2008) would be 500 – 1000 stems. Given that dense stands have 200 – 500 stems per m² (Rutz & Farrar, 1984), the annual production for Brussels only would have required the depletion of c. 0.6 – 3 ha. Such small amounts could easily have been found on Belgian territory, but apparently for reasons of quality or cost, long-distance transport per ship was more attractive.

In the mid-nineteenth century, exports of reeds and rushes to Britain exceeded those to Belgium by about 50% (Departement van Financiën, 1848) and if the export of horsetail may be estimated proportionally to this, the economic value must have been insignificant. John Yeats, an English commercial-geographer, who lived in Holland in 1845/46, compares *E. hyemale*, which “is occasionally imported from Holland” with *Scirpus lacustris* L. (now *Schoenoplectus lacustris* (L.) Palla), saying that “Many vessels laden with this rush arrive annually in England from Holland and Belgium, bringing thirty or forty tons of rushes each voyage. This is a very large quantity considering the lightness of the material. More than 1,000 tons of bulrushes are annually imported into the United Kingdom” (Yeats, 1870). It should be noted, though, that these figures stem from the second half of the nineteenth century, when the use of *E. hyemale* was already on the decline in favour of sandpaper, of which mass production in London had started by 1833. But also much earlier, in 1827, international horsetail trade was but marginal. From an example record of the net produce of customs duties it can be deduced that the total value of polishing rushes legally imported into Britain must have been £13.25 for that year.
CULTIVATION AND CARE IN HOLLAND: COAST PROTECTION

Newman’s statement, that the Dutch grew *E. hyemale* “on the sea ramparts, which are often bound together and consolidated by its strong and matted roots” (Newman, 1844), is repeated with slight variations in later literature. The authors obviously have copied each other in sequence, rephrasing the allegation without adding new observations:

“it is grown on the banks of canals and on the sea ramparts, which are often bound together and consolidated by its strong and matted roots” (Newman, 1844)

“The Dutch are well acquainted with the value of its long and matted roots in restraining the wasting effects of the ocean, which would soon undermine their dykes were it not for the *Equisetum hyemale* which is planted upon them” (Francis, 1851)

“the plant is of immense value in its native country from the extraordinary length and interlaced growth of its root-fibres, which mat together and consolidate the loose and swampy soil in which they grow, and thus form one of the most effectual water-dams of so level a land.” (Wilkinson, 1858)

“this species is planted to support embankments, which it does by means of its branching underground stems” (Moore, 1861)

Along long stretches of the coast of Belgium and Holland, and the outward coasts of the islands in the North, belts of natural and semi-natural sand dunes protect the country from the sea. The hybrid *E. × moorei* Newman (E. *hyemale* × *E. ramosissimum* Desf.) is found in great numbers at several locations in the dunes of Zuid-Holland and southern Noord-Holland. It was first reported by Du Mortier in 1825 (as *E. trachyodon* A.Br.) near Beverwijk (Du Mortier, 1869), where it still can be found today. It is locally abundant between Scheveningen and Hook of Holland, where it has been known since the late 18th century (Anon., 1796; van Hall *et al.*, 1832). It is surprising that J.E. Smith (1793) did not note it when visiting the place. Leiden (L) has a number of specimens collected in the West of Holland during the 19th century; however, until 1870, when the first collection was made from the fore-dunes south of Scheveningen, all the collections originate from the interior dunes around Haarlem.

In 1797, Jan Kops set out to report on the state of the dunes of Holland. He was an eager botanist, paid special attention to any plants that could be used to control shifting sand and, as an agronomist working for the ministry, was also keen to find plants that could be made profitable in any way (Baert, 1943). If Newman with “the consolidation of sea ramparts” referred to the fixation of shifting sands in the dunes, it is hard to imagine that this would have escaped Kops’s attention. Yet Kops explicitly states that no other means are known to him to fix the dunes than marram (*Ammophila arenaria* (L.) Link), straw, and reed mats (Kops, 1798; van Eys *et al.*, 1799). Neither earlier (Montin, 1771; Le Francq van Berkheij, 1780; van Geuns, 1789) nor later authors (Spengler, 1891; Vuyck, 1898) of technical treatments about sand fixation mention *Equisetum* for this purpose, even though Le Francq van Berkheij lists “Paardestaart, *Equisetum*” among the plants of the fore-dunes.

Despite the present abundance of the hybrid in the dunes of southern Holland never, from the 18th century to the present, has its distribution been said to be deliberately promoted by man, and the remarks of Francis and Newman above cannot be attributed to it.

Where dunes are weak or absent, dykes have been built to protect the land, as in the topographically complex estuarine area of Zeeland and Zuid-Holland, along the rivers, along the entire coast of the Wadden Sea, and, before 1932, along the Zuiderzee and its inlets. Sea-dykes must be protected by a hard surface since no vascular plant can form mats firm enough to protect the dyke’s surface from the action of the waves. At the onset of the
19th century such hard-shells were not yet customary.

Reed and rushes were recognised as effective agents to break the action of the waves and to prevent erosion of the banks of lakes (Meese, 1768), but they grow neither in seawater, nor at the high water limits of rivers. Sea-dykes were protected by stapling bundles of straw, eelgrass and rushes to the dyke-surface to form a soft shell that had to be renewed every autumn (Bréval, 1726; Schraver, 1807).

Inland river dykes consist of a core of sand or clay, covered with a water resistant layer of clay and finished with a grass-covered top layer (Fliervoet, 1992). Mats of superficially rooting herbs protect the surface against erosion, but deep-rooting plants weaken the construction (de Haan et al., 2001; 2003) and can perforate the water resistant clay mantle (Technische Adviescommissie voor de Waterkeringen, 1985), without adding much to the stability (Sykora & Liebrand, 1987). High growing dense stands of E. hyemale could outcompete lower herbs that offer better protection. Finally, frequently grazed or mown low vegetation is less than optimal for this woodland species and at present it is not found on dykes, nor are there collections in the National Herbarium from such places. The hybrid E. × moorei has a number of stations along the river Rhine, but it is still rare on dykes. Altogether both technical and historical evidence for Newman’s statement appear to be entirely lacking.

OVER-REPORTING OF DUTCH RUSH DUE TO DUTCH RUSHES

The likely source of the delusion is the 18th century bestseller, *The Gardeners Dictionary* (Miller, 1754, *Ed. 4*) that depicts how species of rush “grow on the Sea-shores, where they are frequently watered by the Salt-water. These two Sorts² are planted with great Care on the Banks of the Sea in Holland, in order to prevent the Water from washing away the Earth; which, being very loose, would be in Danger of removing every Tide, if it were not for the Roots of these Rushes; which fasten themselves very deep in the Ground, and mat themselves near the Surface, so as to hold the Earth closely together. Therefore, whenever the Roots of these Rushes are destroyed, the Inhabitants immediately repair them to prevent farther Damage.” This paragraph is quoted from the section on *Juncus* as “Juncus acutus”.

Miller must have meant the glaucous bulrush *Schoenoplectus tabernaemontani* (C.C. Gmelin) Palla that was cultured in great numbers in the brackish water of the Maas estuary, and possibly to some extent *S. maritimus* (L.) Lye, which was not cultured, but common in the more seaward parts of the estuary and known for its even greater capacity of promoting the sedimentation rate of silt than the former (Clevering & van Gulik, 1990; Weeda et al., 1994).

Miller’s handbook on wild and cultivated plants went into eight editions³, and was copied (e.g. MacFarquhar & Gleig, 1797; Knight, 1833), and translated into French, German and Dutch. As Miller’s text was copied over and over during more than a century, gradually elements changed, and new ones slipped in (Table 2).

Noteworthy is Loudon’s addition that combines the name Dutch rush, and its usability for scouring metals (Loudon, 1829). Rushes (for making chairs) were imported from the

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²i.e. *Juncus acutus, capitulis sorghi* C.B.P. & *J. acutus maritimus Anglicus* Park.

³Cited here is the fourth edition of 1754, but essentially the same text is found in the Dutch translation of 1745 (Miller, 1745) and therefore in earlier English editions not seen by the author.
The first and second sorts grow on the sea shores, where they are frequently washed by the salt water. These two sorts are planted with great care on the banks of the sea in Holland, in order to prevent the water from washing away the earth; which, being very loose, would be in danger of removing every tide, if it were not for the roots of these rushes; which fasten themselves very deep in the ground, and mat themselves near the surface, so as to hold the earth closely together. Therefore, whenever the roots of these rushes are destroyed, the inhabitants immediately repair them to prevent further damage. In the summer-time, when the rushes are fully grown, the inhabitants cut them, and tie them up into bundles, which are dried, and afterward carried into the larger towns and cities, where they are wrought into baskets, and several other useful things, which are frequently sent into England. These sorts do not grow so strong in England, as they do on the Maese, and some other places in Holland, where I have seen them upward of four feet high (Miller, 1754).

The conglomeratus, and acutus or marine rush, are planted with great care on the banks of the sea in Holland (MacFarquhar & Gleig, 1797).

J. acutus and maritimus are planted on the sea-embankments of Holland, and also in some parts of our own coasts, and in America. The roots run deep into the sand, and form a matted body which holds it together. In Holland, when the plants are fully grown and in flower, they are cut down, dried, and bound up like corn. The J. acutus, being very rough, is used for scouring copper and other vessels, and is one of the plants imported into this country for that purpose, under the name of the Dutch rush. The other species, and often both, are plaited into mats, baskets, chair-bottoms, ropes, etc. (Loudon, 1829).

(…) are made of bulrushes; these grow in this country, naturally but not very commonly, in deep slow streams. The demand for them is greater than the home supply, and a considerable quantity is imported from Holland. (Society for the Diffusion of Useful Knowledge, 1833).

Equisetum hyemale: for this purpose it is imported, under the name of “Dutch Rush,” in large quantities, from Holland, where it is grown on the banks of canals and on the sea ramparts, which are often bound together and consolidated by its strong and matted roots. Bundles of this imported Dutch rush are exposed for sale by many London shopkeepers. (Newman, 1842).
of the product offered (Table 3). It must have been this ambiguous use of the name that led Newman to his misunderstanding. Evidently, when writing about *E. hyemale*, he did not make the distinction and copied unreliable sources without checking.

### CULTIVATION AND CARE IN HOLAND: HARVESTING FOR EXPORT

Whereas in English literature it is often stated that Dutch Rushes were cultivated and exported by the Dutch (Newman, 1842, 1844; Francis, 1851; Moore, 1861; Pratt, 1866), the continental literature remains almost taciturn about any culturing activities. The earliest mention of such a trade in Dutch literature is found in the *Flora Batava* (Kops & Van der Trappen, 1846), published shortly after Newman’s publication in The Phytologist (Newman, 1842).

In the Netherlands, *E. hyemale* is not an abundant species. In the east of the country several healthy populations exist, but it is hard to imagine that these could have supported prolonged harvesting for both domestic and export markets. In Holland in the strict sense, i.e. the western part, *E. hyemale* is virtually absent, but the hybrid *E. × moorei* is quite common in the dunes and along railroads (de Winter & Lubienski, 2012). It would be conceivable that present day populations are not representative for the abundance in the 18th and 19th century. Both habitat destruction and harvesting might have caused such a decline. De Gorter (1781) had found the species in forests in the county Zutphen and Kops & Van der Trappen (loc. cit.) say it is abundantly found near Zutphen, at a number of locations in the West, the latter almost certainly to be attributed to the hybrid, and at some riverine locations for many of which the same applies. In the 17th century, Comelin (1683) saw it in stagnant-water ditches near the river Vecht near “*den Bergh*”⁴, at the border of the firm, sandy soil of Utrecht and the fen areas of Holland.

*E. hyemale* is a species of moist woodlands. The area of woodland in the Netherlands has not declined since 1800, but has actually increased (Bijlsma, 2003), as before that time much had been cleared. Older sources often associate the species with ditches rather

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**Table 3.** Advertisements with “Dutch Rushes” or “Holland Rushes” from before 1850.

<table>
<thead>
<tr>
<th>Advertisements where product defined as:</th>
<th>Number</th>
</tr>
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<tbody>
<tr>
<td><em>Equisetum</em></td>
<td>4</td>
</tr>
<tr>
<td><em>Schoenoplectus</em></td>
<td>8</td>
</tr>
<tr>
<td>Unknown</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
</tr>
</tbody>
</table>

**Sources:** *Bury and Norwich Post:* 29 Nov 1815; *Carlisle Journal:* 09 Mar 1833, 24 Aug 1833; *Carlisle Patriot:* 28 Oct 1820; *Chelmsford Chronicle:* 03 Oct 1783; *Chester Chronicle:* 23 Mar 1849; *Hampshire Advertiser:* 15 Sep 1832; *Hampshire Chronicle:* 26 Jul 1819 2x; *Hampshire Telegraph and Sussex Chronicle:* 23 Jul 1832; *Hampshire Telegraph:* 02 Mar 1829, 25 Apr 1831; *Hull Packet:* 07 Jan 1801, 03 Mar 1801, 27 Feb 1835; *Kentish Gazette:* 07 Jan 1845; *Liverpool Mercury:* 13 Jun 1823, 17 May 1844; *Norfolk Chronicle:* 09 Dec 1815; *Salisbury and Winchester Journal:* 19 Sep 1803, 26 Feb 1821; *Stamford Mercury:* 21 Apr 1848; *The Ipswich Journal:* 22 Jun 1805, 14 Sep 1805; *The Times:* 08 Sep 1831

⁴ presumably Nederhorst den Berg, about 25km SE. of Amsterdam
than woodland, which may be explained by its habit of persisting long after the trees have been felled. Altogether it seems unlikely that the magnitude of the population would have permitted large scale harvesting to support an export trade over a long time and it must be questioned whether this country has ever been the source of such trade.

Comelin (loc. cit.) noted that at den Bergh it was collected for turners to polish with it. But later, from 1806, a number of annual reports have been compiled about the Dutch agriculture, including products gathered from uncultivated lands, such as reed (Phragmites), rushes (Schoenoplectus) and herbs (Kops, 1807, 1808, 1809, 1816, 1819, 1821, 1822c, 1822b, 1822a, 1829). No mention is made of any production or harvesting of Equisetums.

Nozeman (1783) expresses his amazement that E. hyemale is imported into the Netherlands from Spain (see below), rather than collected from local stocks. Subsequent botanists agree: “This plant does grow in some places of our home country, in woods and sandy lands, but it is, as far as I have been able to find out, not used” (van Hall, 1854).

“The shave-grass that the cabinet-makers use does not originate from our land, but is imported from elsewhere. From [the Netherlands] it is further transported to England, where it, completely undeserved, is designated by the name Dutch Rushes” (Oudemans, 1862; likewise: de Vries et al., 1870).

An interesting phenomenon is that, analogous to the English “Dutch Rush”, in the Netherlands it was sometimes called “Spanish rush” (Noel Chomel & De Chalmot, 1778; Van Meerten-Schilperoort, 1830; 1843; van Hall, 1843). The epithet “Spanish” intermittently used in the Dutch names of the species does not indicate a Spanish origin. Though it is found in Spain (Prada, 1986; Salvo Tierra, 1990), it occurs too infrequently to allow any significant yield (C. Fraser-Jenkins, pers. com.). Also, historically and regionally the predicate has been applied to at least thirty other species (Heukels, 1907), which seem to have little in common, though for quite a few of them it might be interpreted as “conspicuous” or “foreign”.

A few authors hold the view that that the Netherlands does or could produce commercial horsetails: “This wood [Zalkerbos] also furnishes very good shave-grasses to polish wood” (van der Aa, 1851), without revealing his source nor the scale of the exploitation. In almanacs beggar boys were urged to collect shave-grass (among other products of nature) to earn their own living (Heldring et al., 1837; Heldring, 1845). This may denote more about the low value and little enthusiasm to collect the stems in the Netherlands. Altogether, the conclusion seems justified that if Dutch-produced horsetails were ever imported into England, this must have taken place longer ago than the memory of the 19th century writers cited above, therefore no later than the first half of the 18th century.

CONTINENTAL TRADE AND ORIGIN OF THE SPECIES

Even if the plants have never been cultivated in the Netherlands, nor commercially harvested from the wild, it is still possible that Dutch traders imported them into London from other continental or overseas sources. The question then is where they would have originated.

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5Comelin’s habitat description is more typical for E. fluviatile L. with which E. hyemale was often confused, and so was his description: Equisetum foliis nudum, non ramosum sive junceum. B. pin. Hippiris foliis & nuda Tab. Equisetum majus aquaticum prim. icon J. Bauh. Belg. Groot Paarde-staart. However, his observation on collecting the plant shows that he was at least partially correct.
There is evidence of horsetail trade elsewhere in Europe. Tariffs of the Zollverein (customs union, 1818-1871) name *Schachtelhalm* in near complete lists of items transported through the German lands (Anon., 1824; de Martens & Murhard, 1836; Anon., 1849a, 1849b, 1853, 1858). Likewise, Russia levied a small duty on horsetails (MacGregor, 1850), as did Norway, (Arntzen, 1830), France (Dujardin-Sailly, 1813) and Austria (Klenner, 1822; Anon., 1824, 1838), whereas Illyria (Anon., 1836) let them pass for free.

A number of more incidental sources can be found too. According to an early 19th century encyclopedia, *E. hyemale* was a lucrative product and exports from the southern French department *Bouches-du-Rhone* amounted to 10,000 francs annually (Leman, 1826). The amount in francs suggests the figure applies to statistics postdating 1795, when this currency had been introduced. The 1802 overview of this department, however, makes no mention at all of such an export (Michel, 1802). Moreover, it is stated that the department is very poor in woodland, a condition that has not changed to the present, and this explains the near absence of *E. hyemale* in those parts (J. le Paslier & C. Déliry, pers. com.). Duval-Jouve also expressed his doubt about the figures he had from de Villeneuve (de Villeneuve, 1821), but after investigating the matter he found that they referred to *E. ramosissimum* and *E. palustre* L., of which at least 30,000 bundles were sold each year for kitchen usage (Duval-Jouve, 1864). Local trade in Provence was provided by vendors crying “*Leis fretadous de coussooudo*” (Achard, 1785), who were not likely to be selling the hard skinned *E. hyemale*, since their merchandise would have ruined the kettles.

Abundant sources of *E. hyemale* were found in the forests along the Rhine, where specialised collectors took it to sell it to carpenters and cabinetmakers, or to plait rings of them (the so-called *Schafheukränze*), to clean kitchen utensils and floors (Kirschleger, 1857). These rings, also called *Schlutte* (Acker, 1982), are identical to the one depicted by Bock (1630) and described by Bauhin: *apud nos ancillae circulos (qualis figurae adpositus,) ad suppellectilem expoliendam conficiunt* (Bauhin, 1658). Long distance commercial trade of *E. hyemale* for use by craftsmen to polish wood and metal has been reported from south-west Germany, where they were imported into Württemberg in great masses from the Rhine valley in Baden (von Martens & Kemmler, 1882). French collectors apparently drew a heavy toll on the population, since Hauser (1894) complained that “a few decades ago” at Neubreisach the traders took cartloads of *E. hyemale*, but that at the time of his writing they would have come in vain. Plants collected near Strasbourg (dept. Bas-Rhin) were sold in Nancy, 120 km to the west (Braconnot, 1828; Kirschleger, 1857) and to Paris (Duval-Jouve, 1864). Halfway between Strasbourg and Paris, in the Orient Forest near Troyes, it was available for collecting, but, to the amazement of the Troyans, traders did not know this, or preferred to pull it in other places (des Étangs, 1841). Plumier tells us, presumably from Lyon, that it is “a plant brought to us from the mountains”. In southern France commercial trade existed where Villeneuve functioned as the hub selling horsetails collected in the Lot-valley to the market in Bordeaux (de Saint-Amans, 1821).

6“scouring pads of horsetails”

7at ours the maids make wreaths (illustrated adjacent) to polish the furniture

8The printer as well as the religious authorities approving the book resided in Lyon.
Yet one other possible origin should be considered, viz. North America. For this route I have found no historical data, unless it would account for the surprisingly large export volume of “reeds” from the USA (Wolcott, 1795). However, the species is abundant in the USA to the extent of becoming a nuisance (Millhollon, 1987), and the larger American subspecies would explain observations of Newman and contemporaries on the size of the marketed horsetails.

Newman expressed uncertainty about the Dutch Rushes of the London markets being conspecific with English *E. hyemale*, and was supported in that opinion by other, not explicitly named, botanists. His doubts arose from the “much larger size than any British examples of *E. hyemale*” and “the much greater number of striæ, amounting in some instances to thirty-two” (Newman, 1844). Large size and high number of ridges (Figure 3) better conform to the American *E. hyemale* subsp. *affine* which has 14 – 50 ridges than the European subspecies with 14 – 26 ridges (Hauke, 1963). Johnson (Johnson & Sowerby, 1856), however, disagrees, stating that “British plant from Gamlingay Bog, Cambridgeshire, cultivated in my garden for thirty years, frequently rivals the imported “Dutch Rushes” in these respects, the number of ridges and smaller tubes varying from twenty to twenty-eight in the larger stems”. In the Zalkerbos near Zwolle, the only Dutch population which historically has been associated with usage of the plants as smoothing agents, currently has stems of 1.20m tall with 25 – 26 ribs. Although such thick specimens of *E. hyemale* subsp. *hyemale* are memorable in itself, there is still a gap between Newman’s 32 ridges, and the Dutch and Johnson’s 26-28 ridges.

In the Netherlands, Van Hall (1854) wrote: “This plant does grow in some places of our home country, in woods and sandy lands, but it is, as far as I have been able to find out, not used, and it is by far not as heavy as the one that occurs in commerce and is brought in from elsewhere”. Herman van Hall, a student of Kops, was co-author of earlier volumes of the Flora Batava (Kops & Van Hall, 1828, 1832, 1836, 1844) and therefore likely to be very well aware of the note given there, that *E. hyemale* is exported to England. His 1854 remark reads as if he has double-checked the fact, while bearing in mind that a different, larger taxon might be involved. Oudemans (1862) agreed: “The reason why, both at ours and in England, the foreign shave-grass is preferred, seems to be the latter’s thicker stems”.

**DISCUSSION**

At the onset of the eighteenth century, the name Dutch Rush for *E. hyemale* had become so established that it started to show up in print, at first with authors addressing the artificers and amateurs who would actually be using it and, almost a century later, also in the scientific botanical literature. Something must have happened with the product for people to dub it with that new name. Later authors ascribe the name to imports from Holland, but they all might have been deluded by the *Schoenoplectusus/Equisetum*-misconception, with the possible exception of John Smith (1802), who published in advance of the earliest date so far discovered that shows the entanglement. The best argument that Dutch import does explain the name is given by Aubrey’s 1691 letter. While he writes that the shave-grass comes from Holland, he does not use the name Dutch Rush, and therefore this is not likely to be an *ad hoc* explanation of that name.

As for the scale of the imports during the 150 years between Aubrey and Newman, again we must sift through the mystery caused by the *Schoenoplectusus/Equisetum*-misconception. All the imports of Dutch Rush, in “great quantities” or similar wording, are likely to be ascribed to bulrushes. In the eighteenth century, the Netherlands counted
over 4000 ha of land with culture of bulrushes (Maas, 2000) and this was an important export product. In 1827, 1600 loads of 63 bundles of bulrushes were declared at the British customs, accounting for over 360 times more duty revenues than received on polishing rushes (calculations based on Parnell, 1830). If, in spite of Yeats’s opinion, *E. hyemale* would have been of any observable economic significance, one would have expected it to have been seen in the exceedingly detailed port statistics, where it is not. Evidently, half a year could pass without any horsetails being legally imported. In accordance with these incidental observations, it is absent from English and Dutch trade statistics. The possibility that significant amounts of rushes have deliberately been kept from import registration is not very likely. Smugglers concentrated on low-volume contraband where the import duty exceeded the intrinsic value. It is not unreasonable to assume that anything that was taxed was also smuggled, but 20% is a low tariff compared with that levied on alcohol and tobacco, so the incentive for smugglers to bring in shave-grass must have been slight, given that at some points in British history, smuggling carried the death penalty (Richard Platt, pers. com.). The conclusion must be that even if Holland has been an important source in the horsetail trade, the trade as a whole served but a niche market.

The emerging pattern of several disjunct historic observations is that of trade routes from scattered source regions into the most accessible larger centres of population. For unknown reasons source populations closer at hand were ignored. Like Oudemans, De St.-Amans (loc. cit.) thinks it a matter of quality: “Celle des bords du Lot est belle et plus estimée”. Sustained exploitation sometimes resulted in depletion of local stocks. In the upper Rhine Valley an infrastructure of collectors and transporters had developed who transported *E. hyemale* overland to France and downstream by river, as suggested by the toll-treaties of German rivers (Anon., 1837, 1853). The Dutch had virtually monopolised navigation on the river Rhine (MacGregor, 1843). When brought all the way down to the sea, goods would have ended up in the port of Rotterdam, ideally situated for further transport to Belgium and England. Since the species was “rather common in whole Europe, even in Russia, and especially in Sweden” (de Chalmot & Chomel, 1792), it may have been that the collectors took the product from wherever it was available in north-west Europe, including the eastern part of the Netherlands and Russia (which then included Poland). Tariffs categorising horsetails are available from many north and central European countries.

During the Napoleonic wars around 1800, reciprocal trade embargos caused interruptions in the supply of indispensable commodities. Since the French invasion of the Netherlands in 1794, bulrushes from Holland had become difficult to obtain (Anon., 1819) and consequently become expensive: “during the non-commercial intercourse between Holland and Great Britain, which lasted a number of years, a great scarcity existed in (…) Dutch Rushes, which were not to be had during the war, except in very precarious chance lots, occasionally smuggled over, at any price” (E. Smith, 1841). The same will have happened to *E. hyemale* from the continent and it may be hypothesised that in circumvention a new supply route from the United States was opened. The aberrant size of the plants reported by nineteenth century authors indicates the North American subspecies rather than the slenderer European form. If the shipping cost had allowed a profitable transport, the availability would have been virtually unlimited from the largely uncultivated American lands where the species is abundant. However, doubt exists whether the economic opportunities were recognised: “The rushes used for rubbing down are a valuable article, and grow abundantly in various parts of the United States,
they are found in our own neighbourhood in the State of New Jersey; though extensively used in Europe they are scarcely known to our workmen" (Gill, 1828).

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SHORT NOTE

PLAGIOGYRIA MINUTA IS DISTINCT FROM P. EGENOLFIOIDES VAR. EGENOLFIOIDES

Two fern collections from Gunung Gaharu, Serian District, 1st Division, Sarawak, on the border with Kalimantan in Borneo, have usually been filed with grammitid ferns in herbaria. They are Anderson SAR 15696 and Ilias & Azahari SAR 35673, both seen in K and SAR, and said to be in L also. Both collections grew on sandstone cliffs, at 610 m and 790 m respectively, and are conspecific. They differ from grammitids in numerous morphological characters, however, and actually belong to Plagiogyria.

The Flora Malesiana treatment of Plagiogyria (Zhang & Nooteboom, 1998) provides the most up-to-date account of the genus in Borneo, but the specimens were difficult to key to species because although they had the crenate sterile pinna margins of P. egenolfioides (Baker) Copel., the sterile pinnae were slightly narrowed at base, not auriculate which is a character of this species. The key to varieties of P. egenolfioides indicates that the collections belong to var. egenolfioides, being dwarf and with obscure aerophores only at the stipe base, but the description of this variety also states that most of the sterile pinnae are auriculate. Plagiogyria minuta Copel. is listed in the synonymy of P. egenolfioides var. egenolfioides and examination of the image of the type, MICH 1190812, shows that not only is it the same taxon as the Gunung Gaharu plants, but also that it would not key to P. egenolfioides var egenolfioides either, because of the shape of the pinna bases. Copeland (1915) noted that P. minuta was “an evident and near relative of P. egenolfioides, but much smaller throughout, and the sterile pinnae usually narrowed at the base instead of cordate or auricled”. The Flora Malesiana account needs to be revised to take into account the differences between the two taxa.

REFERENCES


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SHORT NOTE

ACROSORUS NUDICARPUS TRANSFERRED TO XIPHOPTERELLA

Acrosorus nudicarpus P.M.Zamora & Co. is a grammitid fern (Polypodiaceae) known only from ultramafic substrates on Palawan Island in the Philippines (Zamora & Co, 1981) and Mount Kinabalu in Sabah, Malaysia (Borneo) (Parris et. al., 1992 as Xiphopteris nudicarpa (P.M.Zamora & Co) Parris). Although it was described in Acrosorus, it differs from all other member of the genus in having hydathodes at the vein endings on the adaxial surface of the lamina, a character shared with several species of Xiphopterella. The following new combination is proposed:


REFERENCES


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PAPERS should not usually exceed 20 printed pages and are generally expected to be considerably shorter. As well as reports of original research, review articles and short notes, e.g. new records, are encouraged. The senior author should supply an email address to facilitate correspondence.

REVIEWS should cover recent developments in any aspect of research on ferns and lycopods. This can include unpublished research by the author, and/or the possible direction of future research. They should be aimed at the general reader who may not be an expert in the chosen topic. Proposals for a review should be discussed first with the review editors.

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TRICHOMANES SPECIOSUM (HYMENOPHYLLACEAE)
IN SOUTHERN SPAIN

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TWO NEW *Diplazium* (WOODSIACEAE) SPECIES FROM EAST MALESIA

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Keywords: *Diplazium*, taxonomy, rhizome scales, Sulawesi, Seram,

ABSTRACT

Two new species of *Diplazium* are described from Indonesia: *D. walkeri* Hovenk. from Sulawesi, and *D. murkele* Hovenk. from Seram.

INTRODUCTION

During identification of ferns collected by A.C. Jermy and T. G. Walker in 1969 in the Latimojong Mountains, Sulawesi Selatan (Indonesia), a number of specimens of *Diplazium* (Woodsiaceae) could not be identified with any known species. Comparison with the collections in BM, K and L revealed similarities with *Diplazium mutabile* Hovenk. (Hovenkamp & De Joncheere, 1988), described from a different mountain area on Sulawesi, with *D. furculicolom* Alderw. (Van Alderwerelt van Rosenburgh, 1918), described from Seram, and with a number of collections from Seram identified by Kato et al. (Kato, 1994) as *D. vestitum* C.Presl. With *D. mutabile* the unidentified specimens share a characteristic combination of evanescent basal scales and pinnule rachis ornamentation. The basal scales characteristically disintegrate starting with the outer cell walls (Figure 1A), and the abaxial surfaces of the pinna and pinnule rachis are ornamented with a combination of somewhat fleshy hair-like protrusions and appressed flat scales (Figure 1B). The same rachis indument is found in *D. furculicolom*. and in the Seram collections identified as *D. vestitum*, but accompanied by different types of scales. The scales of *D. furculicolom* have the same thin texture as those of *D. mutabile*, but retain their general outline more strongly; the scales of the Seram “*D. vestitum*” specimens are thick, brown and fleshy, but also appear disintegrated somewhat especially at the margin and the surface of the scale, leaving the exact shape and outline of the scales uncertain. *Diplazium vestitum*, as represented in L by collections from the Philippines and Borneo, differs significantly in having basal scales that are translucent with a dark dentate margin and smooth abaxial rachis surfaces and thereby is clearly different from the Seram specimens.

Notwithstanding the general state of ignorance we are in about the exact species delimitation in Malesian *Diplazium*, the four groups of specimens appear to be sufficiently distinct to warrant the description of two new species. Table 1 provides a synopsis of the major morphological similarities and differences between the four species discussed here.

METHODS

Photographs were taken with a Zeiss Discovery V20 microscope equipped with a Zeiss Axioacam MRC5 camera, and processed with Axiovision software (Axiovision Vs40x64 V. 4.9.10). For scanning electron microscopy (SEM) spores were sputter-coated with gold and examined in a JEOL JSM 5300 microscope (Figure 2A) or coated with c. 5 nm Pt/Pd and observed with a Jeol JSM 7600F FEG-SEM (Figure. 2B, C, D).
Figure 1. Diplazium walkeri.
A: Rhizome scale, showing the fibrous transverse cell walls remaining after the disappearance of the superficial walls (rehydrated). Scale bar 2mm.
B: Abaxial surface of penultimate midrib, showing combination of fleshy hairs and appressed flat scales (in sicco). Scale bar 1mm (Walker 12367, BM).

Figure 2. Spores:
A: Diplazium mutabile
B: Diplazium walkeri
C: Diplazium murkele, inset: detail of scaly outer layer
D: Diplazium furculicolum
Table 1. Diagnostic characters for the four taxa.

<table>
<thead>
<tr>
<th></th>
<th>Diplazium mutabile</th>
<th>Diplazium walkeri</th>
<th>Diplazium murkele</th>
<th>Diplazium furculicolium</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rhizome</strong></td>
<td>Ascending or erect</td>
<td>Ascending or erect</td>
<td>Ascending to erect</td>
<td>erect</td>
</tr>
<tr>
<td><strong>Scales on rhizome and base of stipe</strong></td>
<td>Thin, translucent, evanescent</td>
<td>Thin, translucent, evanescent</td>
<td>Thick, spongy, evanescent?</td>
<td>Thin, translucent, more or less persistent</td>
</tr>
<tr>
<td><strong>Reduced pinnae</strong></td>
<td>1-2 (bipinnate specimens)</td>
<td>3-6</td>
<td>1</td>
<td>1-3</td>
</tr>
<tr>
<td><strong>Pinnae length</strong></td>
<td>22-26cm</td>
<td>Up to 27cm</td>
<td>Up to 36cm</td>
<td>7-13cm</td>
</tr>
<tr>
<td><strong>Pinnae width</strong></td>
<td>6-10cm</td>
<td>Up to 10cm</td>
<td>9 -17cm</td>
<td>1-3.5cm</td>
</tr>
<tr>
<td><strong>Pinnules length</strong></td>
<td>3-5cm</td>
<td>c. 5cm</td>
<td>5 - 10.5cm</td>
<td>6-15mm</td>
</tr>
<tr>
<td><strong>Pinnules width</strong></td>
<td>1.5-1.7cm</td>
<td>c. 1.5cm</td>
<td>1.5 - 3mm</td>
<td>1.5-5mm</td>
</tr>
<tr>
<td><strong>Pinnules margin</strong></td>
<td>Serrate-dentate</td>
<td>Deeply incised, to 2-3mm from midrib</td>
<td>Deeply incised, to 1-3cm from midrib</td>
<td>Crenate or rarely more deeply incised</td>
</tr>
<tr>
<td><strong>Basal pinnules free</strong></td>
<td>2-4 pairs</td>
<td>7-10 pairs</td>
<td>7-9 pairs</td>
<td>0-2 pairs</td>
</tr>
<tr>
<td><strong>Lamina apex</strong></td>
<td>Not proliferous</td>
<td>Not proliferous</td>
<td>Not proliferous</td>
<td>Often proliferous</td>
</tr>
<tr>
<td><strong>Spore ornamentation</strong></td>
<td>Weakly rugulate</td>
<td>Weakly rugulate</td>
<td>Weakly regulate, surface eroding</td>
<td>Ornamented narrow wings</td>
</tr>
</tbody>
</table>
DESCRIPTION OF NEW TAXA

**Diplazium walkeri** Hovenk., **spec. nov.**
Type: Trevor G. Walker 12373 (holo BM; iso L), Indonesia, Sulawesi Selatan, Enrekang, above the village of Rantelimo, 25km SE of Kalosi, on the south-western slopes of Mt. Rantemario, 1950-2100m, on ridge above northern branch of R. Pasui, 24 November 1969.
Terrestrial fern, rhizome ascending or erect, incl. stipe-base remnants 2-3cm thick; scaly but glabrous in older parts; rhizome scales basifix, periclinal cell walls very thin and quickly disappearing, often leaving the scale disintegrating into a fibrous network, shape, margin, apex of the scales accordingly not observed. Fronds to c. 130cm long; stipes to c. 46cm, sparsely scaly at base with scales similar to those on the rhizome, upwards with papillose multicellular protrusions; lamina bipinnate, to c. 90 by 50cm, widest at c. 1/3-1/5, gradually narrowing from c. halfway up; free pinnae 12-15 pairs, lowest 1-6 somewhat reduced, alternate to subopposite, largest pinnae to 2 cm stalked, to 37 by 18cm; pinnules alternate to opposite, basal 7-10 pairs free, to 5 by 1.5cm, symmetric at base, incised to 1.5-3mm from midrib, apex obtuse to acute, lobes truncate; pinnae and pinnule rachis on the adaxial surface with two flanges interrupted at the vein junctions, on the abaxial surface with flat translucent scales and papillose multicellular protrusions, veins free. Sori diplazioid, on all veins of a vein-group, running from costule to c. 1mm from the margin, spores c. 50 µm long, very lightly rugulate.
Distribution – Indonesia: Sulawesi Selatan (Enrekang District, Latimojong Mountains).
Ecology – From lowland forest to upper montane forest, in deep shade, in ravines or by streams, 1700-2150m.
Etymology - The species is named after Trevor G. Walker (1927-2006), British pteridologist.
Note - Diplazium walkeri differs from *D. mutabile* mainly in the more highly dissected lamina. *Diplazium furculicolum* differs from both in a much smaller size, basal scales that retain more of their shape and in much more strongly ornamented spores (Figure 2).

**Diplazium murkele** Hovenk., **spec. nov.**
Type: M. Kato, K. Ueda & U.W. Mahjar C 1292 (holo L; iso TI, not seen, BO, not seen), Indonesia, Seram, Tehoru & Seram Utara: Manusela National Park: along a trail between Hatumete (seaside) and Maraina (810 m) in Manusela Valley via Hoale Pass (1770m) in Murkele Ridge, 03° 10-14' S, 129° 35-37' E, 18 Nov. 1983.
Terrestrial fern, rhizome ascending or erect, incl. stipe-base remnants 2-3cm thick; scaly but glabrous in older parts; rhizome scales basifix, to c. 10 by 2mm wide, thick, fleshy, margin entire, in the basal part thin, flaccid; apex obtuse. Fronds to c. 120 or longer; stipes to 75cm, naked or sparsely scaly at base with thick brown scales to 10 by 6 mm, lamina bipinnate, to c. 70 by 50cm, widest at c. 1/4, free pinnae 8-12 pairs, lowest pair slightly reduced, alternate to opposite, largest pinnae to 1.5cm stalked, to 37 by 17cm; pinnules alternate to opposite, basal 7-9 pairs free, to 8.5 (10.5) by 1.5-2 (3)cm, symmetric at base, incised to 1—3 mm from midrib, apex obtuse to acute, lobes truncate; pinnae and pinnule rachis on the adaxial surface with two flanges interrupted at the vein junctions, on the abaxial surface with flat translucent scales and papillose multicellular protrusions, veins free. Sori diplazioid, on all veins of a vein-group, running from costule to c. 1mm from the margin, spores c. 40µm long when well-
formed, many collapsed or otherwise malformed, perispore with a thin almost smooth, eroded, scaly outer layer uncovering a finely irregularly porous inner layer.

Distribution – Indonesia, Moluccas: Seram.

Ecology – Terrestrial, in deep shade, near brooks, in ravines etc., in lowland to montane forest, disturbed forest, often noted for calcareous areas, lowland to 1350m, possibly higher.

Etymology - The epithet is to be treated as a word in apposition and derives from Murkele Ridge, the location of the type specimen and a number of other specimens.

Note – Diplazium murkele differs from D. walkeri mainly in the basal scales that are not thin and translucent but thick and fleshy. However, they often also appear to show signs of disintegration, which may explain the scarcity of intact scales in the collected specimens. The lamina is somewhat smaller, and has distinctly fewer reduced pinnae near the basis, the pinnules on the other hand are a bit larger. The outer layer of the spores appears to erode away, leaving only a partial, scaly cover (Figure 2 C).

Additional Specimens Seen:

Diplazium mutabile

Diplazium murkele.

Diplazium walkeri.
Sulawesi. Rantemario: Walker, T.G. 11958 (BM, L) 1 November 1969; 11959 (BM, L) 11961 (BM, L) 12365 (BM, L) 24 November 1969; 12368 (BM, L) 12373 (BM, L)

Diplazium furculicololum.

ACKNOWLEDGMENTS
A visit to the Natural History Museum, London to identify collections from Sulawesi was supported by Synthesys grant GB-TAF-4095. I gratefully acknowledge the patience of the Curator of Pteridophytes at BM for allowing me the loan of a number of specimens for an extensive period.
REFERENCES


EFFECT OF TEMPERATURE ON THE VIABILITY, LIFESPAN AND VIGOUR, OF CHLOROPHYLLOUS SPORES OF OSMUNDASTRUM CINNAMOMEUM (OSMUNDACEAE)

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ABSTRACT
Short-lived chlorophyllous spores need special treatment to extend their longevity and vigour for ex situ conservation. Germination rates, mean longevity and germination times of chlorophyllous spores of the rare fern Osmundastrum cinnamomeum were determined to assess their viability, lifespan and vigour at 18–25°C (room temperature, RT), 4°C and -80°C during 96 weeks of storage. Results showed that at the low temperatures, the viability and vigour were significantly maintained, and the lifespan of O. cinnamomeum spores was extended. Spores stored at RT had completely lost all ability to germinate within 12 weeks. Some spores retained their viability for more than 96 weeks at 4°C and -80°C. Mean longevities were 6.1, 41.8, and >96 weeks with storage at RT, 4°C and -80°C respectively. The mean spore germination time (MGT), herein regarded as an indicator of vigour, was affected by both storage temperature and time. Spores stored at RT and 4°C began to lose their vigour after three weeks. However, it did not decline at -80°C until 24 weeks of storage. This study demonstrates that low temperature (4°C) and cryopreservation (-80°C) significantly extended the longevity and vigour of chlorophyllous spores of O. cinnamomeum.

Keywords: cryopreservation, mean germination time.

INTRODUCTION

As plants are threatened by habitat loss, pollution, invasive species, and other factors, in situ conservation is considered as the primary focus for conserving threatened species. Ex situ germplasm storage, on the other hand, is an important complementary aspect of plant conservation (Pence, 2004). A germplasm bank, storing living propagules in a small space, can efficiently maintain genetic variability (Dickie et al., 1990; Smith et al., 2003; Pence, 2008). Conservation plans for a number of threatened fern species include propagation from spores (e.g., Estrelles et al., 2001; Lusby et al., 2002; Simabukuro et al., 1998). Spore banks have low financial costs and have been developed for more than 20 years to conserve ferns ex situ (Page et al., 1992; Ibars & Estrelles, 2012).

Most fern spores can typically retain their viability for a long period but there is great disparity among species (Okada, 1929). One of the principal features causing such
variation is the presence of chlorophyll pigments. Fern spores are primarily divided into chlorophyllous spores (also called green spores) and non-chlorophyllous spores (non-green spores), depending on whether or not chlorophyll pigments exist in them (Gabriel y Galán & Prada, 2011). Compared with non-chlorophyllous spores, which retain their viability for several months to years, chlorophyllous spores are relatively short-lived, surviving for only a few weeks or months at room temperature (Lloyd & Klekowski, 1970).

Chlorophyllous spores quickly lose their viability because of a lack of desiccation tolerance, a high metabolic rate, and the absence of dormancy (Kato, 1976; Li & Shi, 2014). Therefore they require specialised treatment for long-term conservation in germplasm banks (Ballesteros et al., 2011). It is well documented that the viability of chlorophyllous spores can usually be extended under low temperatures (Ballesteros, 2011; Gabriel y Galán & Prada, 2011). The longevity of chlorophyllous and non-chlorophyllous spores has been studied for over 50 years, and some protocols have been established to optimise spore preservation in fern spore banks (Ibars & Estrelles, 2012). For example, low temperature (-70°C) storage substantially prolonged chlorophyllous spore viability of *Equisetum hyemale* L. for over 16 months (Whittier, 1996), and at -20°C for more than 28 months for *Osmunda regalis* L. (Magrini & Scoppola, 2012).

The “viability” of spores has been variously defined, such as the length of time that spores retain their ability to germinate (Raghavan, 1989; Gabriel y Galán & Prada, 2011), or the capacity of a spore to germinate (Qunitanilla et al., 2002). The former one is mutually used with the term “longevity” (e.g., Aragón & Pangua, 2004, Pence, 2004). We herein define “spore longevity” (the lifespan of a spore) to be the period from spore harvest to loss of germination ability as per Windham et al. (1986) and Ballesteros et al. (2011). “Spore viability” is defined as the spore germination ability as indicated by the germination rate (%) (Gabriel y Galán & Prada, 2011).

The quality of spores (spore vigour) is related to the germination rate and ageing during storage. Because spore germination time is prolonged with storage time (Smith & Robinson, 1975; Camloh, 1999), germination time was used to indicate spore vigour in this study.

*Osmundastrum cinnamomeum* (L.) C.Presl is a temperate fern, usually growing in swamps and widely distributed from Asia to the Americas (Metzgar et al., 2008). However, the population number is low (≤4) and relatively small (<200 mature individuals in the largest population) in Taiwan (Chiu et al., 2013). It was evaluated to be a vulnerable species based on IUCN red list categories and criteria (Wang et al., 2012). The goal of this study was to determine the effect of various storage temperatures on the longevity and vigour of chlorophyllous spores of *O. cinnamomeum*. The results provide useful information for long-term *ex situ* conservation of this rare fern in spore banks.

**MATERIALS AND METHODS**

Fresh spores of *O. cinnamomeum* were obtained from 12 fertile fronds of six plants growing in a marsh at Tsaopi (24°46'03"N, 121°36'26"E, elev. 809m), Yilan County, northeastern Taiwan, in March 2012. The fertile fronds released spores after being air-dried for three days. Spores were isolated from sporangia and transferred to microtubes (Labcon Screw-Cap Microcentrifuge Tubes, 2.0ml) for dry storage. Microtubes of spores were stored at 18–25°C (room temperature, RT), 4°C or -80°C for up to 96 weeks.
Spores stored at RT, 4°C and -80°C were sown separately onto four sheets of membrane filters (with a pore size of 0.45µm and diameter of 47mm; Gelman Laboratory) after 0 (fresh spores), 1, 2, 3, 4, 8, 12, 24, 48 and 96 weeks of storage. Each sheet was laid on a 4cm thick layer of moist sphagnum moss in a plastic box (114×86×102mm, PHYTATRAY IITM, Sigma). All cultures were incubated under white fluorescent illumination at approximately 25–35µmol m⁻² s⁻¹ for 12h d⁻¹ at 20 ± 1°C.

Germination refers to the breakage of the spore wall and the appearance of the first rhizoid or gametophyte cell (Lloyd & Klekowski, 1970). To calculate the germination rate (%), 200 spores (50 spores/filter × 4 filters) were randomly observed under a microscope (Leica, Wild M8) every week after sowing until no more germination was detected.

The mean germination time (MGT) was calculated for each replication per treatment according to the equation: MGT = ∑(tn)/∑(n), where t is the time in weeks starting from spore sowing and n is the number of spores that had germinated in week t (modified from De Brum & Randi, 2006). The spore lifespan was indicated by the spores’ mean longevity calculated by the equation: ML = ∑(ti)(pi−pi+1), where ti is the spore storage time (ith week), pi is the germination rate in the ith week, and pi+1 is the germination rate for the next storage time (i+1th week).

Germination rates and mean germination times for each temperature were analysed by a one-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test (p<0.05) to estimate significant differences of means among different storage times.

RESULTS

All (100%) fresh spores of O. cinnamomeum germinated within one week. With RT storage, the spore germination rate remained at almost 100% for four weeks in storage. Then it decreased significantly to nearly 50% after eight weeks in storage, and spores had completely lost viability after 12 weeks in storage. When the storage temperature was lowered to 4°C, spores retained almost all of their viability for 24 weeks in storage. Then it decreased significantly to 69.5% and 3.0% after 48 and 96 weeks of storage, respectively. For spores stored at -80°C, the germination percentage was almost 100% during 48 weeks of storage and still remained at 91.5% even after 96 weeks of storage (Table 1).

The MGT of fresh spores was one week. Under RT storage, the MGT increased significantly to 1.4 weeks after three weeks in storage, and up to 2.0 and 2.1 weeks after four and eight weeks in storage, respectively. The MGT of spores stored at 4°C did not increase within two weeks of storage, then slightly (but significantly) increased to 1.3–1.5 weeks during 3–12 weeks of storage, and again significantly increased to 2.4–2.5 weeks after 24–48 weeks of storage. However MGT was found to have decreased to 2.0 weeks after 96 weeks of storage. The MGT was ≤1.2 weeks when spores were stored at -80°C after 12 weeks in storage, but significantly increased to 2.2 weeks after 24 weeks of storage, then decreased to 1.9 and 1.7 weeks respectively when spores were stored for 48 and 96 weeks (Table 2).

Spores had completely lost their viability after 12 weeks of storage at RT, whereas some viability was retained at 4°C and -80°C storage at the end of the observation period (96 weeks) (Table 2). Thus the lifespan of at least some proportion of spores at 4°C and -80°C was more than 96 weeks. The mean longevity of spores significantly
Table 1. Germination rates of spores of *Osmundastrum cinnamomeum* at different storage times and temperatures

<table>
<thead>
<tr>
<th>Storage time (weeks)</th>
<th>Storage temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT(^3)</td>
</tr>
<tr>
<td>0</td>
<td>100(^a)</td>
</tr>
<tr>
<td>1</td>
<td>100(^a)</td>
</tr>
<tr>
<td>2</td>
<td>100(^a)</td>
</tr>
<tr>
<td>3</td>
<td>100(^a)</td>
</tr>
<tr>
<td>4</td>
<td>99.5±1.0(^a)</td>
</tr>
<tr>
<td>8</td>
<td>51.5±4.4(^b)</td>
</tr>
<tr>
<td>12</td>
<td>0(^c)</td>
</tr>
<tr>
<td>24</td>
<td>0(^c)</td>
</tr>
<tr>
<td>48</td>
<td>0(^c)</td>
</tr>
<tr>
<td>96</td>
<td>0(^c)</td>
</tr>
</tbody>
</table>

1) Data are the mean±standard deviation (n=4).
2) Means with the same superscript letter do not significantly differ within a temperature (p<0.05; LSD test).
3) Room temperature of 18–25°C.
Table 2. Mean germination times of spores of *Osmundastrum cinnamomeum* at different storage times and temperatures

<table>
<thead>
<tr>
<th>Storage time (weeks)</th>
<th>Storage temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT&lt;sup&gt;3)&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>1.1±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>1.1±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>1.4±0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>2.0±0.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>2.1±0.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>--&lt;sup&gt;5)&lt;/sup&gt;</td>
</tr>
<tr>
<td>24</td>
<td>--</td>
</tr>
<tr>
<td>48</td>
<td>--</td>
</tr>
<tr>
<td>96</td>
<td>--</td>
</tr>
</tbody>
</table>

1) Data are the mean±standard deviation (<i>n</i>=4).  
2) Means with the same superscript letter do not significantly differ within a temperature (<i>p</i>&lt;0.05; LSD test).  
3) Room temperature of 18−25°C.  
4) -, no data.  
5) --, spores did not germinate.
differed among the three temperature treatments, with 6.1, 41.8, and >96 weeks at RT, 4°C and -80°C storage, respectively.

**DISCUSSION**

The viability of chlorophyllous spores under RT conditions is known to be lost rapidly but there are great disparities among species. For example, longevity of chlorophyllous spores of *Equisetum hyemale*, *Osmunda regalis*, and *Matteuccia* sp. are two weeks, ca. one month, and more than one year, respectively (Stokey, 1951; Lloyd & Klekowski, 1970; Lebkuecher, 1997). This study demonstrated that the lifespan of a proportion of spores of *O. cinnamomeum* at RT reached eight weeks, slightly longer than previous records from Japan (1.4–1.8 months) (Okada, 1929). This difference may have resulted from plant genetic variations and/or spore maturity. Fully mature spores usually have greater longevity than do immature ones (Huang et al., 2014). Some physiological mechanisms and compounds, including the respiration rate, water content, catalase content and granules of lipids and lipoproteins, were documented to affect chlorophyllous spore viability (Okada, 1929; Gullvåg, 1969; Olsen & Gullvåg, 1973). The decay of fern spore viability might also result from the loss of some housekeeping substances, such as sugars, amino acids and proteins (Beri & Bir, 1993; Ballesteros et al., 2004). Spore viability is also related to various kinds of sulphur metabolites, such as glutathione (GSH), glutathione disulphide (GSSG), S-adenosylmethionine (SAM), and S-adenosylhomocysteine (SAH), which are involved in modulating protein activities or other metabolites under stress (Giovanelli et al., 1985; Dixon et al., 2005; Michelet et al., 2005; Loenen, 2006; Roje, 2006). Chang et al. (2013) further documented that GSH/GSSG and SAM/SAH ratios of spores of *O. cinnamomeum* significantly declined as they lost viability. Apparently those physiochemical changes affect the germination rates of *O. cinnamomea* spores and probably are correlated with the loss of viability in other chlorophyllous fern spores.

Chlorophyllous spores tend to germinate quickly because their photosynthetically active chloroplasts can rapidly transform light energy to metabolic activity (Raghavan, 1989). The MGT of chlorophyllous spores at RT is ca. 1.5 days for the Equisetaceae, Osmundaceae, Grammitidaceae, Hymenophyllaceae, *Blechnum nudum* (Labill.) Mett. ex Luerss., *Lomariopsis sorbifolia* (L.) Féé, and some species of *Christiopteris*, *Marginariopsis*, *Matteuccia*, *Onoclea* and *Onocleopsis* (Lloyd & Klekowski, 1970). It is obvious that the germination rate of chlorophyllous spores should be measured at short intervals. Daily observations would provide more accurate data than the weekly observations that were used in this study. Nevertheless, correlations between spore vigour and different storage temperatures were distinguishable.

The results of this study show that the MGT increased before the germination rate declined. However, the MGT of *O. cinnamomeum* spores increased significantly after three weeks of storage at 4°C, then decreased after 96 weeks of storage. A similar situation also occurred with storage at -80°C. The increase and decrease in the MGT may have resulted from the self-regulation of spores in response to stress during storage (Chang et al., 2013). More studies are needed to determine whether the MGT is a good indicator for evaluating the germination vigour of fern spores.

The successful establishment of individuals and populations of a species in a new habitat after spore dispersal depends on how long the spores retain their viability and vigour (Sheffield, 1996; Shorina, 2001; Gabriel y Galán & Prada, 2011). When the spore vigour decreases, subsequent gametophytes may grow abnormally and even
affect subsequent sporophyte production (Smith & Robinson, 1975; Beri & Bir, 1993) and population establishment (Gabriel y Galán & Prada, 2011).

An increase in the storage time results in decline in spore viability and vigour as previously reported for O. cinnamomeum and a few other ferns (Smith & Robinson, 1975; Ballesteros et al., 2011, 2012). Evidence was presented that suggests spore age may be partly attributable to the declining ability of spores to synthesise metabolites essential for germination (Gabriel y Galán & Prada, 2011). It is well known that chlorophyllous spores have shorter lifespans compared with non-chlorophyllous spores (Lloyd & Klekowski, 1970). Using low temperature to prolong their lifespan and vigour was documented (Pence, 2000, 2008; Ballesteros et al., 2006, 2011, 2012; Ballesteros, 2011; Gabriel y Galán & Prada, 2011). It was reported that chlorophyllous spores of Osmunda regalis and O. japonica Thunb. stored at low temperatures (-25 and 4°C) also retained viability for up to 24 and 12 months respectively (Ballesteros et al., 2011; Li & Shi, 2014). This study also showed that spores of O. cinnamomeum rapidly lost viability at RT storage, and the lowest temperature of -80°C provided the best conditions to retain their spore viability and vigour. Cryopreservation is, therefore, recommended for the long-term storage of spores of this species, and this procedure may be applicable to other fern spores in terms of ex situ conservation.

CONCLUSIONS

Chlorophyllous spores of ferns have shorter lifespans at room temperature storage compared with non-chlorophyllous spores. However, cryogenic temperatures might suppress metabolic activities and effectively extend their longevity and retain the vigour of chlorophyllous spores as shown in this study. It is suggested that cryopreservation is a feasible method for establishing long-term germplasm of the chlorophyllous spores of Osmundastrum cinnamomeum.

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PLEOPELTIS ×CERRO-ALTOENSIS (POLYPODIACEAE), A NEW FERN HYBRID FROM ROBINSON CRUSOE ISLAND (JUAN FERNÁNDEZ ARCHIPELAGO, CHILE)

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Keywords : Pleopeltis, hybrid, Polypodiaceae, Juan Fernández, Chile

ABSTRACT
A fern hybrid of the genus Pleopeltis was discovered on Robinson Crusoe Island in the Juan Fernández Archipelago, off the coast of Chile, and is described as P. ×cerro-altoensis. Its putative parents are P. macrocarpa and P. masafuerae, two species present in the archipelago.

INTRODUCTION
Since 1997, the first author has undertaken, with the association ROBINSONIA1, regular expeditions in the Juan Fernández Archipelago, off the coast of Chile, to study...
both the indigenous and the introduced flora of the three islands that constitute this archipelago (Figure 1): the main island, Robinson Crusoe (or Masatierra) and the nearby Santa Clara Island (also called Île aux Chèvres), and the third one, Alejandro Selkirk Island (or Masafuera), located 180 km further west.

During these expeditions, on 29th January 1999, a small isolated population of a polypodiaceous fern was discovered on a cliff of volcanic rocks, at Cerro Alto (Figures 2 & 3) on Robinson Crusoe Island (Masatierra). The presence of peltate scales on both surfaces of the lamina and its axes showed that it belongs to the genus Pleopeltis Humb. & Bonpl. ex Willd. (Figures 4 & 7).

We compared this plant with the Pleopeltis species known from the area, but our review of botanical literature relating to Juan Fernández (Hemsley, 1884; Johow, 1892-93, 1896; Christensen & Skottsberg, 1920; Skottsberg, 1953; Barrera Moscoso, 1997; Marticorena et al., 1998; Boudrie, 2003; Danton & Perrier, 2006) and to continental Chile (Gay, 1853; Looser, 1961, 1962, 1965-66, 1968; de la Sota, 1967; Marticorena & Rodriguez, 1995; Zuloaga et al., 2008) has shown that it was not recorded in any of the floras or floristic catalogues, nor any description applied to it. Therefore, whatever its identity or origin, we believe that it is a taxon new to the Juan Fernández Archipelago.

Two other species of Pleopeltis (previously placed in the genus Polypodium) occur in the Juan Fernández Archipelago (Figure 5):
  Distribution: almost pantropical, America (from Central America to Chile and Argentina, Juan Fernández, Galapagos), tropical southern Africa, India.
- Pleopeltis masafuerae (Phil.) de la Sota, Darwiniana 45(2): 239. 2007.
  Distribution: Juan Fernández Archipelago (only on Alexander Selkirk Island = Masafuera), Chile (province of Antofagasta); mentioned from southern Peru (Marticorena & Rodriguez, 1995), but not confirmed to our knowledge.

Concerning the identity of P. masafuerae, its relationships with several South American Pleopeltis such as P. pinnatifida (Gillies) Hook. & Grev., P. pycnocarpa (C.Chr.) A.R.Sm. and Polypodium mollendense Maxon are unresolved (pers. comm. A.R. Smith, 2015). Although the circumscription of Pleopeltis has recently been enlarged, based both on molecular and on morphological studies (Otto et al., 2009; Smith & Tejero-Diez, 2014), Neotropical Pleopeltis taxa are a taxonomically difficult group, with polyploids and numerous hybrids, and a modern revision is needed to clarify the taxonomy. Tryon & Stolze (1993), treating the Peruvian species (in Polypodium), alluded to these complexities.

Because neither of these two species matched the newly discovered plant, which thus might represent a new taxon (Danton et al. 1999), a more careful study of it was undertaken, as described below.

MATERIAL AND METHODS

The material used for this study comprises:
- Fronds collected in the field at Cerro Alto, now pressed and dried herbarium specimens:
  ● Danton B(652)619 (P, SGO, private herb. Ph. Danton)
- Fresh fronds collected at the Botanical Conservatory of Mulhouse, France, where a *Pleopeltis* plant collected by one of us (Ph. D.) at Cerro Alto on Feb. 27, 2002, has been in cultivation since 2002 under the number *CBM 03-301*, and now, extensively propagated. All the herbarium specimens mentioned below are issued from the *CBM 03-301* plant.

- *Ph. Danton – Mulhouse – 1* (private herb. R. Viane)
- *Ph. Danton – Mulhouse – 2* (private herb. R. Viane)
- *Ph. Danton – Mulhouse – 3* (private herb. R. Viane)
- *Bizot 2905* (private herb. A. Bizot)

**Figure 1:** Location of the Juan Fernández Archipelago and Robinson Crusoe Island, west of Chile. Map base source: © Google maps, 2015

**Figure 2:** Alexander Selkirk Island (left) and Robinson Crusoe Island (right). © Ph. Danton & Ch. Perrier, 2004
Figure 3: *Pleopeltis × cerro-altoensis* in situ at Cerro Alto. © Photo Ph. Danton, 22/01/1999

Figure 4: *Pleopeltis × cerro-altoensis*. © Ph. Danton, 25/01/1999. a - entire plant; b - portion of adaxial laminar surface; c - abaxial surface of fertile frond; d – peltate-based lanceolate laminar scale; e – peltate-based lanceolate rhizome scale.
Figure 5 – Left: *Pleopeltis masafuerae* (Phil.) de la Sota, rocks at the beginning of the Quebrada Las Casas, Alejandro Selkirk Island, 19/12/2003, *Danton G(1319)1083*. – Right: *Pleopeltis macrocarpa* (Bory ex Willd.) Kaulf., epiphytic in forest under the Mirador de Selkirk, Robinson Crusoe Island, 07/12/1998, *Danton B(410)366*. © Photo Ph. Danton, 26/05/2015.
Figure 6: *Pleopeltis × cerro-altoensis* Danton & Boudrie, Cerro Alto, Robinson Crusoe Island, Juan Fernández Archipelago, holotype (SGO). *Danton B(658)619*. © Photo Ph. Danton, 17/11/2014.
Figure 7: Abaxial laminar surface of *Pleopeltis ×cerro-altoensis*, showing peltate-lanceolate scales and sori (from *Bizot 2905*). © Photo A. Bizot, 8/10/2014.

Figure 8: Peltate-based, lanceolate, fimbriate scale of abaxial laminar surface of *Pleopeltis ×cerro-altoensis* (from *Bizot 2905*). © Photo A. Bizot, 10/10/2014.
Figure 9: Sporangium and malformed spores of Pleopeltis ×cerro-altoensis (from Bizot 2905). © Photos A. Bizot, 8/10/2014 & 18/10/2014.
- Dried herbarium specimens issued from the cultivated plant at Mulhouse under number CBM 03-301:
  - Boudrie 3966 (P)
  - Boudrie 4500 (P)
  - Danton Z(hs)1727 (private herb. Ph. Danton)

- Other *Pleopeltis* taxa from Mexico, deposited in the personal herbarium of one of us (R. V.), but used, when freshly collected, for FCM analyses. Only one, *P. guttata*, is in cultivation in R.V.’s garden in Serskamp, near Ghent.
  - Viane 11001: Pleopeltis guttata (Maxon) E.G. Andrews & Windham
  - Viane 11005: Pleopeltis polylepis (Roem. ex Kunze) T.Moore var. erythrolepis (Weath.) Wendt
  - Viane 11013: Pleopeltis polypodioides (L.) E.G.Andrews & Windham var. knoblochiana (Mickel) A.R.Sm. & Tejero

Herbarium specimens of the respective private herbaria of A.B., Ph. D. and R. V., cited above, will be deposited in the future in official institutions.

To establish its characters and identity, several methods have been applied to the material mentioned above.

**Micromorphological observations**

Micromorphological observations were carried out by A.B. using a microscope Bresser triocular TRM-301 microscope at 400x to 1000x enlargements, equipped with a Bresser MicroCamLab-1 ocular camera. Fresh fronds from material in cultivation in the Botanical Conservatory of Mulhouse [Bizot 2905] were used.

- **Laminar scales** were observed (Figures 7 & 8) on fresh fronds. Rhizome scales were not microscopically observed. However, despite their more lanceolate shape than the laminar scales, they show the same clathrate pattern of cells.

- **Stomata**: Using tweezers, three epidermal slices were excised from the abaxial side of the lamina in the middle of a median pinna. The epidermis was observed in glycerin at 1000x enlargement. Eighty (80) stomata were measured.

- **Spores** were observed (Figure 9) in glycerin liquid at 1000x enlargement. They were collected from several (3-4) sori of three different fresh fronds as well as from more than two sori of a frond collected in the field [Danton B(652)619].

Comparison of size of stomata of the Cerro Alto plant with stomata from plants of *P. macrocarpa* and *P. masafuerae* may reveal important information, but this investigation was impossible owing to the lack of adequate fresh material.

**Measurements of rhizomes**

The width (rhizomes are slightly flattened) and the thickness of rhizomes of the 10 specimens of *P. macrocarpa*, four specimens of *P. masafuerae*, and four specimens of the Cerro Alto plant were measured from dried herbarium specimens. Sectioned pieces of rhizomes were observed under a binocular and measured using a plastic ruler with the smallest calibrated unit being 0.5mm.

**DNA content using FCM**

A few fresh fronds (Botanical Conservatory of Mulhouse, France) of the *Pleopeltis* from Cerro Alto (in cultivation since 2002) were sent to the laboratory of Pteridology of Ghent University (Belgium) on 6th October 2014 for flow cytometric measurements (FCM). For this analysis, fresh samples were first chopped, using a sharp razor blade,
in a glass Petri dish containing nucleus-isolation buffer (2.1% [w/v] containing citric acid monohydrate, 0.5% [w/v] Tween 20, and distilled water; this suspension was then filtered through a 50-µm nylon mesh. The nuclear suspension was supplemented with DAPI reagent [6.5% NaH$_2$PO$_4$.2H$_2$O, 40µl DAPI stock solution (5mg DAPI/ml distilled water), distilled water], and each sample was measured three times. The samples were analysed using a flow cytometer (Ploidy Analyser, Partec GmbH, Münster, Germany), following the supplier’s instructions. To calculate the relative nuclear DNA content, Agave striata (2n = 2x = 60, 2C = 7.8pg (Zonneveld et al., 2005) was used as an internal standard.

Fresh material of P. macrocarpa or P. masafuerae was unavailable, as the islands where they grow are difficult of access.

To establish ploidy levels, our FCM results were compared with those of some Mexican Pleopeltis taxa collected in Chihuahua: diploid P. polylepis (Roem. ex Kunze) T.Moore var. erythrolepis (Weath.) Wendt (Viane 11005); P. guttata (Maxon) E.G.Andrews & Windham (Viane 11001); and triploid P. polypondioides (L.) E.G.Andrews & Windham var. knoblochiana (Mickel) A.R.Sm. & Tejero (Viane 11013). To confirm the FCM results, a chromosome count was made for P. guttata, revealing it to be triploid (Van den heede, pers. comm.).

The three specimens from Cerro Alto submitted for FCM have been registered as “Ph. Danton - Mulhouse - 1” to "Ph. Danton -Mulhouse- 3", all sampled from the cultivated plant at Mulhouse under the number CBM 03-301.

RESULTS

Micromorphology, spores and stomata
Spores are either irregular in shape and in size or totally malformed (Figure 9), a condition typical for interspecific fern hybrids. Stomata range from 45 to 63µm long, with an average of 53µm and a standard deviation of 3.1µm.

Rhizome measurements

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Width (mm)</th>
<th>Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min.</td>
<td>mean</td>
</tr>
<tr>
<td>Pleopeltis macrocarpa</td>
<td>1.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Pleopeltis masafuerae</td>
<td>3.0</td>
<td>3.3</td>
</tr>
<tr>
<td>Cerro Alto plant</td>
<td>2.3</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Although the number of measurements is rather low, the values obtained for width and diameter of rhizomes from the Cerro Alto plant are intermediate between those of its putative parents, P. macrocarpa and P. masafuerae.

Flow cytometry
Comparison of FCM values of the Cerro Alto plant with those of Mexican Pleopeltis taxa shows that it is most probably tetraploid (4x). Polyploidy is known within Pleopeltis, and, within P. macrocarpa, three cytotypes, diploid, tetraploid and hexaploid, have been reported (Evans, 1963; Jarret et al., 1968; Walker, 1973; Wagner...
FCM results are presented in the table below:

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Voucher</th>
<th>$RC_{\text{striata}}$*</th>
<th>Ploidy level</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pleopeltis polylepis var. erythrolepis</em></td>
<td>Viane 11005</td>
<td>1.56</td>
<td>2x</td>
</tr>
<tr>
<td><em>Pleopeltis guttata</em></td>
<td>Viane 11001</td>
<td>2.26</td>
<td>3x</td>
</tr>
<tr>
<td><em>Pleopeltis polypodioides var. knoblochiana</em></td>
<td>Viane 11013</td>
<td>2.54</td>
<td>3x</td>
</tr>
<tr>
<td><em>Pleopeltis × cerro-altoensis</em></td>
<td>cult Mulhouse</td>
<td>3.06</td>
<td>4x</td>
</tr>
</tbody>
</table>

*$RC_{\text{striata}}$ : average (based on 3 measurements) of relative DNA content compared with *Agave striata*.

From the value of 3.06 for $RC_{\text{striata}}$ for the new hybrid, we infer that it is tetraploid.

**DISCUSSION**

Interspecific hybrids are well known among species of *Pleopeltis*, as redefined by Smith & Tejero (2014), and are discussed there, as well as in several floras, e.g., Mickel & Beitel, 1987; Mickel & Smith, 2004. Such hybrids, mostly in the Neotropics but a few in southern Africa, have sometimes been treated under the hybrid genus, *×Pleopodium*, because historically they were thought to involve species of two different genera, *Pleopeltis* and *Polypodium*. Our study of the Cerro Alto plant shows that it is a hybrid that clearly belongs to *Pleopeltis*.

Only two species of *Pleopeltis* occur in the Juan Fernández Archipelago: *P. macrocarpa*, with entire, narrowly lanceolate laminae, and *P. masafuerae*, with deltate, pinnatisect laminae and undulate pinnae that are slightly decurrent onto the rachis. The latter species occurs on Alejandro Selkirk Island and on the coasts of Chile (Antofagasta province). Remarkably, *P. masafuerae* has never been found on Robinson Crusoe Island. However, due to the inaccessibility of large areas and infrequency of hybridization, it may have been overlooked.

The overall lanceolate laminar shape of the Cerro Alto plant is similar to that of *P. macrocarpa*, whereas its division, though less incised (pinnatilobed to pinnatifid) and with distinctly decurrent but less undulate pinnae, is more similar to *P. masafuerae*. In addition, we have compared the laminar morphologies of the Cerro Alto plant with the *Pleopeltis* hybrids described by Wagner & Wagner (1975) and Mickel & Beitel (1987), in particular between species with entire, lanceolate laminae, such as *P. macrocarpa* or *P. mexicana* (Fée) Mickel & Beitel, and a species with deltate pinnatisect laminae, such as *P. thyssanolepis* (A.Braun ex Klotzsch) E.G.Andrews & Windham that has laminae similar to those of *P. masafuerae*. Two hybrids, *P. ×leucospora* (Klotzsch) T.Moore and *P. ×tricholepis* (Mickel & Beitel) T.Moore, show a laminar morphology very similar to that of the Cerro Alto plant. An interesting similarity is the irregularity of the shape of the laminae and of the length of the pinnae, pointed out by Wagner (1962), that we notice also in our plant. However, the Cerro Alto plant differs from *P. ×leucospora* by a shorter stipe and a shorter terminal segment, and from *P. ×tricholepis* also by a shorter stipe, broader pinnae and a lamina reduced at its base.

These observations lead us to believe that *P. macrocarpa* and *P. masafuerae* are probably the parents of the Cerro Alto plant. As the hybrid is presumed tetraploid, based
on FCM values, we assume that tetraploid *P. macrocarpa* is involved in the combination, and that *P. masafuerae* is also tetraploid.

We conclude that the Cerro Alto plant is a hybrid new to the Juan Fernández Archipelago.

**Pleopeltis ×cerro-altoensis** Danton & Boudrie, *hyb. nov.*

Abortivis sporis, tetraploideum, hybridum filix est, repente rhizomate, 5-7 cm in longitudine fronde, angustissime alato atrobrunneo petiolo, appressis, fimbriatis peltatisque paleis penitus pinnatifido lanceolato limbo atque infra atrobrunneo et super viridiluteola rhachidi. *A* *P. macrocarpa* pinno-pinnatifido limbo, et *a* *P. masafuerae* basi reducta lanceolato limbo praecipue differt.

**Types:** CHILE. Archipel Juan Fernández, île Robinson Crusoe, Cerro Alto, Quebrada Puerto Inglés, rochers en falaise sur le versant est-sud-est du cerro, 300 m, 22 janvier 1999, Danton B(652)619 [holotype: SGO (Figure 6); isotypes: P, private herb. P. Danton].

**Description:** Epilithic fern. *Rhizomes* creeping, 2.5-3 mm diam. (4-5 mm including the scales), branched, densely covered with oblanceolate, acute, clathrate, bicolorous scales, with dark centres and narrow, light brown, and finely dentate-fimbriate margins. *Fronds* monomorphic, 0.5-1 cm apart, 6-15 × 0.6-4 cm. *Stipes* 2-5 cm, about 1/3 - ¼ as long as laminae, dark brown, with sparse, elongate scales. *Laminae* coriaceous, dark green, matt adaxially, light green to yellowish and slightly glaucous abaxially, narrowly elliptic to lanceolate, deeply pinnatifid, tapering proximally to reduced lobes, and below that long-decurrent (young fronds entire to sinuately lobed), distally ending in a long pointed to obtuse segment; laminar surfaces covered with numerous, scattered, peltate-based, appressed, bicolourous lanceolate scales with dentate or fimbriate margins, scales more abundant abaxially than adaxially. Dark brown rachis colour ending at the base of the apical segment abaxially, adaxially the colour ending at the first or second proximal pinnae and becoming green-yellowish distally. *Pinnae* 5-7 per side, triangular to oblong, 24-26 × 5.5-6 mm (for the longest ones), of variable length, oblique to rachis, alternate to more opposite in the distal half of the laminae, apices obtuse to rounded; laminar margins cartilaginous and slightly recurved. *Stomata* (45)-(53.5)-(63) µm long. *Sori* round to slightly oblong, ca. 0.3 cm diam., exindusiate, mixed with peltate scales, median on both sides of the costae or (in less divided blades) of the rachis. *Spores* whitish to yellowish, malformed and irregular in shape and in size, (53)-73.5-(90) µm long. Tetraploid (*4x*).

**Etymology:** The epithet of the taxon refers to the locality, Cerro Alto, where the plant was found.

**Distribution and habitat:** To our knowledge, endemic to Robinson Crusoe Island (Masatierra) in Juan Fernández Archipelago (Chile), on southern flanks of Cerro Alto. Plants grow in a limited population of four small, relatively close patches, on small rocky edges covered by thin soil, on subvertical unstable volcanic cliffs, exposed to the winds, at 300 m elevation (Figure 3). Due to its ability to revive, it can tolerate periods of drought.
Specimens examined (paratypes): CHILE. Juan Fernández Archipelago, Robinson Crusoe Island, Quebrada Puerto Inglés, rebords de rochers en falaise et balmes sur le versant est-sud-est du Cerro Alto, 300 m, M. Boudrie et session SBF, 20 février 2002, Boudrie 3793 (P).

Specimens ex horto examined (paratypes): CHILE. Juan Fernández Archipelago, Robinson Crusoe Island, Puerto Inglés, rebords de rochers en falaise et balmes sur le versant est-sud-est du Cerro Alto, 300 m, ex horto Conservatoire Botanique de la ville de Mulhouse (Haut-Rhin, France), M. Boudrie et J.-P. Reduron, 22 août 2003, Boudrie 3966 (P); idem, Ph. Danton et M. Hildebrand, 13 juin 2006, Z(hs)1727 (private herb. Ph. Danton); idem, M. Boudrie et J.-P. Reduron, 5 octobre 2009, Boudrie 4500 (P); idem, M. Hildebrand, 29 septembre 2014, Bizot 2905 (private herb. A. Bizot), and P. Danton - Mulhouse - 1, 2 & 3 (private herb. R. Viane).

IUCN Status: “CR”

Conservation ex situ: A plant was previously cultivated, from 1999 to 2004, in the garden of the Administration of the National Park, on Robinson Crusoe Island, but is now dead. Another specimen has been maintained and propagated in the Botanical Conservatory of the town of Mulhouse (France) since 2002.

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A CONTRIBUTION TO THE PHYLOGENY OF *DRYOPTERIS REMOTA* BY GENOTYPING OF A FRAGMENT OF THE NUCLEAR *PgiC* GENE

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Keywords: *Dryopteris remota*, PCR-direct, *PgiC* 15/16 fragment, genotyping, phylogeny

ABSTRACT
Since its discovery by A. Braun in 1834, there has been speculation on the origins of *Dryopteris remota*. The present contribution suggests that none of the previous assumptions are correct. *Dryopteris remota* is triploid and of hybrid derivation. Its parental species are the sexual allotetraploid *D. carthusiana* and an as yet unidentified sexual diploid species, most likely identical with the unknown parent of the apomictic diploid *D. affinis*. These assertions are based on comparison of traits, including habit, apomixis, colour of insertions of pinnae of the species under examination, and fragment length variation of the nuclear *PgiC* gene.

INTRODUCTION
The phylogeny and taxonomic status of *Dryopteris remota* (A.Br.) Druce is probably one of the most controversially discussed of any Central European fern species. When Alexander Braun discovered the plant near Baden-Baden in 1834 (Baden-Württemberg/S-Germany), he thought the new taxon to be a variation of *D. villarii* (Bellardii) Woynar ex Schinz & Tell. As such it was listed in 1843 by J. C. Döll in “Rheinische Flora”. In his book “Betrachtungen über die Erscheinung der Verjüngung in der Natur” of 1851, Braun revised his view in a footnote and described the taxon as a hybrid of *D. filix-mas* (L.) Schott and *D. carthusiana* (Vill.) H.P.Fuchs or *D. dilatata* (Hoffm.) A.Gray. He nonetheless attributed species rank to this taxon by calling it “Aspidium remotum”, i.e. in current nomenclature *D. remotum*. Since then a substantial number of renowned fern experts have speculated on its origin and taxonomic classification. In 1973 Benl and Eschelmüller presented an exhaustive description of this continuing dispute among scientists.

Further confusion had arisen following the discovery of a fern hybrid near Lake Windermere in north-west England in the middle of the 19th century. The morphology of this hybrid resembled very much that of *D. remotum*, but it had abortive spores. In 1859 A. Braun found a single plant of *D. remotum* among a large population of *D. filix-mas* near Aachen (Nordrhein-Westfalen/Germany), which led him to the conclusion that *D. remotum* must be a variation of *D. filix-mas*. He did not realize that this plant was not *D. remotum* but actually the same hybrid as that from Lake Windermere. He transplanted this hybrid to the Botanical Garden of Berlin and for approximatively 15 years he sent incorrectly labelled exsiccate from there to herbaria and to individual scientists before this mistake was discovered (Benl & Eschelmüller,
Further examination of a frond sample in the herbarium at Bonn revealed that Braun’s finding was the second record of the tetraploid hybrid *D. carthusiana × D. filix-mas*, as discovered earlier at Lake Windermere (Krause et al., 2001), and which has now been typified as *D. × brathaica* Fraser- Jenk. & Reichst. (1977). It is now possible to distinguish the two taxa through cytology (Manton, 1950), morphology (Fraser-Jenkins & Reichstein, 1984; Krause et al., 2001; Freigang et al., 2013), chemotaxonomy (Widén et al., 1976) and molecular genetics (Freigang et al., 2013).

*Dryopteris remota* is today recognized as a triploid species of hybrid derivation, but the dispute concerning its origin continues. From fundamental works by W. Döpp (1932; 1933; 1935; 1939; 1955) and I. Manton (1932; 1938; 1939; 1950), Döpp considered *D. remota* to be the hybrid of *D. dilatata × D. affinis* (Lowe) Fraser-Jenk. (Döpp, 1955). Reichstein, Fraser-Jenkins and others are of the opinion that the diploid apomictic *D. affinis* must be one of the parents due to the characteristics of *D. remota* (Fraser-Jenkins & Reichstein, 1984). In consequence the second parent would have to be a diploid sexual taxon that contributes the characteristics known from the *D. carthusiana* group to the genotype of *D. remota*. *Dryopteris expansa* (C.B.Presl) Fraser-Jenk. & Jermy was proposed (Widén et al., 1971, Fraser-Jenkins & Reichstein, 1984), even though chemo-taxonomic results did not match well (Widén et al., 1970; 1971; 1996). Furthermore, in an on-line discussion of the British Fern Group, Fraser-Jenkins (30 December 2008) reiterated doubts as to *D. expansa* being involved: “Although I agree that *D. expansa* is the most likely second ancestor of *D. remota*, it ought to have a ? along that line as it has never been proved in any way and the possibility of some other, even perhaps Asiatic species being involved still remains.”

To shed light on the ongoing discussions, the nuclear *PgiC*-marker of many species of the genus *Dryopteris*, including *D. remota*, has been examined.

**MATERIAL AND METHODS**

**Material**

The samples used in the investigation are listed in Table 1. Herbarium vouchers, currently in the collectors’ private herbaria will be deposited in JE.

**Methods**

Samples were analyzed at the nuclear *PgiC* gene (cytosolic 6-Phosphoglucoisomerase), individual fragments were generated and their lengths compared. The intron fragment between exons 15 and 16 proved especially suitable for comparison as it shows a species specific length for a number of species of the genus *Dryopteris*, especially in the *D. carthusiana* group (Juslén et al., 2011, Freigang et al., 2013). For this fragment Ishikawa et al., 2002 constructed an ‘EPIC’- primer pair (Exon Primed Intron Crossing), which offers almost general applicability. Due to a high frequency of poor PCR yields or complete PCR failures, the method described in Freigang et al. (2013) was partly replaced by the PCR-direct method of ThermoScientific (Phire Plant Direct PCR Kit, F-130WH). This method worked without DNA isolation and PCR yields were often higher and, according to the producer instructions the workflow consisted of only two steps.

1. A disc of 0.3-0.5 mm diameter was punched out from fresh or silica gel-dried leaf material and put into PCR-solution. 2. PCR was carried out according to the producer instructions. The uncleaned amplicon was genotyped in a sequencer (ABI 3500, Applied Biosystems or MegaBace Instrument, Healthcare). Employment of different
sequencers made transformation of results necessary (marked with an asterisk). The analysis of the generated data was first made with Fragment Profiler software (Healthcare), then later with GeneMapper software (Applied Biosystems). Sometimes the sequencing device produced a double peak in consecutive basepairs (bp). This could be an artefact or may be the result of PCR-conditions.

The relative height of signals within an electropherogram depended firstly on the number of sets of chromosomes causing the signal and, secondly, on the relative PCR yield of the 15/16-fragment of the existing alleles.

RESULTS

The genotypes of samples 1 to 24 are summarised in Table 2. Signals of electropherograms which were not separated are noted in the same table-field. Electropherograms of a subset of samples are shown in Figures 1-3 (underlined in Tables 1 and 2).

DISCUSSION

In the case of apomictic reproduction in ferns the sporophyte grows directly from a gametophyte with the unreduced number of chromosomes, and without gametes being involved. The sporophyte produces genetically uniform diplospores through a restitution nucleus; in the case of apomicts of Dryopteris, restitution occurs in the last mitotic cell division before the production of spore mother cells (Manton, 1950), resulting in the production of eight, rather than the usual 16 spore mother cells. Sporogenesis then results in the production of 32 (instead of the usual 64) spores, each with the same number of chromosomes as the sporophyte.

Fischer (1909; 1919) detected apomixis in D. remota and Manton demonstrated that it is a triploid taxon, with three different (non-homologous) chromosome sets (i.e. a heterotriploid fern) as in the occasional production of a spore mother cell of the ‘16 cell type’, the chromosomes are unpaired (non-homologous), with 123 univalents (3x). The heterotriploid character is also shown by the electropherogram of PgiC 15/16 fragments of the species (Figure 1), where three distinct peaks are observed. The electropherogram shows the following: The genome of D. remota contains at least three chromosome sets possessing fragments of different length and therefore derived from different alleles. This supports the results of cytology that the species is heterotriploid. The two shorter fragments of 488 bp and 500 bp correspond to the results seen in D. carthusiana (Figure 2), an allotetraploid species; no other taxon of the European ferns of the genus Dryopteris examined up to now shows this pattern.

Comparison with D. cristata (L.) A.Gray (sexual allotetraploid) and D. intermedia (Mühl. ex Willd.) A.Gray (sexual diploid) reveals that the peak at 488 bp differs from that of D. intermedia (500 bp), and is generated by the chromosome set derived from the common parental taxon of D. cristata and D. carthusiana, referred to as D. “semicristata” (Sc) by Walker (1955), a diploid species as yet unknown. The signal at 500 bp in D. remota is identical with that of D. intermedia (I). The chromosome sets of D. carthusiana can be symbolized by ScScII. The assumption that the parental species of D. carthusiana are D. intermedia and D. “semicristata” is supported (Table 2) and confirms the result of Sessa et al. (2012). The results are also in agreement with chloroplast sequence data by Juslén et al. (2011), which showed that D. carthusiana and D. remota share a maternally inherited set of chromosomes, referred to as “semicristata”. Table 2 shows that the 15/16-fragment length of D. expansa is the same
Table 1: Genotyped samples (Nomenclature according Fraser-Jenkins & Reichstein, 1984 and Fraser-Jenkins, 2007). RF: numbers according to rasters (see Niklfeld, 1971). Abbreviations of the names of collectors: GZ (G. Zenner/ Kirn), HWB (H. W. Bennert/ Ennepetal), JF (J. Freigang/ Bergatreute), SJ (S. Jeßen/ Chemnitz), WB (W. Bujnoch, Trier).

<table>
<thead>
<tr>
<th>Sample no. &amp; species</th>
<th>Origin</th>
<th>Collector/Determination/Date</th>
<th>Internal Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Dryopteris aemula</em></td>
<td>Portugal/ Azores/ São Miguel/ N-side and ridge at Pico da Vara, 1100 m MSL</td>
<td>GZ 28.5.2013 Dr 8</td>
<td>MPb1458, DE807, RUN32/2H</td>
</tr>
<tr>
<td>2. <em>D. affinis</em></td>
<td>Portugal/ Madeira/ N Funchal/ c. 600 m S of restaurant Ribeiro Frio at the roadside EN 103</td>
<td>JF/GZ 25.8.2011 JFV1086</td>
<td>MPb1724, RUN45/7A</td>
</tr>
<tr>
<td>3. <em>D. affinis</em></td>
<td>Germany/ RP/ Palatinate/ NE Wilgartswiesen, rock at Wiligartsburg, ca. 230 m MSL, RF 6713/32</td>
<td>WB/GZ 6.8.1996</td>
<td>DE032, RUN21/11B</td>
</tr>
<tr>
<td>4. <em>D. carthusiana</em></td>
<td>Germany/ NRW/ Märkisches Land, SW Hagen/ near Hasper dam, RF 4603</td>
<td>HWB 27.05.2013</td>
<td>MPb1442, DE791, RUN32/1B</td>
</tr>
<tr>
<td>6. <em>D. carthusiana</em></td>
<td>Germany/ RP/ middle Mosel/ W Trier/ Sirzenicher Bachtal, RF 6205/23</td>
<td>WB 17.7.2005</td>
<td>DE083, RUN22/10B</td>
</tr>
<tr>
<td>7. <em>D. carthusiana</em></td>
<td>Germany/ BW/ Oberschwaben/ NE Ravensburg/ SE Bergatreute/ near Kiliansweiher, RF 8124/32</td>
<td>JF 22.10.2006 JF 2006</td>
<td>DE385, RUN20/12A</td>
</tr>
<tr>
<td>9. <em>D. dilatata</em></td>
<td>France/ Haut–Rhin/ Vosges/ S Col de la Schlucht/ Sentier des Roches/ NE “les Trois Four“, 1050 m MSL, RF 7908/31</td>
<td>GZ/P.Holveck 27.8.2014 Dr 4</td>
<td>MPb1665, RUN40/11D</td>
</tr>
<tr>
<td>11. <em>D. expansa</em></td>
<td>Switzerland/ Wallis/ S Leukerbad/ Dala Valley/ lower Lochwald, 1260 m MSL, RF 9611/23</td>
<td>GZ 18.7.2000 Dr 1</td>
<td>MPb1230 DE696, GWBT182 RUN28/11F</td>
</tr>
<tr>
<td></td>
<td>Scientific Name</td>
<td>Locality</td>
<td>Collection Details</td>
</tr>
<tr>
<td>---</td>
<td>----------------</td>
<td>----------</td>
<td>--------------------</td>
</tr>
<tr>
<td>12.</td>
<td><em>D. intermedia</em></td>
<td>USA/ Vermont/ Rutland/ Leicester Hollow Trail/ NNE Brandon</td>
<td>HWB 15.8.2001 SJ-3476, DE368 RUN25/A11</td>
</tr>
<tr>
<td>13.</td>
<td><em>D. oreades</em></td>
<td>France/ Hérault/ Haut Languedoc/ W Bédarieux/ N Colombières-s-Orb/ near “La Fage”, c. 760 m MSL</td>
<td>GZ/WB 23.7.1994 Dr 1, DE067, GZ-58, RUN18/11C</td>
</tr>
<tr>
<td>16.</td>
<td><em>D. remota</em></td>
<td>Germany/BW/ Northern–Schwarzwald/ SSE Bad Herrenalb/valley of Rothenbach, 440 m MSL, RF 7216/21</td>
<td>GZ/WB 1.8.1990 spore cultured, FZ-90/7, GWBT 32, DE26, RUN19/12G</td>
</tr>
<tr>
<td>17.</td>
<td><em>D. remota</em></td>
<td>Germany/ BY/ W Berchtesgaden/ Ramsau/ SW of Schwarzbachwacht, c. 900 m MSL, RF 8343/31</td>
<td>GZ/JF 5.8.2013 Dr 2, MPb1516, DE 824, RUN35/11B</td>
</tr>
<tr>
<td>19.</td>
<td><em>D. remota</em></td>
<td>Austria/ Salzburg/ Pinzgau/ E Rauris/ valley of Geißbach, c. 1060 m MSL, RF 8744/31</td>
<td>GZ 6.8.2014 Dr 10, MPb1650, RUN44/11H</td>
</tr>
<tr>
<td>21.</td>
<td><em>D. remota</em></td>
<td>Germany/ BY/ West-Allgäu/ NW Scheidegg/ forest N “Lötz”, c. 700 m MSL, RF 8425/11</td>
<td>JF 4.6.2011 JF-V1008, MPb1250, DE710, RUN28/12D</td>
</tr>
<tr>
<td>22.</td>
<td><em>D. remota</em></td>
<td>North–Italy/ Prov. Varese /E Luino/ W Ponte Tresa/ Tresa-Valley near Biviglione, 250 m MSL, RF 0018/21</td>
<td>GZ/JF/SJ 15.6.2014 Dr 33, MPb1580, RUN40/9D</td>
</tr>
<tr>
<td>23.</td>
<td><em>D. remota</em></td>
<td>Northeast-Turkey/ Rize/ E Trabzon, S Ardeşen, Cat -Çamlıhemşin, c. 400m MSL</td>
<td>SJ-3026, TR-4011, CRFJ 4037, 27.08.1973, MPb1738, RUN47/A1</td>
</tr>
</tbody>
</table>
Table 2: Genotyping results for the length of the 15/16 *PgiC* fragment of samples 1 to 24 in base pairs (bp). The sample numbers in Table 2 correspond with those in Table 1. The measurements in Table 2 indicate that the two shorter fragments of *D. remota* correspond with those of *D. carthusiana* and the third fragment of *D. remota* with that of *D. affinis* and *D. oreades*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Fragment length in base pairs (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Dryopteris aemula</em></td>
<td>525</td>
</tr>
<tr>
<td>2*. <em>D. affinis subsp. affinis</em></td>
<td>530</td>
</tr>
<tr>
<td>3. <em>D. affinis subsp. affinis</em></td>
<td>530</td>
</tr>
<tr>
<td>4. <em>D. carthusiana</em></td>
<td>487 499</td>
</tr>
<tr>
<td>5. <em>D. carthusiana</em></td>
<td>486 499 500</td>
</tr>
<tr>
<td>6. <em>D. carthusiana</em></td>
<td>486 487 498 499</td>
</tr>
<tr>
<td>7. <em>D. carthusiana</em></td>
<td>487 500</td>
</tr>
<tr>
<td>8. <em>D. cristata</em></td>
<td>487</td>
</tr>
<tr>
<td>9. <em>D. dilatata</em></td>
<td>499 517</td>
</tr>
<tr>
<td>10. <em>D. expansa</em></td>
<td>499 500</td>
</tr>
<tr>
<td>11. <em>D. expansa</em></td>
<td>499 500</td>
</tr>
<tr>
<td>12. <em>D. intermedia</em></td>
<td>500</td>
</tr>
<tr>
<td>13. <em>D. oreades</em></td>
<td>529 530</td>
</tr>
<tr>
<td>14. <em>D. oreades</em></td>
<td>530</td>
</tr>
<tr>
<td>15. <em>D. pallida subsp. pallida</em></td>
<td>516</td>
</tr>
<tr>
<td>16. <em>D. remota</em></td>
<td>487 488 499 500</td>
</tr>
<tr>
<td>17. <em>D. remota</em></td>
<td>487 500</td>
</tr>
<tr>
<td>18. <em>D. remota</em></td>
<td>487 499</td>
</tr>
<tr>
<td>19*. <em>D. remota</em></td>
<td>489 501</td>
</tr>
<tr>
<td>20. <em>D. remota</em></td>
<td>487 500</td>
</tr>
<tr>
<td>21. <em>D. remota</em></td>
<td>488 500</td>
</tr>
<tr>
<td>22. <em>D. remota</em></td>
<td>489 501</td>
</tr>
<tr>
<td>23*. <em>D. remota</em></td>
<td>489 501</td>
</tr>
<tr>
<td>24. <em>D. villarii</em></td>
<td>532 533</td>
</tr>
</tbody>
</table>

* Employment of different sequencers made transformation of results necessary.
as that of *D. intermedia*. As mentioned in the introduction, the involvement of *D. expansa* in the parentage of *D. remota* has been the subject of much discussion. But this scenario would entail that the second parent of *D. remota* must be a diploid apomictic taxon of the *D. affinis* agg. with signals at 488 bp and 530 bp. Despite study of many taxa of the *D. affinis* agg., this combination has never been found. The same applies to the combination of signals at 500 and 530 bp.

During the first half of the last century *D. remota* was thought to be a hybrid of *D. carthusiana* × *D. filix-mas*, and Döpp endeavoured to backcross *D. remota* with the supposed parental species. He was successful in hybridizing *D. remota* only with *D. carthusiana* (Döpp, 1935). This provides some support for the conclusion that *D. carthusiana* is one parent of *D. remota*.

Already H. Fischer pointed to the fact that such a hybrid should also emerge in nature and he mentioned that the pharmacist H.K. Woynar (Graz) allegedly found intermediate forms in mixed stands of the two species at different sites in Tirol. Since 2011 putative hybrids of *D. remota* × *D. carthusiana* have been found at various locations in the wild (Freigang et al., in prep.).

The remaining question relates to the unknown identity of the third signal in the electropherogram of *D. remota*, at 530 bp. The chromosome set causing this third signal at 530 bp would have to contribute two qualities to *D. remota* that are characteristic of it and cannot be derived from the genetic material of *D. carthusiana* - the obligatory apomixis and the blue-black colour at the insertion of the pinnae (Fraser-Jenkins & Reichstein, 1984). Both features also exist in the *D. affinis* agg., but not in that of *D. filix-mas*. Examination of the diploid taxon *D. affinis* (Figure 3) shows a single peak at 530 bp.

According to current opinion *D. affinis* is a diploid apomict fern that shows non-homology of the two chromosome sets in 16-celled sporangia (Fraser-Jenkins, 2007) and it is presumed to have been derived from a cross between the sexual diploid *D. oreades* Fomin (OO) and a sexual diploid taxon, not yet identified but referred to as *D. “semaniaffinis”* (SaSa) (Krause, 1998). The *PgiC* 15/16 fragments of both taxa correspond to the third peak in *D. remota*. As *D. remota* is triploid and its genome

---

**Figure 1.** Electropherogram of *Dryopteris remota* (Schwarzwald, Smp.No. 16)

**Figure 2.** Electropherogram of *Dryopteris carthusiana* (Bergatreute, Smp. No. 7)

**Figure 3.** Electropherogram of *Dryopteris affinis* (Wilgartswiesen, Smp. No. 3)
contains two sets of chromosomes of allotetraploid *D. carthusiana*, *D. affinis* as the second parent is out of question, because as an apomictic taxon it would contribute both its chromosome sets to *D. remota*, resulting in a tetraploid taxon. This implies that the third peak (530 bp) in *D. remota* must be derived from a diploid sexual taxon with the 530 bp peak, and the only possible candidate from the results presented here are *D. oreades* or the missing diploid taxon *D. “semiaffinis”*. Unfortunately the length of *PgiC* 15/16 fragments of *D. oreades* and *D. “semiaffinis”* cannot be distinguished with the method used here. Of these two, *D. “semiaffinis”* is the more likely parent; *D. oreades* does not exhibit blue-black insertions, nor apomixis. We are left to infer that both diploid *D. affinis* and triploid *D. remota* have inherited these characteristics from *D. “semiaffinis”*, and hence the phylogeny of *D. remota* could be analogous to that of *D. affinis*, as shown in Table 3.

All the evidence suggests that “semiaffinis” induces apomixis in its hybrids and species of hybrid derivation, in contrast to hybrids involving *D. oreades*. This is the case in all heterodiploid as well as heterotriploid taxa of the *D. affinis* complex, which are all fertile but to varying degrees. In hybrids of taxa of the *D. affinis* complex with *D. filix-mas*, apomixis is also expressed, despite that fact the “semiaffinis” chromosome set is only one of four or five sets in the nucleus. The hybrid of *D. carthusiana* and *D. “semiaffinis”* (Table 3) has three non-homologous sets of chromosomes and would be expected to be sterile, in contrast to *D. remota* which is fertile and can produce viable offspring in the wild. This case of *D. remota*, gives further support to the hypothesis that

**Table 3.** Diagram of the hypothetical genesis of *Dryopteris remota* and *D. affinis*

<table>
<thead>
<tr>
<th>Parental Taxa</th>
<th><em>D. oreades</em> homodiploid, sexual</th>
<th><em>D. “semiaffinis”</em> homodiploid, sexual</th>
<th><em>D. carthusiana</em> allotetraploid, sexual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome sets of a somatic cell</td>
<td>OO</td>
<td>SaSa</td>
<td>IScISc</td>
</tr>
<tr>
<td>Chromosome set(s) of a gamete</td>
<td>O</td>
<td>Sa</td>
<td>ISc</td>
</tr>
<tr>
<td>Hybridisation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary hybrid</td>
<td>OSa heterodiploid, sterile</td>
<td>SalSc heterotriploid, sterile</td>
<td>D. affinis</td>
</tr>
<tr>
<td>Development of apomixis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progeny</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>chromosome sets of a somatic cell</td>
<td>OSa heterodiploid, apomictic fertile</td>
<td>SalSc heterotriploid, apomictic fertile</td>
<td></td>
</tr>
</tbody>
</table>
the set of chromosomes from “semiaffinis” has the ability to transfer the qualities of dark insertion of pinnae, and apomixis coupled with fertility.

The distribution of *D. remota* lies between the Pyrenees and the Caucasus and thus falls within the distribution of *D. carthusiana* and *D. affinis* (Fraser-Jenkins & Reichstein, 1984). The genetic variability of *D. remota* was examined by the isozyme-method (Schneller & Holderegger, 1994) and also by RAPDs (Schneller et al., 1998). The first method did not reveal any genetic variability within or between populations, the second only little. The authors suggested that the species developed relatively recently and only once, presumably in Caucasus or the Mediterranean region, and spread over the now populated area by ‘long-distance spore dispersal’. Since spores of apomicts produce sporophytes without the need for sexual fertilization, then an individual spore has the potential to give rise to a new individual or eventually even a new population.

During the past 180 years of discussion about the origin of *D. remota* other taxa have been proposed as possible parents, for example *D. villarii* (Braun in Döll, 1843), *D. dilatata* (Döpp, 1935; 1955), *D. aemula* (Aiton) O.Kuntze (Widén et al., 1971) or *D. pallida* subsp. *pallida* (Bory) Fomin and subsp. *raddeana* Fraser-Jenk. (Peroni et al., 1991). The results in Table 2 rule out almost all of these possibilities, with the exception of *D. pallida* subsp. *raddeana*, as samples of this taxon were not included in the study.

**ACKNOWLEDGEMENTS**

I would like to thank Prof. Dr. M. Veith, head of the Institute of Biogeography at Trier University, for providing me with a working place and also PD. Dr. A. Hochkirch, head of laboratory and the technicians at the laboratory. In particular I would like thank my colleagues, listed in Table 1, who made samples available for me. I would like to thank S. Jeßen and G. Zenner for their discussion and their valuable suggestions, also E. Erfurth and J. Beninde who helped with the translation of this paper into English. Finally I would like to thank the two reviewers and the editor for help and advice.

**REFERENCES**


SHORT NOTE

NOMENCLATURAL NOTE ON HEMIONITIS ARIFOLIA (PTERIDACEAE)

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The fern name Hemionitis arifolia (Burm.f.) T.Moore (Pteridaceae) is based on Asplenium arifolium Burm.f., first proposed by Burmann in 1768. Morton (1974: 316) found a sheet in the Geneva Herbarium (G, barcode G00360112, Figure 1) carrying two specimens and labeled as “Typus”. Alston in August, 1952, had determined these specimens as juvenile Acrostichum aureum L., not Asplenium arifolium. As the type belonged to another taxon Morton (1974) suggested the next available synonym Hemionitis cordata Roxb. ex Hook. & Grev. as the correct name for this taxon. The sheet carries Burmann’s annotation “Arifolium Asplenium Arifolio Petiv Tab 50 Fig 12” indicating that Burmann saw these specimens (Figure 1). But in diagnosis he mentioned “simple ovate auriculate sinuous frond” and these specimens showing non-auriculate, non-sinuous frond (Figure 1), and not the basis of description or diagnosis validating the name (McNeill et al. 2012, Art. 9.3). Thus these specimens are not Types and on that basis the name Asplenium arifolium cannot be reduced as synonym of Acrostichum aureum. Hemionitis arifolia is the correct name for Hemionitis cordata.

In the protologue Burmann mentioned Petiver’s (1702, t. 50, f. 12) and van Rhede tot Drakestein’s (1692-1703, t. 10, Fig. 2zzz) figures showing auriculate sinuous fronds and his diagnosis was based on these figures. Although Morton (1974) used the term “Type” for the specimen in G (Figure 1), he did not accept it, as is clear from his comment “If this really is the type…”; it is not acceptable as lectotype (McNeill et al. 2012, Art. 7.10). Here I select van Rhede tot Drakestein’s figure from Hortus Indicus Malabaricus (1703) as Lectotype. But, as no collection data is available for the specimen on which this drawing was based (Johnston 1970; Wagenitz 1978; McNeill et al. 2012, Rec. 8A.1 & 8A.2) an epitype is selected.

TYPIFICATION
Lectotype (designated here): van Rhede tot Drakestein, Hortus Indicus Malabaricus 12: 21, t. 10, 1703 (Figure 2).
Epitype (designated here): India. West Bengal: Burdwan, Kanchannagar, near bridge above canal, 30 m, 21 Nov 2007, J. Mazumdar 59 (CAL Figure 3).

ACKNOWLEDGEMENTS
I sincerely thank Dr. I. Valette, Secrétaire Herbiers and Conservatoire et jardin botaniques de la Ville de Genève, Switzerland (G) for providing permission for publication of image of G specimens; and Dr. A.R. Smith, University of California, Berkeley, U.S.A. and Mr. N.J. Turland, Botanischer Garten und Botanisches Museum Berlin, Germany for useful discussions.
REFERENCES


Figure 1. Type sheet of Asplenium arifolium Burm.f. from G (barcode G00360112). Image © Conservatoire et jardin botaniques de la Ville de Genève, Switzerland, reproduced with permission.


**Figure 2.** Lectotype of *Asplenium arifolium* Burm.f.: Tab. 10 from van Rhede tot Drakestein’s Hortus Indicus Malabaricus.
Figure 3. Epitype of *Asplenium arifolium* Burm.f. (J. Mazumdar 59) (CAL).
CONFIRMATION OF *LOXOGRAMME LANKOKIENSIS* (POLYPODIACEAE) IN INDIA

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The small epiphytic fern *Loxogramme lankokiensis* (Rosenst.) C.Chr. was first reported to occur in India from the Tirap District, Arunachal Pradesh, by Singh & Panigrahi (1987). Later Singh & Panigrahi (2005) suspected that an earlier record (Bhargavan & Joseph 1978) of *L. grammitoides* (Baker) C.Chr., from Arunachal Pradesh, Lohit District, may also represent *L. lankokiensis* and not *L. grammitoides* (Baker) C.Chr. However, they were unable to locate the specimen at CAL and could not make a definite decision (Singh & Panigrahi 2005). During the taxonomic study of ferns of Eastern India, I found this specimen of *Loxogramme lankokiensis*: INDIA. Arunachal Pradesh: Lohit District (N.E.F.A.), forest around Hayuliang along Dalai river, *J. Joseph 48934* (originally from CAL, now at BURD; Figures 1A–C).

In recent revisions Fraser-Jenkins (2008, 2012) followed the opinion of Dr. M.G. Price (University of Michigan, Michigan) and claimed that the report of *L. lankokiensis* from India was based on the misidentification of small plants of *L. grammitoides*. Close examination of *J. Joseph 48934* showed features that matched with characters of *L. lankokiensis*as described in literature (Singh & Panigrahi 2005, Zhang et al. 2013). *Loxogramme lankokiensis* has dimorphic fronds (Figure 1C) and rhizome scales with entire margins whereas *L. grammitoides* has mostly monomorphic fronds and rhizome scales with slightly dentate margins, according to Zhang et al. 2013. This specimen (*J. Joseph 48934*) confirms the earlier report of *L. lankokiensis* in India, Lohit District, Arunachal Pradesh by Singh & Panigrahi (2005), in addition to China, Thailand and Vietnam.


**ACKNOWLEDGEMENTS**

I sincerely thank the Director, Botanical Survey of India and Prof. R. Mukhopadhyay, Pteridology Section, Department of Botany, The University of Burdwan for kindly providing opportunity to study the specimen and Dr M. Lehnert, University Bonn, Germany for constructive suggestions.

**REFERENCES**


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**Figure 1.** A.–C. *Loxogramme lankokiensis* (J. Joseph 48934 CAL): A. & B. Specimen from Arunachal Pradesh, India, C. Part of plant showing distinct fertile and sterile fronds.

SHORT NOTE

THREE NEW COMBINATIONS IN CTENOPTERELLA (POLYPODIACEAE)

Three new combinations are proposed for Ctenopterella (Polypodiaceae) in preparation for a monograph of the genus.

**Ctenopterella lasiostipes (Mett.) Parris comb. nov.**

**Ctenopterella thwaitesii (Beddome) Parris comb. nov.**

**Ctenopterella undosa (Baker) Parris comb. nov.**

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This festschrift for Wilfried Bennert on the occasion of his 65th birthday is a collection of 11 papers gathered together by Karsten Horn and illustrated with a series of etchings of ferns by Reinhard Böcker. The subjects of the articles reflect Bennert’s wide interests in pteridology and ecology. The volume starts with a description of his life and career in science, the Bochum days, and how he fell under the spell of ferns, horsetails and clubmosses. He carried out fieldwork not only in Europe but also in California, Iceland, Southeast Asia and Russia, and Horn describes the work that culminated in one of his major fern projects, the publication of *Die seltenen und gefährdeten Farnpflanzen Deutschlands* in 1999. This is followed by a full list of his publications.

The subjects and authors of the other papers are summarised below.

Reinhard Bornkamm describes the ferns, horsetails, *Ephedra* and gymnosperms of Egypt and their role in plant communities. Herbert Sukopp looks at three ferns in 19th horticulture around Berlin. Andreas Sarazin et al. describe new records of neophyte fern taxa that have been discovered in Northwest Germany, including species of *Adiantum*, *Cyrtomium* and *Pteris*. Böcker describes the historical and present distributions of ferns, lycophytes and horsetails from the Stuttgart area, and assesses the impact of human activity. Marcus Lubienski provides photographs, detailed descriptions and distributional data for all known hybrids of *Equisetum* in Europe, and their morphological variability is discussed. Caroline Van den heede and Ronnie Viane report the discovery of *Asplenium lolemnamense* and *A. × chasmophillum* and discuss their origins. Wolfgang Jäger reports the occurrence of ascomycete fungi on ferns in the Bergisches Land, Northrhine-Westphalia. Karsten Horn has published an account of the life and scientific output of K.A.J. Milde, a great pteridologist of 19th, with illustrations from some of his significant monographs. Henning Haeupler reviews and clarifies the work on life forms to describe vegetation, first introduced by Humboldt in 1806 and extended by Raunkiaer in 1907. The volume concludes with plant colonisation in shallow waters - plants of the littoral zone, by Klaus Kaplan.

Wilfried Bennert has been a member of BPS since 1975, and is active in the Group of European Pteridologists.

M. Gibby
INSTRUCTIONS FOR AUTHORS

PAPERS should not usually exceed 20 printed pages and are generally expected to be considerably shorter. As well as reports of original research, review articles and short notes, e.g. new records, are encouraged. The senior author should supply an email address to facilitate correspondence.

REVIEWS should cover recent developments in any aspect of research on ferns and lycopods. This can include unpublished research by the author, and/or the possible direction of future research. They should be aimed at the general reader who may not be an expert in the chosen topic. Proposals for a review should be discussed first with the review editors.

MANUSCRIPTS should be submitted in English (British) in electronic format in 10-point Times New Roman font and double spaced. Electronic versions of text and tables should be compatible with WORD, with figures as pdf or jpg files, and sent as email attachments or CDroms. All manuscripts will be refereed.

THE TITLE should reflect the content of the paper and be in BOLD CAPITALS (11-point) and centrally aligned. Generic and specific names should be in italics and any title containing a generic or specific name should be followed by the family name in brackets e.g.

TRICHOMANES SPECIOSUM (HYMENOPHYLLACEAE)
IN SOUTHERN SPAIN

AUTHOR ABBREVIATIONS should follow Pichi Sermolli’s (1996) Authors of scientific names in Pteridophyta, Royal Botanic Gardens, Kew.

MAIN HEADINGS: should be in BOLD CAPITALS (10-point) and centrally aligned.

SUBSIDIARY HEADINGS: should be in bold, the first letter of each word in capitals, the rest in lower case and left-aligned.

AUTHORS’ NAMES AND FULL ADDRESSES (including email address): follow the title and are centrally aligned.

KEY WORDS: up to ten.

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FIGURES: there is no distinction between photographs and line drawings in numbering. All should be presented in a form ready for reproduction, colour, TIF format and 300 dpi (please contact editor with queries), with a scale bar where appropriate. Lettering or numbers (Arabic) should be in the bottom left using uppercase Times Roman. Figure captions should be on a separate sheet.

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REVIEW

MYCORRHIZAL RELATIONSHIPS IN LYCOPHYTES AND FERNS

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ABSTRACT

Mycorrhizae, i.e., symbiotic associations between fungi and plant roots, occur in about 80% of land plant species and have been shown to benefit both the fungus (carbon uptake) and plant (nutrient and water uptake, protection against pathogenic fungi). We here provide a brief overview of the state of knowledge of mycorrhization in ferns. Only about 62% of species studied to date have mycorrhizae, with arbuscular mycorrhizal fungi (mainly Glomeromyctea) being the dominant partners, while other associations are made with ascomycetes and the so-called Dark Septate Endophytes. There is no clear phylogenetic signal in mycorrhization among ferns, with both basal (e.g., Anemiaceae, Gleicheniaceae, Cyatheaceae) and derived families (e.g., non-grammitid Polypodiaceae, Tectariaceae, Aspleniaceae) having <50% of species mycorrhizal. Ecologically, epiphytes have lower degrees of mycorrhization than terrestrial species, with aquatic taxa almost completely lacking mycorrhizae. This probably reflects the requirements of the fungi. There are no experimental studies on the benefits of mycorrhizae for ferns, but field experiments suggest that there is a fine balance between positive and negative effects, so that many fern species have disposed of the fungi either generally or facultatively. Much remains to be learnt about mycorrhization in ferns, especially in an evolutionary context in comparison with bryophytes and seed plants.

INTRODUCTION

The term mycorrhiza (pl. mycorrhizae) was introduced by Frank (1885) for structures in angiosperm roots caused by fungi and translates as “fungus root”. Since then it has been shown that it is one of the most common and important symbioses in the land plants (Brundrett, 2002; 2004; 2009), even in plants like bryophytes and Psilotaceae that lack roots. It is estimated that ca. 80% of all land plants engage either in facultative or obligate mycorrhizal associations (Trappe, 1987; Wang & Qiu, 2006; Brundrett, 2009).

It is likely that the colonization of land by early land plants was facilitated by mycorrhizae (Pyrozinski & Malloch, 1975; Remy et al., 1994; Blackwell, 2000; Selosse et al. 2015), even before roots first arose (Taylor et al., 1995; Brundrett, 2002; Kenrick & Strullu-Derrien, 2014). This assumption is based on the presence of structures very similar to extant symbiotic Glomeromycota in the earliest tracheophyte fossils from the Rhynie Chert (Remy et al., 1994; Strullu-Derrien & Strullu, 2007; Selosse et al., 2015).
and supported by the conserved nature of the genes regulating mycorrhizal associations across all land plant lineages (Wang et al., 2010).

Mycorrhizal fungi are known to provide numerous benefits to their plant hosts (Selosse & Rousset, 2011). They enhance nutrient uptake, especially of phosphorous (Siddiqui & Pichtel, 2008), increase drought tolerance by facilitating water uptake (Faber et al., 1991; Warren et al., 2008), and compete with parasitic fungi thus protecting plants from parasitic fungal infections (Newsham et al., 1995; Brundrett, 2002). In return, the fungal partners receive carbohydrates from the host plant (Selosse & Rousset, 2011). The intensity of this symbiosis may vary with external ecological factors (Allen et al., 2003; Antunes et al., 2011) and apparently can be regulated more actively by the plant host than by the fungal symbiont (Barker et al., 1998; Jones et al., 2004).

Most research on mycorrhizae has been conducted on seed plants because of their dominance, diversity and economic importance (Brundrett, 2004; 2009). In recent years, the scientific focus has shifted more to the origin of this symbiosis and hence to its presence in old land plant lineages like extant bryophytes and lycophytes (Read et al., 2000; Rimington et al., 2015). Mycorrhizae in ferns have been regularly screened for but rarely been in the focus of physiological or synthetic studies combining ecology and phylogeny (Wang & Qiu, 2006; Brundrett, 2009).

In the present paper, we provide a brief overview of the still incomplete knowledge of mycorrhization in lycophytes and ferns (for simplicity called ferns in the following).

**TYPES OF MYCORRHIZAE**

Four major types of mycorrhiza have been distinguished (Barker et al., 1998; Smith & Read, 2008; Siddiqui & Pichtel, 2008) with some characteristic deviations and aberrations in some major clades of host plants (Figure 1; Brundrett, 2002; 2004).

Arbuscular mycorrhiza (AM) is an endosymbiosis, in which the fungi penetrate the cell walls of the root cortex and form characteristic organs in the living cells for nutrient exchange, the so-called arbuscules and vesicles (Figure 2a,b; Bonfante & Genre, 2008). It is the most common, and probably the ancestral form of mycorrhizae (Field et al., 2015a), occurring in ca. 80% of extant land plants (Wang & Qiu, 2006; Brundrett, 2009). The fungal partners are asceptate Eumycota, in the majority Glomeromycota (Schüßler et al., 2001), which are asexual and obligate symbionts (Brundrett, 2004; 2009). In some basal lineages of landplants (hornworts, some liverworts and lycophytes, a few ferns), Mucoromycotina, which are either basal to or sister to Glomeromycota, may also form a similar endomycorrhiza but without the presence of arbuscules (Desirò et al., 2013; Strullu-Derrién et al., 2014; Field et al., 2015a, b).

Ectomycorrhizae (ECM) are mainly formed by the more derived septate Ascomycota and Basidiomycota (Taylor & Alexander, 2005; Taylor et al., 2010) but in a few cases also endogonalean fungi (Mucoromycotina, Hibbet et al., 2007). They build an internal network of hyphae that surround the intact root cells (Hartig net) and an external hyphal sheath around the root tips (Smith & Read, 2008; Brundrett, 2004). ECM occur in 2.0–4.7% of seed plants but dominate among tree species in temperate and boreal zones families (Smith & Read, 2008; Brundrett, 2009). Until now, ECM have not been confirmed for ferns. It has been suggested that ferns shift from AM to ECM in forests where ectomycorrhizal tree species are dominant (Cooper, 1976; Iqbal et al., 1981), but the claim that the investigated roots really belonged to ferns has been strongly contested (Smith & Read, 2008; Brundrett, 2002). Fungal symbioses interpreted as derivations of ECM are the Arbutoid mycorrhiza and the Monotropid mycorrhiza (Brundrett, 2004),
Figure 1. Diagram of the assumed relationships between the different main types of mycorrhizae (AM, ECM, ericoid, orchid) and their modifications (facultative, exploitative, mixed) based on studies of all land plants. For ferns, only AM are confirmed; exploitative AM occur in some gametophytes; ECMF and ericoid MF have been found in some lycophytes and ferns but without expressing the regular phenotype of the mycorrhiza (MF = mycorrhizal fungi). Numbers above arrows indicate instances of transitions ($\alpha =$ many). Adapted from Brundrett (2002)
but these are also not reported for ferns.

Ericoid mycorrhizae are formed with septate Ascomycota, which all are primarily decomposers and saprophytes (Hibbet et al., 2007). They are uncommon but typical of Ericaceae (Cullings, 1996; Brundrett, 2004) and some other plant groups occurring on nutrient-deficient substrates characterized by low primary production (Cairney & Mehar, 2003). Typical of this symbiosis is that the hyphae enter through root hairs and form hyphal coils in cortical cells (Read, 1996). ascomyceta infections of the Ericoid type have been reported for tropical ferns, mainly epiphytes, such as *Elaphoglossum*, grammitid ferns, *Hymenophyllum* and *Trichomanes* (Schmid et al., 1995; Lehnert et al., 2009). Records of the so-called Dark Septate Endophytes (DSE) in ferns (Dhillion, 1993; Jumpponen & Trappe, 1998; Fernández et al., 2008) may include still unrecognized Ericoid mycorrhizae since the taxonomic identity of most of these fungi is unknown (Jumpponen, 2001; Rains et al., 2003). Like the Ericoid mycorrhizal Ascomycota, DSE are more common in epiphytic fern species (Kessler et al., 2010).

Orchid mycorrhizae involve a small group of Basidiomycota (Brundrett, 2002), and are characterized by the initial carbon dependency of the host plant seedling on the saprophytic fungal partner; several orchid lineages have independently developed an exploitative life-style from this condition (Brundrett, 2002; 2004).

Such exploitative mycorrhizae are not known from the sporophytes of lycophytes and ferns, but gametophytes of Lycopsidaceae (Duckett & Ligrone, 1992; Winther & Friedmann, 2008), Ophioglossaceae (Schmid & Oberwinkler, 1994; Field et al., 2015c) and Psilotaceae (Duckett & Ligrone, 2005; Winther & Friedmann, 2009) are well known for being mycoheterotrophic. These mostly involve AMF but recently Horn et al. (2013) reported the steady prevalence of basidiomycetes of the order Sebacinales (as found in orchid mycorrhiza and ECM) in gametophytes of *Diphasiastrum alpinum* (L.) Holub. This had been anticipated because basidiomycetes had already been documented as mycorrhizal endophytes for bryophytes (Pressel et al., 2010).

Mycorrhizae are species-specific regarding their phenotype (i.e., one fungus and one plant always engage in a specific type of interaction) but not regarding the taxa involved: Ferns can host a mixture of up to five different mycorrhizal fungi at the same time but with one usually dominating (Jansa et al., 2008), and one fungus can colonize different

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**Figure 2a.** Internal structures of arbuscular mycorrhiza (vesicles and arbuscules, lower ones partially released from cell due to preparation) in the root cortex of the tree fern *Cyathea tortuosa* R.C.Moran from Ecuador (Prov. Napo, 500 m) (20 x).

**Figure 2b.** The roots of a typical non-mycorrhizal plant with pronounced root hair formation, represented by *Elaphoglossum castaneum* (Baker) Diels from Ecuador (Prov. Napo, 2000 m). (10 x) Photos Ramona Güdel.
plant species (Gemma et al., 1992; van der Heijden et al., 2015).

The true symbiotic nature of a fungal association can only be confirmed by physiological experiments aiming at the flow of nutrients (e.g., Jurkiewicz et al., 2010). As this has not been done for any fern yet, it is uncertain whether most observed associations are really symbiotic or functionally neutral (Brundrett, 2004; 2009). As Glomeromycota are fully dependent on the symbiosis and only known to occur independently from a host plant when they are dormant spores (Brundrett, 2009), it seems likely that a symbiotic relationship is involved in most cases but not necessarily with the roots in which they are found. Under high inoculum pressure in the substrate, typical non-mycorrhizal taxa may harbor AMF as neutral endophytes while the same mycelium probably forms a true mycorrhiza with a typical host plant nearby (Lekberg et al., 2015). Similarly, some typical non-mycorrhizal taxa have been found to be able to host AMF under nutrient deficiency (Brundrett, 2009). Furthermore, a low presence of fungi in the roots must not necessarily imply a low physiological activity between both partners. The categorization into obligate and facultative mycorrhizal plant species can only be evaluated in controlled experiments, e.g., by establishing sterile cultures of both partners and later re-inoculation (Pearson & Read, 1973), and not by visual estimation alone.

TAXONOMIC DISTRIBUTION OF MYCORRHIZAE

The mycorrhizal status is regarded to be ancestral among ferns (Zhao, 2000; Wang & Qiu, 2006), as is likely for all land plants (Pirozynski & Malloch, 1975; Strullu-Derrién et al., 2014; Field et al., 2015a).

The scant reviews of fern mycorrhizae have focused on the sporophyte and have come up with highly diverging results regarding the general presence of fungal symbionts as well as the distribution among the different groups. Newman and Reddell (1987) found 87% to be always mycorrhizal, 7% facultative, and 6% not mycorrhizal (based on 180 fern species), which is within the range of the values found for angiosperms (Brundrett, 2009). Others reported 75% of the studied fern species to have AM (Gemma et al., 1992). Zhao (2000) found only 33% of 256 species from China to be mycorrhizal (17% obligate + 16% facultative), and similarly Moteetee et al. (1996) reported 36% of the species to be mycorrhizal. Cooper (1976) found 100% of the investigated ferns to harbour mycorrhiza, but the sampling included only terrestrials and low epiphytes from humid subtropical to southern temperate forest, i.e., conditions that foster fungal growth. On the other extreme, Lesica & Antibus (1990) focused on epiphytes and found no mycorrhiza in the 12 investigated species. Wang & Qiu (2006) found 52% of all species and 92% of all families to harbour AMF. Brundrett (2009) summarized only more recent reports on mycorrhizae that he could trust, but contrary to his detailed summary on spermatophytes, he could not give an exact evaluation for ferns.

Lehnert (2007) used all available data on fern mycorrhiza worldwide starting from Boullard (1958, 1979), and evaluated simple presence/absence of mycorrhizal colonisations in three classes (aseptate fungi = AMF; septate fungi = DSE/ascomycetes; unspecified mycorrhiza) in 675 taxa (ca. 6% of the global diversity; Smith et al., 2006). The observed average of 62% for fungal colonisation lies in the middle of the range of previous reports for ferns but is lower than the values for seed plants (Brundrett, 2009). AMF are dominant among the fungal symbionts with 66%, whereas the remaining 34% are made up of ascomycetes, DSE and unspecified mycorrhizations. Across the phylogeny at the family level (sensu Smith et al., 2006), below average mycorrhization (≤62%) was found among several families of derived Polypods (non-grammitid
Polypodiaceae 23%, Tectariaceae 25%, Aspleniaceae 45%) but other families of the Polypods such as Davalliaceae and Onocleaceae had 100% mycorrhization. Vice versa, among the other leptosporangiate ferns, several families had below average mycorrhization rates (Anemiaceae 50%, Gleicheniaceae 48%, Cyatheaceae 53%). Among the eusporangiate lineages, only the Equisetaceae showed low mycorrhization (22%) whereas the remainder had average to high values (Psilotacae 67%, Marattiaceae 91%). Families largely restricted to aquatic habitats showed low (Isoetaceae; one of two samples) or no (Marsileaceae, Salviniaceae) fungal colonization. Mycorrhizal epiphytes were found mainly among grammitid ferns (84% of epiphytes and 74% of all species mycorrhizal), Hymenophyllaceae (76% of epiphytes and 76% of all species mycorrhizal) and Dryopteridaceae (63% of epiphytes [mainly Elaphoglossum] and 62% of all species mycorrhizal). Interestingly, only 42% of the investigated sporophytes of Lycopodiaceae had mycorrhizae despite their predominantly mycoheterotrophic gametophytes (Winther & Friedmann, 2008).

Later substantial additions to the survey (Kessler et al., 2009; 2010b; 2014a; Zubek et al., 2010; Ogura Tsujita et al., 2013; Muthukumar & Prabha, 2012; 2013; Lara-Pérez et al., 2015) have increased the species count but have not significantly changed the originally found total average and ratios between the substrates (Lehnert et al., unpublished data).

A caveat to bear in mind when comparing many different reports is the often uneven approaches regarding evaluation and quantification of the presence of fungi, as has been pointed out for AMF by Brundrett (2009). However, considering that it is more likely that fungi present have been over-interpreted as symbionts rather than that true, functioning mycorrhizas have been overlooked, these numbers are more likely to drop than to rise after a thorough re-evaluation. Thus, the observed pattern that lycophytes and ferns have a below-average colonization rate compared with seed plants or land plants in general is expected to hold true.

A similar but even stronger uncertainty lies in the data supporting the mutualistic nature of these colonizations, which would allow them to be addressed undisputedly as mycorrhizae (Brundrett, 2002; 2009). Most reports used only light microscopical preparation techniques to investigate the presence of fungi, which render the sample unsuitable for further ultrastructural and molecular studies (pers. obs. for ferns, but see Ishii & Loynachan, 2004). Even if obligately mycorrhizal fungi abound in the roots, this does not necessarily mean that the partners interact physiologically (Lekberg et al., 2015). Electron microscopy may allow the larger classification of the fungus and could reveal whether the host cell responded more in a symbiotic way or if it just tried to reject and contain the penetrating fungus (e.g. Schmid & Oberwinkler, 1994; Schmid et al., 1995). So far such ultrastructural and/or genetic investigations of mycorrhizae in the sporophyte generation covered only 12 species of lycophytes (Duckett & Ligrone, 1992; Winther & Friedmann, 2007a; Rimington et al., 2015) and 35 species of ferns (Schmid et al., 1995; Schmid & Oberwinkler, 1996; Pazmiño, 2006; Winther & Friedmann, 2007b; 2009; Lehnert et al., 2009; Field et al., 2015c; Rimington et al., 2015), which represents only a small fraction (3.7 %) of the 1,287 species of lycophytes and ferns for which reports are available (ca. 11 % of the global diversity; Lehnert et al., unpibl. data)

**LIFE-STAGE DISTRIBUTION OF MYCORRHIZAE**

Mycorrhizal interactions with fern gametophytes are not as uniform as often stated (Mehltreter, 2010). With a few exceptions (Duckett & Ligrone, 1992), gametophytes of
Lycopodiaceae, Psilotaceae (Duckett & Ligrone, 2005) and Ophioglossaceae are achlorophyllous and mycoheterotrophic, meaning not only do they receive water and minerals but also carbohydrates from the fungi (Schmid & Oberwinkler, 1994). By this means, the gametophytes may tap into the carbon source of another, photoautotrophic host of the fungus (Harley & Harley, 1987; Horn et al., 2013), possibly even the parental sporophyte (Winther & Friedmann, 2008). The anatomy of these mycoheterotrophic gametophytes, which are tuberous and relatively thick textured (Winther & Friedmann, 2007; 2008; 2009), allows an easy colonisation by fungi, whereas across fern lineages with photoautotrophic gametophytes, we see a gradual reduction of size (especially thickness) and longevity along the phyletic sequence (Smith et al., 2006). Mycorrhization rates seem to follow this pattern.

The eusporangiate Marrattiaceae (Bower, 1923; Ogura-Tsuijita et al., 2013) and the basal leptosporangiate Osmundaceae, Plagiogyriaceae, Gleicheniaceae and Cyatheaceae (Ogura-Tsuijita et al., 2015) show relatively constant and high presence of AM fungi (58–97%) in the thick centre of the gametophytes. However, most leptosporangiate families have the common heart-shaped *Polypodium*-prothallium and offer only restricted space for fungal symbionts; accordingly fungal colonization is observed infrequently (e.g., Reyes-Jaramillo et al., 2005; Turnau et al., 2005; Muthukumar & Prabha, 2012) and most taxa are considered to be only facultatively mycorrhizal (Read et al., 2000). In leptosporangiate lineages with strongly dissected to filiform prothallia (e.g., some Hymenophyllaceae, grammitid Polypodiaceae), AM are not observed (Boullard, 1979).

Even if both generations in the life cycle are mycorrhizal, the sporophyte does not directly inherit the fungal partner from the gametophyte but has to acquire it anew from the substrate (Schmid & Oberwinkler, 1995), so that each generation can have a different assortment of fungal partners, although usually with a strong overlap (Winther & Friedmann, 2008).

**HABITAT DISTRIBUTION OF MYCORRHIZAE**

Among ferns, most studies have only considered selected species; whole fern communities have been studied only in Canada (Berch & Kendrick, 1982), China (Zhao, 2000), La Réunion (Kessler et al., 2010), and Ecuador (Kessler et al., 2014a). In general, the mycorrhization rate of soil-growing fern species is typically higher than that of epiphytic species (Gemma et al., 1992; Kessler et al., 2010). Thus, Lehnert (2007) reported putative mycorrhizal fungi mainly in terrestrial and saxicolous species (67% and 59%, respectively) but only in 53% of epiphytic species and in 9% of aquatic species. The percentage in epiphytes included a high presence of ascomycetes + DSE (67% of colonisations), whereas the remaining 33% of epiphytic mycorrhiza represented only 8% of the total of AMF colonisations in all lycophytes and ferns.

The low rate of mycorrhizal infections among epiphytes may be caused by either the fungi or the ferns. Glomeromycetes, in particular, are expected to have a lower colonization potential than fungi with air-borne spores (e.g., ascomycetes, basidiomycetes) because they produce spores subterraneously and require stable substrate conditions for growth and development (Zubek et al., 2010), thus being hardly suited for the dynamic canopy habitat (Janos, 1993). It has also been suggested that epiphytic ferns may be less dependent on mycorrhizae because they have special adaptations (e.g., drought resistance, leaf litter traps, thick storage rhizomes) that limit requirements for mycorrhizae (Mehltreter, 2010).

Among the ferns growing on the ground, there are also slight differences between
substrates, with those on well-developed soil and rocks having high levels of mycorrhization than those on sand – again a habitat that is unsuitable for fungal growth (Brundrett, 2009) – having fewer mycorrhizal fern species (Gemma et al., 1992).

A special case is provided by raw volcanic soils, especially on islands such as Hawai’i. It has been suggested that such soils are more easily colonized by nonmycotrophic or facultatively mycorrhizal fern species due to the paucity of fungal inoculum, perhaps facilitating later colonization of obligate mycotrophic ferns and angiosperms (Gemma & Koske, 1990; Gemma et al., 1992). This contrasts, however, with the regular presence of mycoheterotrophic gametophytes of *Psilotum* in volcanic soils (Holloway, 1938; DUCKETT & Ligrone, 2005) and observations on La Réunion, where the percentage of terrestrial fern individuals with mycorrhizae was higher on shallow, raw soils with high pH values, high base-saturation and low nutrient availability (Kessler et al., 2010). These contrasting results may be due partly to the fact that the Hawai’i studies were based on the species level, whereas the results from La Réunion are based on numbers of individuals.

Mycorrhization is absent to very rare in aquatic environments (Boullard, 1958; 1979; Lehnert et al., 2007; Brundrett, 2009), presumably reflecting the paucity of fungi in such environments. Furthermore, the benefits mycorrhiza could bring are no longer advantageous in a medium in which water and most nutrients are freely available and often in surplus, but – at least in the case of fully submerged plants – assimilation is hampered by lower CO$_2$ partial pressure and decreased light intensity. In some cases, amphibious species that grow both inundated and on dried-out soils are facultatively mycorrhizal, as found in some *Isoetes* (Beck-Nielsen & Madsen, 2001; Radhinka & Rodrigues, 2007). Likewise, species of *Equisetum* are non-mycorrhizal in wetlands, but commonly mycorrhizal in dry environments (Harley & Harley, 1987; Marsh et al., 2000; Read et al., 2000; Brundrett, 2002).

Focussing on climatic gradients, the degree of mycorrhization has been documented to decrease with elevation in Ecuador (Kessler et al., 2010). In addition to the commonly proposed fungus-oriented explanations based on reduced fungal growth at low temperatures (Read & Haselwandter, 1981; Olsson et al., 2004; Schmidt et al., 2008), these authors also proposed a fern-oriented one in that mycorrhization might decrease because the relative costs of allocating carbon to the fungal partner increase with elevation (where photosynthesis decreases: Kessler et al., 2014b) and possibly exceed the benefits of the fungal nutrient supply.

**ECOLOGICAL EFFECTS OF MYCORRHIZATION IN FERNS**

Based on work on seed plants, it is known that fungi provide plants with soil nutrients (especially PO$_4$), water, and other benefits (Blackwell, 2000; Wilkinson, 2001). In exchange, the fungi obtain organic C from the photosynthetic host plants (Smith & Read, 2008; Olsson et al., 2010), which allocate up to 20% of their net photosynthetic C to their fungal partners (Allen et al., 2003). The relative costs of this fungal C use have to be assessed in relation to the benefits derived from increased nutrient uptake, and presumably can be offset partly by higher rates of photosynthesis, if the plants are not light limited (Smith & Smith, 2011). In greenhouse experiments with potted plants, mycorrhizal fungi have been shown to increase leaf tissue nutrient concentrations, most importantly those of P (Stribble et al., 1980; Smith & Smith, 2011).

For ferns, very few data are available, and contrary to seed plants there are no greenhouse experiments. In an early study, Cooper (1976) showed that mycorrhizal plants
of the facultatively mycorrhizal *Pteridium aquilinum* (L.) Kuhn in New Zealand had stronger growth than non-mycorrhizal individuals. More recently, it has been shown both on La Réunion and in Ecuador that terrestrial mycorrhizal fern species are locally more abundant than those lacking mycorrhizae (Kessler et al., 2010; 2014a). For example, on La Réunion, the 78% mycorrhizal species made up 98% of all fern individuals. This suggests a clear ecological advantage provided by mycorrhizae to ferns.

On the other hand, a study in Ecuador found that fern species with mycorrhizae had lower relative biomass increment per year than those without mycorrhizae (Kessler et al., 2010), suggesting a high C cost of mycorrhization. Even more surprisingly, non-mycorrhizal fern species had significantly higher concentrations of N, P, Mg, and Ca in their leaves. Negative growth effects of mycorrhization have previously been documented in a number of cases among seed plants (e.g., Johnson et al., 1992; Smith & Read, 2008; Veiga et al., 2011). These often reflect the parasitic nature of mycorrhizal fungi in situations in which the nutritional benefits for the plant do not outweigh the C loss to the fungi (Johnson et al., 1992; Karst et al., 2008).

The very sparse data available until now thus provide evidence for benefits of mycorrhization in ferns as well as for negative effects. While this may initially be surprising, in fact it makes sense if we consider that not all plant species have mycorrhizae (Brundrett, 2009), even if they do not grow under special habitat conditions that limit fungal growth such as aquatic or epiphytic environments. If we further bear in mind that the ancestral state is most likely the presence of mycorrhization, then the 33% of terrestrial and 47% of epiphytic fern species lacking mycorrhizae (Lehnert, 2007) must have lost them over evolutionary times, suggesting that there must be a benefit of losing mycorrhiza. It thus appears that there is a fine balance between positive and negative effects of mycorrhization among land plants in general and ferns in particular. It is much too early to speculate under which conditions this balance switches either way, especially considering that the balance is likely to be not simply determined by a nutrient-carbon trade-off but also by water relations and the influence of symbiotic fungi on parasitic or pathogenic fungi and other biotic interactions.

**OPEN QUESTIONS**

We conclude this overview of what is known about mycorrhizae in ferns with a series of questions that readily develop from the above text. This list is certainly incomplete, but it may offer some guidelines as to what we would like to know to better understand the role of mycorrhization in ferns and lycophytes:

- Are all visually detectable fungal associations of ferns truly mutualistic? Most of the records are based on light microscopical screenings only and await further microstructural and molecular conformation. Until now, true mycorrhizae with mutual benefits have been confirmed only for lycophytes and basal fern lineages and involve only Glomeromycota and Mucoromycotina.
- Why is the overall degree of mycorrhization lower among lycophytes and ferns (and liverworts) than among seed plants? Does this reflect some basic physiological difference between spore-bearing and seed-bearing vascular plants, e.g., in water use efficiency (Brodribb et al., 2009; Watkins et al., 2010) that tips the balance more in the direction of non-mycorrhization in ferns?
- What is the role of mycorrhization in ferns that are very difficult to cultivate or transplant, such as Gleicheniaceae, Lycopodiaceae and grammitids (Page, 2002)?
- For ferns with facultative or variable mycorrhization, does the degree of
mycorrhization vary with ecological conditions?

- Are there implications of mycorrhizal associations in ferns for insect herbivory or other ecological interactions?

**ACKNOWLEDGEMENTS**

We thank Bridget Laue and Adrian Dyer for the invitation to do this review, and Mary Gibby and the reviewers for helping us improve the manuscript.

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A SHORT BIOGRAPHY OF THE AUTHORS

Michael Kessler was born and grew up in Peru where he spent much of his childhood chasing birds across the forest and mountains of the country. He studied biology at the University of Göttingen, Germany from 1986 onwards, culminating with his PhD thesis on the taxonomy, ecology, and biogeography of the high-Andean tree genus *Polylepis* (Rosaceae) in 1995. He then shifted his focus to the plant diversity of tropical montane forests, and, inspired by the work of Hanna Tuomisto in Amazonia, picked ferns as a study group. Over the years, ferns have become the main focus of his work, with research mainly on diversity patterns and ecology, but also numerous taxonomic studies, especially on Bolivian ferns. Since 2008, he has worked as scientific curator of the botanical garden and researcher at the University of Zurich, Switzerland. Currently, his fern work is mainly concerned with understanding the evolutionary and ecological processes that determine the richness and composition of fern assemblages, involving fieldwork in, e.g., Mexico, the Andes, Uganda, Indonesia, and New Guinea. Other projects involve studies on the diversification of bromeliads and palms in the neotropics and the flora of the canton of Zurich in Switzerland.
Marcus Lehnert was born on 1 October 1975 in Neutstadt/Ostholstein, Germany. He attended Göttingen University, where he received his Diplom (M.S. equivalent) in 2002 and his Ph.D. in 2007. His research was based first at the Albrecht-von-Haller Institute for Plant Sciences, Göttingen (2000–2007), with interludes at the Instituto de Ecologia, Universidad Mayor de San Andres, La Paz, Bolivia (2002–2003) and the University of California, Berkeley (2006–2007). From 2008-2012, he was employed as a Postdoctoral Researcher in the Botany Department of the State Museum for Natural History in Stuttgart. Since 2013 he has been a research assistant at the Nees Institute for Plant Biodiversity, University Bonn. His research interest focuses on the taxonomy, phylogeny, and floristics of ferns but also includes mycorrhiza, general biogeography, and the systematics of several angiosperm families. For his fieldwork, he has visited among others Puerto Rico, Panama, Peru, Argentina, Brazil, and several times Ecuador, Colombia, Bolivia and New Guinea. Marcus Lehnert is currently working on a large-scale projects focusing on the use of DNA barcoding for the rapid assessment of biodiversity (Accelaration of Biodiversity Assessment—ABA Ecuador and German Barcode of Life-GBoL). He has contributed his taxonomical expertise on scaly tree ferns (Cyatheaceae) to floral projects like “Catálogo de las Plantas Vasculares del Cono Sur” and “Ferns of Bolivia.”
ABSTRACT
The last comprehensive study of Turkish Pteridaceae was made over 50 years ago. In this study the family Pteridaceae in Turkey is reviewed according to recent taxonomic and nomenclatural changes and a full description, synonyms and distribution information are given for each taxon and a key provided.

INTRODUCTION
A generic classification of cheilanthoid ferns (Pteridaceae) has eluded taxonomists for more than 200 years (Grusz & Windham, 2013). Several taxonomic and nomenclatural updates have been made in recent decades, based on morphological and molecular methods (Gastony & Rollo, 1995; Sanchez-Baracaldo, 2004; Zhang et al., 2005; Smith et al., 2006; Kamau, 2007; Schuettpelz et al., 2007; Windham et al., 2009; Grusz & Windham, 2013; Schneider et al., 2013; Abotsi et al., 2015). The current family diversity is about 1000 species distributed in 53 genera (Schuettpelz et al., 2007; Christenhusz et al., 2011).

The last comprehensive study of Turkish Pteridaceae was made over 50 years ago (Henderson, 1965) for the Flora of Turkey. A major project was recently initiated to illustrate and describe the flora of Turkey in the Turkish language and the first volume of Resimli Türkiye Florası [Illustrated Flora of Turkey] was published by Güner and Ekim (2014). The second volume, which will include the family Pteridaceae, is scheduled for publication in 2016. The aim of the present study has been to review Turkish Pteridaceae according to recent taxonomic and nomenclatural changes and to provide descriptions and distributions for each taxon. It is based on recent literature on Pteridaceae (e.g. Nardi & Reichstein, 1985; Benlioğlu et al., 1997; Kaynak et al., 2008; Christenhusz et al., 2011; Greuter & Raab-Straube, 2012; Jessen et al., 2012; Güner et al., 2012), and on herbarium specimens from Ankara University Faculty of Pharmacy (AEF), Ankara University Faculty of Science (ANK), Natural History Museum, London (BM), Royal Botanic Garden Edinburgh (E), Geneva (G), Gazi University Faculty (GAZI), Hacettepe University (HUB), Istanbul University Faculty of Pharmacy (ISTE) and Royal Botanic Gardens, Kew (K). All specimens listed below are from E, except where indicated otherwise.

PTERIDACEAE

Key for Turkish Pteridaceae taxa

1. Fertile and sterile leaves or pinnae conspicuously dimorphic ........................................ 2
2. Annual, sori on the lower surface of the leaf, indusium absent ....................................... Anogramma leptophylla
3. Lamina of sterile leaves up to 16 cm long, ovate to deltoid-ovate, spores <60μm long .......... Cryptogramma crispa
4. Pinnules fan-shaped; petiole blackish, glabrous, fine and wiry ........................................... Adiantum capillus-veneris
5. Fertile and sterile leaves or pinnae not, or weakly dimorphic ............................................ 4
6. Leaves 1-pinnate, lanceolate, >10 pinnae pairs ...................................................... Pteris vittata
7. Lamina of sterile leaves up to 7 cm long, broadly deltoid, spores >60μm long .......... Cryptogramma bithynica
8. Pinnules not fan-shaped; petiole not as above ................................................................. 5
9. Fronds pinnate to pinnatisect, weakly dimorphic ............................................................. 6
10. Fronds 2-pinnate, not dimorphic ...................................................................................... 5
11. Fronds pinnate to pinnatisect, frond ovate-triangular, with 7 or fewer pinnae pairs ........... Pteris cretica
12. Pinnules fan-shaped, without a midrib, sori borne on reflexed marginal lobes of lamina. .......
13. Lamina of sterile leaves up to 16 cm long, ovate to deltoid-ovate, spores <60μm long .......... Cryptogramma crispa
14. Pinnules not fan-shaped; petiole blackish, glabrous, fine and wiry .................................... Adiantum capillus-veneris
15. Fronds 2-pinnate, not dimorphic ...................................................................................... 6
16. Leaves 1-pinnate, lanceolate, >10 pinnae pairs ...................................................... Pteris vittata
17. Lamina of sterile leaves up to 7 cm long, broadly deltoid, spores >60μm long .......... Cryptogramma bithynica
18. Pinnules not fan-shaped; petiole not as above ................................................................. 5
19. Fronds pinnate to pinnatisect, weakly dimorphic ............................................................. 6
20. Fronds 2-pinnate, not dimorphic ...................................................................................... 5
21. Leaves 1-pinnate, lanceolate, >10 pinnae pairs ...................................................... Pteris vittata
22. Lamina of sterile leaves up to 7 cm long, broadly deltoid, spores >60μm long .......... Cryptogramma bithynica
23. Pinnules fan-shaped, without a midrib, sori borne on reflexed marginal lobes of lamina. .......
24. Lamina of sterile leaves up to 16 cm long, ovate to deltoid-ovate, spores <60μm long .......... Cryptogramma crispa
25. Pinnules not fan-shaped; petiole blackish, glabrous, fine and wiry .................................... Adiantum capillus-veneris
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27. Leaves 1-pinnate, lanceolate, >10 pinnae pairs ...................................................... Pteris vittata
28. Lamina of sterile leaves up to 7 cm long, broadly deltoid, spores >60μm long .......... Cryptogramma bithynica
29. Pinnules fan-shaped, without a midrib, sori borne on reflexed marginal lobes of lamina. .......
30. Lamina of sterile leaves up to 16 cm long, ovate to deltoid-ovate, spores <60μm long .......... Cryptogramma crispa
31. Pinnules not fan-shaped; petiole blackish, glabrous, fine and wiry .................................... Adiantum capillus-veneris

**Adiantum** L., Sp. Pl. 1094 (1753).


Lectotype: Herb. Linn. 1252.9 (LINN)!

Rhizome densely covered with shiny, narrow lanceolate scales. Fronds bipinnate, (5–)15–30(–45) × (1.5–)4–6(–10) cm, glabrous; stipe (1.5–)8–12(–20) cm long, brown or black, shiny, glabrous. Pinnae 2–7 × 1–4 cm with stalk 0.3–3 cm long. Pinnules fan-shaped, (0.7–)1–2(–3.5) × (0.7–)1–2.4(–3) cm, cuneate at base, without midrib, 2–8 lobed with stalk (1–)2–5(–16) mm long. Sori borne on reflexed marginal lobes of lamina. Habitat: In crevices of limestone and serpentine or tufa and base-rich soil on shady banks, 1–1000 m.

Distribution in Turkey:

Adana: Feke; Göksu gorge, 800 m, 09.vii.1952, Davis 19855!

Antalya: Kemer, Sumakşehir Deresi, kalkerli G. Deresi değil vadisi, P. nigra-C.
**THE FERN FAMILY PTERIDACEAE IN TURKEY**

**BONA & GIBBY:**

**sempervirens** ormani 500-700 m, 06.x.1977, **H. Peşmen** 3569 & **Ş. Yıldırım** HUB 36734! Alanya, 120 m, 04.viii.1982, **Ş. Yıldırım** 4451 HUB 36732! Aksu, Düden Çağlayani, 06.ix.1988, **Ş. Yıldırım** 11596 HUB 36734!

**Artvin:** Çoruh gorge, between Yusufeli and Artvin. 600 m, 01.viii.1966, **Davis** 47690!

**Demirkent nahiyesi,** between Tortum Gölü und Artvin, 620 m, 03.viii.1973, **F. Holtz,** **P. Hanel** & **T. Kesercioğlu** 991!

**Alanya,** 120 m, 04.viii.1980, **Ş. Yıldırım** HUB 36734!

**Denizli:** Pamukkale, 16.x.1980, **Ş. Yıldırım** HUB 36734!

**Düzce:** Akçakoca, Edilli Köyü, orman, 50 m, 50m, 29.ix.2001, **A. Doğu Koca** 1489 (HUB)!

**Hatay:** Dörtyol, Kuzuculu to Bülke on forest road, 1000 m, 04.iv.1965, **M.J.E. Coode** & **B.M.G. Jones** 416!

**Karamanmaraş:** Andırın; 8 miles South of Çatak, 800 m, 17.v.1965, **M.J.E. Coode** & **B.M.G. Jones** 1147!

**Konya:** Ermenek, Kazancı kasabası, Yeşilköy, Göksu Nehri boyunca, 700 m, 12.x.1983, **H. Sümbül** 2536 HUB 36730!

**Konya:** Eşme, Göktepe Kasabası, Darcın Köyü, Ayaşlı mevkii, Göksu Nehri Boyunca, 600 m, 15.ix.1983, **H. Sümbül** 2477 HUB 36729!

**Malatya:** Pütürge, Ferikhan Köyü, dere kenarı, ca. 650 m, 06.v.1981, **A. Güner** et al. (GAZI)!

**Mardin:** Hessana at S foot of Cudi Dağ, 900 m, 10.v.1966, **Davis** 42776!

**Mardin:** Hessana at S foot of Cudi Dağ, 900 m, 10.v.1966, **Davis** 42776!

**Manisa:** Manisa Dağı (Spil), Karaçay boyunca, 150-450 m, 23.iv.1984, **Ş. Yıldırım** 6014, HUB 36739!

**Mardin:** Hessana at S foot of Cudi Dağ, 900 m, 10.v.1966, **Davis** 42776!

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**Mardin:** Hessana at S foot of Cudi Dağ, 900 m, 10.v.1966, **Davis** 42776!

**Mardin:** Hessana at S foot of Cudi Dağ, 900 m, 10.v.1966, **Davis** 42776!
A. Dönmez 16205 & Z. Uğurlu (HUB)!

Şanlıurfa: Urfa-Gaziantep c. 1 km from Urfa, 600 m, 11.ix.1956, McNeill 794!

Trabzon: Maçka, 24 km south of Trabzon, 250 m, 28.vi.1960, Stainton & Henderson 5895!

General Distribution: Widespread in temperate and subtropical regions of the New and Old World, absent from South America.

Anogramma Link, Fil. Spec. 137 (1841).


A. leptophylla (L.) Link, Fil. Spec. 137 (1841).


Lectotype: Herb. Linn. 1251: 56 (LINN)!

Plant delicate, up to 15 cm high, annual, with short rhizomes. Prothallus perennial, reniform, bilobed, 7–10 mm long. Fronds yellow-green, glabrous, young fronds reniform and 3–5 lobed, mature fronds bipinnate 5–15 ×1–1.5 (–2) cm, stipe glabrous 2.5–7 cm long, dark brown-black below and yellow-green above. Pinnae glabrous, 0.5–2 × 0.5–1 cm, dichotomously veined, pinnae stipe 1–2 mm. Pinnule mostly bilobed or lanceolate. Sori linear along veins; no indusium.

Habitat: Dry metamorphic rocks or moist shady serpentine rocks, 30–1000 m.

Distribution in Turkey:
Adana: Feke; Senean dere between Gurumze and Suphandere, 1000 m, 01.vii.1952, Davis 1961, Dodds & Çetik!


Hatay: Dörtyol; Rabat, vertical serpentine flush, c. 100 m, 05.v.1965, M.J.E. Coode & B.M.G. Jones!

Isparta: Ş.Karaağac, Kızildağ Milli Parkı, Kuzgu Tepe, kaya üzerinde, 1350 m, 24.vii.1994, B. Mutlu 1084 HUB 36794!

İçel: Anamur, Nasrettin Köyü üstü, pınar civarı nemli yerlerde, 250 m, 21.iv.1975, M. Koyuncu & M. Coşkun HUB 36797!


General Distribution: Mediterranean area, Switzerland, W. France and Channel Islands, Crimea, N. America; scattered to S. Africa, India and Australasia.

**Allosorus** Bernh., Neues J. Bot. 1(2): 36 (1805).
Rhizome short-creeping or tufted. Fronds persistent, bipinnate; stipe wiry. Sori terminal on veins, protected by a linear pseudoindusium, a recurved and modified leaf margin.


Four plants. (see Nardi & Reichstein. 1985).

Plant up to 20 cm, rhizome brown and covered with long linear, reddish brown scales. Fronds bipinnate, 3–20 × 1–2.5 cm, glabrous, green; stipe dark brown-black 1.5–11.5 cm with a few scales. Pinnae 0.5–3 × 0.3–1 cm, stalk sessile, 1–3 mm long at end of the fronds. Pinnule 0.5–2 mm broad, without midrib. Sori many; pseudoindusium broad, irregularly lobed, ciliate.

Habitat: Crevices in limestone rocks, sea level up to 500 m.

Distribution in Turkey:

Selçuk: Bucağılı, Dere Dağı, Kayalık, kurak yamaçlar, maki, ca.580 m, 25v.1979, M. *Vural* 1873 GAZI!

Muğla: ca. 4 km nw Muğla. Felsen in der Macchie, 03.iv.1974, *K. Lewejohann (Tu-74-137) & F. Holtz*! Dereköy, between Marmaris and Muğla, 10 m, 26.iii.1956, *Davis* 25395 & *O. Polunin*! BM! Fethiye, 30 m, 27.iii.1956, *Davis* 25421 & *O. Polunin*! BM! Kişle to Kozağaç (Milas-Yatağan), 550 m, 13.iv.1965, *Davis* 41056! Marmaris, Sögüt to
Bozburun, sea level–50 m, 15.iv.1965, Davis 41168!
Dalyan-Sülüngür Gölü, Ortaca arası, 100-200 m, 14.iv.1981, A Güner 8571 et al. (HUB)!
Bodrum, Türkbükü-Bodrum, 200-500 m, 15.iii.1984, Ş. Yıldırımlı 5883 HUB 36696!
Köyceğiz, Çandır Köyü, Kaunos Harabeleri, duvar arası, 80 m, 08.xi.1991, A. Güner 10323 (GAZI)!
Köyceğiz, Yangı Köyü, Yangı Deresi, sarp ve derin vadi, kalkerli yamaçlar, 60-80 m, 24.v.1981, A. Güner 8324, M. Vural & H. Şağban (GAZI)!

General Distribution: From Mediterranean area to West Himalayas.

Note: There has been confusion in the past between *Allosorus acrosticus* and *A. pteridioides* (Reichard) Christenh. (see Nardi & Reichstein, 1985). *Allosorus acrosticus* is a tetraploid taxon, whereas *A. pteridioides* is diploid. This latter has a more western Mediterranean distribution and, so far, has not been recorded for Turkey, although there are records from Cyprus and Jordan in BM and from Syria and Palestine in E, and it may have been overlooked in Turkey. The two taxa have similar morphology but can be distinguished; *A. acrosticus* has a pseudoindusium with ciliate margins, with scales on rachis and costae, whereas *A. pteridioides* has pseudoindusium entire, and rachis and costae are ± glabrous.


Lectotype: [Iran] La Perse, parmi les rochers, 1821, C. Bélanger s.n. P00607931 (P)!

Plant up to 20 cm, rhizomes covered densely in narrow dark brown-black scales. Fronds bipinnate, 3–16 × 1–4 cm, green and glabrous above and with brown scales on rachis and costae, eglandular; stipe 1–8 cm, covered with dark brown-black and narrow lanceolate or linear long brown hairs. Pinna (0.3–) 1–3 × 0.2–2 cm; pinna stalk very short c. 1 mm, pinnules lobed, lobes lanceolate. Sori many; pseudoindusium narrow, with many long marginal hairs that cover the lower surface of the segment.

Habitat: Shady crevices in limestone or metamorphic rocks, 200–2300 m.

Distribution in Turkey:

Adana: Feke, Sencan Dere between Belenköy and Sughandere, 1000 m, 02.vii.1952, Davis 19586A, Dodds, Çetik!
Kozan, (Anti-taurus), 300 m, 12.iv.1957, Davis 26614, Hedge! Osmaniyе-Yarpuz, 15 km e Osmaniyе, 800 m, 29.iv.1986, M. Nydegger HUB 36695!

Ankara: mamak, Kibns Köyü vadisi, Dipsiz gölü mevkii, kaya çatlağı, gölgeli yerler, 1000-1100 m, 26.vi.2005, S. Arslan 2352 (GAZI)!


Aydın: 3 km westlich Ortaklar, Abangh am Ovacık Dag oberhalb der Strasse Selçuk-Ortaklar, 200 m, 22.v.1966, G. Bocquet 2472! Söke, piriyen, 400 m, 30.v.1972, N. Tanker, AEF 17!

Denizli: Bozdağ above Geynam yaila, 16.vii.1947, Davis 13432!

Erzincan: Kemaliye, Yaka Köyü, Sazak deresi, kazmofit, 850-1000 m, 18.xi.1980, Ş. Yıldırım 4186 HUB 36708!

Isparta: Ş. Karaağaç, Kızılçağ Milli Parkı girişi, sedir ormanı, kalker, 1200-1300 m, 17.xi.1993, B. Mutlu 163 HUB 36701! Eğridir, Yaka Köy, 2 km güneyi, derin kalker vadisi, *P. nigra-J. excelsa* ormanı, 1800 m, 20.v.1973, Peşmen 3401, Erik, Pamukçuoğlu HUB 36709! Eğridir, Anamas Köyü, Kapiz Deresi, kalkerli sarp ve derin vadi, 1250-
1450 m, 05.viii.1974, H. Peşmen & A. Güner 1836 HUB 36706! Eğridir, Aksu, Karacahisar Köyü, Başak mevkii, kalkerli vadi, 1000 m, 03.vii.1974, H. Peşmen & A. Güner 1653 HUB 36702!

Konya: Ereğli, Aydos Dağı, Delimahmutlu, Kapız, kayalık, 1600 m, 21.vi.1977, S. Erik 1653 HUB 36702!

Konya: Ereğli, Aydos Dağı, Delimahmutlu, Kapız, kayalık, 1600 m, 21.vi.1977, S. Erik 1653 HUB 36702!

Konya: Ereğli, Aydos Dağı, Delimahmutlu, Kapız, kayalık, 1600 m, 21.vi.1977, S. Erik 1653 HUB 36702!

Mardin: Cudi Dağ above Hessana (d. Silopi), 900–1150 m, 11.v.1966, Davis 42862A!

Savur, 900 m, 24.v.1957, Davis 28568, Hedge! Dargeçit, Çelik Köyü, Umutlu mezrası, kaya üzeri, 520 m, S. Arslan 3297 (GAZI)!

Muğla: Kara Dağ, 750 m, 18.v.1988, P. Ainsworth 11916 (HUB)!


Samsun: Vezirköprü at Kızılırmak, 400 m, 06.iv.1969, Tobey 2446!

Şanlıurfa: Yenice Köyü üstü, Nemrut Dağları, kalker kayalığı tabakalar, taşlık, bozkır, 475-675 m, 26.vi.1988, Ş. Yıldırımlı 1388 & Ş. Kaplan HUB 36711!

Sıirt: Botan Çay, by the bridge 19 km from Sırt, 400 m, 17.v.1966, Davis 43220!

Tunceli: Ovacık, Munzur Dağları, Karagöl vadisi, 1300-1500 m, 06.v.1979, Ş. Yıldırımlı 1388 & Ş. Kaplan HUB 36711!

Yozgat: Sorgun to Çekerek, 1200 m, 27.v.1965, M.J.E. Coode & B.M.G. Jones 1598!

General Distribution: Distributed in Europe, Asia, and Africa.


Holotype: [Sicily] “Messina, Cheilanthes odora var. stipites glabrato” Id. 9541 (PAL).

Isotype: Id. 9542 (PAL) (see Nardi, Rasbach & Reichstein, 1979).

Plant up to 25 cm, rhizome covered with brown long scales. Fronds bipinnate 5–25 × 1–2.5 cm, shortly and sparingly pilose when young, later ± glabrate above, scattered 2-5-celled red glandular hairs beneath; stipe densely covered with long scales at base and with a few scattered scales further up when young then almost glabrous, brown-black, 1–10 cm. Pinnae 0.5–1.5 × 0.3–0.5 cm with very short stalks c. 1 mm. Pinnule 0.5–2 mm broad. Pseudoindusium with rounded, entire membranous margin.

Habitat: Coastal, bare rocks, sea level to 1250 m.

Distribution in Turkey:
Istanbul: Büyükada, Eskibağ, kayalıklar, 17.08.1977, H. Demiriz, B. Tütel, A. Aydın (ISTE 31028!)

General Distribution: N.W. Africa, Portugal, S. Europe.


Rhizome creeping to ascending covered with light brown to black long scales. Fronds dimorphic, tripinnate; sori terminal on veins protected by recurved membranous lateral margins of segments.


Plant 10(–22) cm, - covered with light brown long scales. Fronds tripinnate, green, reddish at the end of the fronds, glabrous above, veins visible at and of the lobes in white-yellow colour. Stipe straw-coloured to greenish, glabrous to sparsely covered with light brown scales. Fertile fronds 8–10 × 3–4 cm; stipe 6–7 cm. Pinnae 2 × 1–2 cm; pinna stalks 1–3 mm. Pinnules linear-lobed, convolute at the edge of lobes. Vegetative fronds slightly thick and relatively tough, 8–10(–22) × 3–4.5 cm, broadly deltate; stipe 5–6 cm. Pinnae ovate to orbicular, 1.5–2.5 × 1–2.5 cm; pinna stalks 1–3 mm. Pinnules lanceolate-lobed. Sori terminal on veins protected by recurved margins of lobes.

Habitat: Silicate scree near the cycle roadway, 1700–2300 m.

Distribution in Turkey:

General Distribution: Endemic to Turkey.

C. crispa (L.) R.Br. ex Hook., Gen. Fil. pl. 115B (1842).


Plant to 30 cm, rhizome covered with brown-black long scales. Fronds thin, tripinnate, green, reddish at the end of the fronds, glabrous above, veins visible at and of the lobes in white-yellow colour. Stipe straw-coloured to greenish, glabrous. Fertile fronds 15–30 × 2.5–5 cm; pinna stalks 8–20 cm. Pinnae 2–5 × 1–1.5 cm; stipe 1–6 mm. Pinnules linear-lobed, convolute at the edge of lobes. Vegetative fronds ovate to deltoid-ovate, 15–20 × 3–4 cm; pinna stalks 4–11 cm. Pinnae narrowly ovate, 1.5–3 × 0.7–1.8 cm; pinna stalks 1–4 mm. Pinnules lanceolate-lobed. Sori terminal on veins protected by recurved margins of lobes.

Habitat: Screes, 200–2400 m.

Distribution in Turkey:
Arhavi, above Dikyaça village, 2200 m, 26.vii.1974, M. Çoşkun AEF 19!
Çoruh, Şaval Tepe above Murgul (igneus), 2000 m, 13.viii.1957 Davis 32393 & Hedge!
Şavşat, Kocabey Köşlisi, Camız patlatan mevkii, kayalık alanlar, 1900-1950 m, 14.v.2004, H. Altnözü 3945 (HUB)! Kaçkal Dağı, Pınarlı Üst Yaylası, Cehennem Deresi Mevkii, 1750-3100 m, 12.vii.1984, Demirküş 2641b HUB 36758! Arhavi, Dikyaça Köyü yaylası, Arpaçay civarı, 2200 m, M. Çoşkun AEF!

General Distribution: Europe and Caucasus.


Rhizomes short-creeping to erect with long linear brown scales. Fronds pinnate, both


Lectotype: Portugal, 1777, *F. Masson* s.n. BM000829683 (BM)!

Plant 10–20 cm, rhizomes brown with long linear pale brown scales. Fronds pinnate, 8–16 × 1.5–4 cm, green above and brownish below, both surfaces of the frond covered, densely below, with tangled long whitish multicellular hairs; stipe brown, 1–4.5 cm, densely covered with hairs when young, becoming glabrous when mature, young fronds almost sessile, lobed, 0.5–2 × 0.3–0.8 cm. Sori not discrete, sporangia spread along veins from leaf margin, ± protected by recurved but not modified leaf-margin.

Habitat: Dry limestone, calcareous rocks, sea level to 300 m.

Distribution in Turkey:
- Ankara: Beynam, Karaçam ormanı, 1300-1500 m, 15.v.1986, Ş. Yıldırımlı 9221 HUB 36713!
- Aydın: Priene, 100 m, 09.iv.1965, *Davis* 40788! Milet harabeleri, *R. Lampinen* 7441 ISTE 63662!
- Çankırı: Eldivan, Şeydiköy üstü, step, 1300-1350 m, 09.vii.1984, Ş. Yıldırımlı 7409 HUB 36716!
- Hatay: İskenderun, Musa Dağı, Kale ilerisi, P. brutia ormanı, 200 m, 30.iii.1995, A. Dönmez 4423 (HUB)!
- Isparta: Ş.Karaağaç, Kızıldağ Milli Parkı girisi, Sedir ormanı açıklığı, kalker, 1200-1300 m, 18.xi.1993, *B. Mutlu* 180!
- İçel: Anamur’un üstü, Kayalıklarda 200 m, 06.iii.1977, *M. Koyuncu* HUB 36722!
- Kayseri: Yılanlı Dağ, 1200 m, 12.vi.1975, *M. Coşkun & N. Çelik* HUB 36719!
- General distribution: Macaronesia, Mediterranean region including S. Europe and North Africa and east to Turkey, Egypt, Yemen and Oman.

Note: *Cosentinia vellea* has two subspecies, a tetraploid *C. vellea* subsp. *vellea* and a diploid *C. vellea* subsp. *bivalens* (Reichstein) Rivas Mart. & Salvo. On macromorphological characters the two are indistinguishable, but can be separated by comparison of spore size and stomatal length (see Haou et al., 2011). Of four Turkish
specimens for which spores have been measured, three correspond with subsp. *vellea* [64-(67)-71µm: Davis 25349, 40855, 41147] and one with subsp. *bivalens* [55-(57)-62 µm: Davis 40788], although cytological verification is still lacking.


Rhizome short-creeping, covered with long, linear scales. Fronds pinnate, linear-lanceolate scales along the midrib above and covered with lanceolate brown scales below.


Lectotype: Pierre Magnol s.n. Herb. Linn. 1245.12 (LINN)!

Plant 10–30 cm. Rhizome covered with long, light brown scales. Fronds pinnate-pinnatisect, 10–30 × 1.2–4.5 cm, linear-lanceolate scales only along the midrib above, lower frond surface densely covered with lanceolate brown scales; stipe 4–20 cm, with scattered brown scales. Pinnae 1–2.5 × 0.5–1.2 cm, 3–7 lanceolate or broad triangular lobed, main lobe almost lyre-shaped, edge of pinnae undulate or simple; pinna stalks 1–2 mm or sessile. Sori not discrete, sporangia spread along veins from leaf margin, ± protected by slightly recurved and modified leaf-margin. Habitat: Serpentine rocky slopes, sea level to 1400 m.

Distribution in Turkey:

Adana: Karsantı, Kumbükü, Hizar yolu, ca. 880 m, 05.v.1972, *E Yurdakul* ANK 1768! Feke: Sencan Dere between Belankey and Suphandere, metamorphic rocks, 100 m, 02.vii.1952, *Davis 19586B, Dodds, Cetik* ! Bahçe (N. Amanus), Dumanlı Dağ above Haruniye, shady metamorphic rocks, 1200 m, 19.iv.1957, *Davis 26845, Hedge*!


Erzincan: Erzincan’ın 30 km batiında serpantin boğazı, ca. 1500 m, 18.vii.1958, *H. Birand* ANK 10909! Keşiş dağı above Cimin, among igneous rocks, 2700 m, 28.vii.1957, *Davis 31652, Hedge*!


Hatay: Hassa to Dörttyol, mountain road, slopes above Dörttyol, 1100 m, *A.J. Byfield & D. Pearman* B 2620 ISTE 73362!


Kastamonu: Tosya, Sekiler Köyü, Seki Deresi, kayaların arası, ca. 1100 m, 28.ix.1977, *M. Kılınç* ANK 6866!

Konya: 2 km from Çamlık village, Akseki-Beyşehir area, 1360–1370 m, 03.vi.2003, *R.D. Reeves* 2693 & *N. Adigüzel*!
Fronds pinnate to bipinnate, green and glabrous, midrib and secondary veins distinct.

P. cretica L., Mant. Pl. 130 (1767).

Synonyms: none.

Lectotype: Pietro Arduino s.n. Herb. Linn. 1246.7 (LINN)!

Plant 30–70 cm. Rhizomes short-creeping dark brown with dark brown scales. Fronds pinnate to bipinnate, green and glabrous, midrib and secondary veins distinct. Pinnae ≤ 7 pairs, often with a long basal segment. Fertile fronds serrate only at the end, convolute at the edge of lobes, 40–70 × 15–20 cm; stipe 20–40 cm, pinnae 10–15 cm and sessile or with c. 1 mm stalk. Vegetative fronds serrate at margin, 30–70 × 10–20 cm; stipe glabrous, 20–35 cm. Pinnae 5–20 cm, sessile or with 1–3 mm stalk. Sori submarginal, in a continuous line protected by scarious recurved leaf-margin.

Habitat: Shady banks and shady damp places in deciduous forests, sea level to 800 m.

Distribution in Turkey:


Çamlıhemşin, Aşağı Vice Mahallesi üstü, karışık orman ve nemli çayırlık, 500-600 m, 13.vii.1979, A. Güner 2095 HUB 36749! Çamlıhemşin, Boğazıcık Köyü, Pogina çevresi, karışık orman, 650 m, 04.ix.1978, A. Güner 1820 HUB 36748! Alipaşa Köyü civari, 29.vii.1956, K. Karaosmanoğlu HUB 36749!

Trabzon: Valley above Çaykara, 700 m, 05.vii.1957, Davis & Hedge 32107! Sürmene,
Gökçesu Mahallesi, İdrum tepe çevresi, 350-550 m, 04.vii.1997, H. Duman


P. vittata L., Sp. Pl. 1074 (1753).

Synonyms: P. ensifolia Poir. in Lam, Encycl. 5: 711 (1804); P. diversifolia Sw. Syn. Fil. 96: 288 (1806); P. costata Bory, Sp. Pl. 5: 367 (1810); P. inaequilateralis Poir. Encycl. 4: 601 (1816); Pycnodoria vittata (L.) Small, Ferns Florida 89 (1931).

Lectotype: Herb. Linn. 1246.3 (LINN)!

Plant 10–70 cm. Rhizomes short-creeping, brown with fine long scales. Fronds pinnate, 10–70 × 5–20 cm, green, long thin scales along with midrib; stipe 1.5–10 cm, yellowish brown with thin long scales. Pinnae (2–)6–10 × 0.3–1 cm, ≥10 pairs, longer at apex of frond, reducing in length towards frond base, narrowly lanceolate, undivided, serrate, almost sessile. Sori submarginal, in a continuous line protected by scarious recurved leaf-margin.

Habitat: Shady rocks or serpentine.

Distribution in Turkey:
Hatay: Valley above Dörtyol, S. bank, N. facing, 150 m, 12.viii.1969, J. Darrah 534, 634, 620! Antakya, Harbiye, şelale civarı, 180 m, M. Coşkun 321, B. Şener & F. Ilisulu!
Mersin: Silifke-Anamur arasında Değirmenönü, köy değirmeni yakını, 50 m, 09.ix.1962, H. Kayacık 2958!

Family Pteridaceae

General Distribution: Europe and Asia.

CONCLUSION
In the first volume of the Flora of Turkey (Henderson, 1965) the family currently recognised as Pteridaceae was represented by nine species from five genera and classified in five different families. Since this publication, further new records of Cheilanthes tinaei (Davis, 1988) and Cryptogramma bithynica (Jessen et al., 2012), have been published. In this revision the single family Pteridaceae in Turkey includes 11 species from seven different genera. Families Adiantaceae, Cryptogrammaceae, Gymnogrammaceae and Sinopteridaceae have been combined in the family Pteridaceae (see Christenhusz et al., 2011). The names of the genera Anogramma, Adiantum, Cryptogramma and Pteris have not changed since 1965. However, Turkish Cheilanthes species are now placed in three different genera: Allosorus, Cosentinia and Paragynnopteris. Cheilanthes marantae is a synonym of Paragynnopteris marantae and Cheilanthes catanensis is a synonym of Cosentinia vellea, whilst Cheilanthes persica, Cheilanthes acrostica and Cheilanthes tinaei are now placed within Allosorus (Christenhusz et al., 2011; Greuter & Raab-Straube, 2012).

ACKNOWLEDGEMENTS
We are grateful to Prof. Adil Güner for his help in accessing resources on Pteridaceae, to Prof. Gönül Kaynak who sent images of Allosorus tinaei and to Miss Alison Paul and Dr Fred Rumsey for reviewing the manuscript. This study was supported financially by the Edinburgh Botanic Garden (Sibbald) Trust to M.B.
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THE PUBLICATIONS OF E. “BERT” HENNIPMAN


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1 An obituary for Bert Hennipman by Peter Hovenkamp was published in the British Pteridological Society Bulletin, Volume 8(1), 2014.


PhD Theses supervised


COMPLIED BY P.H. HOVENKAMP
THE PUBLICATIONS OF A.C. JERMY


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1 An obituary for Clive Jermy by Alison Paul was published in the British Pteridological Society Bulletin, Volume 8(1), 2014.


377-405.


JERMY, A.C. & CAMUS, J.M. 1991. The illustrated field guide to ferns and allied
VOGEL, J.C., JESSEN, S., GIBBY, M., JERMY, A.C. & ELLIS, L. 1993. Gametophytes of *Trichomanes speciosum* Willd. (Hymenophyllaceae,


Also abstracts of presentations, obituaries and c. 50 reviews of books in a number of biological and geographical journals.

Compiled by M. Gibby & A.M. Paul
SHORT NOTE

RETYPIFICATIONS OF ADIANTUM INCISUM (PTERIDACEAE) AND PTERIS INTERRUPTA (THELYPTERIDACEAE)

Species-rich eastern India includes the states of Sikkim, West Bengal, Arunachal Pradesh, Meghalaya, Assam, Tripura, Manipur, Nagaland and Mizoram (Ghosh et al. 2004). Rejection of records of a number of new species and new distributional records from this region by Fraser-Jenkins (2008, 2012) necessitate a revision to the treatment by Ghosh et al. As a part of ongoing revision of the ferns of Eastern India, *Adiantum incisum* Forssk. (Pteridaceae) and *Pteris interrupta* Willd. (current name: *Cyclosorus interruptus* (Willd.) H.Ito, Thelypteridaceae) are retypified.


Lectotype (designated here): Yemen. Al Hadiyah ['Hadîe'], 1763, P. Forsskål s.n. (C10001579, Isolectotype C10001580 images!).

The name *Adiantum incisum* was proposed by Linnaeus’s disciple Peter Forsskål (1775) based on his collection from Yemen. The Botanical Garden, University of Copenhagen, Denmark (C) stores two specimens: C10001579 (http://www.daim.snm.ku.dk/digitized-type-collection-details-simple?catno=C10001579) and C10001580 (http://www.daim.snm.ku.dk/digitized-type-collection-details-simple?catno=C10001580).

C10001579 is a complete plant with Forsskål’s field label “Adianthum *incisum Hadîe” , thus undoubtedly original material and type of *Adiantum incisum*. C10001580 is single isolated frond. It does not have Forsskål’s annotation but resembles C10001579 and is most probably from same gathering.

During a revision of the ferns of tropical East Africa the renowned botanist the late Dr. Bernard Verdcourt selected the specimen in C with the original field label (i.e. C10001579) as lectotype (see Verdcourt 2002: 55). Although his lectotype selection is taxonomically correct, it unfortunately did not fulfill requirements specified in the Melbourne Code (McNeill et al. 2012). Lectotypification statements published on or after 1 Jan 2001 must include the phrase “designated here” (hic designatus) or an equivalent (McNeill et al. 2012 Art. 7.10). Verdcourt’s lectotypification was published in 2002 without such statement. Here it is republished to fix the application of the name *A. incisum*.

*Pteris interrupta* Willd., Phytographia 13 t. 10 f. 1. 1794. [Sep 1794].

Lectotype (designated here): Willdenow (1794), Phytographia t. 10, f. 1. [Icon].

Epitype (designated here): India. Tamil Nadu [“Madras”], Aug 1794, Rottler ex Klein s.n. (BW19770011, Isoepitype BW19770012).

*Pteris interrupta* (Cyclosorus interruptus) is the type of the name *Cyclosorus* Link. Fosberg & Sachet (1972) considered a specimen (BW19770011; http://herbarium.bgbm.org/object/BW19770011) in Herb. Willdenow from Herbarium Berolinense, Berlin (B) as the type of *Pteris interrupta* and this has been considered to be a lectotype. Holtttum (1973) also treated it as such.

The name *Pteris interrupta* was published in September 1794 by Willdenow, but the
supposed type sheet (BW19770011) was annotated by Willdenow as “Klein Ind. 1797. W.” This indicates that Willdenow received this specimen in 1797 from Johann Gottfried Klein, a missionary and botanist from South India. Thus this specimen is not original material, rather a later collection.

The putative type (BW19770011) also carries the annotation “Pteris sp. n.? Aug. 1794. Auf den Reise nach Madras” written by Johan Peter Rottler, a missionary and botanist from South India (Dr. Frits Adema pers. comm. 2015). However the annotation “(Klein)” was written by Willdenow. So this specimen was actually collected by Rottler in August 1794 during a trip through Madras (now Tamil Nadu, India) and reached Willdenow through Klein. It is unlikely that Rottler’s collection (in August 1794) was subsequently studied and published by Willdenow within short period of one month (in September 1794).

This sheet does not carry Willdenow’s name *Pteris interrupta*, but Willdenow’s annotation “Asp. obtusatum 1a.” The name *Aspidium obtusatum* Sw. was published in 1801 by Swartz. As explained by Fosberg & Sachet (1972: 8) Willdenow sent a pinna from BW19770011 to Swartz. The pinna, now in the Natural History Museum, London (BM), was identified by Swartz as *Aspidium obtusatum* and he informed Willdenow of this.

Another sheet (BW19770012; http://herbarium.bgbm.org/object/BW19770012) exists in Herb. Willdenow in B carrying two isolated fronds and Willdenow’s annotation “Asp. obtusatum 1b.” These two specimens resemble BW19770011 and were most probably collected by Rottler from same individual or population.

Thus no type specimen for *Pteris interrupta* is known to exist. The illustration “t. 10 f. 1.” of *Pteris interrupta* in the protologue (Willdenow 1794) is the only original material and I select it as lectotype and BW19770011 as epitype.

ACKNOWLEDGEMENTS
I sincerely thank Dr. J. McNeill, Royal Botanic Garden Edinburgh for constructive revision and Dr. F. Adema, Naturalis Biodiversity Center, Leiden for helpful advice.

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BOOK REVIEW


Christopher Fraser-Jenkins has been studying Himalayan pteridophytes for over 30 years and for much of this time also living in Nepal. He is the pre-eminent expert of ferns in Himalayan region and also possesses vast expertise on the ferns of wider Asia and Europe. In this volume (the first of 2) the results of his studies of Nepalese ferns and lycophytes are summarised. The richness of the flora is highlighted by the fact that 580 species and subspecies will be included once the second volume has been completed. By comparison, England and Wales occupying an area of similar size if not topography, has about 80 taxa including naturalised introductions.

The introduction to this volume includes interesting and useful summaries on phytogeography, collectors, collections and publications pertaining to Nepal. Then follows a section outlining the classification utilised (basically that of Smith et al. (2006)), and a longer section on the apparent deficiencies of recent molecular-based phylogenies. Fraser-Jenkins is well known for his scepticism of the utilisation of small sections of DNA as a basis for classification. His frequent strongly stated views and objections of studies that don’t coincide with his ideas based on traditional morphological taxonomies are presented again here. Next are short sections with notes on the history and progress of the current volume and the format of the species accounts. This is followed with a list of accepted species and subspecies included in both Volume 1 and 2. The list of taxa to be published in Volume 2 includes taxonomic and nomenclatural notes on various species as well as new combinations, new species and even a new genus, Katoella (Davalliaceae). This latter example highlights one of the inconsistencies of his morphological approach. Davalliaceae is split into a number of small genera whereas the much larger and morphologically diverse family Thelypteridaceae contains a single genus, Thelypteris. Recent molecular studies are starting to unravel the phylogeny of this family, but will they be acceptable to Fraser-Jenkins? Maybe we will find out when volume 2 is published. Intriguingly, in this list, Dryopsis is lumped back in to Ctenitis without explanation, which seems to be at odds with both morphological and molecular evidence. Hopefully this decision will also be explained in more detail in the second volume.

The main bulk of the book is taken up with the taxonomic accounts of 20 families including those of the Lycophyta and the early branching fern orders through to the Pteridaceae in the Polypodiales (Vittariaceae remains separated and is the concluding family to Volume 1). The amount of information and knowledge collated here is astounding. For example, a page opened at random revealed the treatment of Aleuritopteris bicolor: It starts with a list of synonyms and misapplied names. Then follows a short overview of its confused history, cytology, ethnobotany and a brief comparative description. Next is a list of dozens of examined specimens listed under the different Nepalese regions and finally a note on its total distribution and status in Nepal. It should be emphasised that this is not an identification manual as such and no keys are provided. To confidently identify one of the many different Pteris, Onychium or Aleuritopteris species without prior experience would be difficult. However for smaller genera, such as the difficult Coniogramme, accurate identification should be possible.
from the brief descriptions. Incidentally under Coniogramme and several other genera, Fraser-Jenkins, with some justification, draws attention to the many errors of taxonomy and nomenclature contained within the recent Flora of China Pteridophyta treatment. As we have come to expect, his opinion is strongly presented, finishing with a radical proposal to resolve the problems. In regard to Coniogramme I have spent some time trying to sort out the species in cultivation and in my view his ideas make far more sense than those found in the Flora of China account.

As is often the case in Fraser-Jenkins’ works, many interesting comments and observations are concealed under various species accounts, including unexpected notes on species far away from Nepal. For example, both Adiantum viridimontanum and Pteris longifolia of the New World are reduced to subspecies of Adiantum pedatum and Pteris vittata respectively. Several important accounts clarifying the taxonomy and distribution of difficult genera are included. Onychium is comprehensively revised for the region, with descriptions of two new species and a new hybrid. Selaginella and Pteris are the two largest genera in Volume 1 with 23 and 27 species respectively. I noticed that the name Pteris longipes is considered to have been misapplied and is actually an earlier name for the well-known P. wallichiana. Fraser-Jenkins favours rejection of the former name and I suspect a formal proposal is in the pipeline. Other complex genera such as Huperzia, Adiantum, Aleuritopteris and Microlepia are very fully treated and the latter, in particular, includes much new and useful information. One important name change affects the widespread and common fern, Dennstaedtia scabra. The rules of priority will mean D. zeylanica now takes precedence.

At the end of the book are 138 colour plates that show many of useful discriminating characters for a range of species. Finally there is an appendix explaining in detail the confused identity and nomenclature of Athyrium setiferum and the index.

I noted very few printing or spelling mistakes. Only one error stood out, Sambucus edulis (page 270), presumably meaning S. nigra (Elder). This is an example that demonstrates the author’s intimate knowledge of Asian ferns; he notes that living fronds of Coniogramme procera smell strongly of Elder when crushed. I doubt that this small but useful piece of information has ever been published previously.

Rather frustratingly there is no bibliography in the present volume and presumably this will be included in Volume 2. Also a separate and complete list of new taxa and combinations would be most useful. Although they are included in bold type in the index of this volume I noticed at least one had been overlooked. The book is reasonably well produced although the binding leaves much to be desired and my copy is already showing signs of distress.

In summary, Chris Fraser-Jenkins and his co-authors are to be warmly congratulated on producing this landmark in Asian pteridology. It will provide a detailed baseline of data that will be used widely throughout the Himalayan region and beyond. I and many other pteridologists, both professional and amateur, will eagerly await the arrival of the second volume.

REFERENCES


T. PYNER
INSTRUCTIONS FOR AUTHORS

PAPERS should not usually exceed 20 printed pages and are generally expected to be considerably shorter. As well as reports of original research, review articles and short notes, e.g. new records, are encouraged. The senior author should supply an email address to facilitate correspondence.

REVIEWS should cover recent developments in any aspect of research on ferns and lycopods. This can include unpublished research by the author, and/or the possible direction of future research. They should be aimed at the general reader who may not be an expert in the chosen topic. Proposals for a review should be discussed first with the review editors.

MANUSCRIPTS should be submitted in English (British) in electronic format in 10-point Times New Roman font and double spaced. Electronic versions of text and tables should be compatible with WORD, with figures as pdf or jpg files, and sent as email attachments or CD roms. All manuscripts will be refereed.

THE TITLE should reflect the content of the paper and be in BOLD CAPITALS (11-point) and centrally aligned. Generic and specific names should be in italics and any title containing a generic or specific name should be followed by the family name in brackets e.g.

TRICHOMANES SPECIOSUM (HYMENOPHYLLACEAE)
IN SOUTHERN SPAIN

AUTHOR ABBREVIATIONS should follow Pichi Sermolli’s (1996) Authors of scientific names in Pteridophyta, Royal Botanic Gardens, Kew.

MAIN HEADINGS: should be in BOLD CAPITALS (10-point) and centrally aligned.
SUBSIDIARY HEADINGS: should be in bold, the first letter of each word in capitals, the rest in lower case and left-aligned.

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Alterations from the original text at proof stage will be charged for unless they are minor points of detail. A pdf will be provided free to the senior author.
DECAPLOID GAMETOPHYTE FORMATION FROM SPORES OF A PENTAPLOID CYSTOPTERIS FRAGILIS (CYSTOPTERIDACEAE) COLLECTED IN MONGOLIAN ALTAI

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Key words: Cystopteris fragilis, decaploid, meiosis, pentaploid, sporogenesis

ABSTRACT

Germinating spores were obtained from a pentaploid Cystopteris fragilis collected in Mongolian Altai. DNA content of nuclei in gametophytes showed for the first time that gametophytes that developed from spores of the pentaploid sporophyte had a larger number of genomes than those of the mother sporophyte, and some appeared to be decaploid. Fertile decaploid (10n) spores may be produced in the pentaploid C. fragilis and derived from monad spores observed in sporogenesis. Antheridia were produced on the 10n gametophytes, although antherozooids with swimming ability were not observed. Archegonia were not observed. Apogamous sporophytes with 10n genome content were induced from decaploid gametophytes. The monad spores produced may contribute to the formation of higher polyploid sporophyte series in ferns.

INTRODUCTION

Cystopteris fragilis (L.) Bernh. collected in Mongolian Altai showed three cytotypes, tetraploid, pentaploid and hexaploid, and although numerous univalent and bivalent chromosomes were observed in the spore mother cells of the pentaploid C. fragilis (2n = 5x = ca. 210), some germinating spores were obtained from the sporophyte (Kawakami et al., 2010). In the present paper, therefore, we aimed to investigate the genome contents of germinating spores produced and also to determine how these viable spores are produced in the pentaploid sporophyte. From the results of DNA contents of nuclei in gametophytes raised from spores of the pentaploid sporophyte, it was shown for the first time that the gametophytes produced had not the same but a larger number of genomes than those of the mother sporophyte, and surprisingly, some gametophytes had decaploid...
(10n) genome contents. The results might indicate that 10n spores are produced in the sporophyte and that they derive from monad spores observed in sporogenesis. In the present study, not only decaploid spore formation but also other viable spore formation processes are discussed. Furthermore, since polyploidy is one of the outstanding features in ferns (Manton, 1950; Lovis, 1977; Walker, 1979; Takamiya, 1996; Kato, 1997), we also investigated the gametophytes cultivated on agar to determine whether or not these spores could contribute to the formation of higher polyploid sporophyte series in C. fragilis.

**MATERIALS AND METHODS**

*Cystopteris fragilis* was collected in Mongolia, west of Hovd Province, Erdeneburen sum, N 48° 38’69”, E 091° 07’74”, alt. 2450 m (Kawakami et al., 2010), and then cultivated in Japan. Spores collected were used for axenic culture. Gametophytes were cultivated on 1/4 strength of Murashige and Skoog (1962) (MS) medium supplemented with 0.75 % sucrose and 0.7 % agar. For the apogamous sporophyte formation, gametophytes were transplanted on 1/4 strength of MS medium supplemented with 3 %

![Figure 1](image-url). Sporogenesis in pentaploid *Cystopteris fragilis*, A: sporangia on the underside of the frond; B: meiotic chromosome separation; C: two nuclei formed by chromosome separation; D: dyad spores produced in a sporangium; E: young spores produced in a sporangium, arrow indicates a monad spore; F: four spores in a tetrad; G: sterile spores produced in one sporangium; H: a large round spore with spiked ornamentation produced in a sporangium; I: spores collected from the pentaploid sporophyte, arrow indicates a fertile spore. Scale bars: A: 1 mm; B, C: 10 μm; D, E: 50 μm; F: 25 μm; G, H, I: 100 μm.
sucrose, 0.1 % casamino acid and 0.8 % agar. Cultures were maintained at 25 °C and illuminated by two fluorescent lamps (NEC FL 15BR) to keep 800 lux at the surface. Meiotic chromosomes were observed by fixing sporangia with 3:1 ethanol-acetic acid for 30 min at 5 °C and squashing them in 2 % aceto-orcein solution. The DNA contents of nuclei in fronds were estimated by flow cytometry using a Partec Ploidy Analyzer PA (Partec, Münster, Germany) (Kawakami et al., 2003).

RESULTS

The pentaploid *Cystopteris fragilis* produced sporangia on the underside of the frond (Figure 1A). In spore mother cells, numerous univalent and bivalent chromosomes were observed at meiotic metaphase I. By meiotic chromosome separation (Figure 1B) two nuclei were formed (Figure 1C), and dyad spores were observed frequently in sporangia (Figure 1D). Some of these consisted of both large and small spores. Monad spores (Figure 1E) and spores in tetrads (Figure 1F) were more rarely observed. Mostly, young spores produced in sporangia did not mature and the sporangia did not develop (Figure 1G). In a few expanded sporangia, a few large spores with spiked ornamentation were observed (Figure 1H). Although spores obtained were mostly abortive, some were able to germinate (Figure 1I).

DNA contents of nuclei in 13 gametophytes derived from spores of the pentaploid sporophyte were investigated. Their genome contents were greater than those of the donor pentaploid sporophyte (Figure 2A). Two gametophytes had approximately $8n$ genome content (Figure 2B), another two had approximately $9n$ and nine gametophytes had approximately $10n$ genomes (Figure 2C).

Eight out of nine $10n$ gametophytes produced antheridia (Figure 3A), however, swimming antherozooids were not observed. Archegonia were not observed on any gametophytes during three years of culture. Apogamous sporophytes were induced from two $10n$ gametophytes after one year of culture (Figure 3B). They grew to approximately 10 mm in height but then died.

DISCUSSION

Manton (1950) made a cytological study of the pteridophyta, and following her, similar investigations on pteridophyta were carried out by many researchers (e.g. Wagner, 1954; Lovis, 1964; Sleep, 1966, 2014*; Reichstein, 1981; Pinter, 1995; Ekrt & Koutecky, 2016). In these studies, various suggestions have been proposed about differentiation and development in ferns through observations of meiosis in hybrid species.

Generally speaking, meiosis of sporophytes with many univalent chromosomes is irregular and spores produced are mostly abortive. If fertile spores could be obtained from those sporophytes, one might consider whether they are reduced spores with aneuploid chromosome numbers, as reported in triploid *Osmunda regalis* L. (Manton, 1950) or unreduced spores with the same chromosome number as the mother plant, produced by the pathway of Döpp-Manton or Braithwaite (Manton, 1950; Braithwaite, 1964; Walker, 1979; Kato, 1997; Kawakami et al., 2003). In the present study, however, the genome contents of gametophytes developed from spores of the pentaploid *C. fragilis* were greater than those of the donor pentaploid sporophyte and some gametophytes had

*Sleep examined *Polystichum* and proposed her ideas on the development of this genus. Her studies were not limited to *Polystichum* but also extended to other ferns. Because of her early death, this study (Sleep, 2014) was published posthumously.
10n contents that double the genome contents of the donor sporophyte. Since gametophytes develop directly from spores, their genome content must be considered to be the same as that of the spore from which an individual is derived. The results, therefore, suggest that spores with a higher ploidy level than the mother sporophyte are, surprisingly, produced in the pentaploid *C. fragilis*. The formation of gametophytes with genomes doubled that of the donor sporophyte is reported here for the first time.

From the observation of meiosis, it might be considered that spores with 10n genome

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**Figure 2.** Relative DNA content of gametophytes derived from spores of the pentaploid *Cystopteris fragilis*, A: distribution of relative DNA content of 13 gametophytes; B: sample of gametophytes with approximately 8n genome contents; C: Sample of gametophytes with approximately 10n genome contents.
contents are from monad spores produced in sporangia. They might be produced without meiotic cell division from the spore mother cells with doubled genome contents. When monad spores are produced, restitution nuclei might occur; and if some chromosomes are lost when they occur, monad spores with genomes smaller than 10n, for example, 9n or 8n, might be produced. However, in the formation of spores with 9n or 8n genome contents, especially in the case of 8n spore formation, another pathway might be considered. Since in sporogenesis dyad spores consisting of large and small spores were observed in the present study, the larger spore with 8n might be fertile, though the smaller spore with 2n might be sterile. Although dyad spores are well known to be produced in many ferns, they are unreduced spores with the same genome contents as the donor sporophyte (Walker, 1979; Kato, 1997; Kawakami et al., 2003). Why fertile 5n spores were not obtained in this study is unknown. Further studies of fertile spores may be required to understand sporogenesis of the pentaploid sporophyte. The present study revealed the monad spore formation in the pentaploid C. fragilis.

It is well known that polyploidy is one of the outstanding cytological features in ferns (Manton, 1950; Wagner, 1954; Lovis, 1968; Walker, 1979; Kato, 1997). The highest polyploid fern in Japan is 10x (Nakato, 1987; Takamiya, 1996) and the highest polyploid in the world is reported to be 16x (Walker, 1979). The evolutionary process of how polyploid ferns such as 10x or 16x are produced has not been investigated in detail. Since unreduced spores are well known to play a very important role for polyploid formation (Gastony, 1986; Kato, 1997), the pathway of the decaploid sporophyte (10x) formation could well be that they are produced by fertilization of pentaploid gametes produced from pentaploid gametophytes derived from unreduced spores produced in pentaploid plants. The present study, however, may well indicate another method of decaploid sporophyte formation: the pentaploid sporophytes produce decaploid spores and from decaploid gametophytes developed from spores, decaploid sporophytes could be induced apogamously. Furthermore, if antherozoids with an ability of fertilization could be produced from the decaploid gametophytes, though these were not observed in the present study, it might be possible to produce plants with higher polyploid levels than decaploid, such as 12x plants, by fertilization between decaploid male gametes and female diploid gametes derived from tetraploid sporophytes. Whether the fertile spores produced in pentaploid sporophytes can play a role in the formation of higher polyploid

**Figure 3.** An antheridium with antherozooids (A) and apogamous sporophytes (B) produced on gametophytes with approximately 10n genome content in *Cystopteris fragilis*. Scale bars: A: 25 μm; B: 1 mm.
C. fragilis in nature or not is quite intriguing. If decaploid C. fragilis were discovered in Mongolian Altai, it might have been produced from decaploid gametophytes apogamously. To understand the evolution of polyploidy in these ferns, further studies on C. fragilis in Mongolian Altai may be necessary.

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SHORT NOTE

TWO UPDATES FOR GRAMMITID FERNS (POLYPODIACEAE) ON THE ‘FERNS OF THAILAND’ WEBSITE

The Ferns of Thailand website (http://rbg-web2.rbge.org.uk/thaiferns/Pages/thai-fern-list.htm), updated 23 June 2016, has one wrongly named set of images and one species that has been re-identified amongst the grammitid ferns (Polypodiaceae).

The on-line image of *Prosaptia celebica* is not of that species, but belongs to *Ctenopterella blechnoides*: the voucher specimen is Middleton et al. 5332 (E00700990!). The red-brown rhizome scales characteristic of most species of *Ctenopterella* can be seen in one of the images; in detail they are concolorous and glabrous. The rhizome scales of *Prosaptia celebica* are subclathrate to clathrate, medium to dark red-brown and ciliate.

Thailand material formerly identified as *Prosaptia alata* (Middleton 4400 E00700991!, Middleton E00690558!) has been re-identified as *P. serriformis*. The former has the rachis prominent and concolorous on the adaxial surface and sori in cylindrical pouches, while the latter has the rachis slightly prominent to slightly sunken and concolorous to darker on the adaxial surface and sori in hemispherical or conical pouches. Peninsular Malaysian material of *P alata* has also been re-identified as *P. serriformis*.

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I wish to thank the Curator of the herbarium, Royal Botanic Garden Edinburgh for permission to examine specimens of Grammitids in July 2016.

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TWO NEW RECORDS FOR COLOMBIA OF *PSILOTUM NUDUM* (PSILOTACEAE) FROM THE MAGDALENA VALLEY AND IN THE ANDEAN AMAZONIAN FOOTHILLS

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Key words: Inconspicuous species, Caldas, Caquetá, ferns, distribution.

ABSTRACT
Despite its wide distribution, *Psilotum nudum* has been recorded previously only from the western slopes of the Occidental Andean Cordillera of Colombia, particularly in the Chocó Region, and in the Caribbean Golf of Urabá. As a result of recent exploration, we here expand the distribution of *P. nudum* in Colombia by reporting two new records from the Central and Oriental Andean Cordilleras.

INTRODUCTION

*Psilotum nudum* (L.) Beauv. is a remarkable fern taxon and the simple aerial axis was widely regarded as a possible ancestral shoot design for sporophyte land plants (Figure 1) (Bierhorst, 1977; Gensel, 1977; Schulte et al., 1987). The relationships among Ophioglossales, Marattiales, Psilotales and extinct taxa (Cladoxylales, Zigopteridales, Iridopteridales, Stauropteridales) remain highly ambiguous (Gensel, 1977; Bateman et al., 1998). However, based on more recent molecular analyses, it is currently classified in the family Psilotaceae as a sister group of Ophioglossaceae, with both included in the class Psilotopsida (Smith et al., 2006). These molecular results are supported by evidence from morphology, for example, both families have cylindrical subterranean, non-green, mycorrhizal gametophytes and possess sporangia that are born on the adaxial surface of the leaf (Moran, 2004).

*Psilotum nudum* has a wide distribution, which is principally pantropical (Tryon & Tryon, 1982; Mickel & Smith, 2004), but it can be distributed in the subtropics of Australia and New Zealand (AVH, 2016) and in the more temperate zones of Spain (Molesworth-Allen, 1966; Galan et al., 1996). It can be found from sea level up to 1840m (Idárraga-Piedrahita et al., 2014; Murillo et al., 2016; TROPICOS, 2016). In America, it has been recorded from the south of the United States of America, Mexico, Central America, Colombia, Venezuela, Guayanas, Ecuador, Peru, Bolivia, Brazil, Paraguay, Argentina and in the Antilles (Mickel & Smith, 2004; Murillo et al., 2008; Moran 2011). It can be found either in pristine forest or urban zones (Moran, 2011).

The taxon may have few records in Colombia due to the fact that it does not look like a fern (Moran, 2004) and it prefers to inhabit places in the shade (Galan et al., 1996). Both decrease the probability of its discovery, especially for the non-trained botanist. In
Colombia, until now, it was recorded only from four localities in the Occidental Cordillera and in the Caribbean Golf of Urabá, principally in the Chocó Region (Table 1, Figure 2).

As a result of a botanic exploration in the Magdalena Medio region and in the Andean Amazonian foothills, it was discovered growing as an epiphytic fern in two new locations (Table 1, Figure 2). However, the habit of the plant can be epiphytic, terrestrial or

Figure 1. *Psilotum nudum*. A: Habit of the plant, scale bar (2 cm). B: Stem and leaves, scale bar (5 mm). C: Sporangia, scale bar (1 mm). D: Ramification of the rhizome, scale bar (5 mm). All from *D. Sanín & J. L. Peña 6046* (COL). Photos by J. L. Peña.
lithophytic (Galan et al., 1996; Windisch, 1997). Interestingly, this taxon can be found erect or pendulous, which may depend on the environmental conditions of the locality (see TROPICOS, 2016). A most suitable place to find this species is in the axes of the leaves of palm trees (Windisch, 1997; Moran, 2004), and we have found the new records on Bactris sp. or Elaeis guineensis Jacq. (Figure 1A).

It was suggested that reports of this taxon from Antioquia, Colombia reflected the fact that it was cultivated here (Idárraga-Piedrahita et al., 2014), but these new records provide supporting evidence that it can grow wild here as proposed by Moran (2004).

Figure 2. Distribution of Psilotum nudum in Colombia. The black dots are previous records: 1) Apartadó, 2) Medellín, 3) Chocó, 4) Valle del Cauca and 5) Cauca. The asterisks are the new records in: the Central and Oriental Andean Cordillera of Colombia from 6) Norcasia-Caldas and 7) Florencia-Caquetá.
Table 1. Records of *Psilotum nudum* in Colombia.

<table>
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<tr>
<th>Localities</th>
<th>Altitude (m)</th>
<th>Collection</th>
<th>Collection date</th>
<th>Bibliography</th>
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<td>Antioquia: Apartadó y Medellín.</td>
<td>0-1500</td>
<td>J. Santa 378 (HUA)</td>
<td>Not provided</td>
<td>Idárraga et al. (2014).</td>
</tr>
<tr>
<td>Valle del Cauca: Buenaventura, Bahía de Buenaventura.</td>
<td>0</td>
<td>E. P. Killip 35510 (COL)</td>
<td>05 Apr. 1939</td>
<td>Murillo et al. (2016).</td>
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<td>desviación a mano izquierda de la Quebrada la Tostada.</td>
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<tr>
<td>Caquetá: Florencia, Jardín Botánico Uniamazonia, Sendero de la Anaconda.</td>
<td>269</td>
<td>D. Sanín &amp; J.L. Peña 6046 (COL)</td>
<td>14 Jul. 2015</td>
<td>Current record</td>
</tr>
</tbody>
</table>
The discovery of these populations of *Psilotum nudum* in Caldas and Caquetá department expand its distribution to the Central and Oriental Andean Cordilleras of Colombia (Figure 2), and suggest that the plant may have a wider distribution in Colombia. However, due the difficulties of recording it, its real distribution is still not well known.

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**REFERENCES**


SHORT NOTE

TWO NEW COMBINATIONS IN GRAMMITID FERNS (POLYPODIACEAE): PROSAPTIA HORNEI AND RADIOGRAMMITIS SETULIFERA

Two new combinations in Asian-Pacific Grammitid ferns are proposed: Polypodium hornei (Baker) Parris and Radiogrammitis setulifera (Alderw.) Parris. A lectotype is chosen for Polypodium hornei Baker. Herbarium abbreviations follow Thiers (2016).


Baker’s description mentions two localities for the species, “Nadrau to Babuca, Viti Levu”, and “mountains of Ovalau”. The type sheet at Kew bears two plants; the left hand one (K001044319) is well pressed and bears a label beneath it reading “on trees Nadrau to Babuca”. This plant is chosen as the lectotype. The right hand plant (K000604741) has rolled up fronds with the pinnae pressed together to obscure their abaxial surfaces and it has no label indicating its provenance.

Baker (1879) and Brownlie (1977: 359) both describe the rhizome scales of the species as ciliate, a character common to Prosaptia and some species of Tomophyllum. The type material of Polypodium hornei has a dorsiventral rhizome with stipes in two rows and articulated to prominent phyllopodia, and it also lacks hydathodes on the vein endings on the adaxial surface of the lamina, all of which are characters of Prosaptia. Brownlie’s illustration of part of the frond (1977: 355, pl. XLI, 3, as Ctenopteris hornei) shows the crenulate pinna margin often found in Tomophyllum, but very rarely in Prosaptia, and material collected by him in CHR has the radial stipe arrangement of Tomophyllum. Nakamura & Kokubo (2008: 256, pl. 99, 1) illustrate (as Ctenopteris hornei) a plant that is obviously a species of Tomophyllum; it has the evident hydathodes on the vein endings on the adaxial surface of the lamina characteristic of Tomophyllum, the same slightly crenulate lamina margin that Brownlie illustrated, and rhizome scales with a single apical hair that are found in numerous species of Tomophyllum. Clearly, two species have been confused under the name Ctenopteris hornei; Polypodium hornei = Prosaptia hornei, and an un-named species of Tomophyllum. The confusion is partly due to the fact that Prosaptia hornei is one of a small group of Prosaptia species that have superficial sori (like Tomophyllum), as opposed to sori sunken in steep-walled depressions on the abaxial surface of the lamina or in marginal to submarginal pouches that are typical of Prosaptia.


Detailed examination of the rhizomes of *Matthew* s. n in E and *Matthew* 657 in K shows that they are radial rather than dorsiventral, thus the species belongs to *Radiogrammitis*, rather than *Oreogrammitis*. The species is very similar in frond indumentum and dissection to *R. subpinnatifida* (Blume) Parris, but differs in having fewer and much shorter stipes arranged in a series of looser spirals or whorls on the rhizome.

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BOOK REVIEW


The scientific names of plants are often puzzling and their meanings frequently obscure. Many users of these names find their translation and origin fascinating. Finding information regarding the names of ferns and particularly the myriad of cultivar applications is especially difficult. J.W (Jimmy) Dyce remedied this situation with the publication of this small book in 1988. Proving to be very popular it was reprinted in 1994 but has been difficult to obtain for several years. It is therefore a great pleasure to receive this new edition, greatly expanded and enhanced by Professor John Edgington.

Compared with the first edition, Dyce’s original text is mostly retained unaltered, however there are significant additions that considerably improve the utility of the book. Starting with the introduction Dyce gives a brief explanation of history of using Latin for the naming of plants and why it is still used in the modern era. He also alludes to the particular problem of fern cultivar nomenclature, which even today does not seem to be resolved to the satisfaction of all. (The problem arises because many fern cultivar names are actually brief descriptions or polynominals, and were abandoned in other areas of botanical nomenclature centuries ago). The next section, on the etymology of British fern generic names, has been fully revised and expanded by Professor Edgington. It now includes a brief statement regarding the origin of the name as well as the meaning. (Lycophytes were ignored by Dyce in the first edition but are happily now included despite making the book’s title slightly imprecise).
The next chapter is completely new, an etymology of non-British genera including all those found in Europe and many that are found in cultivation. I found this part particularly interesting. As in the previous section, both the meaning and origin of the name is given. The next two sections, both helpful and informative, listing the word elements at the beginning and end of fern names, are left unrevised.

The next chapter, again new, comprehensively covers most of the specific names of European ferns including hybrids. This is a most useful addition and many obscure names are included, the exceptions being a large number of *Asplenium* hybrids, many only having been found once.

In 1984 Dyce published an updated list of British ferns and their current names and this has been included here, suitably revised taking into account recent research. Under the same heading, Fern nomenclature – old and new, are two new lists. The first covers non-native species that have recorded growing in the wild in the British Isles and the second is a comprehensive list of synonyms of British ferns and lycophytes. Both these lists are of great interest and will prove to be very useful.

There then follows the largest section of the book, a glossary of fern varietal names. This formed the major part of the first edition and is included here unchanged. Many of the terms are also widely used in species names so it is not restricted to horticultural varieties. Finally there is a new, brief glossary of technical terms and an updated list of references.

As I hope will be appreciated from this review this is a much improved publication where John Edgington has managed to retain the content and spirit of Jimmy Dyce’s original but also sought to include much additional relevant and interesting information. He has achieved this most successfully and with the book priced at just £5 it must rank as one of the bargains of the year.

T. Pyner
INSTRUCTIONS FOR AUTHORS

PAPERS should not usually exceed 20 printed pages and are generally expected to be considerably shorter. As well as reports of original research, review articles and short notes, e.g. new records, are encouraged. The senior author should supply an email address to facilitate correspondence.

REVIEWS should cover recent developments in any aspect of research on ferns and lycopsids. This can include unpublished research by the author, and/or the possible direction of future research. They should be aimed at the general reader who may not be an expert in the chosen topic. Proposals for a review should be discussed first with the review editors.

MANUSCRIPTS should be submitted in English (British) in electronic format in 10-point Times New Roman font and double spaced. Electronic versions of text and tables should be compatible with WORD, with figures as pdf or jpg files, and sent as email attachments or CDroms. All manuscripts will be refereed.

THE TITLE should reflect the content of the paper and be in BOLD CAPITALS (11-point) and centrally aligned. Generic and specific names should be in italics and any title containing a generic or specific name should be followed by the family name in brackets e.g. TRICHOMANES SPECIOSUM (HYMENOPHYLLACEAE) IN SOUTHERN SPAIN

AUTHOR ABBREVIATIONS should follow Pichi Sermolli’s (1996) Authors of scientific names in Pteridophyta, Royal Botanic Gardens, Kew.

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REFERENCES: follow the style of a recent issue of The Fern Gazette, e.g.:


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DIVERSITY AND ADAPTATIONS OF RHEOPHYTIC FERNS

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Key words: adaptive strategy, flood zone, heterochrony, Osmunda, recurrent adaptations, gametophyte, streamlined leaf

ABSTRACT
Rheophytes grow in the zone on river beds and banks that are regularly flooded after heavy rains. In the tropics, this zone is of sufficient height (from the lowest to the highest water level) that different rheophytes can occur in the lower and upper subzones. Rheophytic species are obligate or facultative and some woody plants, rarely climbers, are paedorheophytic. I enumerate 141 rheophytes and possible rheophytes in 18 families and subfamilies of rheophytic ferns and lycophytes assigned to a variety of families, suggesting recurrent adaptations. The rheophyte morphology is adapted to the habitat and shared among the lineages, and especially the leaves or leaflets are streamlined with cuneate bases and glabrous, entire, tough blades. In leaf development the mesophyll cells and intercellular spaces, together with the epidermal cells, are smaller in size during a shortened period of ontogeny. The gametophyte of rheophytes, which is independent of the sporophyte, has a different adaptive strategy from the sporophyte: a shortened duration of generation and subtle morphological modification.

ECOLOGY
Plants include 300,000‒330,000 or more species of bryophytes, lycophytes, ferns, gymnosperms and angiosperms (Evert and Eichhon, 2013; Mauseth, 2014). They grow in a wide range of habitats other than terrestrial, including alpine, arid, water, mangrove swamp, epiphytic, lithophytic (e.g., on limestone or ultramafic rocks), and other habitats (Crawford, 2008). The habitats are characterized by abiotic factors (temperature, precipitation, light, etc.) and associated organisms. Remarkable organismal associations are seen in, for example, symbiotic plants (Paracer and Ahmadjian, 2000; Evert and Eichhorn, 2013). This paper reviews our understanding of the diversity and adaptations of ferns that grow in rivers.

A special habitat for plants is provided by rivers. Rivers are often subject to flash floods after heavy rains, even as frequently as once a week in the tropics, and plants called rheophytes grow exclusively on banks and beds of rivers and streams (van Steenis, 1981, 1987; Mehtreter, 2008). Van Steenis (1981) defined rheophytes in ecological terms as follows: rheophytes are “confined to the bank and bed and growing there up to flood level, but not beyond the reach of regularly occurring flash floods” (Figure 1). Rheophytes are largely tropical, but a few extend into temperate zones, e.g., Hokkaido, in northern Japan (43°N) (Iwatsuki, 1992). Most rheophytes live in the air much longer than under relatively brief flooded conditions; this is because water levels return to the
more normal levels shortly after rains stop or abate. A few other rheophytes are hydrophytic and always grow submerged, as in e.g., *Hymenasplenium obliquissimum* (Hayata) Sugim. & Sa. Kurata.

Similar habitats are present in seasonally inundated pools, where semiaquatic ferns, such as *Platyzoma microphylla* R.Br. (=*Pteris platyzomopsis* Christenh. & H.Schneid.) (Tryon, 1964) and *Marsilea* species (Korns, 1988) grow, and in inundated riparian and marshland forests, which are one of the habitats preferred by, e.g., *Osmunda regalis* L. (Lellinger, 1985; Page, 1997). However, the lands are covered by still or slow-running water.

The height of the flood zone, from the normal low level of river water to the highest flood level reached, differs in different climates -- up to 3 metres in humid tropical Borneo (Figure 1; M. Kato, unpubl. data). There are different preferences of rheophytes for the tropical flood zone. Some rheophytes, e.g., *Ctenitis vilis* (Kunze) Ching, grow in the higher zone, which is subject to rarer and shorter floods, while others, e.g., *Microsorum pteropus* (Blume) Copel., prefer the lower zone, which is subject to more frequent and longer floods. *Microsorum paucijugum* (Alderw.) K.Iwats. & M.Kato prefers the further lower zone.

Rheophytes are herbaceous or woody. In herbaceous rheophytes, whole plants are submerged when flooded, while in tall woody rheophytes only the basal part of the plant is submerged while flooded. In tall paedorheophytes, seedlings and low juveniles are rheophytic and the adults are emergent and thus typically not rheophytic. *Alsophila esmeraldensis* R.C.Moran of the tree-fern family Cyatheaceae is regarded as a rheophyte (Moran, 1995), but it may not be paedorheophytic because of the short (up to 80 cm) trunk. Van Steenis (1987) considered *Lomagramma* species as paedorheophytic, because

**Figure 1.** Habitat segregation of rheophytes and land species on river bank in West Kalimantan, Indonesia. Rheophytic *Dipteris lobbiana* occupies the flood zone, and land species such as *D. conjugata* Reinw. and *Blechnum orientale* L. grow above the zone.
the juvenile creeping plants are rheophytic and only later climb trees. Further study is necessary to reveal paedorheophily of ferns.

Rheophytes may be obligate or facultative. Usually, obligate-rheophytic species comprise only rheophytic populations, while facultative rheophytes comprise land-dwelling populations and rheophytic populations. Examples of facultative rheophytic ferns are *Bolbitis heteroclita* (C.Presl) Ching in C.Chr. and *B. sinuata* (C.Presl) Hennipman. *Ampelopteris prolifera* (Retz.) Copel. and *Diplazium esculentum* (Retz.) Sw. may also belong to this category.

**ADAPTIVE MORPHOLOGY**

The impact of flash floods to plants is considerable because non-rheophytic land plants cannot invade the flood zone (Figure 1). Rheophytes have gained particular morphologies to resist or reduce pressure of flooding, although their leaves may be damaged by flooding (Sharpe, 1997). The shoot-branches and leaf-petioles are flexible or tough with developed bark in, e.g., *Salix* or with dense networks of cell walls in *Osmunda* (Imaichi and Kato, 1992). The roots adhere tightly to the river bank and bed. The most remarkable feature of rheophytes is leaf morphology (van Steenis, 1981). The leaves or leaflets are streamlined (usually narrow-lanceolate) with cuneate bases (Figure 2), entire or only minutely toothed at the margins, and hairless or sparsely hairy. Cuneate leaf-bases are particularly important because the bases are under pressure from currents. Thelypteridaceae, a large family generally characterized by having hairy leaves, has the largest number of rheophytes in ferns (Appendix), but the hairs of rheophytic species in this family are usually sparse, short, or antrorse (ascending or appressed toward the distal end of the lamina or pinnae). In paedorheophytes, only the leaves of juvenile plants are streamlined.

Sharpe (1997) found that in the Puerto Rican rheophyte *Meniscium angustifolium* Willd. the leaf growth rate is faster than in other tropical land ferns, and the leaf life-span is shorter. The faster growth rate would reduce exposure of the fragile uncoiling leaves

![Figure 2. Pinnules. A. Land-living Osmunda japonica. B. Rheophytic O. lancea.](image)
to flood, and the shorter life span would allow escape from stresses of flooding. The leaf phenology of rheophytic ferns should be further investigated to clarify the various adaptations (see also section “Adaptations”).

**DIVERSITY**

Rheophytic ferns are most abundant in tropical areas with heavy rainfall, and van Steenis (1981, 1987) enumerated 67 rheophytic ferns and lycophytes. Kato et al. (1991) found about 40 species of rheophytic ferns from Borneo. It indicates that the ever-wet Borneo is remarkably species-rich for rheophytes. At present 141 rheophytic fern taxa are known in the world, and these are assigned to 18 families and subfamilies (Appendix 1). There are other species that require further study with respect to whether they are rheophytic or not.

In many fern genera, only one or a few rheophytic species are known, while there are multiple rheophytes in other genera. This trend is obvious also in flowering-plant rheophytes, believed to be about 800 (0.3% of the total number) species belonging to 68 (17%) families (van Steenis, 1981, 1987). The scattered distribution of rheophytes over many lineages suggests that derivation of rheophytes from land-growing ancestors is markedly recurrent. Some rheophytes are secondarily derived from other rheophytic species in the same genus. I suggest that *Microsorum paucijugum* (Alderw.) K.Iwats. & M.Kato growing in the lower flood zone is derived from another rheophytic *M. pteropus*. Kato (1984) considered that two rheophytes, *Deparia biserialis* (Baker) M.Kato and *D. confluens* (Kunze) M.Kato, are derived from *D. petersenii* (Kunze) M.Kato, although molecular evidence to support these relationships is not available (Kuo et al., 2016). Rheophytic fern species are distributed in a wide range of ferns (PPG I, 2016) from the primitive Osmundaceae through the derived Polypodiaceae, and even in the eusporangiate Ophioglossaceae and the lycophytic family Isoëtaceae. Most fern families with rheophytic members are large (> 200 spp.) and tropical (for example, Thelypteridaceae). The most species-rich genus is *Bolbitis* (14 spp.), which prefers rock surfaces in wet valleys. I suggest that the evolution of rheophytes happened by chance in the tropics where the rheophytic habitats are abundant. However, the basal leptosporangiate families Osmundaceae and Dipteridaceae are small and comprise fewer than 20 species.

**ADAPTATIONS**

Narrow streamlined leaves are characteristic of rheophytes (van Steenis, 1981; Vasco et al., 2013). Kato and Imaichi (1992a) examined 32 tropical rheophytic ferns and related land species, and found a strong correlation between leaf morphology and anatomy. The mesophyll cells and intercellular spaces are smaller in the narrow leaves than in the broad leaves of related species. Correlated with this, the epidermal cells are also smaller (Kato and Imaichi 1992a). In a comparative developmental study, Imaichi and Kato (1992, 1997) showed that the mesophyll cells at the pre-expansion stage are cubical and equal-sized and then expand in both *Osmunda japonica* Thunb. and *O. lancea* Thunb. (Figure 3A, E). However, the expansion time-span is less and the mesophyll cell arms are shorter, surrounding the smaller intercellular spaces, in *O. lancea* (Figure 3). In this study, we interpreted the evolution of the narrow leaves as heterochronic. Kato and Imaichi (1992b) found a broad-leaved variant of the rheophytic *Tectaria lobbii* (Hook.) Copel.in East Kalimantan. This variant grew together with the normal form and differed only in the broad pinnae with irregular lobes (Figure 4A, C). The broad-leaved variant
may have been called *T. jacobii* Holttum (Holttum, 1991). It is anatomically very similar to land-growing species (Figure 4B, D), suggesting atavism.

Cells of petioles of the rheophytic *Osmunda lancea*, like those of the lamina, are smaller and denser, the cuticular layer is thicker, and the epicuticular wax deposits are denser than in *O. japonica* (Imaichi and Kato, 1992, 1993). This leaf construction increases mechanical strength and repels water (Juniper and Walker, 1985). Exceptional flooding (to a height of 10 metres above the normal level of water) rarely reached terrestrial ferns growing well above the flood zone and if this happens, it may decay their leaves (M. Kato, unpublished field observations). This contrasts with rheophytes, which have high resistance to regular flooding in fast-moving, sometimes violent, currents containing dense microbes and soil/rock particles.

There is a clear habitat separation between rheophytes and land plants at the uppermost reaches of flood zones (Figure 1). Land-growing species cannot invade the flood zone, due to their non-adaptive morphology. It is likely that the habitat separation is established in a certain stage of life cycle. Imaichi and Kato (1993) showed that juvenile plants of *Osmunda japonica* grow together with the rheophytic *O. lancea* near the water, and morphological and anatomical differences become prominent at an early ontogenic stage (plants having 5th or 6th youngest leaves). We suggested that *O. japonica*

**Figure 3.** Cross sections of pinnules at different stages of development (A–C, E, F) and paradermal sections of mature pinnules (D, G). A–D. *Osmunda japonica*. A, B. Young pinnules (A, 12 mm long; B, 43 mm). C. Mature pinnule (71 mm). E–G. *O. lancea*. E. Young pinnule (12 mm). F. Mature pinnule (45 mm). Arrowheads indicate veins. *Osmunda japonica* in young stage (A) has as many unexpanded mesophyll cells between veins as has *O. lancea* (E) (Imaichi and Kato, 1992). The mature stage of *O. lancea* (F) is similar to an immature stage of *O. japonica* (B). Intercellular spaces are surrounded by armed mesophyll cells (D, G).
was selected and excluded early from the flood zone and the two species eventually become separated in space and habitat.

In contrast, *O. lancea* cannot extend its habitat above the flood zone. This restriction may be caused by the narrowed leaf area and reduced intercellular space in the mesophyll layer, which may reduce photosynthetic power. This anatomical and the suggested physiological character may be a trade-off. A reduced photosynthetic rate was found in the rheophyte *Farfugium japonicum* var. *luchuense* (Masam.) Kitam. (Asteraceae) (Nomura et al., 2006), although it has a different mesophyll anatomy from fern rheophytes (see below). The isolation of the flood zone from the land zone appears strong, but this is not always strong. The diploid hybrid of *O. japonica* and *O. lancea* [*= O. ×intermedia* (Honda) Sugimoto] occurs in the interface between both habitats and can reproduce F2 or later offspring (Yatabe-Kakugawa et al., 2013).

Ferns and lycophytes are characterized by the independence of the sporophyte and gametophyte. It is likely that the two generations have different adaptive strategies. The gametophyte generation is much smaller, simpler, and shorter-lived. There is little morphological difference between gametophytes of *O. japonica* and *O. lancea* (Hiyama et al., 1992). Hiyama et al. (1992) discovered that in the rheophytic *O. lancea* the life span of gametophytes is shorter and they interpreted that its gametophyte generation is shortened, relative to land ferns, showing a different strategy from the sporophyte.

Compared with the ferns investigated, the leaves of flowering plant rheophytes show different anatomy. Narrow leaves of the facultative-rheophyte *Farfugium japonicum* var.

![Figure 4](image_url)

**Figure 4.** Rheophytic *Tectaria lobbii*. A, B. Normal plants. C, D. Broad-leaved plants. In C, arrowhead indicates normal plants. B, D. Cross sections of pinnules.
*luchuense* have fewer mesophyll cells than the broad-leaved form (Usukura et al., 1994). Similar anatomy was found in other flowering plant rheophytes (Tsukaya, 2002; Setoguchi and Kajimaru, 2004; Okada and Tsukaya, 2010, 2013; Vermeulen and Tsukaya, 2011; Ohga et al., 2012; Matsui et al., 2013). However, a rheophytic orchid ecotype shows fern-type anatomy (Yorifuji et al., 2015).

**EVOLUTION**

The pattern in which rheophytic ferns are scattered in various genera and families indicates that similar modes of adaptations produced rheophytes in a number of independent lineages (Kato et al., 1991). However, the phylogenetic relationships of rheophytic ferns have been clarified in only a few cases (e.g., *Osmunda*, Tsutsumi et al., 2012; *Deparia*, Kuo et al., 2016). Most rheophytes are obligate-rheophytic species, while some others are distinguished at varietal rank, e.g., *Deparia lancea* var. *rheophila* M. Kato, Darnaedi & K. Iwats., *Lindsaea ensifolia* var. *rheophila* K. Iwats. Still others, e.g., *Bolbitis heteroclita* and *B. sinuata* (C. Presl) Hennipman, are facultative and grow in both habitats. Little is known about the evolutionary process of rheophytes. One question to solve is whether facultative rheophytes or rheophytic varieties are transitional toward obligate rheophytes. Another is whether speciation of rheophytes is gradual or abrupt.

**ACKNOWLEDGEMENTS**

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**APPENDIX**

Rheophytic ferns and suggested rheophytes. Family or subfamily names are followed by numbers of taxa.


Blechnaceae (6) --- *Austroblechnum difforme* (Copel.) Gaspe & V.A.O.Dittrich; *Cranfillia fluviatilis* (R.Br.) Gaspe & V.A.O.Dittrich; *Oceaniopteris francii* (Rosenst.) Gaspe & Salino, *Oceaniopteris gibba* (Lab.) Gaspe & Salino; *Parablechnum glaziovii* (Christ) Gaspe & Salino, *Parablechnum rheophyticum* (R.C.Moran) Gaspe & Salino

Cyatheaceae (1) --- *Alsophila esmeraldensis* R.C.Moran

Dennstaedtiaeae (1) --- *Microlepia rheophila* K.Iwats. & M.Kato

Dipteridaceae (1) --- *Dipteris lobbia* (Hook.) T.Moore

Dryopteridaceae subfamily Dryopteroideae (3) --- *Ctenitis vilis* (Kunze) Ching [facultative]; *Dryopteris rheophila* Mitsuta ex Darnaedi, M.Kato & K.Iwats.; *Polystichum yaeyamense* (Makino) Makino

Dryopteridaceae subfamily Elaphoglossoideae (17) --- *Bolbitis fluviatilis* (Hook.) Ching in C.Chr., *Bolbitis heteroclita* (C.Presl) Ching in C.Chr. [facultative], *Bolbitis heudelotti*

Hymenophyllaceae (2) --- *Cephalomanes javanicum* C.Presl var. *asplenioides* (C.Presl) K.Iwats. and var. *sumatranum* (Alderw.) K.Iwats. [facultative]

Isoëtaceae (2) --- *Isoëtes hawaiiensis* W.C.Taylor & W.H.Wagner [facultative], *Isoëtes martii* A.Br. ex Kuhn


Ophioglossaceae (2) --- *Ophioglossum gramineum* Willd. var. *majus* (Alderw.) Wieferring, *Ophioglossum reticulatum* L. var. *dilatatum* (Miq.) Wieferring [facultative]

Osmundaceae (4) --- *Osmunda (Plenasium) angustifolia* Ching, *Osmunda (Plenasium) bromeliifolia* (C.Presl) Copel. [facultative?], *Osmunda hybrida* Tsutsumi et al. [rheophyte?], *Osmunda lancea* Thunb.


Pteridaceae subfamily Vittarioideae (3) --- *Antrophyum vittarioide* Baker [facultative?]; *Austrogramme asplenioides* (Holttum) Hennipman, *Austrogramme decipiens* (Mett.) Hennipman [facultative]


A SHORT BIOGRAPHY OF THE AUTHOR

Masahiro Kato was born in Japan in 1946. He graduated from Kyoto University and received Dr. Sci. in 1976 with a thesis entitled “Morphological and systematic studies of Athyriaceae.” After being employed as research assistant in the Department of Botany, Kyoto University, he moved to the University of Tokyo in 1982 and became professor in 1997. Then he moved to the National Museum of Nature and Science as director of the Department of Botany and director of Tsukuba Botanical Garden. Now he is professor emeritus of the University of Tokyo and researcher emeritus of the National Museum of Nature and Science. He has undertaken a number of fieldwork projects over tropical Asia. His research interest has focused on the fern flora of tropical Asia, classification and systematics of pteridophytes, such as Athyriaceae, Davalliaceae, Ophioglossaceae and Osmundaceae. He has also investigated the evolution of epiphytic Davalliaceae, and explored rheophytes in Borneo and adjacent areas. In extended research of rheophytes, he has studied the flora, morphology and evolution of the torrenticolous rheophytes Podostemaceae (rosid angiosperms). This research extended into the genetic background of their unique morphology. Additionally, he researched the evolution of primitive angiosperms and single-leaf plants (Monophyllaea, Gesneriaceae), and the phylogenetic relationships of bryophytes and pteridophytes.
TWO HYBRIDS OF *EQUISETUM SYLVATICUM* (EQUISETACEAE) 
ON THE ISLAND OF SENJA, TROMS, NORWAY

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**Key Words:** *Equisetum sylvaticum*, hybrids, macromorphology, micromorphology, SEM, Senja, Norway, distribution

**ABSTRACT**

*Equisetum × mildeanum* (*E. pratense* × *E. sylvaticum*) and *E. × lofotense* (*E. arvense* × *E. sylvaticum*) have been discovered on the island of Senja (Troms, Norway). Their appearance in the field and macromorphology are extensively compared and imaged. Additionally micromorphological features of the shoot and stomata of both rare hybrid taxa and the involved parental species have been examined by SEM technology. Distribution maps illustrate the known occurrence for the European Nordic countries. A third taxon, *E. × bowmanii* (*E. sylvaticum* × *E. telmateia*), is included in the comparative study to include all known *E. sylvaticum* hybrids in a single SEM investigation for the first time.

**INTRODUCTION**

Wood Horsetail, *Equisetum sylvaticum* L., belongs to the smooth horsetails (subgenus *Equisetum*) within the family of Equisetaceae (in comparison to the rough textured members of subgenus *Hippochaete* frequently called “scouring rushes”) and is a delicate plant occurring over a wide range of wet woodland communities in the nemoral to boreo-arctic zone of Eurasia and North America (Hultén, 1950; Hultén & Fries, 1986; Jalas & Suominen, 1972).

Of the 11 hybrids that have been described within the subgenus (see overview in Lubienski, 2010; Page & Gureyeva, 2009; 2013) three have *E. sylvaticum* as one parent: *E. × mildeanum* Rothm. (*E. pratense* Ehrh. × *E. sylvaticum*), *E. × bowmanii* C.N. Page (*E. sylvaticum* × *E. telmateia* Ehrh.), and *E. × lofotense* Lubienski (*E. arvense* L. × *E. sylvaticum*).

*E. × mildeanum* was originally described from the Baltic Isle of Rügen of North Eastern Germany by Rothmaier (1944) and has been known to occur there since the 1950s (Lubienski, 2013; Lubienski & Dörken, 2015). More recently the hybrid has been recorded from Scotland (Page, 1988; Acock, 2015), Norway (Lubienski, 2003, 2009), and Sweden (Lubienski & Dörken, 2015). Unfortunately it is often confused with the parents, and its real distribution in the British Isles may be overestimated (see Acock, 2015).

*E. × bowmanii* is recorded from a few localities within the New Forest area of Southern England (Page, 1988). Additionally a second site was reported from Scotland more recently (Acock, 2015), but this likely to be an error and confused with *E. sylvaticum* (H. McHaffie & P. Acock, pers. comm.). Consequently the New Forest sites remain exclusive for this unique plant.
In 2009 *E. ×lofotense*, a similarly remarkable hybrid incorporating *E. sylvaticum*, was detected at a single site in the far north of Norway on the Lofoten island of Austvågøya (Lubienski, 2010).

During a trip to the island of Senja (Troms, Norway) four colonies of *E. sylvaticum* hybrids were found. Two were identified as *E. ×mildeanum* and seem to fit into a series of *E. ×mildeanum* sites lying on the islands of North Western Norway, whereas the latter two represent the second record for *E. ×lofotense*. At first glance the two hybrid taxa are quite similar in habit and certain macromorphological features. Their adjacent sites allowed for study and direct comparison of their macromorphology *in situ*. Additional micromorphological investigation using SEM focused on the silica spikes on the ridges of the shoots and the structure of the stomata. As part of the morphological comparison, the hybrids were compared with *E. sylvaticum* and other taxa to confirm the identity of the second parents.

**MATERIAL AND METHODS**

**Material**

Plant material was collected on the island of Senja (Troms province) that is located northeast of the Lofoten and Vesterålen islands. It is situated in the transition between the Northern and Middle boreal vegetation zone (Sjörs *et al.*, 2004). The islands and coastal mainland of Northwestern Norway display an oceanic climate with high precipitation values (Sjörs *et al.*, 2004). There are two colonies of each hybrid alongside a single fjord (Mefjorden) in the northern part of the island (Figure 1).

**Figure 1.** Mefjorden in the northern part of the island of Senja (Troms, Norway) showing the four sites of the *Equisetum sylvaticum* hybrids (*E. ×mildeanum* 1 & 2, *E. ×lofotense* 3 & 4) (based on Google Earth ©, Google Inc., 26.12.2016).

*Equisetum ×mildeanum* (*E. pratense × E. sylvaticum*)

1. Mefjorden near Svarthola, Berg kommune, Senja, Troms fylke
   N 69°27’53.3” / E 17°34’29.2”, c. 9 m a.s.l.

Growing over a length of approximately 90 metres along the wet western roadside ditch beneath the northeastern slope of Littelehesten mountain, south of Bratthesten tunnel (Figure 2a, b).
2. Mefjorden east of Mefjordvær, Berg kommune, Senja, Troms fylke
N 69°30’54.4” / E 17°28’08.2”, c. 16 m a.s.l.

Occupying both ditches and shoulders of the road east of the town of Mefjordvær and west of Mykjeneset beneath the northern slope of Kyle mountain. The colony stretches over a length of approximately 100 metres (Figure 3a, b).

\[ \text{Equisetum} \times \text{lofotense} (E. \text{arvense} \times E. \text{svylvaticum}) \]

3. Mefjorden near Svarthola, Berg kommune, Senja, Troms fylke
N 69°27’42.2” / E 17°34’51.1”, c. 5-13 m a.s.l.

Growing along a small creek that rises between the northeastern slopes of Store Hesten and Littlehesten mountain, crosses the road south of Brathesten tunnel and flows into Mefjorden just a few metres beneath. The hybrid occupies the wet slope and the roadside ditch west of the road over an area of approximately 40 × 40 metres. The eastern side of the road descends to the sea and is colonized by \( E. \times \text{lofotense} \) over an area of approximately 40 × 5 metres (Figure 4a, b).
4. Mefjorden north of Kvalvika, Berg kommune, Senja, Troms fylke
N 69°28'54.8'' / E 17°33'15.9'', c. 5-13 m a.s.l.

The site is situated by the same road two kilometres to the north, north of Brathesten tunnel. The hybrid grows over an area 10 × 30 metres on the wet slope between Burstind and Bringtinden mountain and in the western roadside ditch; it is also found on the other (eastern) side of the road, where it accompanies a small rivulet to the sea (Figure 5 a, b).

Methods
Freshly collected shoots were fixed in FAA (100 ml FAA = 90 ml 70% ethanol + 5 ml acetic acid 100% + 5 ml formaldehyde solution 37%) before being stored in 70% ethanol. For SEM with AURIGA ZEISS TM the fixed material was dehydrated in FDA (formaldehyde-dimethyl-acetal) and critical-point dried (CPD 030, BALZERS). CP-dried material was sputtered with gold-palladium (thickness 5 nm) with a BALTEC SputterCoater SCD 030.

RESULTS
Comparison of morphology and field observations of *Equisetum ×mildeanum* and *E. ×lofotense* on Senja
In the field both hybrids appear similar to *E. sylvaticum* at first glance and look like odd
Figure 6. SEM images of the micromorphology of the shoot ridges and stomata of *Equisetum arvense* (a-b), *E. sylvaticum* (c-d), *E. pratense* (e-f), and *E. telmateia* (g-h). Arrows and letters indicate ridges (r), furrows (f), mamillae (m) and pilulae (p).
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</tr>
<tr>
<td><strong>Sterile shoot – shape</strong></td>
<td>not bilaterally compressed, variable</td>
<td>often distinctly bilaterally compressed</td>
<td>bilaterally compressed only at exposed sites</td>
<td>often distinctly bilaterally compressed</td>
<td>often slightly bilaterally compressed</td>
</tr>
<tr>
<td><strong>Sterile shoot – branch teeth</strong></td>
<td>spreading away from the internode above (sometimes not spreading)</td>
<td>not or only slightly spreading away from the internode above</td>
<td>spreading away from the internode above</td>
<td>not spreading away from the internode above</td>
<td>not spreading away from the internode above</td>
</tr>
<tr>
<td><strong>Sterile shoot – branches (length ratio of 1st branch internode to nodal sheath)</strong></td>
<td>twice (or more) as long as the adjacent nodal sheath (rarely as long as)</td>
<td>twice as long as the adjacent nodal sheath (very rarely as long as)</td>
<td>twice as long as the adjacent nodal sheath (never shorter)</td>
<td>slightly shorter than to as long as the adjacent nodal sheath</td>
<td></td>
</tr>
<tr>
<td><strong>Sterile shoot – branches (ochreole)</strong></td>
<td>green throughout</td>
<td>green with very short light- to reddish-brown scarious appendices</td>
<td>light-to reddish-brown and scarious throughout</td>
<td>pale green with distinct light-brown scarious appendices</td>
<td>pale green with short brown scarious appendices</td>
</tr>
<tr>
<td><strong>Sterile shoot – nodal sheath</strong></td>
<td>commissure distinct, of a pale green to whitish colour</td>
<td>commissure distinct, of a pale green to whitish colour</td>
<td>commissure obscure</td>
<td>commissure obscure</td>
<td>commissure obscure</td>
</tr>
<tr>
<td></td>
<td>ridge-groove distinct</td>
<td>ridge-groove often distinct</td>
<td>ridge-groove less distinct</td>
<td>ridge-groove less distinct</td>
<td>ridge-groove less distinct</td>
</tr>
<tr>
<td><strong>Spores</strong></td>
<td>green-chlorophyllous, spherical, elater-bearing</td>
<td>non-chlorophyllous, irregularly shaped, without elaters</td>
<td>green-chlorophyllous, spherical, elater-bearing</td>
<td>non-chlorophyllous, irregularly shaped, without elaters</td>
<td>green-chlorophyllous, spherical, elater-bearing</td>
</tr>
<tr>
<td>Sterile shoot – nodal sheath (teeth)</td>
<td>shorter than the sheath</td>
<td>slightly shorter than or as long as the sheath</td>
<td>slightly longer than or as long as the sheath</td>
<td>shorter than or as long as the sheath</td>
<td>shorter than the sheath</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>------------------------</td>
<td>---------------------------------------------</td>
<td>---------------------------------------------</td>
<td>---------------------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>short, dark-brown with a very small white scarious margin</td>
<td>long, with a dark-brown to reddish-brown centre and a distinct white scarious margin</td>
<td>long, with a reddish-brown centre and a very broad light-brown scarious margin</td>
<td>oblong, with a brown to reddish-brown centre and a distinct light-brown scarious margin</td>
<td>short, with a small dark centre and a broad, white, scarious margin</td>
<td>not adhering together</td>
</tr>
<tr>
<td>not or sometimes adhering together</td>
<td>adhering together only at the lowermost sheaths</td>
<td>adhering together throughout the shoot</td>
<td>adhering together only at the lowermost sheaths</td>
<td>not adhering together</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sterile shoot – micromorphology of internodes</th>
<th>ridges rounded</th>
<th>ridges rounded to slightly broadened</th>
<th>ridges broadened with two distinct margins</th>
<th>ridges sharpened to slightly flattened and broadened</th>
<th>ridges sharpened, hardly broadened</th>
</tr>
</thead>
<tbody>
<tr>
<td>silica mamillae indistinct and scattered</td>
<td>silica mamillae indistinct and scattered</td>
<td>silica mamillae thorn-like, forming two rows alongside the margins of the ridge</td>
<td>silica mamillae 2-4, aligned, forming a single row, sometimes individually side by side</td>
<td>silica mamillae 1-3, aligned, forming a single row</td>
<td>silica mamillae indistinct and scattered</td>
</tr>
<tr>
<td>furrows broad and rounded</td>
<td>furrows broad and rounded</td>
<td>furrows broad and rounded</td>
<td>furrows very small and acute, slightly broadened</td>
<td>furrows very small and acute</td>
<td>furrows broad and rounded</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sterile shoot – micromorphology of stomata (visible as two subsidiary cells)</th>
<th>covered with short globose pilulae</th>
<th>sparsely covered with short globose pilulae</th>
<th>sparsely covered with short globose pilulae</th>
<th>densely covered with short globose pilulae</th>
<th>densely covered with short globose pilulae</th>
</tr>
</thead>
<tbody>
<tr>
<td>density of pilulae not thinning towards the margins</td>
<td>pilulae only scattered, often indistinct to nearly absent</td>
<td>pilulae only scattered, often indistinct to nearly absent</td>
<td>pilulae only scattered, often indistinct to nearly absent</td>
<td>density of pilulae not thinning towards the margins</td>
<td>density of pilulae not thinning towards the margins</td>
</tr>
<tr>
<td>surface and pilulae covered with wax crystals or wax cristals absent</td>
<td>surface and pilulae covered with wax crystals</td>
<td>surface and pilulae densely covered with wax crystals</td>
<td>surface and pilulae covered with wax crystals</td>
<td>surface and pilulae covered with wax crystals</td>
<td>surface and pilulae not covered with wax crystals</td>
</tr>
<tr>
<td>bordered by a ring of fused pilulae</td>
<td>bordered by a ring of fused pilulae</td>
<td>bordered by a ring of fused pilulae</td>
<td>bordered by a ring of fused pilulae</td>
<td>ring of fused pilulae absent to indistinct</td>
<td>ring of fused pilulae absent</td>
</tr>
<tr>
<td>pilulae at the stomatal pore globose (sometimes nearly absent)</td>
<td>pilulae at the stomatal pore absent to indistinct globose</td>
<td>pilulae at the stomatal pore absent to indistinct globose</td>
<td>pilulae at the stomatal pore globose, not stalked</td>
<td>pilulae at the stomatal pore stalked with head or globose</td>
<td>pilulae at the stomatal pore absent to indistinct globose</td>
</tr>
</tbody>
</table>
variants of that species. However detailed differences from the parents in macro- and micromorphological aspects have been listed earlier: Page (1988; 1997), Lubienski (2003; 2009; 2013), Lubienski & Dörken (2015) (for *E. ×mildeanum*) and Lubienski (2010; 2013) (for *E. ×lofotense*). Additionally both hybrids show irregular meiotic cell division resulting in aborted spores (for images see Lubienski, 2009; 2010; Lubienski & Dörken, 2015). In both taxa a remarkable discrepancy between their macro- and micromorphological characters is expressed. Traits of the macromorphological dominant parent *E. sylvaticum* are much more obscure in the micromorphology of the main shoot of both hybrids (especially regarding the highly distinctive ridge architecture, Figures 6, 7). Macro- and micromorphological features of both hybrids and their parental species

Figure 7. SEM images of the micromorphology of the shoot ridges and stomata of *Equisetum ×lofotense* (a-b), *E. ×mildeanum* (c-d), and *E. ×bowmanii* (e-f). Arrows and letters indicate ridges (r), furrows (f), mamillae (m) and pilulae (p).
are listed in Table 1.

The characteristic and highly distinct flattened and two-edged ridges bordered by two rows of silica spikes of *E. sylvaticum* are not found in *E. ×mildeanum* and *E. ×lofotense*. Both show a micromorphology more similar to their second parent, *E. pratense* and *E. arvense* respectively (Lubienski, 2010; Lubienski & Dörken, 2015). Interestingly this recessive behaviour of *E. sylvaticum* micromorphology is expressed to a similar degree in the third known *E. sylvaticum* hybrid, *E. ×bowmanii*, also a hybrid clearly indicating Wood Horsetail as one parent in its macromorphological appearance. It therefore is added in Figures 6 and 7, so that the micromorphological characters of all three known *E. sylvaticum* hybrids and their parental species can be compared directly. Because of the rarity of *E. ×lofotense*, in comparison with *E. ×mildeanum*, some further field notes of the hybrid are given, thus supplementing the data given in Lubienski (2010; 2013).

Plants on Senja were detected within the mixed stands with *E. sylvaticum*, because they displayed a characteristic asymmetric overhanging growth habit especially in the exposed roadside ditches and slopes (Figure 8a, b). In this character the hybrid is similar to *E. ×mildeanum* (Figure 8c, d).

Nevertheless in all dimensions it is a more robust and taller plant, reaching heights up to 90 cm (the tallest shoot found on Senja was 78 cm long, Figure 9a), which is never found in *E. ×mildeanum* (max. height approx. 40 cm, Figure 9b). Additionally both *E. sylvaticum* hybrids show distinctly the influence of their second parent in their nodal sheath and branch characters (Figure 10) and can be differentiated by these. An additional task in the field is to distinguish *E. ×lofotense* from its *E. sylvaticum* parent with which it frequently co-occurs. Differences between both are seen in the branches, the branch teeth, the sheath teeth, the shoot surface, and the fertile shoots in general.

The distinct branching pattern of all *E. sylvaticum* hybrids (Lubienski, 2010) seems to be a very stable and reliable character. The side branches are long, spreading, and secondarily branched only in their proximal part, so that a distal unbranched distinctly overhanging part results, giving the whole shoot a somehow irregular habit (Figure 11, 14b; see also image in Lubienski 2010). This differs from the very symmetric and storied branching pattern of *E. sylvaticum* resulting in brush-like side branch clusters (Figure 14a). In comparison with *E. sylvaticum* the teeth of the side branches of *E. ×lofotense* are not, or to a much lesser degree, spreading from the internode above (Figure 12). The sheath teeth are less reddish-brown, shorter or as long as the sheath itself (longer than the sheath in *E. sylvaticum*), only irregular adhering together, becoming darker brown, shorter and more free in the upper parts of the shoot, i.e. *E. arvense*-like. The main shoot surface is not as rough as in *E. sylvaticum*, a direct consequence of the micromorphology, where silica spikes are lacking on the less prominent ridges (Figure 7a). This difference is also clearly seen at the surface of the nodal sheath, which shows shallow ridges with a smooth surface like *E. arvense*, in contrast to prominent ridges with rough silica projections in *E. sylvaticum* (Figure 13).

The fertile shoots of *E. ×lofotense* have a very striking appearance at the Senja sites. These are very sturdy in general habit and bigger in all dimensions than the sterile shoots. Although this is a typical feature of the fertile shoots of all semidimorphic species and hybrids (e.g. *E. sylvaticum*, *E. pratense*, *E. ×mildeanum*, *E. ×bowmanii*), the fertile *E. ×lofotense* shoots from Senja are remarkable in their sturdy conifer-like habit. When viewed from above these shoots appear star-like and distinctly different from the fertile shoots of *E. sylvaticum* with which they grow intermixed. This difference results from
Figure 8. *Equisetum ×lofotense* (a & b) and *E. ×mildeanum* (c & d), showing characteristic overhanging growth habit.

Figure 9. Shoots of *Equisetum ×lofotense* (a) and *E. ×mildeanum* (b), showing differences in size (bar = approx. 10 cm).
Figure 10. Sterile shoots of *Equisetum ×lofotense* (a) and *E. ×mildeanum* (b), showing influence of the second parent species *E. arvense* in *E. ×lofotense* (a) (oblong nodal sheaths and sheath teeth, low number of teeth and side branches) and *E. pratense* in *E. ×mildeanum* (b) (short nodal sheaths and sheath teeth, high number of densely spaced teeth and side branches).

Figure 11. Sterile and fertile shoots of *Equisetum ×lofotense*, showing irregular branching pattern.

Figure 12. Sterile shoots of *Equisetum sylvaticum* (a) and *E. ×lofotense* (b), showing differences in branch teeth, spreading (a) and not spreading (b) from the internode above.
Figure 13. Nodal sheaths of sterile shoots of *Equisetum sylvaticum* (a) and *E. ×lofotense* (b), showing differences in sheath surface and length and sheath teeth colour and length.

Figure 14. Fertile shoots of *Equisetum sylvaticum* (a) and *E. ×lofotense* (b), showing differences in branching pattern.

Figure 15 *Equisetum ×lofotense*, nodal sheaths, sterile (a) and fertile (b) shoot, showing different influence of the parent species *E. arvense* and *E. sylvaticum* in the macromorphological appearance of the hybrid.
the fact that the 1st order side branches of the fertile shoots of E. ×lofotense are in general longer than in E. sylvaticum, while having 2nd order side branches shorter than in E. sylvaticum (Figure 14).

Concerning the sterile and fertile shoots of E. ×lofotense, a different influence of the parent species is also expressed in the macromorphological appearance of the hybrid, making the sterile shoots more E. arvense-like and the fertile shoots more E. sylvaticum-like (Figure 15).

**Distribution of Equisetum ×mildeanum and E. ×lofotense**

Figure 16a shows the known distribution of E. ×mildeanum for whole Norden, and it can be presumed that other populations may be found within this area.

Figure 16b shows the distribution of E. ×lofotense, at present an endemic of northwestern Norway. Further field work in this area should explore whether it is actually more frequent than currently known. The vigour of the hybrid populations on Austvågøya and Senja makes this suggestion likely.

**DISCUSSION**

All four sites of E. ×mildeanum and E. ×lofotense are very similar from a geomorphological and ecological point of view, being situated within a single fjord in the north of Senja over a distance of approximately 7.25 kilometres. The habitat is exclusively a wet, north- to east-facing mountain slope characterised by huge boulders and sparse woodland, interrupted at its base by a road only a few metres above and near to the sea. All sites and the whole area in general harbour extensive stands of the parent species E. sylvaticum, E. arvense and E. pratense.

Horsetail hybrid colonies are often found in disturbed sites such as roadside ditches or railway embankments. This can be caused either by vegetative spreading alongside

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*Figure 16. Distribution of Equisetum ×mildeanum (a) and E. ×lofotense (b) for Norden (arrows indicate two localities, the semi-filled dot indicates the historic occurrence of E. ×mildeanum at its type locality on the Isle of Rügen, Germany*
the traffic system by rhizome or shoot cuttings or through an initial hybridization event due to ecological conditions that favour cross fertilization between *Equisetum* gametophytes. Wet roadside ditches like those on Senja display perfect conditions for germination of the chlorophyllous and short-lived *Equisetum* spores and the establishment of gametophytes. These are open raw soils without higher plant or bryophyte competition, especially after roads are periodically cleared and straightened (Hauke, 1967; Page, 1967; Duckett & Duckett, 1974; Mesler & Lu, 1977; Duckett & Duckett, 1980; Duckett, 1985). Such pioneer habitats fit with the rather narrow ecological requirements of *Equisetum* gametophytes and for this reason can harbour mixed gametophyte populations, that increase the probability of crossing events between different species (Hauke, 1978; Page & Barker, 1985; Husby, 2013).

Intergametophytic fertilization and therefore hybridization in *Equisetum* is additionally favoured by the reproduction biology of the genus in general. *Equisetum* gametophytes are initially unisexual, potentially becoming bisexual under changing growing conditions (e.g. accumulation of metabolites) (Duckett, 1972; Duckett & Duckett, 1980). However such an accumulation rarely takes place under natural growing conditions (Duckett & Duckett, 1980) and therefore bisexuality is obviously rare among wild gametophyte populations (Duckett & Duckett, 1974, 1980; Duckett, 1979). Consequently, in *Equisetum*, intergametophytic fertilization is highly effective in nature, thus outbreeding processes are facilitated. This effect is further emphasized where gametophytes of different species grow together, which in addition favours hybridization events (Duckett & Duckett, 1974; Duckett, 1979).

It seems likely that *E. ×mildeanum* and *E. ×lofotense* both originated in the roadside ditches of Mefjorden and succeeded in colonising the adjacent slopes. Each of the four colonies may have started from an individual crossing event or there may have been just two events with subsequent vegetative spread. As the two sites of *E. ×lofotense* are only two kilometres apart, a single *de novo* hybridization event between the parent species to form this rare hybrid seems to be most likely, followed by vegetative spread as a result of human disturbance.

Nevertheless, the fact that all four colonies stretch over at least several hundred square metres means they are unlikely to be of very recent origin.

**ACKNOWLEDGEMENTS**

We would like to thank Torbjørn Alm (Tromsø, Norway) for help with the map of Norden and Michael Laumann and Lauretta Nejedli (Electron Microscopy Center, Department of Biology, University of Konstanz, Germany) for technical support (SEM). Patrick Acock (St. Mary Cray, Great Britain) and Heather McHaffie (Edinburgh, Great Britain) provided valuable advice concerning *E. ×bowmanii* in the British Isles.

**REFERENCES**


NEW RECORD OF THE RARE SERPOCAULON OBSCURINERVIUM D. SANÍN (POLYPODIACEAE) IN THE EASTERN CORDILLERA OF COLOMBIA

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Key words: Andean-Amazonian foothills; Caquetá; Huila; ferns; Florencia; distribution range; epipetric habit.

ABSTRACT

Serpocaulon obscurinervium is considered as a rare and threatened species, with only four exemplars registered including the type. It was recorded from Colombia and Ecuador, but most commonly reported for central southern Colombia (central west of Chocó and south of Nariño departments). Here, we expand its distribution by reporting new records for this species on both sides of the Eastern Cordillera of the Colombian Andes, specifically from the high Andean forest of Florencia, in the Caquetá department and Suaza in the Huila department. Furthermore, this is the first record of the species as an epipetric plant. Pictures of the plant and an updated distribution map are provided.

INTRODUCTION

Serpocaulon A. R. Sm. (Polypodiaceae) is a neotropical fern genus, which contains 52 taxa (Sanín 2015). These species occur mainly in humid forests (Smith et al. 2006). Colombia harbours 27 species that are distributed in the inter Andean valleys, the Chocó Region, the Sierra Nevada de Santa Marta, Oriental valley and Amazon, but most species occur in the humid forests of the Andes and the Chocó Region (Sanín in press).

Serpocaulon obscurinervium D. Sanín (Figure 1) is an Andean species that was described in 2014 from herbarium specimens deposited in collections of Colombia (CHOCO, COL, CUVC and PSO), France (P) and United States (MO and UC) (Sanín 2014). The first specimen was collected in Ecuador by R. Spruce in 1909. In 1973, other specimens were collected in Nariño at 2500m by O. Hagemann & L. Leist. The most recent collections were recorded in 1988 in the Andean zone of the Department of Chocó by P. Silverstone–Sopkin, and in La Planada Reserve, Nariño Department, by H. Herrera.
(Sanin 2014). Because the distribution of the species within Colombia was restricted to high montane forests outside of any protected area, its conservation status was considered as Endangered (EN) following the IUCN criteria (Sanín 2014). Since its first description, the species had never been found again or reported alive in nature.

During the ongoing botanic exploration of the Caquetá and Huila departments, *Serpocaulon obscurinervium* was recorded growing in sites on both sides of the Eastern Cordillera. The specimens were found along the roadside of Puente Cerro Negro in El Portico, at the western slope in a forest at 2190m and in Gabinete military station at 2050m on the border of Caquetá and Huila Departments on the Eastern slope of the Eastern Andean Cordillera. In Huila Department, it was also found on the road to Neiva, in the municipality of Suaza at 2190m (Figure 2). Two to five vouchers were collected per site and were deposited at the following herbaria: CAUP, COAH, COL, HUA, FAUC & PSO.

The exemplars from Caquetá (Puente Cerro Negro and Gabinete) are the first records of *Serpocaulon obscurinervium* as an epipetric fern. In addition, it is the first time that the species has been collected in the Caquetá and Huila departments (Murillo-P. et al. 2016), the easternmost distribution of the species with its nearest known population at

![Figure 1](image)

**Figure 1.** *Serpocaulon obscurinervium.* A: Habit of the plant, scale bar (5 cm). B: Rhizome, scale bar (1 cm). C: Rhizome bundles, scale bar (3.5 cm). D: Petiole bundles, scale bar (2 cm). E: Fiddlehead, scale bar (1 cm). F: Lamina base, scale bar (3 cm). G: Lamina apex, scale bar (3 cm). H: Immature sori, scale bar (8 cm). I: Mature sori, scale bar (8 cm). J: Pinna veins, scale bar (8 cm). All from *Sanín & Peña 6371* (COL, HUA). Photos by J.L. Peña.
250 km distance on the road of La Victoria–Monopamba, Dept. Nariño, in central south Colombia.

*Serpocaulon obscurinervium* is closely related to *S. eleutherophlebium* (Fée) A. R. Sm., a species that is distributed from Costa Rica to Ecuador and Venezuela (Moran 1995, Smith et al. 2006), and in Colombia is widely recorded in high Andean forest and

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**Figure 2.** Distribution of *Serpocaulon obscurinervium*. The black dots are previous registers of the species: 1) Cerro del Torrá, Valle del Cauca, Colombia; 2) La Planada Reserve, Nariño, Colombia; 3) road La Victoria–Monopamba, Nariño, Colombia; 4) Tungurahua, Mt. Abitagua, Ecuador. The asterisks are the new registers on the Oriental Andean Cordillera of Colombia: 5) El Portico and 6) El Gabinete from Florencia, Caquetá department and 7) From Suaza, Huila department.
The identification of these two taxa has been confused. Both species have similar long-creeping rhizomes, scales with patent apices, concolorous and pinnatisect laminae. However, *S. obscurinervium* differs from *S. eleutherophlebium* in the scales characters, being linear-lanceolate (versus subulate), dark orange (versus dark brown), larger sized 5.2-(6.3)-8.5 × 1.6-(1.7)-2.3 mm (versus 3.5-(5.1)-7.1 × 0.5-(1.1)-1.7 mm), and with longer petioles 24-(2.6)-28 cm (versus 3.4-(12)-25.5 cm), more areoles along the middle segment 19-(20)-28 rows (versus 6-(12)-21 rows), inconspicuous fertile veinlets (versus conspicuous fertile veinlets), and the presence of 2-4 celled, dispersed, catenate, terete and dark brown trichomes on the blades and racishes (versus absent trichomes) (for a complete comparison see Sanín 2014).

Even though three more populations of *Serpocaulon obscurinervium* were discovered in the Caquetá and Huila departments, it is still categorized as Endangered [EN B1ab(i, ii, iii) + B2ab(i, ii, iii)] following the IUCN criteria. Here we present more support for this, and include Criterion D to its IUCN categorization: [EN B1ab(i, ii, iii) + B2ab(i, ii, iii) + D], the latter given that the population size of *S. obscurinervium* is estimated to be fewer than 250 mature individuals.

The sparse and vicariant distribution of the species on the Andean Cordillera of Ecuador and Colombia (Sanín 2014), and the reduction of high montane forest vegetation caused by human settlements and agricultural expansion (Murcia et al. 2010), impose a permanent and increasing danger to the populations of this species, especially as all the records of this species are outwith any protected area. In addition, from five to 10 individuals were recorded in these new localities, including both mature and juvenile individuals (pers. obs.).

**MATERIAL EXAMINED**

*Serpocaulon obscurinervium*—**Caquetá**: Florencia, Via Neiva, vereda El Condor, a mano derecha de la via, a 100m del Puente Cerro Negro [1°45’19.62” N, 75°44’59.98” W], 2050m, 27 Jul 2015, *Sanín & Peña* 6371 (CAUP, PSO, COL, HUA); Florencia, Via Guadalupe, El Gabinete, a mano izquierda de la via, a 100m de la estacion militar [75°40’58.4” O, 1°52’44” N], 2700–2800m, 20 Feb 2016, *Sanín, García & Camargo-García* 6396 (COAH, COL, FAUC, HUA); Huila: Suaza, via Neiva, vereda Campo Hermoso, costado izquierdo de la via, km 40 [1°46’11.71” N, 75°46’37.52” O], 2190m, 1 Apr 2016, *Peña* 542 (COL) **Chocó**: San José del Palmar, Cerro Torrá, Filo de Cumbre, [04°48’ N, 76°29’ W], 2700–2800m, 21 Aug 1988, *Silverstone-Sopkin* 4633 (Paratypes: CHOCO, CUV, MO, UC n.v.). **Nariño**: Reserva Natural La Planada, sendero entre El Honduras y Los Horquetas, Bosque primario, 01°10’ N, 78°00’ W, 1820m, 31 Jan [no year given], *Herrera* 9302, 9193 (Paratypes: PSO, CUV, UC n.v.); Carretera La Victoria–Monopamba, [01°10’ N, 78°00’ W], 2500m, 10 Jan 1973, *Hagemann & Leist* 1898 (holotype: PSO; isotype: COL).

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REFERENCES
**POLYSTICHUM LONCHITIS** (DRYOPTERIDACEAE: PTERIDOPHYTA) A MONTANE-ALPINE FERN SPECIES, MONITORED SINCE 1978 IN THE NETHERLANDS

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Key words: *Polystichum lonchitis*, Kuinderbos, monitoring, mortality

ABSTRACT

*Polystichum lonchitis* was found in 1978 for the first time in The Netherlands on a dry ditch bank, on calcareous sand within a planted woodland (Kuinderbos). In subsequent years six other specimens were found at three other sites, in a woodland, on a scree in a chalk stone quarry and on a wall, and four of these plants became fertile. Mortality in five out of seven plants resulted from a small landslide, habitat destruction, browsing and frost damage. Long term monitoring of two plants showed the impact of rainfall and frost on vitality. No recruitment was observed in the spore shadow of adult plants.

INTRODUCTION

*Polystichum lonchitis* (L.) Roth. is a montane-alpine species, with a Holarctic distribution. In North America it is a species found mostly in boreal and subalpine regions between 0 and 3200 m (Morin, 1993). In Europe the species is found between 900 and 2100 m and rarely at lower altitude up to 300 m, except for the coastal area of Norway (Mutter et al., 1998). It is also found in Greenland, Iceland and in isolated parts of Russia but is rare in Japan (Dostál & Reichstein, 1984). Within the UK it is more widespread in the central Scottish Highlands (c. 610 – 915 m). Occasionally it descends to lower altitudes in steep upland river valleys (c. 150 m) and lower levels near the west coast, with its cool summers (Page, 1982). In 1978, *Polystichum lonchitis* was recorded for the first time in the Netherlands during a survey of 120 km of dry ditches in the Kuinderbos. Two plants were found growing at the sides of two adjacent dry ditches on the bottom of the former Zuiderzee at ca. 1 m below sea level (Bremer, 1980). Since then this small population has been monitored annually for the first 15 years and less intensely in subsequent years. After 1979 the species was found at three other sites in the Netherlands, and monitored annually near Lelystad (Smit, 1989). This paper analyses these records to try to determine whether a montane-alpine plant species is able to build up a viable lowland population.

METHODS

Two plants (plant 1 and 3, both in Flevoland, Table 1) were monitored for 24 years (Kuinderbos) and 27 years (Lelystad) with annual counting of the number of fronds and the maximum frond length and fertility at the end of the summer season or start of autumn, when frond formation had stopped and sori produced. Change in maximum frond length, change in number of fronds and the log transformed product of both parameters (always year t to year t + 1) were used as response variables to rainfall and the number of frost days. The average values in Flevoland were used for annual rainfall
**Table 1.** Some characteristics of the Dutch population of *Polystichum lonchitis*. Nr = plant number, as mentioned in the paper. First = first year of recording, Last = last year of recording, year of death. Fer = Fertility of plant: 0 = not fertile, F = fertile during period of recording, ? = not known, Sl = slope in °, Asp = aspect, Tree layer: Fr = *Fraxinus excelsior*, Qu = *Quercus robur*, Cb = *Carpinus betulus*. Mo = cause of mortality: Ls = small scale land slide, Hd = habitat destruction, Fd = frost damage, Br = browsing.

<table>
<thead>
<tr>
<th>nr</th>
<th>Location</th>
<th>First</th>
<th>Last</th>
<th>Fer</th>
<th>Leg</th>
<th>habitat</th>
<th>Substrate</th>
<th>Sl</th>
<th>Asp</th>
<th>Tree layer</th>
<th>Mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kuinderbos</td>
<td>1978</td>
<td>2014</td>
<td>F</td>
<td>P. Bremer</td>
<td>ditch side, woodland</td>
<td>fine sand</td>
<td>62</td>
<td>NE</td>
<td>Fr</td>
<td>Ls</td>
</tr>
<tr>
<td>2</td>
<td>Kuinderbos</td>
<td>1979</td>
<td>1984</td>
<td>F</td>
<td>P. Bremer</td>
<td>ditch side, woodland</td>
<td>fine sand</td>
<td>80</td>
<td>SW</td>
<td>Fr</td>
<td>Ls Fd</td>
</tr>
<tr>
<td>3</td>
<td>Lelystad</td>
<td>1986</td>
<td></td>
<td>F</td>
<td>A. Smit</td>
<td>ditch side, woodland</td>
<td>sandy clay</td>
<td>45</td>
<td>SSW</td>
<td>Fr-Qu</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Vlaardingen</td>
<td>1994</td>
<td>1994</td>
<td>0</td>
<td>B. van As</td>
<td>wall</td>
<td>cement</td>
<td>90</td>
<td>E</td>
<td>-</td>
<td>Hd</td>
</tr>
<tr>
<td>5</td>
<td>'t Rooth</td>
<td>1998</td>
<td>2011</td>
<td>?</td>
<td>J. Egelsmeer</td>
<td>quarry</td>
<td>loamy soil on chalk</td>
<td>45</td>
<td>N</td>
<td>-</td>
<td>Br, Fd</td>
</tr>
<tr>
<td>6</td>
<td>'t Rooth</td>
<td>1998</td>
<td>1998</td>
<td>0</td>
<td>J. Egelsmeer</td>
<td>quarry</td>
<td>loamy soil on chalk</td>
<td>&gt;30</td>
<td>N</td>
<td>-</td>
<td>Hd</td>
</tr>
<tr>
<td>7</td>
<td>Kuinderbos</td>
<td>2011</td>
<td></td>
<td>F</td>
<td>P. Bremer</td>
<td>ditch side, woodland</td>
<td>fine sand</td>
<td>40</td>
<td>SW</td>
<td>Fr,Cb</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Correlation matrix with doses and effect parameters with correlation (r) and significance (p) shown (and significant levels $p < 0.05$ marked with asterix *). ▲ n = change in number of fronds between year t and t + 1, ▲ Fm = change in maximum frond length between year t and t + 1

### Plant 1, Kuinderbos

<table>
<thead>
<tr>
<th></th>
<th>▲ n</th>
<th>▲ Fm</th>
<th>▲ (nxFr)</th>
<th>log ▲ (nxFr)</th>
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</thead>
<tbody>
<tr>
<td>Rainfall year t</td>
<td>r</td>
<td>0.1</td>
<td>0.01</td>
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</tr>
<tr>
<td></td>
<td>p</td>
<td>0.67</td>
<td>0.96</td>
<td>0.75</td>
</tr>
<tr>
<td>Rainfall April year t+1</td>
<td>r</td>
<td>0.33</td>
<td>0.2</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.17</td>
<td>0.41</td>
<td>0.12</td>
</tr>
<tr>
<td>Rainfall May-July year t+1</td>
<td>r</td>
<td>0.62</td>
<td>0.17</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.01*</td>
<td>0.49</td>
<td>0.07</td>
</tr>
<tr>
<td>Rainfall May-July year t</td>
<td>r</td>
<td>-0.14</td>
<td>0.19</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.95</td>
<td>0.43</td>
<td>0.58</td>
</tr>
<tr>
<td>Number of frost days</td>
<td>r</td>
<td>-0.25</td>
<td>-0.4</td>
<td>-0.32</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.3</td>
<td>0.09</td>
<td>0.18</td>
</tr>
</tbody>
</table>

### Plant 3, Lelystad

<table>
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<tr>
<th></th>
<th>▲ n</th>
<th>▲ Fm</th>
<th>▲ (nxFr)</th>
<th>log ▲ (nxFr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainfall year t</td>
<td>r</td>
<td>-0.43</td>
<td>0.02</td>
<td>-0.36</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.03*</td>
<td>0.93</td>
<td>0.07</td>
</tr>
<tr>
<td>Rainfall April year t+1</td>
<td>r</td>
<td>0.2</td>
<td>0.08</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.32</td>
<td>0.7</td>
<td>0.44</td>
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<tr>
<td>Rainfall May-July year t+1</td>
<td>r</td>
<td>0.09</td>
<td>0.42</td>
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</tr>
<tr>
<td></td>
<td>p</td>
<td>0.66</td>
<td>0.03*</td>
<td>0.29</td>
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<tr>
<td>Rainfall May-July year t</td>
<td>r</td>
<td>-0.38</td>
<td>-0.11</td>
<td>-0.32</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.06</td>
<td>0.58</td>
<td>0.11</td>
</tr>
<tr>
<td>Number of frost days</td>
<td>r</td>
<td>-0.12</td>
<td>-0.45</td>
<td>-0.28</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.56</td>
<td>0.02*</td>
<td>0.17</td>
</tr>
</tbody>
</table>
in year $t$, rainfall in April in year $t + 1$, rainfall in May-July in year $t$ and $t + 1$, as we expected drought in spring or early summer to play a role. The number of frost days per winter (maximum day temperature below 0°C) was used as indicator for the low temperature impact in winters regional data used (www.knmi.nl). These were the same parameters as used by Bremer & Jongejans (2010) in their study on the demography of *Asplenium scolopendrium* L. SPSS 10.1 was used to calculate correlations. Per location data about habitat, substrate, inclination, aspect and composition of the tree layer and herb layer were collected in order to classify the sites and vegetation.

**RESULTS**

*Habitat and vegetation*

Over a period of 39 years seven plants were found at the former sea floor in Flevoland, in the city of Vlaardingen and in a chalk quarry in the southern part of the Netherlands (Figure 1). The Kuinderbos plants grew in dry ditch sides within plantations of *Fraxinus excelsior* L., accompanied by mosses of the *Fissidentietum taxifolii* bryophyte community (Bremer, 1999) and fern species such as *Asplenium scolopendrium* and *Polystichum aculeatum* (L.) Roth. Near Lelystad the plant grew under a canopy of *Fraxinus excelsior* L., with *Oxyrhyynchium hians* (Hedw.) Loeske, *Eurhynchium striatum* (Hedw.) Schimp. and *Geum urbanum* L. The non-native *Potentilla indica* (Andrews) Wolf had invaded the site. This vegetation is related to the *Fraxino-Ulmetum*, a woodland community confined to clayey soils in the Netherlands. Both ‘t Rooth plants were found growing among flint stones deposited at a chalk quarry. The vegetation at this site was characterized by mass growth of *Asplenium trichomanes* L., *Gymnocarpium robertianum* (Hoffm.) Newman and *Asplenium scolopendrium*. In the bryophyte layer *Encalypta streptocarpa* Hedw. was a prominent species. Even *Asplenium ruta-muraria* L. was growing among flint stones, one of the few examples where it shows terrestrial growth in the Netherlands. The vegetation belongs to the *Asplenietum ruto-murario-trichomanes*, a community strongly linked to wall habitats (Segal, 1969) (OV39, British National Vegetation Classification, Rodwell, 1992). The Vlaardingen site was by a wall, which was destroyed two years after the discovery of *Polystichum lonchitis*. The vegetation belonged to the *Filici-Saginetum*, a wall community with *Dryopteris filix-mas* (L.) Schott and *Asplenium scolopendrium*. All sites have a high pH in common and are related to gradients. There was no difference in aspect comparing N-NE with S-SW ($\chi^2 = 0.14 \text{ ns}$) (Table 1).

*Distribution and trend*

The number of plants varied from one to two plants per location. The small population in the Netherlands showed a positive trend until 2010 with decline in recent years (Figure 2). Most plants were found as adults; the Vlaardingen plant was a juvenile/subadult plant, rather small in size, as is often the case with wall dwelling fern species. Five plants died because of landslip, habitat destruction, frost damage and browsing or because of a combination of these factors (Table 1). At Vlaardingen, prior to wall destruction, the plant was conserved and planted in a garden but was subsequently lost through theft within two years. At ‘t Rooth quarry one of the sites was destroyed in favour of pond digging because of a threatened toad species. The other plant died as a result of browsing in combination with frost damage (Bremer & Egelsmeer, 2015). In the Kuinderbos, one plant slipped to the bottom of the dry ditch after a small scale land slip (initiated by heavy rain fall) and drowned as the normally dry ditches contain water (0.1 – 0.3 m depth) in
winter time. Four out of seven plants became adults, producing spores nearly every year. No recruitment took place in the spore shadow.

**The impact of severe winters and rainfall**
The first plant found in the Netherlands (Kuinderbos, plant number 1) has been monitored since 1979. The maximum frond length (Lm) increased exponentially between 1979 and 1985. During the severe winter of 1984 - 1985 it was not harmed at all; number of fronds and maximum frond length increased, despite 22 frost days during that winter. The winter 1985 - 1986 was also long and cold, with the same number of frost days as in the

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**Figure 1.** Distribution of *Polystichum lonchitis* in the Netherlands with number of plants per location (small dot = 1 plant, larger dot = 2 plants). The Kuinderbos is at 52°46’ N 5° 49’ E.
Figure 2. The number of plants during half a century in The Netherlands since 1978.

Figure 3. The change in maximum frond length between year $t$ and year $t+1$ related to the number of frost days per winter between year $t$ and year $t+1$ in the Lelystad plant. (plant number 3).
preceding winter. The plant produced fewer and smaller fronds and the plant was sterile for one year. In subsequent years the plants recovered. Over the whole period the vitality parameters in plant 1 did not correlate significantly with the number of frost days (Table 2). The Lelystad plant however showed a significant negative correlation between change in frond length and the number of frost days per winter (n = 26, r² = 0.18 p = 0.03) (Figure 3).

When analysing rainfall and growth a positive correlation was found in Kuinderbos plant 1 between the change in number of fronds and the May-July rainfall in the year t + 1 (n = 19, r² = 0.38 p = 0.01) (Figure 4) and in plant 3 (Lelystad, Figure 5) between the change in frond length and May-July rainfall in year t + 1 (n = 26, r² = 0.18, p = 0.03). The log transformed product of the change in maximum frond length x change in number of fronds showed a strong correlation in plant 1 (n = 19, r² = 0.36, p = 0.01). The annual rainfall had a negative impact on plant 3 (n = 26, r² = -0.16, p = 0.03).

DISCUSSION

The site in ’t Rooth is an artificial flint stone scree in a chalk quarry, which resembles the more natural habitat. In Scotland stable boulder scree slopes may contain a rich fern vegetation with Asplenium viride Huds., Cystopteris fragilis (L.) Bernh and Dryopters expansa (C.Presl) Fraser-Jenk. & Jermy (Page, 1982). On ledges it grows among low-growing flowering plants in the Dryas octopetala – Silene acaulis ledge community (Rodwell, 1992). Probably the dry ditches under a canopy can at best be compared with the gryke habitat - fissures in exposed limestone habitat that are usually 1 – 2 m deep (Lake et al., 2015). From the syntaxonomical point of view the woodland sites have

**Figure 4.** The increase of number fronds (year t and year t + 1) related to the rainfall in the period May – July in year t + 1 in the Kuinderbos plant (plant number 1).
**Figure 5.** The Lelystad plant (plant number 3) accompanied by *Potentilla indica* in 2007, 21 years after the first record (photo P. Bremer)

**Figure 6.** The plant recorded in the Kuinderbos in 2015 (plant number 7), first discovered four years after the first record (photo P. Bremer).
much in common with, the *Fraxinus excelsior*-Acer campestre-Mercurialis perennis woodland and in particular the Geranium robertianum-subcommunity, where sometimes it is found under a miniaturised version of this woodland in the Carboniferous Limestone grykes in the Yorkshire Dales (Rodwell, 1998).

The montane-alpine species was not expected to colonise a former sea floor. In neighbouring countries lowland sites for this species are not known (e.g. www.floraweb.de). In the year *Polystichum lonchitis* was recorded for the first time, *Asplenium viride* Huds. was also found in the Kuinderbos (Bremer, 1981), and some montane bryophytes had been found on dry ditch sides on the former seafloor [e.g. *Distichium inclinatum* (Hedw.) Bruch & Schimp. (Bremer & Ott, 1979) and fungi (e.g. *Suillus tridentinus* (Bres.) Singer]. The Kuinderbos is the only known site within the Netherlands with a small viable population of the montane *Knautia dipsacifolia* Kreutzer (Meijer & Bremer, 1986). All these species came by long distance dispersal by wind, with *Knautia dipsacifolia* being the exception (probably with humans as vector).

Page (1982) states that the species occasionally descends to lower altitudes near the extreme west coasts of Scotland because of its cool summers. At open sites in the Netherlands the preferred N-E aspect was confirmed. In the Dutch woodlands it thrives with a SW preference, although in April, without the protection of a sprouted canopy, it can be warm in these ditches. A high temperature is probably compensated by evaporation of water, as dry ditches always contain water in this time of the year.

There is a continuous flow of diaspores from mountainous areas spreading over the Netherlands, implicating that under optimal conditions plants can establish from spores. But the chance of establishment is dependent on the extent of an area which is suitable for colonisation, spore density, spore longevity (sporebank) and the ability of intragametophytic selfing (Klekowski 1979). In the Kuinderbos 300 ha of calcareous deposits on peat drained by more than 100 km of dry ditches provided a large area, with colonization by numerous rare fern species (Bremer 2007, de Groot 2012). De Groot (2012) showed in four species [*Asplenium scolopendrium*, *A. trichomanes*, *Polystichum aculeatum*, *P. setiferum* (Forssk.) Woynar] a very high selfing capacity in both diploid and polyploid species. Single-spore establishment following long-distance dispersal may have resulted in selection for selfing genotypes. There was no recruitment from the adult plants of diploid *Polystichum lonchitis*, at woodland sites. This might indicate that selfing is less likely in a second generation and that the species depends on intergametophytic crossing. There is a very low chance that spores from other genotypes are able to establish within the spore shadow of an adult plant because of the large distance to British and Central European populations. *Polystichum lonchitis* does have Arbuscular Mycorrhiza (Harley & Harley, 1987), but it is unknown whether fungi play a crucial role in establishment. With only two plants left, stochastic extinction might occur. In the Netherlands Atlantic species such as *Asplenium scolopendrium*, *Dryopteris borreri* Newman, *D. affinis* (Lowe) Fraser-Jenk. and *Polystichum setiferum* are increasing in number and becoming more common. Global warming with more precipitation in summer and higher temperatures in winter plays a role. With its fluctuating small population *Polystichum lonchitis* remains a threatened species on the Dutch Red List (Sparrius et al., 2012). Landslip within dry ditches, frost damage, habitat destruction and browsing played a role in the mortality of five out of seven plants. One plant lived for ca. 45 years. Northridge writes that one of five Fermanagh plants (N. Ireland) was found as a mature plant in 1979 and was still in excellent condition 32 years later (www.habitas.org.uk/priority/species). Although plants in the UK seem to be long-lived,
no demographic studies are known for this species. In the Netherlands frost damage played a role in vitality and survival. One would expect a montane-alpine species to more adapted to low temperatures, yet effects of severe winters were observed in four of seven plants, with plants becoming smaller, with lower number of fronds per plant, smaller fronds per plant or lower fertility. The same was observed in *Asplenium scolopendrium*, but this species has an Atlantic distribution, and is susceptible to frost. In this species snow cover can protect plants from heavy damage (Bremer & Jongejans, 2010). The more leathery fronds of *Polystichum lonchitis* (see Figure 6) seem to be more protective than those of *Asplenium scolopendrium*. However, our data suggest that snow cover might be crucial, a suggestion also made by Dostál & Reichstein (1984), indicating growth in snow-rich habitats. Mutter *et al.* (1998) did not find a role of extensive snow cover. For many subalpine species snow cover is a prerequisite for survival during winters (Körner & Larcher, 1988) as snow protects from low temperatures. Buried beneath a layer of snow plants are exposed to milder temperatures close to 0°C. Without snow cover desiccation plays a role or excessive natural radiation may result in frost damage. As old fronds might serve as nutrient storage organs frost damage impacts the new generation of fronds (Landi *et al.*, 2014).

Within the Netherlands long term monitoring of a complete plant population is exceptional. While in other groups, e.g. breeding birds, annual monitoring is commonplace, it is much more difficult in plants. Individuals in many species are more difficult to identify [e.g. *Pteridium aquilinum* (L.) Kuhn] and a complete survey of a large area time consuming. Plant species involved mostly have a restricted number of populations, as is the case in *Woodsia ilvensis* (L.) R.Br. (McHaffie, 2010) and more long term monitoring in ferns is needed (Sharpe & Mehlteeter, 2010).

**ACKNOWLEDGEMENTS**

We thank Ben van As, Olaf Opdenkamp and Jan Egelsmeer for their data and Klaus Mehltreter for reviewing the paper.

**REFERENCES**


BOOK REVIEW


This new checklist of Indian pteridophytes is a welcome addition to our knowledge of the diversity of Asian ferns and lycophytes, and adds to the recent publications on pteridophytes from China (Wu et al. 2013) and Nepal (Fraser-Jenkins et al. 2015). This volume covers Indian Lycopodiaceae, Selaginellaceae, Isoetaceae, Equisetaceae and 19 fern families (Psilotaceae to Thelypteridaceae).

In addition to the detailed checklist that forms the bulk of the volume, there is a short introduction that sets the work in context and a summary of the numbers of families, genera and species that are to be included in both Parts 1 and 2. The checklist itself is followed by a series of 37 colour images, that range from some significant herbarium specimens to many field photographs of living plants, either in close-up or as habitat shots, and all of these providing a beautiful visual introduction to the vast range of diversity among Indian pteridophytes.

In generating the information for this detailed checklist, the authors have combined an extensive literature review with detailed studies of herbarium specimens and distributions from both public and private herbaria from India and elsewhere, and sound field knowledge. Critical comparison has been made with records from adjacent areas, and this has led to the inclusion of 34 families, 130 genera, 1107 species and 50 additional subspecies for the whole of the checklist, of which 23 families, 59 genera, 572 species and 32 additional subspecies are covered in Part 1. In addition, for each of the genera, the authors provide lists of excluded taxa; this is very valuable in clarifying the presence/absence of taxa for which past records have proved to be erroneous, often the result of confusion of nomenclature.

The new checklist has a traditional taxonomic framework; the authors have considered recent taxonomic approaches built on phylogenetic analysis of DNA sequence data but have given greater weight to morphological classification. One of the strengths of this checklist is in the detailed citations for families, genera and species, including citation of types and synonyms, and information on distribution within and outwith India. For many of the species there are extensive notes to clarify previous reports, to point out mis-identified material or instances where there has been mis-application of names, sometimes in attempted lectotypification. This detailed clarification of the names used (and those excluded) in the checklist is invaluable for other researchers, and comparison can be made with, for example, fern taxa from elsewhere in Asia.

In a taxonomic work of this detail it is not surprising to find that some 55 nomenclatural changes have been made, for example, *Mickelopteris* Fraser-Jenk., gen. nov. in the family Pteridaceae, and these changes can be identified readily as they are listed in bold in the taxonomically arranged index of accepted and excluded taxa at the end of Part 1.

The authors are to be congratulated on a very welcome addition to current knowledge of the rich diversity of pteridophytes in Asia.
REFERENCES

M. Gibby
INSTRUCTIONS FOR AUTHORS

PAPERS should not usually exceed 20 printed pages and are generally expected to be considerably shorter. As well as reports of original research, review articles and short notes, e.g. new records, are encouraged. The senior author should supply an email address to facilitate correspondence.

REVIEWS should cover recent developments in any aspect of research on ferns and lycopods. This can include unpublished research by the author, and/or the possible direction of future research. They should be aimed at the general reader who may not be an expert in the chosen topic. Proposals for a review should be discussed first with the review editors.

MANUSCRIPTS should be submitted in English (British) in electronic format in 10-point Times New Roman font and double spaced. Electronic versions of text and tables should be compatible with WORD, with figures as pdf or jpg files, and sent as email attachments or CDroms. All manuscripts will be refereed.

THE TITLE should reflect the content of the paper and be in BOLD CAPITALS (11-point) and centrally aligned. Generic and specific names should be in italics and any title containing a generic or specific name should be followed by the family name in brackets e.g.

TRICHOMANES SPECIOSUM (HYMENOPHYLLACEAE)
IN SOUTHERN SPAIN

AUTHOR ABBREVIATIONS should follow Pichi Sermolli’s (1996) Authors of scientific names in Pteridophyta, Royal Botanic Gardens, Kew.

MAIN HEADINGS: should be in BOLD CAPITALS (10-point) and centrally aligned.

SUBSIDIARY HEADINGS: should be in bold, the first letter of each word in capitals, the rest in lower case and left-aligned.

AUTHORS’ NAMES AND FULL ADDRESSES (including email address): follow the title and are centrally aligned.

KEY WORDS: up to ten.

ABSTRACT: should reflect the content of the paper.

FIGURES: there is no distinction between photographs and line drawings in numbering. All should be presented in a form ready for reproduction, colour, TIF format and 300 dpi (please contact editor with queries), with a scale bar where appropriate. Lettering or numbers (Arabic) should be in the bottom left using uppercase Times Roman. Figure captions should be on a separate sheet.

TABLES: can be printed in either portrait or landscape format. Authors should consider this when preparing tables. Authors should ensure that tables fit the printed page size in a legible form.

PAGESIZE: The Gazette has a useable page size of 123 × 204 mm. This can be used in portrait or landscape mode but any supplied artwork should not be larger than this.

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CHECKLISTS: follow the format of Baksh-Comeau, Fern Gaz. 16(1, 2): 11- 122.

REFERENCES: follow the style of a recent issue of The Fern Gazette, e.g.:-


JOURNAL ABBREVIATIONS: should follow Botanico Periodicum Huntianum & Supplements.

Alterations from the original text at proof stage will be charged for unless they are minor points of detail. A pdf will be provided free to the senior author.
REVIEW
SILURIAN-DEVONIAN ORIGINS OF FERNS AND LYCOPHYTES - WHAT WE KNOW, WHAT WE NEED TO FIND OUT

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ABSTRACT
This represents a synopsis of current knowledge of the Siluro-Devonian fossil record concerning evolution of lycophytes and ferns. This is the time period when several taxa or lineages at different grades of organisation existed that may be informative about the origins of these groups or structures typical of these groups. Considerable new data, including earlier first appearances of lineages and plant structures, new data about Siluro-Devonian lycopsids or basal euphyllophytes, and new whole plant reconstructions of small to tree-size plants in both lineages, have been published in recent years. It is not possible to be completely comprehensive, but the taxa discussed are either central to established ideas, or provide new information in relation to phylogenetic relationships and evolutionary trends. It remains difficult to trace the phylogenetic relationships of early plants relative to extant lineages. New data are reviewed which may be important in reassessing homology of characters and/or hypotheses of such relationships or in determining which taxa to exclude. Including fossils in estimates of relationships of these major lineages of plants will provide a more accurate and comprehensive understanding of the past history of seedless vascular plants.

INTRODUCTION
A consensus classification of extant seedless vascular plants by PPG1 (2016), reflecting molecular and some morphological phylogenies (Smith et al., 2006b; Kenrick & Crane, 1997; Pryer et al., 2001; 2009; Schneider et al., 2009; and others) recognises Lycopodiopsida (with three families and collectively referred to as lycophytes) and a grade “euphyllophytes” which consists of two clades - seed plants and Polypodiopsida (Figure 1). The latter includes four subclasses, Equisetidae, Ophioglossidae, Marattiidae and Polypodiidae- all referred to as ferns.

Lycopodiopsida appears to represent a monophyletic group defined by both molecular and morphological characters that diverged at least in the mid-Silurian if not earlier (Kenrick & Crane, 1997; Nickrent et al., 2000; Renzaglia et al. 2000; Pryer et al., 2001; 2004; Qiu et al., 2007; Li et al., 2014) but see Hao and Xue (2013) for a different interpretation. Relationships of several putative stem lycophytes such as some Cooksonia pertoni, Aberlemnia (formerly Cooksonia) caledonica, Renalia and several others, some within more derived extinct clades where critical characters are missing, are poorly resolved (Kenrick & Crane, 1997; Gensel & Berry, 2001; Hao & Xue, 2013). Additional data and more whole plant reconstructions of several Upper Silurian or Lower Devonian polysporangiophytes are needed to address these issues.
The term “fern” or “fern-like” now encompasses the majority of extant and extinct non-lycophytic seed-free vascular plants and several extinct “stem” euphyllophytes (trimerophytes), those with “peripheral permanent protoxylems” such as cladophyllids, iridopterids, and in some cases, rhacophytes and stauropterids (Kenrick & Crane, 1997; Rothwell & Stockey, 2008; Rothwell & Nixon, 2006; Berry & Stein 2000; Berry & Wang 2006). This results in a broad view of what represents a “fern”. This is tied up in considerations of evolution of the so-called megaphyllous leaf via modification of branch systems. However it is clear such leaves evolved many times and possibly from different parts of lateral branch systems. Furthermore, using criteria such as presence/absence of a leaf gap and simple vs branched venation to distinguish microphyll vs megaphyll is not clear-cut. For a more complete discussion of these issues, see Tomescu (2009) and later sections of this paper. The term megaphyll is intentionally not used in this paper.

Fossils were intentionally excluded from the PPG1 classification, because “the phylogenetic affinities of most extinct plants are rather unclear” (p.565). While a practical starting point for names, it, along with molecular-derived phylogenies of only extant forms, implies misleading relationships by ignoring some of the major discoveries in the fossil record of the past several decades. For example, stating that ferns are the closest living relative to seed plants is correct when only living plants are considered, but ignores the existence of extinct plants with unique combinations of characters representing one or more now-extinct lineages, notably progymnosperms, but also others, indicating a more distant relationship between seed plants and ferns. While molecular-based phylogenies may suggest a close relationship among the extant subclasses, incorporation of fossil and morphological data (Figure 2) suggest they are not monophyletic (Rothwell & Nixon, 2006).

The fossil record, while still incomplete, presently suggests each Polypodiopsida subclass (PPG1 2016) differs in time of first appearance and possibly in origin. First appearances of undoubted extinct members of each clade are as follows: equisetophytes -the Late Devonian, filicaleans (extinct Polypodiidae) - Early Carboniferous, Marattiidae -Late Carboniferous, and Ophioglossidae -Cenozoic. Thus time gaps exist between the later appearing taxa/lineages and putative early representatives of those clades. Finding morphologically based synapomorphies to unite extant and extinct putative progenitor

Figure 1. A simplified phylogeny of ferns and lycophytes, showing only the major lineages, modified from Figure 1 of the Pteridophyte Phylogeny Group 1 (PPG1) study.
or sister lineages is difficult. Characters that have been commonly employed among the Devonian age euphyllophytes are 1) branching systems, from which leaves are believed to have evolved and part or all of which are often interpreted as “leaf-equivalents”, 2) stelar anatomy and 3) protoxylem location. Sometimes interpretations are retrospective, with extant leaf morphologies influencing ideas of what constitutes a leaf-equivalent. Not everyone agrees on which part or parts of a branching system constitutes a leaf-equivalent in each lineage, and lastly, for some, all leaves are considered homologous (ignoring the fossil record) rather than evaluating the fossil plants within the context of their time and environment (Berry & Stein, 2000) and taking a more “bottom-up outlook” (Rothwell & Stockey, 2008) of extinct plants relative to extant ones. Recently acquired fossil data and whole-plant reconstructions, coupled with new developmental information or tests of certain character distributions, may improve assessment of homology of structures and character state transformations and ultimately phylogenetic relationships (Tomescu, 2009; Corvez et al., 2012 and authors cited therein). Additional work on understanding variation in anatomy also is needed. As our understanding of certain plant types improve, the fossil record may also indicate which possible progenitor candidates to preclude.

WHAT DOES THE FOSSIL RECORD CURRENTLY TELL US?

Figure 2. From Rothwell and Nixon, 2006 showing a tree based on a combination of fossil and extant plant morphological and molecular characters. Inclusion of fossils causes changes in postulated relationships of so-called “fern-like plants” relative to filicalean (=Polypodiidae) ferns. Terminology is that used by Rothwell and Nixon (2006). Lignophytes refer to progymnosperms and seed plants. OP= ophioglossid ferns and psilotophytes. Palaeozoic leptosporangiate ferns include taxa such as Metaclepsydropsis, Ankyropteris, Psalixochlaena, Botryopteris, and others.
Figure 3. A stratigraphic chart showing major events in plant evolution or first appearances of certain plant groups or plant structures, spanning Ordovician to Tournaisian.
GENERALITIES

1) Everything happened earlier (Figure 3): Dispersed cryptospores and trilete spores provide evidence for Kingdom Plantae in the early Middle Ordovician to late Middle Ordovician respectively (Rubenstein et al., 2014; Steemans et al., 2009; 2010; Wellman et al., 2008; 2015; Kenrick et al., 2012; Edwards et al., 2014, Edwards & Kenrick, 2015, and references cited therein). Cryptospore types and diversity increase until the earliest Devonian, trilete spores diversify from Silurian on. Earliest forms occur in Gondwanan regions, suggesting radiation of cryptospore producers and basal polysporangiates from Gondwanan microcontinents towards Laurussia (Wellman et al., 2015; Steemans et al., 2010). The absence of megafossils during this time span may partly reflect the lack of known terrestrial sediments in the Ordovician and Silurian (Kenrick et al., 2012; Wellman et al., 2015). Some tiny Upper Silurian to Lower Devonian plants produce cryptospores, termed cryptophytes (Edwards et al., 2014), and others produce trilete spores, some possibly representing precursors of a bryophyte or polysporangiophyte grade (Edwards et al., 2014). Unfortunately, only some dispersed spores can be tied to parent plants, which limits establishing evolutionary/phylogenetic links between cryptophyte, bryophyte or polysporangiate lineages, and thus to either lycophytes or fern-like precursors (Edwards et al., 2014).

2) Innovations: The Silurian to Middle Devonian vascular plant diversification demonstrates a greater increase in plant diversity, size and complexity than previously known. Silurian to Early Carboniferous taxa exhibit a trend from considerable morphological/anatomical plasticity to more predictable body plans or architecture in younger, more derived forms (Bateman et al., 1998). Recent research suggests a transition from apparently rhizomatous forms to upright trees by the Middle to late Devonian among lycopsids and euphyllophytes (Figure 3), tree size ranging from 3 to at least 8m tall and of varying trunk diameters (4 to ~20 cm wide). Some formed new vegetation associations, most notably early forests. In euphyllophytes, no laminate leaves and no, or limited, secondary tissues exist (thus having alternative means of mechanical support) (Stein et al., 2007; 2012; Giesen & Berry, 2013; Berry & Marshall, 2015). Archaeopteridalean forests of large trees with abundant secondary tissues dominated some regions in the Late Devonian. Evidence of early evolution of leaves and roots occurs from Late Silurian on, as discussed in greater detail later.

Another major innovation, aligned xylem meeting criteria for secondary xylem and interpreted as an early type of vascular cambium, is demonstrated by the Pragian Amoricaphyton (Gerrienne et al., 2011; Strullu-Derrien et al., 2013; 2014; Gerrienne & Gensel, 2015) and Emsian plants from eastern Canada (Gerrienne et al., 2011; Hoffman & Tomescu, 2013; Gensel in prep.). It is unknown if these possessed uni- or bifacial cambia. That, and lack of fertile remains, renders affinities and potential relevance as to how vascular cambia evolved or to fern and lycophyte vs. lignophyte evolution unclear. Uni- and bifacial vascular cambia originated more than once, as they are known in several extinct lineages from Middle Devonian on, including lycopsids (uni), progymnosperms (bi), early seed plants (bi), and possibly cladoxyls (unclear if secondary xylem or primary, aligned metaxylem).

Heterospory is established by the Middle Devonian in lycopsids and some euphyllophytes (the incipiently heterosporous Lower Devonian Chaleuria; the Upper Devonian archaeopterids and some Lower Carboniferous stauropterids). The Middle Devonian putative ovule precursor, Runcaria (Gerrienne et al., 2004) and numerous pre-
ovules of Late Devonian (Famennian) age indicate an initial radiation of early seed plants at nearly the same time as heterosporous euphyllophytes occurred (Prestianni & Gerrienne, 2010), but prior to appearance of all fern clades except Equisitidae.

LYCOPHYTIE EVOLUTION

The presence of Late Wenlock Cooksonia sp. from Avalonia (Edwards & Feehan, 1980), and the Upper Silurian (Ludlow) occurrences of several zosterophylls, putative stem lycophytes, and the lycopsid Baragwanathia from Bathurst Island, Canada and/or Australia (Kotyk et al., 2004; Rickards, 2000) indicate several grades and clades of rhizomatous, homosporous lycophytes at that time and an early origin for lycophytes. Zosterophylls, stem lycopsids, pre-lycopsids and lycopsids occur widely in the Early Devonian. Strobilate probable zosterophylls such as Distichophyton and plants similar to Bathurstia first appear in the Late Silurian and similar forms are diverse in the Early Devonian.

Zosterophyllopsids are leafless but exhibit similar anatomy and sporangial morphology and dehiscence as lycopsids. Kaulangiophyton, lacking vascularized leaves, Asteroxylon with transfusion tissue in “leaves” (Hueber, 1992), and fertile Drepanophycus spp. all lack association of sporangia with leaves, and sometimes are considered pre-lycopsids (Gensel & Andrews, 1984; Gensel & Berry, 2001). Leaves of differing morphology occur in the Siluro-Devonian Baragwanathia, in the pre-lycopsid Drepanophycus, the putative lycopsid Zhenglia (Hao et al., 2006) and in some Pragian taxa of uncertain affinity from China such as Adoketophyton (Li & Edwards, 1992; Hao et al., 2003). Mid-Upper Devonian and younger lycopsids exhibit mostly laminate univeined lycophylls, some of which are much-divided (Protolepidodendrales) or have laciniate to fimbriate margins (e.g. Longostachys, Wuxia). Ideas about how lycophylls may have evolved vary but are unresolved, ranging from change via progressive vascularization of enations, sterilization of sporangia (Crane & Kenrick, 1997), or by reduction of branch systems (reviewed in Tomescu, 2009).

Growth habit among these basal groups (stem taxa, zosterophylls, pre-lycopsids, early lycopsids) is either rhizomatous or tufted (Gensel et al., 2001; Raven & Edwards, 2001; Gensel & Berry, 2001; Hao et al., 2010). Rooting structures appear to have arisen more than once, ranging from downward trending axes (some Zosterophyllum spp. of earliest to early Devonian age), to part of a bifurcation of a rhizome (H or K branching), with one half being stem-like and bearing enations or leaves, the other portion lacking those structures and appearing root-like (Bathurstia, Drepanophycus - Gensel et al., 2001). An un-named lycopsid from the Lower Devonian of Wyoming differs in that the initial K-branch produces a “root-bearing axis”, from which dichotomizing root-like structures depart (Matsunaga & Tomescu, 2016). These authors suggest rooting structures in lycophytes are de novo structures rather than modified stems. Hetherington and Dolan (2017) note commonalities among lycopsid root morphology as well as disparities, recognizing multiple types of structures that bear the branched roots. None of the early forms are anatomically preserved, thus features that distinguish roots from stems (root cap, root hairs, etc) in younger plants are lacking (Raven & Edwards, 2001; Gensel et al., 2001; Kenrick & Strullu-Derrien, 2014).

The order Protolepidodendrales, plants characterized by much divided or hastate leaves, epiphyllous oval to possibly round sporangia, and faintly to deeply lobed protosteles, is established by the Emsian (Leclercqia spp.) and taxa are much more diverse and widespread in the Middle and early Late Devonian (Gensel & Berry, 2001).
Most specimens may represent more distal or aerial branches and presumably were rhizomatous or in one case, climbing (L. uncinata Xu et al., 2011). Oval to elongate sporangia attach to the upper surface of sporophylls identical to vegetative leaves. Leclercgia complexa from the Middle Devonian Gilboa, NY locality is known to be ligulate; L. complexa, L. cf. complexa and L. uncinata yield spores showing them to be homosporous (Grierson & Bonamo, 1979; Richardson et al., 1993; Gensel & Kasper, 2005; Gensel & Albright, 2006; Wellman et al., 2009; Xu et al., 2011). Spores and ligules are unknown for other genera. Rooting structures, which might aid in better understanding of relationships, are unknown in all taxa. These plants are often considered to be putative precursors to the ligulate heterosporous clades Selaginellales and Isoetales, but better understanding of all plant parts would allow a more precise assessment of their relationship to other extinct and extant clades. As noted below, additional putatively transitional forms exist, although many are incompletely preserved.

A major change in growth habit and reproduction occurred in the late Mid Devonian, as shown by the bipolar/pseudobipolar, approximately half metre tall tree-like taxa Longostachys and Chamaedendron (Cai & Chen, 1996; Schweitzer & Li, 1996), both of uncertain affinity, in China. In situ (and some isolated) lycopsid stems from the upper Middle/lower Upper Devonian of Svalbard, consisting of approximately 1m tall, incomplete trunk pieces, and identified as Protolepidodendropsis pulchra, are interpreted as a paleotropical forest (present in a low paleolatitude) by Berry and Marshall (2015). Sublepidodendron, especially S. songziense Wang et al. 2003, from the Late Devonian of China, was at least a metre tall. A smaller, very slender, upright and unbranched Late Devonian lycopsid, Clevelandodendron ohioensis (Chitaly & Pigg, 1996), exhibits a lobed/branched base and a bisporangiate strobilus reminiscent of some lepidodendrids. Wider stemmed lycopsids such as Cyclostigma, Lepidosigillaria, and the unidentified tree lycopsid from Gilboa, NY (Haughton, 1859; Chaloner, 1968; Kräusel & Weyland, 1949; Stein et al., 2012) also occur in late Mid to early Late Devonian in Laurussia. These taxa not only differ in size (length, width), but also in branching (presence/absence, type), leaf base patterns, leaves, rooting and reproductive structures. They also clearly demonstrate the existence of upright lycopsids of varying heights, diameters, and branching architecture by Middle to Late Devonian time, coexisting with the presumably rhizomatous protelepidodendrids and presaging growth habits and reproductive strategies present among the abundant Carboniferous and younger lycopsid lineages. Various isolated anatomically preserved stem remains, when viewed in comparison to anatomy in more extensively preserved plants, also show differences in some features, not yet well understood. Secondary tissues occur in lycopsids from the Middle Devonian. Tracheid wall pitting among different taxa varies from one or more of the following pitting types: scalariform, pitted, circular bordered pitted, scalariform with fibrils connecting the bars or pitlet sheets. Much remains to be learned about its possible taxonomic implications.

Different types of stem bases with rooting structures occur among these taxa. Longostachys and Chamaedendron stems (and possibly Clevelandodendron) terminate in a series of downward trending, bifurcating root-like structures, but details are poorly understood. The two major rooting structures of arborescent isoetalean lycopsids dominant in the Carboniferous occur by the Middle Devonian. The earliest records of cormose lycopsid bases may be those recorded by Xu and Wang (2016) from the late Middle-Late Devonian Huijiersite Fm, China and the bases of the more extensively preserved Protolepidodendropsis pulchra, of similar age from Svalbard (Berry &
Late Devonian Leptophloem and Otzinachsonia also exhibit cormose bases. A more branched, stigmarian type rooting system (Wang et al., 2003) occurs in Sublepidodendron spp from China. Laurussian forms are less easy to interpret. Pigg (2001) suggests that cormose and stigmarian forms represent variations of a single rooting type, perhaps being influenced by ecological factors.

Some of these Devonian lycopsid stems or branches bear sporangia aggregated into strobili, ranging from mono- to bisporangiate (Longostachys, Chamaedendron, Sublepidodendron, Cyclostigma), while others apparently have alternating fertile and vegetative zones (Monilostrobus Wang & Berry, 2003; Wuxia Berry et al., 2003), as also occurs in younger tree lycopsids. The Upper Devonian Wuxia is interesting in that megasporangiate “cones/fertile zones” occur just below branching points of axes but presumed microsporangiate ones are terminal (Berry et al., 2003). Hoxtolgaya robusta Xu et al. (2012), Middle Devonian of China, is interpreted as arborescent based on 9cm wide stems of unknown length, and exhibits sporangia yielding spores of one size, interpreted as homosporous, but preservation is too incomplete to determine with certainty. In strobili or fertile zones, sporophylls are either identical to vegetative leaves, or modified with a broad fimbriate to spiny base and distal lamina, as in the Middle to Upper Devonian small tree-like lycopsids such as Longostachys (Cai & Chen, 1996), Chamaedendron (Schweitzer & Li, 1996) or Wuxia (and others) (Berry et al., 2003).

Berry et al. (2003) note that variation in fertile regions/structures and in stem anatomical features occurs earlier and more broadly in China than Laurussia, suggesting that perhaps China may have been an important area of early evolutionary innovation in clade(s) from which the major lineages of arborescent, heterosporous lycopsids of the Carboniferous and later arose. More whole plant reconstructions of several of these lycopsids are needed, and particularly, clarification of the organisation of basal regions, presence/absence of branching, anatomical features, and type of reproductive structures among individual plants, in order to better understand their relationships to both earlier and later occurring lineages to resolve relationships of these several plants and other taxa all considered as part of the isoetalean clade.

The record of herbaceous, homosporous lycopsids (e.g. Lycopodiaceae) is far less well known in the Devonian and Carboniferous. Lycopodites oosensis from the Late Devonian of Germany (Kräusel & Weyland, 1937) may represent one, but should be restudied. The Lower Carboniferous herbaceous form Hestia possesses too few characters to assess affinities (Bateman et al., 2007) but might represent an eligulate form. Younger fossils have been assigned to the genus Lycopodites but it is difficult to determine the affinities of these leafy stems; one has been found to represent conifer twigs, others exhibit a mix of lycopodiacean and sellaginellacean characters but are usually allied to Selaginellaceae (Skog & Hill, 1992; Thomas, 1992). Rowe (1988) identified a rhizomatous plant from the late Early Carboniferous Drybrook Sandstone as an early Selaginallalean, Selaginellites resimus, but little is known about early evolution of Selaginellales.

Several questions concerning early lycophyte evolution remain or are only partially answered by recent data. The paraphyly of some Zosterophyllum species and stem lycophytes remains unresolved despite new data. Much remains to be clarified about overall construction and relationships of the numerous Middle-Late Devonian protelepidodendraleans and small tree lycopsids, as discussed above, including how many times have structures such as strobili, ligules, rooting structures, and leaf abscission arisen and in what combinations? How do they relate to later-occurring lineages? And
again, questions remain about the early history of herbaceous, eligulate, homosporous Lycopodiaceae and ligulate, heterosporous Selaginellaceae through much of the Devonian and Carboniferous and even early Mesozoic.

**EUPHYLLOPHYTE EVOLUTION**

Extinct Devonian plants included in the Euphyllophyta mostly exhibit much branched aerial axes, some terminated by mostly fusiform sporangia. Anatomy consists of various protoxyleles (haplo-, actino-, dissected). Pertinent to fern evolution are stem euphyllophytes, such as *Psilophyton* and *Pertica*, the predominantly Mid-Upper Devonian Cladoxylopsida (=Pseudosporochnales [or Cladoxylales] + Iridopteridiales), the enigmatic, but putative iridopterid *Rhacophyton*, and possibly Stauropteridales. The latter groups have been considered by many authors to be derived from a trimerophyte ancestor, and possible fern-like or fern precursors. Reasons vary; some derive from cladistic studies, others from older comparative studies. While lacking laminate leaves, either the whole or part of their lateral branch systems (LBS) are interpreted as showing possible stage(s) in the evolution of a leaf, as mentioned earlier (Kenrick & Crane, 1997; Gensel et al., 2001; Boyce & Knoll, 2002; Sanders et al., 2007; Tomescu, 2008; Galtier, 2010; Corvez et al., 2012). These occurred simultaneously but at different rates in different lineages (Galtier, 2010), and are not homologous within ferns or between “ferns” and seed plants. Secondlly, some of the above taxa exhibit peripherally located protoxylem in various types of actino- or dissected protoxyleles (where anatomy is known) that differs from that present in aneurophytalean progymnosperms and early seed plants. The latter, and some basal euphyllophytes, exhibit radiate protoxylem (Beck & Stein, 1993), in which actinosteles or variants thereof have a central protoxylem, which appears to extend out each arm of the stele to produce lateral traces. These are considered mesarch but this depends on whether the protoxylem at the tips of arms persist, a feature presently not well understood. Anatomy of this type is typical of aneurophytalean progymnosperms, possibly some of the larger (*Pertica*–grade) trimerophytes, stenokolealeans, and other plants of uncertain affinity. However, it remains unclear if these two protoxylem patterns represent a good indicator of phylogenetic relationships. For example, Scheckler et al. (2006), in a re-study of *Langoxylon* Stockmans, a taxon of uncertain affinity, point out that *Langoxylon* stems possess a mixture of these two characters. *Pseudosporochnus hueberi* lacks a central protoxylem, but exhibits peripheral protoxylems plus several separate protoxylems along the midplane of an arm (Stein & Hueber, 1989), thus not all early euphyllophytes can be so easily categorized.

The term “peripheral loop”, initially referring to an anatomical feature of Carboniferous zygopteridalean petioles, has been used to describe protoxylem features in many Devonian plants. As originally defined, a peripheral loop consists of a central rod of parenchyma surrounded by protoxylem (Leclercq, 1970) located near the margin of an elongated vascular bundle. Similar structures are termed the same in stems of Devonian taxa. Several studies show that most of the latter represent protoxylem lacunae, resulting from break-down of thinner walled protoxylem during development or represent a different organisation of protoxylem with associated parenchyma (Scheckler, 1974; Scheckler et al., 2006; Stein, 1982). This suggests that the presence of a so-called peripheral loop should be carefully evaluated in any Devonian plant, and is not phylogenetically useful. Furthermore, care should be taken in use of the term “clepsydroid” and in any phylogenetic implications that might follow, because strap-
shaped strands in main axes or lateral branch traces in Devonian plants expand at their ends in preparation for producing the next higher order traces, thus appearing similar to the dumbbell-shaped petiole trace so termed among some early ferns.

The earliest putative euphyllophyte laminate leaf may date from the Pragian *Eophyllophyton* (Hao, 1988; Hao & Beck, 1993), considered basal and sister to other euphyllophytes by Hao and Xue (2013) but considered of uncertain affinity by this author. While laminate leaves are absent in most other coeval taxa, they occur in the Early to late Devonian *Platyphyllum (= Flabellifolium?)* and the Late Devonian *Ellesmeris* (Hill et al., 1997), enigmatic *Shougangia bella* (Wang DM et al., 2015), archaeopteridaleans and early seed plants, appearing earlier in time in lignophytes than in filicales (Galtier, 2010). Clearly, resolution of what constitutes homologous structures in LBSs among these different much-branched plants, and more documentation of the first appearance of laminae, would address remaining questions concerning number of times and modes of leaf evolution in these groups and non-homology of so-called megaphylls in these groups.

**CLADOXYLS, IRIDOPTERIDS AND RHACOPHYTES—CALLED “FERN-LIKE” BUT?**

Starting in the Mid-Eifelian and extending through the late Devonian, several taxa referable to Cladoxylopsids s.l. (e.g. including iridopterids, Berry & Stein, 2000) and rhacophytes are part of a more complex vegetation, some of the former representing small to large trees recently described as major components of in situ Middle to early Upper Devonian forests in the USA and Germany (Stein et al., 2007; 2012). Concomitantly, major revision of taxon concepts for *Pseudosporochrus* (Berry & Fairon-Demaret, 1997; 2002), *Calamophyton* (now includes *Duisbergia*, *Cladoxylon*, and most species of *Hyenia* - Fairon-Demaret & Berry, 2000; Berry, 2005; Giesen & Berry, 2013) and *Eospermatopteris/Wattieza* (Stein et al., 2007; 2012) has resulted in critical new information about the architecture of these leafless trees.

**Pseudosporochnaleans**

*Pseudosporochrus* trees (Figure 4) produce rows of branches that abscise at their base; the branches are digitately divided and produce higher-order, divided laterals that are three-dimensional to subopposite and alternate, final divisions being vegetative and non-laminate or fertile (Berry & Fairon-Demaret, 2002). *Calamophyton* is a smaller tree, with a swollen base, stems enlarging near apex and bearing vertical rows of lateral branch systems that then divide digitately (Figure 5), and produce higher order helically arranged dichotomizing vegetative or fertile non-laminate units. Older branch systems break off above their base, leaving the *Duisbergia* type morphology (Giesen & Berry, 2013). *Eospermatopteris* is larger, with a swollen base, a wide stem, and crown of digitately divided branch systems of the *Wattieza* type (Stein et al., 2007; 2012), abscising at their base. Terminal fusiform sporangia are known for all of these, being either recurved (*Calamophyton, Wattieza*) or erect (*Pseudosporochrus*). These apparently represent the earliest tree architectural types among euphyllophytes, being similar to some palms, cycads and tree ferns (Stein et al., 2007) in stem and root structure.

Where anatomy is known in the pseudosporochnaleans, it varies from being a multistranded, anastomosing stele, producing similar but smaller multi-stranded steles in lateral branches, as in *Calamophyton* or *Pseudosporochrus* (Figure 6), to being comprised of individual radially oriented strands near the periphery of the stem adjacent
Figure 4. A recent reconstruction of the cladoxylopsid *Pseudosporochnus*, showing its crown of relatively large branch systems, each of which is closely dichotomous so as to appear digitately divided. Reproduced from Berry and Fairon-Demaret, 2002, Figure 9, Int. J. Plant Sci. 163(5) 699-713. © 2002 by The University of Chicago.
Figure 5. Calamophyton primaevum. Drawing of a stem showing two features— the remnant bases of branches along the stem and parts of the attached, digitately arranged branches at the crown. B. Suggested anatomy of Calamophyton (Duisburgia) based on Mustafà (1978) showing primary xylem in black, surrounded by aligned, possibly secondary xylem (lines). Redrawn from Giesen and Berry, 2013; stem is from Figure 5a (in part), anatomy redrawn from Figure 17d.
to a large pith in main trunk (Figure 5) as in the presumed base of Calamophyton stem (Giesen & Berry, 2013) and Eospermatopteris/Wattieza (Boyer & Matten, 1996). Opinion differs as to whether some of these plants exhibit a limited amount of aligned metaxylem or secondary tissues. Coeval progymnosperms possess a eustele and bifacial vascular cambium. Habit, branching, aspects of rooting and internal construction of the large pseudosporochnelean cladoxylopsids might mean they already had diverged to the extent that they have little relationship with extant polypodiid or equisetid lineages, but instead represent a distinct lineage that flourished globally, then went extinct without any survivors (see Rothwell & Stewart, 1993).

Iridopteridales

Numerous Middle Devonian plants from Laurussia, Gondwana, and China comprise another lineage of Middle to Upper Devonian euphyllophytes, the Iridopteridales (Stein, 1982; Fu et al., 2011) based on anatomy, morphology, or a combination of these preservation forms (Figure 7). These plants as presently known are smaller than cladoxylopsids, consisting of stems with a deeply ribbed mesarch actinostele comprised of two to three centrally united arms, each arm dividing one or more times to form six to twenty arms/ribs depending on the taxon. A single protoxylem region exists near the end of each arm (Figure 7 A-C). Branching pattern is essentially whorled, each protoxylem pole emitting a trace that supplies either a lateral branch or a smaller dichotomous appendage, the two types occurring in the same whorl (Figure 7D). Sometimes branching occurs in only part of a whorl, with successive partial whorls offset (Stein, 1982). Sporangia terminate ultimate dichotomizing appendages borne from second order branches- some upright and some recurved (Berry & Stein, 2000, Compsocradus). Other, often less extensively preserved, taxa attributed to the Cladoxylopsida are not treated here. Relationship to Polypodiidae is unclear; depending

**Figure 6.** Anatomy of a lateral branch system of Pseudosporochalus, which is characteristic of many cladoxylopsids; the vascular bundles are arrayed in a manner interpreted as representing the beginning of a dichotomy. Redrawn from Stein and Hueber, 1989.
on what combination of characters are selected, they may be closer to Equisetidae; if others, of uncertain affinity (Stein et al., 1984; Berry & Stein, 2000).

**Rhacophyton and putative relatives**

*Rhacophyton* (several spp) exhibits some characters that presage a fern type of organisation, but also possesses secondary tissues and some morphology reminiscent of progymnosperms or iridopteridaleans. Somewhat rhizomatous to upright axes (?)stems) bearing adventitious roots in *R. ceratangium* (Cornet et al., 1976) produce single or paired much divided lateral branch systems which overall appear three dimensional, with more two-dimensional second order axes. The secondary axes depart oppositely, and range from dichotomous to slightly pseudomonopodial. Fertile lateral branch systems are more complex, each pair with one vegetative and one fertile unit. The vegetative segments are elongate and bear divided higher order non-laminate ultimates. The fertile units are much divided and recurved, forming rounded masses of dividing axes terminating in fusiform sporangia with elongate tips. Anatomy of less extensively preserved, more upright appearing stems of *R. zygopteroides* was described by Leclercq (1951) from poorly preserved specimens, and interpreted as an actinostele with unequally sized arms, although these sections have deterioriated so this cannot be confirmed. Similarly arranged lateral branches produce clepsydroid shaped steles. Both were described as surrounded by secondary xylem. In *R. ceratangium*, both main axis and lateral branches are considered to have produced an elongated clepsydroid-shaped primary xylem, surrounded by secondary xylem with rays (Cornet et al., 1976; Dittrich et al., 1983). Traces to higher order axes also are known. *R. condrusorum* Schultka (1978) is similar. *Ellesmeris* Hill et al. (1997) exhibits a simpler, but similar overall architecture, although the ultimate units are laminate, and fertile structures unknown. Anatomy is not intact, but the authors suggest main axis and lateral branch produce an elongate haplostele, with protoxylems near the tips of the arms. Knowing if all orders of branching possessed the same type of stele, or alternatively that stelar configuration changed from radial to

**Figure 7.** Line drawings of anatomy and stem portion of taxa included in Iridopteridalean cladoxylopsids. A. *Ibyka amphikoma*; B. *Iridopteris eriensis*; C. *Arachnoxylon kopfii*; D. Portion of stem and branching structure of *Anapaulia moodyi*; whorls of branches alternate with dichotomizing structures similar to ultimate branchlets; each lateral branch bears second and ultimate order branches. A-C redrawn from Stein, 1982. D redrawn from Berry and Edwards, 1996.
bilateral from main axis to lateral branch is of some significance in considering relationships and what to interpret as stem vs. leaf in these plants. For the present they usually are considered a separate lineage, Rhacophytaceae, although some authors previously placed them as a basal member of the zygopteridaleans and others consider them more allied to progymnosperms. Berry and Wang (2006) suggest, intriguingly, that Rhacophyton shares some characteristics of iridopteridaleans.

STEM EUPHYLLOPHYTES AND RELEVANCE TO EVOLUTION OF LATER FORMS

Trimerophyte grade euphyllophytes exhibit either dichotomous, anisotomous or strongly pseudomonopodial branching of varying degrees of predictable cladotaxy. Some of these terminate in fusiform sporangia, ranging from loosely (Psilophyton-grade plants) to densely (Pertica) clustered. Anatomy is known from parts of aerial systems of some Psilophyton species, while anatomy is unknown in published Pertica species. However, Gothanophyton and some undescribed plants at the Pertica-grade provide data about possible anatomical variation in these entities, showing some differentiation between ribbed main axes and lateral branches, and possibly indicating evolutionary trajectories different from Pertica, although affinities of most of these are poorly understood.

Psilophyton-grade basal euphyllophytes

Psilophyton (Trant & Gensel 1985), known only from anatomy (no sporangia attached). In this species, more closely spaced branching occurs mainly in lateral branches.

Psilophyton crenulatum Doran (1980), an apparently rhizomatous plant with upright branch systems, exhibits dense regions of mostly vegetative branching, some of which appear immature (apices present) while more distally, spacing of vegetative and fertile lateral branches varies. Psilophyton dawsonii, based mainly on anatomy of a 2.5 cm long specimen (Banks et al., 1975), possesses regions of closely spaced vegetative lateral branches. The more distally located fertile lateral branches exhibit a trace suggested to differ in shape from vegetative ones (Banks et al., 1975). Some short dichotomously divided lateral appendages are considered possible aphlebiae or roots. These features are not known for any other Psilophyton species, including P. conicumum (Trant & Gensel 1985), known only from anatomy (no sporangia attached). In this species, more closely spaced branching occurs mainly in lateral branches.

Comparison of the many other Psilophyton species indicates that relative distribution of vegetative vs fertile regions is unknown, partly because the genus is best recognized
morphologically from fertile specimens. In many instances the number of specimens available or mode of preservation limits information. Where numerous specimens are available, study shows branching is variously spaced. Frequently, lateral branches are incomplete and finding completely vegetative branch systems is difficult.

It was previously questioned whether certain previously described species of *Psilophyton* conform sufficiently to the concept of the genus. *P. kräuselii*, from the Middle Devonian of Bohemia (Obrhel, 1959) is too poorly preserved to determine affinity, being based on fragmentary fertile and sterile remains (Hao & Xue, 2013; this paper). Hao and Xue (2008) also question the generic identity of *P. szaferi*, *P. striatum* (Wang & Berry, 2001) and *P. dapsile* (Kasper et al. 1974) because they are either too poorly known or lack twisted sporangia. Examination of *P. dapsile* sporangia in the author’s collection suggest that while small, they do show some torsion. *Psilophyton szaferi*, described by Zdebska (1986) from the Lower Devonian of Poland, consists of a portion of a lateral branch system with upright sporangia borne in loose clusters more similar to those in *Pertica* and should be restudied. *Psilophyton microspinum* from the Early Devonian of Maine (Kasper et al., 1974) exhibits a more rigid pattern of lateral branches and erect, untwisted sporangia, suggesting its affinity should be re-evaluated.

The few morphologically preserved Pragian *Armoricaphyton* and the Emsian unnamed plant from Canada (Gerrienne et al., 2011; Strullu-Derrien et al., 2014; Gerrienne & Gensel, 2015) with an early type of secondary tissue are similar in stem

**Figure 8.** Diagrammatic drawings of small basal euphyllophytes; predominant mode of branching shown above, anatomy below. Axes approx. 2-5 mm. A. a generalized drawing of branch system of *Psilopyton*. Anatomy a centrarch haplostele, producing circular (or possibly square, if fertile) lateral branches. B. Unnamed plant from Emsian, New Brunswick (Gensel in prep). Ribbed, twice branched axes known. Some stems with elongate haplostele producing lateral trace, others indicate same type of haplostele surrounded by secondary xylem. C. *Armoricaphyton chateaupannense*, Pragian, France (Gerrienne et al., 2011; Gerrienne and Gensel, 2016). Ribbed, twice branched axes. Primary xylem a round haplostele, surrounded by secondary xylem. Basal part of lateral branch with secondary xylem, more distal region without. Dark circles or lines in center of steles indicate protoxylem, white area primary xylem, lines = secondary xylem.
diameter and branching, to *Psilophyton* (Figure 8 B, C). No fertile remains are known. All of these taxa, along with *Pertica* type trimerophytes showing preserved anatomy, exhibit P-type tracheid wall patterning *sensu* Kenrick and Crane, 1997 (Gensel, unpublished data). This character may be less useful at lower taxonomic levels, as the combination of characters evident in these plants suggest broader disparity among these early simpler plants than previously realised.

Morphologically preserved Middle Devonian plants such as *Planatophyton* (Gerrienne et al., 2014), *Pauthecophyton* (Xue et al., 2012), and *Tsaia* (Wang & Berry, 2001) are similar to *Psilophyton* in size and some aspects of branching but differ either in specifics of branching, sporangial orientation and/or dehiscence mode. These may represent distal regions of much larger plants or, alternatively, fragments of plants similar to *Psilophyton* in size. Additional study of these plants may reveal features useful in determining if they represent one, or several, Lower to Middle Devonian genera and/or lineages and clarify their postulated phylogenetic relationships.

**Larger basal euphyllophytes similar to *Pertica***

*Pertica* and similar-appearing plants (Figure 9A-C) are taller and more robust in width, with a dominant main axis and variously arranged lateral branches. *Pertica* spp. exhibit mostly isomitously divided lateral branches with terminally borne, closely spaced, erect fusiform sporangia. Anatomy is unknown in published *Pertica* species.

A new genus, initially considered *Pertica*-like but definitely an unrelated taxon (Figure 9A), has a dominant main axis, helically arranged lateral branches that are single or paired, and, in contrast to *Pertica*, dichotomously- divided ultimate units located along their length, some sterile and some terminating in fusiform sporangia. Main axis anatomy is an actinostele (usually) with a single central protoxylem and a protoxylem near the end of each arm (Gensel, 1984). The lateral branch trace(s) are ovoid. There is no evidence of secondary xylem.

An undescribed plant from the Lower Devonian of New Brunswick is similar to *Pertica* spp. in morphology (Figure 9B), with a dominant main axis, helical lateral branches, and dichotomous laterals terminating in tight clusters of fusiform sporangia (Gensel, in prep.). Anatomy is unusual, consisting of a “winged” stele in which four major arms emanate from an elongate central region, but with one arm dividing further (Figure 9B). At least two types of lateral branch traces are formed, differing from main axis stelar configuration, but preservation is incomplete. Do other perticas have similar anatomy or might there be variation?

*Gothanophyton* Remy et Hass (Figure 9 C), with a 1 cm wide main axis and single or paired lateral branches, exhibits a “winged” stele, consisting of an elongated central region with two major arms extending from either end (Remy & Hass, 1986a). One or more of the arms divide again, so that 4-7 armed steles are known. Protoxylems are located at either end of the central region near departure of major arms and near the end of each arm (Figure 9C). Shape and/or lobing of lateral branch traces differ from the main axis configuration. Morphology is poorly preserved, but the authors suggest lateral branches departed singly, in pairs or in threes. Much more needs to be learned about this complex Lower Devonian plant.

In all of these plant types, protoxylems emanate from one or more of the main axis arms to supply lateral branches and only primary xylem is present. Architecture of these plants is morphologically and anatomically plastic, although with some common patterns, but evidence is building that these taxa exhibit enough differences to not be closely
Figure 9. Diagrammatic drawings of larger basal euphylllophytes, similar in size to *Pertica* (main axes approx. 1 cm) - note that none show evidence of secondary xylem. A. Plant described by Gensel (1984), with large main axis, single or paired lateral branches, dichotomously divided ultimates arranged along lateral branches. Anatomy a three-lobed stele with central protoxylem and protoxylem at end of each arm. First order lateral trace (or pair of traces) oval, second order trace circular. (Gensel, in prep) B. A *Pertica*-like plant with large main axis, helically arranged predominantly dichotomously divided lateral branch, terminating in bunched sporangia (not shown). Anatomy consists of slightly elongate central region from which four arms emanate, one of these divides again to produce five arms total. Protoxylem located at each end of central area, and ends of arms. First order traces are elongate to v-shaped or oval and in pairs as shown. Gensel (in prep). C. *Gothanophyton zimmermanni* Remy and Hass. Large main axis with single or paired lateral branches (poorly preserved), bearing at least second order ones. Main axis stele consists of an elongate central region from which 4-7 arms emanate. Protoxylem located at each end of central region, along and at ends of arms. Lateral traces change shape after departure to become 4-lobed. Protoxylem in black. Clear area in stele is primary xylem. C. redrawn from Remy and Hass, 1986.
related. They are similar in showing a change in shape between main axis and first-order lateral branches which conforms more closely to that seen in many ferns or extinct fern-like plants than anything known in progymnosperms, despite apparently comparing more closely to the radiate protoxylem group.

Basal regions or rooting structures for *Psilophyton* and the taller euphylllophytes mentioned above are unknown. The fossils appear to represent aerial shoots extending from a rhizome, but it also is possible that some represent the main part of a non-rhizomatous plant that may have had a basal rooting system. Fairly wide, branched but detached rooting structures running perpendicular to bedding surfaces occur in sediments near some of the larger taxa in northern New Brunswick, while one long, downward extending axis was discovered by Elick (1998) in Gaspé which may represent a rooting structure.

**Basal euphylllophytes incertae sedis**

Several upper Lower Devonian plants (late Emsian) have been excluded from phylogenetic studies, largely because their anatomy is unknown and branching pattern and other features are unique. These include *Oocampsa catheta*, interpreted as a trimerophyte-progymnosperm intermediate (Andrews et al., 1975), *Chaleuria cirrosa* (Andrews et al., 1974) and an undescribed plant with similar branching architecture from northern Maine (Gensel, in prep). They all exhibit a robust, fairly long, greater than 1 cm wide dominant main axis with densely spiraled lateral branches. In *Oocampsa*, higher order branches are several times irregularly dichotomous, bearing clusters of ovoid sporangia. *Chaleuria* and the new plant from Maine exhibit isotomous second-order branches, some terminating in fusiform to ovoid sporangia. Basal regions and thus growth habit(s) are unknown. One might expect either a multilobed or cladoxyl-like stelar configuration based on the major branching patterns. *Oocampsa* differs from the other two in higher order branching and sporangial morphology, while the other two represent one or more as yet unrecognized lineages, or may be early members of one of the later-occurring lineages such as cladoxyls or iridopterids.

Stenokolealeans are still poorly known, but recent studies indicate a possible affinity to early seed plants (Moment et al., 2016).

**SUMMARY**

Significant new data now exist concerning the time of appearance of plants and morphology and anatomy of some taxa. Exceptionally preserved plant remains have indicated an earlier advent of plants, plant structures and habit, including transitions to upright, even arboreal types by the Middle Devonian. Limited evidence indicates a wider array of growth strategies among these. Although the euphylllophyte and lycophyte lineages separate very early in time, with each demonstrating acquisition of major plant structures, gaps in preservation preclude establishing evolutionary trends or clear phylogenetic relationships within each lineage, especially early vs later Devonian plant types, and between Devonian and Carboniferous or younger lineages that appear more directly related to extant ferns or lycophytes (similar situation for seed plants). As whole plant reconstructions are made and new approaches employed, with new discoveries or re-investigation of earlier material, assessment of character homology will be more possible.

Several putative candidate lineages leading to equisetids or polypodiids remain, but it is clear that some, such as cladoxyls, are already highly specialized in development.
and construction by the Middle Devonian. Were more basal taxa similar, as suggested by Lorophyton Fairon-Demaret et Li (1993), Foozia minuta Gerrienne (1992) or perhaps as yet unrecognized taxa? Basal euphyllophytes at the Psilophyton, Pertica, and Gothanophyton grade are increasingly interesting in that it is among plants of this type that early differentiation of anatomical configurations of main axis- lateral branch is established. Within this group one might possibly be able to discern early divergence of the fern sensu lato vs seed plant lineages. Further examination of anatomy and organisation of lateral branch architecture, incorporating signals of developmental differences, may aid in recognizing early stages in evolution of leaves- these clearly will be different in various lineages. Similarly, the questions of transitions between predominantly rhizomatous and upright habit, and between types of rooting and of organisation of reproductive structures in both major lineages may become clearer with future discoveries or new approaches.

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A SHORT BIOGRAPHY OF THE AUTHOR

Patricia G. Gensel is a professor in the Biology Department, University of North Carolina at Chapel Hill, having started there in 1975. She received her B.A. at Hope College, Holland, Michigan, then she worked as a research assistant in the Geology Department, King's College, Univ. of London, England, for Dr. John Richardson in the area of Devonian palynology. Patricia returned to the U.S.A. and embarked on graduate studies of fossil plants and their spores with Professor Henry Andrews at the University of Connecticut, Storrs, CT., where she earned a M.S. and then Ph.D. After a few years of post-doctoral work, again with H. N. Andrews, she joined the faculty of (initially) the Botany Department, and later, Biology Department, at UNC.

Patricia continued work on both Devonian (from U.S. and Canada) and Early Carboniferous plants (from US), studying both megafossils and where possible, in situ spores. She also had students who worked on Cretaceous fossils from North Carolina and prepared a field guide to Triassic Plants of the Deep River Basin, NC. She co-authored the book Plant Life in the Devonian with H.N. Andrews (1984), and co-edited a book of contributed chapters entitled Plants Invade the Land with Dianne Edwards (2001).
She has authored or co-authored at least 68 peer-reviewed publications, the majority on Devonian plants, but also some about in situ spores, spore ultrastructure, and Early Carboniferous plants, several book chapters, reviews or field guides. Patricia has organized many symposia, mostly about early land plant evolution, trained several MS students and a few PhD students. Her major interests center on producing a better understanding of the morphology, structure, evolutionary relationships or patterns of evolutionary change, and where possible ecology, of early land plants, mainly of Devonian and Early Carboniferous age. She continues her interest in palynology, especially in situ spores or spore ultrastructure, in terms of what they may contribute relative to systematics or phylogenies of early plants. Recent publications include a report of early wood from the very Early to Early Devonian of France and New Brunswick (Gerrienne et al., 2011, Gerrienne & Gensel, 2016, work in prep), a revision of sporangial morphology and attachment in the iconic zosterophyll Sawdonia ornata (Gensel & Berry, 2016) and paleoenvironmental interpretations of some of the major plant-bearing deposits in New Brunswick or early coal (Kennedy et al., 2012; Kennedy et al., 2013). Her on-going work focuses on elucidating anatomy and morphology of basal euphyllophytes (formerly trimerophytes), additional work on Sawdonia spp., and other Devonian or Carboniferous plants.

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A NEW RECORD OF BROWN ROT DISEASE IN WATER FERN AZOLLA MICROPHYLLA (AZOLLACEAE): LOSS OF IMPORTANT BIO-RESOURCE

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Key words: cottony white mycelium; yellow pigmentation; macroconidia; microconidia; chlamydospores; pathogenic fungus; Fusarium thapsinum

ABSTRACT
Severely infected water fern, Azolla microphylla Kaulf., has been observed in natural habitats in different districts in the lower Gangetic plain of West Bengal, India. The infected plants have turned dark brown and been rotted entirely within 5-7 days, causing loss of natural resource as the plant species has immense commercial value. Specific growing colonies with cottony white mycelium, yellow pigmentation on the cultured medium, macro- and micro-conidial features, and absence of chlamydospore indicate the causal pathogen to be Fusarium thapsinum Klittich., Lesile, Nelson, Marases. The identification of the causal pathogen has been authenticated by standard pathogenicity test (Koch’s Postulates). Extensive literature survey reveals that this is a new record of brown rot disease in A. microphylla.

INTRODUCTION
Azolla Lam. is a free-floating, fast growing water fern. The plant is widely distributed in tropical, subtropical, and warm temperate zones in different countries of the World including India (Wagner, 1997). Here Azolla spp. are naturally available, mostly on moist soils, freshwater ditches, ponds, lakes, sluggish rivers and marshy lands. Azolla is treated as a ‘green gold mine’, because of its high nutritive value and it is considered as ‘super plant’ due to its fast-growing capacity (Wagner, 1997). They can even grow in nitrogen deficit areas and can double the biomass in 3-5 days because of its unique symbiotic relationship with the nitrogen fixing endophytic blue-green cyanobacterium, Anabaena azollae Sterberger (Wagner, 1997).

High nutritive values (Buckingham et al., 1978; Alcantara & Querubin, 1985; Paoletti et al., 1987; Lejeune et al., 2000; Alalade & Lyayi, 2006) and huge productivity through easy cultivation methods (Dao & Tran, 1979; Lumpkin & Plucknett, 1982; Liu et al., 2008) are the key factors for utilizing Azolla as one of the most promising aquatic plants for livestock feed (Becerra et al., 1995; Alalade & Lyayi, 2006; Cagauan & Pullin, 1991; Shiomi & Kitoh, 2001; Fiogbe et al., 2004), and as an effective natural fertilizer to increase rice yield successfully (Peters, 1978; Tung & Shen, 1985; Watanabe & Liu, 1992; Yadav et al., 2014). The plant has been employed in experiments during space and planetary travel (Liu et al., 2008; Carrapico, 2002; Katayama et al., 2008), and used for the production of non-polluting high energy biofuel (Peters et al., 1976; Newton, 1976; Das et al., 1994; Hall et al., 1995), especially biodiesel (Salehzadeh et al., 2014). The plant can act as reducer of various greenhouse gases by ammonia volatilization.
(Watanabe & Liu, 1992), by inhibiting methane production from rice field (Prasanna et al., 2002), and noticeably by sequestering atmospheric CO$_2$ (Brinkhuis et al., 2006). In addition, weed control (Krock et al., 1991), mosquito repellence (Ansari & Sharma, 1991), phyto-remediation from waste water (Jain et al., 1989; Saxena, 1995; Costa et al., 1999; Antunes et al., 2001; Khosravi et al., 2005; Umali et al., 2006; Rai, 2008; Costa et al., 2009; Rai & Tripathi, 2009; Elmachliy et al., 2010; Rai, 2010a, b; Sood et al., 2012) and soil (Umali et al., 2006; Cohen et al., 2002 and Mashkani & Ghazvini, 2009) are added approaches for utilizing this vital bio-resource.

With view to the many valuable uses of _Azolla_, the present authors have attempted to explore the species under various abiotic stresses through experimental set-up by culture of collected specimens from natural habitats of the lower Gangetic plains of West Bengal, India. During field survey, it has been observed that among the available _Azolla_ species, _A. microphylla_ Kaulf. grows dominantly over others and it can resist direct sunlight in summer. However, the species has been found to be severely infected by a fungal pathogen at the end of winter season (January-March) in most of the collection sites, which disrupts normal growth of the plant and ultimately causes death within 5–7 days.

From the available literature, it seems that pathogenic reports on _Azolla_ spp. are rather meagre (Sasi et al., 1982; Kannaiyan, 1985; Kannaiyan & Kumar, 2005; Lee et al., 2011; Banihashemi, 2014). The present communication describes a new causal organism for _A. microphylla_, which may help in identifying suitable measure(s) to protect this important natural resource.

**MATERIAL AND METHODS**

Fresh and infected samples of _Azolla microphylla_ Kaulf. were collected from different sites of three districts, namely, North 24 Parganas (22.936 N, 88.392 E; 22.825 N, 88.786 E), South 24 Parganas (22.021 N, 88.615 E), and Nadia (22.983 N, 88.445 E) in the lower Gangetic plain of West Bengal.

The morphological studies of infected and non-infected plants were made by hand lens and stereo zoom microscope (model LEICA S8APO). The anatomical details of infected plants and that of the isolated pathogen were thoroughly studied under light microscope (model Leitz Laborlux S). Macro and micro photographic documentations were made using Nikon D3200 DSLR and EC3 scientific cameras. To identify the pathogen from morphological characteristics, first the isolations were made from the frond of the naturally infected _A. microphylla_ plant using a sterile inoculating loop and plated onto 2.25% Potato Dextrose Agar (PDA). A total of five replicas of culture and three replicas of control plates were incubated at 25˚C temperature in complete darkness. After 2–3 days of inoculation, the fungal colony was observed to grow in each of the five replications of cultured plates but no changes were observed in control plates. The colony morphology, growth pattern, size and shape of macro- and micro- conidia were characterized thoroughly.

To confirm the identity of the pathogen, the standard pathogenicity test (Koch’s Postulates) was performed stepwise. Student’s $t$-test was done to compare the variations in two sets of data of pathogens detected from inoculated and re-inoculated pure cultured plates prepared through the pathogenicity test. The tests were performed by inoculating naturally-collected, healthy _A. microphylla_ plants of a single genotype, because the disease was observed in _A. microphylla_ only in natural habitats. A total of 45 experimental plants were surface sterilized through rinsing in 70% ethanol for 30 sec. followed by 1%
sodium hypochlorite solution for 1 min., and sterile distilled water for three times. Out of 45 surface sterilized plants, 30 healthy plants were wounded on the fronds by sterile needle. A small mycelial plug taken from a month-old colony of the pathogen was inserted into the wounds using a sterile toothpick. Both 30 inoculated and 15 non-inoculated plants were then placed onto nutrient medium, distributing five plants in each plate so that six replicas were made for inoculated plants and three for non-inoculated ones. The nutrient media was prepared using HIMEDIA PTO10 Murashige and Skoog medium with 17.04 gm./lit. and 10 gm. of agar. The plates were kept in a growth chamber at 25˚±2˚C temperature and 1800 lux intensity of light in 16:8 hr day and night cycle.

RESULTS AND DISCUSSION

Severe infection was observed in *A. microphylla* during the months of January to March in the field (Figure. 1A, B), as well as in a tank placed in the experimental garden of the Botany Department, Kalyani University (Figure 1C). In a fresh individual plant (Figure 1D), at first the infection was observed in roots (Figure 1E), followed by dark brown-coloured spots on the leaf (Figure 1F). Later, the infected areas sequentially enlarged (Figure 1G), and eventually the whole leaf turned into brown, showing bloch notch symptoms (Figure 1H). Finally, the plant rotted within 5-7 days (Figure 1I). Outwardly protruding, cottony, white mycelial growth covering the entire leaf surface was observed with disease severity (Figure 1J). The transverse section of infected leaves at the initial stage showed loosening of leaf tissues with distinct entrance of hyphae (Figure 1K). Anatomical section of leaf was not possible at the final stage of infection due to total disintegration of leaf tissues. The browning and rotting of leaves and roots are characteristic symptoms produced by the pathogen in *A. microphylla*. The dark brown coloration of the infected parts of the plant is because of the degradation of chlorophyllous pigments and probably production of phenolic compounds as resistance to pathogens (Lattanzio et al., 2006). By comparing the symptoms of rot diseases (Agrios, 2005), the studied infection in present investigation has been designated as brown rot disease of *A. microphylla*.

Each replica of cultured PDA media produced fungal colonies with very thick, abundant, cottony white mycelium, finally attaining a diameter of 48.71±3.0 mm (Figure 1L) after seven days of incubation. The growth pattern of the mycelium was radial. A characteristic yellow pigmentation was found to appear on the agar plate (Figure 1L) after 3-4 days of incubation. After 5-6 days, a slight purplish pigmentation was observed to ooze out and persisted for 1-2 days only. No sporodochia was observed in any of the cultured plates.

Under the microscope, the fungal hyphae appeared hyaline and septate (Figure 1M). Conidiogenous cells were mostly monophialidic (Figure 1N) and rarely polyphialidic. Monophialidic conidiogenous cells were long (range 10.53-33.94 µm), with an average length of 21.43±7.41 µm (Table 1). The microscopic examination of the fungi taken from the pure culture plates revealed hyphae with characteristic macro- and microconidia (Table 1). Microconidia were produced in relatively short chains (Figure 1O) or in a false head; conidiophores were branched or unbranched, bearing hyaline, monophialidic, conidiogenous cells. Three different forms of microconidia [oval (Figure 1P), fusiform (Figure 1Q), and napiform (Figure 1R)] were found, which were hyaline, thin-walled, and yellowish in colour. Fusiform shaped microconidia had a single septum. The average sizes of the oval, fusiform, and napiform shaped microconidia were
Figure 1(A.-W.): Caption opposite page
10.73±3.31 x 2.48±0.33 µm, 13.27±1.98 x 4.85±0.48 µm, and 7.17±0.77 x 3.37±0.18 µm respectively (Table 1).

The macroconidia were found abundantly in aerial parts of the hyphae of the infected leaf; 3-5 septate, but rarely 6; straight to falcate, thin walled, hyaline; slightly curved and tapering in a banana shape (Figure 1S). The average size of macroconidia is of 33.10±5.92 x 3.62±0.28 µm (Table 1). The apical cell of each macroconidia was conical, whereas the basal cell was relatively ill-developed and not the usual foot-shaped. The average length of the apical cell (6.47±0.77 µm) was longer than the penultimate cell (5.29±0.83 µm) (Table 1). It is significant to note that no chlamydospore was found in any one of the culture plates.

The growth pattern of the cultured pathogen onto PDA media and the occurrence of distinctive banana-shaped macroconidia and specific types of microconidia indicate that the causal pathogen is *Fusarium* Link (Moretti, 2009). *Fusarium* is a well-known plant pathogenic fungus, which is soil borne in nature and causes severe plant diseases around the world (Agrios, 2005). The *Fusarium* strain that produces diffusing yellow pigment was separated as a new species, *F. thapsinum* Klittich., Leslie, Nelson, Marases (Teleomorph, *Gibberella thapsina*) (Klittich et al., 1997, Summerell et al., 2003; Leslie & Summerell, 2006).

The characteristic features of cottony white mycelium, yellow pigmentation onto pure culture PDA medium, absence of chlamydospores, shape and size of macro- and microconidia corroborate with the diagnostic features of the holotype BPI 737885 (Klittich et al., 1997) and hence identify the species epithet of the present causal pathogen as *F. thapsinum*.

After seven days incubation in the pathogenicity tests, identical disease symptoms with brown-coloured lesions and protruding cottony white mycelium were observed only on the inoculated plants (Figure 1V) and the fungus was consistently re-isolated from lesions. The identity of the re-isolated pathogen was confirmed as *F. thapsinum* again by pure culturing onto PDA medium (Figure 1W) maintained at similar conditions mentioned before and subsequent microscopic examinations of the hyphae with

**Figure 1(A.-W.):** Non-infected, and infected *Azolla microphylla* plant showing symptoms of brown rot disease, cultured colony of causal pathogen, and microscopic observation of different features of causal organism (bar= 0.1 mm except otherwise mentioned). A. & B. Naturally infected plant on pond (A) and in rice field (B); C. Infected plant grown in tank at experimental garden of Botany Department, Kalyani University; D. Non-infected plant; E. Infected root showing brown coloration; F. Brown coloured spots on leaf (arrow marked) at initial stage of infection; G. Gradual enlargement of infected area on leaf; H. Leaf showing bloch notch symptom; I. Entirely rotten plant at final stage of infection; J. Cottony white mycelial growth on infected leaf; K. Transverse section of infected leaf showing hyphal infiltration; L. Growth pattern of pure cultured colony showing white mycelium and diffuse yellow pigmentation (dyp); M. Septate fungal hyphae; N. Monophialidic conidiogenous cells; O. Occurrence of microconidia in short chain; P.-R. Microconidia of different shapes- P. oval, Q. fusiform, R. napiform; S. Banana-shaped macroconidia; T. Experimentally diseased plants in pathogenicity test; U. Control plants in pathogenicity test; V. Magnified view of experimentally diseased plants showing same disease symptoms; W. Growth pattern of pure cultured colony prepared from re-isolated pathogens from experimental disease plants showing white mycelium and diffuse yellow pigmentation (dyp).
Table 1: Characterization of macro- and micro- conidia grown in pure cultures prepared from the isolated inoculum of naturally diseased plant (NDP) and the re-isolated inoculum of experimentally diseased plant (EDP) of Azolla microphylla

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Measurement (in µm) of macro- and micro- conidia</th>
<th>*t value at 18 df</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NDP</td>
<td>EDP</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>MACROCONIDIA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of septation</td>
<td>3 - 6</td>
<td>5.00± 0.79</td>
</tr>
<tr>
<td>Length of apical cell</td>
<td>4.82 – 7.58</td>
<td>6.47 ± 0.77</td>
</tr>
<tr>
<td>Length of penultimate cell</td>
<td>3.89 – 6.51</td>
<td>5.29 ± 0.83</td>
</tr>
<tr>
<td>Ratio of apical to penultimate cell</td>
<td>0.95 – 1.58</td>
<td>1.25 ± 0.22</td>
</tr>
<tr>
<td>Size [length (l) x width (w)]</td>
<td>19.43 – 39.23 (l) 3.06 – 3.98 (w)</td>
<td>33.10 ± 5.92 x 3.62 ± 0.28</td>
</tr>
<tr>
<td>Length width ratio</td>
<td>7.63 – 11.50</td>
<td>9.18 ± 1.70</td>
</tr>
<tr>
<td>MICROCONIDIA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of conidiogenous cell</td>
<td>10.53 – 33.94</td>
<td>21.43 ± 7.41</td>
</tr>
<tr>
<td>Size of oval shaped microconidia [l x w]</td>
<td>7.22 – 16.08 (l) 2.05 – 2.92 (w)</td>
<td>10.73 ±3.31 x 2.48 ± 0.33</td>
</tr>
<tr>
<td>Size of napiform shaped microconidia [l x w]</td>
<td>6.18 – 8.26 (l) 3.14 – 3.68 (w)</td>
<td>7.17 ±0.77 x 3.37 ± 0.18</td>
</tr>
<tr>
<td>Size of fusiform shaped microconidia [l x w]</td>
<td>10.08 – 16.86 (l) 3.97 – 5.41 (w)</td>
<td>13.27 ±1.98 x 4.85 ± 0.48</td>
</tr>
</tbody>
</table>

* p>0.01 in all cases
characteristics macro and micro conidia (Table 1). The control plants, however, did not show any disease symptoms and no traces of *F. thapsinum* were recovered from them.

Thorough literature survey reveals that previously reported diseases in *Azolla* are black rots in *A. pinnata* from India (Kannaiyan, 1985), *Rhizoctonia* blight in *A. japonica* from Korea (Lee et al., 2011), and *A. filiculoides* from Iran (Banihashemi, 2014). Kannaiyan and Kumar (2005) identified a fungal complex as a causative organism for black rot disease in *A. pinnata*, with *Rhizoctonia solani* as the primary infecting organism.

**CONCLUSION**

The present investigation reports a new record of brown rot disease in *Azolla microphylla* caused by the fungal pathogen *Fusarium thapsinum*. Because of the immense value of this plant, it will be essential to explore preventive measure(s) to protect this important bio-resource from destructive fungal attack.

**ACKNOWLEDGMENTS**

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**ABSTRACT**

A taxon in the *Cystopteris fragilis* (L.) Bernh. complex that occurs in Britain appears up to now to have been overlooked there. It is proposed that this fern, which was recently rediscovered in Snowdonia, North Wales, should be referred to a subspecies of *C. fragilis*, namely subspecies *huteri* (Hausm. ex Milde) C. Prada & Salvo, that occurs in mainland Europe, but had not previously been recognised in the British Isles. The Snowdonia fern is distinct in its morphology from other British taxa in the *C. fragilis* complex and is described here together with its historic records and present distribution, updated from information given in an earlier paper in *Watsonia* (Tennant, 2010), and its relationship to affiliated taxa is discussed.

**INTRODUCTION**

The *Cystopteris fragilis* complex is perhaps the most difficult one to resolve of all fern groups. In the British Isles it presently includes the species *C. fragilis* (L.) Bernh., *C. alpina* (Lam.) Desv., *C. diaphana* (Bory) Blasdell, and *C. dickieana* R. Sim. It is generally accepted that more than one original diploid parent was probably involved in the ancestry of the present species recognised in the complex. One result of which is the large variation between and within the species, making it especially difficult to recognise the different cytotypes based solely on their gross morphology. Over many decades, a study was made of the species within the complex, and especially their variants, both in the field and in herbarium collections. This led to earlier papers being published on *C. alpina* and *C. dickieana* in Britain (Tennant, 1995; 1996; 2010), but two especially distinctive taxa were noted in Britain, which in their morphology did not readily conform to the appearance or descriptions of any of the known presently extant British taxa within the complex.

The first of these was found in 2008 in Snowdonia, V.C. 49, North Wales, where it was extremely local and scarce. A search in herbarium collections revealed that a morphologically identical taxon had been collected previously there. A paper published in *Watsonia* (Tennant, 2010) gave most of the records of this fern, which are updated and relisted in this paper, together with its historical references, description, illustrations (Figures 1 & 2), and habitat, and evidence for the presently proposed identity of this Snowdonia fern is given.

The second distinctive *Cystopteris* taxon discovered during the course of these studies occurs in northern Scotland, and a similar taxon also in North Wales. These ferns appear to be affiliated to certain variants currently included under *C. alpina* (Lam.) Desv., previously as “*C. regia*” auct., non (L.) Desv. Morphologically these Scottish ferns
closely match certain *Cystopteris* forms found by the author recently in alpine regions of mainland Europe in three different countries (Figures 3 & 4). Living material and details of these Scottish ferns, which were grown from spores, have been donated to the Natural History Museum in London for further study and will be the subject of a later paper.

**Figure 1.** *Cystopteris fragilis* subsp. *huteri*. Cultivated examples from Snowdonia, V.C.49, North Wales illustrating the degree of variation. (Further illustrations were given previously in Tennant (2010)).
Figure 2. *Cystopteris fragilis* subsp. *huteri*. Snowdonia, V.C. 49, North Wales. Cultivated specimen.
Figure 3. *Cystopteris alpina* (Lam.) Desv. s. str. From the European Alps, A – D. 
A. France. B. Spain. C. Austria. D. Italy. (British examples from Yorkshire 
collected by J. Backhouse were illustrated in Tennant (1995)). The *Cystopteris* 
variant taxon, which is intermediate between *C. alpina* and *C. fragilis*, E – G. 
E. From the Italian Dolomites. F. French Alps. G. Northern Scotland.
Figure 4. Cystopteris variant from Northern Scotland, V.C. 105, which is intermediate between C. alpina and C. fragilis.
MATERIALS AND METHODS
The fieldwork described below on *Cystopteris* in Britain dates back over many decades through an early interest of the author, particularly in the British records for *C. alpina*. More recently, following several examinations of herbarium specimens at the Natural History Museum (NHM) London (BM) and at the Royal Botanic Garden, Edinburgh (E) between 2004 and 2006, details of all relevant specimens were abstracted and compared with recently found examples of *Cystopteris*. This was followed by further fieldwork in North Wales and in Scotland. Permission was obtained from the Countryside Council for Wales to collect a small amount of material on Snowdon, V.C. 49, which was undertaken in 2009, following which this Snowdonia fern was cultivated in Yorkshire. Living examples of the Snowdonia *Cystopteris* were then donated to NHM in London for further studies. This work is being undertaken by Dr F.J. Rumsey at NHM, and so far has included a detailed examination of spores and some plastid DNA studies.

Meanwhile, historic publications were examined and information dating back to the 17th century was abstracted, after it was discovered that the Snowdonia *Cystopteris* had first been collected at that time. Field studies on *Cystopteris* were more recently extended in alpine regions of mainland Europe, namely Norway (Tromsø 2009), France (Vanoise Alps 2010), Spain (Central Pyrenees 2012), Italy (Dolomites 2014, 2015), and Austrian Tyrol (2016). Material found there was compared with examples from Britain.

RESULTS

Description of the Snowdonia *Cystopteris*
This fern was largely described and illustrated in an earlier paper (Tennant, 2010) from samples that had been found in Snowdonia in 2008, and cultivated from 2009, although one crucial character was then omitted. During examination of the cultivated material it was noted that the fronds were distinctly glandular, having scattered, capitate glandular hairs on the pinnae, a character unique in British *Cystopteris*. A further more detailed description is as follows, with illustrations given in Figures 1 and 2.

Stipe slender, 1.5 – 6 cm. brownish, with scattered pale brown linear scales, frond (lamina) lanceolate, 4 – 10 x 2 – 5 cm, pinnae mainly narrowly ovate, 1.5 – 2.5 x 0.7 – 1.3 cm, with scattered, capitate glandular hairs, which are most visible on the margins, pinnules stalked, the lower oblong-obdeltoid, subtruncate at the apex, often with short, sharply acute points, the upper narrower, linear-obdeltoid, more acute at the apex, the veins mainly terminating in the acute apical points. Sori scattered, situated adjacent to the veins, spores echinate, very large, mean c. 50 microns (F.J. Rumsey, pers.comm.).

The juvenile sporophytes have well-separated (divaricate), linear-oblong, blunt pinnules, unlike those of typical *C. fragilis*, but very similar to those of *C. alpina*. The immature, intermediate fronds usually have either obdeltoid (wedge-shaped) or linear pinnule lobes, again similar to those seen in *C. alpina*. The mature fronds are more finely dissected, sometimes with pinnae obliquely inclined to the plane of the lamina. The Snowdonia ferns, although sharing several characters with *C. alpina* are readily distinguished from it by the acute points at the apex of the pinnule lobes into which the majority of the veins terminate, and the unique presence of glandular hairs on the pinnae. Plastid DNA examination has also confirmed that the ferns are not *C. alpina* (F.J. Rumsey, pers. comm.). The relationship of these Snowdonia ferns to a described, glandular subspecies of *C. fragilis*, which occurs in southern, central and western montane regions of mainland Europe is discussed below.
Habitat
The Snowdonia fern is a plant of the alpine zone in Britain, probably not occurring at altitudes below 700m. In the two localities in which it was rediscovered in 2008 it was extremely local and scarce. It occurred on basic rocks in a north-facing vertical crevice and in a recess on the cliff face. No other vascular plants were present, but *Cochlearia pyrenaica* subsp. *alpina* (Bab.) Dalby, *Minuartia verna* (L.) Hiern and *Silene acaulis* (L.) Jacq. occurred nearby, and typical *C. fragilis* was not far distant.

Records of the Snowdonia *Cystopteris*
Specimens in British herbarium collections and references in historical literature showed that the fern that had been rediscovered in Snowdonia in 2008 had been collected very much earlier. Identical material was located by the author in 2004-2006 at the Natural History Museum (NHM) in London (BM) and other relevant *Cystopteris* specimens were found in the herbarium of the Royal Botanic Garden (RBGE) in Edinburgh (E), and the Snowdonia records are given below. This fern was first collected in the late 1680s by Edward Lhwyd in Snowdonia, and two of Lhwyd’s original specimens were recently located by F.J. Rumsey in the Sloane herbarium at BM; these specimens were identical to those found on Snowdon by the author in 2008. Further specimens collected by Lhwyd, which are probably the same, are present in the herbarium of Oxford University (OXF) (Edgington, 2013). Lhwyd’s specimens were also sent to John Ray and details were included in Ray’s *Synopsis* (Ray, 1724). Lhwyd’s record and others from Snowdonia were later included by Sir J.E. Smith (1828; 1834) in his *English Flora*, but under *Cystea regia* Smith, possibly the first, but illegitimate, binomial name given to this fern, which is discussed later in this paper.

The Snowdonia *Cystopteris* would meet the I.U.C.N. Threat Category of ‘critically endangered’, and is especially vulnerable to collecting, therefore precise details of its locations are withheld, but otherwise its presently known records are as follows:

Snowdon, SH65, Caernarvonshire, V.C.49 circa 1688, Edward Lhwyd (Sloane herb., BM; OXF)

Snowdon, SH65, V.C.49, 1824, collector unknown, herb.Rev. William Newbould as *C. fragilis* (BM)

Snowdon, 1826, William Wilson (?herb. J.T.I. Boswell-Syme), as *Cyathea regia* (in the *C. fragilis* folders) (BM)

Snowdon, 1847, collector uncertain, herb. F.J. Hanbury, as *C. fragilis* var. (BM)

Near Glyder Fawr (Cwm Idwal), SH65, V.C.49, 1853, collector’s name illegible, in *C. alpina* folder (BM)

Cwm Glas, Snowdon, 1874, collector uncertain (same sheet gives H. & J. Groves also W.R. Linton), as *C. fragilis* (BM)

Snowdon, 1890. J. Lloyd Williams, in herb. Arthur Bennett, as *C. alpina* (later renamed *C. fragilis*) (BM)


In several cases not all of the specimens on the sheets for the above records were the same taxon; as few as a single specimen conformed to the Snowdonia *Cystopteris* in question in at least one case. A single specimen on the sheet which represents the 1853 record given above, and those representing the 1853 record are morphologically identical to the others, but require to be re-examined for the presence of glandular hairs. Glandular hairs were present on specimens representing the remaining seven records given above.

A specimen marked ‘W.J. Hooker’ in BM, but undated, was collected at Castell Dinas
Bran, Denbighshire, V.C.50, and named *Cyathea regia* by Hooker, but later renamed as a variety of *C. fragilis*. This record was listed by Tennant (2010), but is not the Snowdonia Cystopteris for which records are given above. This interesting specimen bears resemblance to those found in northern Scotland, which were mentioned in the Introduction, and require further investigation. A careful search by the author at Castell Dinas Bran and adjacent rocks failed to locate Hooker’s fern, which is probably extinct there. Similarly, a recent but lesser search in Cwm Idwal did not reveal the Snowdonia Cystopteris taxon.

Following the discovery that the Snowdonia taxon was glandular, it was drawn to the attention of the author by another botanist, Brian Burrow, that *Flora Iberica* (Prada, 1986) describes an infra-specific form of *C. fragilis* that is distinguished by the presence of glandular hairs, namely subspecies *huteri* (Hausm. ex Milde) C. Prada & Salvo. The description, illustration and spore size given (44-55 (60) microns) for this subspecies matched those of the Snowdonia Cystopteris very well. Subsp. *huteri* is a taxon found mainly in montane regions of southern and western mainland Europe, with a general distribution similar to that of *C. alpina* (Lam.) Desv., and has been linked to the latter in Scandinavia (F.J. Rumsey, pers. comm.), although the extent of its further geographic limits is not certain.

In 2014 and 2015 fieldwork was carried out by the author in the central Italian Dolomites, which is the centre of distribution of subsp. *huteri* (F.J. Rumsey, pers. comm.). Subsp. *huteri* was found to be frequent there, enabling a comparison to be made with the Snowdonia Cystopteris, and material was donated to the NHM for further study by F.J. Rumsey. In the Dolomites *C. fragilis* subsp. *huteri* occurred mainly between 1600 and 2400m on dolomitic limestone in open, rocky, alpine woodland, extending to boulders and scree at the base of cliffs well above the tree-line, both habitats with a very rich alpine vascular flora. The other ferns often present were *Cystopteris fragilis* subsp. *fragilis*, *C. alpina*, *C. montana* (Lam.) Desv., *Gymnocarpium Robertianum* (Hoffm.) Newman, *Asplenium trichomanes* L. subsp. *quadralens* D.E. Meyer emend. Lovis, *A. trichomanes-ramosum* L., *A. ruta-muraria* L. (including subsp. *dolomiticum*) and occasionally *Woodsia glabella* R. Br. ex Richards. *Cystopteris fragilis* subsp. *huteri* in the Dolomites was more variable than the Snowdonia fern, but close matches to the latter were found there, the majority being intermediate between *C. fragilis* and *C. alpina* in general appearance. F.J. Rumsey has agreed that these Snowdonia ferns should be referred to *C. fragilis* subsp. *huteri*.

A very small population of another morphologically distinctive *Cystopteris* was also found by the author in the Dolomites, very locally at 2300m, and this matched the fern mentioned in the Introduction that occurred in Scotland (Figures 3 & 4). The herbarium studies at RBGE (E) showed that this Scottish fern had been collected earlier there, and was re-found in Orkney, V.C. 111, in 2010, and later in 2014 by the author in West Ross, V.C. 105, then cultivated from spores. Morphologically similar ferns had also been seen by the author in the French Vanoise and Norwegian Alps. All of these ferns are somewhat intermediate in their morphology between *C. fragilis* and *C. alpina*. Those from mainland Europe occur there with *C. alpina* s. str., although often at lower altitudes, and at least some of these are currently referred to *C. alpina*. Living examples from both recently found localities in Scotland that resemble the ferns from mainland Europe have been donated to NHM for biosystematic analyses in order to study their phylogenetic relationships and to establish ploidy levels.
DISCUSSION

Various authors have commented on the difficulties of diagnosing taxa in the *Cystopteris fragilis* complex by use of morphological characters alone, for example Vida (1974) and Rothfels et al. (2013). Blasdell (1963), however, had successfully developed an evolutionary structure, which in most cases showed a good correlation with recent phylogenetic analyses (Rothfels et al., 2013). The main problems arise because of multiple ancestral origins, the existence of many polyploids, and in Europe, the absence of the ancestral diploid parent. In Europe *C. fragilis* is tetraploid, hexaploid, or octoploid, but in the British Isles the last is so far unknown. Intermediate ploidy levels have arisen through intraspecific hybridisation, and pentaploid examples are said to be frequent (Vida, 1974). It should therefore be emphasised that the main conclusion in this paper, namely the recognition of *C. fragilis* subsp. *huteri* in the British Isles, is very largely based on morphological characters. In this case, however, the morphology of this Snowdonia fern is quite distinct from other British variants of *C. fragilis*; the spores are exceptionally large, and the glandular condition is presently a unique character in European *Cystopteris*. These characters provide the confidence to refer the Snowdonia *Cystopteris* to this European taxon.

As stated above, diploid *Cystopteris* is not recorded in Europe, but in North America several glandular species of *Cystopteris* are known and their glandular condition has resulted from the donation of a maternal genome from the diploid plant, *C. bulbifera* (L.) Bernh. (Rothfels et al., 2003). One glandular North American allohexaploid species, *C. laurentiana* (Weatherby) Blasdell, which possibly arose as a cross between *C. fragilis* and *C. bulbifera*, has been suggested to be synonymous with *C. fragilis* subsp. *huteri* (F.J. Rumsey, pers. comm.), but that would imply that *C. bulbifera* at one time had occurred in Europe, which at present is not proven.

The Snowdonia fern here referred to *C. fragilis* subsp. *huteri* is clearly responsible for all of the previously published erroneous records in Wales for *C. alpina* s. str. (but see comments above in Results, and below regarding a Welsh fern named by W.J. Hooker). The Snowdonia fern does share some characters found in *C. alpina*, which may have led to the confusion, namely the close similarity of its juvenile sporophytes and the morphology of its immature pinnules (see under Results above and Tennant (2010)). These characters probably arose from an ancestral genome donated from *C. alpina*, which may therefore have once occurred in Wales. The very large spore size given for *C. fragilis* subsp. *huteri* in *Flora Iberica* was suggested by the authors of this publication to indicate that this taxon is probably octoploid. Spores of Snowdonia examples were found to be similar in size (F.J. Rumsey, pers. comm.), suggesting that these ferns were likely to be at least hexaploid, if not octoploid, a ploidy level not recorded for *C. fragilis* in the British Isles. Blasdell (1963) had suggested that spore size in *Cystopteris* could be used in the determination of the different cytotypes, but Vida (1974) found that, contrary to that opinion, spore size was not always reliable in this respect. R.H. Roberts measured the spores of two different examples of *C. alpina* for the author. These both had almost identical finely dissected fronds with linear pinnule segments that are associated with classic *C. alpina* s. str. (Figure 3), but were found to differ widely in spore size (Tennant, 1995), supporting Vida’s findings, which require further investigation using the various cytotypes. Vida (1974) also found that a deliberately induced apogamous diploid of *C. fragilis* had a much more finely dissected frond than the natural tetraploid plants from which it had been derived, and that can clearly be seen in his Plate 2, fig. 9, which might suggest that degree of frond dissection may not necessarily increase with an increasing
ploidy level.

Historic *Cystopteris* nomenclature used by British authors is very relevant to *C. fragilis* subsp. *huteri* from Snowdonia. This particularly applies to the names ‘*incisa*’, ‘*regia*’ and ‘*alpina*’, and is also relevant to the related taxa from Scotland and other taxa from Wales, which are discussed earlier in this paper. Smith (1794) in Volume 3, p.163 published the name *Cyathea incisa* Sm., ‘Laciniated Cup-fern’ when describing and illustrating a fern collected pre 1790 by E. Forster at a single locality near Walthamstow in South Essex, V.C. 18. Smith stated that this fern was not the *Polypodium regium* of Linnaeus and was also different from *P. alpinum* Jacquin. Smith’s description of this fern gives ‘frond bipinnate’, (the pinnae lobes) ‘obtuse with no bristly point’, the last character clearly seeming to distinguish it from the Snowdonia subsp. *huteri*. Smith (1828; 1834) later published the name *Cystea regia* Sm., and here he cited Lhwyd’s late 17th century Snowdon record, two further records from Snowdon, namely one by the Rev. Hugh Davies in the early 19th century and one by W. Wilson in 1826, and a further record by Mr. Griffith from Cwm Idwell (Idwal), which all almost certainly referred to the Snowdonia subsp. *huteri*. Smith, however, surprisingly also added here the Essex fern (*Cyathea incisa* and *C. regia* Forster), thus seeming to suggest that these were synonymous with the Snowdonia ferns, even though their descriptions differed. Smith also stated that *Cystea regia* Sm. was unquestionably distinct from every other British fern, and from the ‘*regia*’ of alpine stations (in Europe), presumably meaning the fern now referred to *Cystopteris alpina* (Lam.) Desv., although confusingly he included *Aspidium regium* Sw. (1802) in the synonymy.

W.J. Hooker (1842) then grouped together all of Smith’s taxa under *C. alpina* Desv., and added ‘tripinnate’ to Smith’s earlier ‘bipinnate’ description of these taxa. Hooker had earlier (before 1842) confirmed the name of one of the specimens from Essex collected by Forster as *Cyathea regia* (Stansfield, 1930), and in the same period had also given the same name to an undated specimen in BM that had been collected in Denbighshire. V.C. 50. These Essex and Denbighshire records, in the sense of Hooker (1842), therefore represent the first and second British records respectively for *C. alpina*. J. Backhouse’s record from Upper Teesdale in Yorkshire, V.C. 65 in 1872 (Tennant, 2010) would therefore be the third British record. In spite of Smith (1828; 1834) and Hooker (1842) including the Snowdonia ferns under the species which is now referred to *C. alpina* (Lam.) Desv., *C. fragilis* subsp. *huteri* from Snowdonia is shown here to be different, and its first name, *Cystea regia* sensu Sm. (parte) should be retained only in synonymy.

The use of the name ‘*regia*’ for some of these ferns is, however, incorrect on the assumption that the name ‘*regia*’ was entirely derived from *P. regium* L. The type sheet of *P. regium* in LNN contains more than one species and *P. regium* was therefore a ‘nomen ambiguum’. None of the species on this sheet represent the taxa discussed in this paper, including *C. alpina* (Lam.) Desv.. This has long been known (Stansfield, 1929), and much earlier, for example *P. regium* L. was included by J.T. Syme in the synonymy under *C. fragilis* in the third edition of *English Botany*, Vol. 12 (1886), which would be correct, although an individual specimen still requires to be selected from the type sheet and formally lectotypified as such.

Other authors also considered ‘*regia*’ to be distinct. Milde made ‘*regia*’ a variety of *C. alpina* (Kestner, 1930), and Stansfield (1929) proposed the name *C. alpina* var. *obtusa* for Forster’s *Cystopteris* from Essex. After examining the Essex specimen, Stansfield pointed out that it was bipinnate, with only shallowly notched pinnule lobes, many of
the veins terminating in an apical point, unlike *C. alpina* s.str., which is at least tripinnate with virtually all the veins ending in notches. Kestner (1930) had stated that the position of the vein-endings was the only reliable character in the determination of *C. alpina*, additionally the *Cystopteris* key given in Murphy and Rumsey (2005) similarly relies on this character for the determination of *C. alpina*.

The first name given to the Essex fern, *Cyathea incisa* Sm. (1794), would be a legitimate name at specific level, and for the Denbighshire *Cystopteris* as this appears to belong to the same taxon. It is doubtful, however, whether these ferns could be justified as a species distinct from *C. alpina*, in which case the name would be reduced to synonymy under the latter. The Essex and Denbighshire ferns are both thought to be extinct at their original localities. A similar Welsh taxon from Merionethshire, V.C. 48, has not been collected since 1928, so none of these ferns are presently available for biosystematic studies that might elucidate their correct status. The *Cystopteris* taxon from Scotland discussed briefly above also seems to have affinities with *C. alpina*. Two examples of this and a morphologically identical fern from Italy are in cultivation by the author, and material is now at NHM for further studies to establish their status.

Several recently confirmed specimens of *C. alpina* seen by the author in the Tromsø Museum herbarium had exceptionally broad pinnule lobes. Following the fieldwork by the author, uniformly similar *Cystopteris* forms were also encountered in alpine regions of France, Italy, and in Scotland, some of which are presently determined as *C. alpina* (Lam.) Desv. This might suggest that the concept of *C. alpina* s.str. as a fern restricted to one with a finely dissected frond and linear pinnule lobes, and with the veins virtually all ending in notches, unlike the ‘regia’ of some authors, may be one that is too narrow. An alternative suggestion may be that these ferns with broader pinnules truly represent a distinct taxon, which is affiliated to *C. alpina* through the presence of a genome from this species. Hopefully biosystematics will resolve this.

*Cystopteris fragilis* subsp. *huteri* is presently known in Britain only from two localities in North Wales, where it is very local and scarce. It meets the I.U.C.N. Threat Category of Critically Endangered (CR) and should be considered for inclusion in the British Red Data List, its main vulnerability being one of collecting.

ACKNOWLEDGEMENTS

I would especially like to thank Alison Evans for assistance in the preparation of the manuscript, Clive Stace for general advice, Brian Burrow for information on the Spanish publication, Clive Jermy for details of previous papers, Eric Meek for organising collection of specimens from one of the Scottish sites, Fred Rumsey for information, comments, opinions, and undertaking the preliminary studies at NHM, Arve Elvebakk, Torstein Engelskjøn and Douglas McKean for access to herbarium material, and John Ratcliffe at the Countryside Council for Wales for permission to collect material.

REFERENCES


JERMY, A.C. & HARPER, L. 1971. Spore morphology of the *Cystopteris fragilis*
INSTRUCTIONS FOR AUTHORS

PAPERS should not usually exceed 20 printed pages and are generally expected to be considerably shorter. As well as reports of original research, review articles and short notes, e.g. new records, are encouraged. The senior author should supply an email address to facilitate correspondence.

REVIEWS should cover recent developments in any aspect of research on ferns and lycopsods. This can include unpublished research by the author, and/or the possible direction of future research. They should be aimed at the general reader who may not be an expert in the chosen topic. Proposals for a review should be discussed first with the review editors.

MANUSCRIPTS should be submitted in English (British) in electronic format in 10-point Times New Roman font and single spaced. Electronic versions of text and tables should be compatible with WORD, with figures as TIFF files, and sent as email attachments. All manuscripts will be refereed.

THE TITLE should reflect the content of the paper and be in BOLD CAPITALS (11-point) and centrally aligned. Generic and specific names should be in italics and any title containing a generic or specific name should be followed by the family name in brackets e.g. **TRICHOMANES SPECIOSUM** (**HYMENOPHYLLACEAE**) IN SOUTHERN SPAIN

AUTHOR ABBREVIATIONS should follow Pichi Sermolli’s (1996) Authors of scientific names in Pteridophyta, Royal Botanic Gardens, Kew.

MAIN HEADINGS: should be in BOLD CAPITALS (10-point) and centrally aligned.

SUBSIDIARY HEADINGS: should be in bold, the first letter of each word in capitals, the rest in lower case and left-aligned.

AUTHORS’ NAMES AND FULL ADDRESSES (including email address): follow the title and are centrally aligned.

KEY WORDS: up to ten.

ABSTRACT: should reflect the content of the paper.

FIGURES: there is no distinction between photographs and line drawings in numbering. All should be supplied in mono or colour, TIFF format, 300 dpi and CMYK. (please contact the Production Editor with queries), with a scale bar where appropriate. Lettering or numbers (Arabic) should be in the bottom left using uppercase Times Roman. Figure captions should be on a separate sheet.

TABLES: can be printed in either portrait or landscape format. Authors should consider this when preparing tables. Authors should ensure that tables fit the printed page size in a legible form.

PAGESIZE: The Gazette has a useable page size of 123 × 204 mm. This can be used in portrait or landscape mode but any supplied artwork should not be larger than this.

MEASUREMENTS: follow the metric system.

CHECKLISTS: follow the format of Baksh-Comeau, Fern Gaz. 16(1, 2): 11- 122.

REFERENCES: follow the style of a recent issue of The Fern Gazette, e.g.:-


JOURNAL ABBREVIATIONS: should follow Botanico Periodicum Huntianum & Supplements.

Alterations from the original text at proof stage will be charged for unless they are minor points of detail. A pdf will be provided free to the senior author.
TAXONOMIC SURVEY OF OCCURRENCE, DIVERSITY AND ETHNOBOTANY OF PTERIDOPHYTES IN SOME PARTS OF NASARAWA STATE, NIGERIA

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Key words: Pneumatopteris afra, Nasarawa, Pteridophytes, Taxonomic survey

ABSTRACT

The study was carried out to investigate the taxonomic occurrence of Pteridophytes in Lafia and Doma Metropolis of Nasarawa State, Nigeria. A total of 16 locations were sampled in Lafia and Doma respectively. These locations include wetlands, swamps, forest shade and trees. From these locations, ferns were collected, pressed and identified. Their relative abundances in each location were calculated and their ethnobotanical uses by the local dwellers were documented using structured questionnaires. Ferns were observed only in 12 locations in Lafia and nine locations in Doma. Seven species of ferns were identified namely, Pneumatopteris afra, Nephrolepis biserrata, N. undulata, N. cordifolia, Adiantum capillus-veneris, Adiantum sp. and Pityrogramma calomelanos. Pneumatopteris afra was found to be the most widely distributed fern. Of the ferns observed, terrestrial ferns had the highest occurrence (85.7%) in Lafia with only 50% in Doma. Pneumatopteris afra was found to have the highest relative frequency while Nephrolepis biserrata and N. undulata had the lowest relative frequency in Lafia and Doma. All these species have been reported by the local dwellers to have ethnobotanical uses such as in the treatment of ulcer, fever, typhoid, chest pain, stomach pain and diabetes. Also, Pityrogramma calomelanos had the highest percentage awareness by the local dwellers. It is concluded that Lafia is more rich and diverse in fern species than Doma. Locations where ferns were absent are due to the long-term alterations in the microclimatic conditions by various human activities in those places.

INTRODUCTION

In most parts of Africa ferns are more diverse in the eastern than in the western mountains (Moran & Smith, 2001). Ecological and physiological factors such as habitat, seasonality, elevation, soils, rainfall, cloud cover and temperature have been identified as the determinants for the diversity, richness and distribution of ferns in most parts of the world (Kornas, 1993).

Nigerian’s first biodiversity report stated that ferns and fern allies have been observed to grow in most ecological zones but different habitats (Akinsoji et al., 2016). Although, ferns have wide coverage in Nigeria, due to habitat change by factors such as unplanned urbanization, over-exploitation of minerals and farming, many species have been reduced, endangered or are on the verge of extinction (Dixit, 2000).

Pteridophytes are often restricted to specific micro-habits. However, as a result of serious threats faced by these plants coupled with the fact that they are underutilized...
and overlooked in this part of the world because of their relatively small size and crytogramic nature, there is need for adequate survey and documentation of their occurrences, locations and economic values. Therefore, this research aimed to investigate the occurrence, diversity and ethnobotany of ferns in Lafia and Doma metropolis, Nasarawa State, Nigeria.

MATERIALS AND METHODS
This study was carried out at Lafia and Doma Local Government areas of Nasarawa State, Nigeria. Habitats assessed for the various diversities of pteridophytes include river banks, streams, forest (under shade), arboreal (top of trees), swamps and some other wet lands. The study was conducted towards the end of rainy season in 2016 and early dry season of 2017. Plants specimens were collected using Random sampling method. Various fern species parts were collected which included leaflet, fronds and roots. The samples collected were all recorded in the field. Representative of all collected fern species or specimens, were also photographed. These specimens were later used for identification and to provide permanent records for future use. The geographical coordinates of each sampling location were taken by using a GPS device.

Mature fern species were collected in each location and pressed, dried, poisoned then mounted on standard herbarium sheets and classified according to standard procedures. Identifications were made with the aid of taxonomic literature (Oloyede & Odu, 2011). In the field proper care was taken in selecting the plant materials to enable correct identification. Specimens were collected in a good condition, free of insect damage or diseases. Voucher specimens are preserved in the Department of Botany Herbarium, Federal University, Lafia.

Ethnobotanical Uses
A total of ninety (90) structured questionnaires were administered to local dwellers of all the locations visited to gather information on the knowledge of the plants by the people, the uses of the plants and the part of the plant that had been used. The relative frequency of occurrence of each fern species was determined to assess the distribution of the species.

\[
RF = \frac{\text{No of occurences of individual fern species}}{\text{No of occurences of all ferns}} \times 100
\]

Sørensen Similarity Coefficient was calculated to indicate their floristic similarities between the two towns (Lafia and Doma) as follows:

\[
Sc = \frac{2W}{a + b} \times 100
\]

where Sc is the similarity coefficient, W is the species occurring in both communities under consideration, a is the number of species occurred in Lafia and b is the number of species occurred in Doma.

The relative abundance of the ferns at each site was determined according to Bongers et al. (1988) and Kayode (1999): less than five individuals as Rare; 5 to 10 as Occasional; 11 to 30 as Frequent; 31 to 100 as Abundant and over 100 individuals as Very Abundant.

RESULTS
Ferns occurrence and distribution in Lafia
A total of 16 locations were sampled in Lafia, of which ferns were observed in only 12
Table 1: Sampling Locations In Lafia L.G.A

<table>
<thead>
<tr>
<th>S/N</th>
<th>DESCRIPTION OF LOCATION</th>
<th>LOCATION CODE</th>
<th>LATITUDE</th>
<th>LONGITUDE</th>
<th>PRESENCE / ABSENCE OF FERNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Akurba osanya</td>
<td>AKB</td>
<td>8.47104N</td>
<td>8.58747E</td>
<td>Present</td>
</tr>
<tr>
<td>2</td>
<td>Opposite triple Z farm</td>
<td>OTF</td>
<td>8.45695N</td>
<td>8.57489E</td>
<td>Present</td>
</tr>
<tr>
<td>3</td>
<td>Gandu</td>
<td>GAN</td>
<td>8.45116N</td>
<td>8.57304E</td>
<td>Absent</td>
</tr>
<tr>
<td>4</td>
<td>Agudu</td>
<td>AGD</td>
<td>8.51771N</td>
<td>8.52483E</td>
<td>Absent</td>
</tr>
<tr>
<td>5</td>
<td>Akunzan tabori</td>
<td>AKTR</td>
<td>8.4105N</td>
<td>8.56368E</td>
<td>Present</td>
</tr>
<tr>
<td>6</td>
<td>Beside college of Agric</td>
<td>BCA</td>
<td>8.55723N</td>
<td>8.54292E</td>
<td>Present</td>
</tr>
<tr>
<td>7</td>
<td>Opposite college of Agric</td>
<td>OCA</td>
<td>8.51061N</td>
<td>8.52016E</td>
<td>Absent</td>
</tr>
<tr>
<td>8</td>
<td>Inside college of Agric</td>
<td>ICA</td>
<td>8.53292N</td>
<td>8.53659E</td>
<td>Present</td>
</tr>
<tr>
<td>9</td>
<td>Between lafia and shabu river</td>
<td>B/W LSSR</td>
<td>8.58883N</td>
<td>8.5559E</td>
<td>Present</td>
</tr>
<tr>
<td>10</td>
<td>Between lafia shabu river</td>
<td>B/W LSSR2</td>
<td>8.56688N</td>
<td>8.54802E</td>
<td>Present</td>
</tr>
<tr>
<td>11</td>
<td>Shabu one</td>
<td>SHBI</td>
<td>8.57557N</td>
<td>8.55119E</td>
<td>Present</td>
</tr>
<tr>
<td>12</td>
<td>Shabu two</td>
<td>SHB2</td>
<td>8.56688N</td>
<td>8.54802E</td>
<td>Present</td>
</tr>
<tr>
<td>13</td>
<td>Shabu three</td>
<td>SHB3</td>
<td>8.57405N</td>
<td>8.55062</td>
<td>Present</td>
</tr>
<tr>
<td>14</td>
<td>Akunzan tabo well</td>
<td>AKTW</td>
<td>8.49018N</td>
<td>8.53358E</td>
<td>Present</td>
</tr>
<tr>
<td>15</td>
<td>FUL Auditorium</td>
<td>FUL AUD</td>
<td>8.47066N</td>
<td>8.58688E</td>
<td>Present</td>
</tr>
<tr>
<td>16</td>
<td>Tudun amba</td>
<td>TUA</td>
<td>8.49216N</td>
<td>8.50355E</td>
<td>Absent</td>
</tr>
</tbody>
</table>
Table 2: The locations occupied by the ferns and the respective abundance status in Lafia

<table>
<thead>
<tr>
<th>S/N</th>
<th>Species</th>
<th>Location / Abundance status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AKB</td>
</tr>
<tr>
<td>1</td>
<td>Adiantum sp.</td>
<td>X</td>
</tr>
<tr>
<td>2</td>
<td>Adiantum capillus-veneris</td>
<td>X</td>
</tr>
<tr>
<td>3</td>
<td>Pneumatopteris afra</td>
<td>√ VA</td>
</tr>
<tr>
<td>4</td>
<td>Nephrolepis bisserata</td>
<td>X</td>
</tr>
<tr>
<td>5</td>
<td>Nephrolepis cordifolia</td>
<td>X</td>
</tr>
<tr>
<td>6</td>
<td>Nephrolepis undulata</td>
<td>X</td>
</tr>
<tr>
<td>7</td>
<td>Pityrogramma calomelanos</td>
<td>X</td>
</tr>
</tbody>
</table>

KEY: √ means present, X means absent  
A – abundant, VA – very abundant, F – frequent, O – occasional, R – rare
Figure 1: Relative frequency of Fern species in Lafia town

Figure 2: Relative frequency of Fern species in Doma town
Table 3: Sampling Locations in Doma LGA

<table>
<thead>
<tr>
<th>S/ N</th>
<th>DESCRIPTION</th>
<th>LOCATION CODE</th>
<th>LATITUDE</th>
<th>LONGITUDE</th>
<th>PRESENCE / ABSENCE OF FERNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Omenza 1</td>
<td>Omz1</td>
<td>8.39514N</td>
<td>8.35005E</td>
<td>Present</td>
</tr>
<tr>
<td>2</td>
<td>Omenz2</td>
<td>Omz2</td>
<td>8.39017N</td>
<td>8.34801E</td>
<td>Present</td>
</tr>
<tr>
<td>3</td>
<td>Osota1</td>
<td>OST1</td>
<td>8.39013N</td>
<td>8.3481E</td>
<td>Absent</td>
</tr>
<tr>
<td>4</td>
<td>Osota 2</td>
<td>OST2</td>
<td>8.39278N</td>
<td>8.35039E</td>
<td>Absent</td>
</tr>
<tr>
<td>5</td>
<td>GSS Doma</td>
<td>GSSD</td>
<td>8.39188N</td>
<td>8.35039E</td>
<td>Present</td>
</tr>
<tr>
<td>6</td>
<td>Govt. college Doma</td>
<td>GCD</td>
<td>8.39024N</td>
<td>8.35762E</td>
<td>Absent</td>
</tr>
<tr>
<td>7</td>
<td>Arumangye</td>
<td>ARG</td>
<td>8.39004N</td>
<td>8.35762E</td>
<td>Absent</td>
</tr>
<tr>
<td>8</td>
<td>Federal Science and Technical College</td>
<td>FSTC</td>
<td>8.39131N</td>
<td>8.34863E</td>
<td>Absent</td>
</tr>
<tr>
<td>9</td>
<td>Ogbobula1</td>
<td>OGB1</td>
<td>8.39695N</td>
<td>8.34863E</td>
<td>Present</td>
</tr>
<tr>
<td>10</td>
<td>Ogbobula2</td>
<td>OGB2</td>
<td>8.39074N</td>
<td>8.34881E</td>
<td>Absent</td>
</tr>
<tr>
<td>11</td>
<td>Okpuripu</td>
<td>OKPU</td>
<td>8.39145N</td>
<td>8.3498E</td>
<td>Absent</td>
</tr>
<tr>
<td>12</td>
<td>Okussupa</td>
<td>OKSP</td>
<td>8.3906N</td>
<td>8.34861E</td>
<td>Present</td>
</tr>
<tr>
<td>13</td>
<td>Ipuigigu</td>
<td>IPG</td>
<td>8.39102N</td>
<td>8.3492E</td>
<td>Present</td>
</tr>
<tr>
<td>14</td>
<td>GGSSD formal hostel</td>
<td>GGDFH</td>
<td>8.4043N</td>
<td>8.36809E</td>
<td>Present</td>
</tr>
<tr>
<td>15</td>
<td>Campo 1</td>
<td>CMP1</td>
<td>8.46461N</td>
<td>8.43512E</td>
<td>Present</td>
</tr>
<tr>
<td>16</td>
<td>Campo 2</td>
<td>CMP2</td>
<td>8.44435N</td>
<td>8.41107E</td>
<td>Present</td>
</tr>
</tbody>
</table>
Table 4: The locations occupied by the ferns and the respective abundance status in Doma

<table>
<thead>
<tr>
<th>S/ N</th>
<th>Species</th>
<th>OMZ1</th>
<th>OMZ2</th>
<th>OST1</th>
<th>OST2</th>
<th>GSSD</th>
<th>GCD</th>
<th>ARG</th>
<th>FSTC</th>
<th>OGB1</th>
<th>OGB2</th>
<th>OKPU</th>
<th>OKSP</th>
<th>IPG</th>
<th>GGFH</th>
<th>CMP1</th>
<th>CMP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Pneumatopteris afra</em></td>
<td>√ VA</td>
<td>√ A</td>
<td>X</td>
<td>X</td>
<td>√ F</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>√ A</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>√ VA</td>
<td>√ A</td>
</tr>
<tr>
<td>2</td>
<td><em>Nephrolepis undulata</em></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>√ VA</td>
<td>√ VA</td>
<td>√ O</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

KEY: √ means present, X means absent
A – abundant, VA – very abundant, F – frequent, O – occasional, R - rare
Table 5: Ethnobotanical uses of the fern species in Lafia

<table>
<thead>
<tr>
<th>S/N</th>
<th>SPECIES</th>
<th>% AWARENESS</th>
<th>ETHNOBOTANICAL USES</th>
<th>PARTS USED</th>
<th>MODE OF PREPARATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Pneumatopteris afra</em></td>
<td>90</td>
<td>Typhoid, fever, headache, ulcer, piles, stomach pain, strengthening of bones, healing of wounds caused by guinea worm</td>
<td>Leaflets, fronds, roots &amp; fronds, whole plants</td>
<td>boiling, Drying, drying &amp; boiling</td>
</tr>
<tr>
<td>2</td>
<td><em>Pityrogramma calomelanos</em></td>
<td>100</td>
<td>Enhancement of male sexual cells, treatment of chest pain, healing of stomach pain, fever, treating shortage of blood, reducing prolonged labour, breast cancer</td>
<td>Leaflets, fronds, leaflets</td>
<td>boiling, air drying, boiling</td>
</tr>
<tr>
<td>3</td>
<td><em>Nephrolepis undulata</em></td>
<td>30</td>
<td>Snake bite, convulsion, high blood pressure, poisoning, goitre, bone fracture, sickle cell anemia, diabetes</td>
<td>Whole plants, roots</td>
<td>boiling, drying, freshly pounded, boiling</td>
</tr>
<tr>
<td>4</td>
<td><em>Adiantum capillus-veneris</em></td>
<td>40</td>
<td>Convulsion, diabetes, headache, pile, Gonorrhea, scorpion bite, dysentery, Measles</td>
<td>Whole parts, roots &amp; fronds, leaflets only</td>
<td>air drying, freshly pounded, boiling, Boiling</td>
</tr>
<tr>
<td>5</td>
<td><em>Adiantum sp</em></td>
<td>33.3</td>
<td>fibroid, shortage of blood, diabetes, abnormal menstruation, stomach pain, piles, back pain, waist pain &amp; joint cases</td>
<td>whole parts</td>
<td>Boiling &amp; air drying, Boiling &amp; air drying</td>
</tr>
<tr>
<td>6</td>
<td><em>Nephrolepis cordifolia</em></td>
<td>20</td>
<td>sickler cases, high blood pressure, bone fracture, goiter, snake/scorpion bite, skin rashes &amp; diabetes, headache &amp; rheumatism</td>
<td>whole parts &amp; roots, leaf</td>
<td>Boiling, freshly pounded, air drying, air drying</td>
</tr>
<tr>
<td>7</td>
<td><em>Nephrolepis biserrata</em></td>
<td>30</td>
<td>Eyesight issues, bone fracture, goitre, high blood pressure, snake / scorpion bite &amp; diabetes</td>
<td>Whole parts &amp; roots</td>
<td>Boiling, freshly pounded &amp; drying</td>
</tr>
</tbody>
</table>
Table 6: Ethnobotanical uses of the fern species in Doma

<table>
<thead>
<tr>
<th>S/N</th>
<th>SPECIES</th>
<th>% AWARENESS</th>
<th>ETHNOBOTANICAL USES</th>
<th>PARTS USED</th>
<th>MODE OF PREPARATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Pneumatopteris afra</em></td>
<td>50</td>
<td>increases male sexual stamina</td>
<td>Leaf, stem &amp; whole parts</td>
<td>Boiling</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rheumatism, stomach pains, tuberculosis, fever</td>
<td>Leaf, stem &amp; whole parts</td>
<td>Boiling</td>
</tr>
<tr>
<td>2</td>
<td><em>Nephrolepis undulata</em></td>
<td>50</td>
<td>Healing of wounds, asthma, pneumonia, cough &amp; ulcer</td>
<td>Leaf, whole parts</td>
<td>Drying, boiling &amp; freshly pounded</td>
</tr>
</tbody>
</table>
Seven species of ferns were identified from the sampled locations in Lafia: *Pneumatopteris afra* (Christ) Holttum, *Nephrolepis undulata* (Afzel.) J.Sm., *Nephrolepis cordifolia* (L.) K.Presl, *Nephrolepis biserrata* (Sw.) Schott, *Adiantum sp.*, *Adiantum capillus-veneris* L. and *Pityrograma calomelanos* (L.) Link (Table 2). *Pneumatopteris afra* was found to be the most widely distributed fern in Lafia where it was found in seven locations. Of the ferns observed, terrestrial ferns were most frequent with 85.7% occurrence while epiphytes were least frequent at 14.3%. *Pneumatopteris afra* was found to have the highest relative frequency at 65% while *Nephrolepis biserrata* and *N. undulata* had the lowest (5%) (Figure 1). The similarity coefficient between the two towns is 22.2%.

### Ferns occurrence and distribution in Doma
In Doma, from a total of 16 locations sampled ferns were observed in only nine locations (Table 3). However, in Doma, only two species of ferns were recorded, one terrestrial and the other epiphytic, namely *Pneumatopteris afra* and *Nephrolepis undulata* (Table 4). *Pneumatopteris afra* was found to be the most widespread fern in Doma where it was found in six locations. The relative frequency of *Pneumatopteris afra* was greater (66.67%) than that of *N. undulata* (33.34%) (Figure 2).

### Ethnobotanical Uses of Ferns in Lafia
The results of the survey of ethnobotanical uses and awareness of the ferns in Lafia are presented in Table 5. Every species was discovered to have ethnobotanical uses among the local population. *Pneumatopteris afra* had 90% percentage awareness and it was used for treating ulcer, fever, typhoid and piles. *Pityrogramma calomelanos* had the highest percentage awareness (100%) and was used for enhancement of male sexual performance and treating chest pain. The lowest percentage awareness (20%) was recorded for *Nephrolepis cordifolia* which was used for treating high blood pressure and skin rashes.

### Ethnobotanical Uses of Ferns in Doma
In Doma, *Pneumatopteris afra* had 50% percentage awareness and was used for treating rheumatism, stomach pains and tuberculosis (Table 6). *Nephrolepis undulata* also had 50% percentage awareness and was used for treating wounds, asthma and pneumonia.

### DISCUSSION AND CONCLUSION
The occurrence of ferns in the two towns studied had only two species in common. As reported by Jones et al., (2011), some fern species are highly flexible and are found in nearly all habitats and also have various life forms. High species occurrence in areas with suitable environmental conditions showed that such species have adapted to the environment (Richard et al., 2000). Consequently, the relatively high number of ferns species found in Lafia compared with Doma might be the result from more favourable microclimatic conditions than in Doma, where conditions have been affected by human activities such as deforestation and farming. This is supported by Sumesh et al. (2014) who stated that human activities usually increase pressures on plants.

The occurrence of some species in Lafia such as *Pneumatopteris afra*, *Nephrolepis undulata* and *Adiantum capillus-veneris* with high relative frequencies suggests that these species will persist in this area if the environmental conditions remain favourable. But species like *Adiantum sp.*, *Nephrolepis cordifolia*, *Nephrolepis biserrata* and *Pityrogramma calomelanos* with low relative frequencies are more likely to become
threatened in the future. Also in Doma town, only two ferns were recorded, and *Pneumatopteris afra* was more frequent than *Nephrolepis undulata*. The paucity and often total absence of ferns in some locations sampled in Lafia (four sites) and Doma (seven sites) is a clear indication of the accumulated long-term anthropogenic activities in the areas resulting in loss of ferns species at these sites.

It is concluded that, in comparison, Lafia has a more rich and diverse ferns flora than Doma, and that despite the presence of relatively few species, all have been used by the local dwellers for various economic purposes. Therefore, conservative measures should be put in place by the Government to avoid future extinction of these ferns.

**REFERENCES**


SUMESH, N.D., SUBASH, M.D.C. & RAMACHANDRA, T.V. 2014. Conservation of Pteridophytes to maintain vital link between lower and higher group of plants. Energy and Wetlands research group, center for Ecological Sciences, Indian Institute of Science, Bangalore, pp. 560012
ERRATUM

The correct title and citation of this article should be:
PHYTOSOCIOLOGICAL NOTES ON THE FERN-MEADOW VEGETATION OF MID-WEST SCOTLAND AND THE NETHERLANDS

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Keywords: Argyll, Melampyro-Holcetea mollis, Dryopteris, Oreopteris, climate, Luzulo luzuloides-Thelypteridetum limbospermae, phytosociology

ABSTRACT
On the basis of five phytosociological relevés, the fern-meadow vegetation with Oreopteris limbosperma and Dryopteris cambrensis from the region around Oban, Argyll, West Scotland is described and compared with similar communities in the Netherlands and West Germany. It is concluded that these fern-meadows can be assigned to the Luzulo luzuloides-Thelypteridetum limbospermae, which in Central and continental West Europe is restricted to forest edges. In West Scotland this community is also common in open landscapes, due to the hyperatlantic climate. In comparison with the fern communities in the Netherlands and Germany, the Scottish examples are very rich in fern species, illustrating the optimal development of the community in West Argyll. Possible future development of this community in the Netherlands are discussed in the light of the increase in records of Oreopteris limbosperma and the ecological requirements of Dryopteris cambrensis.

INTRODUCTION
For a continental phytosociologist, one of the striking features of the vegetation of the coastal lowlands of West-Argyll is the omnipresence of ferns in open landscapes (Figure 1). Well-known is the invasive character of Pteridium aquilinum (L.) Kuhn in extensively managed grasslands all over the British Isles (Rodwell et al., 1992), but many other fern species are found in the dense tall-forb vegetation on slopes in the hyperatlantic lowland around Oban. After Pteridium, probably Blechnum spicant (L.) Sm. is the most frequent species, but also Dryopteris cambrensis (Fraser-Jenk.) Beitel & W.R.Buck can be found on virtually every slope and this species is much more abundant than Blechnum. Other species which can be found often are, in no particular order, Dryopteris filix-mas (L.) Schott, D. borreri (Newm.) Newm. ex Oberh. & Tavel, D. dilatata (Hoffm.) A.Gray, D. carthusiana (Vill.) H.P.Fuchs, Athyrium filix-femina (L.) Roth, Phegopteris connectilis (Michx.) Watt, and Oreopteris limbosperma Holub.

In West-Europe, fern-dominated forest edge communities from lowland, hills and lower mountain areas were recently placed in a newly described alliance, the Holco mollis-Athyriion felicis-feminae (Royer et al.) Haveman & Weeda (class Melampyro-Holcetea mollis Passarge ex Weeda & Haveman; Weeda & Haveman, 2017). The first author who drew the attention to these fern-rich forest edge communities was Wittig (2000), who described Oreopteris-rich forest-edges from the German lower mountain ranges in the Rothhaargebirge, Taunus, and Schwarzwald as Luzulo luzuloides-Thelypteridetum limbospermae Wittig. Apart from Oreopteris limbosperma (syn.:
Thelypteris limbosperma (All.) H.P.Fuchs also Athyrium filix-femina, Phegopteris connectilis, Blechnum spicant, Gymnocarpium dryopteris (L.) Newman, Dryopteris carthusiana, D. dilatata, and D. filix-mas are fern species mentioned for this vegetation type. This Oreopteris-community is not strictly oreophytic (growing in the mountain belt): Bremer (2016) and Weeda & Haveman (2017) recorded the Luzulo-Thelypteridetum also from the Netherlands, i.e. from the lowlands of Northwest-Europe. Rodwell et al. (1992) described a similar community as Thelypris limbosperma-Blechnum spicant community (U19) from moist base-poor peaty soils on steep, sheltered banks at low to moderate altitudes through the wetter west and north of Britain. Remarkably enough, other fern species are rare in this community: only Polypodium vulgare L. and Athyrium distentifolium Tausch ex Opiz are mentioned in the table published by these authors. Weeda & Haveman (2017) included this British community in the Luzulo-Thelypteridetum.

During a three-weeks summer holiday in July 2017, I was able to study some of the fern-meadow communities in the wider surroundings of Oban, West-Argyll, Scotland. I was especially interested in the communities with the alleged mountain-dwelling Oreopteris limbosperma and Dryopteris cambrensis, because the latter is very rare in the Netherlands. From the species composition, it was immediately clear that the Scottish fern-meadow community bears a very close resemblance to the Luzulo-Thelypteridetum, but also that it had characteristic features of its own. In this paper, I will describe this community on the basis of a few phytosociological relevés. It will be compared with the continental fern communities of the aforementioned Holco-Athyrion and more in particular the Luzulo-Thelypteridetum, in order to gain a better understanding of these communities, both in hyperatlantic Scotland and the subatlantic Netherlands.

Figure 1. Fern-meadow on a lowland hillside in West-Argyll near Loch Fyne (photo: R. Haveman)
METHODS

On July 6th and 7th 2017, I made five phytosociological relevés of fern-rich forest-edges in Ardcastle Forest at the shore of Loch Fyne and the area of Glencruitten House just outside Oban, according to the methods of the French-Swiss school (Mueller-Dombois & Ellenberg, 1974; Westhoff & Van Der Maarel, 1978). In floristically homogeneous parts of the vegetation of 8-20 m², all species were recorded, and their abundance was estimated using the 9-level extended scale of Braun-Blanquet (Westhoff & Van Der Maarel, 1978) in every single layer (tree, shrub, field, and moss layer). The relevés were digitised and stored in TurboVeg (Hennekens & Schaminée, 2001) in the personal database of the author. After export from TurboVeg, the relevés were imported in JUICE (Tichý 2002) for further analysis. In JUICE, all species were ordered according to their phytosociological position according to Weeda & Haveman (2017). Both a full and a (heavily abbreviated) synoptic table (in frequency percentages) of constant species (frequency > 40%) were exported, and the synoptic table compared with the synoptic tables published by Weeda & Haveman (2017) and Wittig (2000) by arranging them in a longitudinal order (Scotland—the Netherlands—Germany). In the full table, all pteridophytes are included, as well as all species occurring in more than one relevé, or in one relevé with a cover ≥ 5%. In the synoptic tables, only the species with a frequency > 10% are included in at least one column.

RESULTS

In the Scottish relevés (Table 1), 11 fern species were recorded in total, and one fern hybrid. The most frequent fern species in the table is Blechnum spicant (in all five relevés), followed by Oreopteris limbosperma, and Dryopteris cambrensis (both in four relevés). In all relevés, Dryopteris species have an abundance prevalence over the other fern species. Other species recorded in at least four out of five relevés are Luzula sylvatica (Huds.) Gaudin, Holcus mollis L., Hylocomium splendens (Hedw.) Schimp., Rhytidiadelphus squarrosus (Hedw.) Warnst. and Polytrichum formosum Hedw.. In all relevés, more than one fern species was found, and relevé 1 in Table 1 is especially rich in ferns: in the vegetation sample of 20 m² 9 pteridophytes were recorded.

There are clear differences between the Scottish fern-communities with Oreopteris limbosperma and Dryopteris cambrensis (Table 2, column 1), and the Luzulo-Thelypteridetum in the Netherlands (Table 2, column 2) and Germany (Table 2, column 3). Especially striking is the richness in ferns in the Scottish community: on average, 6.2 fern species are recorded in the Scottish relevés, a number that declines strongly when going to the suboceanic regions in the Netherlands and Germany (viz. 3.9 and 3.4 respectively). Also their absolute number in the Scottish dataset is higher than in the other two (12, vs. 7 and 9 respectively), although the number of Scottish relevés is evidently lower than in the other two groups. Dryopteris cambrensis, D. borreri and Pteridium aquilinum are exclusively or, in case of the latter, mainly recorded in the Scottish relevés, although it cannot be ruled out that Dryopteris affinis Fraser-Jenk., mentioned in one relevé from Germany, is in fact D. borreri. Also Blechnum spicant and Dryopteris flix-mas have a clear optimum in the Scottish relevés, and the same holds true for Holcus mollis, Luzula sylvatica, Epilobium montanum L., Betula pendula Roth (juveniles), and the mosses Hylocomium splendens and Rhytidiadelphus squarrosus. Typical for the Dutch relevés are Molinia caerulea (L.) Moench and Quercus robur L. (juveniles), whereas the German relevés are differentiated by Luzula luzuloides (Lam.) Dandy & Wilmott. However, the three regional groups of relevés have many taxa in
Table 1. Full table of relevés of fern-rich communities in West-Argyll.  
Location: G = Glencruitten House, Oban; A = Ardcastle Forest.

<table>
<thead>
<tr>
<th>Table number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relevé number (RH 17.)</td>
<td>167</td>
<td>164</td>
<td>165</td>
<td>168</td>
<td>166</td>
</tr>
<tr>
<td>Date (July 2017)</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Location</td>
<td>G</td>
<td>A</td>
<td>A</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>Area (m²)</td>
<td>20</td>
<td>8</td>
<td>10</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Exposition</td>
<td>NW</td>
<td>N</td>
<td>Z</td>
<td>N</td>
<td>W</td>
</tr>
<tr>
<td>Inclination (degrees)</td>
<td>45</td>
<td>40</td>
<td>45</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Total cover (%)</td>
<td>100</td>
<td>99</td>
<td>95</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cover herb layer (%)</td>
<td>95</td>
<td>90</td>
<td>90</td>
<td>95</td>
<td>80</td>
</tr>
<tr>
<td>Cover moss layer(%)</td>
<td>80</td>
<td>100</td>
<td>10</td>
<td>80</td>
<td>70</td>
</tr>
<tr>
<td>Avg. height herb layer (cm)</td>
<td>60</td>
<td>80</td>
<td>60</td>
<td>60</td>
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<tr>
<td>Max. height herb layer (cm)</td>
<td>120</td>
<td>120</td>
<td>90</td>
<td>120</td>
<td>80</td>
</tr>
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**Pteridophytes**

<table>
<thead>
<tr>
<th>Species</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blechnum spicant</strong></td>
<td>+</td>
<td>2a</td>
<td>+</td>
<td>3</td>
<td>2a</td>
</tr>
<tr>
<td><strong>Oreopteris limbosperma</strong></td>
<td>+</td>
<td>3</td>
<td>2b</td>
<td>2a</td>
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</tr>
<tr>
<td><strong>Dryopteris cambrensis</strong></td>
<td>2a</td>
<td>3</td>
<td>3</td>
<td>.</td>
<td>2b</td>
</tr>
<tr>
<td><strong>Pteridium aquilinum</strong></td>
<td>2b</td>
<td>2b</td>
<td>2a</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td><strong>Dryopteris filix-mas</strong></td>
<td>+</td>
<td>2a</td>
<td>2b</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td><strong>Dryopteris dilatata</strong></td>
<td>3</td>
<td>+</td>
<td>.</td>
<td>.</td>
<td>2a</td>
</tr>
<tr>
<td><strong>Dryopteris borreri</strong></td>
<td>+</td>
<td>.</td>
<td>.</td>
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<td>.</td>
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<tr>
<td><strong>Athyrium filix-femina</strong></td>
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<td>.</td>
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<td>2a</td>
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<tr>
<td><strong>Dryopteris carthusiana</strong></td>
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<td>4</td>
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</tr>
<tr>
<td><strong>Phegopteris connectilis</strong></td>
<td>2a</td>
<td>.</td>
<td>.</td>
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<tr>
<td><strong>Polypodium vulgare</strong></td>
<td>.</td>
<td>+</td>
<td>.</td>
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</tr>
<tr>
<td><strong>Dryopteris x deweveri</strong></td>
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**Melampyro-Holcetea**

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<td><strong>Holcus mollis</strong></td>
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<td>2a</td>
<td>+</td>
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<tr>
<td><strong>Luzula sylvatica</strong></td>
<td>2b</td>
<td>+</td>
<td>2b</td>
<td>4</td>
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<tr>
<td><strong>Oxalis acetosella</strong></td>
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<td>.</td>
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**Epilobietea**

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<tbody>
<tr>
<td><strong>Epilobium montanum</strong></td>
<td>+</td>
<td>.</td>
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<td>+</td>
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<tr>
<td><strong>Digitalis purpurea</strong></td>
<td>1</td>
<td>.</td>
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</table>

**Scrubs and juvenile trees**

<table>
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<tr>
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<tr>
<td><strong>Rubus subgen. Rubus</strong></td>
<td>2a</td>
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<tr>
<td><strong>Betula pendula</strong></td>
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<td><strong>Rubus spectabilis</strong></td>
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**Mosses**

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<tr>
<td><strong>Hylocomium splendens</strong></td>
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<td>2b</td>
<td>2a</td>
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<tr>
<td><strong>Rhytidiadelphus squarrosus</strong></td>
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<td>1</td>
<td>2m</td>
<td>4</td>
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<td><strong>Polytrichum formosum</strong></td>
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<td>2a</td>
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<td><strong>Lophocolea heterophylla</strong></td>
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<td><strong>Sphagnum capillifolium</strong></td>
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<td><strong>Peltigera didactyla</strong></td>
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<td><strong>Lophocolea bidentata</strong></td>
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<tr>
<td><strong>Dicranum scoparium</strong></td>
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<td><strong>Campylopus pyriformis</strong></td>
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<td><strong>Kindbergia praelonga</strong></td>
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**Other species**

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<tr>
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<tr>
<td><strong>Deschampsia cespitosa</strong></td>
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<td><strong>Ranunculus repens</strong></td>
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<tr>
<td><strong>Potentilla erecta</strong></td>
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<tr>
<td><strong>Agrostis capillaris</strong></td>
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</table>
Table 2. Synoptic table of Oreopteris limbosperma communities in Scotland (column 1, source: Table 1), the Netherlands (column 2, source: Weeda & Haveman 2017), and Germany (column 3, source: Wittig 2000).

<table>
<thead>
<tr>
<th>Pteridophytes</th>
<th>Column 1</th>
<th>Column 2</th>
<th>Column 3</th>
</tr>
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<tbody>
<tr>
<td>Dryopteris cambrensis</td>
<td>80</td>
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<td>Dryopteris borreri</td>
<td>40</td>
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</tr>
<tr>
<td>Polypodium vulgare</td>
<td>20</td>
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<td>..</td>
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<tr>
<td>Dryopteris x deweveri</td>
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<td>..</td>
<td>..</td>
</tr>
<tr>
<td>Pteridium aquilinum</td>
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<td>9</td>
<td>..</td>
</tr>
<tr>
<td>Blechnum spicant</td>
<td>100</td>
<td>64</td>
<td>24</td>
</tr>
<tr>
<td>Oreopteris limbosperma</td>
<td>80</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Dryopteris dilatata</td>
<td>60</td>
<td>55</td>
<td>57</td>
</tr>
<tr>
<td>Athyrium filix-femina</td>
<td>40</td>
<td>64</td>
<td>76</td>
</tr>
<tr>
<td>Dryopteris carthusiana</td>
<td>40</td>
<td>64</td>
<td>14</td>
</tr>
<tr>
<td>Phegopteris connectilis</td>
<td>20</td>
<td>36</td>
<td>33</td>
</tr>
<tr>
<td>Dryopteris filix-mas</td>
<td>60</td>
<td>..</td>
<td>14</td>
</tr>
<tr>
<td>Gymnocarpium dryopteris</td>
<td>..</td>
<td>..</td>
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</tr>
<tr>
<td>Dryopteris affinis</td>
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<td>..</td>
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<tr>
<td>Hylocomium splendens</td>
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<td>..</td>
</tr>
<tr>
<td>Pseudoscleropodium purum</td>
<td>20</td>
<td>55</td>
<td>..</td>
</tr>
<tr>
<td>Potentilla erecta</td>
<td>40</td>
<td>45</td>
<td>..</td>
</tr>
<tr>
<td>Holcus mollis</td>
<td>100</td>
<td>27</td>
<td>43</td>
</tr>
<tr>
<td>Atrichum undulatum</td>
<td>20</td>
<td>45</td>
<td>10</td>
</tr>
<tr>
<td>Oxalis acetosella</td>
<td>40</td>
<td>9</td>
<td>43</td>
</tr>
<tr>
<td>Digitalis purpurea</td>
<td>60</td>
<td>9</td>
<td>43</td>
</tr>
<tr>
<td>Rhytidiadelphus squarrosum</td>
<td>100</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Polytrichum formosum</td>
<td>80</td>
<td>64</td>
<td>43</td>
</tr>
<tr>
<td>Rubus species</td>
<td>60</td>
<td>73</td>
<td>52</td>
</tr>
<tr>
<td>Agrostis capillaris</td>
<td>40</td>
<td>45</td>
<td>67</td>
</tr>
<tr>
<td>Juncus effusus</td>
<td>20</td>
<td>45</td>
<td>33</td>
</tr>
<tr>
<td>Luzula sylvatica</td>
<td>100</td>
<td>..</td>
<td>52</td>
</tr>
<tr>
<td>Epilobium montanum</td>
<td>80</td>
<td>..</td>
<td>24</td>
</tr>
<tr>
<td>Betula pendula</td>
<td>60</td>
<td>..</td>
<td>5</td>
</tr>
<tr>
<td>Rubus idaeus</td>
<td>20</td>
<td>..</td>
<td>71</td>
</tr>
<tr>
<td>Molinia caerulea</td>
<td>..</td>
<td>73</td>
<td>..</td>
</tr>
<tr>
<td>Quercus robur</td>
<td>..</td>
<td>55</td>
<td>..</td>
</tr>
<tr>
<td>Deschampsia flexuosa</td>
<td>..</td>
<td>27</td>
<td>51</td>
</tr>
<tr>
<td>Dicranella heteromalla</td>
<td>..</td>
<td>45</td>
<td>5</td>
</tr>
<tr>
<td>Galium saxatile</td>
<td>..</td>
<td>18</td>
<td>48</td>
</tr>
<tr>
<td>Pellia epiphylla</td>
<td>..</td>
<td>55</td>
<td>14</td>
</tr>
<tr>
<td>Luzula luzuloides</td>
<td>..</td>
<td>..</td>
<td>81</td>
</tr>
</tbody>
</table>
common, like *Dryopteris dilatata*, *Athyrium filix-femina*, *Phegopteris connectilis*, *Atrichum undulatum* (Hedw.) P.Beauv., *Polytrichum formosum*, *Rubus* L. subgen. *Rubus*, *Agrostis capillaris* L., and *Juncus effusus* L. Several other species are common to two regions, whereas they are lacking from the third. *Potentilla erecta* (L.) Raeusch. is an example, which was found in both the Scottish and the Dutch communities, and not in the German.

**DISCUSSION**

**Phytosociological Assignment**

The here described Scottish fern-meadows are at least for the greater part to be assigned to the *Luzulo luzuloides-Thelypteridetum limbospermae*, on the basis of the presence of the characteristic species *Oreopteris limbosperma* as well as the constant species, with many pteridophytes and other species which are found in both the Scottish relevés as well as in the German and/or the Dutch plots. The last relevé, without *Oreopteris*, probably represents a fragmentary example of this association. As was shown though, the Scottish fern-meadows have their own characteristics, with several species not found in the suboceanic forms of the association. Especially typical is *Dryopteris cambrensis*. According to Fraser-Jenkins (2007), this species has its distribution centre in oceanic Europe. In the British Isles it is common in Scotland, contrary to its name less so in Wales, and rather rare elsewhere (Page 1997; Trewren 2014). Considering this, the Scottish fern-meadows could probably be described as a separate subassociation (*Luzulo luzuloides-Thelypteridetum dryopteridetosum cambrensis* prov.) but, at this stage, a formal description of such a subassociation is rejected, since the available dataset is too small for a proper assessment.

![Figure 2. Abundant fern growth in a forest edge, Oban, Argyll. Apart from *Pteridium aquilinum*, the vegetation is formed mainly by *Dryopteris* species and *Oreopteris limbosperma* (photo: R. Haveman)](image)
Ecology
In the German lower mountain belt, the *Luzulo-Thelypteridetum* is almost strictly confined to forest edges (Wittig, 2000), and the same applies to the situation in the Netherlands (Weeda & Haveman, 2017). In Scotland, the community has a much broader ecological amplitude. Although it is frequently found in forest edges (relevés 3-5 in Table 1, Figure 2), it also develops frequently at forest clearings on suitable soils (relevés 1 and 2 in Table 1, Figure 3). Typical species of woodland clearings (grouped under the heading of *Epilobietea* and Scrubs and juvenile trees in Table 1) are not confined to the latter environment: they are equally present in the relevés from forest edges, and also in the relevés from Germany (Table 2 column 3, less so in the Dutch relevés). Unfortunately, no relevés were made in the stands on the third environment where the community is found, viz. abandoned and extensively managed grasslands (Figure 1). However, several short checks of the stands in this situation made clear that the species composition of the vegetation here is very similar to the community which develops in forest edges or at forest clearings.

The abundant occurrence of the combination of *Oreopteris limbosperma*, *Blechnum spicant*, *Athyrium filix-femina*, and several *Dryopteris* species outside the buffered forest micro-climate indicate the high air-humidity and annual rainfall in this part of the UK, where water availability is never a problem (see also Knaben 1950, who drew the same conclusion after study of the fern-communities of West-Norway). According to the climate maps of the Met Office (www.metoffice.gov.uk), the region directly around Oban has an average annual rainfall of 1,700-2,200 mm, which is 2.5 to 3 times the amount in the Netherlands. The German lower mountains receive more rain than the Netherlands, but not as much as the Oban region: the higher regions of the Rothaargebirge e.g., have an average annual rainfall of 1,000 mm (www.wetter.de). Where the *Luzulo-
Thelypteridetum grows in zonation with Pteridium-dominated communities, the first is confined to the steep banks, or the shelter of woodlands (Figure 4). Probably this is caused by difference in water demand, which is higher in Oreopteris than in Pteridium (Weeda & Haveman, 2017). In this respect, a striking description of the edaphic demands of Oreopteris limbosperma is given by Page (1997): "... niches where trickling acidic peaty water provides the most constant surface run-off or subterranean seepage, creating cool and constantly moist but well-aerated edaphic conditions ..."

In West-Argyll, the Luzulo-Thelypteridetum can form complexes with different types of grasslands. On the least fertile situations, the grasslands can be assigned to the class Nardetea Rivas Goday et Borja Carbonell. On soils with a somewhat better availability of nutrients, the main grassland type that is found in complex with the fern-meadow community is the Lolio-Cynosuretum Tüxen (alliance Cynosurion cristati Tüxen, class Molinio-Arrhenatheretea Tüxen). Usually, both Nardion and Cynosurion grasslands are grazed by cattle or sheep. Where the grassland is mown, especially at the bottom of valleys, the grassland vegetation resembles the Crepido-Juncetum acutiflori Oberdorfer, a grassland community characteristic for peaty soils with laterally water movement and seepage. It is remarkable that this last grassland type is also mentioned by Bremer (2016) when he discusses the landscape in which the Luzulo-Thelypteridetum is found.

In The vegetation of Scotland, the classic book edited by Burnett (1964), Oreopteris limbosperma is mentioned only in the context of sub-alpine scrubs and fern-meadows (p. 162-163, and p. 516). Only one remark in the chapter on the history and regional patterns in Scottish vegetation (p. 574) associates the species with burning on north-exposed slopes in the Northern Highlands. The occurrence of fern-dominated forest edges

Figure 4. Abandoned hillside meadow with fern encroachment. Large areas are covered by Pteridium aquilinum (in the background and in the lower right corner). Steep banks are invaded by Oreopteris limbosperma and Dryopteris species (lower left corner, and the linear structure in the centre of the photo). Oban, Argyll (photo: R. Haveman)
and fern-meadows in the lowlands (other than those dominated by *Pteridium aquilinum*) like those in the studied part of Argyll, are completely neglected. The same holds true for the description of the fern-dominated communities in Rodwell's *British Plant Communities* (Rodwell et al., 1992). The already mentioned *Thelypteris limbosperma*-Blechnum spicant community (U19), which is placed in the Dryoptero-Calamagrostidion Nordh. alliance (class Betulo-Adenostyletea Br.-Bl.) by the authors with some reserve, is also considered to have its main distribution at higher altitudes, although it was recorded as low as 245 m above sea level. According to Page (1997), *Oreopteris limbosperma* might reach its highest abundance in western Scotland anywhere on a world scale, and here it can be found nearly from sea-level to about 880 m.

**The Dutch *Oreopteris* Community In Comparison**

Bremer (2016) and Weeda & Haveman (2017) characterised the *Luzulo luzuloides-Thelypteridetum limbospermae* in the Netherlands as an association in development (an association in statu nascendi; Westhoff 1990). The current distribution of *Oreopteris limbosperma* in the Netherlands is the result of a rather recent increase (Figure 5). In the period before 1950, the species was only found in 45 standard grid cells (5x5 km). This increased in a short time to 71 in 1955, possibly as a result of a better recognition of the species or intensified surveys. Until 2000, the number of grid cells in which the species was found increased gradually to 103, in order to increase exponentially in the following years, to 176 in 2015 (verspreidingsatlas.nl). This expansion of *Oreopteris* can be attributed to the development of the landscape in the 20th Century. Most of the recent population growth of *Oreopteris* in the Netherlands is observed in forest plantations (mostly of Scots pine, *Pinus sylvestris*) in former heathland areas from the late 19th and early 20th Century (Bremer, 2016; Weeda & Haveman, 2017). Apparently, site conditions

![Records of Oreopteris limbosperma in the Netherlands](image)

*Figure 5.* Cumulative numbers of grid cells (5x5 km) with *Oreopteris limbosperma* in the Netherlands between 1940 and 2015. (Data source: verspreidingsatlas.nl)
are developing into a favourable state for the establishment of Oreopteris in these landscapes after about 100 years, especially in ditch sides in these plantations. In most sites where the species occurs it is only present in very small populations, sometimes only consisting of one single plant. This is in sharp contrast with the situation in West Scotland, where the species usually forms dense stands, and where a mature fern community is developed.

The difference between the mature Scottish fern-meadows and the juvenile Dutch Oreopteris community is expressed in particular in the average number of fern species: 6.2 in the Scottish relevés, and only 3.9 in the Dutch ones. Remarkably, in the only relevé that was published by Bremer (2016) which can compete with the Scottish one in this respect (relevé 2003.20 in the table published by Bremer), Dryopteris borreri was recorded, the only observation of a species of the Dryopteris affinis complex in the association in the Netherlands.

Considering all available data, including vegetation structure, fern species numbers, as well as the ominipresence of the described fern-meadow vegetation, the Luzulo-Thelypteridetum may be optimally developed in the lowlands and low mountain areas of West Scotland. In the Central European lower mountain regions, O. limbosperma can form dense stands locally, but the number of fern species drops notably in comparison with the fern species numbers in the Scottish fern-meadows. In the Netherlands, the distribution area of Oreopteris is still developing, and it is only rarely found in a dense fern vegetation rich in pteridophytes.

The major absent in the Dutch relevés published by Bremer (2016) and Weeda & Haveman (2017) is Dryopteris cambrensis, which is so prominently present in the Scottish relevés. This species was discovered only recently in the Netherlands, viz. in 2010 by Koos Ballintijn in the Purmerbos just north of Amsterdam. The species is recorded in eight grid cells now, although their identity is not yet verified in all cases (oral comm. Sipke Gongrijp). Whether the species is really as rare as the online map (verspreidingsatlas.nl) suggests is not clear. The apomicts in the Dryopteris affinis aggregate are difficult to separate, although it is possible after some experience. Considering the distribution pattern in the UK, where the species seems to be much more restricted in the east than in the west, D. cambrensis may be and remain a rare species in the Netherlands. It remains an open question whether this species will be able to establish in the Luzulo-Thelypteridetum. Its dispersion might be adequate to reach the sites eventually, but whether the conditions for the establishment of the species are appropriate is not certain. The current occurrence of the species in dense (young) forest plantations suggest that D. cambrensis is confined to rather high levels of air humidity. In this respect, the environmental conditions in which the Luzulo-Thelypteridetum develops might be favourable also for the growth of D. cambrensis too. The future will show whether this and other fern species can become established in this association in the Netherlands. I expect however, that the Luzulo-Thelypteridetum never will become as species rich and luxuriant as in the coastal areas of Argyll.

REFERENCES

HAVEMAN: FERN-MEADOW VEGETATION

MARSILEA AEGYPTIACA (MARSILEACEAE) ON THE MEDITERRANEAN ISLAND OF ELAFONISOS (LACONIA, PELOPONNESE, GREECE)

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Key Words: Marsilea aegyptiaca, Elafonisos, Greece, European species

ABSTRACT
The water-clover species Marsilea aegyptiaca was first detected on the Mediterranean island of Elafonisos (Peloponnese, Greece) nearly 25 years ago. This was the first record for the species as part of the European flora. Recent work has shown that M. aegyptiaca still occurs at the site, and data are presented concerning its identification, habitat and distribution. Morphological characters of all known European species within the genus are compared.

INTRODUCTION
Water-clovers (Marsilea L.) are heterosporous ferns and the most species rich group within the Marsileaceae. The family additionally comprises the pillworts (Pilularia L.) and the monotypic genus Regnellidium Lindm. All three genera are aquatic to semi-aquatic rhizomatous plants with roots and leaves born at nodes and sori arranged in sporocarps (Kramer, 1990; Nagalingum et al., 2006). Recent phylogenetic studies have revealed that the Clover ferns (Marsileaceae) together with the Floating ferns (Salviniaceae; Salvinia Seg. and Azolla Lam.) represent a monophyletic group of heterosporous ferns within the fern clade, which evolved in the Mesozoic (Pryer, 1999; Smith et al., 2006; Nagalingum et al., 2006). Twentieth century treatments of the genus mainly focussing on morphology have been published for Africa (Launert, 1968, 1970, 1971, 1984), Australia (Jones, 1998), India (Gupta, 1962), and the Americas (Johnson, 1986). Recent molecular studies have thrown some light on the phylogenetic relationships within Marsilea (Nagalingum et al., 2007, 2008; Whitten et al., 2012).

During extensive field work on the island of Elafonisos in 1991 (Laconia, Peloponnese, Greece; see Jagel, 1992) the semi-aquatic Water-clover species Marsilea aegyptiaca Willd. was found growing abundantly in a seasonal pool. This was not only the first record for Greece but also the first true European occurrence of the species known at that time; its only known station at the time being from the Lower Volga in Russia (Jalas & Suominen, 1972; Tutin et al., 1993; Tzvelev, 2012) and an erroneous record for Spain (Alcober et al., 1980). The site was reinvestigated in 2016 and M. aegyptiaca confirmed to be still thriving at its obviously undisturbed habitat.

In regard to the biogeographical importance of the record, more detailed and comprehensive data are given below.

BIOLOGY AND IDENTIFICATION
Water-clover species (Marsilea L.) are in general a variable and frequently confusing group of plants and depending on the life phase, are nearly impossible to separate from each other. Most members of the genus inhabit seasonally dry areas inundated only
during the rainy season and like most aquatic plants are characterized by a phenotypic plasticity, which renders identification on a species level quite difficult. This is emphasized when plants are in their sterile, vegetative growth stage with submerged rhizomes and floating leaves on the water surface. Sporocarps are formed only in the terrestrial life stage, but are essential for species determination. Sterile, aquatic plants lacking sporocarps are nearly impossible to identify, because the different species in that life stage are morphologically very similar.

*Marsilea aegyptiaca* (Figure 1) belongs to a group of xerophytic members within the genus and is adapted to extreme dry conditions. Crouch *et al.* (2011) report that the species in Southern Africa inhabits arid semideserts and is obviously able to exist for several years without being submerged. Consequently *M. aegyptiaca* is quite small in its overall appearance (stipes in the dry land form max. 5 cm long with pinnae max. 1 cm long) and shows hairy leaves (Figure 2 a-d), a feature often displayed by xerophytic *Marsilea* species. Nevertheless this is a very unstable and variable character, because hairs on the leaves and petioles readily occur in hydrophytic species too, when being in their terrestrial life stage. The pinnae of the land form are narrowly cuneate and pilose with the distal margin being slightly truncate and shallowly bilobed. The sporocarp of *M. aegyptiaca* is quite distinct (Figure 3): usually appearing in clusters of two or more on short, free pedicels (2-8 mm), almost squarish in lateral view, with a distinct lateral furrow, densely pilose at first, becoming subglabrous later, lacking an inferior but mostly having a prominent superior tooth. In its aquatic life stage *M. aegyptiaca* is less distinct (see above). The floating leaves have stipes up to 30 cm long, broadly obovate, glabrous and larger pinnae (max. 2.5 cm) with rounded and shallowly sinuate distal margins (Figure 4 a, b).

Figure 1. *Marsilea aegyptiaca* at its growing site on the island of Elafonisos.
Table 1 shows the morphological characters of all known European species within the genus *Marsilea*. The former listed Azorean endemic species *M. azorica* Launert & Paiva has recently been shown to be a misidentification and synonymous with the invasive *M. hirsuta* R. Br. from Australia and Asia (Schaefer *et al.*, 2011) and therefore is excluded here.

**GEOGRAPHY, HABITAT, AND ECOLOGY**

Elafonisos is located in the region of Laconia, an administration unit of the Peloponnese. The island lies just off the Southwest coast of Malea Peninsula and is separated from it by a 600 m wide strait. The history of Elafonisos as an island is short, being part of the peninsula in former times. Therefore, it is not surprising that the largest part of the flora is typical for the South Peloponnese. However, some species show relations to the floristic region of the South Aegean (Jagel, 1992).

In the Northwest part of Elafonisos at Leptos Kavos a seasonally moist habitat of about 1000 m² exists (Figure 5 a, b), at a distance of not more than 100 m from the Mediterranean sea. From late autumn until very early spring the shallow depression is

![Image](image_url)

**Figure 2 a, b, c, d.** *Marsilea aegyptiaca*, fertile dry land plant in terrestrial life stage with hairy leaves.

<table>
<thead>
<tr>
<th></th>
<th><em>M. quadrifolia</em></th>
<th><em>M. strigosa</em></th>
<th><em>M. batardae</em></th>
<th><em>M. aegyptiaca</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arrangement</strong></td>
<td>clustered, 2-3, rarely solitary</td>
<td>mostly solitary crowded in two regular imbricating rows along the rhizome</td>
<td>solitary or in loose groups of 2-3, not crowded along the rhizome</td>
<td>clustered, usually 2, rarely solitary</td>
</tr>
<tr>
<td></td>
<td>stalked</td>
<td>sessile</td>
<td>stalked</td>
<td>stalked</td>
</tr>
<tr>
<td><strong>Shape</strong></td>
<td>oblong-ellipsoid, bean shaped</td>
<td>oblique-elliptic, obliquely squarish or rectangular, obovoid</td>
<td>irregularly squarish to subcircular</td>
<td>squarish</td>
</tr>
<tr>
<td></td>
<td>longer than high not laterally flattened</td>
<td>longer than high laterally usually flattened</td>
<td>as long as high laterally usually flattened</td>
<td>as long as high laterally flattened</td>
</tr>
<tr>
<td><strong>Size</strong></td>
<td>3-6 mm</td>
<td>3-5 mm</td>
<td>3-4.5 mm</td>
<td>2-3 mm</td>
</tr>
<tr>
<td><strong>Teeth</strong></td>
<td>superior tooth 0-1 (short to indistinguishable)</td>
<td>superior tooth 1 (short to indistinguishable)</td>
<td>superior tooth 1 (prominent)</td>
<td>superior tooth 1 (prominent, blunt to acute)</td>
</tr>
<tr>
<td></td>
<td>inferior tooth 0</td>
<td>inferior tooth 0-1</td>
<td>inferior tooth 0-1</td>
<td>inferior tooth 0</td>
</tr>
<tr>
<td><strong>Surface</strong></td>
<td>glabrous (hairy only when young)</td>
<td>strigose or silky pubescent, later subglabrous</td>
<td>strigose or silky pubescent, later subglabrous</td>
<td>densely pilose later subglabrous</td>
</tr>
<tr>
<td></td>
<td>without a furrow on the lateral sides</td>
<td>without a furrow on the lateral sides</td>
<td>without a furrow on the lateral sides</td>
<td>with a distinct curved vertical furrow on the lateral sides</td>
</tr>
<tr>
<td><strong>Pedicel (stalk of sporocarp)</strong></td>
<td>2-20 mm</td>
<td>1-2 mm</td>
<td>2-8 mm</td>
<td>2-8 mm</td>
</tr>
<tr>
<td></td>
<td>2- to 4-branched, rarely unbranched</td>
<td>unbranched</td>
<td>unbranched</td>
<td>unbranched</td>
</tr>
<tr>
<td><strong>Rhizome</strong></td>
<td>long, creeping, profusely branched</td>
<td>short, caespitose, sometimes branched</td>
<td>long, creeping, profusely branched</td>
<td>long, creeping to subcaespitose</td>
</tr>
<tr>
<td></td>
<td>internodes more than 10 mm</td>
<td>nodes crowded</td>
<td>internodes 5-20 mm</td>
<td>internodes 5-10 mm (sometimes longer)</td>
</tr>
<tr>
<td><strong>Pinnae</strong></td>
<td>obovate-cuneate</td>
<td>obdeltate-cuneate</td>
<td>obdeltate-cuneate</td>
<td>narrowly cuneate to obdeltate</td>
</tr>
<tr>
<td></td>
<td>apex entire to undulate, rounded</td>
<td>apex entire to crenulate, rounded</td>
<td>apex entire to crenate</td>
<td>apex deeply crenate to sinuate or entire, rounded</td>
</tr>
<tr>
<td><strong>Pubescence</strong></td>
<td>glabrous</td>
<td>glabrous to sparsely hairy</td>
<td>glabrescent</td>
<td>hairy or glabrous</td>
</tr>
</tbody>
</table>
filled with fresh water not influenced by salt water.

*Marsilea aegyptiaca* is very frequent in this small area, with hundreds or thousands of fronds being found. A smaller population exists more or less 50 m east of the pond at the border of a scrub. The sub-surface of the site is formed by limestone rocks (Symeonidis, 1969), covered by a shallow layer of sandy fine soil. After the beginning of the rainy season in autumn e.g. when *Narcissus serotinus* L. is flowering and when the shallow depression is filled up with rainwater, *Ranunculus peltatus* Schrank subsp. *fucoides* (Freyn) Munoz Garmendia (= *Ranunculus saniculifolius* Viv.) can be found as a water plant in the pond. At this time of year the *Marsilea* grows in its aquatic life stage (Figure 4). The pond is framed by tufts of *Juncus heldreichianus* Parl. and *Schoenus nigricans* L. At the end of winter or in early spring, usually at the end of March, the pond rapidly dries out within a few weeks. Species found growing together with *M. aegyptiaca* are *Anagallis foemina* Miller, *Briza minor* L., *Isoetes phrygia* (Boiss.) Hausskn., *Isolepis cernua* (Vahl) Roem. & Schult., *Juncus capitatus* Weigel, *Juncus hybridus* Brot., *Lythrum borysthenicum* (Schrank) Litv., *Lythrum hyssopifolia* L., *Mentha pulegium* L., *Plantago weldenii* Rchb., *Polypogon maritimus* Willd., *Ranunculus muricatus* L., *Romulea ramiflora* Ten. *Sagina maritima* G.Don, *Solenopsis lauritina* (L.) C.Presl, *Trifolium lappaceum* L. *Trifolium resupinatum* L., and *Triglochin barrelieri* Loisel. Most of these are highly specialized and characteristic for such sites in the Peloponnese.

**DISTRIBUTION**

*Marsilea aegyptiaca* is mainly an African species, recorded for Ethiopia, Botswana,

![Figure 3 a, b, c, d. Marsilea aegyptiaca showing characteristic clustered, squarish, pilose sporocarps with lateral furrow and blunt superior tooth on short pedicels.](image-url)
Namibia, South Africa, Sudan, Egypt, Tunisia, Algeria, Libya, and Madagascar (Launert, 1984; Crouch et al., 2011). The species also extends its range to the east with occurrences on the Indian subcontinent (Gupta, 1962; Sharma, 2005).

Until the 1990s *M. aegyptiaca* was known from Europe only from the river Volga and its delta near the Caspian Sea in Russia (Jalas & Suominen, 1972; Tutin et al., 1993; Tzvelev, 2012). A record for Spain (Alcober et al., 1980) turned out to be a misidentification and the plants are now known to belong to a different species, *Marsilea batardae* Launert, occurring in Southwest Spain and Southern Portugal (Launert, 1983; Castroviejo et al., 1986; Rosselló-Graell et al., 2000; Prelli, 2001; Delgado Vázquez & Plaza Arregui, 2010).

The discovery of *M. aegyptiaca* on the Peloponnesian island of Elafonisos in 1991 therefore was a remarkable addition to the knowledge concerning the range of the species now extending into the Eastern Mediterranean (Jagel, 1992) and representing the first record for Greece and the European Union. Later a second occurrence for the region has been reported from Cyprus (Hand, 2011), validating the species as an element of the European mediterranean flora.

**HABITAT AND THREATS**

In former times sites of *M. aegyptiaca* on Elafonisos were used for agricultural purposes.

![Figure 4 a, b. Marsilea aegyptiaca, sterile plant with floating leaves in aquatic life stage.](image1)

![Figure 5 a, b. Habitat of Marsilea aegyptiaca on Elafonisos, dried up in spring (a) and flooded in winter (b).](image2)
perhaps because of the good water supply, with evidence of several low walls of collected field stones. Nowadays there is no noticeable human influence, neither huts, houses nor stables are in the proximity so no current threat for the fern is perceived. The fact that the species has been known there for nearly 25 years now with no obvious change to the site supports this assessment.

A large area of similar seasonally wet ponds is situated nearby on the mainland in Viglafia north of the beach of Punta. The area seems to be comparable with the pond of Elafonisos but is quite overgrazed and in parts more brackish. Although it has been investigated intensively over several years no plants of the Water-clover could be found although most accompanying plants of the Elafonisos site were present. However the site is nevertheless remarkable for hosting some very rare plant species which could not be found on Elafonisos: *Callitriche brutia* Petagna, *Cicendia filiformis* (L.) Del., *Crassula vaillantii* (Willd.) Roth, *Isoetes gymnocarpa* (Gennari) A.Braun, *Isoetes histrix* Bory, and *Myosurus heldreichii* Heldr. ex H.Lév. Additionally a third Quillwort species, *Isoetes phrygia* (Boiss.) Hausskn., is present here (as it is at the *Marsilea* site on Elafonisos), together with a hybrid probably involving this taxon.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. H. W. Bennert (Ennepetal [formerly of Ruhr-University of Bochum], Germany) for the initial confirmation of *Marsilea aegyptiaca* in 1992. He also contributed with literature and helpful discussions. Dr. V. M. Dörken (University of Konstanz, Germany) also supplied us with literature. Dr. A. Troia (University of Palermo, Italy) is gratefully acknowledged for his help with the determination of the different *Isoetes* species at the sites.

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SHORT NOTE

HYPODEMATIUM CRENATUM SUBSP. CRENATUM (HYPODEMATIACEAE): A NEW DISTRIBUTIONAL RECORD FOR GUJARAT STATE

The state of Gujarat is divided into five zones viz., Kutch, Saurashtra, North, Central and South Gujarat. These zones are based on climate and geographic location. The district Junagadh is located on the Kathiawar peninsula in western Gujarat (Saurashtra zone). It contains the Gir National Park and Girnar wildlife sanctuary, which is the only home for Asiatic lions. Girnar hills are also declared as a forest reserve for bamboos (Nakar & Jadeja, 2015), and ‘Girnari Giddh’, the long-billed vulture, is found only in the Girnar hills. The Girnar region alone accounts for about 25 per cent of the species in Gujarat state (Patel et al., 1961). However, lycophyte and fern diversity of the whole of the Gujarat state including Saurashtra regions (particularly Junagadh) is less understood, although recently there have been a few reports on the pteridophyte diversity of Central, North and South Gujarat (Kachhiyapatel et al., 2015, 2016; Rajput et al., 2016). The present investigation was carried out to study pteridophytes from Girnar hills. During this survey an interesting pteridophyte was collected which, after critical observation of morphological characters, was identified as Hypodematium crenatum (Forssk.) Kuhn. subsp. crenatum and it is reported here as new distributional record for Gujarat state. The identification was confirmed by comparing generated nucleotide sequences with the GenBank database, and the sequence data were submitted to BOLD.

The genus Hypodematium in the family Hypodematiaceae is represented by 20 species in the world (PPG-I, 2016). A sole species of Hypodematium (divided into three subspecies viz., H. crenatum subsp. crenatum, H. crenatum subsp. loyalii Fraser-Jenk. & Khullar and H. crenatum subsp. mehrae Fraser-Jenk.) has been reported from India (Fraser-Jenkins, 2008). We have found a single subspecies, H. crenatum subsp. crenatum, for Gujarat state. Its description, range of distribution, ecology, conservation status and specimen examined is given.

Systematic Treatment


Hypodematium crenatum (Forssk) Kuhn. Manickam and Irudayaraj, Pterid. FI. W.


Plant lithophytic, perennial herb; rhizome long creeping, densely red-brown scaly, giving woolly look to rhizome; scales 1-2 cm × 1-3 mm, red-brown, oblong lanceolate-lanceolate, glossy, apex long acuminate, margin entire; frond 20-30 cm × 15-20 cm, green-dark green, bipinnatified-tripinnatifid; stipe 10-18 cm long, brown, densely scaly at base, grooved, glabrous above; lamina 5-12×15-20 cm, bi-pinnatifid-tri-pinnatifid, ovate, apex acute, base cuneate, basal half tri-pinnatifid, distal half base bi-pinnatifid, distal most part simply pinnatifid; pinnae 6-10 pairs, subopposite, lanceolate, apex acute, base decurrent, pale brown, stiff, hairs sparsely-densely distributed; texture herbaceous; vein forked 3-4 times, indistinct above and below, free, reaching up to margin; sori median on the vein lets in two rows; indusia reniform, entire, densely covered with short stiff hair; spores 45-55 µm in dia., reniform to ellipsoid, exine with prominent
protuberances (and see Figure 1).

**Distribution:** World: Africa, China, India, Japan, Malaysia, Myanmar, Philippines and Thailand

**India:** Himachal Pradesh, North East India, Punjab, Gujarat, Maharashtra, Kerala.

**Gujarat:** Junagadh

**Figure 1.** A: Habit of *Hypodematium crenatum* subsp. *crenatum*. B: Frond C: Rhizome D: Stipe base showing dense scales E: Stipe base scale F: Pinna G: Pinnules showing arrangement of sori H: Enlarged view showing hairs on costules I: Sorus J: Indusium K: Sporangium L: Spore. Scale bar: A-3 cm, B-3 cm, C- 1cm, D-0.5 cm, E-2 mm, F-2 cm, G-5 mm, H-1mm, I-0.5 mm, J-0.5 mm, K-100 µM, L-25 µM.
**BOLD ID:** MIPDG013-15  
**Phenology:** Vegetative phase: June-September; Reproductive phase: October-December  
**Ecology:** Lithophytic, collected from rock crevices at Girnar hill slopes (on the way to Sitavan), between 650-700 m.  
**Specimens Examined:** INDIA-Maharashtra: Kolhapur Dt., Pankala, alt 1000 m., 26/10/1962, Bhandare, BLAT; 02/01/1952, V. D. Vartak (BLAT205), BLAT; Satara Dt., Wai Tehasil; Panchagani, alt. 1100 m., Oct. 1918, Blatter (BLAT1211), BLAT; Satara Dt., Panchgani, alt. 1200 m., Sept. 2014, (SMP10071) SUK; Gujarat: Junagadh dt, Girnar hills, on the way to Sitavan, 680 m, (21°32'03.50" N, 70°31'28.10" E, at 668 m), R.N. Kachhiyapatel & K.S. Rajput 129, 26/12/2014, BARO.  

**ACKNOWLEDGMENTS**  
The authors are grateful to the Gujarat Biodiversity Board (GBB) and Forest Department, Government of Gujarat for providing necessary permission to visit the area and permit collection of the specimens, and to an anonymous reviewer and Dr Mary Gibby for valuable suggestions on the previous version of the manuscript.  

**REFERENCES**  

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SHORT NOTE

ELEVEN NEW COMBINATIONS FOR MALESIAN FERNS

The preparation of a checklist of pteridophytes from Gunung Mulu National Park, Sarawak, necessitates eleven new combinations to bring the generic treatment into line with PPG 1 [Pteridophyte Phylogeny Group] (2016).

NEW COMBINATIONS

DAVALLIACEAE


HYMENOPHYLLACEAE

1. Abrodictyum saxatile (T.Moore) Parris comb. nov., based on Trichomanes saxatile T.Moore, Gard. Chron. 1862: 45 (1862). Type: Borneo, collector not specified (K?).

2. Abrodictyum setigerum (Backh. ex T.Moore) Parris comb. nov., based on Trichomanes setigerum Backh. ex T.Moore, Gard. Chron. 1862: 45 (1862). Type, Borneo, collector not specified (K?).


POLYPODIACEAE

1. Leptochilus bolsteri (Copel.) Parris comb. nov., based on Polypodium bolsteri Copel., Philipp. J. Sci. 1, Suppl. 4: 257, pl. 4a (1906). Type: Philippines, Mindanao, Surigao, April 1906, Bolster s. n. (MICH).
THELYPTERIDACEAE


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PAPERS should not usually exceed 20 printed pages and are generally expected to be considerably shorter. As well as reports of original research, review articles and short notes, e.g. new records, are encouraged. The senior author should supply an email address to facilitate correspondence.

REVIEWS should cover recent developments in any aspect of research on ferns and lycopsods. This can include unpublished research by the author, and/or the possible direction of future research. They should be aimed at the general reader who may not be an expert in the chosen topic. Proposals for a review should be discussed first with the review editors.

MANUSCRIPTS should be submitted in English (British) in electronic format in 10-point Times New Roman font and single spaced. Electronic versions of text and tables should be compatible with WORD, with figures as TIFF files, and sent as email attachments. All manuscripts will be refereed.

THE TITLE should reflect the content of the paper and be in BOLD CAPITALS (11-point) and centrally aligned. Generic and specific names should be in italics and any title containing a generic or specific name should be followed by the family name in brackets e.g.

TRICHOMANES SPECIOSUM (HYMENOPHYLLACEAE) IN SOUTHERN SPAIN

AUTHOR ABBREVIATIONS should follow Pichi Sermolli’s (1996) Authors of scientific names in Pteridophyta, Royal Botanic Gardens, Kew.

MAIN HEADINGS: should be in BOLD CAPITALS (10-point) and centrally aligned.

SUBSIDIARY HEADINGS: should be in bold, the first letter of each word in capitals, the rest in lower case and left-aligned.

AUTHORS’ NAMES AND FULL ADDRESSES (including email address): follow the title and are centrally aligned.

KEY WORDS: up to ten.

ABSTRACT: should reflect the content of the paper.

FIGURES: there is no distinction between photographs and line drawings in numbering. All should be supplied in mono or colour, TIFF format, 300 dpi and CMYK. (please contact the Production Editor with queries), with a scale bar where appropriate. Lettering or numbers (Arabic) should be in the bottom left using uppercase Times Roman. Figure captions should be on a separate sheet.

TABLES: can be printed in either portrait or landscape format. Authors should consider this when preparing tables. Authors should ensure that tables fit the printed page size in a legible form.

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MEASUREMENTS: follow the metric system.

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REFERENCES: follow the style of a recent issue of The Fern Gazette, e.g.:-


JOURNAL ABBREVIATIONS: should follow Botanico Periodicum Huntianum & Supplements.

Alterations from the original text at proof stage will be charged for unless they are minor points of detail. A pdf will be provided free to the senior author.
GLOBAL REVIEW OF RECENT TAXONOMIC RESEARCH INTO ISOETES (ISOETACEAE), WITH IMPLICATIONS FOR BIOGEOGRAPHY AND CONSERVATION

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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

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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. pietmontana* (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 × *hyemalis* D.F.Brunton, b) heptaploid *I. xharveyi* A.A.Eaton, pro sp. (*I. echinospora* ssp. *muricata* (Durieu) Löve & Löve × *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. xbrittonii* D.F. Brunton & W.C. Taylor (*I. appalachiana × septentrionalis*) (Brunton & Taylor, 1990; Brunton, 2015), *I. xbruntonii* D. Knepper & Musselman (*I. appalachiana × hyemalis*) (Musselman et al., 1996; Brunton, 2015) and hexaploid *I. xfairbrothersii* J.D. Montgomery & W.C. Taylor (*I. engelmannii × lacustris*) (Montgomery & Taylor, 1994). In addition, 19th Century taxa *I. xfoveolata* A.A. Eaton, pro. sp. (*I. engelmannii Dur. × tuckermanii A. Braun*) and *I. xeatonii* R. Dodge, pro. sp. (*I. echinospora × engelmannii* and its synonym *I. xgravesii* 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

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**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 & Stutzsel 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 (Entwhisle, 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 µm.
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 \textit{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 \textit{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 \textit{I. jamaicensis} J.R.Hickey from its namesake island (Hickey, 1981), the lowland \textit{I. pallida} J.R.Hickey of the South American \textit{I. triangula} Weber s.l. complex found in southern Mexico (Hickey, 1988), and a bedrock outcrop pool taxon \textit{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 \textit{Isoetes coromandalina} L.f. s.str. was one of the first \textit{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, \textit{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 \textit{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, \textit{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, \textit{Isoetes ×paratunica} D.F.Brunton, O.Mochalova & A.A.Bobrov (\textit{I. asiatica} (Makino) Makino x \textit{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 at 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.

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
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 exits *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.

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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 duriei*. 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).
Two new combinations in New Guinea Grammitid ferns (Polypodiaceae) are proposed: *Prosaptia ledermannii* (Brause) Parris and *Radiogrammitis habbemensis* (Copel.) Parris. Herbarium abbreviations follow Thiers (2018).


A detailed examination of the second collection of this species (*Kessler* 14049 & *Abrahamczyk*, Indonesia, Papua Barat, road from Wamena to Lake Habbema, 6 km before summit, 4°08’S 138°47’E, 3000 m, 20 March 2011, UC) shows that the rhizome is radial with stipes arranged in whorls, characteristic of *Radiogrammitis* Parris rather than dorsiventral, with stipes in two rows, characteristic of *Oreogrammitis* Copel., so the species needs to be transferred to the former from the latter.

**ACKNOWLEDGEMENTS**

I wish to thank the Keepers of A, K, L, MICH and UC for loan of material, the Keeper of BO for permission to examine Grammitid holdings in 2013 and Ewen Cameron (AK) for expediting the loans.

**REFERENCES**


Continued on page 347
GROWTH OF FERN GAMETOPHYTES AFTER 20 YEARS OF STORAGE IN LIQUID NITROGEN

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Key words: cryopreservation, ex situ conservation, gametophyte; in vitro; long-term storage

ABSTRACT
In vitro grown gametophytes of six species of ferns, which had been cryopreserved using the encapsulation dehydration procedure, were evaluated for survival after 20 yrs of storage in liquid nitrogen. Tissues were rewarmed and transferred to a recovery medium with the same methods originally used to test pre-storage viability. All six species resumed growth. Post-storage viability was not consistently higher or lower than pre-storage viability of LN exposed tissues, likely reflecting the small sample sizes. However, these results demonstrate that long-term storage in liquid nitrogen is a viable option for preserving gametophytes of at least some fern species and could be utilized as an additional tool for preserving valuable gametophyte collections and for the ex situ conservation of fern biodiversity.

INTRODUCTION
For many species of ferns, gametophyte tissues have proven to be highly adaptable to growth in vitro (Table 1). Most of these have been initiated through the aseptic germination of spores, although the aseptic germination of gemmae has also been demonstrated (Raine & Sheffield, 1997). As in vitro cultures, gametophytes can provide tissues for research and for propagation, both for ornamental ferns as well as for ferns of conservation concern. The ex situ conservation of ferns has traditionally relied on living collections and spore banks (Ballesteros, 2011). However, there are situations where it may be desirable to conserve other types of fern tissues, particularly if spores are few or are difficult to access. If only a few spores are available, they can be germinated in vitro and multiplied to provide a theoretically unlimited supply of gametophytic tissue, which can be used to generate sporophytes.

Tissues of such in vitro-grown gametophytes have also been shown to be adaptable to storage using cryopreservation, or storage in liquid nitrogen (LN) (Barnicoat et al., 2011; Makowski et al., 2016, 2015, Mikula et al., 2010; Mikula, Jata & Rybczyski, 2009; Pence, 2015; 2000; Wilkinson, 2002). This can serve as an alternative to maintaining active cultures, which requires a constant input of labor and resources. Gametophytes appear to be particularly adaptable to cryostorage, largely due to their simplicity of form and high regenerability (Maeda & Ito, 1981). However, results thus far have demonstrated the ability of gametophytic tissues to survive only short-term exposure to LN, generally 1 hr-3 days (Mikula et al., 2010; Pence, 2000).

In this lab in the 1990s, tissues from in vitro cultures of gametophytes of several fern species were shown to survive LN exposure (Pence, 2000). These common species were chosen because they provided readily accessible material for experimentation, but three
<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Method(^a) if LN Exposed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiantum capillus-veneris L.</td>
<td>Adiantaceae</td>
<td>Somer et al., 2009</td>
<td></td>
</tr>
<tr>
<td>Adiantum tenerum Sw.</td>
<td>Adiantaceae</td>
<td>ED</td>
<td>Pence, 2000</td>
</tr>
<tr>
<td>Adiantum trapeziforme L.</td>
<td>Adiantaceae</td>
<td>ED</td>
<td>Pence, 2000</td>
</tr>
<tr>
<td>Anemia phillitidis (L.) Sw.</td>
<td>Schizaeaceae</td>
<td>Kaźmierczak, 2003</td>
<td></td>
</tr>
<tr>
<td>Asplenium adiantum-nigrum L.</td>
<td>Aspleniaceae</td>
<td>Somer et al., 2009</td>
<td></td>
</tr>
<tr>
<td>Asplenium scolopendrium L.</td>
<td>Aspleniaceae</td>
<td>ED</td>
<td>Pence, 2015</td>
</tr>
<tr>
<td>Ceratopteris richardii Brongn.</td>
<td>Parkeriaceae</td>
<td>Kamachi &amp; Noguchi, 2012</td>
<td></td>
</tr>
<tr>
<td>Ceratopteris thalictroides (L.) Brongn.</td>
<td>Parkeriaceae</td>
<td>ED</td>
<td>Makowski et al., 2015</td>
</tr>
<tr>
<td>Ceterach officinarum DC.</td>
<td>Aspleniaceae</td>
<td>Somer et al., 2009</td>
<td></td>
</tr>
<tr>
<td>Cibotium glaucum (Sm.) Hook. &amp; Arn.</td>
<td>Dicksoniaceae</td>
<td>ED, EV, V</td>
<td>Mikula et al., 2010; Pence, 2000</td>
</tr>
<tr>
<td>Cibotium schiedei Schltdl. &amp; Cham.</td>
<td>Dicksoniaceae</td>
<td>ED, EV, V</td>
<td>Mikula et al., 2010</td>
</tr>
<tr>
<td>Ctenitis pauciflora (Kauff.) Holttum</td>
<td>Dryopteridaceae</td>
<td>ED</td>
<td>Barnicoat et al., 2011</td>
</tr>
<tr>
<td>Cyathea australis Domin.</td>
<td>Cyatheaceae</td>
<td>ED, EV, V</td>
<td>Mikula et al., 2010; Mikula, Jata &amp; Rybczyski, 2009</td>
</tr>
<tr>
<td>Cyathea dealbata (G.Forst.) Sw.</td>
<td>Cyatheaceae</td>
<td>ED, EV, V</td>
<td>Mikula et al., 2010</td>
</tr>
<tr>
<td>Cyathea delgadii Sternb.</td>
<td>Cyatheaceae</td>
<td>ED, EV, V</td>
<td>Mikula et al., 2010</td>
</tr>
<tr>
<td>Cyathea smithii Hook.f.</td>
<td>Cyatheaceae</td>
<td>ED, EV, V</td>
<td>Mikula et al., 2010</td>
</tr>
<tr>
<td>Davallia canariensis (L.) Sm.</td>
<td>Davalliaceae</td>
<td>Somer et al., 2009</td>
<td></td>
</tr>
<tr>
<td>Davallia fejeensis Hook.</td>
<td>Davalliaceae</td>
<td>ED</td>
<td>Pence, 2000</td>
</tr>
<tr>
<td>Dicksonia fibrosa Colenso</td>
<td>Dicksoniaceae</td>
<td>ED, EV</td>
<td>Mikula et al., 2010</td>
</tr>
</tbody>
</table>

\(^a\) LN: Liquid Nitrogen
<table>
<thead>
<tr>
<th><strong>Dicksonia sellowiana</strong> (Pr.) Hook.</th>
<th>Dicksoniaceae</th>
<th>Fiori, Santos &amp; Randi, 2009</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drynaria quercifolia</strong> (L.) J.Sm.</td>
<td>Polypodiaceae</td>
<td>ED Pence, 2000</td>
</tr>
<tr>
<td><strong>Dryopteris dilatata</strong> (Hoffm.) A.Gray</td>
<td>Dryopteridaceae</td>
<td>Somer et al., 2009</td>
</tr>
<tr>
<td><strong>Dryopteris filix-mas</strong> (L.) Schott</td>
<td>Dryopteridaceae</td>
<td>Somer et al., 2009</td>
</tr>
<tr>
<td><strong>Hymenophyllum tunbrigense</strong> (L.) Sm.</td>
<td>Hymenophyllaceae</td>
<td>ED Wilkinson, 2002</td>
</tr>
<tr>
<td><strong>Lepisorus longifolius</strong> (Blume) Holttum</td>
<td>Polypodiaceae</td>
<td>ED Barnicoat et al., 2011</td>
</tr>
<tr>
<td><strong>Macroglossum smithii</strong> (Rac.) Campbell</td>
<td>Marattiaceae</td>
<td>ED Barnicoat et al., 2011</td>
</tr>
<tr>
<td><strong>Notholaena</strong> R.Br. ‘Sun Tuff’</td>
<td>Adiantaceae</td>
<td>Rogers &amp; Banister, 1992</td>
</tr>
<tr>
<td><strong>Osmunda regalis</strong> L.</td>
<td>Osmundaceae</td>
<td>ED, EV, V Makowski et al., 2016 Mikula et al., 2010</td>
</tr>
<tr>
<td><strong>Osmundastrum cinnamomeum</strong> (L.) C.Presl</td>
<td>Osmundaceae</td>
<td>Hollingsworth et al., 2012</td>
</tr>
<tr>
<td><strong>Phlebodium aureum</strong> (L.) J.Sm.</td>
<td>Polypodiaceae</td>
<td>ED Pence, 2000</td>
</tr>
<tr>
<td><strong>Phyllitis (Asplenium) scolopendrium</strong> (L.) Newman</td>
<td>Aspleniaceae</td>
<td>ED, EV, V Mikula et al., 2010</td>
</tr>
<tr>
<td><strong>Polypodium cambricum</strong> L.</td>
<td>Polypodiaceae</td>
<td>Bertrand et al., 1999; Somer et al., 2009</td>
</tr>
<tr>
<td><strong>Pteridium aquilinum</strong> (L.) Kuhn</td>
<td>Dennstaedtiaceae</td>
<td>Whittier &amp; Steeves, 1960</td>
</tr>
<tr>
<td><strong>Pteris adscensionis</strong> Sw.</td>
<td>Pteridaceae</td>
<td>ED Barnicoat et al., 2011</td>
</tr>
<tr>
<td><strong>Tectaria devexa</strong> (Kunze) Copel.</td>
<td>Dryopteridaceae</td>
<td>Ashmore et al., 2011</td>
</tr>
<tr>
<td><strong>Thelypteris confluens</strong> (Thunb.) C.V.Morton</td>
<td>Thelypteridaceae</td>
<td>Sara &amp; Manickam, 2007</td>
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<td><strong>Pronephrium triphyllum</strong> (Sw.) Holttum</td>
<td>Thelypteridaceae</td>
<td>Marimuthu &amp; Manickam, 2011</td>
</tr>
<tr>
<td><strong>Sphaerostephanos unitus</strong> (L.) Holttum</td>
<td>Thelypteridaceae</td>
<td>Marimuthu &amp; Manickam, 2011</td>
</tr>
<tr>
<td><strong>Trichomanes speciosum</strong> Willd.</td>
<td>Hymenophyllaceae</td>
<td>Raine &amp; Sheffield, 1997</td>
</tr>
<tr>
<td><strong>Woodwardia radicans</strong> (L.) Sm.</td>
<td>Blechnaceae</td>
<td>DeSoto et al., 2008</td>
</tr>
</tbody>
</table>

*aMethods: ED = encapsulation dehydration; EV = encapsulation vitrification; V = vitrification*
of the species are also congeneric with rare species (Kang et al., 2008; Kishida, 2015; Yu et al., 2017). In those studies, in addition to testing the initial survival of the gametophytes (Pence, 2000), samples were banked in the Frozen Garden™ of CREW’s CryoBioBank™ for long-term storage. In a recent study to evaluate the viability of numerous samples from the Frozen Garden after years of storage, several samples of gametophytes of these six species were removed and evaluated for their viability after 20 yrs in LN. The results of that study are presented here.

**METHODS**

In vitro gametophyte cultures of *Davallia fejeensis* Hook., *Drynaria quercifolia* (L.) J. Sm., *Cibotium glaucum* (Sm.) Hook & Arn., *Adiantum trapeziforme* L., *Adiantum tenerum* Sw., and *Phlebodium aureum* L. were originally initiated by the aseptic germination of spores in vitro, as previously described (Pence, 2000), and maintained on a medium of half-strength Murashige and Skoog salts and minimal organics (Linsmaier and Skoog, 1965) with 1.5% sucrose and 0.22% Phytage (gellan gum, Sigma Chemical Co., St. Louis, MO) (=½ MS medium), in 60 x 15 mm Petri plates (Corning™ Falcon™, Durham, NC), approximately 15 ml of medium/plate. Cultures were maintained at 26°C under cool white fluorescent lights at 10 – 20 µmol m−2 sec−1 PPFD, with a 16:8 hr light:dark cycle.

Tissue banking took place between 6/21/1995 and 7/26/1995. Fragments of gametophyte tissue were isolated by cutting the gametophyte with a scalpel into small pieces from throughout the gametophyte thallus. Pieces ranged in size from 1-3 mm in length and width. Gametophyte fragments were prepared for cryopreservation by culturing for seven days on ½ MS medium with the addition of filter-sterilized 10 µM abscisic acid (ABA). They were then cryopreserved using the encapsulation dehydration procedure (Fabre & Dereuddre, 1990), in which the fragments were encapsulated in alginate beads before an 18 hr incubation in a 0.75 M sucrose solution, with more than one fragment being encapsulated per bead. The encapsulated tissues were then dried for 3 or 5 hrs under the airflow of a laminar flow hood. The dried beads were stored in 2 ml polypropylene cryovials (Corning®, Corning, NY), 6 to 19 beads per vial, the number of beads varying depending on the amount of tissue available. The samples were stored in a MVE XC 47/11, 900 vial capacity LN storage tank (Chart MVE, Ball Ground, GA), submerged in LN (-196°C). As a control at the time of banking, one vial of each species from each time of banking was rewarmed after a one-day exposure to LN and cultured on ½ MS medium for recovery. Recovery growth was scored as the number of beads showing green growing tissues, and data for the samples were taken between nine and 28 days after rewarming. In the case of three species, survival was also measured after 4.5 months of growth, with data taken after four weeks of growth. Samples from each species were also removed after 3.5 yrs of storage in LN, and recovery data was taken after two and seven weeks of growth. The data taken seven weeks after rewarming were reported previously (Pence, 2000).

Samples were removed from LN between 7/16/2015 and 8/14/2015 to evaluate 20-yr survival. Recovery conditions in the present study replicated as much as possible the original recovery conditions, using ½ MS medium, only with modification in the source of the prepared MS salt mixture (Phytotechnology Laboratories, Overland Park, KS) and the source and amount of the gellan gum (Gelzan, Caisson Labs, Smithfield, UT), added at 0.33%. One vial representing each species and each banking time for that species was removed (9 vials total) and rewarmed at ambient temperature (22°C) for 15 min. The
beads were then plated on 1 – 4 plates of ½ MS medium, depending on the number of beads, 4-6 beads/plate. Cultures were maintained under the same temperature and lighting conditions originally used for growth.

At the time of banking and at 3.5 yrs, survival was scored as the number of beads showing some recovery growth of encapsulated tissues, regardless of the number of fragments per bead. When tissues were evaluated after 20 years, survival was scored both as the percent of fragments and the percent of beads showing green, growing tissue at 3 – 4 weeks.

RESULTS

Growth from cryopreserved gametophyte tissue occurred from all of the species of ferns tested in this study (Table 2). The values for the original survival data reported here are those recorded for the specific samples that were banked and are different from those reported previously (Pence, 2000), which were collected from experiments done in preparation for the long-term banking of samples. In all but two species, the percent of tissues showing growth after storage was greater than that observed before long-term storage. In *Drynaria quercifolia* and both samples of *A. trapeziforme*, post-storage growth was less than that observed originally, and in one sample of *A. trapeziforme*, there was no post-storage growth. However, that sample produced fungal growth on a few beads within the first few days of culture, and the remaining beads were transferred to fresh plates. The transferred beads did not show any contamination, but they also did not show any growth. Specific moisture levels of the banked samples used in this study are not available, except for two of the three samples, *D. quercifolia* and *A. trapeziforme*, which were dried for 5 hrs to 17.5% and 18.9% moisture, respectively.

Samples recovered at 20 yrs were scored at 3 – 4 weeks. When these were compared with samples recovered at 3.5 yrs, the percent survivals were, in most cases, more similar to those recorded at two weeks from the 3.5 yr samples than with survivals recorded at seven weeks for those samples, which in all cases were higher than two week survival values (Table 2).

Since pre-storage viability was based on growth per bead, post-storage measurements were calculated both on growth per bead and on growth per fragment for seven of the nine samples. The percent survival was very similar between the two measurements (Table 2). Most fragments showed some damaged areas, but ultimately, those with any surviving areas went on to regenerate gametophyte tissues (Figure 1).

DISCUSSION

These results demonstrate that *in vitro*-grown gametophytes of at least some fern species can survive up to 20 yrs in LN storage without appreciable deterioration using the encapsulation dehydration procedure. This method has been applied by others in the intervening yrs with good success (Table 1). There also have been reports of some survival of fern gametophytes using the encapsulation vitrification method (Makowski *et al.*, 2016; Mikula *et al.*, 2010), although when it was compared with encapsulation dehydration, the latter resulted in higher survival. Studies using vitrification without the protection of encapsulation have uniformly reported severe plasmolysis of the gametophyte tissues and no survival after exposure to LN (Makowski *et al.*, 2016; Mikula *et al.*, 2010).

When the successful cryopreservation of fern gametophytes was reported originally, 3 – 4 hrs of drying brought samples to 19-27% moisture (wet weight basis) (Pence, 2000).
TABLE 2
Percent survival of six species of fern gametophytes, dried for 3 or 5 hrs, through LN exposure before storage and after 4.5 mos, 3.5 yrs, and 20 yrs of LN storage. Day of observation is given in adjacent column, if different for different samples, or in the heading, if the same for all samples.

<table>
<thead>
<tr>
<th>Species</th>
<th>Hrs of Drying</th>
<th>Pre-storage</th>
<th>4.5 Mos</th>
<th>3.5 Yrs</th>
<th>20 Yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day</td>
<td>Survival</td>
<td>aSurvival (at 4 wks)</td>
<td>Survival (at 2 wks)</td>
</tr>
<tr>
<td>Adiantum tenerum</td>
<td>3</td>
<td>12</td>
<td>41.7</td>
<td>63.0</td>
<td>88.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>16</td>
<td>60.0</td>
<td>73.3</td>
<td></td>
</tr>
<tr>
<td>Adiantum trapeziforme</td>
<td>3</td>
<td>12</td>
<td>25.0</td>
<td>33.0</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4</td>
<td>84.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cibotium glaucum</td>
<td>3</td>
<td>16</td>
<td>46.2</td>
<td>80.0</td>
<td>75.0</td>
</tr>
<tr>
<td>Davallia fejeensis</td>
<td>5</td>
<td>8</td>
<td>46.2</td>
<td>80.0</td>
<td>100.0</td>
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<tr>
<td></td>
<td>3</td>
<td>9</td>
<td>6.7</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>Drynaria quercifolia</td>
<td>5</td>
<td>6</td>
<td>91.7</td>
<td>50.0</td>
<td>67.0</td>
</tr>
<tr>
<td>Phlebodium aureum</td>
<td>3</td>
<td>27</td>
<td>19.3</td>
<td>67.0</td>
<td>90.0</td>
</tr>
</tbody>
</table>

aData available for three species only.
bSeven week survival data from Pence, 2000.
The moisture contents available for two of the samples in this study that had been dried for five hrs were 18 – 19%. With seeds, a number of studies have been directed at defining optimum storage moisture levels, which are related to the seed oil content (e.g. Pritchard, 1995; Hor et al., 2005). Whether the moisture levels used in this study are optimum for fern gametophytes will require further research, but they were sufficient to allow regeneration of at least some of the tissues after cryostorage.

The fact that survival after long-term storage was greater in most cases than pre-storage survival may have resulted from data being taken much earlier in the case of some of the pre-storage samples, as these were done by different operators, at different times, for various goals. The results of the 3.5 yr samples indicate that the initiation of growth continued for several weeks, with more fragments growing at seven weeks than at two weeks. Alternatively, the sample sizes in this study were small and may not be large enough to clearly track any small changes in viability over the time of storage. Similar increases in viability after LN exposure have been noted with small samples of other species, by others, as well as in this laboratory (Mix-Wagner, Schumacher & Cross, 2003; Vanhove, Philpott & Pence, 2016).

The highly regenerative nature of fern gametophytes has been noted for several species (Maeda & Ito, 1981; Ong & Ng, 1998), and this ability makes these tissues well adapted to cryopreservation. Even if some tissues are damaged in the process of either cryoprotection or LN exposure, only a small amount of tissue is required (possibly only one cell) to regenerate the gametophyte tissue (Maeda & Ito, 1981). However, this ability makes it more difficult to evaluate survival quantitatively, because fragments are of different sizes and only portions of them may survive. Having more than one fragment encapsulated in an alginate bead can increase the number of fragments available for regrowth and minimize the number of beads needed, and survival per bead appears to

Figure 1. Encapsulated gametophytes from two fern species resuming growth after 20 yrs of storage in liquid nitrogen. A. Adiantum tenerum gametophytes, as well as fragments that did not grow (arrow); B. Phlebodium aureum gametophytes. Scale bars = 0.5 cm.
correlate well with fragment survival. When survival of the 20-yr old samples was measured by both methods, the results were very similar.

Cryopreservation of fern gametophytes has thus far been applied to in vitro cultures of gametophytes. This requires the initiation of gametophyte cultures by the aseptic germination of spores or gemmae, and in vitro fern gametophyte cultures initiated in this way have been established and reported for a number of species (Table 1). However, for species known only from the gametophyte in nature, in vitro cultures would need to be established directly from gametophytic tissue. Methods for this have been reported for gametophytes of bryophytes (Rowntree & Ramsay, 2005; Segreto et al., 2010) but not yet for fern gametophytes. Gametophytes of bryophytes are more complex structures than those of ferns, and further research is needed to determine whether fern gametophytes can survive the surface sterilization required for initiating in vitro cultures.

The results of this study indicate gametophyte cryopreservation is an option for long-term conservation of in vitro collections of fern gametophytes that have been developed for horticultural or experimental purposes, as well as an option for the ex situ conservation of fern biodiversity. Thus, it is a tool that can contribute to the ex situ conservation goals (Target 8) of the Global Strategy for Plant Conservation (Convention on Biological Diversity, 2010). Further research is needed to test the applicability of encapsulation dehydration to more species with different habits and adaptations in order to more clearly define the breadth of its potential use. In addition, more long-term studies should be initiated to evaluate the effectiveness of cryopreservation in preserving tissues over the course of decades. However, these results with six species clearly demonstrate the potential of this method for fern conservation in the future.

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REFERENCES


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