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ANNOUNCING A NEW FEATURE – FERN GAZETTE REVIEWS

With this issue, we are introducing a new feature. The Fern Gazette will be publishing a series of reviews on developments in a wide range of research topics relating to ferns and lycopods, written by international authorities in their fields. Every issue of the Fern Gazette will contain at least one review.

Each review will provide an easily accessible introduction to a comprehensive list of recent research-based publications and is intended for those new to the subject or who wish to bring themselves up-to-date with the literature. For a fast-developing subject featuring in frequent publications, 'recent' might mean the last 5-10 years, while when progress is slower, and especially when the subject has not been reviewed for many years, it might represent a much longer period.

While taxonomic and floristic themes will continue to be covered, any research topic which has ferns or lycopods as its central subject will be eligible for consideration in a review, whether it is concerned with conservation or classification, development or demographics, gametophytes or genetics, phenology or physiology. Some historical topics, like the exploitation of ferns as a resource, or the development of ideas, illustration methods or investigative techniques, might be suitable for a review. A review can contain an account of some original, unpublished, research by the author, but if original work forms the major part and the literature survey is merely the introduction, it should be submitted to the senior editor, Professor Mary Gibby, as a paper.

The review topic should normally be one that can be adequately covered in a review of 3-4000 words plus figures and references, though longer or shorter reviews might be acceptable for some topics. The review will be refereed, and the editors may subsequently request clarification or modification but no editorial changes will be made without the author's agreement. Publication will usually be within six months of receipt of the agreed final version.

Those reviews that will appear over the next two years have been written in response to invitations from the Review Editors but offered contributions are welcomed.

Offers of reviews, or requests to discuss possible topics or to obtain further details of instructions for authors, should be sent to the Fern Gazette Review Editors:
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RECENT DEVELOPMENTS IN *EX SITU* AND *IN SITU* CONSERVATION OF FERNS

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Key words: fern conservation, *in situ*, *ex situ*, *quasi in situ*, spore bank, wet and dry storage, cryopreservation, *in vitro* tools.

ABSTRACT

Some members of the monilophytes, an ancient plant group with worldwide distribution, show significant population decline which is frequently correlated with the vulnerability of their habitat. Many species are today under some level of threat, mainly by the disappearance of natural habitats or climate change. In evaluating the threat to species survival, the IUCN indicate that the coverage of the pteridophyta is insufficient, and the lack of information concerning the conservation status of Pteridophytes has been highlighted. Protection measures are demanded and developed for most of them to counteract the drastic reduction of genetic diversity of natural populations. In this review, *in situ*, *ex situ* and *quasi in situ* conservation techniques applied during the last decade are surveyed and future research needs identified.

The different types of conservation are not exclusive but complementary. Fern research requires higher commitment, mainly to increase knowledge of actual biodiversity, species richness and the occurrence of threatened species, soil spore banks, reproductive biology and population dynamics, threat identification and conservation management priorities and procedures. Open access to knowledge will enhance the conservation and rational utilization of ferns.

INTRODUCTION

Chapman (2009) estimates that there are c. 12,000 species of ferns and fern allies. These plants were previously grouped together as Pteridophyta but are now classified as monilophytes and lycophytes respectively. They are both ancient plant groups with long fossil records (Pryer *et al*. 2004, 2009; Christenhusz *et al*. 2011). Research suggests that the last common ancestor of extant monilophytes and lycophytes existed about 400 million years ago in the early-mid Devonian (Becker *et al*. 2002; Pryer *et al*. 2004). Ferns were dominant from about 380 million to 290 million years ago in a tropical and subtropical environment, but many of the current families and species did not appear until roughly 145 million years ago in the early Cretaceous.

Distributed worldwide, this plant group, growing mainly in tropical and temperate regions, comprises a significant plant biodiversity. Anthelme *et al*. (2011) highlights the diversity of the pteridological flora of arid zones because ferns are more frequent than initially thought in arid environments thanks to efficient dispersal, elevation refuges, physiological adaptations and the presence of local abiotic refuges.

Ferns are not of major economic importance, but they have potential as commercial and environmental resources; some are grown or gathered for food, as ornamental plants, and for the phytoremediation of contaminated soils, as is the case with *Pteris vittata* L., the first fern identified as a naturally evolving arsenic hyperaccumulator (Xie

et al. 2009). Ferns also have been the subject of research for their ability to remove some chemical pollutants from the air (Kim *et al*. 2010), or because some of them are significant weeds. They also play a role in mythology, medicine and art (Keller *et al*. 2011; Molina *et al*. 2009).

During recent decades, many reports indicate a decline in the fern population, maybe linked to the vulnerability of their habitats. Their sexual reproductive system, strictly tied to water, gives them great sensitivity to environmental changes. Many species are today under some level of threat, mainly by the disappearance of natural habitats or climate change. The Red List of Threatened Species (IUCN, 2012) includes 167 species of fern and fern allies in the threatened category (critically [CR]+endangered [EN[+vulnerable [VU]), 2 extinct (EX) and 19 near-threatened (NT), but there is insufficient coverage of the group, as only 3% of described species are evaluated. Protection measures have been demanded and developed for most of them to counteract the drastic reduction of genetic diversity of natural populations, but additional efforts are still needed. Blackmore & Walter (2007) emphasized the lack of information concerning the conservation status of Pteridophytes and so the need for experts committed to the improvement of understanding of the conservation needs of ferns and fern allies is one of the challenges for the Global Strategy for Plant Conservation (GSPC) 2002-2010. Today, from the perspective of GSPC 2011-2020, this necessity remains valid.

The main serious threats for ferns are: loss of habitats due to climate or microclimate change or designation of land for agriculture, commerce related to ornamental and ethnobotanical uses, invasive species and overexploitation of natural resources. Ranil *et al*. (2011) denounce the illegal use of *Cyathea* Sm. plants collected from the wild. The utilization of ferns is not usually properly regulated. For this reason, research into cultivation of the species is an essential requirement.

In situ versus *ex situ* conservation is a question that often arises when dealing with the conservation of species. It is universally accepted that the most effective and efficient mechanism in conservation is habitat protection, but it is also accepted that *ex situ* conservation techniques are critical components in a global conservation programme.

In contrast to *in situ*, *ex situ* conservation aims to preserve the genetic integrity of populations and individuals.

These two types of conservation are not mutually exclusive but complementary. In the special case of threatened species, *in situ* conservation measures are complemented by *ex situ* ones, increasing long-term security.

The GSPC 2011-2020, in its target 3, emphasizes the need to encourage research, particularly to develop methodologies and techniques oriented towards the optimization of plant conservation practices, mainly integrating *in situ* and *ex situ* conservation and, as well, to make all information available to practitioners of plant conservation.

In 2001, during the International Symposium celebrated at the University of Surrey, Guildford (UK) entitled: "Fern Flora Worldwide Threats and Responses", a wide view of practices, experiences, techniques and studies on fern conservation was provided by specialists from all around the world. The link of this relevant event with the Species Survival Commission of the World Conservation Union (IUCN) gave wide awareness of the most significant aspects of fern conservation through numerous communications highlighting different research lines on fern conservation (e.g. Dyer *et al*. 2002). These publications in turn stimulated new work on these topics during the last decade.

Today, many conservation actions have been developed, but we found large differences between geographical areas and between programmes aimed at ferns. Many areas are well covered but others not at all, and this is not always related to the wealth of biodiversity present in them.

With these circumstances, most fern research today is designed to increase knowledge of actual biodiversity, species richness and the occurrence of threatened species, soil spore banks, reproductive biology, and population dynamics, to identify threats and to establish *ex situ* and *in situ* conservation management priorities and procedures. Finally, studies on spore germination, gametophyte development and cultivation of sporophytes constitute the basis of recovery programmes for damaged populations in nature.

The aim of this review is to provide an over-view of research during the last decade into the conservation of ferns and their allies, and to identify future research needs in this area.

IN SITU CONSERVATION ACTIONS

In situ conservation is a dynamic conservation, since the evolution of species continues in the same environment in which plants grow, involving gene pools and also the process of co-evolution (Maxted *et al.* 1997).

In situ conservation actions could have different approaches oriented to preserve genetic, species and ecosystem diversity. The main objectives of the published research or activity reports are genetic characterization, re-enforcements, re-introduction, recovery of populations or establishment of natural reserves and protected areas to preserve fern species. Practical actions usually include some study of, or are developed as a result of, previous research on threatened species.

The management of protected areas constitutes a central element of a strategy to conserve biodiversity. Usually these plans are governmental programmes, national or regional. The preservation of threatened species in their natural habitats is the principal aim of an *in situ* conservation programme. The first step is to know exactly which components of the biodiversity within the territory under consideration need protection action, by determining the conservation status of the species. IUCN Standards and Petitions Subcommittee (2010) established the guidelines to assess the conservation status of species. Benniamin *et al.* (2008) indicate various difficulties of applying IUCN methods to the ferns, and proposed new criteria, using biological characters, for determining the conservation status when they lack sufficient population data.

Strategic plans for fern *in situ* conservation

When an *in situ* conservation plan must be developed, it is recommended that available information of previous activities is checked and the priorities are discussed with experts in order to select the best methodology. Afterwards it is useful to share the results and conclusions through open access publishing which enables future improvement of *in situ* techniques. We have found diverse recovery, management or strategic plans or programmes for fern species conservation, etc., with diverse structure and contents; most of them are mono-specific studies.

Sarkis (2010) gives us a relevant model of a recovery plan with a very detailed description of priorities and tasks needed to implement actions to protect and recover a natural population of ferns. Ranil *et al.* (2011) established conservation priorities for tree-ferns of the Cyatheaceae family and indicated that limited information is a major

barrier to conservation and management of these species. Some other practical examples are the management plans for *Chingia australis* Holttum (Herbert, 2006), for *Thelypteris quelpaertensis* (H.Christ) Ching (Wildlife Division, 2011) and for *Dicksonia sellowiana* Hook. (Alfonso-Moreno *et al*. 2011).

Studies led by Aguraiuja et al (2008) conclude that the analysis of population stage structure, together with data about population size, gives useful information about the relative status and regional dynamics of populations in cases where precise demographic and distribution data are lacking.

In experiments with *Diellia pallida* W.H.Wagner, Aguraiuja (2005) described the sowing of spores directly on soil, and concluded that the unpredictability of environmental changes in the natural habitat creates difficulties for the study of gametophyte establishment. This observation is in agreement with McHaffie (2004), who indicated that a microhabitat with stable conditions is required for successful gametophyte establishment for *Woodsia ilvensis* (L.) R.Br. Aguraiuja (2005) stated the relevance of increased knowledge on the timing of germination in natural conditions and, as well, on the ecology of gametophyte development for ferns.

In situ conservation also requires monitoring to check the evolution of the population and the success of the methodology applied in order to improve this type of protocol.

Natural soil spore banks

To complete this survey of different options for the conservation of ferns, we must consider the existence of banks of spores in the soil. Soil spore banks have a potential role in the conservation of endangered fern species (Dyer & Lindsay, 1992, 1994; Dyer, 1994). Ramírez-Trejo *et al.* (2004) emphasized the spore bank in the soil as a potential source for *in situ* regeneration. Ranal (2004) has also proposed tree bark as another kind of *in situ* spore bank that could contribute to fern conservation.

Hock *et al.* (2006) studied seasonal patterns of soil spore banks of ferns in grasslands on dolomite rock and stated that, after one year of storage, the number of emerging prothallia in some soil samples increased, presumably because some spores were initially dormant.

Soil spore banks can be very useful for population reinforcements and to increase the genetic diversity, especially in threatened species with very small populations, and it is the first option for the recovery of spores for the reintroduction of species in places in which the disappearance of populations is observed.

Practical examples are the recovery of a lost population of *Marsilea quadrifolia* L. (Figure 1) in the Natural Park of the Ebro Delta, Catalonia, Spain (Estrelles *et al*. 2001) and the recuperation of *Christella dentata* (Forssk.) Brownsey & Jermy in the Natural Park of los Alcornocales, Andalusia, Spain (Rodríguez-Hiraldo *et al.* 2006).

The persistence of soil spore banks determines the natural capacity for *in situ* regeneration of wild populations. Besides, from soil spore banks additional *ex situ* actions could be developed for propagation and conservation of germplasm.

EX SITU CONSERVATION

Ex situ conservation could be developed as living collections (sporophytes), usually maintained in Botanic Gardens, or through germplasm banks which include conservation of vegetative propagules, gametophytes or spores. Storage of spores

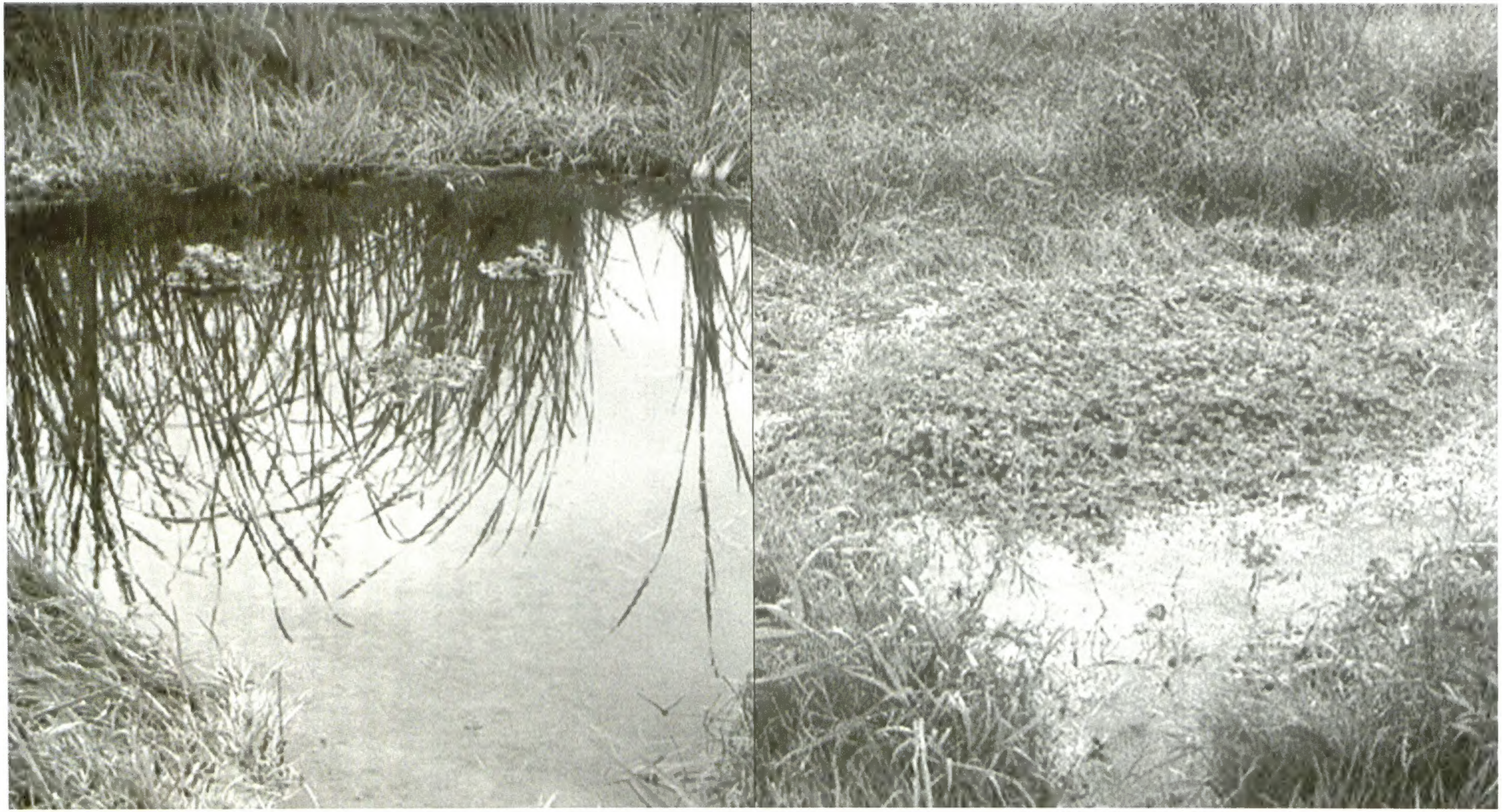

Figure 1. Recovery of *Marsilea quadrifolia* L. in the Natural Park of Park of the Ebro Delta, Catalonia, Spain. Left. Reintroduction of cultivated plants. Right. Coverage of the plants after five months since their plantation.

preserves large quantities of germplasm with high genetic variation in small spaces with low financial and technical costs. *Ex situ* conservation activities also include research into *in vitro* germination from spores and the cultivation of sporophytes from stored germplasm (Figure 2).

The use of *ex situ* conservation techniques for endangered plants in germplasm banks has increased since its consideration in the Convention on Biological Diversity (UNEP, 1992) and in the Global Strategy for Plant Conservation (UNEP, 2002). It is widely employed for seed-bearing plants but needs further efforts in Pteridophytes. Already in 1992, Page *et al*. provided a first approach to spore storage in germplasm banks as an *ex situ* conservation tool. Today *ex situ* measures are widely recognized as an effective tool for fern preservation, complementary to *in situ* conservation programmes.

The study of the longevity of chlorophyllic (green) and non-chlorophyllic (non-green) spores has evolved during the past 50 years. Methodologies have been improved and some protocols established to optimize spore preservation in fern spore banks.

Green spores show a rapid deterioration which has been attributed to their lack of tolerance to desiccation, their high metabolic rate, and their absence of dormancy (Kato, 1976). They have traditionally been treated similarly to recalcitrant seeds, while non-green spores have been treated in the same way as orthodox seeds.

Gabriel y Galán & Prada (2011) analyze and discuss the concept of variation in fern spore viability between species, the factors influencing it (ploidy level, age, temperature or spore physiology), and the best methods to determine it. The study of all these factors helps to increase the understanding of spore viability loss under different storage conditions and the optimal conditions to preserve it. Ballesteros (2011) analyzed different techniques for conserving spore viability and reviewed the background research.

Magrini (2011) retrieved viable spores of the endangered species, *Dryopteris tyrrhena* Fraser-Jenk. & Reichst., from herbarium specimens. With a maximum germination around 20%, spores remain viable up for to three decades after placing them in storage. Fern propagation from herbarium spores is proposed as a useful method for preserving rare, threatened or extinct species.

For green spores, herbaria are also a valuable source of living germplasm with potential in the recovery threatened plant species. Magrini *et al*. (2010) propagated *Osmunda regalis* L. using spores collected from exsiccate (dried herbarium specimens) stored in herbaria for five and 17 years.

Long term viability in ambient herbarium conditions has been reported for many species. However, storage conditions of herbarium specimens are highly variable, especially with respect to the relative humidity, depending on geographical location and available facilities. Spore longevity is closely correlated with temperature and humidity, and varies between species (Ballesteros *et al*. 2011), so longevity of spores in uncontrolled conditions is unpredictable.

Artificial Spore Banks – spores in storage facilities

There are different approaches to setting up a new bank for spore storage. Short (1-10 years), mid (10-30 years) and long term storage (>30 years) options are available for different objectives. These options are established under a range of temperatures and relative humidity values; wet and dry storage and cryopreservation are widely used.

The selection of the particular conditions to use in a spore bank depends basically

on the type of material to be preserved, the conservation action to be achieved and the available facilities. Lower temperature and lower humidity tend to prolong spore longevity. But water content must be strictly controlled and the optimal value determined because there seems to be a minimum value below which viability is lost.

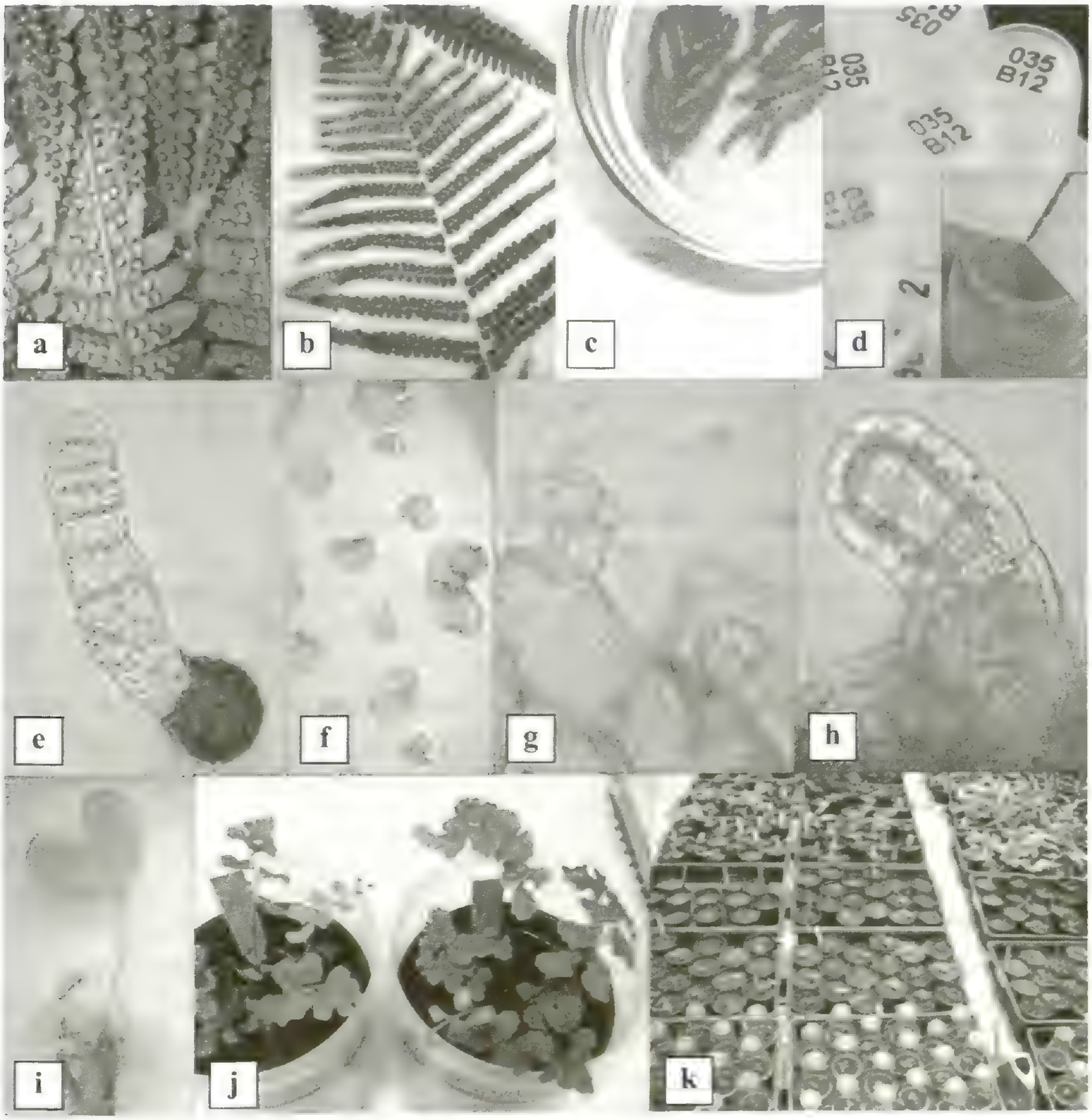

Figure 2. Details on fern propagation and cultivation for conservation purposes, from the spore collecting to growing of the sporophytes. **a.** Selection of mature fronds with preferably closed sporangia. **b.** Drying of fronds till opening of sporangia and releasing of spores on glossy paper sheets. **c**. Elimination of indusia, scales and remnant sporangia or leaf material with a sieve in order to collect clean spores. **d.** Dosage of spores in vials for cryopreservation. **e.** Linear phase of gametophyte development (*Cosentinia vellea* (Aiton) Tod.). **f.** Cordate phase of gametophyte development (*Asplenium marinum* L.). **g.** Detail of antheridia and liberated antherozoids in the gametophyte of *Asplenium majoricum* Litard. **h**. Detail of archegonium in the gametophyte of *Asplenium majoricum*. **i.** First leaf of a young sporophyte (*Athyrium filix-femina* (L.) Roth). **j.** Growing of sporophytes of *Asplenium marinum* in plastic containers for plant culture in incubators. **k.** Acclimatization of adult sporophytes in the greenhouse.

Cryogenic conditions are aimed at long-term collections.

The main objective of long-term conservation spores of ferns in germplasm banks is to maintain viability and the capability of producing sporophytes that can later be used in restoration of habitats.

Wet storage

Lindsay *et al.* (1992) established the effectiveness of wet spore storage, particularly recommended for green spores and where low temperature facilities are not available.

Quintanilla *et al.* (2002) and Aragón & Pangua (2004) compared germination of spores of different species stored under wet and dry conditions. Their results indicate tolerance to desiccation and different preferences for the species studied. Quintanilla *et al.* (2002) identify some advantages of dry storage, such as the shorter preparation time and smaller space requirements, and the higher efficacy at lower temperatures. Aragón & Pangua (2004) found some correlation between ecological requirements of species and spore viability behaviour, and therefore proposed that the ecology of the species be considered when developing spore conservation programmes.

Dry storage

Dry storage has been practised under different temperatures; 5°C, -10°C and -20°C are the usual temperatures in gene banks. Longevity studies under diverse storage temperatures and humidity were conducted in a wide range of species. In a first approach to provide a general scheme of fern spore behaviour, Ballesteros *et al.* (2006) checked the response of spores from ten species collected in diverse habitats after six months of storage. Ballesteros *et al.* (2012) provide relevant data on behaviour during a longer, 3-year, storage period. These studies showed great variation of spore longevity between different species. Temperatures below zero, such as – 25°C, require an accurate control of water content in order to avoid negative effects on viability. Practical procedures for spore bank management were summarized in Estrelles *et al.* (2003) and Ibars *et al.* (2011), including collecting and spore preparation details.

Other parameters have been recently analyzed, such as water affinity, calorimetric properties of water and triacylglycerols, to increase physiological understanding of the spore ageing processes. Ballesteros & Walters (2007a) suggest that the affinity of fern spores for water is low and that they dry quickly but rehydrate more slowly. As with seed storage, the longevity of fern spores and the optimal conditions of storage will be closely related to the moisture content of the spores.

Ballesteros & Walters (2007b) demonstrated that crystallization of triacylglycerols (TAG) appears to be associated with fern spore response to low temperature. This research suggests that fern spores exhibit a storage physiology that has been described as intermediate between recalcitrant and orthodox. They determined that there is a narrow range of water contents appropriate for low temperature storage of fern spores, such that the water content of the spore must be adjusted carefully for achieving maximum longevity.

The behaviour of green spores continues to receive attention. Lebkuecher (1997) confirmed their recalcitrant behaviour, while Pence (2000b) suggested that green spores could be more tolerant to desiccation than was originally thought. The recent work of Magrini & Scoppola (2012) studied the influence of long term storage on the viability of wet and dry spores of *Osmunda regalis* stored under different temperatures. New information on the viability of chlorophyllous spores was provided, and, in contrast to

Pence (2000b), they concluded that dried spores of *O. regalis* showed the lowest survival percentages. However their results do not confirm the beneficial effects of wet storage for this species. The optimal result (fast germination and gametophyte development) in this study was obtained at sub-zero temperatures (-20°C) with no desiccation.

Cryopreservation

Cryopreservation is typically used for the long-term storage at -80 °C to -196 °C, of germplasm of plants in which the previously described methods are not suitable (Benson, 1999). It has been widely reported that the spores of diverse species remain viable longer at these temperatures than at higher temperatures (Quintanilla *et al.* 2002; Pence, 2000b; Ballesteros *et al.* 2004; 2006). Cryogenic preservation and *in vitro* culture appear to be the best option for spores with recalcitrant behaviour, but also for optimal conservation of Pteridophytes in general, due to its simplicity and effectiveness for a wider range of species and for longer periods. Ballesteros (2006, 2011, 2012) showed the optimal and homogeneous responses of ultra-freezing temperatures for all the species studied. Overall, cryopreservation appears to be the most effective method for long term conservation of fern spores.

Pence (2008a, b) incorporated into her work various protocols for cryopreservation of spores and gametophytes of Pteridophytes. Cryopreservation of gametophytes of Pteridophytes is maybe less used than in Bryophytes, although processes described for mosses can also be applied to ferns (Pence, 2002; Wilkinson, 2002). Fern gametophytes have a high capacity for regeneration but a lower tolerance to desiccation. Mikula *et al.* (2011) report diverse practices for cryopreservation of gametophytic tissues. These techniques are specially recommended for species with short-lived spores.

In vitro techniques

In vitro techniques are mainly needed to rescue plants that produce recalcitrant spores/seeds or propagate only by vegetative means, but also to complement other *ex situ* methods for saving plants from extinction (Barnicoat *et al.* 2011). Menéndez *et al.* (2011a) review the different *in vitro* options and summarize some protocols to obtain sporophytes in the laboratory.

Somer *et al.* (2010) provide relevant data on *in vitro* culture techniques, mainly relative to the influence of growth rate, the formation of sexual organs, and the production of sporophytes from homogenised gametophytes or sporophytes of several species. Numerous aspects of the regulation of sexual expression of gametophytes were studied to provide optimal protocols for *ex situ* propagation. Menéndez *et al.* (2011b) summarize many questions about sexual reproduction in pteridophyta, and the hormonal control in sexual differentiation in the gametophyte and also introduce proteomic studies as a valuable tool to gain knowledge on plant reproduction.

The application of *in vitro* techniques has been assessed for different fern groups. Ranil *et al.* (2008) provide encouraging results on effective spore germination media, gametophyte morphology and the successful raising of sporophytes from gametophytes and their transfer to general growth media in *Cyathea walkerae* Hook. Rybczyński & Mikuła (2011) summarize published studies related to the application of biotechnology methods to obtain tree fern sporophytes from freshly collected or stored spores. Camloh & Ambrožič-Dolinšek (2011) reviewed *in vitro* and cryopreservation techniques for propagation and conservation of *Platycerium* Desv. species. Marszał-Jagacka &

Kromer (2011) evaluate these *in vitro* techniques for serpentine fern species of the genus *Asplenium* L.

Asexual propagation, also known as vegetative techniques, is only recommended for conservation purposes in special situations; e.g. for taxa where sexual reproduction through spore germination presents difficulties. In consequence, it is not the first option. The most usual protocols use rhizome cuttings, bulbils, stolons and root buds.

The stipule is an optional method efficiently applied to *ex situ* propagation of marattioid species as their spores are hard to germinate and gametophytes grow slowly (Chiou *et al.* 2006; Chou *et al.* 2007; Huang *et al.* 2011). The only limitation of this method is for those species with low production of fronds, in which case the material for asexual propagation is strictly limited (Huang *et al.* 2011). Another approach is given by Martin *et al.* (2006) for the propagation of *Pityrogramma calomelanos* (L.) Link through the induction of apospory and apogamy.

Chá- Chá *et al.* (2005) and Barnicoat *et al* (2011) highlight *in vitro* culture procedures and cryopreservation methods for the integrated conservation of threatened ferns and indicate the importance of proper collection measures. The sampling strategy for germplasm collecting must be always carefully determined prior to developing any *ex situ* action. Distribution, genetic structure of population, autecology and population biology are the main factors determining the optimum sampling strategy.

QUASI IN SITU CONSERVATION: A NEW CONCEPT

When we apply *ex situ* techniques we preserve the genetic structure of the population at the moment of sampling. Evolution and co-evolution, the engine driving new diversity, are interrupted (Jaramillo & Baena, 2002). Integration between *in situ* and *ex situ* strategies are needed for plant conservation.

In 2010, a new concept for plant conservation was introduced by Volis & Blecher, the "*Quasi in situ*" conservation. This approach basically proposes the maintenance of living-plant *ex situ* collections in a natural or semi-natural environment. *Quasi in situ* preservation is considered a novel strategy for biodiversity conservation, with the aim of maintaining interactions between individuals, species and environment, in order to allow the evolution and adaptation of wild genotypes to continue during conservation actions. Detailed guidelines for representative sampling, collection maintenance and utilization for *in situ* actions, as well as advantages of this new strategy over traditional *ex situ* living collections, are compiled by Volis & Blechner (2010). In summary, the advantages include less restriction on available space, high suitability of environment, natural processes of maintenance of plants and, consequently, low costs. Specific details and guidelines on this new strategy are given in the original paper and seem to be relevant for fern conservation.

Some conservation projects, developed some years ago, included in their approach the essence of this new strategy before the appearance of the concept. This applies especially to those developed as a consequence of the disappearance of the natural habitat, where the relocation of wild populations is required (Lusby *et al.* 2002. McHaffie, 2007). Aguraiuja (2011) discusses the experimental methods used and the first results of an introduction action with *Woodsia ilvensis*, with 10 years monitoring of plants. The results of plant establishment and subsequent successful recruitment confirm that the rarity of this species in Estonia is mainly due to the limited distribution of suitable habitats. This author indicates the relevance of future research on population ecology of ferns.

Laguna *et al.* (2012) applied the term "safety neopopulations" to new populations, created in areas distant from the natural populations, which are not jeopardized by the factors that reduced or caused the disappearance of the species.

But, in most studies, the proposed establishment of *ex situ* collections in a natural or semi-natural environment is only the final option of several. Sometimes, after the genetic survey, artificial propagation and transplantation programmes to establish populations in suitable habitats were recommended. In many instances, the eventual action depends on a state decision (national or local government of the territory) and, finally, on availability of funds. It would be interesting to conduct a survey to determine the number of conservation plans, based on previous research funded for several years, that have been subsequently carried out by competent authorities.

GENETIC DIVERSITY OF POPULATIONS

Preservation of genetic diversity of a particular population is, finally, the main aim of any conservation strategy. Both *in situ* programmes and *ex situ* collections require the evaluation of genetic diversity to achieve an accurate management and establish priorities.

A number of recent examples, Liu *et al.* (2007), Dong *et al.* (2007, 2010), Kang *et al.* (2008), Hunt *et al.* (2009, 2011), Cheng *et al.* (2010), Izuno *et al.* (2011, 2012), all refer to the conservation implications for the evaluated species.

Jiménez (2011) proposes microsatellite technology as a high-quality procedure to determine fern genetic biodiversity including a review of other methods as well as the advantages and disadvantages of this technique. Peredo *et al.* (2011) recommend that dominant or unspecific markers (RAPD, ISSR, REMAP, IRAP, AFLP, MSAP) as useful tools to answer genetic questions concerning diversity, reproductive isolation, or conservation actions in ferns.

Genetic conservation includes the maintenance of the level of genetic variability of the species and of their natural populations, as well as respect for, and maintenance of, genetic structure within and between existing populations, in order to avoid losing the adaptations already in place. Small populations are more affected by drift and gene flow than large ones; this makes it essential to take into account genetic considerations.

De Groot *et al.* (2012a, 2012b) evaluated the genetic structure in young populations of four species, from two fern genera, with different ploidy level and mating system, and correlated it with long- distance colonization events. They prove that establishment from a single spore, only possible for genotypes capable of self-fertilization, are possible and frequent, even at long distance, for all studied species. These populations showed low genetic variation and high inbreeding coefficients. Evidence for inter-population gene flow was low. They indicate that limited gene flow can conserve the genetic signature of multiple colonization events for decades. This is an important consideration for conservation of genetic diversity, as extinction of a single population (e.g. by habitat destruction) will result in a considerable loss of genetic diversity. These findings will be highly relevant for future decisions concerning the conservation of pteridophyte diversity and the development of strategic plans for its conservation.

Field studies and actual experiences configure the base on which to model future actions oriented to reduce human pressures on the natural populations of threatened fern species.

The basic principles of fern conservation seem to be the protection of the natural habitats, and the main action to mitigate the decline or disappearance of natural

populations is the investigation of propagation techniques, among which spore germination is recommended.

AN APPROACH TO FUTURE FERN CONSERVATION

The only certainty is that fern conservation requires multiple efforts, a compendium of actions as the result of the application of all the knowledge raised through the scientific research of different groups from several disciplines all around the world.

In the last decade, among the referenced papers, we found many works focused on germplasm conservation, followed by propagation studies and restoration activities (Figure 3). It would be desirable to increase the number of studies on the other, less well explored topics, in order to have complete and balanced view studies of pteridophyte conservation. The detected deficiencies may be due to the high cost, in time and experienced technical personnel, of the field work and the further monitoring required after the initial conservation actions.

As we could observe in many conservation programmes already developed, coordination between scientists and government departments is necessary to develop, and especially to complete, effective conservation or recovery programmes. The necessary steps are summarized in a basic diagram of actions (Figure 4).

A collaborative network of institutions involved in fern biology and biodiversity studies and, as well, a responsible implementation of conservation practices, could help to improve methodologies by exchange of experiences. Arcand & Ranker (2008) encourage biologists, land managers, and conservationists to expand knowledge of biology of ferns and lycophytes as one of the relevant contributions to their conservation.

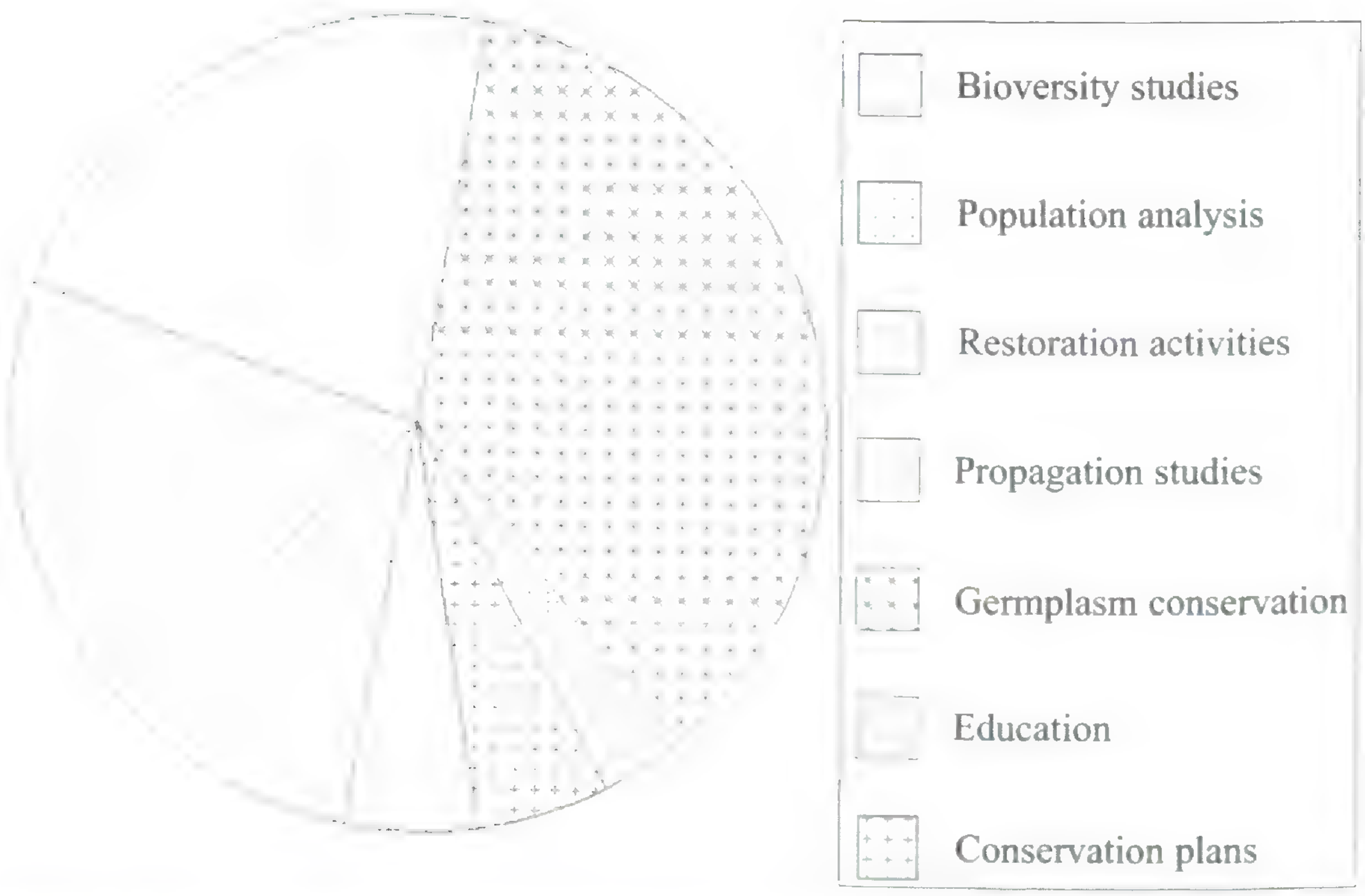

Figure 3. Proportion of the different aspects of fern conservation covered in the recent works and studies referenced.

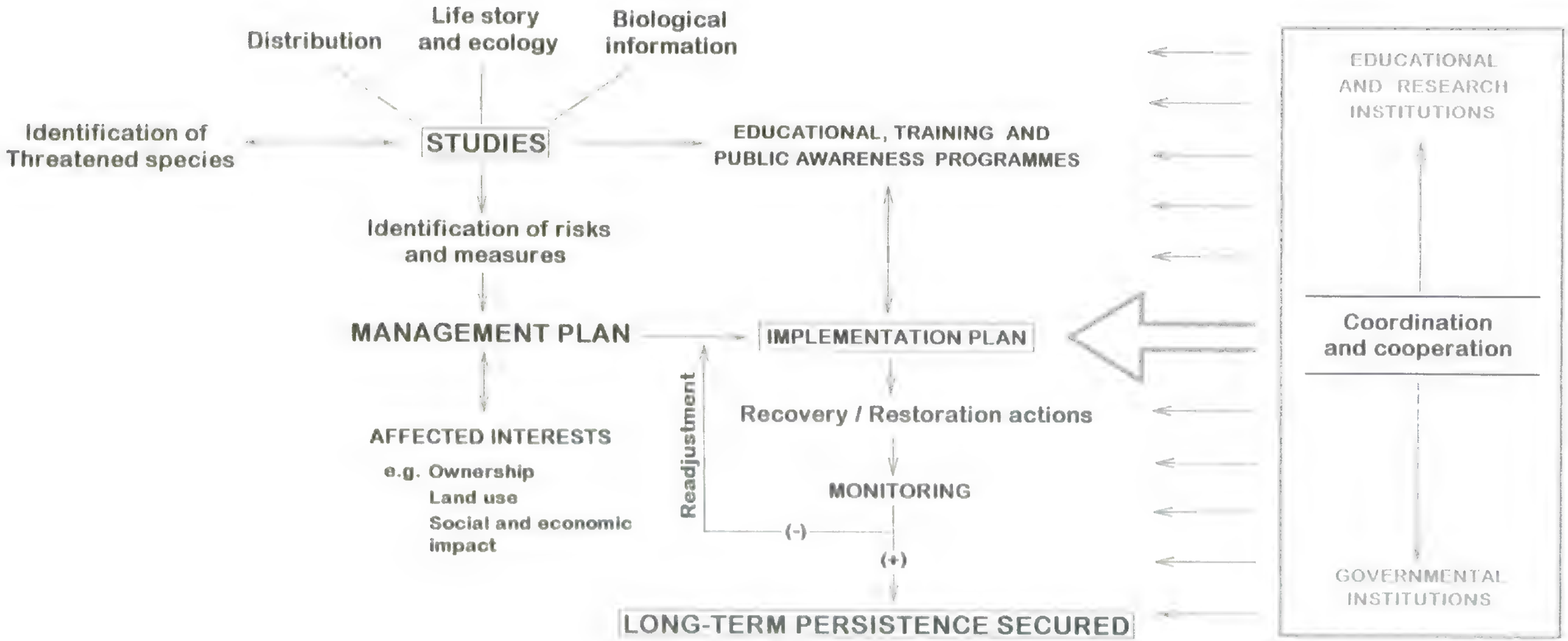

Figure 4. 4. Sequence diagram showing actions for securing the long-term persistence of natural populations, developed under coordination and cooperation by Governmental, educational and/or research institutions.

As a result of reviewing numerous conservation works published in the last decade we summarize a 10- step approach to plant conservation that is applicable to ferns:

1. Revision of morphological characters and confirmation of the taxonomic identification.

2. Compilation of complete information of distribution; revision of published records, herbaria and field data.

3. Characterization of ecological and phenological features of the species in the different locations. Identification of singular ecotypes (especially relevant when the genetic structure of populations is not well established).

4. Determination of the conservation status of each population (number of individuals, mature individuals proportion, actual and potential threats, IUCN categories, etc…).

5. Research and compilation of data on reproductive biology (vegetative and sexual) of the species. Identification of optimal protocols.

6. Research and compilation of data on genetic diversity of populations in order to establish conservation priorities.

7. Revision of legal aspects (regional, national or international laws) related to the species or their habitats, and those related to natural protected areas when they are included in any category.

8. Development of an *ex situ* conservation programme.

9. Revision and research of possible restoration techniques applicable to each species.

10. Proposal of a sustainable management plan for the taxa.

Complementary actions to assure fern conservation are: training of local staff in spore germination, development of comprehensive research into the biology of the species, creating educational guides and leaflets, and coordination between institutions.

Open access to knowledge will be the key to future sustainability of mankind. Public awareness programmes on the conservation and sustainable utilization of ferns should be initiated promoting *in situ* and *ex situ* conservation.

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A SHORT BIOGRAPHY OF THE AUTHORS

Photograph of Elena (to the left) and Ana (to the right)

The Germplasm Bank of the Botanical Garden of the University of Valencia is working today for the conservation of wild flora of the Valencian Community in Spain. Its main aim is the long-term preservation of spores and seeds.

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ELENA ESTRELLES, received her Ph.D. in Botany from the University of Valencia. From 1991 she has been Senior Technical Officer in the Botanical Garden of the University of Valencia. She is now curator of the Germplasm collection in this institution. She has coordinated the activities of our centre in different European projects related to Plant Conservation including Ensconet, Genmedoc and Semclimed. Today, she is collaborating with Ana M. Ibars in the study of spore ageing in order to optimize the maintenance of viability.

CTENOPTERELLA GABONENSIS, A NEW SPECIES OF GRAMMITID FERN (POLYPODIACEAE) FROM GABON, AFRICA

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Key Words: *Ctenopterella gabonensis,* Gabon, Grammitidaceae, Polypodiaceae

ABSTRACT

A new species of grammitid fern, *Ctenopterella gabonensis* (Polypodiaceae), is described from Gabon. The only grammitid ferns previously reported from Gabon are *Cochlidium serrulatum* (Sw.) L.E.Bishop (syn. *Xiphopteris serrulata* (Sw.) Kaulf.) and *Zygophlebia villosissima* (Hook.) L.E.Bishop (syn. *Ctenopteris villosissima* (Hook.) W.J.Harley).

INTRODUCTION

During the preparation of the account of Grammitidaceae for the Flora of Tropical East Africa (FTEA) (Parris, 2005) loans were made of material from countries beyond the FTEA area to ascertain the wider distribution of FTEA species. Amongst material sent from WAG was a specimen of an unknown species of *Ctenopterella* from Gabon, described below as *C. gabonensis*. Only two species of grammitid fern have been reported from Gabon (Tardieu, 1964): *Xiphopteris serrulata* (Sw.) Kaulf.) and *Ctenopteris villosissima* (Hook.) W.J.Harley, now known as *Cochlidium serrulatum* (Sw.) L.E.Bishop and *Zygophlebia villosissima* (Hook.) L.E.Bishop respectively. *Cochlidium serrulatum* has laminae differentiated into a fertile ± entire apical portion and a sterile lobed basal portion with one vein per lobe. *Ctenopterella gabonensis* and *Zygophlebia villosissima* have deeply pinnately divided laminae, with several pairs of veins in the pinnae; the former has glabrous rhizome scales, pale yellow-brown to pale red-brown non-catenate simple eglandular hairs and hydathodes on the vein endings on the abaxial surface of the lamina while the latter has marginal and apical hairs on the rhizome scales, medium to dark red-brown non-catenate simple eglandular hairs and vein endings lacking hydathodes.

Three species of *Ctenopterella* have been described from the Africa-Madagascar-Mascarene region: *C. macrorhyncha* (Baker) Parris, *C. parvula* (Bory ex Willd.) Parris and *C. zenkeri* (Hieron.) Parris. *Ctenopterella gabonensis* differs from these species by the presence of short pale non-catenate simple eglandular hairs on the stipes and laminae. Table 1 summarises the hair types on the stipes and lamina of *C. gabonensis*, *C. macrorhyncha, C. parvula* and *C. zenkeri*. The very short veins of *C. gabonensis*, extending only half way between the margin and the costa, are similar to those of *C. parvula*, which lacks the simple eglandular hairs and branched hairs with simple eglandular branches found in *C. gabonensis*, however, and sometimes has branched hairs with catenate branches, a hair type not found in *C. gabonensis*.

TYPIFICATION AND DESCRIPTION

Ctenopterella gabonensis *Parris,* **sp. nov.**

Holotype

Gabon, Chantier CEB, Monts Doudou, c. 20 km WSW of Doussala, 2º25'S 10º30'E, c.

Table 1. Stipe and lamina hairs of *Ctenopterella gabonensis, C. macrorhyncha, C. parvula* and *C. zenkeri*.

	C. gabonensis	*C. macrorhyncha*	*C. parvula*	*C. zenkeri*
Stipe hairs	Non-catenate simple eglandular hairs and 1-3-forked hairs with non-catenate simple eglandular branches.	Catenate simple eglandular or glandular hairs 3 or more cells long, sometimes also with simple glandular hairs 2 cells long.	Simple glandular hairs 2 cells long, sometimes also with catenate simple glandular hairs 3 or more cells long and/or 1-forked hairs with non-catenate simple eglandular branch.	Glabrous or with catenate simple glandular or eglandular hairs 3 or more cells long and/or simple glandular hairs 2 cells long and/or1-2-forked hairs with glandular branches or catenate glandular or eglandular branches.
Lamina hairs	As stipe, sometimes also with catenate simple glandular hairs 3 or more cells long.	Catenate hairs as on stipe.	As stipe.	Catenate simple glandular or eglandular hairs 3 or more cells long and/or simple glandular hairs 2 cells long, sometimes with 1-2-forked hairs with catenate glandular or eglandular branches.

650-700 m, 20 May 1985, *J M & B Reitsma, Breteler & A M Louis* 1081 (WAG 0014897!).

Etymology
From Gabon, Africa.

Description
Rhizomes 3.1-4.2 mm diam. including scales, 1.4-1.7 mm diam. without scales, short-creeping, not branched, dorsiventral, stipes in 2 rows, articulated to rhizome, phyllopodia 0.2-0.3 mm high, stipes 0.3-0.9(-1.1) mm apart in each row; scales (1.6-)1.9-3.6 x 0.3-0.5 mm, narrowly lanceolate, acuminate at apex which is terete, cordate at base, pale yellow-brown to pale red-brown, glabrous, not clathrate, not iridescent, subglossy, cells in centre of scale 1-2 x longer than broad, cells not turgid. Stipes (7-)8-11(-12) x (0.4-)0.5-0.7(-0.8) mm, dull dark brown; with dense ± patent pale yellow-brown to pale red-brown non-catenate simple eglandular hairs 0.1-0.2 mm long and sparse to scattered ± appressed 1-3-forked pale yellow-brown to pale red-brown hairs with catenate base, simple eglandular branches and glandular apex 0.1-0.2 mm long. Laminae (80-)89-136(-149) x (8-)9-12(-13) mm, narrowly elliptic in outline, bluntly acute at apex, long-attenuate at base, pinnate, pinnae (30-)32-42(-48) pairs, at (60-)62-70° to rachis, 0-2 mm apart midway, lowest 1-2(-3) pairs reduced to auricles, longest pinnae 5-7 x 2-3 mm, narrowly triangular-oblong, bluntly acute to acute at apex, sessile to slightly surcurrent on acroscopic margin, decurrent on basiscopic margin at base, entire; texture thinly coriaceous; with ± patent pale yellow-brown to pale red-brown non-catenate simple eglandular hairs c. 0.1 mm long occasional to sparse on abaxial surface of rachis and ± appressed 1-3-forked pale yellow-brown to pale red-brown hairs 0.1-0.2 mm long with catenate base, simple eglandular branches and glandular apex sparse to scattered on abaxial surface of rachis and occasional to scattered on adaxial surface of rachis especially in sinuses, sometimes occasional to sparse on abaxial surface of costae, sometimes with ± appressed pale red-brown catenate simple glandular hairs up to 0.1 mm long occasional to sparse on abaxial surface of lamina, occasional to sparse on abaxial surface of costae and sparse to scattered on abaxial surface of rachis; rachis slightly sunken between two flanges on adaxial surface of lamina, prominent on abaxial surface, darker than lamina on both surfaces; costae slightly prominent and concolorous on both surfaces; veins invisible in transmitted light, sometimes slightly prominent and concolorous on adaxial surface, pinnately branched, 1st acroscopic and basiscopic branches from axil of rachis and pinna mid-vein, 2-3 pairs of branches in longest pinnae, branches simple, short, reaching half way between costa and margin, not extending beyond sorus, each branch ending marked by a dark elongate hydathode 0.2-0.3 x 0.1 mm on adaxial surface of lamina, free. Sori (1.2-)1.4-1.7 x (1.1-)1.2-1.5(-1.6) mm, ± circular to broadly elliptic in outline, on surface of lamina or slightly sunken in broad shallow depressions in lamina which may be slightly prominent under hydathode on adaxial surface, confluent within and between rows when mature, covering under-surface of lamina at maturity and extending beyond margin, on c. 11 pairs of pinnae, in apical 1/3 of lamina, to c. 7 mm below lamina apex, 1-2 rows per pinna, 1 on each side or on either side of costae, 1-2 in each row on longest pinnae, in basal 1/2 of pinnae, to 2-3 mm below pinna apex, midway between costa and margin, oblique to costa. Sporangia (220-)230-250(-260) µm, glabrous; indurated cells of annulus 10-11. Spores (37-)39-48 µ m diam.

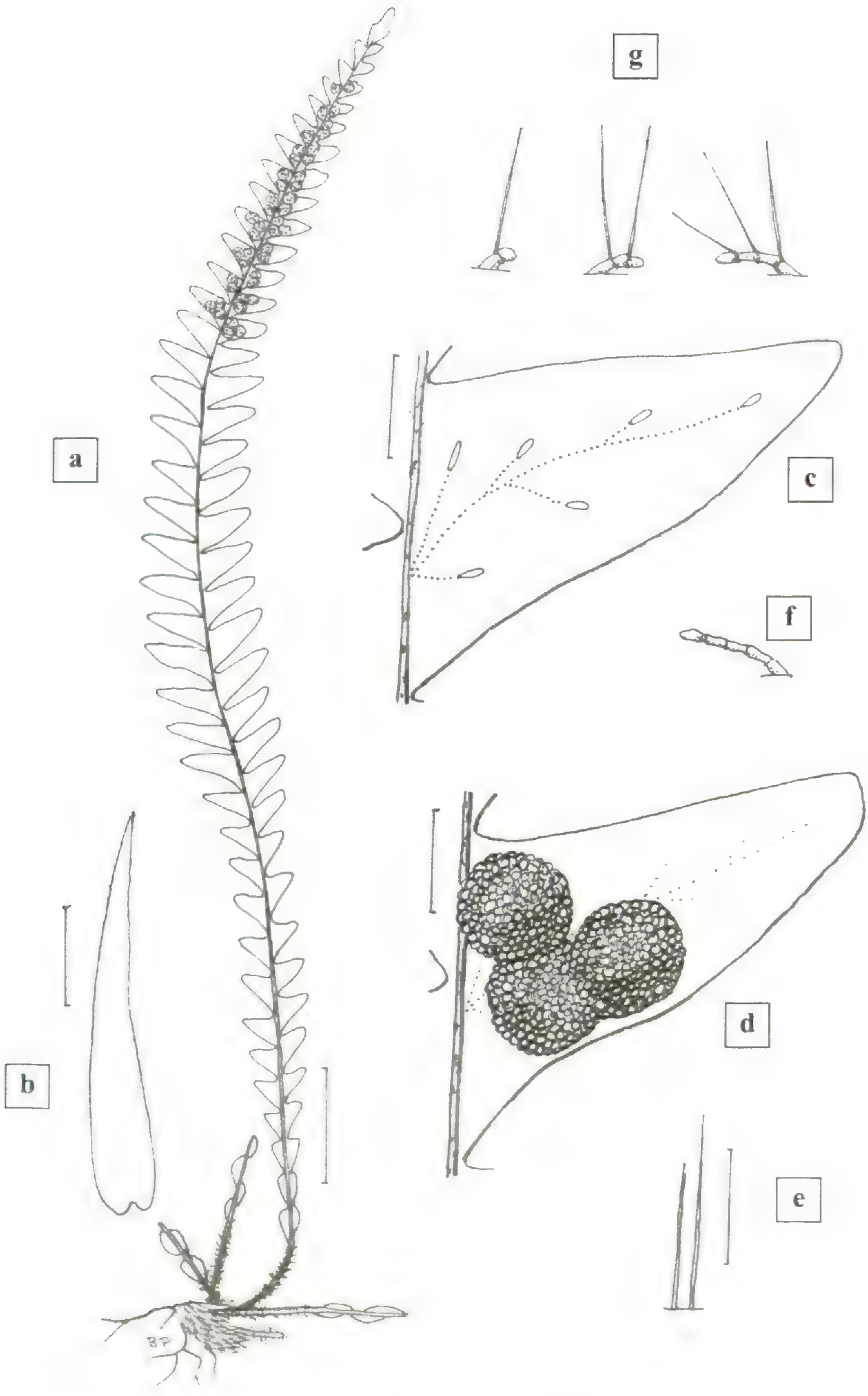

Figure 1. *Ctenopterella gabonensis*. **a.** whole plant: scale 1 cm. **b.** rhizome scale: scale 1 mm. **c.** adaxial surface of pinna showing venation and hydathodes: scale 1 mm. **d.** abaxial surface of lamina showing sori: scale 1 mm. **e.** simple eglandular hairs from stipe. **f.** catenate simple hairs from abaxial surface of rachis. **g.** branched hairs from abaxial surface of rachis. e-g: scale 0.1 mm. All from *Reitsma, Reitsma, Breteler & Louis* 1081 (WAG).

ECOLOGY AND DISTRIBUTION

Epiphyte in mossy forest, growing with *Hymenophyllum kuhnii* C.Chr., between c. 650 and 700 m alt. Known only from the type locality in Gabon.

ACKNOWLEDGEMENTS

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BOOK REVIEW

LES FOUGÈRES D'ALSACE, D'EUROPE ET DU MONDE. ACTES DU COLLOQUE EN HOMMAGE À CLAUDE JÉRÔME (1937-2008). Publ. Société Botanique d'Alsace, 2012, 198pp., hard-back, recommended price €29.

Claude Jérôme, photo by Patrick Acock.

Claude Jérôme, the *eminence grise* of French Pteridology – a seemingly curmudgeonly figure of few words (even fewer English ones), cheroot permanently dangling from the lips of a well-worn face. A fixture at the annual excursions of the Group of European Pteridologists (GEP), he was well-known to all who attended these fascinating meetings. But behind the slightly intimidating exterior was a very special person. A man with extensive knowledge of the Alsace – a region he lived and worked in all his life – a fluent speaker of French and German (and no doubt the Alsatian dialect), he only pretended as a matter of principle not to speak English, and he freely extended his knowledge and friendship to everyone, with the added benefit of a delightfully wicked sense of humour. Claude died in 2008, and this volume reports a symposium held as a tribute to him, in 2009. The contributors are mainly from the Alsace (which includes the University of Strasbourg) and many will be well-known to those in the BPS who have attended meetings of the GEP. French pteridology has few genuine amateurs as we do, but it has a core of highly knowledgeable specialists. Their contribution, and that of the University

of Strasbourg, together with the influence of the French Office National des Forêts (ONF), which actively sponsors a knowledge of the botany and environment of forests, can be seen in this book.

By the nature of symposia, it is something of a mixed bag. A short portrait of Claude (and what a delight to see photos of Kurt Rasbach and André Labatut) opens the volume, and a list of species found on a fern excursion at the end of the symposium closes it. Of course they found four *Diphasiastrum* species! Many of the papers relate to the north-east of France, and there are three papers dealing with the herbaria (and their collectors, some of whom brought specimens from French colonies overseas) at Strasbourg, and an account of ferns in the botanic garden of the Col de Saverne (there's a place to visit!). A number of papers deal with rare or interesting plants in the region (including Franche-Comté and Luxembourg). There is an addendum to the ferns of the north-east of France which describes and illustrates, *inter alia, Asplenium trichomanes* subsp. *hastatum* and its hybrid with subsp. *quadrivalens, A. viride* var. *incisum*, and *Dryopteris affinis* subsp. (sic) *pseudodisjuncta* from the Vosges.

Fern allies are represented, and there are articles on populations of *Botrychium matricarifolium* in the Vosges. A very comprehensive paper by Michel Boudrie and Ronnie Viane deals with the lectotypification and authorities for *Asplenium foreziense*. Jakob Schneller presents a paper (in English!) in which he compares the growth at low altitude of *Athyrium filix-femina* plants collected from mountain sites.

There are a number of papers concerning tropical ferns. Serge Muller and Roger Etcheberry discuss the ecology of four species of Ophioglossaceae from Saint-Pierre et Miquelon; Michel Boudrie and Georges Cremers discuss, with illustrations, seven species (including the charming *Schizaea incurvata*) from French Guiana; there is a revision (in Spanish) of two *Blechnum* species from central and south America; and a short article on the decorative use of *Dicksonia sellowiana* in Brazil.

Nine posters were presented at the meeting, but only four were not covered elsewhere in the proceedings. I single out a poster by Gerard de Groot and others, whose results supported the idea that selection for selfing genotypes may occur during long-distance colonisation, even in normally outcrossing diploid ferns, and one by Elke Bellefroid and Ronnie Viane who report a new base number (x = 35) for "loxoscaphoid" Aspleniums.

Finally I cannot resist mentioning the charming cartoons in the paper by Jean-Baptiste Gallé, who discusses the multiple, not just medicinal, uses of ferns.

For anyone who has ever attended meetings of the GEP, or who has acquaintance with at least some of the authors, and this part of France, this book is a "must-have". Although most of it is in French, its largely technical content will appeal even to the most monoglot. It is not unreasonably priced, is well-illustrated, beautifully produced, and a fitting tribute to a lovely man.

Paul Ripley

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STEVENSON, D.W. & LOCONTE, H. 1996. Ordinal and familial relationships of pteridophyte genera. In: CAMUS, J.M., GIBBY, M. & JOHNS, R.J. (Eds) Pteridology in perspective, pp. 435-467. Royal Botanic Gardens, Kew.

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