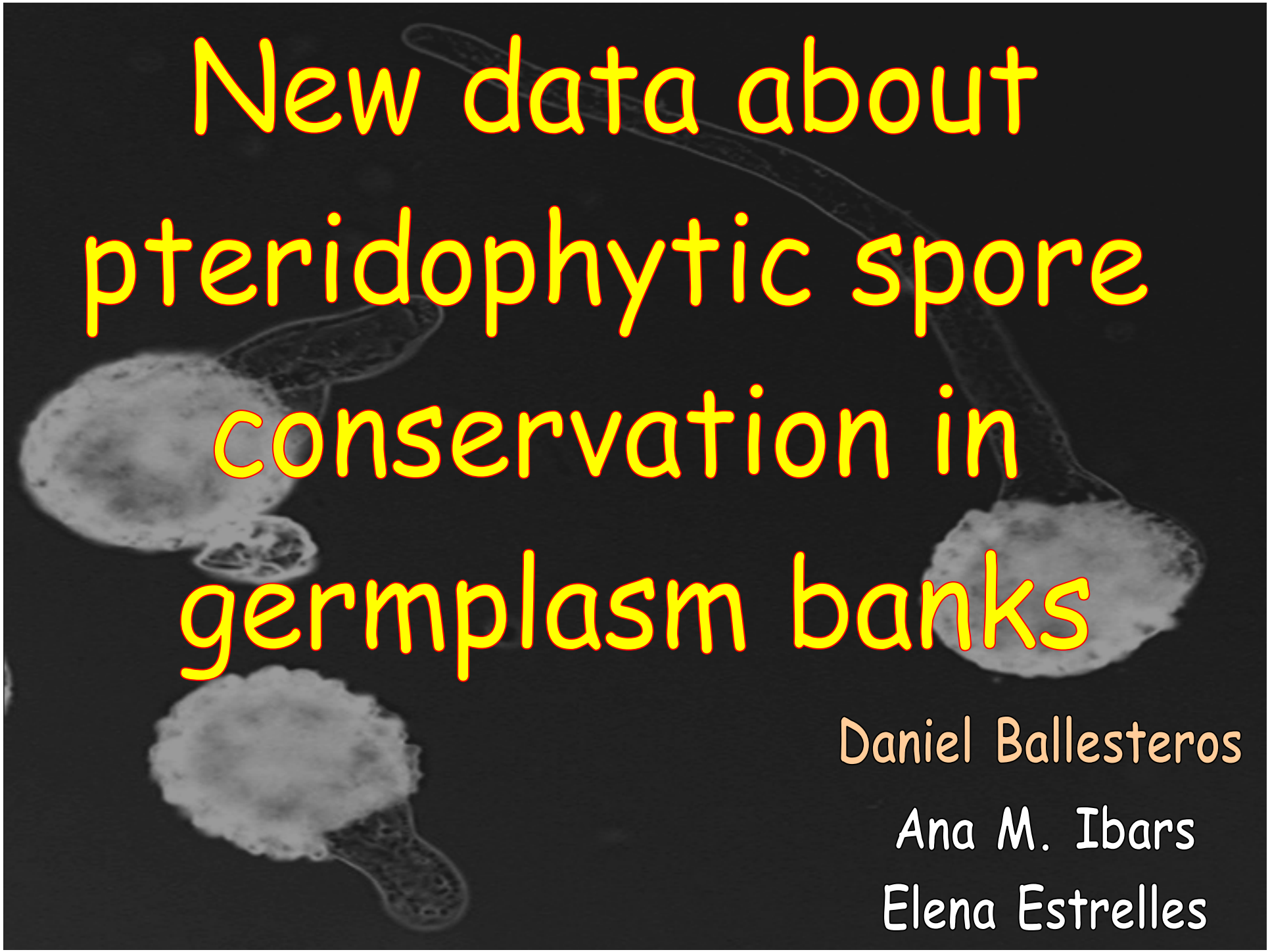


New data about pteridophytic spore conservation in germplasm banks

Daniel Ballesteros
Ana M. Ibars
Elena Estrelles

Biological Diversity or Biodiversity

The variety of life in all its forms, levels and combinations. Includes ecosystem diversity, species diversity, and genetic diversity (IUCN, UNEP and WWF, 1991).

Pteridophyte's habitats are fragile and liable to suffer damage


Forest fires

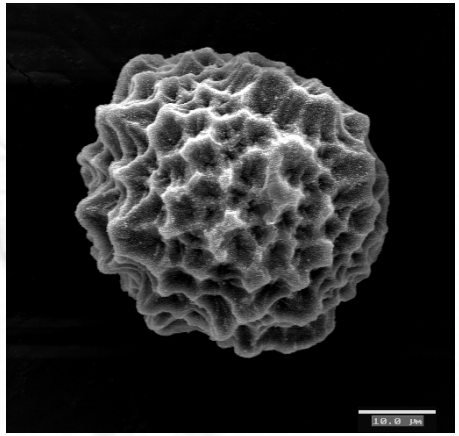

Illegal or abusive trade


Abusive forest felling


Habitat alteration-spring canalization

Ex situ Conservation

Germplasm Banks

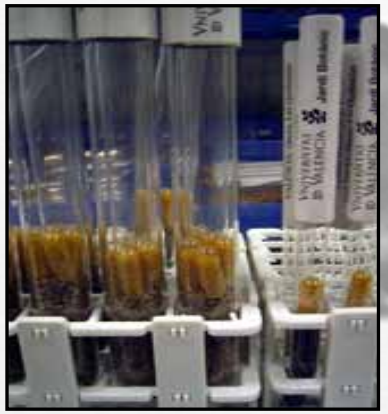


Seeds:

Ellis et al., 1985;
Dickie et al., 1990;
Gomez-Campo, 2001.

Spores:

¿?



GENERALITAT VALENCIANA

CONSELLERIA DE TERRITORI I HABITATGE

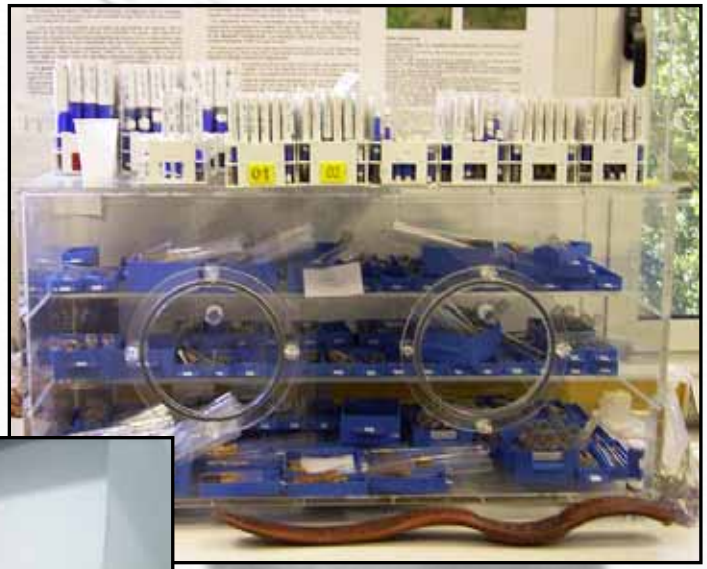
Life

VNIVERSITAT DE VALÈNCIA

Jardi Botànic

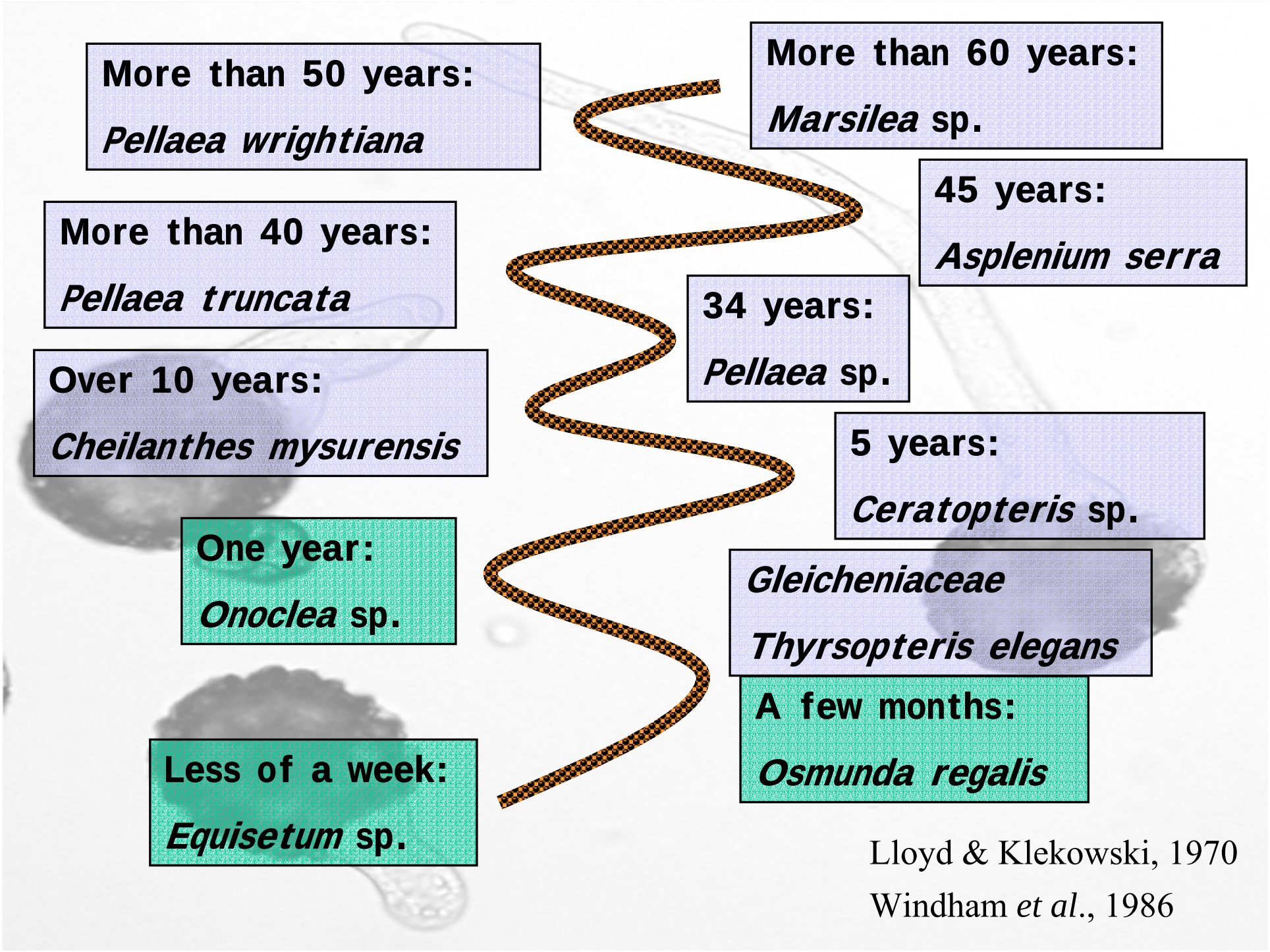
Valencia Botanical Garden (Universitat de Valencia)

Germplasm bank



Spore bank

Nowadays it's one of the few active in all the world



Lloyd & Klekowski, 1970

Windham *et al.*, 1986

Posible causes

Deficiency of respiratory substrata.

Irreversible loss of membrane permeability.

Inactivation of various enzymes and growth substances.

Chromosomic mutations.

Beri & Bir, 1993

Page *et al.*, 1992

High respiratory rate.

Loss of the capacity for recuperation the photosintetic activity after desication.

Lloyd & Klekowsky, 1970

Lebkuecher, 1997

Tested techniques for spore conservation:

Extending viability:

Refrigeration (4-5°C)

2 or 3 years in *Equisetum* sp.

(Jones & Hook, 1970)

Freezing (-12°C, -20°C, -70°C)

Cryopreservation (-196°C)

No loss of viability during 12 or 24 months in different temperate forests ferns.

(Lindsay et al. 1992; Quintanilla et al., 2002)

Dry storage

Dry storage with silica gel

Viable spores after 75 months at –196ºC

(Pence, 2000)

Principal problems

in fern spore conservation

Few quantitative dates for long term conservation

Incomplete knowledge of the causes of viability's loss in fern spores

What we want in spore conservation?

Viability conservation, growth conservation & genetic integrity conservation

Page *et al.*, 1992

Work's Objectives

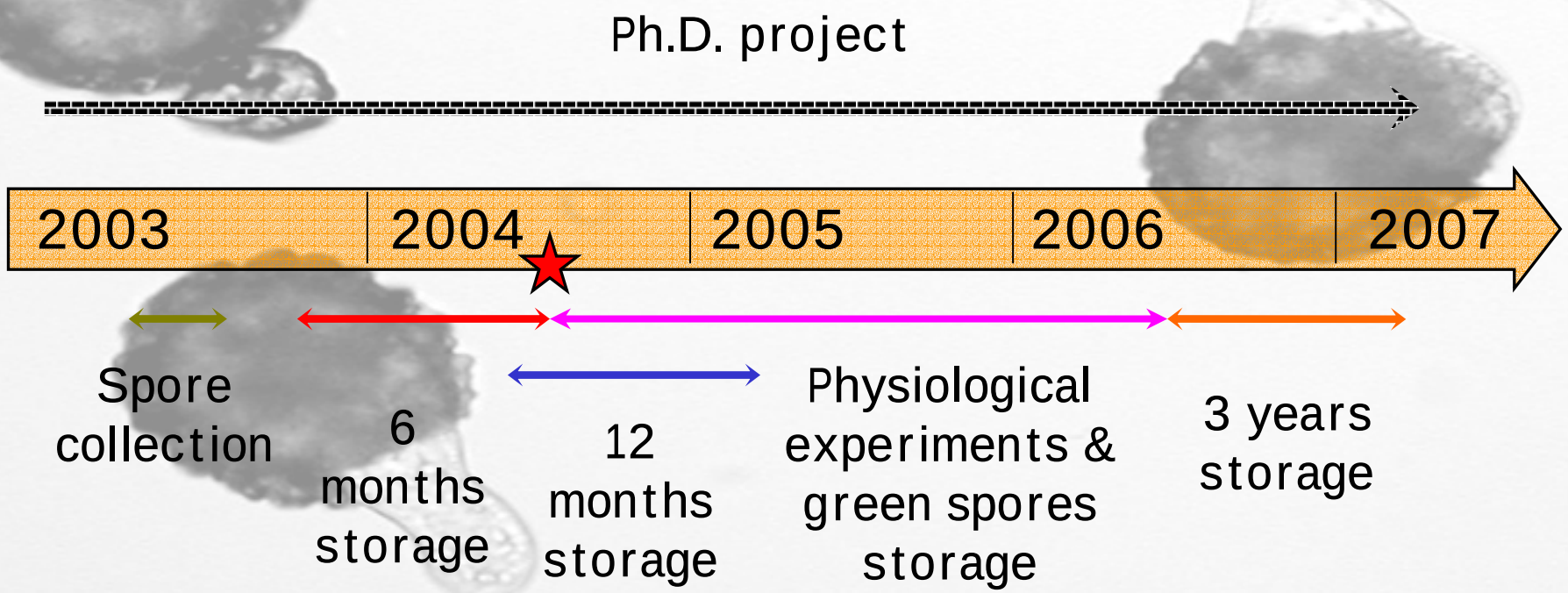
★ To establish the best conditions for the long-term storage of spores.

★ To verify the effects of the medium-long time ultrafreezing on:

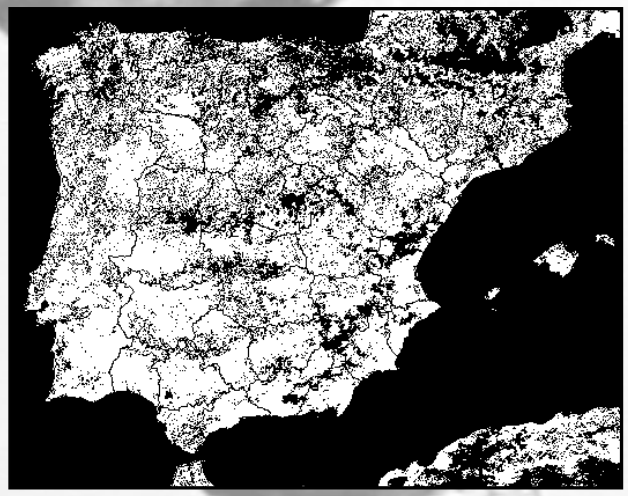
- Spore germination / spore viability.
- Gametophyte early development.
- Gametophyte late development.

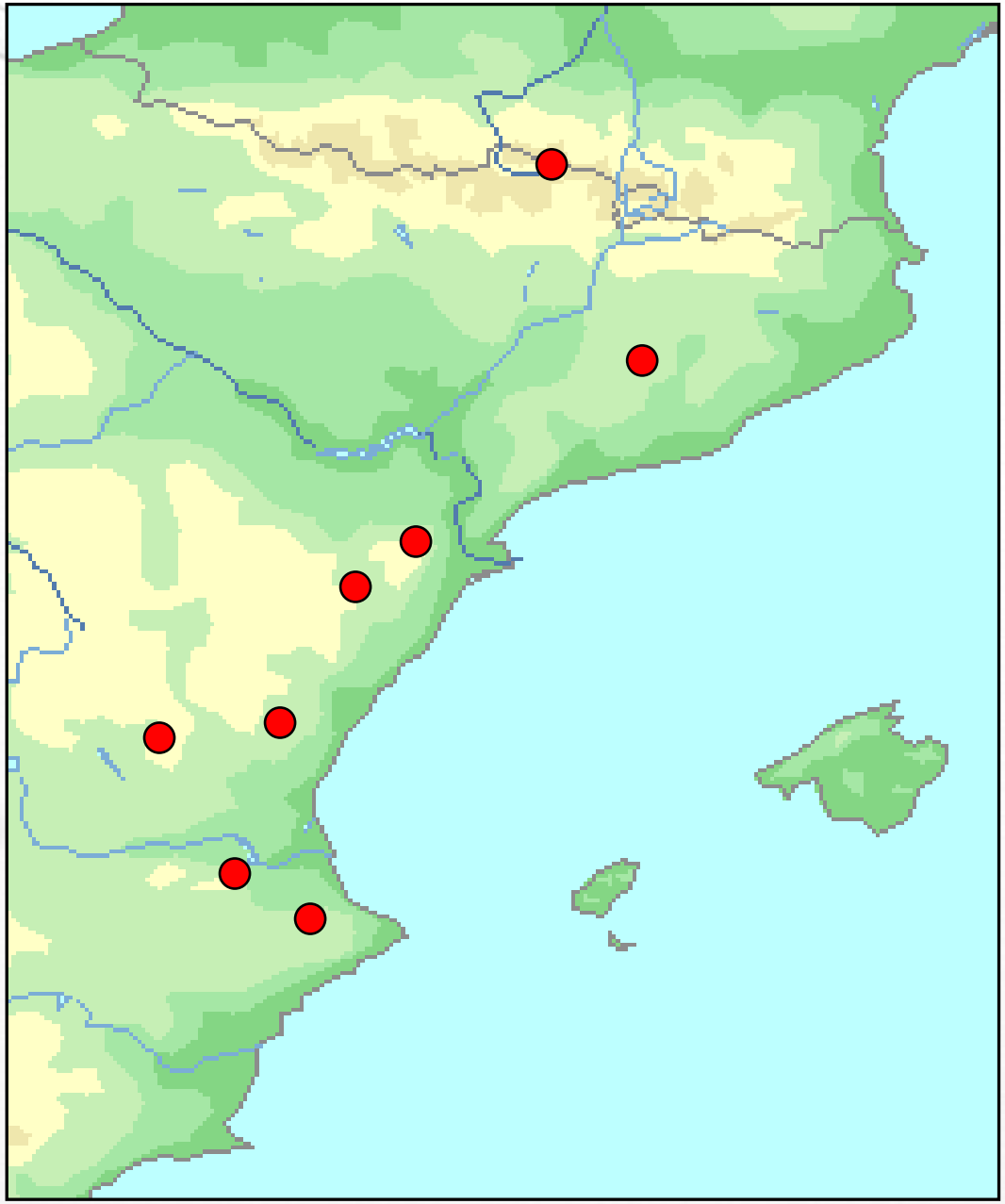
★ To know the causes of the loss of viability of the spores.

This work has been funded by the MINISTERIO DE CIENCIA Y TECNOLOGÍA (Grant nº REN2002-03697 / GLO), by the GENERALITAT VALENCIANA (CONSELLERÍA DE TERRITORI I HABITATGE) and by the European Commission (LIFE 99 NAT/E/006417. Project LIFE CONSERVACIÓN HABITATS PRIORITARIOS).



SPECIES USED





Xerophytic

***Ceterach officinarum* Willd.**

Notholaena marantae (L.) Desv.

Higrofilous

Thelypteris palustris Schott.

***Athyrium filix-femina* (L.) Roth.**

Tropical and wet habitat

Cyrtomium falcatum (Lf) K. Presl.

Pteris vittata L.

High mountain

Polystichum lonchitis (L.) Roth.

Underwoods

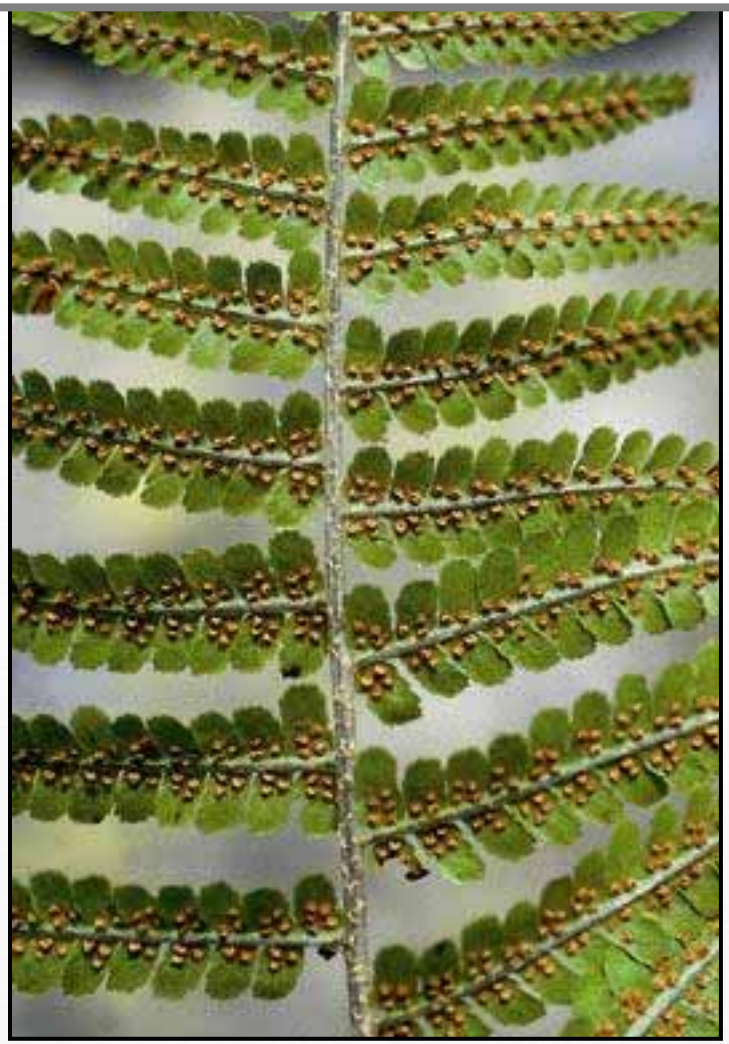
Cystopteris fragilis (L.) Bernh.

Asplenium onopteris L.

Underwoods

Polystichum aculeatum (L.) Roth.

Dryopteris filix-mas (L.) Roth.



Metodology

Spores collection, on satin paper
25ºC, 1 week.
>20 individuals per population.

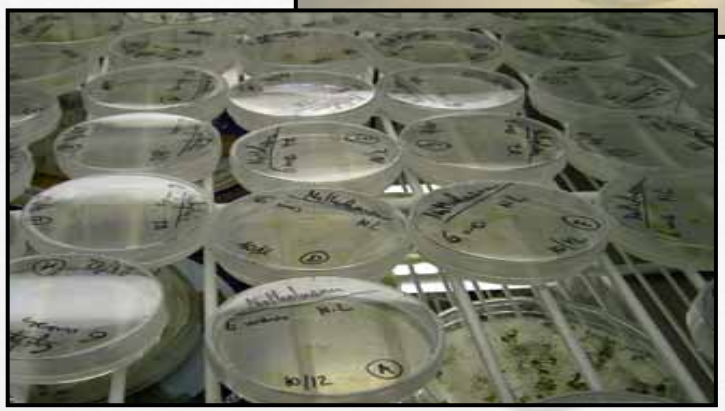
Spores that were dryed:
1 week (at 4ºC) with
silica gel

Spores stored at laboratory temperature (25ºC aprox.), 4ºC & –25ºC.

Spores stored at –80ºC

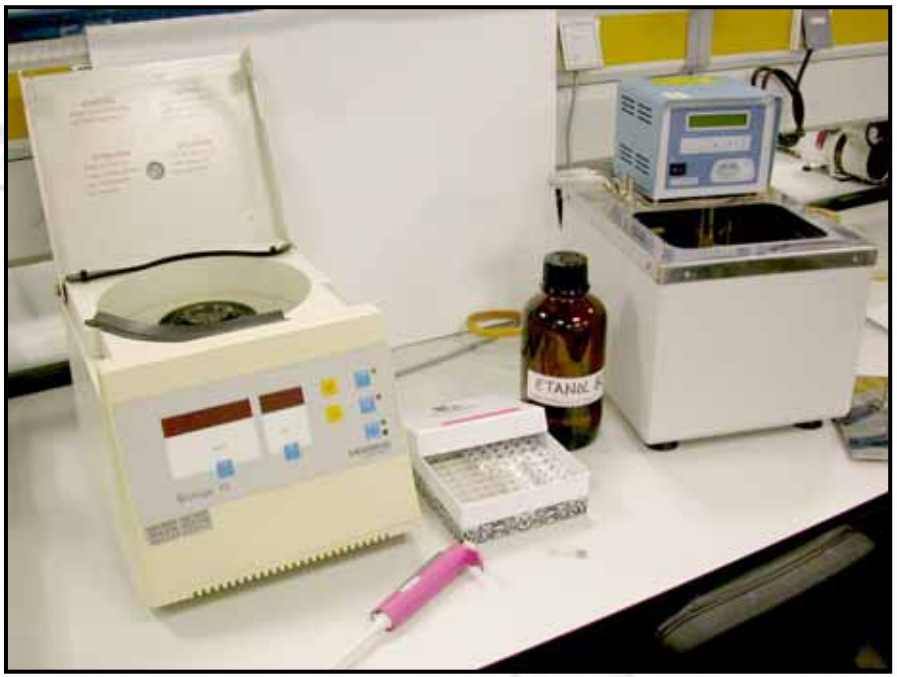
Spores stored in liquid nitrogen (-196ºC)

After 6 or 12 months



Spores were sowed in petri dishes, with Dyer's culture medium (1% agar) 20ºC, 12h. light.

Sugars and aminoacids alcoholic extraction (50 mg. spores)

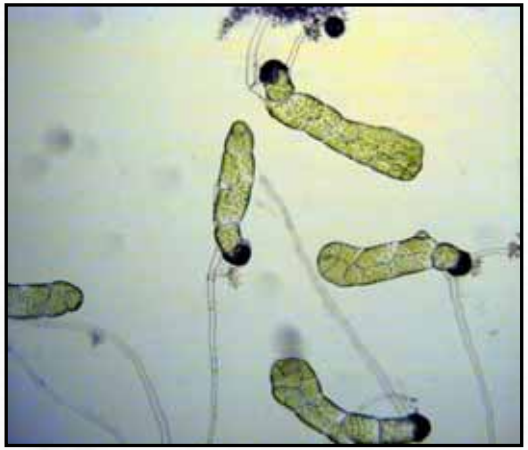


Sugars determination: anthrone-sulfuric acid reagent (Mc Cready *et al.*, 1950)

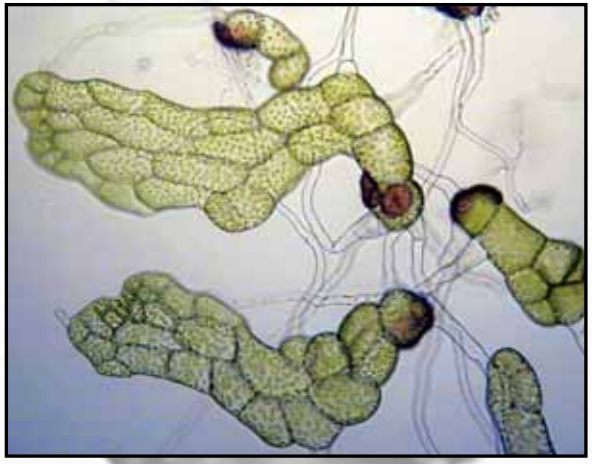
What we consider?

The day of germination's beginning

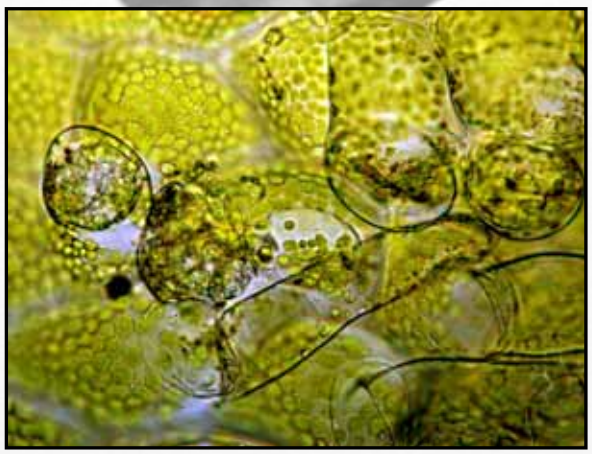
The germination rate at 30 days



2D gametophyte's rate at 30 days



The number of petri dishes with any sexual gametophyte



Statistical analysis:

ANOVA α=0.95

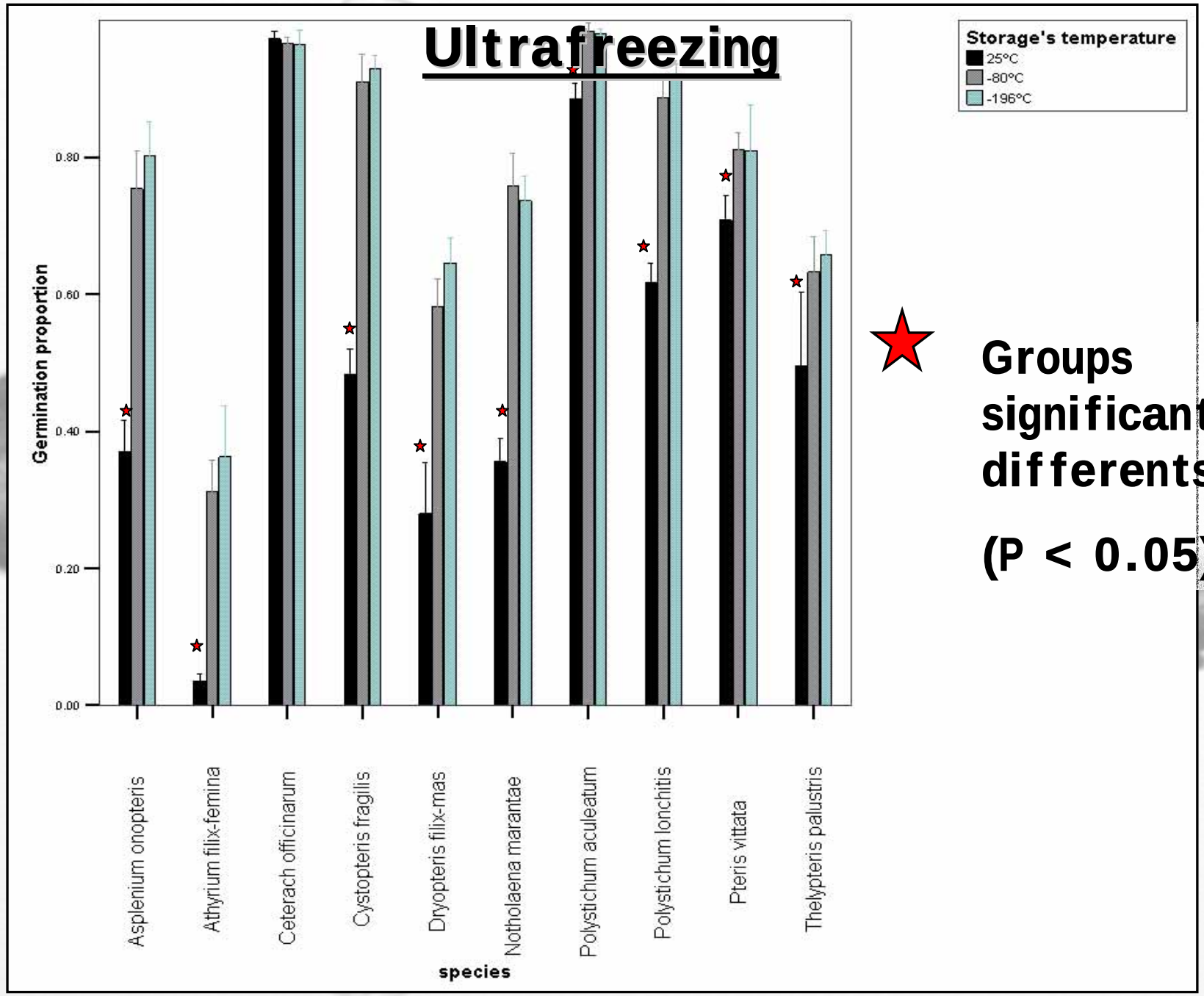
Test-t α=0.95

Tuckey α=0.95

SPSS 11.0

Germplasm banks traditional storage methods

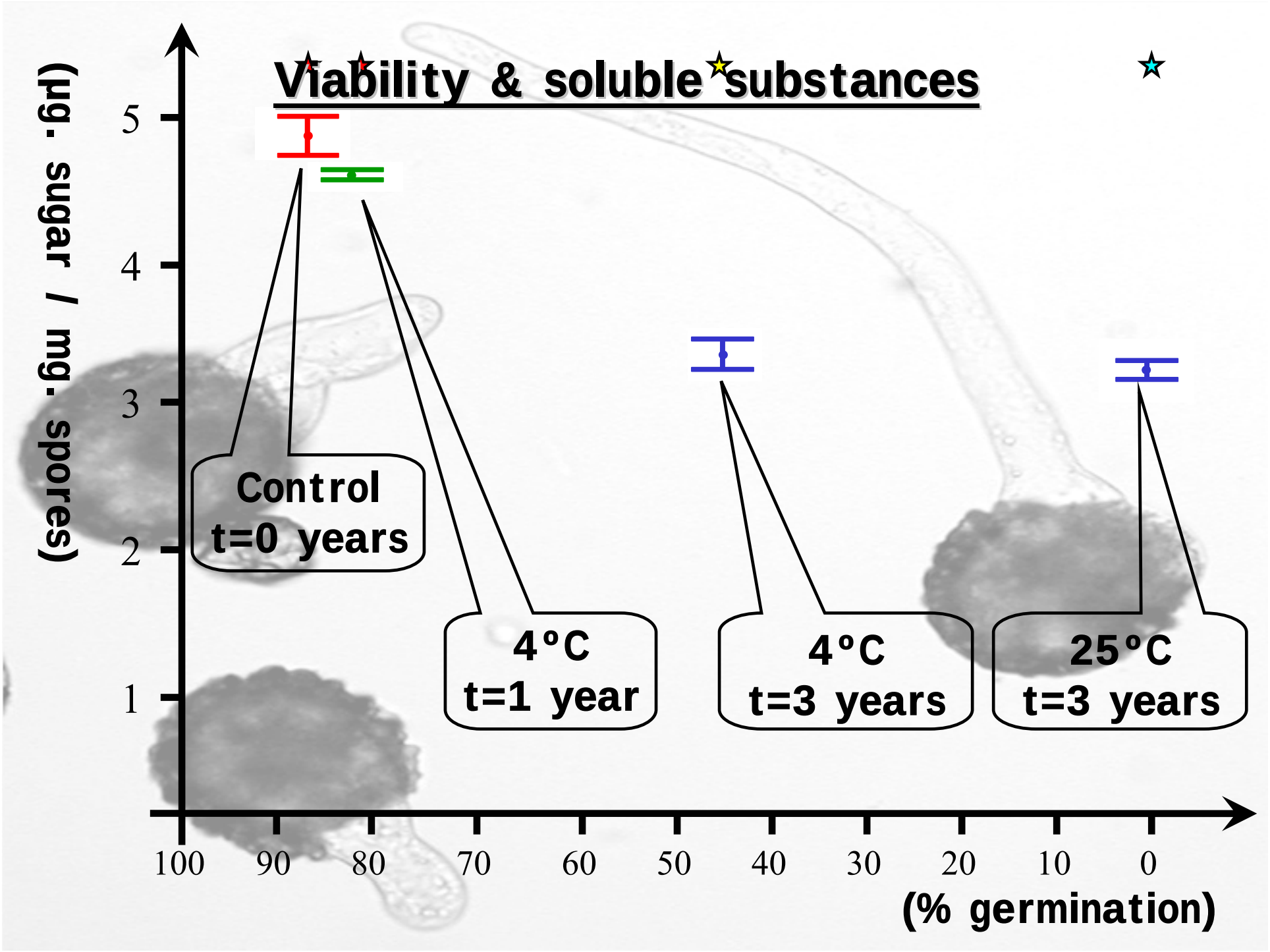
Storage	*Arachis stenosperma*			*Arachis villosulicarpa*	
	Germination (%)	Laminar fase 30 days (%)	Laminar fase 60 days (%)	Germination (%)	Laminar fase 30 days (%)
4°C, Dry	81.9±2.3	97.0±0.9	97.0±0.9 ♥	95.9±0.9	85.7±2.8 ⚡
4°C; Non dry	85.4±1.2	98.7±0.6	98.7±0.6 ♥	95.6±0.5	73.4±7.8 ⚡
-25°C; Dry	79.7±1.5	94.4±0.9	94.4±0.9 ♥	95.9±0.9	85.4±5.0 ⚡
-25°C; Non dry	60.3±2.7	66.3±3.6	90.1±1.8 ♥	96.6±0.6	88.7±3.1
-25°C; Dry; Defrost monthly	65.7±1.4	87.7±0.8	97.1±0.5 ♥ ★	92.9±3.9	76.4±9.2 ⚡
-25°C; Non dry Defrost monthly	56.7±2.3	74.3±3.9	95.0±0.8 ♥ ★	95.1±0.8	57.3±9.6 ⚡



★ **Groups significantly differents**

(P < 0.05)

Taxon	Temperatura almacenado	Día inicio germinación	Porcentaje gametofitos en fase laminar	Grupos homogeneos (prueba tuckey $\alpha=0.05$)	Placas con gametofitos sexuados		
					60 días	90 días	120 días
Asplenium onopteris	25ºC	5	98.4 ± 0.3	*	7/7	7/7	7/7
	-80ºC	4	99.1 ± 0.7	*	7/7	7/7	7/7
	-196ºC	4	99.6 ± 0.2	*	7/7	7/7	7/7
Athyrium filix-femina	25ºC	6	91.6 ± 1.8	*	7/7	7/7	7/7
	-80ºC	4	95.6 ± 0.6	*	7/7	7/7	7/7
	-196ºC	4	95.0 ± 1.5	*	6/7	7/7	7/7
Ceterach officinarum	25ºC	7	86.9 ± 8.4	*	5/7	5/7	5/7
	-80ºC	7	52.3 ± 12.9	*	1/7	3/7	3/7
	-196ºC	7	71.4 ± 12.3	*	2/7	5/7	5/7
Cystopteris fragilis	25ºC	6	98.4 ± 0.4	*	5/7	6/7	7/7
	-80ºC	5	98.3 ± 0.5	*	5/7	5/7	7/7
	-196ºC	5	99.0 ± 0.4	*	5/7	5/7	7/7
Dryopteris filix-mas	25ºC	5	99.3 ± 0.3	*	1/7	7/7	7/7
	-80ºC	4	99.3 ± 0.4	*	3/7	7/7	7/7
	-196ºC	4	99.3 ± 0.2	*	3/7	7/7	7/7
Notholaena marantae	25ºC	6	97.0 ± 0.6	*	7/7	7/7	7/7
	-80ºC	6	95.4 ± 0.8	*	7/7	7/7	7/7
	-196ºC	6	96.6 ± 0.6	*	7/7	7/7	7/7
Polystichum aculeatum	25ºC	5	98.1 ± 0.3	*	6/7	6/7	7/7
	-80ºC	4	98.1 ± 0.6	*	5/7	5/7	6/7
	-196ºC	4	98.3 ± 0.6	*	5/7	7/7	7/7
Polystichum lonchitis	25ºC	6	93.7 ± 1.8	*	2/7	5/7	5/7
	-80ºC	6	95.1 ± 0.9	*	2/7	4/7	5/7
	-196ºC	6	98.1 ± 0.4	*	6/7	7/7	7/7
Pteris vittata	25ºC	4	99.3 ± 0.3	*	7/7	7/7	7/7
	-80ºC	3	98.6 ± 0.6	*	7/7	7/7	7/7
	-196ºC	3	93.4 ± 0.9	*	7/7	7/7	7/7
Thelypteris palustris	25ºC	6	91.0 ± 1.4	*	5/7	6/7	6/7
	-80ºC	5	94.0 ± 1.1	*	3/7	7/7	7/7
	-196ºC	5	90.3 ± 2.0	*	3/7	4/7	7/7


Viability & soluble substances
(µg. sugar / mg. spores)
5
4
3
2
1
Control
t=0 years
4°C
t=1 year
4°C
t=3 years
25°C
t=3 years
100
90
80
70
60
50
40
30
20
10
0
(% germination)

Main work's conclusions

- After 6 months at 4ºC: temperature and spores drying haven't got effects in spore viability and gametophyte late development.

- After 6 months: Freeze spores (-25ºC) has got negative effects in spore viability and gametophyte late development, except for dry spores.

- Repeated defrost are negative for spore viability and gametophyte development, even when spores are dry.

- After 6 months: None of the treatments haven't got negative effects in spore viability of *Ceterach officinarum*.

Main work's conclusions

- After 6 months: Ultrafreezing hasn't got negative effects in spore viability and gametophyte late development.
- At room temperature spores loss their viability (germination capacity), but there aren't effects in gametophyte late development (except *C. officinarum*).
- At room temperature spores delay their germination beginning, except spores of species that lives in habitats with water deficiencies. These are the latest species who starts germination too.
- Differences in the quantitative loss of viability haven't got relation with ecology of the species, lessure type, ploidy level or taxonomic group.

Main work's conclusions

- There are a significant soluble sugar loss along the time in spores. This loss is independent of the storage method.

- A significant soluble sugar loss in stored spores isn't synonymous with a significant viability loss or spores death.

- Other factors, moreover soluble substances loss (Beri & Bir, 1993), should be implicated in viability loss and death of spores.


Thank you!