In-vitro germination of two endemic species from Berlengas Archipelago, *Pulicaria microcephala* and *Armeria berlengensis*

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1 Introduction

The Berlengas archipelago is located in the Atlantic Ocean, on the Portuguese continental shelf, on the western side of the Iberian Peninsula, close to Cape Carvoeiro (Peniche). It distances approximately 5.7 miles from mainland. It is a protected area since 1981.

In 2011 Berlengas became part of the list of patrimony of UNESCO, aiming to conserve representative terrestrial and marine ecosystems of the Portuguese coast.

As a result to insular isolation, and to very difficult climatic and edaphic conditions, terrestrial flora evolved differently from the species that growth in mainland.

Due to human interference, namely in the introduction of the rabbit and mouse in the island, to the invasive plant Hottentot-fig (*Carpobrotus edulis) and the Yellow-legged Gull* (*Larus michaellis*) rapid population growth some species are endangered. To be precise, of the three endemic species *Herniaria lusitanica* subsp. *berlengiana* and *Armeria berlengensis* are critically endangered, and *Pulicaria microcephala* is considered vulnerable.

Therefore, the control of seagulls has been undertaken for more than ten years and in 2014 a project for the recovery of the Berlengas ecosystems has begun – LIFE BERLENGAS, including the removal of rabbits, rats and Hottentot-fig.

The main goal of this work was to establish seed germination protocols for *P. microcephala* and *A. berlengensis, and* to determine the viability and natural regeneration capacity of these species through seed propagation.

2 Materials and Methods

Collection of specimens and infructescences

- Infructescences of the two species collected from the wild, from different individuals, of similar maturation.
- In *P. microcephala*, flowers extracted from the infructescences and full achenes were collected.
- In *Armeria berlengensis*, due to their larger size, seeds were collected.

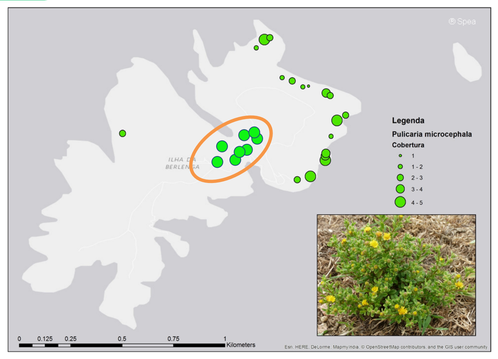


Fig. 1 Map indicating the distribution of *Pulicaria microcephala.* The circle represent the areas were the specimens were collected.

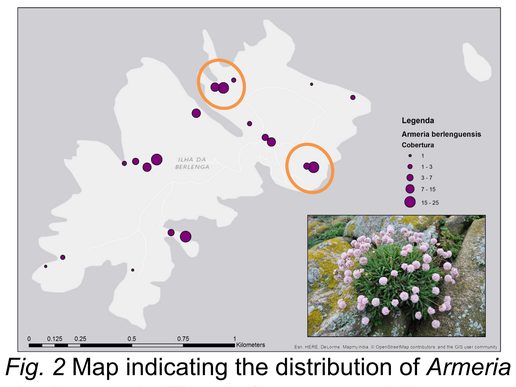
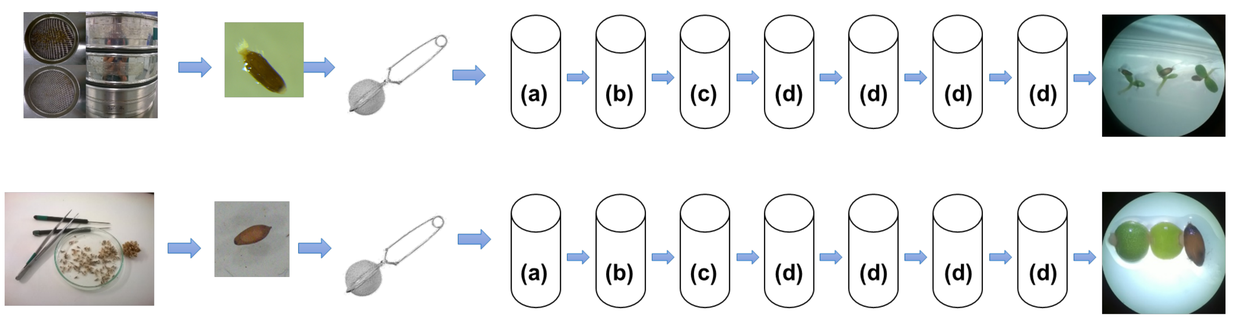


Fig. 2 Map indicating the distribution of *Armeria berlengensis.* The circles represent the areas were the specimens were collected.

Sterilization of the seeds

- Seeds were sterilized at varying concentrations and times of exposure to ethanol and/or sodium hypochlorite.
- Immersion for 24 hours in distilled water or fungicidal solution were also tested.
- Seeds were placed in Murashige and Skoog culture medium supplemented with B5 vitamins, 0.3% (w/v) sucrose and 1% (w/v) Phyto-agar.
- Seeds were maintained in a 16h photoperiod growing chamber under white fluorescent lamps (60 µmol.m-2.s-1) at a temperature of 21°C.



Component	
(a)	Ethanol
(b)	Sodium Hypochlorite
(c)	Fungicide
(d)	Sterilized Distilled Water

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(a)	Distilled Water
(b)	Ethanol
(c)	Sodium Hypochlorite
(d)	Sterilized Distilled Water

Fig. 3 Sterilization process.

3 Results and discussion

Table I. Best disinfection process used in *Armeria berlengensis* seeds.

Process 3		Process 5	
-		2 hours	Submerged in distilled water
1 min	Ethanol 96%	1 min	Ethanol 70%
20 min	Sodium hypochlorite <5%, 10%	5 min	Sodium hypochlorite <5%, 10%
4x4min Sterilized distilled water			

Fig. 4 Seddling of *A. berlengensis.*

Success Rate: 20.0%

Table II. Best disinfection process used in *Pulicaria microcephala* seeds.

Process 6	
1min	Ethanol 96%
20min	Sodium hypochlorite <5%, 20%
1min	Mancozebe 64%, 2,5g/L
1 min	Tirame 80%, 0.2g/L
4x4min Sterilized distilled water	

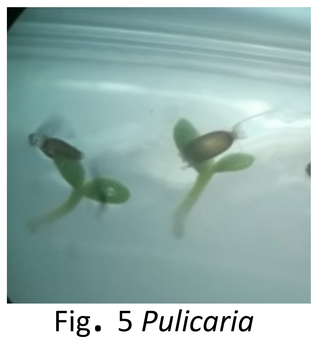

Fig. 5 *Pulicaria microcephala* seedling and seed sowing.

Success Rate: 73.0%

Table III. Total number and mean number of fruiting heads of *Armeria berlengensis* and characterization of the flowers that compose them.

	#	mean values /fruiting head
Fuiting heads analysed	98	-
Total number flowers analyzed	4721	48,173
Total number seeds collected	164	**1,673**

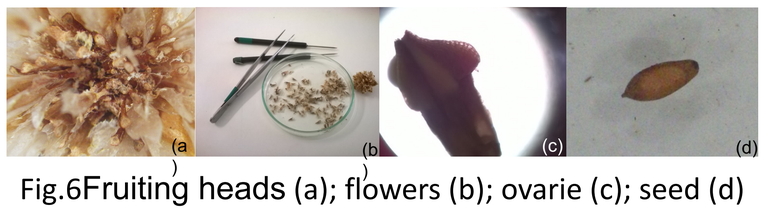

Fig.6Fruiting heads (a); flowers (b); ovarie (c); seed (d)

4 Conclusion

Regarding seed production, *A. berlengensis* exhibited a remarkably low fertility rate: having opened 4721 flowers, we obtained only 164 seeds .

The results indicate that it is possible to germinate *P. microcephala* in vitro with high success rates (up to 70%) whereas for *Armeria berlengensis* more studies need to be carried out to surpass the low germination rates observed (up to 20%), and to try to determine the causes of the reduced fertility rate of this species.

These findings are probably correlated with the critically endangered status this species currently holds

5 References

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6 Acknowledgements

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