



Article

# A Common Approach to the Conservation of Threatened Island Vascular Plants: First Results in the Mediterranean Basin

Giuseppe Fenu <sup>1</sup>, Gianluigi Bacchetta <sup>1,2</sup>, Charalambos S. Christodoulou <sup>3</sup>, Donatella Cogoni <sup>1,\*</sup>, Christini Fournaraki <sup>4</sup>, Giusso del Galdo Gian Pietro <sup>5</sup>, Panagiota Gotsiou <sup>4</sup>, Angelos Kyratzis <sup>6</sup>, Carole Piazza <sup>7</sup>, Magdalena Vicens <sup>8</sup> and Bertrand de Montmollin <sup>9</sup>

<sup>1</sup> Centro Conservazione Biodiversità (CCB), Dipartimento di Scienze della Vita e dell'Ambiente (DISVA), Università degli Studi di Cagliari, 09123 Cagliari, Italy; gfenu@unica.it (G.F.); bacchet@unica.it (G.B.)

<sup>2</sup> Hortus Botanicus Karalitanus (HBK), Università degli Studi di Cagliari, 09123 Cagliari, Italy

<sup>3</sup> Department of forests, Ministry of Agriculture, Rural Development and Environment, 1516 Nicosia, Cyprus; floracy@primehome.com

<sup>4</sup> CIHEAM Mediterranean Agronomic Institute of Chania (MAICh), 73100 Chania, Crete, Greece; flora@maich.gr (C.F.); yiota@maich.gr (P.G.)

<sup>5</sup> Department of Biological, Geological and Environmental Sciences, University of Catania, 95025 Catania, Italy; g.giusso@unict.it

<sup>6</sup> Agricultural Research Institute, Ministry of Agriculture, Rural Development and Environment, 1516 Nicosia, Cyprus; a.kyratzis@ari.gov.cy

<sup>7</sup> Office de l'Environnement de la Corse (OEC)—Conservatoire Botanique National de Corse, 20250 Corte, Corsica, France; Carole.Piazza@oec.fr

<sup>8</sup> Jardí Botànic de Sóller Foundation (JBS), 07100 Sóller, Balearic Islands, Spain; mvicens@jardibotanicdesoller.org

<sup>9</sup> Mediterranean Plant Specialist Group (IUCN/SSC), 2000 Neuchâtel, Switzerland; bertrand@montmollin.me

\* Correspondence: d.cogoni@unica.it; Tel./Fax: +39-070-675-3509

Received: 24 March 2020; Accepted: 16 April 2020; Published: 18 April 2020



**Abstract:** The Mediterranean islands represent a center of vascular plant diversity featuring a high rate of endemic richness. Such richness is highly threatened, however, with many plants facing the risk of extinction and in need of urgent protection measures. The CARE-MEDIFLORA project promoted the use of ex situ collections to experiment with in situ active actions for threatened plants. Based on common criteria, a priority list of target plant species was elaborated, and germplasm conservation, curation and storage in seed banks was carried out. Accessions were duplicated in the seed banks of the partners or other institutions. Germination experiments were carried out on a selected group of threatened species. A total of 740 accessions from 429 vascular plants were stored in seed banks, and 410 seed germination experiments for 283 plants species were completed; a total of 63 in situ conservation actions were implemented, adopting different methodological protocols. For each conservation program, a specific monitoring protocol was implemented in collaboration with local and regional authorities. This project represents the first attempt to develop common strategies and an opportunity to join methods and methodologies focused on the conservation of threatened plants in unique natural laboratories such as the Mediterranean islands.

**Keywords:** CARE-MEDIFLORA project; ex situ conservation; in situ conservation; insular vascular flora; threatened plant populations; Mediterranean islands

## 1. Introduction

The preservation of biodiversity is a well-established priority at the global level, but unfortunately, despite the efforts of institutions and conservationists over the last decades, biological diversity faces serious threats, and continues to be lost when attempting to implement rates at a global level [1–4]. The current situation indicates that the (overambitious) targets to reduce biodiversity loss set by several international conventions will not be reached in the scheduled deadlines.

Currently, plant conservation has mostly concentrated on the passive protection of fragmented natural habitats. Nevertheless, this strategy is deemed to be insufficient in the aim to reduce the increasing losses of both species and natural habitats around the world [5–7]. Over the last few years, great efforts have been made to preserve plant diversity in seedbanks; seed banking is largely recognized as a main *ex situ* conservation tool for the dependable and capable conservation of wild plant genetic resources [8]. Germplasm conservation, including seed banking, pollen/tissue storage and vegetative cloning, allows the preservation of most of the genetic material in a small space, and thus seed banks should be considered an adequate wealthy biological resource with high quality germplasm, considerable taxonomic diversity and vast geographic coverage of accessions, which represent important natural capital and population worth [9,10]. Despite several objective constraints (e.g., plants producing few viable seeds, plants circumscribed to peculiar habitats or unorthodox seeds, etc.), the main purpose of these structures is to ensure any possible effort for the long-term conservation of the highest number of plant taxa [9–11]. Optimistically, *ex situ* conservation could reach significant levels in forthcoming years (at least in some territories worldwide), and with accessions representative of the natural variability. Conversely, *in situ* conservation practices—which are commonly recognized as the main and most relevant approach to preserve biodiversity, since they are centered on populations in their natural habitats—remain far from being widely achieved, for a large number of reasons [12–18].

Plant translocation is one of the most recently developed *in situ* activities, and it represents a relevant tool for lessening extinction risks for endangered species and to ameliorate their conservation status [12,17–20]. The importance of conservation translocations, including reintroduction and population reinforcement, is notably considerable when it is part of a combined *ex situ* and *in situ* conservation strategy; the tight connection between the *in situ* and *ex situ* conservation approaches is the emerging instrument in the preservation of plant diversity [18,21,22].

The Mediterranean Basin is a priority center of plant diversity. Despite representing 1.6% of the Earth's surface, approximately 7% of the whole world's plants can be found in this region, and for this reason it has been recognized as one of the world's biggest biodiversity hotspots [23]. Nevertheless, this plant richness is irregularly distributed [24,25]; in particular, plant diversity occurs on the large Mediterranean islands (i.e., Sicily, Sardinia, Cyprus, Corsica and Crete), as well as the archipelagos (Balearic, Tuscan, Aeolian, etc.) that have an endemism rate of more than 40% [26]. In fact, the characteristic Mediterranean insular traits, such as different geographic situations (some islands are close, and others far away from the mainland), as well as their geographic and paleogeographic evolution (some islands have been isolated for a long time, while others have not), show particular features of plant diversity and association, so that as a result, within the Mediterranean Basin, islands and islets (and archipelagos) possess flora of exceptional diversity and represent the principal centers of plant diversity, particularly due to the restricted range of most of their flora [23–27]. Concurrently, it is also accepted that such plant diversity is seriously threatened due to numerous factors (physical, biological and anthropogenic); therefore, many plants on these islands need pressing measures, including, but not restricted to, protection.

Plant richness in the Mediterranean islands is correlated with many anthropogenic activities that have a negative effect on plant distributions and dynamics. Over the last four millennia, the Mediterranean Basin has been the cradle of several of the world's greatest civilizations, a condition which has resulted in the overexploitation of soil and the conversion of natural habitats into agricultural landscapes [27]. Moreover, the Mediterranean Basin is one of the most susceptible regions to climate

change, exposing the Mediterranean islands to unique challenges [27–29]; in addition, it is also documented that climate change will exacerbate these threats.

In this context, the CARE-MEDIFLORA project supported by the MAVA Foundation, is an enterprise guided by the conservation institutions (mostly botanical gardens) of six Mediterranean islands and the IUCN/SSC Mediterranean Plant Specialist Group. Involved institutions have great experience of the regional flora and their preservation, and possess considerable knowhow with ex situ conservation. All institutions worked together to manage both short-term and long-term requirements for insular threatened plants, comprising: (1) in situ conservation through active management activities, particularly translocations; and (2) ex situ conservation through the seed banking of accessions indicative of the overall diversity, as a tool for plant duplication for future in situ conservation measures [26].

The adopted approach is primarily based on the use of ex situ actions and knowledge as a tool to ameliorate the in situ conservation of threatened plant species. For example, genetic material (seeds) and knowhow from previous ex situ activities (e.g., seed collections, germination experiments, living plant collections, etc.) were utilized in field work to conserve endangered plants in situ, especially with translocation programs. This overture is coherent with the need to incorporate ex situ and in situ strategies, which has been long acknowledged [30–32]. The CARE-MEDIFLORA project takes advantage of the long experience acquired from a number of protection programs that have employed material conserved and/or propagated in botanic gardens for in situ translocations [19–21,33]. The CARE-MEDIFLORA strategy can be framed as a conservation-oriented restoration idea [22,34], since it assumes the same two major assumptions: (1) there are no options to actively control populations of endangered species to stop their disappearance; and (2) wide-scale plant introductions of endangered species—both within and outside of known historical ranges for the species—are necessary. The main aims of this paper were to summarize the results obtained during the CARE-MEDIFLORA project.

## 2. Materials and Methods

### 2.1. Study Area

The Mediterranean Basin, with about 10,000 islands and islets, encompasses one of the largest “archipelagos” in the world [35]. Some eastern Mediterranean countries, such as Croatia or Greece [36,37], include a remarkable number of these islands; however, the largest Mediterranean islands (Sicily and Sardinia), as well as around 1100 islets, are located on the western side [35]. For historical and geographical reasons, but also for peculiar species-species interactions, the Mediterranean’s insular conditions determine specific plant diversity and assemblages; in particular, the rate of plant endemism reaches high levels in these territories, at generally around 10–12% of the total vascular flora [24,35,38]. In particular, the endemism rate is considerably higher in mountain ranges and in satellite uninhabited islets, where endemics represent about 35–40% of the total vascular flora [24,25,37–41].

The six largest Mediterranean islands can be divided into the Tyrrhenian (Balears, Corsica, Sardinia and Sicily) and the East Mediterranean (Crete and Cyprus). These islands cover an overall surface of approximately 91,250 km<sup>2</sup>, including more than 600 satellite islets, and host a peculiar flora consisting of ca. 1500–1600 taxa in the Balearic Islands and Cyprus, [42,43], 2100 taxa in Crete [44–46], 2800 taxa in Corsica [47] and more than 3000 taxa in the biggest islands, Sardinia and Sicily [38,48,49]. According to the updated flora, a relevant number of endemic plants occur in all islands: 140 in the Balearic Islands [42]; 142 in Cyprus [43]; 228 in Crete [44–46]; 302 in Corsica [47]; 347 in Sardinia [38]; and 370 in Sicily [49].

### 2.2. Target Plant Selection

In order to select the plant species that will be targeted during the project, four common criteria to prioritize the conservation actions were preliminarily agreed on, in order to develop knowledge and common methodologies among the islands. The following four criteria were established: (1) the threat

degree, referring to the inclusion of a plant species into a threat category of the global/regional IUCN (International Union for Conservation of Nature) Red List as at least “threatened” (thus corresponding to the IUCN categories CR, EN and VU), and the DD plant species has been additionally included as a precautionary approach [50–53]; (2) the “regional responsibility” criterion, indicating the greatest importance given to those species whose distribution is restricted to a specific territory, such as endemic plant species and plants deserving conservation interest for a given island (e.g., peripheral and isolated plant populations, PIPPs, etc.) [51,53–56]; (3) the so-called “policy species”, which include those taxa listed in the European (i.e., Habitat Directive), or in other specific national or regional laws/regulations [7,56]; and finally, (4) vascular plants linked to wetlands, habitats severely threatened by climate change or climatic instability and hosting species intrinsically unable to migrate to other sites [57].

For each plant, the distribution type was compiled as follows: ENE = Extremely Narrow Endemic (only one population); NE = Narrow Endemic ( $\leq$  five populations); RE = Regional Endemic (only one island); IE = Insular Endemic (more than one island); and W = distributed in more islands or in a wider area.

The six island lists of the target plants elaborated have been incorporated into a global list of Mediterranean insular plants, which require urgent *in situ* and *ex situ* conservation actions. This priority list identifies the populations in which seed collection should have been performed for germplasm conservation, and the populations that need urgent *in situ* conservation actions (such as translocation, alien species eradication or control, fencing, etc.).

### 2.3. *Ex Situ Conservation and Plant Multiplication*

*Ex situ* actions, such as seed collection, curation and storage for germplasm conservation, are a relevant action of the project. Germplasm collection and curation have been carried out with consideration to the national and international rules and standards (such as those defined by Genmeda, Ensconet, etc.). The germplasm has been collected following criteria pointing to the optimization of the representativeness of the genetic diversity of the populations in each island; where possible, collections of the same plant species were realized in more than one population [58,59]. In addition, each partner guaranteed the plants’ availability for recovery or restoration programmes, and at the same time, seed collection was partly dedicated to an “active collection” to be used for producing plants. As a preventive measure aimed at guaranteeing the conservation of the collected seed material, several seedlots were duplicated in other seed banks, both from the project’s partners and/or public institutions.

Moreover, data regarding the germination eco-physiology of the collected germplasm was archived by seed germination tests performed following the national and international regulations and standards [58,59]. The species to be tested were chosen on the basis of their availability, especially in terms of the number of seeds that allow for both *ex situ* conservation and seed germination tests. The optimal protocol obtained from these experiments, or those obtained in previous projects [60], were used for plant multiplication in both laboratories and/or public and private nurseries.

### 2.4. *In Situ Conservation*

*In situ* conservation includes all the *in situ* measures aiming to ameliorate the conservation status of the target species/populations. Translocations represent the best method (comprising reintroduction and/or population reinforcement), and correlative active management actions were also implemented (as passive defence measures which may consist of the erection offences around the area where the endangered species/populations grow, eradicating or controlling invasive alien plants, or restoring the natural vegetation within or around the area, etc.).

In order to define whether the translocation of the target species was possible and feasible, a preliminary survey—chiefly based on historical data, the current distribution range of the species, the distance from the nearest natural population(s) and the availability of the potential growing sites—was performed. In addition, the availability of scientific studies on the biological (e.g., life cycle,

reproductive biology, etc.) and ecological requirements of the target species or plant group were verified, and all this information was considered as crucial data for having a reliable in situ action [12,13,21,22,33,34,61]. A translocation plan was elaborated for each plant, mainly taking into account the IUCN guidelines [13], but also considering the available updated scientific literature [12,14,17,20,21,33,62].

A similar science-based approach was adopted in order to plan and implement passive or other management activities. In addition, for each in situ action, a specific monitoring plan was elaborated and regularly implemented to control the effectiveness of such in situ measures. In the effort to make in situ activities more effective, local authorities and stakeholders were actively included in the action implementation, as well as all the monitoring processes.

### 3. Results

Based on the selected common criteria, six local lists (one per partner) and a global list of target plants needing conservation were compiled. The global list includes a total of 735 target vascular plants, which in turn consist mostly of plants chosen with the Regional Responsibility Criterion (630 taxa) and plants included as threatened in the global and/or regional IUCN Red List (343 taxa). Plants recorded in the Habitat Directive, national or regional laws, and plants correlated to wetland habitats accounted for 80 and 71 taxa, respectively.

The global list is mainly composed of vascular plant species that are widely distributed in more than one island, as well as in mainland territories (377 taxa; 51.29%), while the Narrow and Extremely Narrow Endemics together account for the 34.83% of the total (see Table 1 for details at the island level). During the project, for a total of 436 taxa, one or more conservation actions were accomplished; 429 and 51 vascular plants were included in ex situ and in situ conservation actions, respectively.

**Table 1.** Detailed report of the priority list of the selected plants at the island level. One plant could have been selected on the basis of one or more criteria.

Priority List Details	Balearic Islands	Corsica	Sardinia	Sicily	Crete	Cyprus
Total number of taxa	159	86	125	221	126	108
Threatened plants (global/regional Red List)	42	28	22	96	109	88
Regional Responsibility criterion	156	84	125	206	93	38
Policy plant species	15	15	11	20	11	11
Plants linked to wetlands habitats	14	5	20	24	8	0
Extremely Narrow Endemic (only one population)	19	5	13	40	16	8
Narrow Endemic ( $\leq$ five populations)	21	2	29	71	52	4
Regional Endemic (only one Island)	9	19	12	6	23	40
Insular Endemic (more than one island)	19	27	25	1	11	11
Plants distributed in more islands or in a wider area	91	33	46	103	24	45

#### 3.1. Ex Situ Conservation

A total of 740 accessions (seedlots) from 429 vascular plants were collected and stored in partners' seed banks during the project, and 410 seed germination experiments for 283 target plants were completed, providing data on the optimal germination protocol of the species and useful information for their multiplication (Table 2; see Tables S1 and S2 for details).

In addition, a total of 359 accessions (seedlots) belonging to 260 vascular plants were duplicated in other seed banks for security reasons and as a precautionary measure (Table 2).

Over 27,000 new plants (belonging to 162 target species) were produced for the needs of the in situ conservation actions, for availability in future restoration actions and for ex situ conservation in botanical gardens (Table 2). Plants were multiplied in botanical gardens and/or in public and private nurseries, according to the local needs, while the total outplants cultivated changes based on the type of plant (e.g., life form, seed availability, etc.), the presence/absence of biological or ecological restrictions

(e.g., problems with the multiplication of the species, high mortality of the seedlings, etc.) and/or the translocation importance considered as the number of plants to be reintroduced (Table S3).

**Table 2.** Overall number of the conservation actions, both ex and in situ, at the island level.

Ex Situ and In Situ Conservation Actions	Balearic Islands	Corsica	Sardinia	Sicily	Crete	Cyprus	Overall Project
Seedlots collected	133	103	127	154	102	121	740
No. taxa collected	35	55	85	107	63	82	457
Germination tests	102	50	23	113	43	79	410
No. taxa tested	53	46	20	47	38	79	283
Duplicata	50	50	52	55	50	102	359
No. taxa duplicated	42	29	40	47	31	71	260
Plant production	5871	1904	10,522	2040	5327	1362	27,026
In situ conservation action	10	11	11	11	10	10	63
No. taxa involved	7	7	10	7	10	10	51
Monitoring plan	10	11	11	11	10	10	63

### 3.2. In Situ Conservation

Overall, a total of 63 in situ conservation actions (related to 51 plant species) were implemented, mainly represented by plant translocations (51 actions; Table 2 and Table S4); conversely, management measures are represented by the erection of protective fences (nine actions), followed by the removal of invasive/ornamental species (three actions).

Many translocations were planned as reinforcement for existing endangered populations (49.02%), while other translocations included reintroductions in places where the plant had disappeared a short time ago (due to anthropogenic causes; 13.72%); new populations at localities with no data of species' presence were recorded previously, but with appropriate ecological conditions (37.26%). In most of the last two situations, the choice of the microsites was based on expert-based criteria.

Translocations have been carried out with distinct plant material, primarily juvenile plants (64% of the total cases) and seeds (40%), then reproductive plants, seedlings, and finally (but seldom) bulbs (24%, 14% and 2%, respectively). Some translocations (40% of the total) have been implemented using a distinct material type (e.g., seeds and juvenile plants, juvenile and reproductive plants).

As expected for highly threatened plants with small population(s), many translocations were made with very few outplants, with 43.14% using <100 outplants and only 17% using >250 individuals.

Considering all the translocations carried out, 80% of them needed management actions such as the erection of fences, and the eradication of alien species or weeding; these management actions are needed both before (41.18%) and after translocation (74.51%). Even though there are many threatened plants growing on private localities (and outside of the protected areas), in situ conservation actions were carried out on legally protected sites managed by public administrations (76% of the total), rather than on private land (12% of the total).

Finally, for each in situ action, a species-specific monitoring protocol was scheduled and implemented to guarantee its sustainability; specifically, the monitoring actions were scheduled and implemented on a monthly basis for 75% of the actions completed. Preliminary data for translocation performance, calculated by the number of surviving plants, was extremely variable between the plant life forms and outplants used, with woody and shrub species (phanerophytes/nanophanerophytes) reaching the best performance after one year (survivorship rate > 60%), followed by chamaephytes and geophytes (survivorship rate around 45%), and the worst performance being obtained for hemicryptophytes (survivorship rate < 25%). After one year, it is very difficult to evaluate the results obtained for annual plants.

The first data highlight the absence of relevant differences between the translocations carried out using juvenile and reproductive plants (survivorship rate > 60% in both cases), while considerable differences were observed in the seedlings (survivorship rate < 25%); for translocations carried out

using seeds, the results obtained cannot yet be evaluated, since seeds could perhaps be entering the soil seed bank and germinating after many years, and/or may need specific ecological requirements such as natural scarification in the soil in order to germinate.

#### 4. Discussion

The disappearance of biodiversity is continually expanding worldwide, mostly due to human-related impacts (i.e., pollution, global change, industrialization, urbanization, etc.) [2–4]. In fact, despite the adoption of several directives aiming at the protection of plant species and areas featuring remarkable biodiversity, such tools do not seem to be as effective as expected. Mediterranean islands do not differ from this situation, and indeed, considering the high endemism rate of these territories, the situation is rather worrying. Although great efforts have been made in recent years to take concrete conservation actions and develop the structures dealing with ex situ conservation, in situ conservation actions are minimal when compared to the huge number of threatened plant species. In general, there are several constraints that may hamper the realization of in situ conservation measures, such as the significant economic and time costs, the availability of optimal sites, the difficulties (or more likely impossibility) of implementing these activities on private localities, and the great uncertainty of success chiefly related to natural stochastic events [12,18,22,34,63]. These limitations are common to a wide spectrum of human-mediated conservation actions, from those that are extremely complicated, such as translocations, to less demanding ones, such as fencing (to avoid or reduce grazing and protect the most critical life-cycle step for the population survival), removing/eradicating invasive alien plants or controlling pest plants.

In this challenging context, the CARE-MEDIFLORA project, an initiative carried out in the biggest Mediterranean islands, aimed to address both short-term and long-term needs using a common strategy mostly based on the use of ex situ actions; thus, knowledge as a tool to ameliorate the in situ conservation status of endangered plants should be inserted. All conservation actions implemented during this project have been recently carried out, and therefore it is not possible to provide a thorough evaluation of their success—however, some general indications arise from this experience.

First of all, the selection of target vascular plants highlighted a general point: in all islands, a relevant proportion of the selected plant species needing urgent conservation measures is represented by plants distributed in a wider area, while narrowly distributed endemic plants (including Extremely Narrow Endemic and Narrow Endemic) reach a third of the total, except in Sicily, where they represent half of the selected plants. This suggests that the hypothesis according to which endemic species are more threatened in respect to more widely distributed species is not supported by local experts' knowledge. In addition, such observation conflicts with the common use of only endemic species to define the areas of priority interest for conservation. A second general indication comes from the island priority lists: the same plant can be extremely widespread in one area at the same as being extremely threatened with extinction in another territory (e.g., peripheral and isolated plant populations (PIPPs), ecological disjunctions, etc.). Such evidence leads us to affirm that the priorities of conservation should vary at the small local level. Therefore, a common priority list (e.g., at the biogeographical level such as the Mediterranean Basin) may not be effective for plant diversity conservation in a very heterogeneous context, such as that represented by the Mediterranean islands. In fact, a plant species could need different conservation interest depending on the particular conditions of the island or the locality on which it grows, and thus, the regional responsibility criterion should be the main guide to plan conservation measures at the restricted local scale.

Despite in situ conservation actions being the best option to conserve plant diversity, ex situ conservation allows a complementary method to avoid prompt extinction [9,10,26,63]. Currently, ex situ conservation focuses on seed banking, but this strategy is inappropriate for several endangered plants; new ex situ techniques that are currently available, such as cryopreservation (and in vitro methods) and collections of living specimens, should be implemented in order to limit this gap [64]. As a general rule, conservation in a seed bank should only be taken as a temporary measure, aimed to preserve a sufficient quantity of germplasm for future in situ actions. Nevertheless, the integration

of the ex situ approach in an in situ program, a strategy which was recognized as pivotal in the conservation of threatened plants, remains sporadically adopted.

Conservation translocation (including population reinforcement, reintroduction and introduction) are finalized to increase survival and avoid the extinction of plant species, and their usefulness in promoting the recovery of endangered populations is enhanced when done as a step in an integrated in situ and ex situ conservation plan [12–14,19–22,34]. In the Mediterranean Basin, several national and international initiatives promoted the implementation of seed bank collection, but conversely, not many initiatives/projects based on the use of ex situ expertise as an instrument to promote the in situ conservation of endangered plants have been funded or implemented. Indeed, in spite of the critical need for such activities for many localized endemic species, very few recorded translocations have been performed in these areas [21,62,65,66]. One of the main outputs of CARE-MEDIFLORA responds precisely to this need; it is represented by several in situ conservation actions that are implemented, which also exploit the knowledge acquired in ex situ conservation. In fact, all conservation translocations were carried out by introducing ex situ propagated material using different protocols, which varied in their type of translocation, locality selection process, provenance of the genetic material, type of material (seeds or cuttings) and/or planting method [18]. Preliminary data on translocation performance was highly variable between plant life forms and outplant type, as previously highlighted [9,17].

Several translocations carried out during the project involved extremely small numbers of outplants, as expected when working with highly threatened plants that survive in small populations, and often present significant reproductive limitations; surprisingly, the percentage of small conservation actions compared to the total translocations implemented is similar to that which was recently observed in Australia [17].

CARE-MEDIFLORA experiences confirm that many restrictions stay in the execution of these conservation actions. Generally, translocations are recognized as time-consuming activities, as they need much preparatory analysis, a constant commitment to propagate the outplants, realization control of the activities and an extended monitoring plan to check their efficacy; also, translocations are intended as economically costly activities, due to the relevant costs of the pre- and post-management actions. The factors that made translocation challenging, as well as stimulating, included limited human resources and the availability of adequate sites, administrative difficulties encountered by working on both private and public lands, and the high uncertainty of success due to stochastic events [18].

In general, the effectiveness of the in situ actions carried out during the project can be evaluated in a few years, while those obtained in ex situ conservation represent a guarantee in the event of any extinctions in nature. The experience of CARE-MEDIFLORA has consolidated awareness among the partners that when initiating in situ plant conservation actions, the following aspects must be carefully evaluated:

- A clear idea of what is being planned (biological and ecological implications, but also time and costs involved)
- Adequate knowledge of the seed germination, propagation and growing of the species both in the nursery/botanical garden and in situ
- Adequate economic resources not only for the transplants production and reintroduction, but also for site management actions, as well as for long-term monitoring activities
- Availability of a suitable site for the species that is preferably not too difficult to manage and control
- Consideration of the fact that the actions could take a very long time
- Acceptance from/collaboration with local institutions and stakeholders
- Regular monitoring activities, which should be continuous in order to check the effectiveness of the actions over time



## 5. Concluding Remarks

The Mediterranean insular areas, which share an exceptional rate of endemism, combined with an extraordinary level of environmental and human-related dangers, provide a good occasion to associate distinct methodologies within a common conservation approach for threatened plants. Moreover, owing to their restricted surface and discrete nature, islands could be considered as “natural laboratories” for ecological studies, including translocation experiments. The CARE-MEDIFLORA project represents a step forward in the conservation of the Mediterranean flora, and is probably a good basis for implementing conservation programs for other species under threat, if our experiences are to be repeated on a larger scale, as well as in other regions featured by analogous environmental conditions. Moreover, in situ actions play a relevant social and cultural role, and they could appreciably contribute to reinforcing cooperation among national and regional institutions, as well as NGOs, and—what is likely to be most important—local stakeholders. For this reason, a significant activity of the CARE-MEDIFLORA project was aimed at sharing knowledge and experiences among partners and adopting common protocols; in actual fact, project participants shared different approaches regarding in situ protocols, technical issues, successful or unsuccessful methodologies, and so on. At the same time, local and regional authorities and local stakeholders (particularly during the monitoring stage) were actively involved by each partner to make conservation actions, such as translocations, more effective. Finally, the conservation actions carried out during the CARE-MEDIFLORA project represent an important contribution to the implementation of the in situ conservation measures in highly endemic-rich and threatened areas, such as the biggest Mediterranean islands.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/1424-2818/12/4/157/s1>, Table S1: Germplasm accessions conserved in the CARE-MEDIFLORA project, Table S2: Germination tests carried out during the CARE-MEDIFLORA project, Table S3: Plant produced in CARE-MEDIFLORA project, Table S4: In situ conservation actions carried out during the CARE-MEDIFLORA project.

**Author Contributions:** Conceptualization, A.K., B.d.M., C.F., C.P., C.S.C., D.C., G.B., G.F., G.d.G.G.P., P.G. and M.V.; Data curation, A.K., B.d.M., C.F., C.P., C.S.C., D.C., G.B., G.F., G.d.G.G.P., P.G. and M.V.; Writing—review & editing, D.C. and G.F. All authors have read and agreed to the published version of the manuscript.

**Funding:** This project was supported by the MAVA Foundation.

**Acknowledgments:** The MAVA Foundation is gratefully acknowledged for financially supporting (80%) the CARE-MEDIFLORA project (<http://www.CARE-MEDIFLORA.eu/>). The authors wish to thank all those people who gave their contribution to the CARE-MEDIFLORA project. The authors also thank Maria Verivaki for the linguistic revision of the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Pimm, S.L.; Russel, G.J.; Gittleman, J.L.; Brooks, T.M. The future of Biodiversity. *Science* **1995**, *269*, 347–350. [[CrossRef](#)] [[PubMed](#)]
2. Butchart, S.H.M.; Walpole, M.; Collen, B.; van Strien, A.; Scharlemann, J.P.W.; Almond, R.E.A.; Baillie, J.E.M.; Bomhard, B.; Brown, C.; Bruno, J.; et al. Global biodiversity: Indicators of recent declines. *Science* **2010**, *328*, 1164–1168. [[CrossRef](#)] [[PubMed](#)]
3. Ceballos, G.; Ehrlich, P.R.; Barnosky, A.D.; García, A.; Pringle, R.M.; Palmer, T.M. Accelerated modern human-induced species losses: Entering the sixth mass extinction. *Sci. Adv.* **2015**, *1*, e1400253. [[CrossRef](#)] [[PubMed](#)]
4. Le Roux, J.J.; Hui, C.; Castillo, M.L.; Iriondo, J.M.; Keet, J.H.; Khapugin, A.A.; Médail, F.; Rejmánek, M.; Theron, G.; Yanneli, F.A.; et al. Recent anthropogenic plant extinctions differ in Biodiversity Hotspots and Coldspots. *Curr. Biol.* **2019**, *29*, 2912–2918. [[CrossRef](#)]
5. Heywood, V.H. In situ conservation of plant species e an unattainable goal? *Isr. J. Plant Sci.* **2016**, *63*, 211–231. [[CrossRef](#)]
6. Heywood, V.H. Plant conservation in the Anthropocene – Challenges and future Prospects. *Plant Divers.* **2017**, *39*, 314–330. [[CrossRef](#)]

7. Fenu, G.; Bacchetta, G.; Giacanelli, V.; Gargano, D.; Montagnani, C.; Orsenigo, S.; Cogoni, D.; Rossi, G.; Conti, F.; Santangelo, A.; et al. Conserving plant diversity in Europe: Outcomes, criticisms and perspectives of the Habitats Directive application in Italy. *Biodivers. Conserv.* **2017**, *26*, 309–328. [[CrossRef](#)]
8. Liu, U.; Breman, E.; Cossu, T.A.; Kenney, S. The conservation value of germplasm stored at the millennium seed bank, royal botanic gardens, Kew, UK. *Biodivers. Conserv.* **2018**, *27*, 1347–1386. [[CrossRef](#)]
9. Godefroid, S.; Rivièrè, S.; Waldren, S.; Boretos, N.; Eastwood, R.; Vanderborght, T. To what extent are threatened European plant species conserved in seed banks? *Biol. Conserv.* **2011**, *144*, 1494–1498. [[CrossRef](#)]
10. Mattana, E.; Fenu, G.; Bacchetta, G. Regional Responsibility for Plant Conservation: The 2010 GSPC Target 8 in Sardinia. *Plant Biosyst.* **2012**, *146*, 649–653. [[CrossRef](#)]
11. Krigas, N.; Menteli, V.; Vokou, D. Analysis of the ex Situ Conservation of the Greek Endemic Flora at National, European and Global Scales and of Its Effectiveness in Meeting GSPC Target 8. *Plant Biosyst.* **2015**, *150*, 573–582. [[CrossRef](#)]
12. Godefroid, S.; Piazza, C.; Rossi, G.; Buord, S.; Stevens, A.-D.; Agurauja, R.; Cowell, C.; Weekley, C.W.; Vogg, G.; Iriondo, J.M.; et al. How successful are plant species reintroductions? *Biol. Conserv.* **2011**, *144*, 672–682. [[CrossRef](#)]
13. IUCN: Guidelines for Reintroductions and Other Conservation Translocations. Available online: <https://www.iucn.org/theme/species/publications/guidelines> (accessed on 1 June 2013).
14. Commander, L.E.; Coates, D.J.; Broadhurst, L.; Offord, C.A.; Makinson, R.O.; Matthes, M. (Eds.) Guidelines for the Translocation of Threatened Plants in Australia. Available online: <https://www.anpc.asn.au/translocation/> (accessed on 29 August 2018).
15. Volis, S. Complementarities of two existing intermediate conservation approaches. *Plant Divers.* **2017**, *39*, 379–382. [[CrossRef](#)] [[PubMed](#)]
16. Heywood, V.H. Conserving plants within, outside and beyond protected areas. *Plant Divers.* **2019**, *41*, 36–49. [[CrossRef](#)]
17. Silcock, J.L.; Simmons, C.L.; Monks, L.; Dillon, R.; Reiter, N.; Jusaitis, M.; Vesk, P.A.; Byrne, M.; Coates, D.J. Threatened plant translocation in Australia: A review. *Biol. Conserv.* **2019**, *236*, 211–222. [[CrossRef](#)]
18. Fenu, G.; Bacchetta, G.; Christodoulou, C.S.; Fournaraki, C.; Giusso del Galdo, G.P.; Gotsiou, P.; Kyratzis, A.; Piazza, C.; Vicens, M.; Pinna, M.S.; et al. An early evaluation of translocation actions for endangered plant species on Mediterranean islands. *Plant Divers.* **2019**, *41*, 94–104. [[CrossRef](#)]
19. Maschinski, J.; Duquesnel, J. Successful reintroductions of the endangered long-lived Sargent’s cherry palm, *Pseudophoenix sargentii*, in the Florida Keys. *Biol. Conserv.* **2006**, *134*, 122–129. [[CrossRef](#)]
20. Menges, E.S.; Smith, S.A.; Weekley, C.W. Adaptive introductions: How multiple experiments and comparisons to wild populations provide insights into requirements for long-term introduction success of an endangered shrub. *Plant Divers.* **2016**, *38*, 238–246. [[CrossRef](#)]
21. Cogoni, D.; Fenu, G.; Concas, E.; Bacchetta, G. The effectiveness of plant conservation measures: The *Dianthus morisianus* reintroduction. *Oryx* **2013**, *47*, 203–206. [[CrossRef](#)]
22. Volis, S. Conservation meets restoration—rescuing threatened plant species by restoring their environments and restoring environments using threatened plant species. *Isr. J. Plant Sci.* **2016**, *63*, 262–275. [[CrossRef](#)]
23. Médail, F.; Quézel, P. Hot-spots analysis for conservation of plant biodiversity in the Mediterranean Basin. *Ann. Missouri Bot. Gard.* **1997**, *84*, 112–127. [[CrossRef](#)]
24. Thompson, J.D. *Plant Evolution in the Mediterranean*; Oxford University Press: Oxford, UK, 2005; pp. 1–304.
25. Cañadas, E.M.; Fenu, G.; Peñas, J.; Lorite, J.; Mattana, E.; Bacchetta, G. Hotspots within hotspots: Endemic plant richness, environmental drivers, and implications for conservation. *Biol. Conserv.* **2014**, *170*, 282–291. [[CrossRef](#)]
26. Fenu, G.; Giusso del Galdo, G.P.; de Montmollin, B.; Gotsiou, P.; Cogoni, D.; Piazza, C.; Fournaraki, C.; Kyratzis, A.; Vicens, M.; Christodoulou, C.S.; et al. Active management actions for the conservation of the endangered Mediterranean island flora: The CARE-MEDIFLORA project. *Plant Sociol.* **2017**, *54*, 101–110. [[CrossRef](#)]
27. Vogiatzakis, I.N.; Mannion, A.M.; Sarris, D. Mediterranean island biodiversity and climate change: The last 10,000 years and the future. *Biodivers. Conserv.* **2016**, *25*, 2597–2627. [[CrossRef](#)]
28. IPCC WGI. Climate Change 2013: The Physical Science Basis. In *Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M., Eds.; Cambridge University Press: Cambridge, UK, 2013.

29. Cramer, W.; Guiot, J.; Fader, M.; Garrabou, J.; Gattuso, J.-P.; Iglesias, A.; Lange, M.A.; Lionello, P.; Llasat, M.C.; Paz, S.; et al. Climate change and interconnected risks to sustainable development in the Mediterranean. *Nat. Clim. Chang.* **2018**, *8*, 972–980. [[CrossRef](#)]
30. Falk, D.A. Integrated conservation strategies for endangered plants. *Nat. Areas J.* **1987**, *7*, 118e123.
31. Heywood, V.H. The role of botanic gardens and arboreta in the ex-situ conservation of wild plants. *Opera Bot.* **1993**, *121*, 309–312.
32. Maunder, M.; Higgins, S.; Culham, A. The effectiveness of botanic garden collections in supporting plant conservation: A European case study. *Biodivers. Conserv.* **2001**, *10*, 383–401. [[CrossRef](#)]
33. Fenu, G.; Cogoni, D.; Bacchetta, G. The role of fencing in the success of threatened plant species translocation. *Plant Ecol.* **2016**, *217*, 207–217. [[CrossRef](#)]
34. Volis, S. Species-targeted plant conservation: Time for conceptual integration. *Isr. J. Plant Sci.* **2016**, *63*, 232–249. [[CrossRef](#)]
35. Pons, E.C.; Clarisó, I.E.; Casademont, M.C.; Arguimbau, P.F. *Islands and Plants: Preservation and Understanding of Flora on Mediterranean Islands*; Institut Menorquí d’Estudis: Menorca, Spain, 2013; pp. 1–409.
36. Nikolic, T.; Antonic, O.; Alegro, A.L.; Dobrovic, I.; Bogdanovic, S.; Liber, Z.; Rešetnik, I. Plant species diversity of Adriatic islands: An introductory survey. *Plant Biosyst.* **2008**, *142*, 435–445. [[CrossRef](#)]
37. Kougioumoutzis, K.; Thalassini Valli, A.; Georgopoulou, E.; Simaiakis, S.M.; Triantis, K.A.; Trigas, P. Network biogeography of a complex island system: The Aegean Archipelago revisited. *J. Biogeogr.* **2017**, *44*, 651–660. [[CrossRef](#)]
38. Fenu, G.; Fois, M.; Cañadas, E.M.; Bacchetta, G. Using endemic-plant distribution, geology and geomorphology in biogeography: The case of Sardinia (Mediterranean Basin). *Syst. Biodivers.* **2014**, *12*, 181–193. [[CrossRef](#)]
39. Brullo, S.; Giusso del Galdo, G.; Guarino, R. The orophilous dwarf-shrub vegetation of Mt. Troodos (Cyprus). *Bot. Chronika* **2005**, *18*, 63–73.
40. Trigas, P.; Panitsa, M.; Tsiftsis, S. Elevational gradient of vascular plant species richness and endemism in Crete—The effect of post-isolation mountain uplift on a continental island system. *PLoS ONE* **2013**, *8*, e59425. [[CrossRef](#)]
41. Fois, M.; Fenu, G.; Bacchetta, G. Global analyses underrate part of the story: Finding applicable results for the conservation planning of small Sardinian islets’ flora. *Biodivers Conserv.* **2016**, *25*, 1091–1106. [[CrossRef](#)]
42. Sáez, L.; Fraga, P.; Lopez-Alvarado, J. The Flora of the Balearic Islands. In *Islands and Plants: Preservation and Understanding of Flora on Mediterranean Islands*; 2nd Botanical Conference in Menorca; Pons, E.C., Clarisó, I.E., Casademont, M.C., Arguimbau, P.F., Eds.; Consell Insular de Menorca: Col·leció Recerca Menorca, Spain, 2013; pp. 91–103.
43. Flora of Cyprus—A Dynamic Checklist (Continuously Updated). Available online: <http://www.flora-of-cyprus.eu/> (accessed on 3 January 2017).
44. Dimopoulos, P.; Raus, T.; Bergmeier, E.; Constantinidis, T.; Iatrou, G.; Kokkini, S.; Strid, A.; Tzanoudakis, D. *Vascular Plants of Greece: An Annotated Checklist*; Botanic Garden and Botanical Museum Berlin-Dahlem: Berlin, Germany; Hellenic Botanical Society: Athens, Greece, 2013; pp. 1–31.
45. Dimopoulos, P.; Raus, T.; Bergmeier, E.; Constantinidis, T.; Iatrou, G.; Kokkini, S.; Strid, A.; Tzanoudakis, D. Vascular plants of Greece: An annotated checklist. Supplement. *Willdenowia* **2016**, *46*, 301–347. [[CrossRef](#)]
46. Strid, A. Atlas of the Aegean flora. *Englera* **2016**, *33*, 1–1578.
47. Jeanmonod, D.; Gamisans, J. *Flora Corsica*, 2nd ed.; Koeltz Scientific Books: Koenigstein, Germany, 2013; pp. 1–921.
48. Bartolucci, F.; Peruzzi, L.; Galasso, G.; Albano, A.; Alessandrini, A.; Ardenghi, N.M.G.; Astuti, G.; Bacchetta, G.; Ballelli, S.; Banfi, E.; et al. An updated checklist of the vascular flora native to Italy. *Plant Biosyst.* **2018**, *152*, 179–303. [[CrossRef](#)]
49. Giardina, G.; Raimondo, F.M.; Spadaro, V. A catalogue of plants growing in Sicily. *Boccone* **2007**, *20*, 5–582.
50. IUCN (2020) The IUCN Red List of Threatened Species. Version 2019-2. Available online: <http://www.iucnredlist.org> (accessed on 16 January 2020).
51. Orsenigo, S.; Montagnani, C.; Fenu, G.; Gargano, D.; Peruzzi, L.; Thomas, A.; Alessandrini, A.; Bacchetta, G.; Bartolucci, F.; Bovio, M.; et al. Red Listing plants under full national responsibility: Extinction risk and threats in the vascular flora endemic to Italy. *Biological Conserv.* **2018**, *224*, 213–222. [[CrossRef](#)]

52. Liste Rouge Régionale de la Flore Vasculaire de Corse. Available online: [https://www.researchgate.net/publication/313887878\\_Liste\\_rouge\\_regionale\\_de\\_la\\_flore\\_vasculaire\\_de\\_Corse](https://www.researchgate.net/publication/313887878_Liste_rouge_regionale_de_la_flore_vasculaire_de_Corse) (accessed on 30 November 2015).
53. Orsenigo, S.; Fenu, G.; Gargano, D.; Montagnani, C.; Abeli, T.; Alessandrini, A.; Bacchetta, G.; Bartolucci, F.; Carta, A.; Castello, M.; et al. Red list of threatened vascular plants in Italy. *Plant Biosyst.* **2020**, 1–31. [[CrossRef](#)]
54. Bacchetta, G.; Fenu, G.; Mattana, E. The checklist of the exclusive vascular flora of Sardinia and its priority settings for conservation. *Anales. J. Bot. Madrid* **2012**, *69*, 81–89. [[CrossRef](#)]
55. Gauthier, P.; Debussche, M.; Thompson, J.D. Regional priority setting for rare species based on a method combining three criteria. *Biol. Conserv.* **2010**, *143*, 1501–1509. [[CrossRef](#)]
56. Rossi, G.; Orsenigo, S.; Montagnani, C.; Fenu, G.; Gargano, D.; Peruzzi, L.; Wagensommer, R.P.; Foggi, B.; Bacchetta, G.; Domina, G.; et al. Is legal protection sufficient to ensure plant conservation? The Italian red list of policy species as a case study. *Oryx* **2016**, *50*, 431–436. [[CrossRef](#)]
57. Tickner, D.; Opperman, J.; Abell, R.; Acreman, M.; Arthington, A.H.; Bunn, S.E.; Cooke, S.J.; Dalton, J.; Darwall, W.; Edwards, G.; et al. Bending the Curve of Global Freshwater Biodiversity Loss—An Emergency Recovery Plan. *Bioscience* **2020**, *70*, 330–342. [[CrossRef](#)]
58. Bacchetta, G.; Fenu, G.; Mattana, E.; Piotta, B.; Virevaire, M. *Manuale per la Raccolta, Studio, Conservazione e Gestione ex Situ del Germoplasma*; APAT: Roma, Italy, 2006; pp. 1–248.
59. Bacchetta, G.; Bueno Sánchez, A.; Fenu, G.; Jiménez-Alfaro, B.; Mattana, E.; Piotta, B.; Virevaire, M. *Conservación ex Situ de Plantas Silvestres*; Principado de Asturias/La Caixa, Ed.; Obra Social La Caixa y Gobierno del Principado de Asturias: Asturias, Spain, 2008; p. 378.
60. Manual for the Propagation of Selected Mediterranean Native Plant Species. Available online: [http://www.ecoplantmed.eu/en/publications/propagation\\_manual](http://www.ecoplantmed.eu/en/publications/propagation_manual) (accessed on 11 November 2015).
61. Menges, E.S. Restoration demography and genetics of plants: When is a translocation successful? *Aust. J. Bot.* **2008**, *56*, 187–196. [[CrossRef](#)]
62. Laguna, E.; Navarro, A.; Pérez-Rovira, P.; Ferrando, I.; Ferrer-Gallego, P. Translocation of *Limonium perplexum* (Plumbaginaceae), a threatened coastal endemic. *Plant Ecol.* **2016**, *217*, 1183–1194. [[CrossRef](#)]
63. Fenu, G.; Fois, M.; Cogoni, D.; Porceddu, M.; Pinna, M.S.; Cuenca Lombraña, A.; Nebot, A.; Sulis, E.; Picciau, R.; Santo, A.; et al. The Aichi Biodiversity Target 12 at regional level: An achievable goal? *Biodiversity* **2015**, *16*, 120–135. [[CrossRef](#)]
64. Wyse, S.V.; Dickie, J.B.; Willis, K.J. Seed banking not an option for many threatened plants. *Nature Plants* **2018**, *4*, 848–850. [[CrossRef](#)]
65. Piazza, C.; Hugot, L.; Richard, F.; Schatz, B. Bilan des opérations de conservation in situ réalisées entre 1987 et 2004 en Corse: Quelles leçons pour demain? *Ecol. Médit.* **2011**, *37*, 7–16.
66. Rita, J.; Cursach, J. Creating new populations of *Apium bermejoi* (Apiaceae), a critically endangered endemic plant on Menorca (Balearic Islands). *Anales. J. Bot. Madrid* **2013**, *70*, 27–38. [[CrossRef](#)]

