



Prioritizing Plants around the Cross-Border Area of Greece and the Republic of North Macedonia: Integrated Conservation Actions and Sustainable Exploitation Potential

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Abstract: Plants know no political borders and some of them are restricted to small geographical territories of different countries in which they are endemic. In this study, we prioritized plants (PPs) of the crossborder area of Greece and the Republic of North Macedonia that are already threatened or nearly so (PPA), those which occur exclusively on either side of borderline and/or nearby countries (PPB), or those which are uncommon and rare in this region (PPC) with the aim to document in-situ the species-specific risksthreats; offer ex-situ conservation for them as a back-up solution for future re-introductions and sustainable exploitation; and raise public awareness and alertness about the importance of local biodiversity. In the framework of the project Conse-pp, 20 botanical expeditions were performed in 75 selected areas to collect samples and suitable propagation material from 130 PPs (147 accession numbers), also recording all types of threats-identified in-situ for each of them. No ex-situ conservation was detected for 40 PPs worldwide while for another 12 PPs only limited attempts have been made to date. The fully documented PPs are currently maintained under ex- situ conservation and acclimatization in the Balkan Botanic Garden of Kroussia (BBGK). In total, 156 propagation trials (sexual or asexual) have been made for these PPs to develop species-specific propagation protocols. Consequently, the production of new plant stocks raised ex-situ was achieved (n = 3254 individuals; first-time ex-situ conservation for 40 taxa), and this has enabled the establishment of three awareness-raising sites with PPs: (i) 1000 plants of 70 PPs in the newly designed Kardia Botanical Park in Thermi (Greece); (ii) 850 plants of 104 PPs in the phytodiversity awareness spot in BBGK and 130 individual PPs in the Garden of Environmental Awareness; and (iii) 42 plants of 14 PP taxa delivered to the botanic garden of Ss. Cyril and Methodius University in Skopje. This project outlines the necessity of the development of common plant conservation strategies for threatened plants in cross-border areas of neighboring countries and presents an integrated approach allowing for sustainable development and future in-situ protection measures and actions in the studied cross-border area.

Keywords: Conse-pp (INTERREG); in-situ conservation; ex-situ conservation; propagation protocols; Balkan vascular flora; threatened plant populations; local endemic plants; Mediterranean



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1. Introduction

Biodiversity loss has been a complex problem for decades, while the conservation of biological diversity still remains a global priority issue [1]. Despite the measures taken by organizations, countries, institutions and scientific groups, global biodiversity still seems to be threatened. Although we have not reached the fulfillment of the targets of the Convention on Biological Diversity (CBD) by 2020, the post-2020 goals appear to be ambitious and challenging as well [2–6]. Although conservation efforts focused on plant diversity are taking place over the last decades worldwide (including the Mediterranean basin and its surrounding areas), still the results seem to be affected by social, scientific, and political factors coupled with complexities arising from target species prioritization and/or focal area selection schemes [1,7].

Region-wise, biodiversity in Europe is legally protected across numerous in-situ conservation sites of the Natura 2000 Network (https://natura2000.eea.europa.eu/, accessed on 13 June 2022) and the Emerald Network (https://www.coe.int/en/web/bern-convention/emerald-viewer/, accessed on 13 June 2022), and in some cases in Important Plant Areas (IPAs) Important Bird Areas (IBAs), or Key Biodiversity Areas (KBAs) [8]. Species-wise, the IUCN's Red Listing system is broadly considered the most authoritative source for assessing the species' extinction risk at global, regional, or national levels [9]. This system includes a set of objective criteria, decision rules, and threshold values that lead to a proper match of taxa (species or subspecies) to a number of well-defined IUCN extinction risk categories [10] of escalating conservation interest and concern (i.e., Data Deficient, Least Concern, Near Threatened, Vulnerable, Endangered, Critically Endangered, and Extinct). In this way, the IUCN system provides a worldwide-used tool that supports conservation planning, management, and monitoring [11].

Both Greece and the Republic of North Macedonia are considered biodiversity hotspots, hosting a high amount of flora and fauna of the Balkan Peninsula. The Greek flora is represented by at least 6760 plant taxa, out of which 1461 taxa are national endemics of Greece or parts thereof [12], while North Macedonia hosts nearly 3200 vascular plants, 120 of which are local endemics [8,13,14]. Both countries have a remarkable variety of habitat types, ecosystems and landscapes quite rich in rare, local endemic and/or threatened species. The cross-border area of Greece and North Macedonia is defined by mountain ranges, river valleys, and lakes that render this region as an area of high interest for investigation and conservation of the flora. As mountain-massifs represent natural geomorphological barriers, they are often used for mutual demarcation of states designating political or administrative limits [13]. However, wild-growing plant populations may cross such limits. Natural ecosystems in both mountain and lowland areas are often degraded by man-made interventions such as construction works and infrastructure, urbanization, wild fires, grazing, erosion and invasion of alien species [9]. Aiming to identify cases in Greece that need attention, the risks-threats as well as the protection measures associated with the rare and threatened taxa of the national Red Data Books of Greece [15,16] has been performed during the last decade [17]. This investigation delivered a hierarchically organized, expandable, and scale-independent system classifying concomitant risks-threats as well as protection measures and outlined the most threatening process for these conservation priority plants.

In general, in-situ plant biodiversity conservation should be undoubtedly supported by ex-situ actions involving seedbank storage and different propagation trials allowing for future reintroduction and/or population reinforcement of targeted species [18–20]. However, there are severe knowledge gaps regarding the biological cycle and the speciesspecific propagation and ex-situ cultivation of most of the conservation important plants such as local endemics of small geographical areas (e.g., [21–29]). Previous studies on sexual reproduction via seeds of several conservation priority plants have focused on the conditions of favourable seed germination, thus proposing seed propagation protocols (e.g., [20,24–26,30–32]). However, seeds of many species may present either physical or physiological dormancy [33], and in such cases a plethora of stratification and pre-treatment regimes have been proposed in the literature to overcome this [34–37]. Although asexual propagation does not offer genetic diversity to the offspring [38], when applied properly (namely careful selection of initial plant materials (selected genotypes), season, and methodology) it can be more successful from seed propagation in terms of plant production. Therefore, asexual propagation can be considered a useful tool in the ex-situ production of valuable plant material for conservation and natural population reinforcement [39,40] as it can be used to increase adequately the number of identical individuals per selected genotype to meet the needs of defined conservation targets or actions [20]. Such approaches most often use softwood cuttings and external hormone application at different concentration under mist and experimentation performed at various seasons following the growth pattern of the studied species (e.g., [40–43]). Additionally, plant division can be also employed, depending on the morphology of the studied species (i.e., plants forming rosettes and/or rhizomes).

In terms of gardening or landscapes, native plants are important elements that can be used to create fascinating and unique plant displays for visitor attraction in botanic gardens and urban thematic parks, representing an essential tool for raising environmental awareness on biodiversity issues [21,44,45], while new design approaches of plant displays incorporating range-restricted plants are useful for establishing stronger relationships between people, plants, and associated environmental issues [46,47]. To date, several studies indicate the importance of landscaping with native plants [45,48,49], giving preference to local ecotype plant stock [45,50]. Over the last few decades, the Balkan Botanic Garden of Kroussia of the Institute of Plant Breeding and Genetic Resources (IPGRB-BBGK) is engaged in gardening and landscaping applications with selected native plants of Greece that have been domesticated directly from the wild after propagation and acclimatization in man-made environments [45,51,52]. This novel approach promotes design installations that utilize the local phytogenetic resources sustainably to create new garden aesthetics based on consolidated plant identity and biological characteristics (e.g., rarity, endemism, habitat specificity, extinction risk assessment) combined with naturally enhanced plant resilience and minimized maintenance cost compared to exotic horticultural plants [21,45].

In the context of biodiversity conservation and implementation of CBD goals at international scales, the core idea of the research project Conse-pp (INTERREG IPA CBC Program Greece—Republic of North Macedonia 2014–2020) was to improve the conservation status of the prioritized plants occurring in the cross-border area of Greece and the Republic of North Macedonia through targeted actions. The aim of the current study was four-fold: to define priority plants in the cross-border study area, creating a geo-referenced database for the targeted priority plants (PPs); to identify the risks-threats of PPs, permitting the development of a future conservation strategy with protection measures; to collect suitable propagation material and develop species-specific propagation protocols for effective ex-situ conservation, facilitating future reinforcement of natural populations; and finally, to create original and open-air plant displays with PPs, thus raising environmental awareness and public engagement for the targeted PPs and their habitats.

2. Materials and Methods

2.1. Study Area and Botanical Collections of Target Plants

In general, the study is floristically classified into the Scardo-Pindic province, which is part of the Balkan sub-region, belonging ultimately to the Sub-Mediterranean region [53] in which the mountainous flora is part of the Oro-Mediterranean floristic region [54]. In the north-central floristic region of Greece (NC Greece, [55]), the study was conducted in certain regions of Macedonia, which is the largest geographical unit of Greece, located in the north of the country (regional unities of Thessaloniki, Florina, Pella, Kilkis, and Serres), comprising of a wide variety of habitats, vegetation zones, and floristic elements. In the Republic of North Macedonia, the focal study areas were located across the mountainous borderline with Greece and in the steppic central part of the country, including the catchment area of the Treska River (region of Porece, Alshar in Kozjak mountain, Mariovo region, Pletvar and gorge of Raec river, Galicica mountain range, Belcishko wetland in

Municipality of Debrca and Mokra mountain, which are floristically rich areas with many high mountain, gorge and lowland endemic plants [8].

Plant propagation material was collected from specific study areas located on both sides of the borderline between Greece and the Republic of North Macedonia. In total, 20 botanical expeditions were conducted in 2019–2021 in the frame of the research project Conse-pp (Supplementary Materials Table S1). The authorized collections of plant material were performed using the official permit of the Institute of Plant Breeding and Phytogenetic Resources, Hellenic Agricultural Organization Demeter (Permit 82336/879 of 18 May 2019 & 26895/1527 of 21 April 2021) which is issued yearly by the Greek Ministry of Environment and Energy. A principal task during the in-situ filed work was to be able to localize the wildgrowing populations based on literature data whenever available and to collect suitable plant propagation material (seeds, bulbs, rhizomes, cuttings, and/or few entire plants or all of them whenever possible) from as many targeted plant taxa (species and subspecies) as possible; this task was performed with the aid of specific working lists per visited site and locality, following ecological information retrieved from literature. In case of absence of data, randomized exploring of possibly suitable wild habitats was performed in the visited cross-border areas. During in-situ fieldwork, detailed ecological observations were performed for each taxon regarding its biotic and abiotic conditions in its original habitat. More specifically, we recorded the geographical coordinates, the elevation, the geological substrate, and the habitat type for each taxon. The documentation for each taxon was substantiated with deposited herbarium specimens in the registered BBGK herbarium and photos of the taxon in its wild habitat.

All of the collected plant materials originated from wild-growing populations, and they were taxonomically identified based on standard plant diagnostic keys for Greece and the Republic of North Macedonia (see full scientific names with authorship of taxa in Supplementary Materials Table S1). Consequently, the collected plant materials (seeds, cuttings or entire living plants) were transferred to the facilities of the IPBGR-BBGK of the Hellenic Agricultural Organization Demeter (Thermi, metropolitan Thessaloniki, Greece), where a unique IPEN (International Plant Exchange Network) coded accession number was assigned to each population sample collected from a single locality on a specific date. The IPEN number is used for plant exchange and indicates the country of origin (GR or RNM), the terms of use (1: restrictions of use or 0: no restriction), the collecting institution (BBGK), the collection year (two digits) and the basic number identifier (https://www. bgci.org/wp/wp-content/uploads/2019/04/Manual_IPEN_Numbers.pdf, accessed on 13 June 2022). For example, in the IPEN accession number GR-1-BBGK-19,123 the basic accession number is 19,123 which is used solely as plant identifier and corresponds to an individual plant collection from a given site on a specific date by certain person(s). This documented original propagation material was used to establish customized propagation protocols, aiming to enhance the ex-situ conservation of the targeted plants. All data were recorded and elaborated in respective Excel files (Supplementary Materials Table S1).

2.2. Hierarchical Prioritization of Target Plants

The collected priority plants (PPs) were divided into three main priority categories (PCA, PCB, PCC) based on combined biological features of conservation importance such as extant extinction risk assessments used only in PCA; degree of endemism used in PCA and PCB; distribution range and range-restriction used in PCB and PCC; and apparent rarity or uncommonness used only in PCC. The designated hierarchical prioritization categories were as follows (Supplementary Materials Table S1):

Priority category A (PCA)—This category was based on the adoption of the combined IUCN extinction risk criterion (national and/or global) assigned to plants. Data Deficient (DD) plant taxa as well as taxa assigned with a 'Rare' status (old IUCN characterization) have been also included in this group in the frame of a precautionary approach [1,56,57]. The PCA included two main groups of plants, i.e., Group A1 (Threatened or Near Threatened endemics with distribution restricted either to Greece, Republic of North Macedonia,

or either countries, or restricted to neighboring Balkan countries and/or Anatolia), and Group A2 (Threatened or Near Threatened non-endemic taxa extending further beyond the Balkan range).

Priority category B (PCB)—This category was based on the adoption of the combined criterion of narrow distribution range (range-restriction) and endemism as proxies of regional responsibility and irreplaceability or uniqueness, respectively [1,8]. In other words, the selected PPs in this group are range-restricted to Greece and/or the Republic of North Macedonia and/or extending to adjacent Balkan countries, lacking however any IUCN extinction risk status. PCB comprised of four main groups: Group B1 (Greek endemic taxa, encountered exclusively in Greece), Group B2 (North Macedonian endemic taxa, encountered exclusively in the Republic of North Macedonia), Group B3 (endemics of both countries, thus restricted to Greece and Republic of North Macedonia), Group B4 (Balkan endemics encountered in Greece and/or Republic of North Macedonia but also found in other Balkan countries and/or adjacent Anatolia).

Priority category C (PCC)—This category adopted the combined criterion of apparent regional rarity or uncommonness of occurrence of taxa in Greece and/or the Republic of North Macedonia incorporating motivation criteria of the Natura 2000 and Emerald Networks. Obviously, this category included taxa with wider distribution range than those outlined in previous categories (PCA and PCB) extending beyond the Balkan countries. Three groups were defined in PCC: Group C1 (other rare taxa of Greece and/or North Macedonia with distribution extending just beyond the Balkan-Anatolia area not included in Other Important Species of Natura 2000), Group C2 (other rare taxa of Greece and/or North Macedonia with distribution extending beyond the Balkan area assigned with motivation B in the Natura 2000 or Emerald Networks), and Group C3 (uncommon taxa of Greece and/or North Macedonia with distribution extending beyond the Balkan area assigned with motivation D in the Natura 2000 and Emerald Networks).

After taxonomic identification, to designate each collected taxon (species or subspecies) to the PCA, PCB or PCC prioritization categories (Figure 1), we consulted the following sources:

- 1. Threatened taxa included in the Greek national Red Data Books [15,16] found in Greek sites of INTERREG eligible region,
- 2. Taxa assessed as threatened in the Global IUCN Red List website (https://www. iucnredlist.org/, accessed on 13 June 2022) and/or the 1997 Global IUCN Red List [58] present in sites of the INTERREG eligible region,
- 3. Other Important Species of the NATURA 2000 Network (EU Directive 92/43/EEC) of the Greek INTERREG eligible regions [59],
- 4. Species of special conservation interest of the Emerald Network Areas of (http:// emerald.eea.europa.eu/#, accessed on 13 June 2022) of sites of the Republic of North Macedonian included in the INTERREG eligible region,
- 5. Trigger species of Important Plant Areas (IPA) and Key Biodiversity Areas (KBA) of the Republic of North Macedonian [8] included in the INTERREG eligible region,
- 6. Other local Balkan endemic taxa (some extending to Italy, Anatolia and/or Romania) present in the INTERREG eligible regions according to widely scattered literature sources (e.g., [60], etc.).

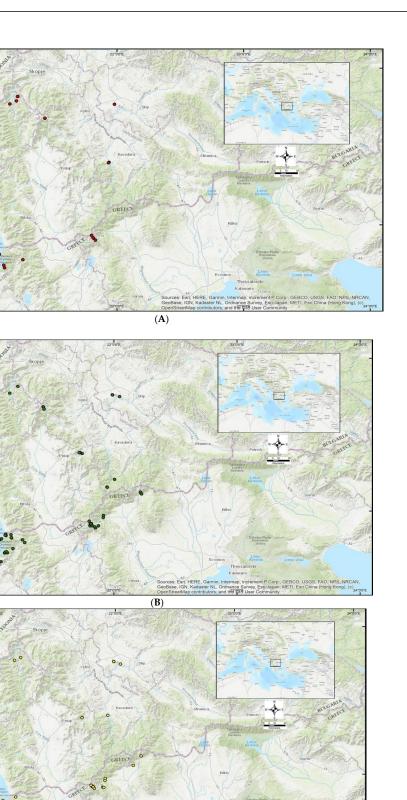


Figure 1. Botanical collections performed in Greece and the Republic of North Macedonia of plants of different conservation priority categories. (**A**) PCA, Threatened and Near Threatened (red dots); (**B**) PCB, Local Balkan endemics and subendemics (green dots); (**C**) PCC, Regionally rare and/or uncommon plants (yellow dots).

(C)

The nomenclature of taxa, the distribution range and the endemism status of all of the plant records was retrieved from a variety of literature sources and databases and data was cross-checked with the aid of the Euro + Med Plantbase (http://www.emplantbase.org/home, accessed on 13 June 2022) and/or the official Flora of Greece (http://portal.cybertaxonomy.org/flora-greece/, accessed on 13 June 2022). In cases of divergence in plant nomenclature or distribution range of taxa, the following hierarchy was considered:

- (i) The Euro + Med Plantbase (http://www.emplantbase.org/home, accessed 13 June 2022) and/or the official Flora of Greece (http://portal.cybertaxonomy.org/flora-greece/, accessed 13 on June 2022),
- (ii) The Global IUCN Red List website (http://www.iucnredlist.org/, accessed on 13 June 2022),
- (iii) The Kew Botanic Garden's database (http://plantsoftheworldonline.org/, accessed on 13 June 2022).

All such data were recorded and elaborated in respective Excel files and are presented in Supplementary Materials Tables S1, S3, S6 and S8.

2.3. In-Situ Documentation of Threats-Risks of Target Plants

Furthermore, the potential and actual threats-risks that each taxon faces in its wild habitat were recorded in-situ for 130 PPs by examining their wild-growing populations within a radius of around 300 m around every collection locality (Figure 2, Supplementary Materials Table S2). Signs of herbivore activity (traces of excrements or herbivory signs by rabbits, deers, cattle or goats and sheep), nearby land uses, and habitat degradation indications such as erosion or fire were recorded for each PP. The purpose was to create a profile for each collected taxon, with respect to their in-situ risks-threats. The taxonspecific inventory was focused on 'yes/no' cross-check regarding all possible threats-risks identified in the hierarchical classification system developed for the rare and threatened plants of Greece [17]. This system was originally constructed on four levels including 56 possible types of risks-threats in total. Out of all of the aforementioned types of threats-risks (Supplementary Materials Table S2), 11 are classified as endogenous and are related to traits that reflect in situ sensitivity/vulnerability to external stress factors such as ecological specificity, hybridization, low genetic diversity, low fecundity, etc. The remaining 45 types of risks-threats are classified as exogenous and these are further divided into those deriving by natural processes and those by man-made interventions. Risks of natural processes are related to phenomena such as erosion, floods, or landslides. Risks prompted by man-made interventions include mechanical type events such as deposition of debris, application of agrochemicals, changes in land management and uses, and are also associated with invasion of alien species, and multi-effect threats such as climate change or fires. All data were recorded and elaborated in respective Excel files (Supplementary Materials Table S2).

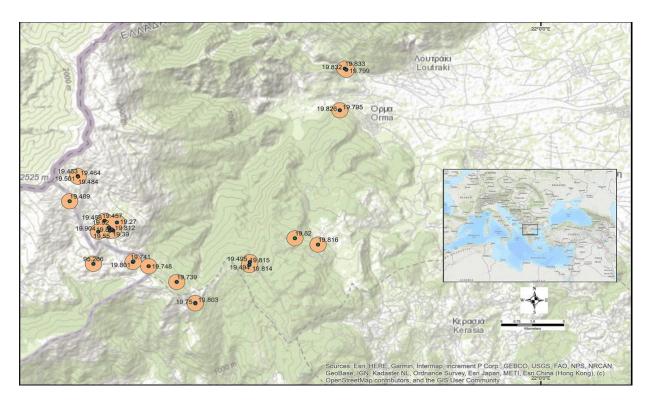


Figure 2. Overview of the botanical collections performed on Mt Voras (altitude 2521 m above sea level) in NW Greece indicating the area examined (around 300 m around every collection locality) for actual risks-threats that each taxon faces in its wild habitat. PCA: Threatened and Near Threatened plants (red central dots), PCB: Local Balkan endemics and subendemic plants (green central dots), PCC: Rare and/or uncommon plants (yellow central dots). The numbers correspond to abbreviated accession numbers documenting each collection. For individual accession numbers, see Supplementary Materials Table S1.

2.4. Spatial Analysis of the Habitats of Target Plants

Spatial analysis was carried out aiming to generate useful insights facilitating sustainable spatial planning and landscape management. The collected plants' habitats were categorized according to the EUNIS habitat classification (level 2), and their priority category. The EUNIS habitat classification is a comprehensive pan-European system, covering all types of habitats from natural to artificial (https://www.eea.europa.eu/data-and-maps/ data/eunis-habitat-classification-1/documentation/eunis-2004-report.pdf/download, accessed on 7 July 2022). We also calculated the surface explored in-situ of level 2 EUNIS habitat types per conservation priority category of plants (PCA, PCB, PCC) excluding possible overlap among collection sites.

2.5. Worldwide Ex Situ Conservation of Target Plants

To investigate the presence of collections of 130 targeted PPs in seed banks and botanical gardens worldwide, numerous taxon-specific queries were made in the PlantSearch facility of the Botanic Garden Conservation International (BGCI) database (https://tools.bgci. org/plant_search.php, accessed on 13 June 2022) to estimate how many institutions host propagation material of the targeted 130 PPs (for methodology, see [19]). The PlantSearch tool performs automatic queries based on a plant's scientific name among 1,580,137 collection records representing 645,778 taxa hosted by 1193 contributing institutions worldwide (https://tools.bgci.org/plant_search.php, accessed on 13 June 2022). All data were recorded and elaborated in respective Excel files (Supplementary Materials Table S3).

2.6. Seed Germination Trials of Target Plants

Seeds were collected directly from wild individuals of 86 PPs in the study area during full maturity and were unsheathed from inflorescences, cleaned, photographed, counted, and weighted for each taxon (species or subspecies). Seeds were stored in the seedbank of the IPBGR-BBGK until used for propagation trials. All seed handling and sampling for germination trials (Supplementary Materials Table S4) was conducted according to ISTA specifications [61]. Seed propagation trials were conducted in open trays using a substrate consisted of peat: vermiculite: fine soil at 2:2:0.5 v/v/v. Germination conditions were under greenhouse mist (85% relative humidity, RH) and ambient temperature. When physical or physiological dormancy was evidenced by literature in members of the same genus, pre-treatments were applied prior to seed germination of these taxa such as immersion in water for 12–24 h at 30 °C or cold stratification at 4 °C for 30 days in the dark, respectively [34,35,62,63]. Depending on the available material collected from the wild and concomitant limitations [20], 50, 100 or 200 seeds per seedlot were used for each propagation trial divided into two repetitions of 25 seeds or four repetitions of 25 or 50 seeds, respectively, and the highest germination percentage is presented following ISTA guidelines [61]. Germination frequency was assessed on a weekly basis. According to the highest seed germination rates observed, each taxon was allocated to three distinct classes of seed propagation success (Low < 25%, Average 25–55%, and High > 55% germination, Supplementary Materials Table S4). Seedling establishment immediately after germination was facilitated through transplanting in 0.2 L trays with a mixture of peat (Terrahum): perlite at 1:1 v/v under mist for another 1–2 weeks. According to the observed rates of seedling survival during the establishment period, each taxon was allocated to three distinct seedling establishment success classes (Low < 25% survival, Average 25–75% survival and High > 75% survival, Supplementary Materials Table S4). Following successful establishment, the new plants were kept within a greenhouse under partial shade with regular watering for two weeks, with successive transplanting from 1 L to 2 L pots containing a mixture of nutrient enriched peat (Klasmann, KTS 2): perlite (3:1 v/v). All data were recorded and elaborated in respective Excel files (Supplementary Materials Table S4).

2.7. Cutting Propagation Trials of Target Plants

When possible, cuttings were obtained from 45 PPs collected in the study during various seasons following each taxon's growth pattern to obtain fresh, softwood cuttings of primary growth with extant apical meristem. The number of cuttings used for each trial varied according to the volume of the obtained material of donor plants originally collected in the wild. For example, the mother plants of the PCB taxon Trifolium medium subsp. *balcanicum* provided only six (6) cuttings which were divided into two repetitions of three cuttings each, whereas the collected mother plants of the PCB taxon Achillea *chrysocoma* provided in total 168 cuttings which were divided into two repetitions of 84 cuttings each. In every case, the median value between the two repetitions is presented in Supplementary Materials Table S5. Similarly, various external hormone application treatments of indole-3-butyric acid (IBA) were applied at concentrations of 1000-4000 ppm according to the level of lignification of the obtained cuttings from the PPs using the quick dip method [41–43]. Cuttings were set for rooting under mist with relative humidity (RH) > 85% within a greenhouse at ambient temperature. The substrate used was peat (Klasmann, KTS 1): perlite at 1:3 v/v. Cuttings were attended weekly to assess their rooting capacity. According to the rooting rates observed, each taxon was allocated to three distinct classes (Low < 30%, Average 30–60% and High > 60% rooting, Supplementary Materials Table S5). The rooted cuttings were kept ex-situ at the IPBGR-BBGK's nursery under partial shade for two weeks to assess plant acclimatization (Supplementary Materials Table S5). Consequently, each tested taxon was allocated to three distinct plant acclimatization success classes (Low < 40%, Average 40–60% and High > 60%, Supplementary Materials Table S5). The acclimatized plants were irrigated regularly and were maintained in 3 L pots using a

substrate composed of fertilized peat (Klasmann, KTS 2): perlite (3:1 v/v). All data were recorded and elaborated in respective Excel files (Supplementary Materials Table S5).

2.8. Plant Division Trials of Target Plants

Plant division was applied in rhizomatous PPs or those forming rosettes, i.e., species with crown buds connected to lateral root sections were available [64]. At least two mother plants were used in plant division trials and the total number of individuals produced is presented in Supplementary Materials Table S5. The effectiveness of plant division was scored after the median (for two replicates) or the mean (for >3 replicates) number of daughter plants produced from a single mother plant originally collected in the wild (Low < 5 plants, Average 5–10 plants and High > 10 plants, Supplementary Materials Table S5). Plant division was conducted within a greenhouse using a transplanting substrate of peat: perlite at 3:1 v/v, and acclimatized in a non-heated greenhouse under partial shade (Supplementary Materials Table S5). The effectiveness of plant division was scored based on the number of daughter plants produced from a single mother plant division second based on the number of daughter plants produced from a single S5).

2.9. Statistical Analysis of Datasets

The calculated coverage of explored level 2 EUNIS habitat types per conservation priority category (PCA, PCB, PCC) as well as the number of identified threats-risk for the PCA, PCB, and PCC categories were compared, respectively, through pairwise Pearson Chi-Square tests in IBM-SPSS 23.0 software (IBM Corp., Armonk, NY, USA).

The seed germination success rates per conservation priority category (PCA, PCB, PCC) were calculated from the number of taxa successfully germinated out of the total collected taxa for each priority category and were compared through pairwise Pearson Chi-Square tests in IBM-SPSS 23.0 software (IBM Corp., Armonk, NY, USA).

The cutting propagation success rates per conservation priority category (PCA, PCB, PCC) were calculated from the number of taxa that were successfully rooted out of the total collected taxa for each priority category and were compared through pairwise Pearson Chi-Square tests in IBM-SPSS 23.0 software (IBM Corp., Armonk, NY, USA).

Similarly, the plant division success rates among the tested taxa were calculated from the number of taxa that were successfully divided out of the total collected taxa for each priority category and these were also compared through pairwise Pearson Chi-Square tests in IBM-SPSS 23.0 software (IBM Corp., Armonk, NY, USA).

2.10. Ornamental Characteristics of Target Plants for Gardening-Landscaping Applications

All of the collected material was evaluated and studied for the first time in terms of its ornamental value and its ability to be maintained ex-situ in specially designed man-made environments (Supplementary Materials Table S6). Most of the targeted PPs are perennial herbaceous plants, with flowering periods extending mainly from May to September due to their natural occurrence in high altitudes; they mostly grow naturally in sunny and/or partially shaded places, on stony or rocky soils, thus having rather low demands. These features are associated with low inputs needed in man-made environments such as irrigation, fertilization and plant protection. In particular, about 30 taxa have aromatic and/or medicinal properties such as the different species of thyme (Thymus spp.), sage (Salvia spp.) and Greek mountain tea (Sideritis spp.), and another 25 taxa have associated commercial value according to European nurseries catalogues such as Achillea clypeolata, Dianthus pinifolius subsp. pinifolius, Erodium absinthoides subsp. guicciardii, Geranium subcaulescens, Geum coccineum, Iris reichenbachii, Jovibarba heuffelii, Lilium martagon, and Salvia jurisicii (data not shown, widely available over the internet). Although some PPs can be available widely through specialized nurseries on the internet, it is worth noting that most of these taxa remain unknown to horticulturists, gardeners and landscape designers; this is especially true for Greece and the Balkans, and such PPs are absent in catalogues of domestic (or even international) nurseries.

Four fundamental criteria were set in order to decide which of the 130 studied PPs should be presented to the public in organized thematic displays: (a) their estimated ornamental and/or aromatic-pharmaceutical value, (b) the existence of sufficient plant material raised ex-situ from propagation trials, (c) their successful reproduction allowing further propagation, and (d) their effective preservation in pots at the lowland nursery during the acclimatization process in the man-made environment. In combination with previous experience and knowledge in landscaping with Greek and Balkan autochthonous plants [19,45,51], special designs were performed incorporating rock formations in various scales and gravel for better plant acclimatization, drainage and enhanced aesthetics alluring to natural elements, as well as woven geotextile fabric as an underlay ground cover for effective weed suppression and/or reduction. The main scope of the planting designs was to deliver sustainable planting solutions with PPs, both attractive and with conservation awareness interest. The latter was accomplished by focusing on: (i) certain botanical ornamental characteristics of PPs such as growth form, flower and leaf color and size, flowering period and plant height providing alternating patterns; (ii) specific biological features such as life-form, habitat preferences, and natural altitudinal occurrence in the wild providing long adaptation success locally; and (ii) specific priority categories of escalating conservation concern (from PCC to PCB and PCA). All data were recorded and elaborated in respective Excel files (Supplementary Materials Table S7).

3. Results

3.1. Overview of Botanical Collections

The botanical expeditions made in the context of the Conse-pp project has led to 130 plant taxa collected from Greece (n = 77), the Republic of North Macedonia (n = 38), or from both countries (n = 15). In total, 83 PPs have been collected from a single locality (one accession number per taxon), two accession numbers have been collected for 23 PPs, 3–4 accession numbers for 16 PPs, and 5 accession numbers for *Achillea holosericea* Sm. and *Saxifraga federici-augusti* subsp. *grisebachii*, thus resulting on a total of 147 designated accession numbers with an average of 1.6 accessions per PP (Supplementary Materials Table S1).

The PCA category included 23 collected taxa (PCA1 = 19 and PCA2 = 4), the PCB included 75 taxa (PCB1 = 5, PCB2 = 7, PCB3 = 4, and PCB4 = 59) and the PCC comprised of 32 collected taxa (PCC1 = 28, PCC2 = 2, and PCC3 = 2). All taxa are shown with their full scientific names and priority category in Supplementary Materials Table S1.

The surface of the sites explored in the natural environment of the cross-border studied area was 35.8 km², excluding any overlapping among different collection sites. In terms of conservation priority categories, the surface explored for PCA PPs was 9.7 km², 25.8 km² for PCB PPs, and 12.7 km² for PCC PPs.

The collected PPs were aggregated in 10 different level 2 EUNIS habitat classes (Figure 3). Predominately, most of the prioritized taxa were collected from the EUNIS level 2 habitat type 'broadleaved deciduous woodlands' (PCA = 32%, PCB = 40%, PCC = 40%) followed by the types 'dry grasslands' (PCA = 20%, PCB = 18%, PCC = 21%). Other level 2 EUNIS habitat types with high representativity of collected PPs were the 'seasonally wet and wet grasslands' (PCA = 4%, PCB = 16%, PCC = 18%), 'anthropogenic woodlands including recently felled woodland, early-stage woodland and coppice' (PCA = 12%, PCB = 16%, PCC = 6%) and the 'Maquis, arborescent matorral and thermo-Mediterranean brushes' (PCA = 12%, PCB = 1%, PCC = 6%) (Figure 3).

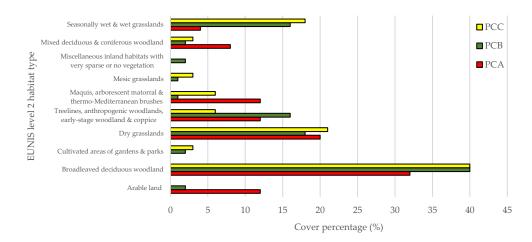


Figure 3. Collected plant taxa (species and subspecies) per priority category (PCA: Threatened and Near Threatened, PCB: Local Balkan endemics and subendemics, PCC: Regionally rare and/or uncommon plants) and level 2 EUNIS habitat type.

3.2. Extinction Risk Assessments

The majority (*n* = 96) of the PPs have never been evaluated (NE) against the IUCN extinction risk criteria (Supplementary Materials Table S3). Among those that have been evaluated (*n* = 34), *Carex echinata, Convallaria majalis* and *Sempervivum marmoreum* subsp. *marmoreum* are considered globally as Least Concern (LC). In total, 17 taxa were previously assessed as "Rare" [58] while *Allium cyrilli, Galium rhodopeum* and *Rumex balcanicus* are currently assessed as Data deficient (DD). *Galanthus nivalis, Lilium candidum, Sideritis scardica* are assessed as Near Threatened (NT). *Centaurea immanuelis-loewii* and *Ranunculus cacuminis* are assessed as Vulnerable (VU) while *Teucrium flavum* subsp. *hellenicum* has also been recently assessed as Vulnerable (VU, [65]). *Petrorhagia graminea* has recently been assessed as Endangered [65], *Crepis arcuata* was previously assessed as Endangered (EN) [14,15] but more recently as Critically Endangered [65]. *Centaurea devasiana, Dianthus murtinervius* subsp. *caespitosus* (previously only Rare) and *Viola voliotisii* have all been recently assessed as Critically Endangered (CR, [65]), while *Thymus oehmianus* was previously considered as Extinct (EX, [58]). Among the 130 PPs studied herein there are three NT and three VU taxa, one EN taxon and four CR taxa (Supplementary Materials Table S3).

3.3. Threats-Risks Analysis

The profiling of taxon-specific threats-risks identified in-situ in the studied crossborder region is illustrated in Supplementary Materials Table S1. Assessments of threatsrisks for the selected PPs were performed only in Greece (n = 72), only in the Republic of North Macedonia (n = 42) or in both countries (n = 16). Out of various types of risks-threats reported for Greek threatened plants [17], 35 types were in-situ detected for the target 130 PPs, varying from 5 to 12 threats-risks per taxon (on average 8.15). The highest number of threats-risks (n = 12) was recorded for *Alkanna noneiformis* followed by a group of another 12 PPs with one less (n = 11); the fewer ones (n = 5) were recorded for *Alkanna pulmonaria*, *Anthemis pindicola*, *Bruckenthalia spiculifolia* and *Centaurea immanuelis-loewii* (Supplementary Materials Table S2). Endogenous risks or threats were identified for all (100%) of the PPs (Figure 4) with habitat specificity, competition with other native taxa and low dispersal potential being the most frequent ones (70%, 52.3% and 13.8%, respectively). Exogenous threats were reported for all PPs as well (100%). In total, 82.3% of the taxa were affected by threats originating from natural processes (Figure 4), namely natural phenomena (51.5%) and change of habitat conditions (86.9%).

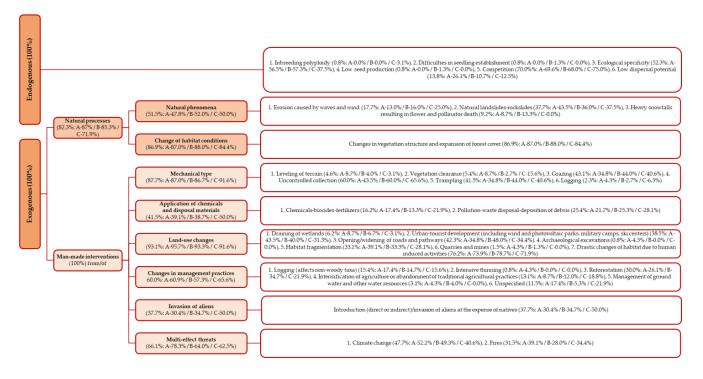


Figure 4. Classification of risks and threats identified in-situ for the 130 priority plants in the crossborder area of Greece and the Republic of North Macedonia (Supplementary Materials Table S2). In parentheses, the percentage of plant taxa affected is shown (first number for all 130 PPs; A, for priority category A taxa; B, for priority category B taxa; C, for priority category C taxa).

On the other side, man-made interventions affected all (100%) of the PPs examined, with six sub-categories discerned (Figure 4): (i) Interventions of mechanical type: they affected 87.7% of PPs and the prominent types included grazing (43.1%), uncontrolled collection (60.0%) and trampling (41.5%); (ii) Application of chemicals and disposal of materials: they affected 41.5% of the PPs and included use of chemicals, biocides or fertilizers and concomitant pollution (16.2%), waste disposal and deposition of debris (25.4%); (iii) Land use changes: they affected 93.1% of the PPs including, among others, urban-tourist development (38.5%), opening and/or widening of roads and pathways (42.3%), habitat fragmentation (33.1%) and drastic changes of habitat due to human-induced activities (76.2%); (iv) Changes in management practices: this sub-category affected 60.0% of the PPs and covered practices such as logging (15.4%), reforestation (30.0%), intensification of agriculture or abandonment of traditional agricultural practices (13.1%) or unspecified activities (11.5%); (v) Invasion of aliens: this was recognized as a threat to 37.7% of the PPs; (vi) Multi-effect threats: They are recognized as a threat-risk for 66.1% of the PPs, namely climate change (47.7%) and fires (31.5%).

PCA taxa seem to face both exogenous and endogenous risks-threats. The most frequently reported endogenous risks-threats were ecological specificity (56.5%), and competition with other species (69.6%). A great part (87.0%) of the PPs in PCA is affected by natural processes, involving natural phenomena and changes in habitat conditions. The weightiest threat seems to be the changes in vegetation structure and the expansion of forest cover which seems to affect 87.0% of the PPs. Man-made interventions affect all of the taxa belonging to PCA. These plants seem to undergo threats from all sub-categories, the most prominent of which are interventions of mechanical type (87.0%), land-use changes (95.7%), changes in management practices (60.9%) and multi-effect threats (78.3%, Figure 4).

Similarly, plants of PCB are mostly controlled by endogenous pressures linked to ecological specificity-inability to adjust (57.3%) and competition with other species (68.0%). All taxa belonging to PCB were found to be threatened by exogenous factors, both natural processes (85.3%) and man-made activities (100%). Most PCB taxa that are affected by the

latter and seem to be prone to mechanical-type interventions (86.7%), land-use changes (93.3%), changes in management practices (57.3%), and multi-effect threats (64.0%, Figure 4).

Plants belonging to PCC face intrinsic risks-threats mostly associated with competition (75.0%) and, secondarily, with ecological specificity and inability to adjust to environmental conditions (37.5%). Exogenous threats affect all PCC taxa; 71.9% of them are affected by natural processes and all of them by man-made interventions with the most frequent sub-categories the mechanical type of interventions (91.6%), land-use changes (91.6%), changes in management practices (65.6%), and multi-effect threats (62.5%). Application of chemicals and disposal materials as well as invasion of alien species, seem to affect 50% of the PCC taxa (Figure 4).

3.4. Worldwide Ex-Situ Conservation

According to the PlantSearch facility of the BGCI network including ex-situ conservation institutions with their botanical collections around the globe, 40 out of the 130 studied PPs of the Conse-pp project (30.77%) were not included in any ex-situ collection (Supplementary Materials Table S3). For these PPs (Alkanna noneiformis, A. pulmonaria, Anthemis pindicola, Astragalus sericophyllus, A. lacteus, Cardamine acris subsp. acris, Centaurea devasiana, C. finazzeri, C. grbavacensis, C. leucomalla, C. prespana, C. deustiformis, Cerastium rectum subsp. petricola, Crepis arcuata, Cynoglossum pustulatum subsp. parvifolium, Euphorbia glabriflora, Galium rhodopeum, Iris reinchenbachii, Micromeria cristata subsp. kosanii, Minuartia glomerata subsp. macedonica, Myosotis alpestris subsp. mrkvickana, Nepeta ernesti-mayeri, Petrorhagia graminea, Pilosella echioides subsp. proceriformis, P. pavichii, Ranunculus cacuminis, Rumex balcanicus, Scabiosa webbiana, Sempervivum galicicum, Thymus oehmianus, T. stojanovii, Trifolium medium subsp. balcanicum, Verbascum glandulossum, Veronica linearis, Viola allchariensis, Viola eximia subsp. eximia, V. herzogii, V. arsenica, V. doefleri and V. voliotisii; for full scientific names and authorship of taxa see Supplementary Materials Table S1), BBGK is the first botanic garden worldwide offering ex-situ conservation for them. In total, 10 of these PPs belong to PCA, 26 PPs to PCB and 4 PPs to PCC (PCC1).

Overall, information about 1511 accession numbers was retrieved for the rest of PPs (90 taxa). For 12 PPs there seems to be a single accession under ex-situ conservation worldwide and the BBGK is only the second botanic garden offering ex-situ conservation for them. In total, 46 PPs were found in 2–10 institutions of 1–5 countries, 12 PPs in 11–20 institutions of 2–7 countries, 6 PPs in 21–30 institutions of 4–9 countries, and 8 PPs in 31–47 institutions of 6–9 countries; *Atropa bella-dona, Geum coccineum* and *Lilium candidum* were detected in 53, 60, and 89 institutions of 7, 15 and 14 countries, respectively, whereas *Galanthus nivalis* and *Convallaria majalis* were found in 162 and 201 institutions of 16 and 19 countries, respectively (Supplementary Materials Table S3). Overall, the distribution of ex-situ accessions found in botanical gardens and/or seed banks of a single country accounts for 33 cases of PPs, 17 PPs were found in two countries and 8 PPs in three countries, while 32 PPs are found in ex-situ facilities of more than four countries.

To date, all of the 130 PPs have been successfully established under ex-situ conservation at the grounds of IPBGR-BBGK, for further propagated and use in environmental awareness spots.

3.5. Ex-Situ Propagation Trials

The ex-situ propagation of PPs was successful in 92 out of 130 taxa (species and subspecies) collected for ex-situ conservation (Table 1). Consequently, this allowed their direct utilization in awareness-raising sites after fast-track acclimatization, i.e., 16 PPs in PCA (threatened and near threatened plants), 48 in PCB (local Balkan endemic plants) and 28 taxa in PCC (rare and/or uncommon plants) (Table 1). In total, 38 PPs presented limited ex-situ propagation success which consequently resulted in restricted use in awareness-raising sites, i.e., 8 PPs in PCA, 26 in PCB, and 4 in PCC (Table 1).

PPs in Ex-Situ Conservation	Taxa	PCA Taxa	РСВ Таха	PCC Taxa
Successfully propagated and planted in awareness sites	92	16	48	28
Ex-situ maintained with restricted use in awareness sites	38	8	26	4
Total	130	24	74	32

Table 1. Overview of plant taxa of different conservation priority categories (PCA, Threatened and Near Threatened; PCB, Local Balkan endemics and subendemics; PCC, Regionally rare and/or uncommon plants) used in different awareness-raising sites after ex-situ propagation and acclimatization.

Table 2 presents an overview of the propagation success for the above-mentioned PPs per propagation method used (or combinations thereof) and showcases a slightly higher success pattern of asexual propagation via cuttings for all conservation priority categories (PCA, PCB, PCC) but also a higher success pattern for PCB (local Balkan endemic and subendemic plants).

Table 2. Overview of different propagation methods (or combinations thereof) used for the priority plants (PPs) of different categories (PCA, Threatened and Near Threatened; PCB, Local Balkan endemics and subendemics; PCC, Regionally rare and/or uncommon plants) planted in awareness-raising sites.

Propagation Methods	Type of Propagation Method	Total PPs	PCA Taxa	PCB Taxa	PCC Taxa
	Seed germination trials	34	6	19	9
One	Cutting trials	45	7	21	17
	Plant division	31	6	19	6
T	Seed germination trials and cutting trials	3	-	2	1
Two	Seed germination trials and plant division	6	1	3	2
	Cutting trials and plant division	11	2	7	2
Three	Seed germination trials, cutting trials, plant division	2	-	1	1

As a result of the pilot propagation actions implemented, a total of 3247 plant individuals were raised ex-situ (Table 3) and 1939 of them were established in the awareness-raising sites, while the rest of them are maintained in the nursery of the IPBGR-BBGK (Table 3). Out of the total number of ex-situ produced individuals, 427 belong to PCA, 1940 to PCB and 880 to PCC (Table 3).

Table 3. Numbers of plant individuals raised per propagation method during ex situ conservation from priority plants (PPs) of different categories (PCA, Threatened and Near Threatened; PCB, Local Balkan endemics and subendemics; PCC, Regionally rare and/or uncommon plants) planted in awareness-raising sites.

Propagation Method	Total Individuals	PCA Individuals	PCB Individuals	PCC Individuals
Seed germination trials	1044	40	822	182
Cutting trials	1617	231	842	544
Plant division	586	156	276	154
All methods	3247	219	1940	880

Concerning the establishment rates of seedlings after seed germination, 29 out of the 34 PPs that were successfully propagated via seeds presented very high establishment

rates (>75% survival/trial), with 12 taxa also presenting high germination rates (>55% germination/trial), 10 of which being local Balkan endemic plants of PCB (Table 4). In contrast, the establishment rates of plantlets after rooting of cuttings were equally high for 38 out of 45 PPs that were asexually propagated presenting high acclimatization rates (>60%/trial), 35 of which also presented high rooting rates (>60% rooting/trial); most of them (29 taxa) belong to PCB and PCC, representing local Balkan endemic plants and rare-uncommon plants, respectively. The proportion of PPs that presented high rooting and acclimatization rates out of the total number of PPs that were successfully propagated via cuttings was higher than the corresponding proportion observed in seed propagation trials (Tables 4, S4 and S5).

Table 4. Overview of propagation success and survival success per method employed during the ex-situ conservation of priority plants (PPs) of different categories (PCA: Threatened and Near Threatened, PCB: Local Balkan endemics and subendemics, PCC: Regionally rare and/or uncommon plants). High Survival Success (HSS): Seedling establishment rates > 75%, or rooted cuttings acclimatization > 60%, or divided plants survival > 90%; High Propagation Success (HPS): Seed germination > 55%, or rooting of cuttings > 60%, or division effectiveness > 10 plants from a single mother plant (see Tables S2 and S3).

Propagation Method	Total Taxa Propagated	HSS	HSS and HPS	HSS and HPS—PCA Taxa	HSS and HPS—PCB Taxa	HSS and HPS—PCC Taxa
Seed germination trials	34	29	12	1	10	1
Cutting trials	45	38	35	6	16	13
Plant division	31	10	10	3	6	1

The Figures 5 and 6 below illustrate representative examples of different wild-growing PPs in different conservation priority categories during their ex-situ asexual propagation at the grounds of IPBGR-BBGK.

In asexual propagation via plant division, 10 out of 31 PPs showed high propagation effectiveness (>10 plants produced from a single mother plant) with 6 of these taxa being in PCB (Tables 4 and S5).

Figure 7 illustrates representative examples of different wild-growing PPs in different conservation priority categories during their ex-situ sexual propagation at the grounds of IPBGR-BBGK.

In general, the overall propagation effort provided useful information on the propagation and seedling/plantlet behavior, and thus, allowed the development of working propagation protocols. Supplementary Materials Tables S4 and S5 contain details of the propagation protocols including the least and most successful cases of taxa for each propagation method and priority category (PCA, PCB, PCC).

Concerning seedbank storage (in addition to those used in propagation trials, Figure 7), 85 seedlots of PPs were collected in total, 64 of which were associated with successfully propagated PPs during ex-situ conservation and used in awareness-raising sites and 21 with PPs of limited propagation success and restricted use in awareness-raising sites (Table 5). A total of 592,412 seeds were counted and stored in BBGK's seedbank, apart from those used in seed propagation trials. Out of these seedlots, 16 accessions (66,365 seeds) belong to PCA, 48 (269,948 seeds) belong to PCB and 21 (256,099 seeds) belong to PCC (Table 5).



(a)



(**b**)



(c)

Figure 5. Representative photos of asexual propagation via cuttings (cutting preparation from mother plants, rooted cuttings, transplanted young individuals) of wild-growing plants in different conservation priority categories. (a) Priority A1 taxon *Thymus oehmianus* (Lamiaceae) with accession number 19,365; (b) Priority B4 taxon *Betonica scardica* with accession number 19,451; (c) Priority C3 taxon *Cerastium rectum* subsp. *petricola* with accession number 19,493.



(a)



(b)



(c)

Figure 6. Representative photos of asexual propagation via plant division (mother plant, divided daughter plants) of wild-growing plants in different conservation priority categories. (**a**) Priority A1 taxon *Galium rhodopeum* (Rubiaceae) with accession number 19,373; (**b**) Priority B4 taxon *Ranunculus sartorianus* (Ranuculaceae) with accession number 1955; (**c**) Priority C2 taxon *Sempervivum ruthenicum* (Crassulaceae) with accession number 19,489.



⁽c)

Figure 7. Representative photos of sexual propagation via seeds (germinated seedlings, young seedling root development, transplanted seedlings, mature individuals) of wild-growing plants in different conservation priority categories. (a) Priority A1 taxon *Centaurea immanuelis-loewii* (Rosaceae) with accession number 19,583; (b) Priority B4 taxon *Anthemis macedonica* subsp. *macedonica* (Asteraceae) with accession number 19,964; (c) Priority C1 taxon *Potentilla astracanica* subsp. *astracanica* (Rosaceae) with accession number 19,441.

PPs in Ex-Situ Conservation	Number of Taxa	Total Seeds Counted	Number of Seedlots (Counted Seeds) of PCA Taxa	Seedlots (Counted Seeds) of PCB Taxa	Seedlots (Counted Seeds) of PCC Taxa
Successfully propagated and planted in awareness sites	64	480,246	12 (9090)	33 (215,122)	19 (256,034)
Ex-situ maintained with restricted use in awareness sites	21	112,166	4 (57,275)	15 (54,826)	2 (65)
Total	85	592,412	16 (66,365)	48 (269,948)	21 (256,099)

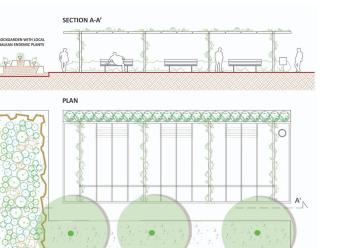
Table 5. Overview of seedlots and ex-situ stored in the Balkan Botanic Garden of Kroussia from priority plants (PPs) of different categories (PCA, Threatened and Near Threatened; PCB, Local Balkan endemics and subendemics; PCC, Regionally rare and/or uncommon plants).

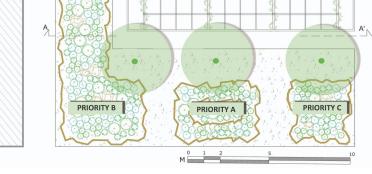
3.6. Establishment in Awareness-Raising Sites

The focal PPs reproduced ex-situ constitute to date the living collections of three awareness-raising sites Tables 6 and S7): (i) The new thematic section in the Balkan Botanical Garden of Kroussia (BBGK) with establishment of a total of 850 individuals belonging to 104 PPs from 26 plant families (Table 6: 17 of PCA, 60 of PCB, 27 of PCC, Figures 8 and 9); (ii) The Botanical Awareness Public Park (BAPP) in Kardia, Munipalicity of Thermi, metropolitan Thessaloniki, North Greece with establishment of a total of 1000 individuals belonging to 70 PPs from 23 plant families (Table 6: 12 of PCA, 42 of PCB, 16 of PCC, Figures 10 and 11); and (iii) The new small-scale rock garden in the Garden of Environmental Awareness of IPBGR-HAO Demeter with 130 PPs (totaling 130 individuals, Table 6). In addition to other donations during events (e.g., funding authorities or citizens), 39 well-acclimatized plant individuals of 13 PPs (Supplementary Materials Table S7) have already been delivered to the botanic garden of Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia for ex-situ conservation and local awareness-raising about these plants, together with another 3 individuals of the newly propagated and acclimatized *Linaria peloponesiaca* (Supplementary Materials Table S8).

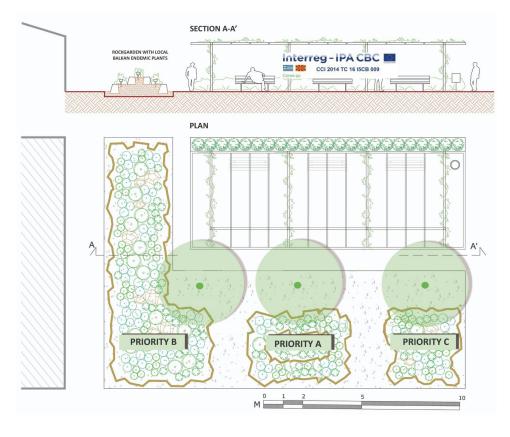
Table 6. Floristic composition in priority plants (planted individuals of taxa, representation of different families) of different awareness-raising sites (BBGK, Balkan Botanic Garden of Kroussia; BAPP, Botanical Awareness Public Park in Kardia; GEA, Garden of Environmental Awareness in Thermi, all in Northern Greece). PCA, Threatened and Near Threatened plants; PCB, Local Balkan endemics and subendemic plants; PCC, Regionally rare and/or uncommon plants.

Awareness Site	Total Taxa (Total Individuals)	Families	PCA Taxa	PCB Taxa	PCC Taxa
BBGK	104 (850)	26	17	60	27
BAPP	70 (1000)	23	12	42	16
GEA	130 (130)	37	26	74	30





(a)



(b)

Figure 8. (a) Plan of the new thematic section targeting priority species of the cross-border area of Greece and the Republic of Northern Macedonia presented in three rock gardens in front of a sitting area under a metal pergola in the Balkan Botanic Garden of Kroussia, Northern Greece. (b) Planting plan of the Priority A rock garden (approximate size $3 \text{ m} \times 3 \text{ m}$) displaying 16 threatened and near threatened plants with numbers in parentheses indicating the plant individuals established in each section of the plant display (e.g., five individuals of *Thymus ochmianus*).



Figure 9. (a) Representative overview of the new thematic display 'Rock gardens with A, B, C conservation priority plants' as implemented at the grounds of the Balkan Botanic Garden of Kroussia, Northern Greece within two years, using material of wild-growing plants that have been consequently propagated and acclimatized in man-made environments; Priority C2 plants rocky bed with *Sempervivum ruthenicum* (b) and *Thymus thracicus* (c).



Figure 10. Masterplan of the Botanical Awareness Public Park (BAPP) in Kardia, Munipalicity of Thermi, metropolitan Thessaloniki, Northern Greece. (**a**) Rock formation of the spiral garden for the exhibit of the aromatic-pharmaceutical section (August 2020); (**b**) planting on the ground of *Rumex balcanicus* (Priority A1); (**c**) Planting the spiral garden (September 2020); (**d**) Planting on geotextile *Alchemilla serbica* (Priority B4); (**e**) planting of three different local Balkan priority plants in circular raised concrete beds in September 2020.

In terms of implementation, the first attempt to cultivate ex-situ and acclimatize the selected PPs was attempted at the grounds of GEA of IPBGR by the end of 2019. The construction works of the thematic plant displays were completed by the beginning of September 2020 and all plantings were finished by the end of the same month in all awareness-raising sites. After two-years of establishment of the PPs in the three thematic plant displays, the acclimatization process of the plants and their growth in the respective soils of the ex-situ sites have been very encouraging despite the pilot nature of this endeavor. The development status of the PPs at the grounds of BBGK (650 m above sea level) differs considerably from those in the lowland BAPP due to different management, maintenance, soil and climatic conditions. Despite local differences, all plant materials used in environmental awareness sites (Figures 8–11) have reached maturity and bloomed. To date, many PPs previously unknown to horticulturists, gardeners and landscape designers for their aesthetic value such as Achillea holosericea, Alchemilla serbica, Alyssum doerfleri, Centaurea immanuelis-loewii, Centaurea prespana, Cerastium banaticum subsp. speciosum, Dianthus petraeus subsp. orbelicus, Dianthus viscidus, Salvia ringens and others have presented rich flowering of ornamental-horticultural interest in both awareness sites.

The above-mentioned applications which are shown in Figures 8–11 may outline that significant gaps have been bridged in a quite short period of time regarding the possibility of landscaping with conservation priority plants which are native to the cross-border area of Greece and the Republic of North Macedonia. Moreover, a documented catalogue of newly proposed plant species for ornamental use in contemporary garden design has been delivered herein (Supplementary Materials Table S7), thus opening the ground for new advancements in terms of sustainable exploitation of the domestic phytogenetic resources.



Figure 11. Representative views of different plants displayed at the grounds of the Botanical Awareness Public Park (BAPP) which was created in Kardia, Munipalicity of Thermi, metropolitan Thessaloniki, Northern Greece. (a) Priority plants with medicinal and/or aromatic value; (b) *Erysimum microstylum* (Prority B4); (c) *Geum coccineum* (Priority B4); (d) *Salvia jurisicii* (Priority A1); (e) *Viola arsenica* (Priority B2); (f) Priority plants in circular raised concrete beds; (g) *Alyssum dorfleri* (Priority A1) and *Lilium candidum* (Priority A2); (h) *Dianthus stenopetalus* (Priority C1).

The approach followed in this study falls within the concise sustainable exploitation framework developed by the IPBGR-BBGK which involves and promotes at the international level the sophisticated concept of using selected native plants of different regions

with ornamental potential in different gardening and landscaping applications in connection with the knowledge generated during their initial domestication process [19,45,51,52]. This initial domestication process is based on authorized collections of limited original plant material from wild-growing populations of prioritized taxa for ex-situ conservation and propagation purposes which is further propagated in multiple ways and long-acclimatized in different man-made environments before being used in gardening applications. This novel approach suggests that the native phytogenetic resources can be considered to date as key-elements in contemporary garden and landscape design of specific areas. This becomes possible when authorized applied research and sustainably performed approaches are implemented beforehand, thus permitting new knowledge to be tested for the development of themed-displays with consolidated identity, impressive biological features, alternating scenery, high natural resilience, and minimized maintenance cost [19,21,45].

4. Discussion

4.1. Conservation Concern and Plant Prioritization Process

Several studies exist on the plant diversity of different areas across the borderline of Greece with the Republic of North Macedonia (e.g., [60]). However, almost none of them has focused on which plant species should be prioritized in terms of cross-border conservation concern at an international scale. While some conservation priority plants have been proposed for protection and/or monitoring on the Greek side of the borderline, this is mainly due to their national-scale extinction risk assessments as documented in Greek red data books [15,16] or after their global extinction risk designations (e.g., Sideritis scardica, [66]. Otherwise, their inclusion of taxa among Other Important Species of the Natura 2000 Network of protected areas in Greece [59] may qualify them as eligible priority plants for their conservation value. Although some prioritization schemes exist in Greece, red data books for rare and threatened plants are still absent in the Republic of North Macedonia, and only some trigger species have been proposed for the nationally defined Important Plant Areas and Key Biodiversity Areas [8]. The focal PPs of this study are to be found in 10 different basic habitat types but predominately in broadleaved deciduous woodlands, dry grasslands, seasonally wet and wet grasslands. No apparent connection was found between different habitat types and plant categories of conservation concern (data not shown).

The study herein has proposed three coherent hierarchical plant prioritization categories -namely Priority Category A, B and C (PCA, PCB, PCC). In the same line with other studies (e.g., [1]), the first one (PCA) is centered on the plants' diagnosed extinction risks with IUCN criteria, either nationally or globally. At this point, it should be noted that the IUCN extinction risk assessments of taxa are assigned by combining and applying five different criteria (population reduction; restricted range; small population size and decline; very small or restricted population; extinction probability analysis), each with numerical threshold values and decision rules to be met [10]. The second priority category defined herein (PCB) is centered on the restricted-range criterion [10] and the regional responsibility criterion [1], thus combining narrow distribution range and endemism status as proxies for regional uniqueness of irreplaceability. In other words, the selected taxa in PCB were all range-restricted either to Greece (B1, local endemics) or the Republic of North Macedonia (B2, local endemics) or parts in these countries thereof (B3, local subendemics). Otherwise, their range just extends to adjacent Balkan countries (B4, local Balkan endemics), and no IUCN extinction risk status is known for them. The last priority category (PCC) has combined the criterion of local (regional) rarity and uncommonness of occurrence of taxa in the wild of Greece and/or the Republic of North Macedonia (C1) incorporating also motivation criteria of the Natura 2000 and Emerald Networks (C2, C3). Apparently, this category included PPs with wider ranges (extending beyond the Balkan countries) than those outlined in previous categories (PCA and PCB). In our opinion, these three plant priority categories as defined herein with 130 PPs may serve as prioritization example outlining hierarchically the most valuable plant taxa in terms of conservation concern in

the borderline of Greece and the Republic of North Macedonia. Undoubtedly, many more PPs can be added in the future in these three priority categories and it is estimated that at least another 600 plant taxa (species and subspecies) of the cross-border area of Greece and the Republic of North Macedonia may be eligible as PPs as defined herein. For this reason, the botanical expeditions in the frame of this project are still ongoing with additional areas explored for taxon-specific threats-risks and collection of propagation material and associated data from more PPs (see examples in Supplementary Materials Table S8).

4.2. Facilitating Informed In-Situ Conservation Efforts in the Cross-Border Area

To the best of our knowledge, this study is the first to define comprehensively and report back on the threats-risks of numerous priority plants (n = 130) of the cross-border area of Greece and the Republic of North Macedonia after in-situ exploration of their wild habitats in an area of at least 35.8 km² (see overall collections in Figure 1 and an example of area studied in Figure 2). Generally, risks-threats derived from man-induced activities represent at least 83% of those imperiling the threatened plants across the Mediterranean biodiversity hotspots in different continents [67], a trend that has been verified for the cross-border area of Greece and the Republic of North Macedonia. When someone attempts to compare the most frequently identified threats-risks for different target plant groups such as the rare and threatened plants of Greece [17], the European threatened plants [9] or the threatened flora in the Mediterranean biodiversity hotspots [67], interesting matches but also mismatches can be revealed. Grazing (and associated intensive livestock farming) as well as urbanization are usually recognized as major threats for the threatened plants of Greece [17], of Europe [9] and of the Mediterranean biome [67], a trend also evident in the study herein. In contrast, although uncontrolled harvesting of wild-growing threatened plants is a major issue in Greece [17] or the cross-border area of Greece and North Macedonia as revealed herein (60% of the PPs are affected), this has not been considered as a major threat-risk neither for the threatened European plants [9] nor for those of the Mediterranean biodiversity hotspots [67]. In the same line, ecological specificity (narrow niche) may represent a major endogenous threat-risk for the threatened plants in Greece or the cross-border PPs studied herein. However, this has not been considered as a major threat-risk neither for the threatened European plants [9] nor for those of the Mediterranean biome [67]. The comparison of identified threats-risks related to PPs of different conservation priority categories (PCA, PCB, PCC) has not revealed statistically important differences (data not shown).

Conservation of biodiversity implies that the threats-risks affecting species of conservation concern are well-known and identified in the first place. This is an important issue allowing for comprehensive conservation planning beforehand. In this way, threat analysis such as the one discussed herein may lead to a more complete design of conservation strategies and protection measures as well as more to effective future listing assessments and assigning of more informative threat classifications and extinction risk designations [67,68]. This can be extremely useful for cross-border areas such as the one examined herein.

During the last few decades, the identification of threatening processes that should drive the in-situ conservations actions implemented locally as well as the observed absence of objective and quantitative criteria for such procedures have been strongly criticized [69,70]. This is mainly due to the fact that such procedures may generate biases leading to discrepancies between threats-risks diagnosed and recommended protection measures [17]. The threats-risks identified for the conservation priority plants studied in the cross-border area of Greece and the Republic of North Macedonia may be used to propose both general and specific protection measures to alleviate the threatening factors affecting the wild-populations of cross-border PPs. Several general types of conservation measures/actions may be proposed as beneficial for target PPs including the creation of micro-reserves at local scales, repeated awareness-raising actions on conservation important plants locally as well as repeated propagation trials and maintenance of priority plants in botanic gardens and seed banks. These general measures, in conjunction with related actions are essential such as designation of Important Plant Areas (see [8]) also in

the cross-border region, spatial extension of extant protected areas of the Natura 2000 or Emerald Networks to offer in-situ protection of targeted populations of selected PPs, and the development of species-specific management plans.

The threat-risk analysis performed herein for the cross-border PPs has revealed some intrinsic characteristics related to many PPs that render them vulnerable to external stresses and constrains such as competition with other species, ecological specificity (narrow niche) and low colonization levels. To alleviate such taxon-specific risks, the ecological demands and reproductive behavior of selected PPs should be studied and monitored along with repeated censuses of their population size and their ability to colonize new areas. Another valuable approach would be the creation of targeted seed reserves for assisted seed dispersal of selected PPs possibly in the future with the aim to induce the recovery or reinforcement of documented declined populations (e.g., Greek mountain tea plants such as Sideritis scardica and S. raeseri subsp. raeseri, see relevant discussion in [19]). Monitoring and assessment of the grazing effect on current vegetation is also suggested as a way to control undesirable changes in vegetation affecting several PPs in the studied cross-border area. Wild forest and meadow fires affecting the habitats of some PPs can be dealt by intensifying patrols of the domestic forestry departments and rangers to improve their overall operational capacity; the latter can certainly be improved with engagement of early warning systems such as drones and smart monitoring technologies. Large-scale construction works should be avoided in areas with wild-growing populations of PPs since this may be detrimental for several wild populations leading to clearance of vegetation cover, vegetation changes and erosion. Erosion effects imperiling some PPs in the cross-border area can also be mitigated or even be avoided by measures such as the control of grazing, the prevention of deforestation, and the maintenance of the forest cover.

4.3. Ex-Situ Conservation Efforts for Future In-Situ Actions

Apart from the limited knowledge concerning the biological cycle of conservation important plants, severe knowledge gaps exist to date regarding the species-specific propagation and ex-situ cultivation of most of them. Such gaps can usually be bridged with targeted applied research efforts serving both conservation needs and/or sustainable exploitation strategies (e.g., [20–28,71,72]). To date, propagation for conservation and/or commercial purposes has been implemented in a plethora of taxa (e.g., [29,73–75], but only for a very small part of the cross-border priority plants dealt herein (e.g., [42]). Consequently, the development of species-specific propagation protocols for 92 PPs stemming from the study herein constitutes a considerable input and may serve as a valuable tool for the implementation of targeted future conservation actions such as ex-situ propagation of individuals for reinforcement of threatened wild-growing populations. Of course, this knowledge can also be applied for sustainable exploitation of valuable cross-border PPs on the basis of agroalimentary value (e.g., Crepis spp., Lactuca spp., Rumex spp.), specific ornamental interest (e.g., Achillea spp., Crocus spp., Dianthus spp., Sempervivum spp., etc.) and/or medicinal-cosmetic potential (e.g., Achillea spp., Thymus spp., Salvia spp., Sideritis spp., *Stachys* spp., etc.).

Local endemic taxa usually occur in limited population sizes in the wild and form range-restricted populations. This fact highlights the need for ex-situ conservation actions including propagation of individuals for population re-enforcement, as seed storage in seed banks may not always be sufficient [1,76]. In this context, the propagation protocols via seeds, cuttings, or plant division that have been developed herein create a valuable knowledge that can aid in future the in-situ conservation actions in the Balkans and around the Eastern Mediterranean region. To this end, the use of ex-situ actions and concomitant knowledge has been proposed as a tool to ameliorate the in-situ conservation status of threatened plants [1]. Modern conservation studies propose novel regimes including restoration plantings as an essential part of conservation planning and management (e.g., [77]) and these efforts usually have to engage ex-situ conservation techniques [1]. In some cases of PPs, the asexual propagation via cuttings or plant division as tested herein

may complement extant in vitro propagation knowledge for these PPs produced in the past [72,78,79] and may lead to faster production of individuals raised ex-situ with much lower cost and equipment requirements, albeit at lower volumes. It is known that asexual propagation can overcome the problem of seed dormancy often occurring in many priority plants of conservation concern [34], but also can provide plant material for conservation in cases of taxa with recalcitrant seeds which cannot be stored for long periods of time [76,80].

Asexual propagation via cuttings presented in this study the highest success rates compared to the other two propagation methods employed across all three conservation priority categories. For example, 30.4% of all PCA taxa were successfully propagated via cuttings and this was quite higher compared to 8.7% of PPs propagated via seed germination and 13% via plant division (p < 0.05); a similar pattern was observed in PCB and PCC taxa with 28% and 50% of all PCB and PCC taxa successfully propagated via cuttings, respectively (p < 0.05). Concerning the other two methods, the Balkan endemics (PCB) showed comparatively higher success (21.3%, p < 0.05) in seed propagation than the other two categories, whereas a similar success rate was observed in plant division across all priority categories (12–13%). Consequently, the current data indicate that complementing seed propagation with asexual propagation via cuttings can be a powerful tool in terms of acquiring valuable material for population reinforcement of conservation important plants. Undoubtedly, the selection of appropriate genotypes per employed propagation method is a major issue in attempts to create highly diverse and viable neopopulations destined for population reinforcement actions [20]. In general, it is known that different species, even members of the same genus, can present contrasting propagation behavior. In the current study, Centaurea immanuelis-loewii presented very low seed germination rates (2.5%) after cold stratification and water imbibition pre-treatment, but the raised individuals were able to be reproduced asexually via plant division. On the contrary, Centaurea grbavacensis presented 15% seed germination without any pre-treatment but failed to be propagated asexually via cuttings. Similarly, seed germination of Saxifraga federici-augusti subsp. grisebachii failed in our tests but new individuals were produced asexually via plant division. Other PPs with unsuccessful seed germination trials include Saxifraga rotundifolia subsp. chrysospleniifolia, Gentiana verna subsp. balcanica, Dianthus petraeus subsp. orbelicus and Allium cyrilli; all of them have been propagated asexually. In our study, Alkanna noneiformis and Minuartia glomerata subsp. macedonica presented very low asexual propagation potential but still some individuals were raised and maintained ex-situ. On the contrary, in cases such as Petrorhagia graminea and Potentilla astracanica subsp. astracanica, successful propagation was achieved with all three methods employed (seed trials, cuttings and plant division). The above-mentioned, may highlight that the combined use of propagation methods (sexual and asexual) in a biodiversity conservation program can overcome diverse intrinsic taxon-behaviors and such trends can be present in a plethora of taxa with unknown biological cycles and habits.

On the other hand, even though many local Balkan endemic taxa of PCB have been successfully propagated as shown herein, several other PPs of PCA, PCB, and PCC categories have proved very difficult to propagate despite the efforts made (Table S1). In such cases, long ex-situ acclimatization periods are required and further research is needed on species-specific propagation techniques [76,81] including in vitro propagation as well [82]. For example, it has been shown in Greek native small tree species that when trying to propagate a species directly from the wild during the early stages of domestication, inevitably we may come across difficult-to-propagate populations or genotypes and, consequently, long-term monitoring and conservation is essential to explore additional reproduction methods [83,84]. In other parts of the world such as China, some studies have shown that species-related eco-physiological traits may vary between in-situ and ex-situ growing environments, thus implying the complexity of ex-situ acclimatization [85]. In conclusion, the propagation data furnished herein can be used in future conservation actions transcending the boundaries of the area covered by the current study and the production of stock plant

material referred herein may facilitate the re-enforcement of wild populations of priority plants in an ad-hoc basis, permitting additionally public awareness actions.

4.4. Priority Plants in Awareness-Raising Sites

In this study, the focal PPs reproduced ex-situ were used to create three awarenessraising sites (i.e., a new thematic section in the BBGK, a new urban park (BAPP), and a new small-scale rock garden within a small botanical garden (GEA)). In terms of ecological plantings [44,86], the original idea behind these landscape applications in the frame of the Conse-pp project was to bring garden visitors into close contact with threatened, local endemic and rare floristic elements of the cross-border area of Greece and the Republic of North Macedonia. In this way, awareness was aimed to be raised through the naturalistic rock displays designed to incorporate priority plants as emotive representations of specific natural ecosystems, thus offering to people the possibility to experience the feeling of visiting these areas before actually being there.

The results of this pilot conservation project have shown that landscaping with priority plants can deliver promising and well-established gardens and parks even within short time-frames (two years), which are low in maintenance cost due to limited nutrient requirements and minimal water needs of the selected PPs. For the first time, in only twoyears-time, numerous priority plant species from the wild have been collected, evaluated in terms of their ornamental value, successfully and accordingly propagated, and maintained in 2.5 L pots in the mother plantations of IPBGR-BBGK (https://ipgrb.gr/, accessed on 14 March 2022) for a 10-month period to acclimatize in man-made environments through periodic watering, mild nutrition, and soft plant protection measures, and have finally been planted in specially designed thematic displays for public view and awareness-raising. In fact, the close collaboration of various scientists with different expertise (taxonomy and plant ecology, plant conservation, propagation and exploitation of native plants, agronomy, horticulture and forestry) has actually proved this endeavor a successful story. To the best of our knowledge, this endeavor has never been attempted before [86,87]. Therefore, comparison with other studies is not possible. Such ambitious and risky gardening initiatives with native plants of special conservation concern may contribute to both plant conservation and environmental education, enhancing the public's awareness on the native biodiversity [44,45,52,86,87]. IPBGR-BBGK aims to diffuse and expand the idea to a greater scale through future installations and collaborations.

5. Conclusions

Conservation management requires a comprehensive strategy with clear targets and measurable goals. The three plant priority categories as defined herein with one hundred and thirty PPs exemplified for the cross-border area of Greece and the Republic of North Macedonia may serve as a clear prioritization scheme outlining hierarchically the most valuable plant taxa in terms of conservation concern. This scheme can be further enriched and expanded to cover at least another 600 eligible PPs of the studied cross-border area. From this study, it becomes evident that the clear matching of threats-risks diagnosed for cross-border PPs with general and specific protection measures and actions to alleviate their detrimental effect may outline a powerful tool for the development of impactful conservation strategies, and may facilitate the identification of issues needing more attention (and, hence, the allocation of resources).

The development of species-specific propagation protocols for 92 priority plants (PPs) stemming from the study herein constitutes a considerable input. Undoubtedly, this outcome represents only the first successful steps made towards more propagation trials regarding the conservation important plants of this cross-border biodiversity hotspot. Additionally, this output as outlined herein may serve as a valuable tool for sustainable spatial planning and landscape management as well as for the implementation of integrated and targeted conservation actions in the future. The latter may range from prioritization of genotypes of PPs for selective propagation and reproduction of individuals for

targeted reinforcement of threatened or declined wild-growing populations to coherent cross-border plant conservation strategies. The propagation data furnished herein can also be used in future conservation actions transcending the boundaries of the area covered by the current study and the production of stock plant material may facilitate or empower public awareness actions. This emerging potential has actually allowed the design and creation of three awareness-raising displays with selected PPs (two new garden sections and a botanical park) in different areas of Greece within only two years. Of course, such knowledge as furnished herein can further be applied and exploited for the sustainable exploitation of valuable cross-border PPs on the basis of their species-specific ornamental-horticultural, agro-alimentary, or medicinal-cosmetic interests, thus offering new grounds and possibilities in economic sectors.

Supplementary Materials: The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/d14070570/s1. Table S1: Overview of the collection details of the priority plants collected in the frame of the integrated conservation actions of the project Consepp from Greece (GRE) and the Republic of North Macedonia (NMK), Table S2: Overview of the taxon-specific threats-risks identified for priority plants in the frame of the integrated conservation actions of the project Conse-pp. The numbers in parenthesis correspond to the classification of [16], Table S3: Extinction risk assessments with criteria of the International Union for the Conservation of Nature (IUCN) and overview of the worldwide ex-situ conservation of the priority plants collected in the frame of the integrated conservation actions of the project Conse-pp, Table S4: Overview of the ex-situ sexual propagation via seeds conducted within the research project Conse-pp, Table S5: Overview of the ex-situ asexual propagation trials (rooting of cuttings and plant division) and their success achieved in the frame of the project Conse-pp, Table S6: Overview of the 130 priority plant taxa (species and subspecies) collected during the project Conse-pp with collection details, priority categories, biological information and active URL links for additional data, Table S7: Overview of the produced individuals raised ex-situ per awareness-raising site created in the frame of the project Conse-pp. BBGK: new thematic section in the Balkan Botanical Garden of Kroussia (Pontokerasia, Kilkis prefecture, North Greece); BAPP: Botanical Awareness Public Park in Kardia (Munipalicity of Thermi, metropolitan Thessaloniki, North Greece); GEA: new small-scale rock garden in the Garden of Environmental Awareness, Institute of Plant Breeding and Genetic Resources, Hellenic Agricultural Organization Demeter (Thermi, Thessaloniki, North Greece), Table S8: Examples of additional priority plants of the cross-border area of Greece and the Republic of North Macedonia targeted for botanical collections in the frame of the integrated conservation actions of the project Conse-pp.

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