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A Comprehensive Approach to Improving Endemic Plant Species Research, Conservation, and Popularization

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Abstract: Scientific research is the main driver to push forward and disseminate botanical knowledge. Despite many institutions having this fundamental aim as a core activity, many of them do not have a complete set of facilities, expertise, staff, and resources to cover all the steps involved in the study, management, conservation, and popularization of plant diversity. Accordingly, we propose a workflow formalizing the cooperation between a botanical garden and a botanical research center, focused on the study of plant endemic species. Specifically, the cooperation was implemented between the PLANTSEED Lab of the Department of Biology and the Botanic Garden and Museum of the University of Pisa. We present seven representative case studies (*Armeria arenaria* complex, *Bellevalia webbiana*, *Crocus etruscus* and *C. ilvensis*, *Dianthus virgineus* complex, *Pulmonaria hirta* complex, and *Santolina chamaecyparissus* complex) to disentangle the approaches and opportunities arising from cooperative approaches, from laboratory to cultivation. We analyze the emerging properties derived from this synergistic cooperation by promoting open research questions and answering them using a comprehensive approach to improving endemic plant species research, conservation, and popularization in the botanical garden. In this manuscript, we show how a cooperative approach between heterogeneous botanical institutions can constitute an effective and easy-to-implement approach to achieve the goals of each partner involved in the cooperation.

Keywords: horticulture; plant diversity; systematics; seed bank; taxonomy

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1. Introduction

Today, botany is a science that integrates longstanding traditional research lines with the most updated knowledge and tools. This integration allows us to understand plant evolution [1–3] and is the background for good conservation practices [4–6], especially for what concerns endemic plants, which have a high evolutionary significance in any given territory [7–9]. Endemic taxa tend to be particularly prone to extinction risks, and they hence represent fundamental targets of conservation efforts, from the regional to local scale [10,11]. Accordingly, without a detailed scientific knowledge of the systematics, distribution, and ecology of endemic taxa coupled with planned conservation priorities, the safeguarding of these plants may be jeopardized [10,12]. To deal with this great challenge and further enhance the study and conservation of endemic plants, cooperation between institutions with botanical knowledge is needed. In this context, botanical gardens and other plant science research centers play a crucial role.

Since their creation in 1543 in Pisa, Italy [13], academic botanical gardens have evolved to encompass a wide range of activities, ranging from horticulture and science to education, public engagement, social wellness promotion, and sustainability [14–18]. The balance between these activities is usually determined by the historical vocation and governance of the garden [14,19]. Besides botanical gardens, scientific research on plants is today carried out by other academic and non-academic institutions. To date, the range of scientific investigations directly performed or supported by botanical gardens and plant science centers is vast, including several fields such as biotechnology, climate change research, conservation biology, ethnobotany, horticulture, plant ecology, restoration ecology, phytochemistry, and reproductive and seed biology [17,18].

Botanical gardens play a strategic role for species conservation [20]; as highlighted by Paul Smith [21], the rise of threats to plant biodiversity, at both the global and local level, have promoted a great boost to plant conservation in botanical gardens [19,22].

Species with narrow distributions are particularly relevant for plant scientists, not only as a focus of conservation actions [23], but also because they represent valuable case-studies for investigating evolutionary and biogeographical processes. Sharrock et al. [23] reported that 42% of the "most threatened European plants" are frequently single-country endemics (90%) and are included in ex situ conservation projects in European botanical gardens and/or seed banks.

We present here seven case studies on endemic plants focused on integrative taxonomy [24] and evolutionary ecology [25], arising from the cooperation between the PLANt Taxonomy, Systematics, Evolution, Ecology, and Distribution Lab of the Department of Biology (PLANTSEED Lab hereafter) and the Botanic Garden and Museum of the University of Pisa (BGM-PI hereafter). Accordingly, we summarize the workflow—from laboratory to cultivation—aimed at performing scientific investigations, ensuring ex situ conservation and promoting public awareness of endemic plants.

2. Materials and Methods

2.1. The Involved Institutions

The BGM-PI currently covers about 25,000 m^2 and hosts in cultivation about 2000 species belonging to 168 families. All the specimens displayed to the public, accompanied by accession metadata, are freely available for consultation on the online portal U-Plant DISCOVER (https://uplantdiscover.sma.unipi.it/, accessed on 1 June 2023). The research interests of the PLANTSEED Lab deal with the systematics, ecology, and evolution of vascular plants. The research group operates at different geographical scales, including a global perspective and a focus on Mediterranean and Italian endemics.

2.2. General Procedures

Experimental designs and research activities were coordinated by the PLANTSEED Lab. Plant material was sampled in the wild: adult plants and cuttings were managed by the horticultural staff of BGM-PI for cultivation, whereas seeds were moved to the Germplasm Bank of the PLANTSEED Lab to (1) store the germplasm for ex situ conservation, (2) carry out studies on germination requirements, and (3) obtain seedlings to be used for cultivation at BGM-PI (Figure 1a–c). The seven case studies presented here contributed to the implementation of a standardized workflow in order to optimize and make explicit the cooperation between a plant research institute and a botanical garden. This workflow is intended to cover all the existing steps involved in research and conservation activities on endemic species, from the field sampling to popularization. Specifically, the case studies fully covering the previous range of activities and fitting our purposes are the following: montane *Aquilegia* (Ranunculaceae) species from Northern Apennines and Western Alps, *Armeria arenaria* (Pers.) F.Dietr. complex (Plumbaginaceae), *Bellevalia webbiana* Parl. (Asparagaceae), *Crocus etruscus* Parl. and *C. ilvensis* Peruzzi & Carta (Iridaceae), *Dianthus virgineus* L. complex (Caryophyllaceae), *Pulmonaria hirta* L. complex (Boraginaceae), and *Santolina chamaecyparissus* L. complex (Asteraceae). For all these studies, the main findings are summarized below, and we describe the horticultural and management aspects. The cultivation trends were analyzed via Local Polynomial Regression (LOESS fitting) using the 'ggplot2' package in R (version 3.4.2) [26].

Figure 1. Germination of *Armeria arenaria* subsp. *praecox* in the Germplasm bank of the PLANTSEED Lab (Department of Biology, University of Pisa) (**a**) and subsequent cultivation at the Botanic Garden and Museum (**b**); a detail of a pot in cultivation (*Aquilegia reuteri* Boiss.) in nursery with the registration strip (**c**).

2.3. *Aquilegia* Species from N Apennines and W Alps

The systematics of *Aquilegia* (i.e., columbines) was traditionally determined on a morphological basis with wide margins of subjectivity in the choice of the taxonomic ranks. This aspect has determined a high heterogeneity in European floristic works [27]. Physiological processes that regulate seed dormancy and germination usually differ among species [28–30], and germination patterns in columbines are scarcely explored [31]. A comparative study of seed morphological features [32] and seed germination analysis in multiple populations of 5 montane species (*Aquilegia alpina* L., *A. bertolonii* Schott, *A. lucensis* E.Nardi, *A. ophiolithica* Barberis & E.Nardi, and *A. reuteri* Boiss.) was performed. Some seeds were exposed to different temperatures shortly after harvesting, while others were pre-treated either with warm stratification or cold stratification.

2.4. *Armeria arenaria* Complex

The *Armeria arenaria* complex includes 13 subspecies distributed from Austria to Spain [33]. In Italy, three subspecies were recorded as native [34], while the French endemic *Armeria arenaria* subsp. *praecox* (Jord.) Kerguélen ex Greuter, Burdet & G.Long was indicated as doubtfully occurring [35]. Moreover, the taxonomic value of the two Italian endemic taxa of this complex—*Armeria arenaria* subsp. *apennina* Arrigoni and *Armeria arenaria* subsp. *marginata* (Levier) Arrigoni—was considered as doubtful by Bartolucci et al. [36]. Tiburtini et al. [37] addressed these issues using an integrated taxonomic approach. Morphological, karyological, molecular, cypsela morpho-colorimetrical, and ecological niche data were collected from 12 populations from Italy and France to test the taxonomic hypotheses formulated by Arrigoni [34]. New, more robust, taxonomic hypotheses were formulated, considering *A. arenaria* subsp. *apennina* as a heterotypic synonym of *A. arenaria* subsp. *marginata* and *A. arenaria* subsp. *praecox* as the only other subspecies occurring in Italy.

2.5. *Bellevalia webbiana*

Amongst Italian narrow endemic plants, Webb's hyacinth (*Bellevalia webbiana*) is one of the most evolutionarily relevant [38,39] and threatened [10] species. Chiarugi [38] hypothesized an autopolyploid origin from *B. boissieri* Freyn, but Borzatti von Loewenstern et al. [39] clearly showed an allopolyploid origin for this species. Gestri et al. [40] reconstructed the range of this bulbous plant, which is restricted to a pre-Apennines area in Tuscany and Emilia-Romagna (Central Italy), with two disjunct population groups separated by the Northern Apennines. During the last century, Webb's hyacinth disappeared from several localities due to the development of human settlements, leading to this species currently being listed in the global IUCN Red List of Threatened Species as Endangered (EN) [41], and may possibly be even more threatened in the future due to climate change [42]. Considering these conservation issues, analyses of genetic and reproductive features, as well as CSR strategies [43], were carried out [44–46] in relation to this species.

2.6. *Crocus etruscus* and *C. ilvensis*

In the Italian peninsula, 15 species are recorded in the genus *Crocus* L. (Iridaceae), 7 of which are endemic to Italy [36]. Elba Island (Tuscan Archipelago) populations were tentatively referred to *C. vernus* (L.) Hill. [47], *C. etruscus* [48,49], or *C. corsicus* Vanucchi [50], but Carta et al. [51] questioned these attributions and hypothesized that these plants may be a distinct systematic unit. In a later paper, Peruzzi and Carta [52] finally described *C. ilvensis* based on morphological and karyological analyses. The divergence of this species with respect to *C. etruscus* was later confirmed on phylogenetic and functional grounds [53–56].

2.7. *Dianthus virgineus* Complex

Among the several species groups of European wild carnations (*Dianthus* L.), the *Dianthus virgineus* complex [57] is one of the most taxonomically debated. It includes around 30 taxa distributed on cliffs and meadows in Southern Europe and Northern Africa, spanning coastal areas to alpine summits [36,58], delimited mostly on qualitative morphological grounds. In the Central Mediterranean region, mostly in Peninsular Italy, Sardinia, and Sicily, 21 closely related taxa are currently recognized, many of which are endemic to restricted areas [36,58]. Genotypic and phenotypic variation of natural populations collected along an elevation gradient was characterized and individuals from different populations were cultivated at BGM-PI (Table 1; Figure 2a). Common gardening allowed us to carry out observations and measurements (Figure 2b) that are crucial to understand whether phenotypic variation of natural populations is the result of phenotypic plasticity and/or local selection. Moreover, during flowering season, cultivated plants were used to explore the effect of different sexual strategies (gynodioecy, gynomonoecy, and gynodioecy–gynomonoecy) on reproductive output (e.g., number and mass of seeds) under homogeneous growing conditions. Currently, an integrated systematic workflow is being applied in a broader taxonomic framework to test morphometric, cytogenetic, and genomic differences among 25 taxa of the complex.

Table 1. The complete list of the endemic taxa, their endemicity status, and IPEN (International Plant Exchange Network) codes included in this study.

Name before Study	Name after Study (If Different)	Endemic to (Before Study)	Endemic to (After Study, If Different)	Ex Situ Cultivation (IPEN Number)
Aquilegia reuteri		SW Alps		IT-0-PI-2021-0242
A. ophiolithica		N Apennines		IT-0-PI-2021-0241
A. lucensis		N Apennines		IT-0-PI-2021-0238 IT-0-PI-2021-0239 IT-0-PI-2021-0240
A. bertolonii		Apuan Alps		IT-0-PI-2021-0235 IT-0-PI-2021-0236 IT-0-PI-2021-0237
A. alpina		W Alps		IT-0-PI-2021-0232 IT-0-PI-2021-0233 IT-0-PI-2021-0234

Table 1. *Cont.*

Name before Study	Name after Study (If Different)	Endemic to (Before Study)	Endemic to (After Study, If Different)	Ex Situ Cultivation (IPEN Number)
Armeria arenaria subsp. *apennina*	*A. arenaria* subsp. *marginata*	N Apennines		IT-0-PI-2021-0041
A. arenaria subsp. *arenaria*		France, W Alps	France	FR-0-PI-2021-0043
A. arenaria subsp. *marginata*		N Apennines		IT-0-PI-2021-0040
A. arenaria subsp. *praecox*		SW Alps	W Alps	FR-0-PI-2021-0042
Bellevalia webbiana		N Italian peninsula		IT-0-PI-2020-0728
Crocus etruscus		N Italian peninsula		IT-0-PI-2020-0737
C. etruscus	*C. ilvensis*	N Italian peninsula	Elba Island	IT-0-PI-2020-0734
Dianthus virgineus		C Mediterranean		IT-0-PI-2020-2441
D. virgineus		C Mediterranean		IT-0-PI-2020-2455
D. virgineus		C Mediterranean		IT-0-PI-2020-2451
D. virgineus		C Mediterranean		IT-0-PI-2020-2457
D. virgineus		C Mediterranean		IT-0-PI-2020-2442
D. virgineus		C Mediterranean		IT-0-PI-2020-2450
D. virgineus		C Mediterranean		IT-0-PI-2020-2443
D. virgineus		C Mediterranean		IT-0-PI-2020-2444
D. virgineus		C Mediterranean		IT-0-PI-2020-2452
D. virgineus		C Mediterranean		IT-0-PI-2020-2456
D. virgineus		C Mediterranean		IT-0-PI-2020-2454
D. virgineus		C Mediterranean		IT-0-PI-2020-2453
Pulmonaria apennina	*P. hirta*	Italian peninsula	Italy, SE France	IT-0-PI-2021-0124
P. apennina	*P. hirta*	Italian peninsula	Italy, SE France	IT-0-PI-2021-0415
P. hirta		NW Italy, SE France	Italy, SE France	IT-0-PI-2021-0426
P. hirta		NW Italy, SE France	Italy, SE France	IT-0-PI-2021-0416
P. hirta		NW Italy, SE France	Italy, SE France	IT-0-PI-2021-0125
P. hirta		NW Italy, SE France	Italy, SE France	IT-0-PI-2021-0127
P. vallarsae	*P. hirta*	N Italy	Italy, SE France	IT-0-PI-2021-0126
Santolina benthamiana		E Pyrenees		FR-0-PI-2021-0017
S. benthamiana	*S. intricata*	E Pyrenees		FR-0-PI-2021-0018
S. chamaecyparissus subsp. *tomentosa*	*S. villosa*	Spain		ES-0-PI-2021-0211 ES-0-PI-2021-0403
S. corsica		Sardinia, Corsica		FR-0-PI-2021-0035
S. decumbens	*S. decumbens* subsp. *decumbens*	SE France		FR-0-PI-2021-0034
S. decumbens	*S. decumbens* subsp. *diversifolia*	SE France		FR-0-PI-2021-0402
S. decumbens	*S. decumbens* subsp. *tisoniana*	SE France		FR-0-PI-2022-0024
S. ericoides		S France, Spain		ES-0-PI-2021-0212 FR-0-PI-2021-0230
S. etrusca		N Italian peninsula		IT-0-PI-2020-2517 IT-0-PI-2020-2518
S. insularis	*S. corsica*	Sardinia	Sardinia, Corsica	IT-0-PI-2021-0003 IT-0-PI-2022-0025
S. ligustica		NW Italy		IT-0-PI-2021-0033
S. magonica		Baleares		ES-0-PI-2021-0015 ES-0-PI-2021-0016
S. neapolitana		S Italy		IT-0-PI-2020-2539
S. pinnata		Apuan Alps		IT-0-PI-2020-1776 IT-0-PI-2021-0231
S. vedranensis		Baleares		ES-0-PI-2021-0002
S. virens		N Spain		ES-0-PI-2022-0023

Complete scientific names for taxa which have not yet appeared in the main text: *Santolina benthamiana* Jord. & Fourr., *S. chamaecyparissus* L., *Santolina chamaecyparissus* L. subsp. *tomentosa* (DC.) Arcang., *S. decumbens* Mill., *S. ericoides* Poir., *S. etrusca* (Lacaita) Marchi & D'Amato, *S. ligustica* Arrigoni, *S. magonica* (O.Bolòs, Molin. & P.Monts.) Romo, *S. neapolitana* Jord. & Fourr., *S. pinnata* Viv., *S. vedranensis* (O.Bolòs & Vigo) L.Sáez, M.Serrano, S.Ortiz & R.Carbajal, *S. villosa* Mill., and *S. virens* Mill.

Figure 2. (**a**) Specimens of *Dianthus virgineus* L. complex in cultivation at the Botanic Garden and Museum of the University of Pisa; (**b**) measurements carried out on plants cultivated on the ground in a collection displayed to the public.

2.8. *Pulmonaria hirta* Complex

Pulmonaria apennina Cristof. & Puppi, an Italian endemic widely distributed along the Apennines, overlaps in the northern portion of its range (Tuscan–Aemilian Apennine) with *P. hirta*, a species endemic to the Tyrrhenian area of NW Italy and SE France, where a high morphological convergence and intermediate chromosome numbers have been observed [59,60]. *Pulmonaria vallarsae* A.Kern. is instead restricted to a small area in NE Italy. Furthermore, the ranges of all these taxa partially overlap with that of *P. officinalis* L., a species widely distributed in Europe with $2n = 16$ chromosomes. Astuti et al. performed a study of the complex including these three species, aimed at disentangling the fuzzy assembly of lineages reported for the three taxa [61–63], which were thought to be distinct in their shape, maculation, and hair pattern of basal leaves [59,64,65], as well as in chromosome numbers: $2n = 28$ (22, 26) in *P. hirta* s.str., $2n = 22$ in *P. apennina*, and $2n = 22$ in *P. vallarsae* [59]. For the analyses, plants in cultivation were used, allowing the evaluation of leaf features (Figure 3), chromosome numbers, and molecular data on the same individuals (about 20 per population). Additionally, morphological analysis made on cultivated plants allowed the evaluation of the morphological variation from spring to summer, which were thought to occur in basal leaves [59,65,66], and the effect of common garden on different populations. The three taxa were shown to be conspecific.

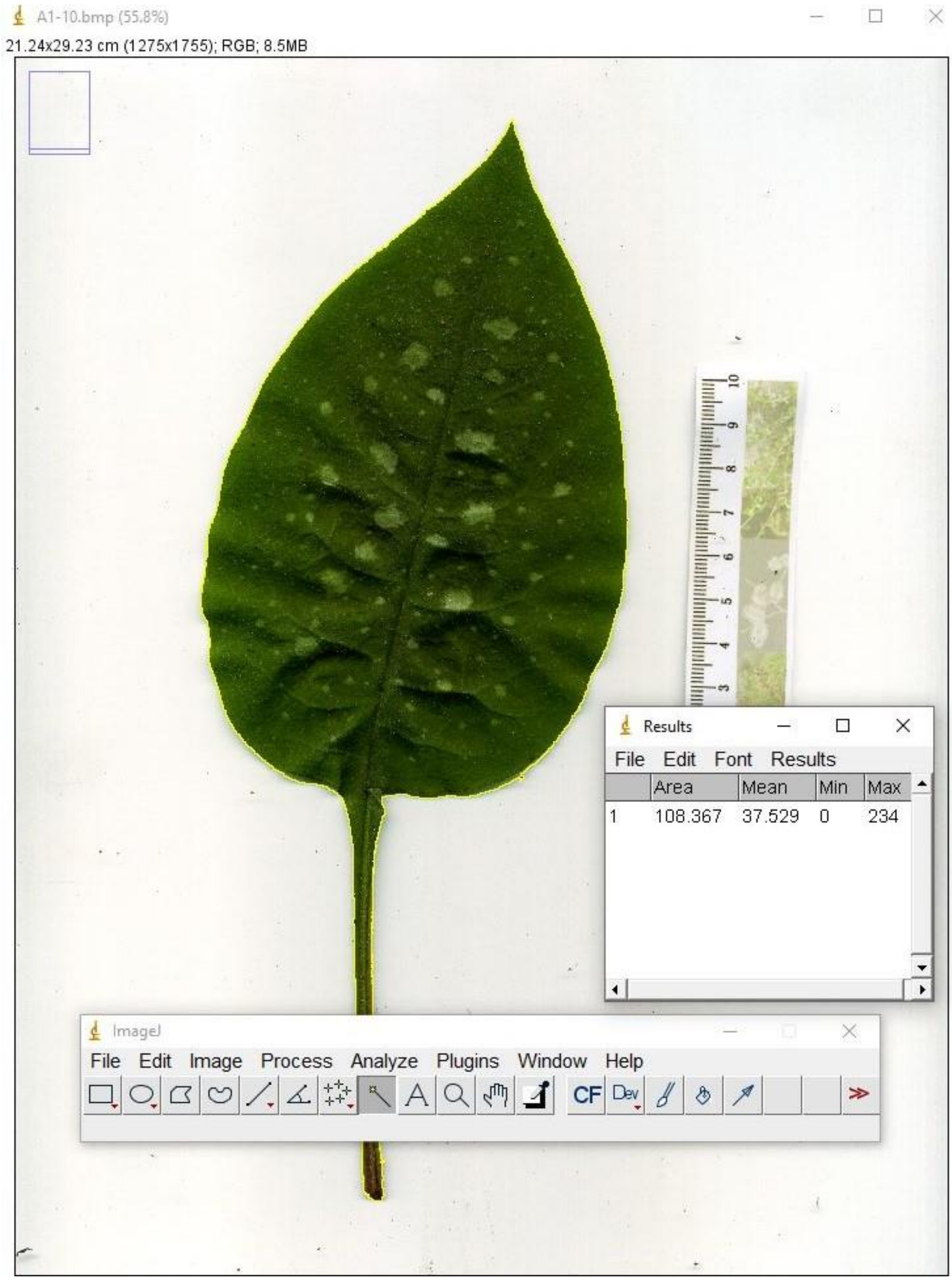

Figure 3. Morphometric analyses on *Pulmonaria* leaves using the software ImageJ version 1.47 [67]. The leaves were collected from potted plants cultivated at the Botanic Garden of the University of Pisa. These plants originated from specimens sampled in the wild during the sampling campaigns conducted by the PLANTSEED Lab.

2.9. Santolina chamaecyparissus Complex

The *Santolina chamaecyparissus* complex (Asteraceae, Anthemideae) includes 14 species of aromatic shrubs endemic to the western Mediterranean region [68]. All the species within this complex are known for their ornamental, ethnobotanical, and pharmaceutical uses [69]. However, the taxonomy of this complex was very unstable in the past, mostly because all species were circumscribed based only on qualitative morphological observations. A research project was recently conducted to clarify the systematics and taxonomy of the *S. chamaecyparissus* complex using an integrative taxonomic approach, involving nomenclature, cytogenetics, morphometry, cypsela morpho-colorimetry, molecular systematics, and niche analysis [70–74]. For the purposes of the project, all species were sampled for a total amount of 27 populations, including all known type localities and populations with an uncertain taxonomic position. While some species have been synonymized (i.e. *S. insularis* (Gennari ex Fiori) Arrigoni with *S. corsica* Jord. & Fourr.), other taxa were recognized as distinct (i.e. *S. decumbens* Mill. subsp. *diversifolia* (Jord. & Fourr.) Giacò & Peruzzi, *S. decumbens* subsp. *tisoniana* Giacò & Peruzzi, *S. intricata* Jord. & Fourr.).

3. Overview of the Main Results Achieved in Horticulture and Management

Overall, 31 taxa endemic to the Alps, Pyrenees, Apennines, and Central Mediterranean were studied by the PLANTSEED Lab. Among them, 13 taxa names were revised after taxonomic investigations, and 12 also had new geographic distribution ranges as a consequence of the taxonomic revisions. The complete list of plant names and their endemic status is presented in Table 1.

Concerning the ex situ management of plants, *Aquilegia* showed more than 90% of loss in cultivated plants, with temporal trends for each plant accession reported in Figure 4a. *Armeria* showed 22% of loss in cultivated plants (Figure 4b), while *Santolina* showed ca. 94% of loss in cultivated plants (Figure 4c).

In *Dianthus*, a relatively higher mortality of montane plants (1874 m a.s.l.) with respect to other lowland populations was observed in cultivation at 4 m a.s.l. (Figure 5). In this genus, the differences in some morphological features (e.g., calyx length and leaf width) are maintained in common garden.

Except for *Aquilegia*, for which plant material was not adequate in terms both of quantity and quality, all the other six study cases were displayed to the public. *Armeria* (Figure 6), *Bellevalia*, *Crocus*, *Dianthus* (Figure 7), and *Pulmonaria* (Figure 8) were put on the ground for permanent cultivation. Concerning *Bellevalia*, some specimens collected from five different wild populations were grown together with the aim of evaluating the putative effects of this admixture at the genetic level.

Plants from six of the populations studied of *Pulmonaria* are currently being grown on the ground in six contiguous plots (Figure 8). The populations were selected to best display both the morphological variation of basal leaves (from unspotted to spotted, from more rounded to more elongated) and the karyological diversity (all cytotypes represented). In *Pulmonaria*, differences between spring and summer were found to be faint and the potential homogenizing effect of common garden conditions was not detected.

A planting design scheme for a dedicated bed is currently being planned to display and conserve ex situ the species belonging to the *Santolina chamaecyparissus* complex (Table 1) at BGM-PI.

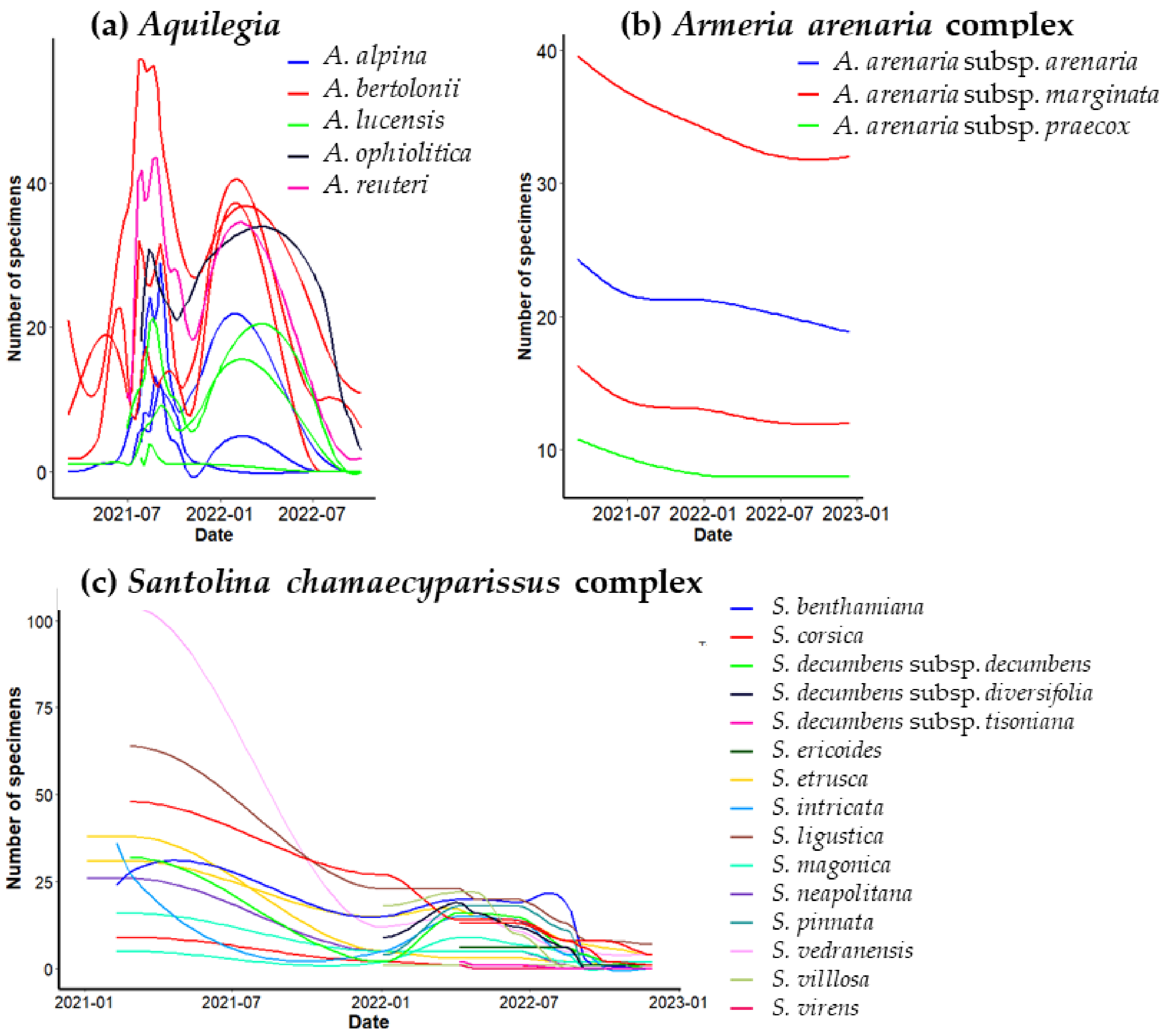

Figure 4. Cultivation trends at the Botanic Garden and Museum expressed by the number of individuals in cultivation (y-axis) over time (x-axis). Each line corresponds to a single accession, while taxa are represented by different colors. An increase in the curve means that new material arrived from the PLANTSEED Lab. Cultivation data for: (**a**) montane *Aquilegia* from N Apennines and W Alps, (**b**) *Armeria arenaria* complex (top line: former circumscription of *A. arenaria* subsp. *apennina*; bottom line: former circumscription of *A. arenaria* subsp. *marginata*), and (**c**) *Santolina chamaecyparissus* complex.

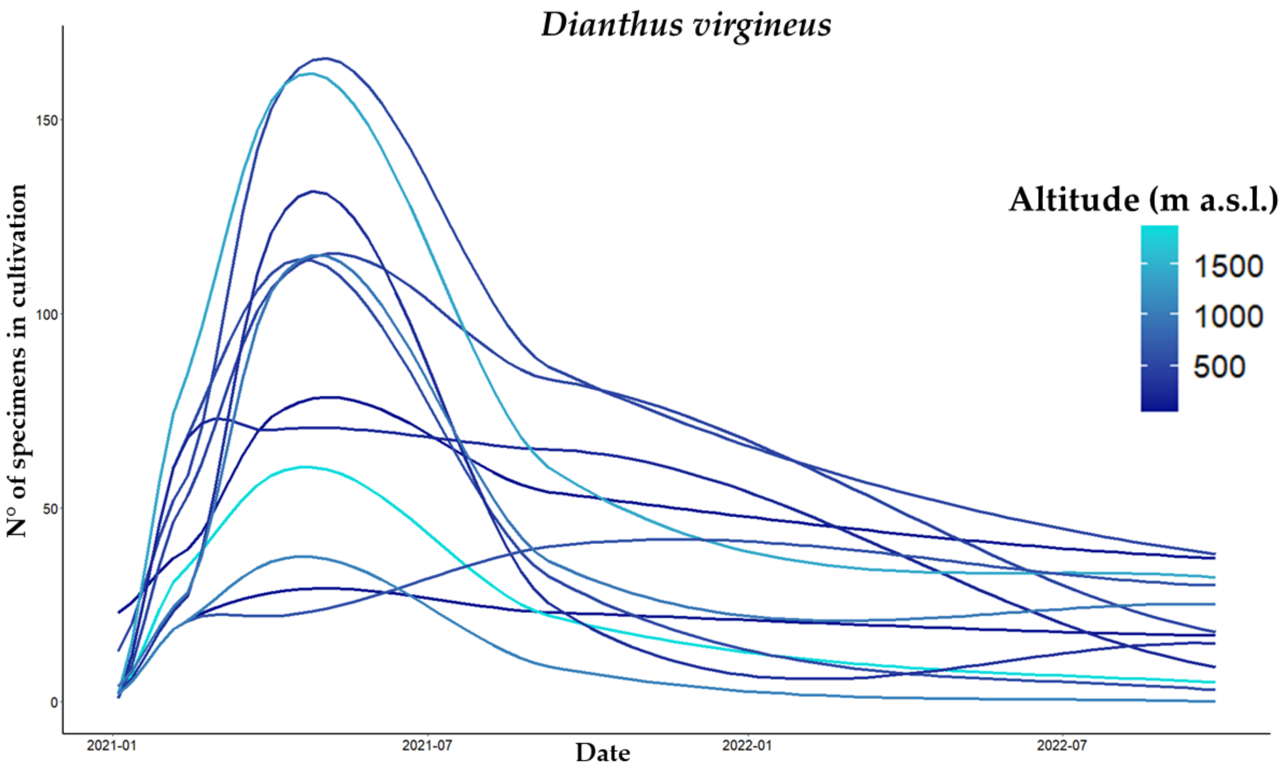

Figure 5. The cultivation trends at the Botanic Garden and Museum of the University of Pisa for different populations of *Dianthus virgineus* collected from Tuscany (Central Italy). Lines correspond to different populations (accessions), while the color expresses the altitudinal gradient (higher elevations in blue, and low elevations in red. An increase in the curve means that new material arrived from the Germplasm bank of the PLANTSEED Lab (Department of Biology, University of Pisa).

Figure 6. Specimens of *Armeria arenaria* complex prepared during the plantation phase at the Botanic Garden and Museum of the University of Pisa (**a**) and the labeled plants later displayed to the public (**b**).

Figure 7. Individuals from Tuscan populations of *Dianthus virgineus* planted on the ground and arranged according to increasing elevation in December 2021 (**a**); the collection later displayed in June 2022 (**b**).

Figure 8. Potted specimens (**a**) of *Pulmonaria hirta* complex prepared during the common garden cultivation in the Botanic Garden and Museum of the University of Pisa and the collection later displayed (**b**).

4. Discussion

A Workflow for Cooperation between Botanical Institutions

Botanical gardens contribute—directly or indirectly—to enhancing scientific research in botany, safeguarding plant diversity against the biodiversity crisis, and engaging and sensitizing people to conservation issues [4,14,75]. Research centers in botany are, by definition, highly specialized establishments where basic and applied investigations on various aspects of plant science are undertaken. Taking advantage of the experience accumulated by managing several case studies deriving from a structured interaction among PLANTSEED Lab and BGM-PI for research, conservation, and popularization purposes, we here present a workflow implemented to formalize all the significant steps operating in this cooperation process (Figure 9).

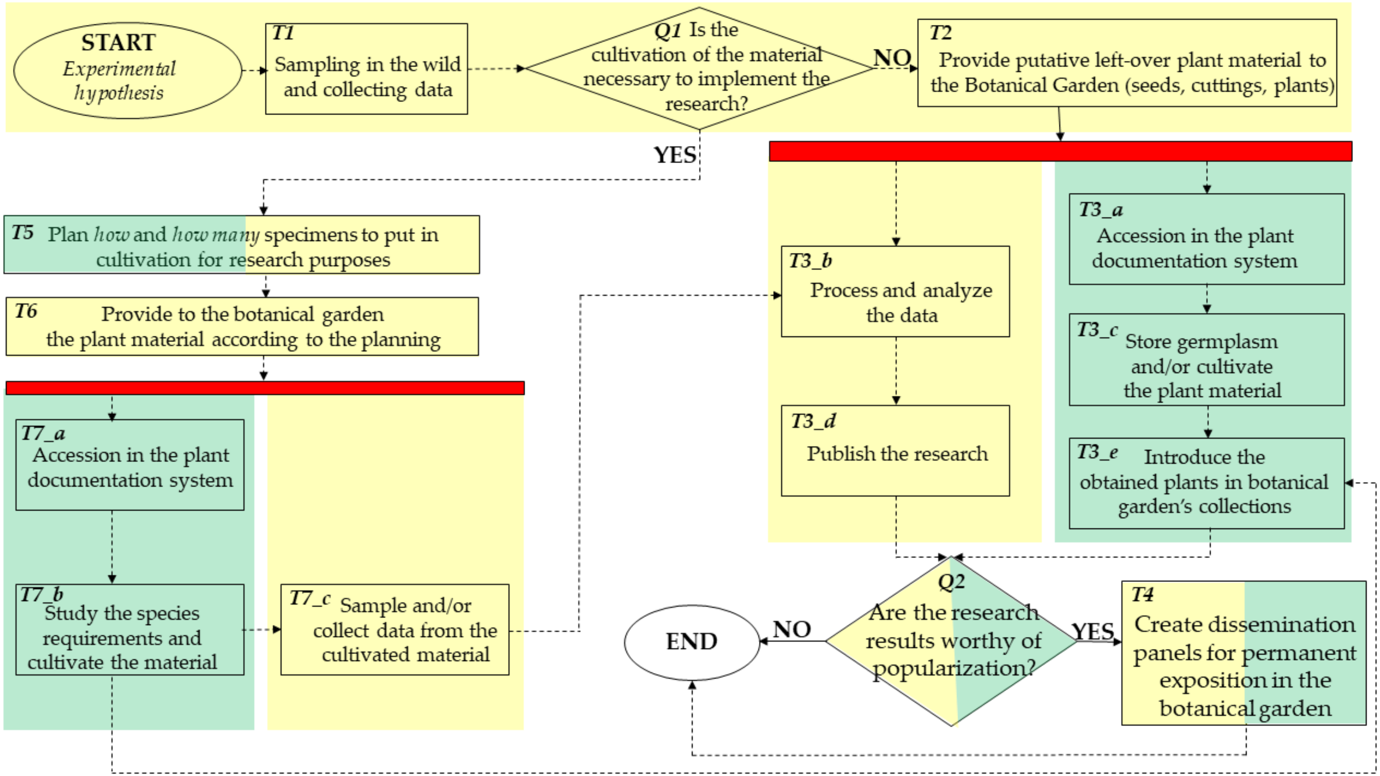

Figure 9. Workflow showing the cooperation between the Botanical Garden of the University of Pisa (BGM-PI) and the PLANTs Taxonomy Systematics Evolution Ecology and Distribution Lab of the Department of Biology (PLANTSEED Lab). Rectangles in the workflow represent tasks, while rhombi represent queries. A node colored in light yellow indicates a task performed by the PLANTSEED Lab, while light green is a task performed by BGM-PI; double-colored nodes express parts in which both institutions contribute. The red bar indicates when the workflow splits into two parallel paths.

The workflow is composed of queries and tasks, performed either by one institution or by both. The start point originates from a given experimental hypothesis, ending with different outputs depending on the specific case. From the workflow, several emerging properties arise for both the institutions involved, as well operational advantages. Indeed, a prolific intellectual environment where new ideas arise from the interaction of different skills, attitudes, and points of view (according to the peculiarities of PLANTSEED and BGM-PI, respectively) is created, boosting the process from the experimental hypothesis up to the expected outcomes (scientific research publishing, conservation, and popularization). For a research-focused institution such as the PLANTSEED Lab, cooperation with a botanical garden can ensure space, facilities, and expertise to cultivate and manage the material under study (Tasks T5, T6, and T7_c; Figure 9), hence providing access to well-documented and organized specimens which are easy to sample and measure (Task T7_c; Figure 9) for the following analyses (Task T3_b; Figure 9). Again, through the user base of the botanical garden, in terms of both visitors and social networking, the PLANTSEED Lab can popularize its research activity to a vast public, thus promoting the importance of plant diversity and contribute to overcoming the so-called 'plant blindness' [76] in society. On the other hand, through this cooperation the BGM-PI is able to acquire rare and high-quality wild plant material, collected through appropriate scientific standards which ensure a good representation of its genetic diversity (Task T1; Figure 9). Accordingly, BGM-PI significantly fosters the implementation and the scientific value of its collections (Task T3_e; Figure 9), fulfilling its scientific institutional mission. As with the majority of small- or medium-sized botanical gardens, the staff of BGM-PI are not exclusively assigned to research tasks, so that support from a scientific institution can overcome these limitations.

All the tasks described here are subtended by two basic queries. The first query of the workflow (query Q1; Figure 9) is meant to evaluate whether the plant material is necessary or not for the implementation of the research (Query Q1; Figure 9). If the answer is NO (see case studies: *Bellevalia webbiana*, *Crocus*, and *Pulmonaria*), after the donation of plant material to the botanical garden (Task T2; Figure 9), the workflow splits into two parallel steps (BGM-PI: Tasks T3_a, T3_c, T3_e; PLANTSEED Lab: Tasks T3_b, T3_d) which eventually converge on the second query, which is meant to evaluate if the research is worthy of popularization (Query Q2; Figure 9). If the answer is YES (see case studies: *Aquilegia*, *Armeria*, *Dianthus*, and *Santolina*), the BGM-PI and PLANTSEED Lab set up a detailed plan for the ex situ cultivation (Task T5; Figure 9).

Since a large number of endemic plants have never been grown or managed ex situ, horticulturists need to acquire comprehensive information on the biological requirements of these plants (Task T7_b; Figure 9). The right amount of plant material to manage is determined by the trade-off between the carrying capacity of the garden and the research purpose to be satisfied (see Task T5; Figure 9). We experienced different percentages of plant material loss over the cultivation phases among taxa, reaching extremely low survival ratios in some cases (e.g., *Aquilegia* < 10%, *Santolina* ca. 6%). These high ratios of mortality were likely due to problems concerning cultivation in pots instead of direct cultivation on the ground (*Santolina*), narrow ecological requirements hard to replicate in a botanical garden (*Aquilegia*, mountain populations of *Dianthus virgineus* complex), and forced staff turnover for significant periods, highlighting again the important of horticultural staff continuity when managing challenging taxa. In addition to the standard horticultural management, botanical gardens need to undertake meticulous record-keeping to ensure accurate records of provenance (Task T3a or T7a; Figure 9), and that the genetic diversity of cultivated plant material is maintained over time.

Research lies at the core of the institutional mission of a botanical garden. It is a mandate of all academic botanical gardens to provide information and knowledge about their collections and make them available to the community [15]. The availability of space to cultivate plants and the cultivation of plants over time provide an effective advantage for comparative approaches; specimens may be regularly and repeatedly monitored and sampled for various purposes [77,78].

Some of the studies on endemic taxa presented in this paper led to taxonomic and nomenclatural changes (Table 1), often implying a redefinition of species distribution ranges. It is well recognized how taxonomy directly influences conservation issues on plant diversity [79,80], highlighting again the primary link interconnecting botanical gardens, research, and conservation. In actuality, the conservation of threatened endemic plants in ex situ collections is a key feature of botanical gardens and seedbanks, which also involves research tasks and education programs to promote plant conservation issues among the public [81]. To contribute to the conservation of the endemic species mentioned in this paper, and more generally to highlight the importance of scientific research to visitors, the BGM-PI recently created a collection named 'Plants in research' (Figure 10a). In addition, in the Germplasm Bank of the PLANTSEED lab, seeds under appropriate conditions for long-term conservation are stored. Despite their undeniable contribution to ex situ conservation of plant diversity, botanical gardens face several issues in cultivating wild plants, such as a limited genetic diversity, potential hybridization between closely related species or breeding between different populations, and adaptation to conditions different from those experienced in the wild [82,83].

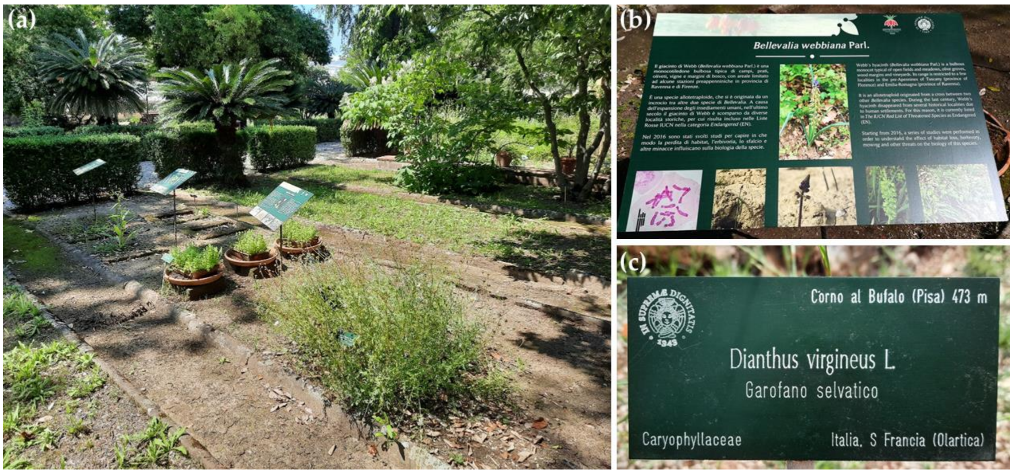

Figure 10. (**a**) A view of the collection 'Plants in research' at the Botanic Garden and Museum of the University of Pisa; (**b**) informative bilingual (Italian and English) panel about the research conducted on *Bellevalia webbiana*; (**c**) a label sample of *Dianthus virgineus* complex modified from the standard template, showing the population data on the top right.

Educational purposes represent another main goal of botanical gardens [84]. Over 300 million visitors per year are estimated to visit botanical gardens worldwide [85], which has significant potential to greatly improve people's knowledge and attitudes. The 'Plants in research' collection in BGM-PI, which receives more than 80,000 visitors/year, is specifically planned to connect people, researchers, and botany. Accordingly, all the panels of this collection are designed to show the research carried out on the species displayed using lay language (Figure 10b). This is the last fundamental outcome of the workflow summarized in Figure 9 (Task T4). When useful, labels with additional information compared to the standard template are used. For example, the planting design of *Dianthus virgineus* follows an increasing elevational gradient, so the label reports the sampling locality and the elevation (Figure 10c).

The cooperation between heterogeneous botanical institutions can constitute an effective and easy-to-implement approach to achieve research, conservation, and popularization of endemic plant species.

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