The cultivation of *Primula palinuri* Petagna for *ex situ* conservation: lessons learned from the Royal Rotterdam Zoological and Botanical Gardens

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Abstract

Primula palinuri Petagna is an endangered species endemic to a tiny coastal area in southern Italy. Investigating the possibility of growing and propagating this endangered species with *ex situ* actions should be encouraged, as this could provide a second line of security for rare plants by allowing specimens to be grown in the absence of natural environmental challenges. Currently, the Royal Rotterdam Zoological and Botanical Gardens (RRZBG) hold 31 accessions of *P. palinuri*, which represents an *ex situ* collection with potential value for conservation programmes. Over the years, horticulturists at RRZBG have created a solid protocol to produce viable seeds through hand-pollination techniques, thus allowing conservation programmes for this species to become more effective. In this article the cultivation of the species is described, including information on seed germination, general maintenance, cultivation to flowering stage and propagation from the resulting seeds.

The National Collection of Primula at the Royal Rotterdam Zoological and Botanical Gardens

Primula is one of the largest and most popular plant genera in temperate gardens, with approximately 430 species mainly distributed in the mountain areas of the Holarctic Floristic Kingdom (Crema *et al.*, 2013; Richards, 2003). *Primula* species are often found in montane areas, but they can also be present in specific habitats such as open meadows (*P. farinosa*), closed-canopy forests (*P. veris*) and cliffs (*P. apennina*, *P. involucrata*, *P. cusickiana* var. *maguirei* and *P. scotica*).

The collection of the genus *Primula* at the Royal Rotterdam Zoological and Botanical Gardens (RRZBG) was established in 2005 as part of the National Plant Collection in co-operation with the Dutch Association of Botanic Gardens. The *Primula* collection at RRZBG consists of 166 accessions with a total of 667 individuals, encompassing 84 *Primula* taxa (including 3 varieties, 8 subspecies and 4 cultivars). According to data in BGCI's PlantSearch database (BGCI, 2023), RRZBG ranks in the

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top 10 global collections of *Primula* for number of taxa of 296 botanic gardens. When cultivars are excluded, RRZBG ranks seventh for living collections and ninth when seedbanks are excluded. The *Primula* collections at RRZBG are well above the national average of 17 species and the global average of 15. Notably, RRZBG has the largest *Primula* collection in the Netherlands.

RRZBG is currently divided into areas each representing a continent, and animals and plants are placed together to create an ecosystem as close as possible to the natural one. Because of this, most of the *Primula* species can be found in the Asian area and in the European Rockgarden. For instance, many specimens of *P. bulleyana* can be seen on the shores of the Pelican Pound and *P. viallii* along the public trails. Other areas of the zoo, such as the Chinese Garden, exhibit specimens of *P. capitata*, *P. denticulata*, *P. forrestii*, *P. prolifera*, *P. pulverulenta*, *P. rosea* and *P. sieboldii*, either in small groups or in natural-looking flowerbeds. The relatively small European Rockgarden contains *P. auricula*, *P. clusiana*, *P. daonensis* and *P. wulfeniana*.

However, not all the *Primula* species are exhibited and accessible to the public. Among those that are kept behind the scenes year-round – all of which have at least three specimens of each accession – is the rare *P. palinuri* Petagna, which represents an unusual and precious contribution to the Dutch National Collection of Primula.

Primula palinuri

Primula palinuri is a rare endemic species and the only Mediterranean and maritime species in section Auricula. This section is endemic to the mountain ranges of central and southern Europe and includes 25 species, with the highest species richness (22 species) in the Alps (Zhang & Kadereit, 2004). *P. palinuri* most likely developed from *P. auricula* during a cool, wet episode – probably in a cold period of the Quaternary – and later evolved to resist the hot, dry summers currently experienced in southern areas, with subsequent geographical isolation (Aronne *et al.*, 2014; Crema *et al.*, 2013). The natural habitat of *P. palinuri* is limited to the vertical cliffs along the Tyrrhenian coast of southern Italy, between Palinuro and Scalea, with a fragmented distribution along a narrow area of less than 100 km (Fig. 1). It predominantly grows in soil pockets inside rock crevices on north-facing sea cliffs (Fig. 2). During the hot, arid Mediterranean summers, northern exposure and verticality ensure a cooler and wetter microclimate compared with the surrounding areas. Access to these sites is difficult, and new approaches like the use of small unmanned aerial vehicles to monitor *P. palinuri* and other species on coastal cliffs have been proposed (Strumia *et al.*, 2020).

Currently, this species is included in the International Union for Conservation of Nature (IUCN) Red List and classified as Endangered, with a decreasing population trend in natural areas (Gangale *et al.*, 2011). According to the European Environment Agency, 'the species is not as critical as being unfavourable-bad, but still requires significant conservation and restoration measures to make it viable in the long-term, or to enlarge its current range, or to improve the quality and availability of its habitat' (EEA, 2023).

Morphological description

Primula palinuri shows similarities to some of the larger forms of *P. auricula*, with stems up to 25 cm tall and leaves that are about 20 cm long by 7 cm wide, obovate, fleshy, irregularly toothed and that sometimes bear

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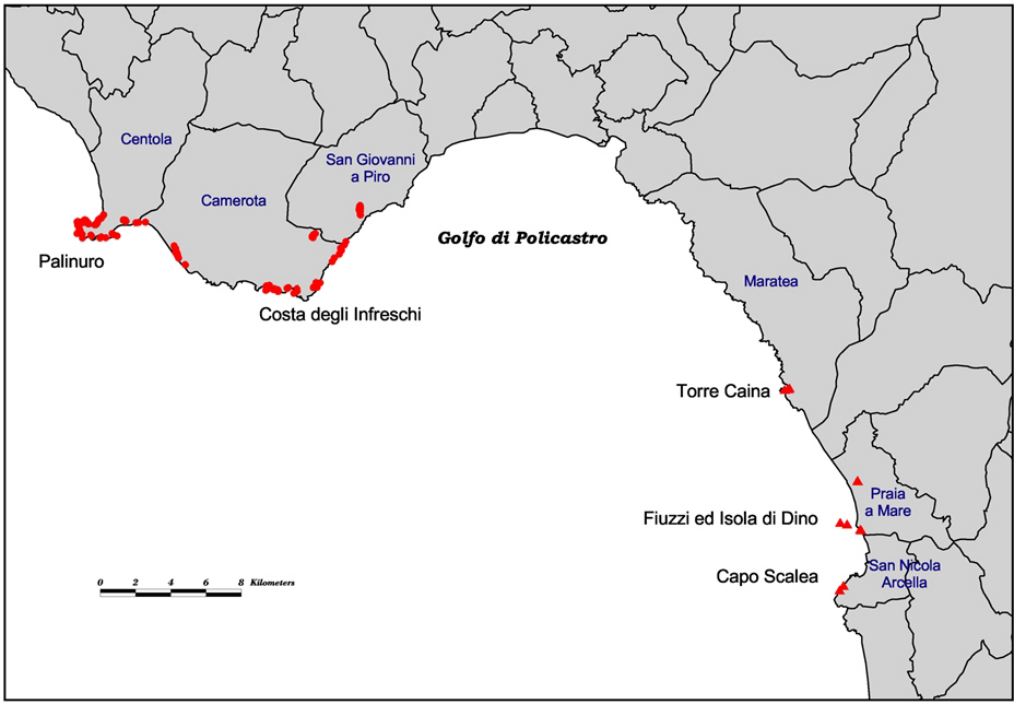

Fig. 1 Distribution (in red) of *Primula palinuri* along the Tyrrhenian coast of southern Italy, between Palinuro and Scalea. The red dots indicate populations in Cilento and Vallo di Diano National Park; red triangles indicate populations outside the Park. Map drawn by G. Aronne.

short glandular hairs (Fig. 3). With its toothed, fleshy leaves, the leaf rosette somewhat resembles a cabbage (Richards, 2003; Fig. 4). The starchy stem develops indefinitely with a seasonal rhythm: vegetative growth starts at the end of summer, continues throughout the winter and stops at the end of spring. New leaves develop together with the stem elongation and dry up in summer. Consequently, plants assume a winter habit, with green and fleshy leaves during the cool and wet seasons (Fig. 5), and a summer habit, where they shed their withered leaves. Such seasonal behaviour does not occur if plants are grown in a humid and cool environment outside their natural habitat. The rhizomes elongate about 1 cm each year and gradually hang down from the steep cliff faces through gravity. In the wild, plants with rhizomes longer than 1 m have been found, suggesting that individual plants can survive for many decades (De Micco & Aronne, 2012).

At the end of the winter period, a floral axis with flowers clustered in an inflorescence develops from most of the rosettes (Fig. 3). Each flower is funnel-shaped and has a white, waxy calyx. The corolla is yellow, with a white ring on the throat and a white-farinose calyx. As with many primroses (Richards, 2003), long-styled and short-styled flowers are present (Fig. 6), of which the orientation changes from pendent to vertical, corresponding to three successive flowering stages – young-pendulous, mature-horizontal and senescent-vertical (Aronne *et al.*, 2014). In the wild, flowering starts at the end of January and lasts until mid-April. Longevity of single flowers is about 20 days. Pollen viability is affected by time, relative humidity and temperature (Aronne *et al.*, 2014).

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Fig. 2 Predominant natural habitats of *Primula palinuri* on the vertical cliffs of the Tyrrhenian coast. Photo: G. Aronne.

Fig. 3 Potted *Primula palinuri* at the Royal Rotterdam Zoological and Botanical Gardens. Photo: R. van Vught.

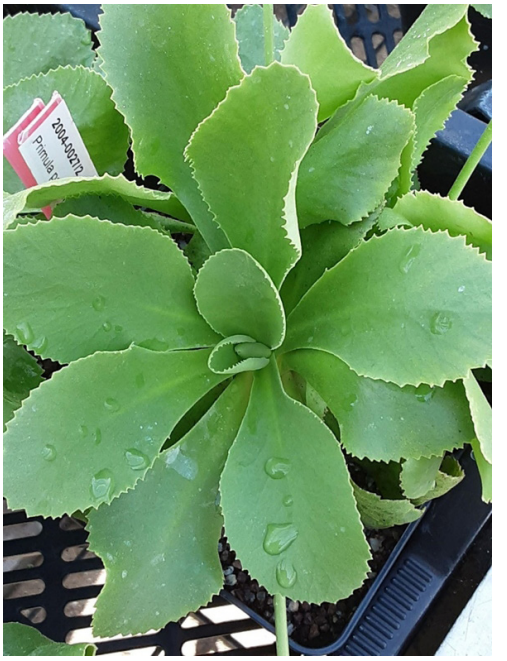

Fig. 4 Detailed view of the leaf rosette with the typical 'cabbage' look. Photo: S. Mugnai.

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Fig. 5 *Primula palinuri* plants assume a winter habit with green and fleshy leaves during the cool and wet season. Photo: G. Aronne.

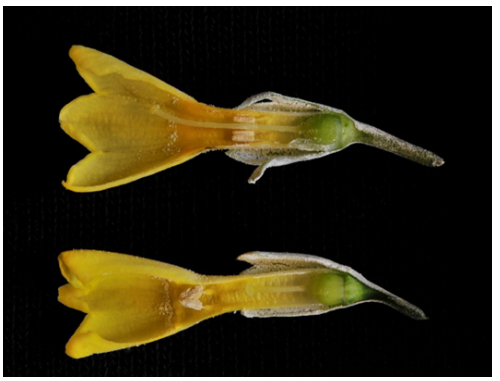

Fig. 6 Longitudinal sections of long-styled flowers (above) and short-styled flowers (below) of *Primula palinuri*. Photo: R. van Vught.

The natural habitat and impact on conservation efforts

Glacial advancement across Europe during the Pleistocene epoch fragmented once-continuous populations, which later differentiated in isolation (Casazza *et al.*, 2012). Upon glacial retreat, many relict species remained; nowadays some can be found in the Mediterranean region on cliffs that once served as glacial refugia. *Primula palinuri* is a well-known example of this geo-evolutionary process. Vertical cliffs are habitats generally restricted to high and very steep rock walls, characterised worldwide by a great geomorphological heterogeneity on small surface areas, such as cracks, fissures, pockets, ledges, corners, overhanging rock and roofs (Figs 2 & 5). As a result, cliffs comprise several plant microhabitats. The interactions among the environmental factors typical of cliffs have probably exerted a strong selective pressure on the traits of

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plants living in this habitat, such as *P. palinuri*. The results of this evolutionary process successfully promote the survival of many cliff-dwelling taxa when compared to their congeners.

The species living in these habitats require specific action plans for conservation management, which demand species- or ecotype-specific knowledge. In this context, it is important to identify the critical phases in their life cycles and the bottlenecks for their conservation, and to improve our understanding of their regeneration traits (Aronne, 2017). These traits comprise seed production, dispersal, germination, seedling emergence and establishment, all also relevant to the understanding of community dynamics. Regarding the endangered *Primula palinuri*, the possibility of growing and propagating it *ex situ* could provide a second line of conservation by allowing specimens to be grown in the absence of natural environmental challenges. Botanic gardens can play a major role in preventing species extinction through integrated conservation action and offering the opportunity to conserve genetic diversity *ex situ* (Oldfield, 2009; Mounce *et al.*, 2017).

There is therefore an increasing need to integrate *in situ* and *ex situ* conservation planning to ensure that, whenever appropriate, *ex situ* conservation of *Primula palinuri* is used to support *in situ* conservation to the best effect possible. The aim of this article is to investigate and report the horticultural requirements for *P. palinuri* in an *ex situ* collection in order to provide further data for eventually expanding the species' conservation programmes for *in situ* projects within protected areas.

Cultivation of *Primula palinuri* at the Royal Rotterdam Zoological and Botanical Gardens

Establishment of the Primula palinuri *collection*

According to the BGCI PlantSearch database (BGCI, 2023), RRZBG is one of the 46 institutions worldwide that host *Primula palinuri* in their collection. The *P. palinuri* collection there currently comprises 31 accessions. The collection was initially established in 2004, with 17 founder accessions obtained after the germination of *P. palinuri* seeds (IPEN number XX-0-M-G/0405) provided by the Botanischer Garten München-Nymphenburg (Germany) on 18 February 2004. A further eight items were added to the list of founder accessions after the germination of seeds received from the Alpengarten Innsbruck (Austria) on 6 March 2019. These seeds came from the *Index Seminum 2018* catalogue of HBLFA (the Higher Education and Research Institute for Agriculture and Nutrition, Food and Biotechnology) in Tyrol (Austria), and have IPEN number XX-0-BGAT-0000048. The last founder accessions derive from six juvenile plants received from Hortus Botanicus Delft (the Netherlands) on 6 October 2022. It should be made clear that RRZBG does not intend to use the *ex situ* population, which is derived from other *ex situ* collections, for reintroduction or genetic rescue programmes. This would require more detailed knowledge on the genetic provenance of the collection. Moreover, multiple generations in cultivation may introduce a risk of habituation to the *ex situ* environment as a result of (usually unintended) artificial selection (Enßlin *et al.*, 2011; Nagel *et al.*, 2019).

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General maintenance

In order to create the existing collection, seeds of *Primula palinuri* were sown in a cool greenhouse in April 2023. They germinated after approximately 30 days at temperatures of around 10–15 °C. Seedlings and young plants were then placed in a sun-protected, cool greenhouse until May. As soon as seedlings started forming their true leaves, they were pricked out in small groups and planted in pots (9 × 9 × 9 cm) filled with a universal potting soil and with added slow-release organic fertilisers for 100 days. The top layer of the pots was slightly covered with horticultural grit (grain size < 4 mm) to prevent young plants becoming covered by soil particles when watered. Once a year, all *P. palinuri* individuals are repotted by adding new soil before the flowering season starts (late winter/early spring).

Plants are regularly watered during summer months and kept slightly moist and cool during winter months. The accessions of *Primula palinuri* are placed in a cold greenhouse, with temperatures between 4 °C and 15 °C, from October to April to prevent damage from frost and excessive moisture. During spring and summer, *P. palinuri* plants are moved outside and placed underneath a sheltered, open structure, where during this period solar radiation can be adequately controlled by screening, as can optimal moisture. Plants of *P. palinuri* are placed along the edges of the benches, which allows for a few hours of direct solar irradiation in the morning but protects them from direct sunlight in the afternoon and evening. *Ex situ* adult plants take two years to start developing flowers.

Pests and diseases

Primula palinuri seems rather unaffected by vine weevils (*Otiorhynchus sulcatus*) compared with other *Primula* species, in which damage appears rapidly, often resulting in the sudden death of individuals. However, once a year, when temperatures are above 15 °C, *P. palinuri* plants are given a solution of water and nematodes (*Steinernema* spp.) to control damage. During late summer, *P. palinuri* plants can be vulnerable to infestation with the mealybug *Pseudococcus viburni*, especially at the base of the leaf rosette and the rhizome. Mealybugs can be removed either manually or by using the adults or larvae of the natural enemy *Cryptolaemus montrouzieri*, which is sold as a biological pest control by Entocare, Wageningen as CRYPTOS. Generally, adult plants are developed from seed to prevent contamination from other sources. When adult plants are bought from EU nurseries, the associated plant passport ensures traceability and compliance with plant health regulations. Exchange of adult plants between botanical gardens is rare. When it does occur, frequent health checks are conducted by an external plant health expert.

Phenological stages, flowering and pollination

Primula palinuri is unexpectedly hardy in colder, wetter climates, and grows well without protection in any well-drained site. During hot summers, plants in the RRGBZ collection experience a period of dormancy. *P. palinuri* is an early flowering species (December to February), and it can flower reliably over a long period in a protected environment. Despite the original Mediterranean habitat, the pollen functional traits of *P. palinuri* remain more associated with cold mountain habitats than with warm coastal cliffs (Aronne *et al.*, 2014). At present, the species has found refugia in crevices of the north-facing cliffs. However, given the

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rapid trend of climate warming, migration and new adaptive processes in *P. palinuri* are unlikely to occur in the near future.

Like many *Primula* species, *P. palinuri* is heterostylous with a diallelic self-incompatible mating system (Aronne *et al.*, 2014). Consequently, it relies on pollinators to cross-transfer pollen between pin and thrum flowers or manual cross-pollination among style morphs to achieve good seed set. Manual cross-pollination has been conducted on the specimens at RRZBG. Although seed germination and seedling establishment have been widely investigated in the wild (De Micco & Aronne, 2012; Aronne *et al.*, 2014), viable seed production and the successive germination and seedling growth in *ex situ* conditions has, to our knowledge, never been investigated and reported in scientific literature. According to Richards (2003), the flowers rarely set seeds in *ex situ* cultivation. After an extensive search on scientific databases, we can conclude that there is no peer-reviewed research evidence that *P. palinuri* cultivated from seed can produce viable seeds themselves. In our experience, however, manual cross-pollinations were successful in producing large numbers of fertile seeds.

The production of seeds by *Primula palinuri* at RRZBG was achieved as follows. Flowering stalks start appearing at the end of December in the cool greenhouse, with a peak in flower production from February to March. To enlarge the genetic diversity of the collection at RRZBG, cross-pollination between different accessions of *P. palinuri* was carried out by using a small paintbrush to transfer the pollen between long- and short-style flowers belonging to different accessions (chosen haphazardly). Because the flowering peak of *P. palinuri* occurs a few weeks before other species of *Primula* open their flowers, and there are no insects in the greenhouse, the interspecific hybridisation rate is zero (because viable seeds are never produced without manual pollination). By using manual cross-pollination among existing accessions, new accessions of *P. palinuri* have been created in our collection. It is quite likely that mixing our founder accessions has generated new genetic combinations, but genetic analysis is needed to confirm this.

All cross-pollination events have been recorded for each accession in the plant records database. Information about the parent accessions has been registered. At RRZBG, all mother plants were haphazardly pollinated by mixing the 2014 group with the 2009 group, with the aim of spreading the pollen of both groups as much as possible over the two founder populations. Pin flowers were used as mother plants, whereas thrum flowers were used as pollen donors. After pollination, the seed pods of all accessions were collected. These seeds were given 2022 accession numbers and parent accession numbers were noted in the database. Finally, we noted in the database which offspring (child accessions) the mother plants from both founder groups have produced.

Vegetative propagation

The species is not easily propagated by vegetative propagation methods, such as leaf cuttings. Survival of different types and ages of leaf cuttings seems to be unsuccessful so far. Several attempts with young and old leaves, cut through or planted out using the base of the leaf in moist peat or gritty substrates failed, even when using root-promoting hormones such as Rhizopon 1% cutting powder.

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Conclusions

Primula palinuri is the only Mediterranean and maritime *Primula* species (all the others being alpine and montane) and still survives the hot and dry conditions of the sea cliffs of southern Italy. Furthermore, it depends strongly on cool and humid environmental conditions during its flowering and seedling establishment. Such conditions represent a major concern for the survival of the remnant populations in southern Italy but simplify cultivation protocols and maximise the plant survival rate during *ex situ* conservation. During the past decade, RRZBG has established a solid protocol for effective cultivation and propagation of this species by seed. With this specific horticultural knowledge, *ex situ* conservation programmes can be potentially supported.

Using *ex situ* populations that directly derive from seeds harvested in wild populations allows the *ex situ* production of a large number of seeds or seedlings, which can subsequently be introduced either into unoccupied habitats deemed suitable for the species or to augment or reinforce existing populations. However, *Primula* seeds in general are thought to have relatively short viability, and further study on long-term seed storage could provide valuable data to support *ex situ* conservation. Such *ex situ* conservation programmes ideally use only a single generation to avoid problems of habituation (Enßlin *et al.*, 2011; Nagel *et al.*, 2019). This has been done successfully with, for example, *Primula vulgaris* in the Netherlands (Barmentlo *et al.*, 2018).

Biosecurity risks associated with seeds must be assessed and managed appropriately before reintroducing *ex situ* species into their natural habitats (Redstone & Fox, 2021). Examples of such risks are pest and disease transmission, ecological risks (a translocated species may have major impacts on ecosystem functions) and risk of hybridisation with closely related species or subspecies, with a potential lower fitness of offspring and/or loss of species integrity (IUCN, 2013). By carefully assessing these biosecurity considerations and risks before moving seeds from *ex situ* conservation to important plant areas, conservationists can help minimise the potential negative impacts and maximise the effectiveness of their conservation efforts.

Finally, together with its importance for *ex situ* conservation programmes, the *Primula palinuri* collection can also be used to increase support for plant conservation with visitors, highlighting flagship species unique to a specific territory. For this reason, *P. palinuri* is frequently displayed in the RRZBG rock gardens.

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