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One year after the first reintroduction of the Malinverni’s quillwort in the Ticino River Natural Park, Italy

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Introduction
The Malinverni’s quillwort (Isoëtes malinverniana Ces. & De Not.), is an endemic aquatic pteridophyte occupying a very restricted range in the western Po Valley (Piedmont and Lombardy, Northern Italy). It occurs in lowland streams, channels and small river branches originally characterized by oligotrophic waters and the presence of Fontinalis antipyretica Hedw., an indicator of clean waters. Nowadays, most populations are found in a highly impacted area, well known for the production of rice. Major reasons for decline are the agricultural practices connected with rice cultivation, in particular, the use of fertilizers and herbicides that changed the water chemistry of the area from oligotrophic to meso- or eutrophic and the mechanized channel reshaping and cleaning (Abeli et al., 2012; Barni et al., 2013). I. malinverniana is included in annex II of the European Directive 92/43/EEC among the species requiring special areas of conservation and in annex I of the Bern Convention. Moreover, this species is listed as Critically Endangered in the Red List of the European Union and in the Red List of Italy for the strong range decline observed in the last 15 - 20 years (Minuzzo et al., 2016). The project described represents the first reintroduction attempt for this species.

Main Goals
• Goal 1: Investigate and define the threats affecting the species.
• Goal 2: Create an ex situ population to be used as a nursery for the final translocation in the wild.
• Goal 3: Establish some viable (self-sustaining) populations of the species in suitable areas within its native range.

Success Indicators
• Indicator 1: Threats affecting the species understood.
• **Indicator 2**: Establishment of a safety *ex situ* population of at least 300 plants from sexual reproduction.
• **Indicator 3**: Creation of at least two new populations of the species in suitable sites.

**Project summary**

**Feasibility:** Given the rapid decline in extent of occurrence recorded at the end of 2000s, conservationists realized that urgent conservation measures were needed for this species. The past species distribution was quite well known from 1960s and 1970s field survey. Recent (2007 - 2009) surveys revealed that the species was still growing only in nine sites (88% of its original range). However, knowledge on the species ecology and reproductive biology were scarce or lacking, preventing the realization of proper conservation actions. For this reason, since 2008, the species was the subject of several studies aimed at investigating the population genetics (Gentili *et al.*, 2010), the ecological requirements (Abeli *et al.*, 2012; Barni *et al.*, 2013) and the reproductive phenology (Abeli & Mucciarelli, 2010) of the target species. These studies made it possible to understand the reasons for the species decline. In particular, the ecological studies highlighted the negative effect of water eutrophication and channel management on the species survival. Moreover, data obtained during these studies were of key importance for the reintroduction project described here, and for future reintroductions. In particular, knowledge gained were useful to identify suitable release sites for the species and to develop a spore cultivation protocol for the *ex situ* propagation of the species.

**Implementation:** Genetic analyses performed on nine known populations revealed a moderate within-population genetic variation and a low between-population genetic differentiation, so *ex situ* propagation of the species was aimed at increasing the genetic variation by crossing male and female spores from different wild populations. A spring-fed artificial channel within a protected area (Parco Naturale della Valle del Ticino) was chosen as reintroduction site. This site was prepared for the reintroduction. In particular, the channel was reshaped and the spring (partially covered by soil and rotten vegetation) was restored, increasing the water flow. The water chemistry was not as good as in other pristine-like wild populations of the species, but the general conditions of the habitat were excellent (the channel crosses one of the few relict pristine *Alnus glutinosa* woodlands). Moreover, constant monitoring from the Park staff could be guaranteed. In spring 2016, 20 individuals of *I. malinverniana* were placed in the middle of the channel as a first trial. Further 20 individuals were transplanted in spring 2017.

**Post-planting monitoring:** The post-release monitoring is still active and consists of monthly visits to the reintroduction site. Variable collected are the mortality (18% after one year and including the 20 plants planted in 2017) and the length of the longest sporophyll in each plant as a measure of performance. Mortality was mainly due to anomalous sediment deposition on the plants. In autumn 2016, we checked for mature spores and we found that they were mature (grey) and ready for dispersal. A complete survey of the downstream sector of the channel in spring 2017 did not reveal any sporelings. So, one year after reintroduction we did not observe the second generation. For this reason, we can conclude that our translocation has been partially successful. It should be
considered that the number of released plants was low and this likely affected the reproductive potential of the overall population (low probability of establishment). The monitoring activity will continue in the next years.

**Major difficulties faced**

- **Low number of individuals**: The low number of wild individuals prevented the identification of lethal thresholds for nutrients and pesticides through experimental manipulation. This also limited spore for *in vitro* fecundation and reproduction.
- **Low growing rates**: Plants reproduced *in vitro* grew very slowly (about 8 - 9 months from spores to individuals of 8 - 10 cm). However, one year old plants can potentially reproduce.
- **Scarcity of suitable release sites**: based on the model developed by Abeli *et al.* (2012), an analysis of nine apparently pristine channels and streams revealed the none were suitable for the species.

**Hydrological modification of the selected channel**: Changes in water speed and sediment accumulation threaten the reintroduced population. Constant monitoring and management are required. Further 20 individuals were released in early 2018 after sediment accumulation was halted.

**Major lessons learned**

- Although the success of a translocation is linked to the next generation establishment, the outcome of our project is encouraging and a key aspect of our success is the deep knowledge of the species developed with *ad hoc* studies.
- Constant monitoring is essential after transplanting to ensure rapid corrections should problem arise.
- Small-scale trials and gradual transplanting allow to highlight unforeseen problem and correct the project before the massive release of individuals.
Success of project

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Reason(s) for success/failure:
- The reintroduced population is still alive and healthy one here after its release, yet sporelings were not observed.
- The reintroduced population require constant management and monitoring.
- A suitable site for a second reintroduction has not been found yet.

References


