

## Guarding European biodiversity: Molecular early-warning tools for invasive alien plants of Union concern

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### Abstract:

Invasive alien species (IAS) are among the main drivers of biodiversity loss and can deeply alter the structure and functioning of terrestrial and aquatic ecosystems, with cascading effects on ecosystem services and management costs. In this context, early detection is essential to intercept invasions at their initial stages, when eradication or targeted containment actions are still feasible. Among the available tools, the use of eDNA is particularly promising for increasing the capacity to detect IAS in the early stages of invasion.

In this context, the aims of this project are: (i) to build a molecular reference dataset based on standard DNA barcode regions (ITS2, *rbcL*, *matK*, *trnH-psbA*, *rpoB*), carefully curated for a set of invasive alien plant species of Union concern (*sensu* EU Regulation no.1143/2014); (ii) to design and validate species-specific couple of primers on standard markers for a representative subset of these plant species; and (iii) to develop and validate a field-oriented assay based on eLAMP (environmental Loop-mediated Isothermal Amplification) for the early detection of *Pontederia crassipes* Mart.

The results include a curated reference sequence library enabling reliable molecular identification of the target plant species, a panel of species-specific primers ready for operational deployment, and an eLAMP test for *Pontederia crassipes* targeting the *rpoB* gene whose primers were highly specific to the target species and showed no amplification of non-targets. The assay will be applied to environmental water samples collected in the central-western part of the Mediterranean island of Sardinia (Italy), in the Province of Oristano, to evaluate its performance under field conditions.

The project outcomes can be adopted by environmental agencies, protected area managers, regional environmental protection agencies (ARPAs), port and customs authorities, and diagnostic/control laboratories to strengthen surveillance, prevention and early-warning systems for invasive alien plant species of Union concern. Overall, the project aims to provide concrete tools to support European policies for the management and containment of invasive alien species.