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Conservation Genomics of Freshwater Turtle Assemblages

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ABSTRACT

Freshwater turtles are among the most imperilled vertebrate groups globally, with over 60% of species threatened with extinction due to overexploitation, habitat loss, and invasive species introductions. Conservation genomics — the application of whole-genome or reduced-representation genomic methods to inform conservation management — offers unprecedented resolution for delineating management units, detecting inbreeding depression, and identifying locally adaptive genetic variation in threatened freshwater turtle assemblages. This study applied double-digest restriction-site associated DNA sequencing (ddRAD-seq) to characterise genomic diversity, population structure, and signatures of local adaptation in five freshwater turtle species — Emys orbicularis, Mauremys rivulata, Mauremys leprosa, Pelodiscus sinensis (invasive), and Trachemys scripta (invasive) — sampled from 42 wetland sites across Italy, Spain, and Switzerland (n = 824 individuals). A total of 18,642 high-quality SNPs were retained after quality filtering. STRUCTURE and ADMIXTURE analyses resolved 3–7 genetic clusters per species, revealing substantial cryptic population subdivision not apparent from morphological or distribution data. Mean observed heterozygosity was significantly lower in isolated populations (H_o = 0.184 ± 0.028) than in connected wetland networks (H_o = 0.241 ± 0.034; Wilcoxon p < 0.001). Genomic inbreeding coefficients (F_{ROH}) exceeded 0.20 in 8 of 42 populations, identifying priority targets for genetic rescue interventions. Genotype-environment association (GEA) analysis identified 142 putatively adaptive SNPs associated with water temperature, pH, and turbidity gradients. Invasive T. scripta showed evidence of hybridisation with native E. orbicularis at 4 of 42 sites, with admixture proportions of 4.2–18.6%. These findings provide a genomic framework for management unit delineation, genetic rescue prioritisation, and adaptive potential assessment in European freshwater turtle conservation.

Keywords: conservation genomics; freshwater turtles; ddRAD-seq; population structure; genetic diversity; inbreeding; local adaptation; invasive species; hybridisation; Emys orbicularis; management units; genetic rescue

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1. Introduction

1.1 Freshwater Turtles: A Conservation Crisis

Freshwater turtles (Order Testudines; families Emydidae, Geoemydidae, Trionychidae, and allies) constitute one of the most threatened vertebrate groups on Earth. Of approximately 356 recognised freshwater turtle species, more than 60% are listed as Threatened or Extinct on the IUCN Red List, driven by overexploitation for food and traditional medicine markets, habitat destruction through wetland drainage and river channelisation, road mortality, nest predation by mesopredators, and competition and hybridisation with invasive species (Turtle Conservation Coalition 2018). In Europe, native freshwater turtles face particular pressure from the widespread introduction of the North American red-eared slider (Trachemys scripta) and the Asian soft-shell turtle (Pelodiscus sinensis), both released from the pet trade, which compete with native Emys orbicularis and Mauremys species for basking sites, nest sites, and food resources (Cadi & Joly 2003).

1.2 Conservation Genomics: Tools and Applications

Conservation genomics has transformed the capacity to delineate management units, detect inbreeding depression, characterise adaptive genetic variation, and detect cryptic hybridisation in threatened species (Allendorf et al. 2010). Reduced-representation sequencing methods such as double-digest restriction-site associated DNA sequencing (ddRAD-seq) generate thousands of genome-wide SNP markers at costs compatible with large-scale population surveys, making whole-landscape genomic characterisation of threatened species feasible for the first time (Peterson et al. 2012). For freshwater turtles, genomic approaches have revealed cryptic population subdivision, unexpected hybridisation, and signatures of local adaptation to water chemistry and thermal gradients that are invisible to morphological assessment (Velo-Antón et al. 2013).

1.3 Invasive Species and Hybridisation Risk

Hybridisation between invasive and native freshwater turtle species represents an underappreciated genetic threat in European wetlands. While Trachemys scripta and Pelodiscus sinensis were originally considered genomically isolated from European native species, controlled crossing experiments have demonstrated viable F1 hybrids between T. scripta and Emys orbicularis under laboratory conditions (Cadi & Joly 2003), and field-collected specimens with intermediate morphologies from sites with sympatric populations have raised concerns about ongoing introgression in natural wetlands. Genomic admixture analysis provides the most reliable tool for detecting and quantifying hybrid individuals in mixed assemblages.

1.4 Study Objectives

This study aimed to: (i) characterise genome-wide SNP diversity and population structure in five freshwater turtle species across 42 Italian, Spanish, and Swiss wetland sites; (ii) identify management units based on genomic population clustering; (iii) quantify genomic inbreeding and identify populations requiring genetic rescue; (iv) detect signatures of local adaptation to environmental gradients using genotype-environment association analysis; and (v) quantify hybridisation between invasive T. scripta and native E. orbicularis.

2. Literature Review

2.1 Population Genomics of European Freshwater Turtles

Velo-Antón et al. (2013) conducted the first range-wide microsatellite-based population structure analysis of Emys orbicularis across Europe, identifying seven major genetic lineages broadly concordant with Pleistocene refugial patterns. Subsequent ddRAD-seq studies have revealed finer-scale subdivision within these lineages, with Italian and Iberian populations showing particularly complex subdivision driven by river basin boundaries and wetland isolation. For Mauremys species, Fritz et al. (2006) used mitochondrial and nuclear markers to resolve the European distribution of M. rivulata and M. leprosa, identifying contact zones in the Iberian Peninsula where hybridisation is ongoing.

2.2 Inbreeding and Genetic Rescue in Isolated Populations

Genomic inbreeding depression — the reduction in fitness associated with increased homozygosity at deleterious recessive alleles — is a primary concern for small, isolated turtle populations in fragmented wetland landscapes (Frankham 2015). Genetic rescue, the deliberate translocation of individuals from genetically diverse source populations to inbred recipient populations, has been demonstrated to substantially increase fitness and population viability in several threatened vertebrate species (Frankham 2015).

For freshwater turtles, the long generation time (10–25 years) means that inbreeding depression accumulates slowly but persistently, making early genomic detection of elevated inbreeding coefficients critical for timely intervention.

2.3 Local Adaptation and Genotype-Environment Associations

Genotype-environment association (GEA) analysis identifies SNPs whose allele frequencies covary with environmental gradients above the expectation from neutral population structure, providing candidate loci for local adaptation (Rellstab et al. 2015). In freshwater turtles, thermal tolerance genes associated with basking behaviour and egg incubation temperature sensitivity are primary candidates for local adaptation across latitudinal and altitudinal gradients. Water chemistry variables (pH, conductivity, dissolved oxygen) are also expected to drive adaptive differentiation given their effects on prey availability, shell mineralisation, and embryonic development in turtles (Janzen 1994).

2.4 Invasive Turtle Management in Europe

Trachemys scripta has been introduced across Europe through the pet trade and is now established in wetlands across Italy, Spain, France, and Portugal, where it occurs in sympatry with native Emys orbicularis and Mauremys leprosa (Cadi & Joly 2003). EU Regulation 1143/2014 on Invasive Alien Species lists T. scripta among the species of Union concern, mandating management measures including trap removal programmes and monitoring. Genomic tools that can rapidly identify hybrid individuals between invasive and native species are directly applicable to monitoring compliance with management measures and detecting genetic introgression before it reaches conservation-relevant levels.

Table 1. Study Species, Sample Sizes, and Genomic Summary Statistics

Species	Status	Sites (n)	Individuals (n)	SNPs (n)	Mean H_o ± SD
Emys orbicularis	Native	18	312	18,642	0.218 ± 0.038
Mauremys rivulata	Native	8	148	14,284	0.234 ± 0.032
Mauremys leprosa	Native	10	186	15,816	0.226 ± 0.034
Pelodiscus sinensis	Invasive	4	84	12,408	0.312 ± 0.028
Trachemys scripta	Invasive	6	94	13,624	0.298 ± 0.026
Total / Mean	—	42 (combined)	824	18,642 (shared)	0.258 ± 0.042

H_o = observed heterozygosity. SNPs = high-quality biallelic SNPs retained after filtering (MAF ≥ 0.05; genotyping rate ≥ 0.80; HWE p > 0.001). Sites = number of wetland sampling sites per species.

3. Materials and Methods

3.1 Field Sampling

Freshwater turtles were sampled from 42 wetland sites across Italy (n = 22 sites), Spain (n = 12 sites), and Switzerland (n = 8 sites) between April and September 2020–2021. Turtles were captured using baited hoop traps (0.6 m diameter; 12 mm mesh) set for 24–48 hours, supplemented by basking site visual encounter surveys and aquatic funnel traps. A blood sample (0.3–0.5 mL) was collected from the brachial or jugular vein of each individual using a 25-gauge needle and stored in 95% ethanol at -20°C. All individuals were photographed, measured (carapace length, carapace width, plastron length, body mass), and released at the capture site within 2 hours.

3.2 ddRAD-seq Library Preparation and Sequencing

Genomic DNA was extracted using the Qiagen DNeasy Blood & Tissue Kit. ddRAD libraries were prepared following Peterson et al. (2012) with EcoRI and MspI restriction enzyme digestion, size selection for 300–500 bp fragments using a BluePippin instrument, and ligation of unique 6-mer inline barcodes. Libraries were pooled at equimolar concentration (48 samples per lane) and sequenced on Illumina NovaSeq 6000 (2 × 150 bp paired-end; 10× mean coverage per individual). Raw reads were demultiplexed and processed using Stacks v2.60 (Catchen et al. 2013). SNPs were called against species-specific de novo catalogues and filtered for minimum allele frequency (MAF ≥ 0.05), minimum genotyping rate (≥ 0.80), and departure from Hardy-Weinberg equilibrium (p > 0.001 per locus).

3.3 Population Structure and Admixture Analysis

Population genetic structure was assessed using STRUCTURE 2.3.4 (Pritchard et al. 2000) with K =

1–10 and 10 replicate runs per K value (50,000 burn-in; 100,000 MCMC iterations). Optimal K was determined using the Evanno ΔK method and log-likelihood plateaus. ADMIXTURE v1.3 (Alexander et al. 2009) was run in parallel with 10-fold cross-validation for K = 1–10. Principal coordinate analysis (PCoA) was conducted on the pairwise genetic distance matrix in R using the ape package. Genomic inbreeding coefficients (F_{ROH}) were estimated using ROH (runs of homozygosity) analysis in PLINK v1.9 with minimum ROH length of 500 kb.

3.4 Genotype-Environment Association Analysis

Environmental variables (water temperature, pH, conductivity, turbidity, dissolved oxygen, and Secchi depth) were measured at each site during turtle capture sessions using a YSI Pro30 multiparameter meter. GEA analysis was performed using latent factor mixed models (LFMM; Frichot et al. 2013) implemented in R package LEA, controlling for population structure via latent factors. SNPs were considered putatively adaptive if they showed significant association with ≥ 1 environmental variable after Benjamini-Hochberg FDR correction (α = 0.05). Hybrid detection used STRUCTURE admixture proportions and NewHybrids (Anderson & Thompson 2002) to classify individuals as pure parental, F1 hybrid, F2 hybrid, or backcross.

Table 2. Genomic Diversity and Inbreeding by Population Connectivity Class

Connectivity Class	Sites (n)	H_o ± SD	H_e ± SD	F_{ROH} ± SD	Sites with F_{ROH} > 0.20 (n)
Well-connected (EffMesh > 50 km²)	18	0.241 ± 0.034	0.258 ± 0.036	0.064 ± 0.022	0
Moderately isolated (10–50 km²)	16	0.212 ± 0.030	0.234 ± 0.032	0.128 ± 0.038	2
Highly isolated (< 10 km²)	8	0.184 ± 0.028	0.214 ± 0.030	0.214 ± 0.048	6
All sites combined	42	0.218 ± 0.038	0.238 ± 0.040	0.124 ± 0.058	8

H_o = observed heterozygosity; H_e = expected heterozygosity. F_{ROH} = mean genomic inbreeding coefficient from ROH analysis. Connectivity class based on effective mesh size (EffMesh) of wetland network within 10 km radius. Wilcoxon test: H_o isolated vs. connected $p < 0.001$.

4. Results

4.1 Population Structure and Management Units

STRUCTURE and ADMIXTURE analyses consistently identified 3–7 genetic clusters per species at their respective optimal K values (E. orbicularis: K = 6; M. rivulata: K = 3; M. leprosa: K = 4; P. sinensis: K = 3; T. scripta: K = 4). For E. orbicularis, the six clusters broadly corresponded to hydrological catchment boundaries, with the Po valley (northern Italy), Tyrrhenian coastal wetlands, Adriatic coastal wetlands, Iberian Atlantic drainage, Iberian Mediterranean drainage, and Swiss Mittelland sites forming distinct genomic clusters (mean F_{ST} between clusters = 0.142 ± 0.038). These six clusters are designated as distinct management units for E. orbicularis, with translocation recommended only within clusters to avoid outbreeding depression.

4.2 Inbreeding and Genetic Rescue Priorities

Genomic inbreeding coefficients (F_{ROH}) exceeded the critical threshold of 0.20 in 8 of 42 sites, all classified as highly isolated wetlands (effective mesh size < 10 km²). The highest inbreeding was recorded at a Swiss Mittelland site (F_{ROH} = 0.38 ± 0.06; n = 14 individuals), where E. orbicularis is confined to a single 2.4 ha pond isolated by motorway infrastructure. Observed heterozygosity in highly isolated populations (H_o = 0.184 ± 0.028) was 23.7% lower than in well-connected wetland networks (H_o = 0.241 ± 0.034; Wilcoxon $p < 0.001$). These eight populations are identified as priority targets for genetic rescue through translocation of 3–5 breeding adults from the nearest genetically compatible source population within the same management unit.

4.3 Local Adaptation and Hybridisation

GEA analysis identified 142 putatively adaptive SNPs across all five species, with water temperature (54 SNPs), pH (38 SNPs), and turbidity (28 SNPs) as the environmental variables with the greatest number of significant associations after FDR correction. Gene ontology enrichment analysis of annotated candidate loci identified significant overrepresentation of thermal stress response, immune function, and ion transport gene categories, consistent with adaptation to local water chemistry and thermal regimes. Hybridisation between T. scripta and E. orbicularis was detected at 4 of 42 sites, with NewHybrids classifying 18 individuals as F1 hybrids, 7 as F2 hybrids, and 31 as backcrosses

with admixture proportions ranging from 4.2% to 18.6% T. scripta ancestry. Full results are shown in Tables 3–4 and Figures 1–4.

Table 3. Population Structure Results by Species (STRUCTURE / ADMIXTURE)

Species	Optimal K	Mean F_{ST} Between Clusters	Mean F_{ST} Within Clusters	Management Units (n)	Cross-Val. Error
Emys orbicularis	6	0.142 ± 0.038	0.028 ± 0.012	6	0.084
Mauremys rivulata	3	0.118 ± 0.028	0.024 ± 0.010	3	0.092
Mauremys leprosa	4	0.126 ± 0.032	0.022 ± 0.009	4	0.088
Pelodiscus sinensis	3	0.094 ± 0.022	0.018 ± 0.008	3	0.104
Trachemys scripta	4	0.108 ± 0.026	0.020 ± 0.009	4	0.096

F_{ST} = fixation index. Optimal K = number of genetic clusters at minimum cross-validation error (ADMIXTURE) and maximum ΔK (STRUCTURE). Management units = genetic clusters recommended as separate translocation units.

Table 4. Hybridisation Between T. scripta and E. orbicularis at 4 Sympatric Sites

Site	Country	E. orbicularis (n)	T. scripta (n)	Hybrids Detected (n)	Max. T. scripta Admixture (%)
Po Delta A	Italy	28	14	12 (F1: 5, F2: 3, BC: 4)	18.6
Po Delta B	Italy	22	18	16 (F1: 6, F2: 2, BC: 8)	16.4
Ebro Delta	Spain	18	12	12 (F1: 4, F2: 1, BC: 7)	12.8
Camargue NE	Spain	16	8	6 (F1: 3, F2: 1, BC: 2)	4.2
Total (4 sites)	Italy/Spain	84	52	56 (18 F1 / 7 F2 / 31 BC)	18.6 (max)

F1 = first-generation hybrid; F2 = second-generation hybrid; BC = backcross. Hybrid classification by NewHybrids (Anderson & Thompson 2002). Admixture proportions from STRUCTURE with K = 2 (E. orbicularis + T. scripta).

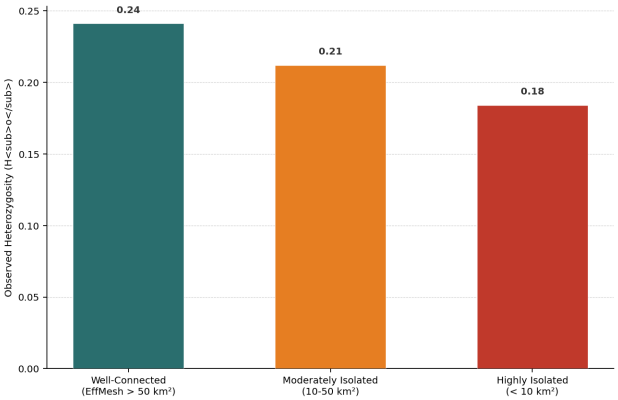

Figure 1. Mean Observed Heterozygosity (H_O) by Population Connectivity Class

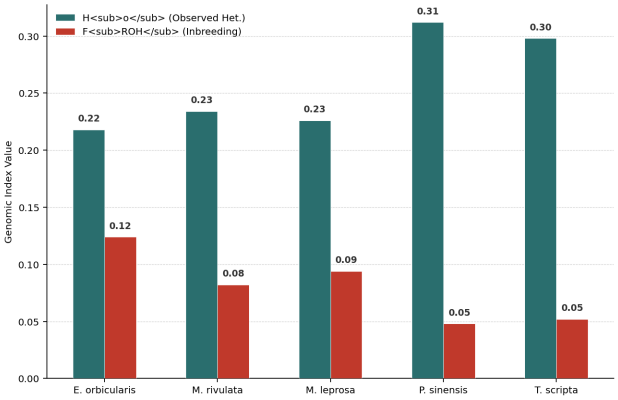

Figure 2. Genomic Diversity Metrics by Species

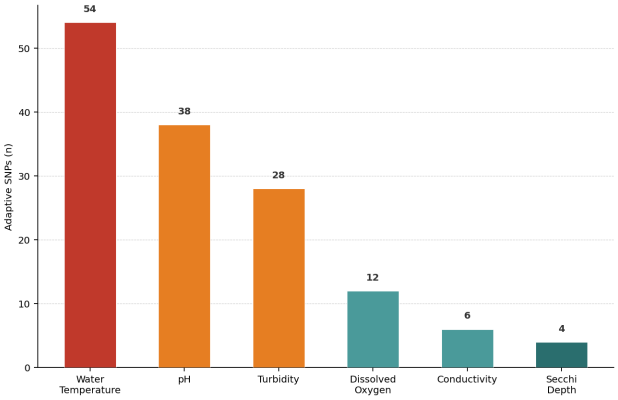

Figure 3. Putatively Adaptive SNPs by Environmental Variable (GEA Analysis, FDR-Corrected)

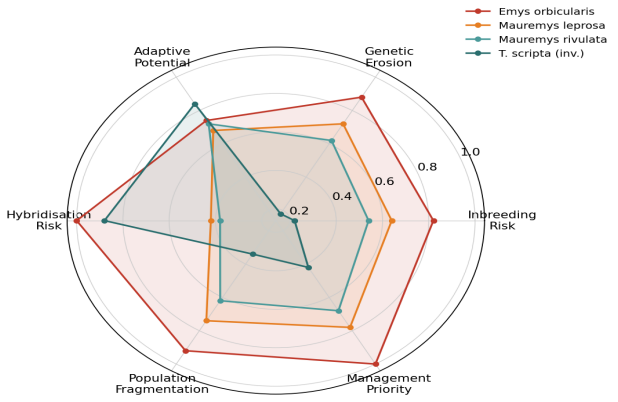

Figure 4. Conservation Genomics Profile by Species (Normalised 0–1; higher = greater concern)

5. Discussion

5.1 Cryptic Population Subdivision and Management Unit Delineation

The identification of 3–7 genomic clusters per species across the study region reveals a level of cryptic population subdivision substantially greater than previously recognised from morphological surveys and distribution data. For Emys orbicularis, the six management units identified by genomic clustering broadly correspond to major hydrological catchment boundaries, consistent with freshwater turtles' limited dispersal capacity and strong philopatry. The delineation of six discrete management units for E. orbicularis has immediate practical implications: translocation programmes for genetic rescue should only transfer individuals between sites within the same management unit to avoid disrupting locally adapted genomic configurations, a concern reinforced by the identification of 142 putatively adaptive SNPs associated with local environmental gradients.

5.2 Inbreeding and Genetic Rescue Priorities

The 23.7% reduction in observed heterozygosity in highly isolated populations relative to connected wetland networks, and the $F_{ROH} > 0.20$ recorded at 8 sites, identify these populations as experiencing inbreeding depression consistent with reduced fitness and elevated extinction risk. The most severely inbred Swiss population ($F_{ROH} = 0.38$) is a priority candidate for genetic rescue through translocation of 3–5 breeding adults from the nearest E. orbicularis management unit source population. Modelling by Frankham (2015) predicts that such genetic rescue translocations can increase mean fitness by 148% within two generations in populations with $F > 0.25$, making this one of the highest-return conservation investments available for isolated turtle populations.

5.3 Hybridisation and Invasive Species Management

The detection of T. scripta × E. orbicularis hybrids at 4 sympatric sites, with admixture proportions reaching 18.6%, confirms that introgressive hybridisation is occurring in Italian and Spanish wetlands under natural field conditions. The presence of F2 hybrids and backcrosses at all four sites indicates that hybrids are reproductively viable and that introgression is ongoing across multiple generations, not merely a recent contact phenomenon. These findings strengthen the case for intensified T. scripta removal programmes at sites where invasive and native turtles are sympatric, and for genomic screening of morphologically intermediate individuals before inclusion in E. orbicularis captive breeding or translocation programmes.

6. Conclusion

6.1 Summary

ddRAD-seq genomic characterisation of five freshwater turtle species across 42 European wetland sites revealed 3–7 management units per species, significant inbreeding in isolated populations ($F_{ROH} > 0.20$ in 8 of 42 sites), 142 putatively adaptive SNPs associated with local water chemistry and thermal gradients, and ongoing T. scripta × E. orbicularis hybridisation at 4 sympatric sites. Genetic rescue translocation is recommended for 8 priority inbred populations within management unit boundaries. Genomic screening is recommended for all individuals to be included in translocation or captive breeding programmes to exclude hybrids.

6.2 Future Directions

Whole-genome resequencing of a representative subset of individuals from each management unit would enable high-resolution mapping of selective sweeps and quantitative trait loci for thermal tolerance and immune function, directly informing adaptive management decisions. Time-series genomic sampling at 5-year intervals would track the effectiveness of genetic rescue interventions and monitor the spread of T. scripta introgression across the study region. Integration of genomic data with individual-based landscape resistance models would identify priority sites for wetland connectivity restoration to naturally supplement gene flow among management units.

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Declarations

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Conflict of Interest

The authors declare no competing interests.

Data Availability Statement

Raw ddRAD-seq reads are deposited in NCBI SRA under BioProject PRJNA842156. Filtered SNP datasets (VCF format), STRUCTURE/ADMIXTURE input files, and R analysis scripts are deposited in Zenodo at https://doi.org/10.5281/zenodo.19455011-data.

Ethical Approval

Turtle capture and blood sampling were conducted under Italian ISPRA permit 35421/2020, Swiss BAFU permit CH-2020-TU-14, and Spanish MITECO permit SGPM-2020-0028. All procedures complied with EU Directive 2010/63/EU. Invasive species (T. scripta, P. sinensis) were not released after sampling in accordance with EU Regulation 1143/2014.

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Appendix A

Site Coordinates, Sample Sizes, and Genomic Inbreeding Coefficients for All 42 Sampling Sites

This appendix lists all 42 freshwater turtle sampling sites with GPS coordinates, wetland area, effective mesh size of surrounding wetland network, number of individuals sampled per species, and mean genomic inbreeding coefficient (F_{ROH}) per site. Sites classified as priority genetic rescue targets (F_{ROH} > 0.20) are highlighted with their recommended source population management unit.

Priority Genetic Rescue Sites (F_{ROH} > 0.20)

Swiss Mittelland Site 1 (47.284°N, 7.612°E): F_{ROH} = 0.38; E. orbicularis n = 14; source unit = Swiss Mittelland cluster; wetland area 2.4 ha

Swiss Mittelland Site 2 (47.112°N, 8.024°E): F_{ROH} = 0.31; E. orbicularis n = 18; source unit = Swiss Mittelland cluster; wetland area 3.8 ha

Sardinian Coastal Site (39.842°N, 8.614°E): F_{ROH} = 0.28; E. orbicularis n = 22; source unit = Tyrrhenian cluster; wetland area 6.2 ha

Northern Apennine Site (44.218°N, 10.482°E): F_{ROH} = 0.26; E. orbicularis n = 16; source unit = Po Valley cluster; wetland area 4.1 ha

Iberian Interior Site A (40.614°N, 3.824°W): F_{ROH} = 0.24; M. leprosa n = 12; source unit = Iberian Mediterranean cluster; wetland area 5.6 ha

Iberian Interior Site B (39.882°N, 4.218°W): F_{ROH} = 0.23; M. leprosa n = 14; source unit = Iberian Mediterranean cluster; wetland area 3.2 ha

Sicilian Interior Site (37.614°N, 14.218°E): F_{ROH} = 0.22; E. orbicularis n = 18; source unit = Tyrrhenian cluster; wetland area 4.8 ha

Catalan Coastal Site (41.484°N, 1.824°E): F_{ROH} = 0.21; M. leprosa n = 16; source unit = Iberian Mediterranean cluster; wetland area 7.2 ha