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BRIDGING THE GAP: ADVANCING eDNA METABARCODING FOR BIOMONITORING IN SARDINIAN COASTAL LAGOONS

COLMARE IL DIVARIO: eDNA METABARCODING PER IL MONITORAGGIO DELLE LAGUNE COSTIERE DELLA SARDEGNA

Abstract - Coastal lagoons are dynamic ecosystems that provide essential ecological and socio-economic services in the Mediterranean Sea. However, these habitats face increasing anthropogenic pressures, therefore requiring effective biomonitoring strategies. Environmental DNA (eDNA) metabarcoding is a promising approach, but its accuracy depends on how complete the genetic reference databases are. In Sardinia, lagoon biodiversity has been studied to varying extents, but there is no comprehensive synthesis on macrozoobenthic diversity and its genetic representation. We did a systematic review compiling species records and assessing their representation in global repositories for 18S rRNA and Cytochrome c oxidase I genes. A total of 536 taxa were reported in 42 lagoons, with 401 identified at the species level. Only 69% had COI barcodes, and 42% 18S rRNA sequences, with major gaps in Mollusca, Nemertea and Arthropoda (18S) and Echinodermata (COI). Expanding reference libraries is crucial for improving eDNA-based monitoring and conservation of Mediterranean coastal lagoons.

Keywords: environmental DNA, genetic reference databases, macrozoobenthos

Introduction - Coastal lagoons are transitional ecosystems shaped by complex physical, chemical, and biological gradients, supporting high biodiversity and providing important ecological services such as nutrient cycling and nursery areas (Newton *et al.*, 2014). In the Mediterranean, they also support fisheries, aquaculture, and recreational activities, playing a crucial role in the regional economy (Pérez-Ruzafa *et al.*, 2019). Many lagoons are included in the Natura 2000 network, which highlights not only the ecological value, but also the ecological, cultural, and socio-economic significance. In Sardinia, these environments represent long-standing biodiversity reservoirs and important areas for artisanal fisheries and aquaculture (De Falco & Piergallini, 2003), with several identified as Allocated Zones for Aquaculture (AZA). Despite their ecological and economic importance, these ecosystems are increasingly threatened by anthropogenic pressures, including eutrophication, habitat alteration, climate change, and biological invasions (Newton *et al.*, 2014). Effective monitoring and management are therefore essential to safeguard ecosystem integrity while sustaining traditional and productive activities. Traditionally, the biomonitoring of biodiversity in lagoons has been based on the morphological identification of benthic macroinvertebrates, which are robust indicators of both biodiversity and environmental quality. However, morphological identification is often time-consuming and requires specialised taxonomic expertise (Ferraro *et al.*, 1989; Troudet *et al.*, 2017). Environmental DNA (eDNA) metabarcoding use has recently emerged as a complementary tool for biodiversity assessment. It offers higher sensitivity for detecting both common and rare taxa, and potentially can improve the accuracy and efficiency of monitoring (Hering *et al.*, 2018). However, the precision of molecular identification strongly depends on the availability and completeness of genetic reference databases, which are incomplete across different taxonomic groups (Weigand *et al.*, 2019). Although biodiversity in Sardinian lagoons

has been the subject of numerous ecological studies (De Falco & Piergallini, 2003; Gravina *et al.*, 2020; Brundu & Magni, 2021), a regional synthesis of macrozoobenthic diversity and its representation in global genetic repositories is still lacking. This study aims to evaluate the extent of this gap by providing a comprehensive synthesis of macrozoobenthic diversity across Sardinian lagoons and assessing their coverage in major genetic databases (NCBI GenBank, BOLD, SILVA). The identification of taxonomic and molecular gaps forms the basis for developing effective eDNA-based monitoring programmes and contributes to conservation and management strategies for Mediterranean coastal lagoons.

Materials and methods – A systematic review of the scientific literature was conducted to compile records of macrozoobenthic species inhabiting Sardinian coastal lagoons. The review included peer-reviewed articles, technical reports, and grey literature published up to March 2025. Search strategy was implemented in Web of Science, Scopus, and Google Scholar, exploring titles, abstracts, and keywords, and using combinations of the following terms "Sardinia", "lagoon", "macrozoobenth*", "benthic invertebrate*", "benth*", "macrobenth*", "macroinvertebrate*", "benthic fauna" and "biodiversity". For each study, we recorded the name of the lagoon, the list of reported macrozoobenthic taxa, and the corresponding taxonomic resolution. Taxa were standardised according to the World Register of Marine Species (WoRMS) database to correct synonymies and ensure consistency in nomenclature. The compiled dataset included all macrozoobenthic taxa reported from Sardinian lagoons. Only records identified at the species level were included in subsequent genetic coverage analyses. Higher-level identifications (family and genus) were excluded from barcode searches to avoid misinterpretations.

Each species was cross-referenced against three widely used genetic repositories: GenBank (NCBI) for Cytochrome c oxidase I (COI) and 18S rRNA (18S) sequences; BOLD Systems (Barcode of Life Data System) for COI barcodes and the SILVA database for 18S sequences. For each species, we recorded the presence/absence of COI and 18S sequences in the respective repositories. The proportion of species with available barcodes was calculated for the entire dataset and major taxonomic groups (e.g., Mollusca, Arthropoda, Annelida, Echinodermata, Nemertea). Gaps in genetic coverage were summarized by phylum and illustrated.

Results – The literature review yielded 41 articles reporting macrozoobenthic data from 42 coastal lagoons in Sardinia. A total of 536 taxa were documented, 401 of which were identified at the species level and considered for the analysis of genetic coverage. When cross-referenced with global repositories, substantial variability in sequence availability emerged. A higher representation was observed in BOLD compared to SILVA. Overall, 69% of the species had at least one COI barcode in BOLD, whereas 42% were represented by 18S sequences in SILVA. GenBank searches confirmed these proportions, with minor discrepancies due to duplicated entries or incomplete metadata. Taxonomic groups differed markedly in their representation. Annelida showed the greatest coverage, with 128 taxa recorded in BOLD and 97 in SILVA. Mollusca and Arthropoda also exhibited substantial representation in BOLD (64 and 75 taxa, respectively), but their coverage decreased considerably in SILVA (36 and 21 taxa, respectively). Conversely, Bryozoa, Chordata, and Cnidaria were consistently underrepresented in both databases, with fewer than 10 taxa each. Nemertea showed minimal representation, with only a single taxon present in both repositories (Table 1).

Tab. 1 – Number of macrozoobenthic species from Sardinian lagoons represented in reference databases, and percentage of species lacking records (Gap %) for BOLD and SILVA.

Numero di specie macrozoobentoniche delle lagune sarde rappresentate nelle banche dati di riferimento e percentuale di specie prive di sequenze (Gap %) per BOLD e SILVA.

Phylum	BOLD		SILVA	
	Sequenced species	Gap %	Sequenced species	Gap %
Cnidaria	4	33%	5	16%
Bryozoa	4	20%	3	40%
Nemertea	1	66%	1	66%
Annelida	128	28%	97	45%
Mollusca	64	34%	36	63%
Arthropoda	75	24%	21	79%
Chordata	8	-	4	50%

The largest gaps were observed in Mollusca and Nemertea, where over half of the species lacked both COI and 18S sequences. Annelida displayed intermediate gaps, with 35% of species missing COI barcodes and 52% lacking 18S sequences. Arthropoda showed relatively small gaps for COI (24%) but a very large gap in SILVA (79%). Echinodermata showed the opposite pattern, with a wide gap for COI (71%) but a narrower one for 18S (29%) (Table 1). These patterns are further shown by the distribution of species with and without barcode coverage across phyla (Figure 1a).

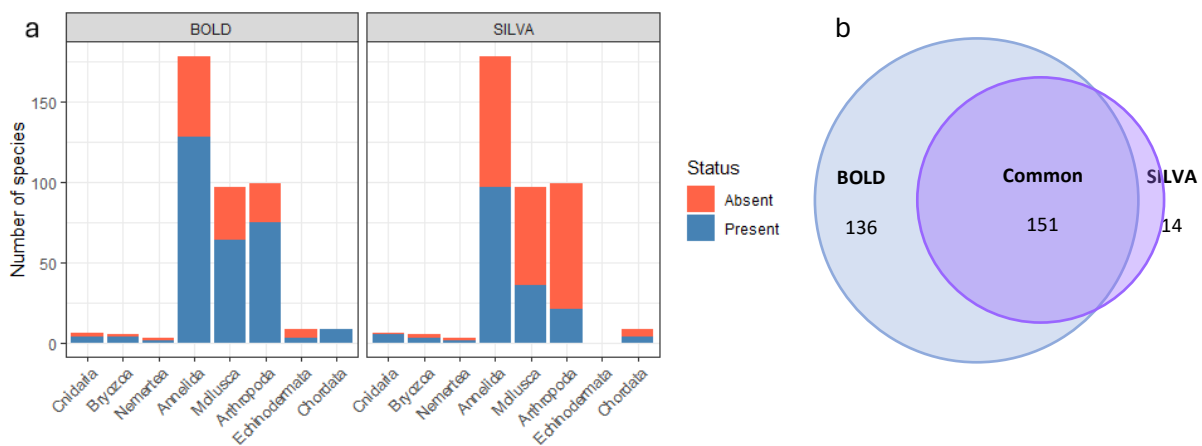


Fig. 1 - Representation of macrozoobenthic species from Sardinian lagoons in reference databases BOLD and SILVA (a) Bars show the number of species with available sequences in genetic repositories ("Present", blue) and those lacking records ("Absent", red) for each phylum; (b) Venn diagram showing the overlap of macrozoobenthic species in the reference databases.

Rappresentazione delle specie macrozoobentoniche delle lagune sarde nelle banche dati di riferimento (a) I grafici a barre mostrano il numero di specie con sequenze disponibili nelle banche dati ("Present", blu) e quelle prive di sequenze ("Absent", rosso) per ciascun phylum; (b) Diagramma di Venn che illustra la sovrapposizione delle specie macrozoobentoniche tra i due database.

The comparison between the two reference databases revealed a substantial overlap, with 151 macrozoobenthic species being represented in both BOLD and SILVA. However, database-specific records were also evident: 136 species were found exclusively in BOLD, whereas only 14 species were unique to SILVA (Figure 1b).

Conclusions - This study provides the first synthesis of macrozoobenthic diversity in Sardinian coastal lagoons and its representation in global genetic repositories. Despite the high taxonomic richness reported across 42 lagoons, our results highlight significant gaps in the availability of DNA barcodes, particularly for Mollusca, Nemertea, and to a lesser extent, Arthropoda. These gaps represent a major limitation for the effective application of eDNA metabarcoding, which depends on comprehensive and curated reference libraries for accurate taxonomic assignment. The uneven coverage across phyla emphasises the urgent need for targeted sequencing efforts to improve representation in databases such as BOLD and SILVA. Addressing these deficiencies will not only increase the reliability of eDNA-based biodiversity assessments but also facilitate their integration into the monitoring frameworks required by the EU Habitats Directive (92/43/EEC).

Moreover, it is important to underscore that morphological identification remains the foundation of biodiversity studies, as it provides the baseline knowledge needed to validate molecular data, describe new species, and resolve ambiguities where reference sequences are unavailable. Therefore, a complementary approach that combines morphological and molecular identification methods is crucial, since biodiversity studies relying solely on metabarcoding may have insufficient resolution, especially at lower taxonomic levels. By bridging molecular and morphological approaches, future biomonitoring programmes can achieve more robust and cost-effective biodiversity assessments. The expansion of genetic libraries, together with strengthening traditional taxonomy, represents a crucial step towards effective conservation and sustainable management of the Mediterranean coastal lagoons.

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