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Advancing subterranean conservation through Global Research on eDNA in Groundwaters (GReG)

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If water is life, then groundwater is its lifeblood—essential, but hidden, it forms the largest reservoir of unfrozen freshwater globally. Populated by highly specialized, often relictual lineages across the tree of life, groundwater ecosystems not only support remarkable taxonomic, functional and phylogenetic diversity (Griebler and Lueders 2009; Martínez et al. 2018; Marmonier et al. 2023), but also play a crucial role in sustaining the functioning of the global water cycle (Griebler and Avramov 2015; Saccò et al. 2024). Consequently, the protection of groundwaters as ecosystems, which can be affected by overextraction, warming or pollution, is essential. Despite this, groundwater is largely absent from policy frameworks and overlooked by large initiatives such as the European Water Framework Directive (Di Lorenzo et al. 2024) that are designed to protect the environment (Fiser et al. 2022, 2025; Griebler et al. 2023). While the human impact on above-ground global biodiversity has triggered widespread conservation responses, subterranean ecosystems continue to suffer from a lack of visibility, research attention, and regulation (Saccò et. al., 2025a). Current global knowledge on groundwater biodiversity and ecological dynamics is far from complete, hindering effective assessment and conservation efforts (Mammola et al. 2024). Regrettably, groundwater species are facing escalating cumulative threats including climate and land use change, contamination, over-extraction, heating and salinization, amongst many others (Manenti et al. 2021; Becher et al. 2022; Couton et al. 2023a; Nanni et al. 2023; Vaccarelli et al. 2023). Left unaddressed, these threats compromise not only biodiversity but also the vast number of services that groundwater ecosystems unnoticeably provide to both nature and human society (Boulton et al. 2008; Griebler and Avramov 2015; Mammola et al. 2025; Saccò et al. 2025b).

The development of conservation strategies for groundwater and groundwater-dependent ecosystems (GDE, ecosystems that rely on groundwater for some or all of their water needs) requires an improved understanding of subterranean ecological dynamics, particularly under ongoing climatic changes (Boulton et al. 2023; Griebler et al. 2023; Vaccarelli et al. 2023). To address these challenges, increased collaborative efforts based on new technologies, scientific rigour, interdisciplinarity and inclusivity are required (Saccò et al. 2025b). In this regard, the analysis of environmental DNA (eDNA) is a technique proving to be an effective bridge connecting researchers, stakeholders and citizens over a myriad of aquatic ecosystems (Deiner et al. 2017; Takahashi et al. 2023; Blackman et al. 2024). Broadly defined as the total pool of DNA isolated from environmental samples (Pawlowski et al. 2020), eDNA has been successful at unveiling biodiversity patterns from regional (Nakagawa et al. 2018) to global scales (Ficetola et al. 2024), and has been applied in taxonomy-free (Apothéloz-Perret-Gentil et al. 2017) and functional (Condachou et al. 2023; Cantera et al. 2025) approaches. Moreover, eDNA is being increasingly combined with other large-scale monitoring tools (e.g., satellite data, Zong et al. 2024) and broader biodiversity datasets (Abarenkov et al. 2023; Takahashi et al. 2025) to advance a rich, diverse research agenda. Concerning groundwater, eDNA research is gaining similar momentum (e.g., Koch et al. 2022; Saccò et al. 2022; Couton et al. 2023a, b; Al-Rashidi et al. 2025; Guzik et al. 2025a, b), despite a lag in protocol validation, application, and DNA-assay development compared to other biomes (e.g., oceans or rivers - Altermatt et al. 2025).

With the intention of disrupting the current inertia in the global conservation of groundwater ecosystems - where many calls for action have gone unheeded (e.g., Sánchez-Fernández et al. 2021; Wynnee et al. 2021; Di Lorenzo et al. 2024) - we present here a research initiative defined by the acronym of GReG: Global Research on eDNA in Groundwaters. The first pitch for this collaborative global eDNA study in groundwater ecosystems was proposed during the 26th International Conference on Subterranean Biology (ICSB) held in Sardinia in September 2024. Nine months later, on the 5th of June 2025, GReG was launched *via* an online seminar, led by Dr Mattia Saccò, Dr Michelle Guzik and Dr Kathryn Korbel. A gathering of 56 representative researchers and regulators across the globe took place, and guest speakers provided insights regarding global eDNA studies in oceans (Citizens of the Sea; Pochon et al. 2025), open and FAIR (Findable, Accessible, Interoperable and Reusable) data practices for sharing eDNA data (Takahashi et al. 2025), taxon-free eDNA approaches in groundwater studies, and how to utilize eDNA to address global biodiversity targets (Altermatt et al. 2025). The second part of the seminar was dedicated to the planning of a self-funded project in collaboration with partners such as Biodiversa+ projects (DarCO - Mammola et al. 2024; Sub-BioMon - Zagmajster et al. 2024), research laboratories (TrEnD Lab - https://research.curtin.edu.au/scieng/research/trend-lab/; Altermatt Lab - https://www.altermattlab.ch/) and global datasets (Stygofauna Mundi - Martínez et al. 2018).

Planned to be carried out in two phases, GReG is conceived as a unifying platform for researchers and diverse stakeholders from government and industry to meet and significantly advance groundwater science primarily through eDNA and genomics. Phase 1 (June 2025–June 2026) will involve fieldwork at over 50 well-known sites (karst, alluviums and anchialine caves) across four continents, using standardized sampling and analytical protocols targeting biodiversity across the entire Tree of Life. In addition, a survey on methodological approaches in groundwater eDNA research has been circulated to all the collaborators, aimed at understanding current practices and limitations for eDNA analysis in groundwater environments. Further projected research outputs include peer-reviewed papers on groundwater eDNA protocols and publicly available DNA barcode quality assessments for stygofauna. Phase 2 (from July 2026) will build on the lessons learned from Phase 1 to expand the project to the Global South and other under-investigated areas of the world *via* participatory science approaches. This Forum Paper also serves as an opportunity to make a public call to the researchers and stakeholders from those regions, with an ambition of widening novel groundwater-based research beyond well-known global biodiversity hotspots. The outcomes derived from this collaborative effort will involve scientific publications, data sharing following the FAIR eDNA guidelines, workshops and media articles, with the objective to advance subterranean aquatic eDNA research. Last but not least, GReG also aims to raise awareness regarding the importance of groundwater ecosystems - a notoriously arduous task for an out of sight, out of mind environment (Boulton et al. 2023).

A shared vision amongst GReG collaborators is that the key to effectively preserving unique subterranean biota while using groundwater resources sustainably is not yet lost; we just need the right team of locksmiths (researchers, regulators, stakeholders, citizens) to come together and cut the right set of biomonitoring keys and conservation guidelines. GReG and its multidisciplinary approach provide the first step towards preserving subterranean biota at the global level, and thus utilising groundwater resources sustainably—an ambitious but vital goal for future water security.

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