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Global assessment of aquatic *Isoëtes* species ecology

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Abstract

1. *Isoëtes* are iconic but understudied wetland plants, despite having suffered severe losses globally mainly because of alterations in their habitats. We therefore provide the first global ecological assessment of aquatic *Isoëtes* to identify their environmental requirements and to evaluate if taxonomically related species differ in their ecology.

2. The assessment resulted in an extensive new database on aquatic *Isoëtes*, ecological niche analyses, and descriptive species accounts. We compiled a global database that includes all known environmental data collected from 1935 to 2023 regarding aquatic *Isoëtes*. We then evaluated the environmental drivers of 16 species using 2,179 global records. Additionally, we used hypervolume analysis to quantify the ecological niches of the two species with the greatest number of records, finding significant differences and evidence that *Isoëtes echinospora* occupies a wider ecological niche than *Isoëtes lacustris*.

3. Fifty-nine species (30% of the *c.* 200 *Isoëtes* species known today) were categorised as aquatic and were mainly reported in the Americas and northern Europe. About 38% of the aquatic species are threatened with extinction or are endemic to a small region, according to the International Union for Conservation of Nature's

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Red List in 2023. Many species were determined to be sensitive to certain water physical and chemical factors, generally preferring oligotrophic conditions such as low total phosphorus, moderate total nitrogen, moderate to low pH, and low conductivity.

4. This analysis includes ecological data in the assessment of rare/threatened aquatic plants globally. This new database and the ecological analyses completed defined the ecological requirements of several species and identified knowledge gaps, which can aid management actions and future research.

5. This paper highlights ecological significance and environmental sensitivities of aquatic *Isoëtes*. The current level of knowledge is inadequate for a large proportion of known taxa. We affirm the extreme need to support global, collaborative initiatives on which to build future conservation strategies.

1 | INTRODUCTION

Species within the genus *Isoëtes*, commonly known as quillworts, are plants that are detected globally. They are vascular, heterosporous lycophytes belonging to the class *Lycopodiopsida* (PPG I, 2016), sharing a simple morphology consisting of: a compact stem portion (corm); leaves that are typically arranged in a spiral and form a rosette; sunken sporangia; and leaves with four gas-filled chambers and one large ligula (Taylor & Hickey, 1992). *Isoëtes* is the only genus of the *Isoëtaceae* family with modern-day forms already present during the Jurassic (Pigg, 2001). The age of the living group of *Isoëtes* is highly debated (Larsén et al., 2022; Wikström et al., 2023). The genus is the only survivor of an ancient group of plants that diverged from its living sister group *Selaginella* (=*Selaginellales*) in the Late Devonian at 359–383 million years ago (Kenrick & Crane, 1997), but the estimated ages of the living genus are most likely to be from the Jurassic–Cretaceous boundary (Larsén & Rydin, 2016; Pereira et al., 2017) or Cenozoic (Pereira et al., 2021; Wood et al., 2020).

Because of difficulty in interpreting their phenotypic plasticity and subtle and sometimes variable morphological distinctions, *Isoëtes* taxonomy was substantially neglected until recent years. At the end of the 19th century, 46 species were known (Baker, 1880). The most recent global enumeration lists 188 accepted taxa, with South America hosting the highest diversity of this genus (Troia et al., 2016). Recent findings (e.g., Brunton et al., 2021; Pereira & Prado, 2022) increase *Isoëtes* taxonomy diversity to more than 200. The database *Plants of the World Online* from the Royal Botanical Gardens, Kew, reports 197 accepted species (POWO, 2024).

The genus was first described in 1751 by Linnaeus, based on observations of the morphological and ecological characteristics of *Isoëtes lacustris* ("*habitat in Europae frigidae fundo lacuum*"—living at the bottom of cold European lakes). Botanists who described new species throughout the 19th century began to point out a much wider ecological niche of the genus than that outlined by Linnaeus (Baker, 1880; Engelmann, 1882; Montelay & Vendryes, 1883). The only monograph on the genus *Isoëtes* was written in 1922 by Norma Etta Pfeiffer (Pfeiffer, 1922) that formalised the distinction of the three ecological categories proposed by Engelmann (1882): submersed (i.e., aquatic), amphibious, and terrestrial. This study focuses on aquatic *Isoëtes* species, which are defined as aquatic species living in perennial waterbodies (such as rivers, lakes, or perennial ponds) that only produce spores if the sporophyte is permanently submerged. Amphibious species are those that are adapted to live in environments where water is present for only part of the year (such as temporary ponds, vernal pools, and swales), and thus can live submerged for long periods, but can also produce spores when they emerge. Finally, terrestrial species are all those that can live submerged for short periods during the wet/colder season but do not benefit or require submergence to produce spores. Theses definitions are based on those suggested by Pfeiffer (1922) and Troia and Greuter (2015). We prefer the term *aquatic* over *submerged* as used in Pfeiffer (1922) to denote this group of taxa because not all aquatic *Isoëtes* live submerged in water throughout the year and can partially emerge at the end of the dry season. Even though *Isoëtes* are adapted to different edaphic conditions and are documented in many different habitats worldwide (except for Antarctica), they are closely associated with waterlogged conditions. Like all lycophytes, the male gametes (i.e., antherozoid) must cross water and *swim* to reach the archegonia to complete reproduction (La Motte, 1937). Accordingly, both the sporophyte and gametophyte require water during fertilisation (Hilger et al., 2002).

Aquatic ecosystems are threatened worldwide (Dudgeon, 2019; Reid et al., 2019; Westveer et al., 2022) and have the highest rates of decline among other ecosystems in terms of area and biodiversity. By 2000, only 13% of the wetlands present in the 1700s remained (IPBES, 2019) and thus aquatic *Isoëtes* species are of conservation concern due to habitat loss. Some *Isoëtes* are

very rare and presented in only in a single site (e.g., *Isoëtes cangae*, *Isoëtes sabatina*, *Isoëtes taiwanensis*; Pereira et al., 2016; Troia & Azzella, 2013; Yu et al., 2013, respectively), and many have seen their habitat range shrink to a few known populations (e.g., *Isoëtes boryana*, *Isoëtes malinverniana*, *Isoëtes philippinensis*; Abeli et al., 2020; Amoroso et al., 2022; Bertrin et al., 2013, respectively). Other species have a wide distribution, such as *I. lacustris* (s.l.) and *Isoëtes echinospora*, which are reported throughout the Holarctic region and are often used as aquatic bioindicators (e.g. Romero & Onaindia, 1995; Schaumburg et al., 2007; Seddon, 1972). Based on those latter two relatively common species, Den Hartog and Segal (1964), while classifying macrophytes' life forms, identified isoëtids as "rhizophytes with a short stem, a rosette of stiff radical leaves and with or without stolons." Other authors later referred to this category of macrophytes (small, slow-growing, generally evergreen species with thick leaves and a proportionately large below-ground biomass; Boston et al., 1987; Hutchinson, 1975) when describing aquatic plants showing a shape similar to that of the *Isoëtes*. Thus, species such as *Eleocharis acicularis* (L.) Roem. & Schult., *Littorella uniflora* Asch., *Lobelia dortmanna* L., and *Subularia aquatica* L. fall into the isoëtid category. Subsequent studies indicated that isoëtids and *Isoëtes* species are linked to well-defined ecological conditions. Jensen (1979) classified lakes in southern Sweden and noted that isoëtids dominate under conditions of highly transparent water and low conductivity and pH (soft waters), which he called *isoëtids-type lakes*. This categorisation of lakes was then widely used in the analysis of northern European lakes (e.g., Free et al., 2009). In the framework of Habitat Directive 92/43/EEC, isoëtids and *Isoëtes* species are characteristics of a habitat of European interest, protected under Habitat Directive 92/43/EEC, the "oligotrophic waters containing very few minerals of sandy plains (*Littorelletalia uniflorae*)" (code 3110).

Many authors have related the *Isoëtes* and isoëtid vegetation to soft-water oligotrophic conditions (e.g., Rørslett & Brettum, 1989; Smolders et al., 2002; Vöge, 2004) and low carbon dioxide (CO_2) availability in water, to which the plants have adapted by developing crassulacean acid metabolism photosynthesis (Gacia & Ballesteros, 1993; Keeley, 1981; Wickell et al., 2021), high porosity of root aerenchyma (Smits et al., 1990), elevated root biomass and slow leaf turnover (Gacia & Ballesteros, 1994), and arbuscular mycorrhizal symbiosis (Sudová et al., 2020). As an indirect result, isoëtids provide ecosystem services such as sediment stabilisation and radial oxygen loss, favouring aerobic processes including methanotrophy and phosphorus precipitation (Ribaudo et al., 2017; Smolders et al., 2002). Water temperature could regulate spore germination: *I. echinospora* spores need at least 3 months of cold stratification to break megaspore dormancy and germinate (Čtvrtlíková et al., 2012). In contrast, *I. sabatina* has adapted to the warm waters of a Mediterranean lake, and in an ex-situ germination experiment, no cold period was needed (Magrini et al., 2020). Edaphic characteristics are poorly investigated, but some studies have pointed out that the availability of dissolved organic carbon and inorganic carbon (as CO_2) is very important for aquatic *Isoëtes* growth (Lucassen et al., 2012; Madsen et al., 2002).

Many of the *Isoëtes* habitats have been lost or are at risk of degradation. Among the anthropogenic impacts, the increase of sedimentary organic matter favours the substitution of different communities, for example, in Europe, dominated by *Juncus bulbosus* L. (Bertrin et al., 2018; Smolders et al., 2002). The creation of swimming areas and associated shoreline is also recognised as potentially damaging for *Isoëtes* vegetation within shallow areas (Bertrin et al., 2018; Mochalova et al., 2015). In gentle sloped lakes, water-level drawdowns are expected to frequently occur under various climate change scenarios and could substantial negatively affect isoëtids (Hellsten, 2002). Further, eutrophication of freshwaters worldwide may negatively affect many *Isoëtes* species that are sensitive to high nutrient concentrations (Klimaszyk et al., 2020).

An ecological reference framework for the aquatic species is necessary to inform *in-situ* and *ex-situ* conservation programmes (Prado et al., 2023) given the concerning conservation status of many taxa. As of 2024, the International Union for Conservation of Nature (IUCN) Red List includes 68 assessed quillworts (out of the total of about 200 species), so more than 60% of species are unstudied for conservation status assessment. Further, 26 species are considered threatened (IUCN status: vulnerable, endangered) and 12 are critically endangered, which is more than 20% of all *Isoëtes* species known today (also see Table 1).

Our study reviews global ecological knowledge concerning aquatic *Isoëtes* species and provides an updated list of those that can be considered *aquatic*. In taking this on, an updated global map of aquatic taxa based on the latest available ecological data is provided. Secondly, we have formalised a Global aquatic *Isoëtes* Database (acronym GaID) that contains both published and unpublished data (i.e., grey literature) on the physical and chemical conditions of freshwaters hosting aquatic *Isoëtes*. Our paper aims to explore whether the current body of knowledge is robust enough to confidently assert that we have a comprehensive understanding of the ecological requirements of aquatic *Isoëtes* species. Alternatively, we aim to investigate if there are nuanced differences among the species' requirements that were not detected in prior studies. Based on available data, we aim to answer specific questions: which water and substrate features are shared or unique among aquatic *Isoëtes* species? Do all aquatic quillworts prefer slightly acidic, oligotrophic soft waters? Are there ecological factors explaining the presence of different species in the same waterbody? Answering these questions is important for the preservation of these species and understanding the ecological drivers that may have contributed to differentiation and phylogenetic diversity.

2 | DATA AND METHODS

2.1 | Species selection and taxonomy

We developed a database, called GaID, containing records collected between 1935 and 2023 for aquatic quillwort species reported in Table 1 (the GaID and the appendixes are available in Larson

TABLE 1 List of aquatic species belonging to the genus *Isoëtes*.

Species	Status	Type of waterbody	Distribution
Isoëtes alpina T. Kirk	NE	Lakes	New Zealand
Isoëtes asiatica (T. Makino) T. Makino	NE	Lakes	Far Eastern Russia–Japan
Isoëtes azorica Durand In: Milde, Fil. Eur.: 278. 1867	VU	Lakes	Azores
Isoëtes baculata R. J. Hickey & H.P.Fuchs	NE	Rivers	Colombia-Brazil border
Isoëtes bolanderi G. Engelmann	LC	Lakes, rivers	Western U.S.A.–Canada
Isoëtes boryana M. Durieu	EN	Lakes	Southwestern France
[a] *Isoëtes cangae* J. B. S. Pereira, Salino & Stützel	CR	Lakes	Brazil (Pura State)
Isoëtes chubutiana Hickey, Macluf & W. C. Taylor	NE	Streams and lakes	Chile–Argentina
[a] *Isoëtes cipoensis* J. B. S. Pereira	NE	Lakes	Brazil (Minas Gerais state)
Isoëtes creussensis J. J. Lazare & S. Riba	LC	Alpine lakes	Pyrenees (Spain, France, Andorre)
Isoëtes cubana G. Engelmann Ex Baker	NE	Shallow ponds	Belize–Cuba
[a] *Isoëtes dubsii* J. B. S. Pereira	NE	Ponds	Brazil (Mato Grosso do Sul)
Isoëtes echinospora M. Durieu	LC	Lakes	Europe; western Asia
Isoëtes elatior F. Mueller & A. Braun	NE	Rivers	Australia (Tasmania)
Isoëtes fluitans M. I. Romero	EN	Rivers	Spain
[a] *Isoëtes fuliginosa* R. L. Small & R. J. Hickey	NE	Shallow lakes	Colombia
Isoëtes gigantea U. Weber	NE	Rivers and streams	Brazil (Bahia State)
Isoëtes gunnii A. Braun	NE	Lakes and river	Australia (Tasmania)
Isoëtes habbemensis Alston	NE	High altitude lakes	Indonesia
Isoëtes heldreichii Wettstein	CR	Lakes	Greece
Isoëtes hemivelata R. L. Small & R. J. Hickey	NE	Lakes	Colombia
Isoëtes humilior F. Mueller ex A. Braun	NE	River and lakes	Australia (Tasmania)
Isoëtes hypsophyla Hand.-Mazz	NE	Ponds and marshes	Southeastern Tibetan Plateau of China
Isoëtes japonica A. Braun	NE	Lakes	Japan
Isoëtes karstenii A. Braun	NE	Ponds and lakes	North Andes (Colombia)
Isoëtes kirkii A. Braun	NE	Lakes	New Zealand
Isoëtes lacustris Linnaeus	LC	Lakes	Europe
Isoëtes laurentiana D. F. Brunton	NE	Tidal marshes	Canada (Quebec)
[a] *Isoëtes longifolia* J. B. S. Pereira & Prado	NE	Rivers	Brazil (Bahia state)
[a] *Isoetes longpingii* Y. H. Z. Yan, Y. F. Gu & J. P. Shu	NE	Pools	China (Hunan Province)
Isoëtes macrospora M. Durieu	NE	Lakes	Northern North America
Isoëtes malinverniana Cesati & De Notaris	CR	River and streams	Northern Italy
Isoëtes maritima L. Underwood	LC	Fresh to slightly brackish lakes	Western Canada, Alaska–Far Eastern Russia
Isoëtes mattaponica L. J. Musselman & W. C. Taylor	NE	Tidal rivers	U.S.A. (eastern VA)
Isoëtes maxima R. J. Hickey, C. Macluf & M. Link-Pérez	NE	Pools	Brazil (Rio Grande do Sul)
Isoëtes mourabaptistae J. B. S. Pereira, P. G. Windisch, Lorscheitt. & Labiak	NE	Rivers	Southern Brazil
Isoëtes muelleri A. Braun	NE	Rivers and lakes	Australia
Isoëtes neoguineensis Baker	NE	Lakes	Papua New Guinea
Isoëtes occidentalis L. F. Henderson	NE	Lakes	Pacific Coast (Canada – U.S.A.)
Isoëtes ovata N. Pfeiffer	NE	Rivers	Brazil, French Guiana, Guyana
Isoëtes philippinensis E. D. Merrill & L. M. Perry	NE	Rivers	Philippines and Indonesia
Isoëtes pirinica D. F. Brunton, D. Ivanova & P. C. Sokoloff	NE	Subalpine lakes	Bulgaria
Isoëtes prototypus D. M. Britton	NT	Lakes	Eastern Canada–adjacent U.S.A.

(Continues)

TABLE 1 (Continued)

Species	Status	Type of waterbody	Distribution
Isoëtes quiririensis J. B. S. Pereira & P. H. Labiak	NE	Rivers and streams	Brazil (Santa Catarina state)
Isoëtes riparia G. Engelmann Ex A. Braun	NE	Tidal flats and rivers	U.S.A. (East Coast)
[a]***Isoëtes sabatina*** A. Troìa & M. M. Azzella	CR	Lakes	Italy
Isoëtes savatieri Franchet	NE	Streams and lakes	Argentina-Chile
Isoëtes septentrionalis D. F. Brunton	NE	Rivers and Lakes	Canada-U.S.A.
Isoëtes storkii T. C. Palmer	NE	Lakes	Costa Rica
Isoëtes taiwanensis De Vol	CR	Lakes	Taiwan
Isoëtes tennesseensis Luebke & Budke	NE	Rivers	U.S.A. (TN)
Isoëtes tenuissima Boreau	NE	Ponds	Central France
Isoëtes tripus A. Braun	NE	Pools	Southeast Australia
Isoëtes tuckermanii A. Braun ssp. *acadiensis* (L. Kott) D. F. Brunton	NT	Rivers and lakes	Eastern U.S.A.–Canada
Isoëtes tuckermanii A. Braun Ex Engelmann	LC	Rivers and lakes	Eastern U.S.A.–Canada
Isoëtes vermiculata R. J. Hickey	NE	Streams	Venezuela
[a]*Isoëtes viridimontana* M. A. Rosenthal & W. C. Taylor	NE	Lake	Vermont
Isoëtes weberi W. Herter	NE	Ponds	Brazil-Uruguay
Isoëtes wormaldii R. Sim	CR	Ponds	South Africa

Note: Conservation status, according to the IUCN Red List, type of waterbody colonised, and distribution are given for each species. The species for which ecological data were also collected are listed in bold.

Abbreviations: CR, critically endangered; EN, endangered; LC, least concern; NE, not evaluated; NT, near threatened; VU, vulnerable.

[a]Identifies species for which only one site is known.

et al., 2024; see appendixes *01_Aquatic Isoëtes* for the justification of the species selection). The taxonomy of *Isoëtes* species has undergone many changes over this time frame. Species names used in this paper refer to the most recent and comprehensive taxonomic updates (POWO, 2024). *Isoëtes creussensis* data, for example, were collected from populations attributed to *I. lacustris* until 2020 (Brunton et al., 2021). Thus, the original scientific work did not refer to *I. creussensis* but to *I. lacustris* (Ballesteros et al., 1989; Gacia et al., 2018). Most of the GaID records are part of previous databases made available by the authors of this study. Three hundred and two records are from a global macrophytes database already used for global community composition assessments (Alahuhta et al., 2017), where species were recorded using standardised national protocols. Seven hundred and twenty records belong to the Norwegian Institute on Water Research (NIVA) database. Five hundred and seventy-six records were collected from the State of Wisconsin, U.S.A., using standardised protocols (Hauxwell et al., 2010; WIDNR, 2021) and merged with lake habitat assessment data according to the same collection site and date (APPI [Aquatic Plants PI Database], 2023; SWIMS, 2023). In the latter database, the records registered as *I. macrospora* from Wisconsin in the GaID, in the original source, are registered as *I. lacustris*. Moreover, in 376 cases, the determination does not reach the species level, and therefore, data should be associated with both species (*I. macrospora* and *I. echinospora*). The Appendixes *02_Data references* contains the complete list of bibliographic references from which the data presented here were retrieved.

2.2 | Ecological data collection and construction of the global aquatic *Isoëtes* database (GaID)

Ecological data were obtained from scientific literature retrieved through a Google Scholar web search engine query (https://scholar.google.com/) using the word "Isoëtes" associated with "ecology", "pH", "conductivity", and "depth" (e.g., "Isoëtes AND ecol*", "Isoëtes AND pH", "Isoëtes AND conductivity", "Isoëtes AND depth"). Ecological information was also obtained by the core study-proposing group (composed of the Italian authors), who contacted experts in quillwort taxonomy and ecology using the published literature as a guide, personal contacts, and cross contact networks. All the respondents are coauthors of the study. Targeted environmental data included in the GaID are geographic setting (latitude, longitude, altitude of the site, and minimum and maximum growing depth), water variables (pH, conductivity, Secchi disk depth, alkalinity, dissolved oxygen concentration, dissolved carbon dioxide concentration, concentrations of total phosphorus [TP], total nitrogen [TN], ammonia, nitrate, silicate, and ions), and edaphic characteristics (pH, porosity, density, TP, organic matter, and organic carbon concentration). To be included in the GaID, each information set must offer species data and some of the environmental data. Accordingly, we excluded records with only species occurrence data, such as those commonly documented via herbarium specimens.

The GaID data set and metadata are permanently and publicly archived (Larson et al., 2024). The data set was constructed so that

it can be easily updated with new information in the future. We encourage the data to be used for new scientific and conservation purposes and request that users contact the authors who uploaded the data for collaborations.

2.3 | Data analyses

The data collected in GaID are heterogeneous because, unfortunately, not all scientific literature on the ecology of the *Isoëtes* genus contains information that can be used for data analyses because data are presented in graphical form or as averaged data (e.g. Rørslett & Brettum, 1989; Vestergaard & Sand-Jensen, 2000). Each record refers to the presence of one *Isoëtes* species; thus, the data are of the *presence/absence* type. Data on species abundance, cover, and frequency of occurrence were not collected because the methods for evaluating these metrics were heterogeneous. For ecological variables, only data referring to measurements of water or substrate variables made within a single waterbody and a narrow time frame (either one sample or an average of samples collected within 2 years at the same waterbody where the presence of an aquatic *Isoëtes* has been reported) were used for statistical analysis. Average data referring to many waterbodies and different years were therefore excluded from the analyses but recorded in the data set. To assess the difference among species in the physical and chemical variables of water and substrate, and thus answer the first three questions posed in the introduction, a nonparametric Kruskal–Wallis test was completed, followed by a post hoc Dunn test after assessing that normality and homogeneity assumptions of ANOVA were not met. Species with only one record could not be considered in the analysis. Then, the concept of ecological niche was applied (Devictor et al., 2010) to the two species holding the greatest number of records (*I. echinospora*, *I. lacustris*) to explore how these species occupy the environmental space. The results are presented as an analysis of the *ecological space of the species* and not of a strictly *ecological niche analysis* because we only had sufficient data for five environmental variables on which this analysis was completed. In fact, the concept of niche is more complex, and it would be reductive to refer to it by analysing only a few ecological variables in the results. The environmental variables were selected with the aim of maximising the number of records to be included while maintaining a balanced number of observations between the two species. Then, collinear variables were omitted and a principal component analysis (PCA) was carried out with the remaining variables, which were centred and scaled. Water pH, conductivity, and ammonium, TN, and TP concentrations were selected for the ecological space analysis. We used 148 records for *I. echinospora* and 124 records for *I. lacustris*. The first three PCA axes (together explaining more than 80% of the variation in the data) were selected to calculate the ecological niche size and the uniqueness of the two target species, namely the portion of the niche not shared with the other species. The hypervolume approach for niche size and uniqueness was implemented using the *hypervolume* package in R (Blonder et al., 2023). We used the

preferred Gaussian kernel method, which is considered the most appropriate for niche applications because it uses elliptic random sampling to create clouds of data points around the observed data points to build the niche (Blonder et al., 2018). To obtain relative values rather than absolute values of niche size and uniqueness, we used the *hypervolume_n_occupancy* function to subset hypervolumes of each species to the same random density. Mean absolute error and root mean squared error were used to evaluate the accuracy of the subset hypervolumes (Laini et al., 2023). The relative niche size was calculated as the absolute volume of each species divided by the volume of the union of both hypervolumes. Relative niche uniqueness was calculated as the absolute niche uniqueness of a species divided by its absolute niche size. To obtain uncertainty measures of niche size and uniqueness, bootstrapping was implemented on 199 permutations (to optimise the accuracy of results and computation performance) of each species niche, which were then used to calculate 199 relative total occupancies. Significant differences of niche size and uniqueness between species was assigned by comparing quantiles with a significance level of $\alpha = 0.05$. Graphical representations of all results were created with packages *ggplot2* (Wickham, 2016), *ggbiplot* (Vu, 2011), and *rgl* (Murdoch & Adler, 2023). All analyses were carried out in the R environment (R Core Team, 2022).

2.4 | Specific descriptions

Descriptive sheets of the 16 species for which ecological data could be collected were made and are provided in the Appendixes *04_Species descriptions*. The sheets also include brief descriptions of the habitats in which the species live, observations not included in the GaID (for example, the growing depth of the species is generally roughly estimated and not systematically measured), and references to their conservation status.

3 | RESULTS

3.1 | Towards the GaID

A total of 2,179 records are stored in the data set (Larson et al., 2024) and refer either to: (1) measurements directly collected at the station (population) of the species (228 cases); or (2) values collected within a single waterbody (1,935 records) or multiple waterbodies (17 records) where the target species is reported. In the latter case, these are exclusively data obtained from the literature and not subjected to statistical analysis. The literature search produced 150 records; thus, most of the records were unpublished data (1,367 records) or public data belonging to an existing database devoted to a monitoring system (655 records), and only a few records (seven) belong to grey literature. Most confirmed aquatic species are from the Americas (19 in South America and 12 in North America). According to the IUCN Red List in 2024, six aquatic species that we included in the GaID (Table 1) are critically endangered. In addition, seven

species are known to occur at only one site. Figure 1 shows the geographical distribution of the records included in the GaID.

Overall, ecological data with at least one ecological driver could be retrieved for only 16 species (bolded species names in Table 1) of the 59 species selected as aquatic. Only one record is available for two of the species (*I. philippinensis* and *Isoëtes maritima*). Moreover, the record for *I. maritima* refers to exceptional lake conditions because of the human-made inflow of geothermal water and probably is not representative of the species' requirements. The species with the largest number of records are *I. echinospora* (779 records) and *I. lacustris* (665 records). Among the various variables capable of describing the physical and chemical characteristics of an aquatic ecosystem, water pH and conductivity were the most measured, showing at least one measured value for all the species investigated. Other available data for a representative number of species are the concentrations of NH_4^+ (11 species), Ca^{2+} and Cl^- ions (10 species), alkalinity, TP, and TN (nine species; Figure 2). Table 2 shows, for each species, the mean values and standard deviation of the minimum and maximum depth of colonisation, pH, conductivity, alkalinity, dissolved oxygen, Secchi disk, dissolved carbon dioxide, and the concentrations of TN, TP, ammonium, nitrate, phosphate, chlorophyll, Ca^{2+}, Mg^{2+}, Na^+, K^+, SO_4^{2-}, and Cl^-.

The ecological characteristics of sediments turn out to be the least known. The percentage composition (sand, silt, and clay content) is estimated for only five species (*Isoëtes asiatica*, *I. cangae*, *I. echinospora*, *I. lacustris*, and *I. malinverniana*). Data on organic matter content, porosity, and density were determined only for *I. boryana* and *I. sabatina*. Only 22 records referring to *I. lacustris* and *I. echinospora* report organic carbon percentage in the sediment.

3.2 | Global ecological characterisation of aquatic *Isoëtes*

Eleven of the 16 aquatic *Isoëtes* species assessed prefer approximately neutral water pH conditions (between 6.50 and 7.50 pH units). Documented exceptions are *I. malinverniana* (mean 7.51 pH units), *I. boryana* (mean 7.62), and *I. sabatina* (mean 8.53), which seem to colonise slightly alkaline waters, and *I. cangae* (4.90), typical of acidic waters. Analysis of variance among species associations with pH is statistically significant ($\chi^2_{[df=15]} = 184.4$, $p < 2.2^{-16}$). The difference of *I. sabatina* from the other species, tested with the post hoc Dunn test, is statistically significant, except against *Isoëtes alpina*, *Isoëtes azorica*, *Isoëtes bolanderi*,
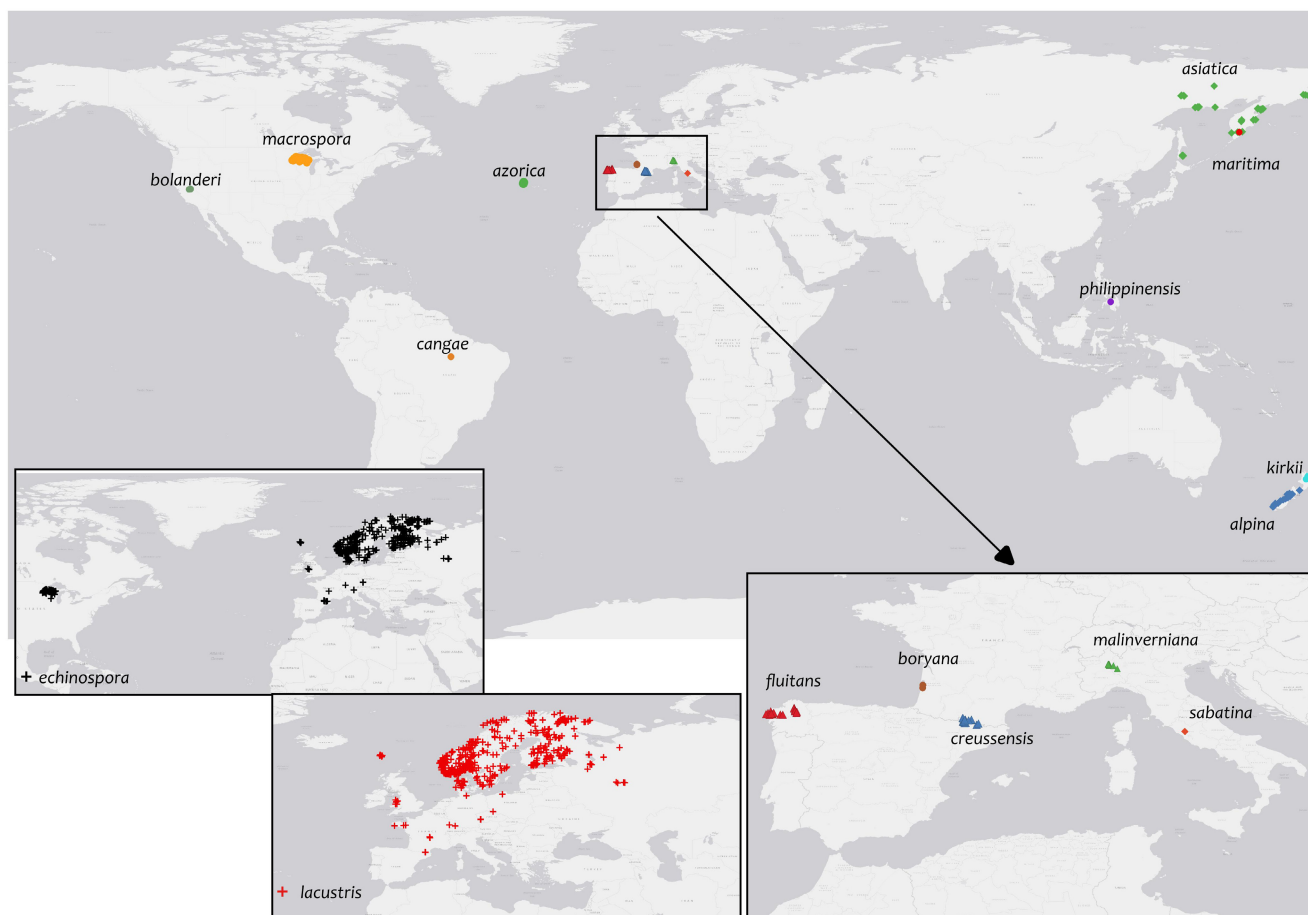

FIGURE 1 The geographical distribution of the *Isoëtes* species records included in the Global aquatic Isoëtes Database (GaID) as of 2024.
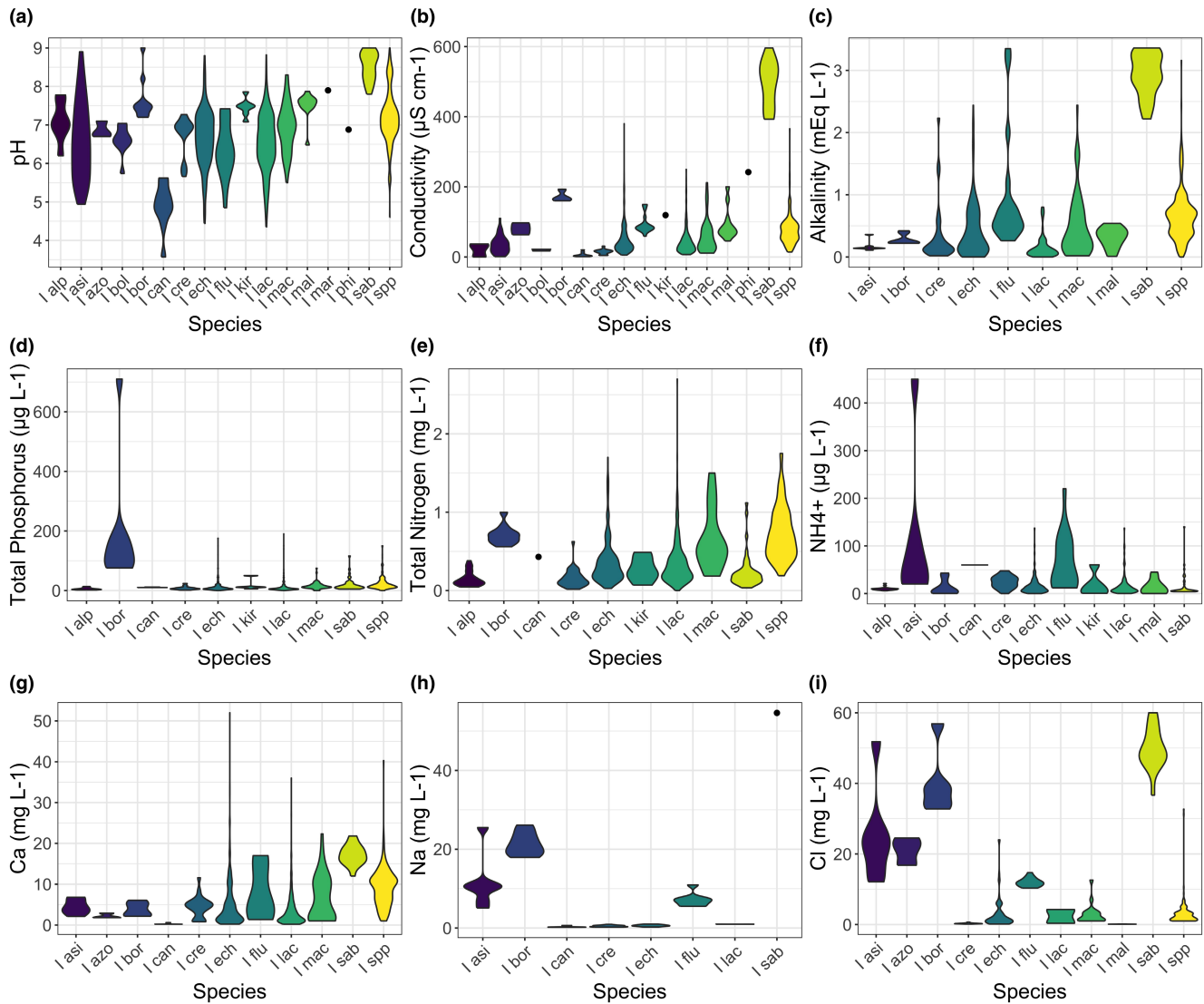
FIGURE 2 Violin plots representing variation of main environmental variables for each species. *I alp* = *Isoëtes* (*I.*) *alpina*, *I asi* = *I. asiatica*, *I azo* = *I. azorica*, *I bol* = *I. bolanderi*, *I bor* = *I. boryana*, *I can* = *I. cangae*, *I cre* = *I. creussensis*, *I ech* = *I. echinospora*, *I flu* = *I. fluitans*, *I kir*, *I. kirkii*, *I lac* = *I. lacustris*, *I mac* = *I. macrospora*, *I mal* = *I. malinverniana*, *I mar* = *I. maritima*, *I phi* = *I. philippinensis*, *I sab* = *I. sabatina*, *I spp* = *I. species plures*.

Isoëtes kirkii, and *I. malinverniana* (Figure 3). Similar results were obtained for *I. cangae* (pH values are significantly different from those of other species except for the comparisons with *I. azorica*, *I. bolanderi*, and *Isoëtes fluitans*) and *I. malinverniana* (pH values are significantly different from those of *I. asiatica*, *I. cangae*, *I. echinospora*, *I. fluitans*, and *I. lacustris*). There is not a significant difference between *I. echinospora* and *I. asiatica* (from the *I. echinospora* species complex) and *I. lacustris* and *I. macrospora* (the North American species from the *I. lacustris* complex).

Regarding water conductivity, most species live in water with low conductivity (Figure 2; mean values <100 μS/cm), except for *I. boryana* (mean 172 μS/cm), *I. sabatina* (495 μS/cm), and *I. maritima* (although we have only one value for the latter species, equal to 775 μS/cm). Analysis of variance (ANOVA) among species associations with conductivity is statistically significant ($\chi^2_{[df=14]} = 264.68$,

$p < 2.2^{-16}$). Many specific differences tested with the post hoc Dunn test are significant (Figure 3), but there are no significant differences between *I. lacustris* and *I. macrospora* or between *I. asiatica* and *I. echinospora*.

The data collected identify aquatic *Isoëtes* species able to grow in waters with a relatively high concentration of chloride ions ([Cl⁻] > 20 mg/L; *I. asiatica*, *I. azorica*, *I. boryana* and *I. sabatina*) and species adapted to concentrations below 20 mg/L (*I. creussensis*, *I. echinospora*, *I. fluitans*, *I. lacustris*, *I. macrospora* and *I. malinverniana*). Overall, the ANOVA confirms that the variance among species is statistically significant ($\chi^2_{[df=9]} = 148.48$, $p < 2.2^{-16}$), but not all Dunn's post hoc tests are significant (Figures 2 and 4).

Aquatic *Isoëtes* are confirmed to prefer waters with low TP concentrations with a global average of 15.8 μg/L TP, and average species values ranging from 5.8 by *I. alpina* to 181 μg/L TP by *I. boryana*,

TABLE 2 Summary of ecological data collected in the Global aquatic Isoëtes Database y, variables for which at least four values are reported.

Species	Records	Altitude (m)	Min depth colonisation (m)	Max depth colonisation (m)	Temperature (°C)	pH
Isoëtes alpina	23	627 (148)	0.4 (0.5)	6.0 (2.8)	No data	7.09 (0.53)
Isoëtes asiatica	50	174 (327)	0.2 (0.3)	1.1 (0.7)	18.8 (2.3)	6.61 (1.02)
Isoëtes azorica	3	No data	No data	No data	No data	6.87 (0.21)
Isoëtes bolanderi	10	2672 (245)	No data	No data	17.3 (4.2)	6.61 (0.37)
Isoëtes boryana	29	15 (9)	No data	No data	18.2 (5.3)	7.62 (0.53)
Isoëtes cangae	11	701	No data	7.0	27.5 (1.0)	4.90 (0.58)
Isoëtes creussensis	33	2248 (98)	0.5 (0.4)	3.6 (1.1)	No data	6.73 (0.51)
Isoëtes echinospora	696	332 (547)	0.4 (0.2)	0.9 (0.6)	15.0 (3.4)	6.69 (0.70)
Isoëtes fluitans	18	311 (100)	No data	No data	16.9 (2.1)	6.39 (0.72)
Isoëtes kirkii	12	242 (90)	0.1 (0.1)	6.2 (1.2)	16.5 (0.8)	7.46 (0.24)
Isoëtes lacustris	579	236 (327)	0.5 (0.5)	2.6 (1.3)	14.9 (4)	6.59 (0.71)
Isoëtes macrospora	74	500	No data	2.3 (1.2)	No data	6.93 (0.61)
Isoëtes malinverniana	12	No data	0.1	0.7	15.6 (1.5)	7.51 (0.35)
Isoëtes maritima	1	No data	0.3	0.8	No data	7.90
Isoëtes philippinensis	1	No data	1.0	2.0	24.0	6.88
Isoëtes sabatina	48	162	0.3	2.5	18.9 (6.3)	8.53 (0.36)

Species	Conductivity (µS/cm)	Alkalinity (meq/L)	O_2 (mg/L)	O_2 (%)	Secchi disk (m)	Dis CO_2 (mg/L)
I. alpina	20 (19)	No data	No data	No data	8.05 (3.44)	No data
I. asiatica	35 (25)[a]	0.17 (0.08)	14.53 (0.88)	No data	No data	7.0 (1.15)[c]
I. azorica	80 (17)	No data	No data	No data	No data	1.70 (0.44)
I. bolanderi	20 (2)	No data	No data	87.78 (16.18)	No data	3.95 (1.92)
I. boryana	172 (13)	0.28 (0.08)	9.23 (1.10)	97.00 (5.92)	3.81 (1.29)	0.28 (0.20)
I. cangae	6 (6)	No data	8.06 (1.99)	97.24 (6.67)	4.34 (0.23)	2.52 (0.12)
I. creussensis	15 (7)	0.33 (0.48)	No data	No data	No data	No data
I. echinospora	48 (39)	0.36 (0.40)	7.75 (1.12)	84.21 (17.52)	2.71 (1.75)	No data
I. fluitans	94 (26)	0.97 (0.92)	8.80 (0.65)	91.97 (8.24)	No data	No data
I. kirkii	119	No data	No data	89.46 (0.77)	7.54 (4.39)	1.67 (0.05)
I. lacustris	43 (34)	0.16 (0.15)	7.82 (2.32)	100.07 (6.60)	3.15 (2.00)	No data
I. macrospora	59 (47)	0.49 (0.50)	No data	No data	6.38 (1.38)	No data
I. malinverniana	92 (45)	0.32 (0.16)	No data	No data	No data	No data
I. maritima	775	No data	No data	No data	No data	No data
I. philippinensis	242	No data	5.59	No data	No data	No data
I. sabatina	495 (63)	2.93 (0.35)	9.60 (1.4)	101.37 (17.13)	10.06 (2.76)	No data

Species	Total nitrogen (mg/L)	Total phosphorous (µg/L)	NH_4^+ (µg/L)	NO_3^- (µg/L)	PO_4^{3-} (µg/L)	Chl-*a* (µg/L)
I. alpina	0.15 (0.1)	5.81 (3.66)	9.95 (3.28)	No data	No data	No data
I. asiatica	No data	No data	108.57 (155.18)[a]	1940 (1095.08)	78.17 (44.34)	No data
I. azorica	No data	No data	No data	353.33 (83.27)	26 (2)	No data
I. bolanderi	No data	No data	No data	No data	No data	No data
I. boryana	0.71 (0.12)	181.05 (190.54)[a]	12.78 (15.84)	235.67 (262)[b]	5.39 (12.72)	No data
I. cangae	0.43	10.5 (0.86)	60	270 (197.99)	No data	1.13 (0.06)
I. creussensis	0.15 (0.11)	8.17 (5.59)	24.26 (12.92)	396.57 (306.02)[c]	No data	2.64 (2.74)
I. echinospora	0.39 (0.28)	13.77 (17.70)[a]	15.59 (17.97)	131.17 (185.78)	3.83 (18.21)	4.98 (7.56)
I. fluitans	No data	No data	65.39 (56.98)	1114.78 (751.86)	0.11 (0.12)	No data
I. kirkii	0.27 (0.16)	17.94 (18.11)	18.35 (24.84)	No data	No data	No data
I. lacustris	0.38 (0.28)	12.04 (16.17)	15.01 (20.15)	119.69 (337.14)	3.91 (17.89)	4.97 (9.4)
I. macrospora	0.66 (0.39)	15.45 (12.04)	No data	No data	No data	6.01 (6.29)

TABLE 2 (Continued)

Species	Total nitrogen (mg/L)	Total phosphorous (µg/L)	NH_4^+ (µg/L)	NO_3^- (µg/L)	PO_4^{3-} (µg/L)	Chl-a (µg/L)
I. malinverniana	No data	No data	13.64 (15.18)[a]	1675 (898.86)[a]	274.09 (189.73)[a]	No data
I. maritima	No data	No data	No data	No data	No data	No data
I. philippinensis	No data	No data	No data	No data	No data	No data
I. sabatina	0.24 (0.22)	19.37 (20.34)	14.68 (23.47)	64.93 (75.34)	8.63 (10.82)	1.63 (1.39)

Species	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)	Na^+ (mg/L)	K^+ (mg/L)	SO_4^{2-} (mg/L)	Cl^- (mg/L)
I. alpina	No data	No data	No data	No data	No data	No data
I. asiatica	4.5 (1.81)	1.23 (1.01)	11.46 (6.58)	No data	1.46 (0.97)	24.86 (13.04)
I. azorica	2.17 (0.64)	1.6 (0.69)	No data	No data	8.47 (0.31)	20.97 (3.89)
I. bolanderi	No data	No data	No data	No data	No data	No data
I. boryana	3.86 (1.6)	3.47 (0.53)	21.35 (2.83)	2.61 (0.35)	7.66 (2.76)	39.92 (8.75)
I. cangae	0.28 (0.15)	0.04 (0.02)	0.30 (0.16)	0.14 (0.1)[a]	16.17 (21.21)	No data
I. creussensis	4.42 (2.60)	0.22 (0.13)	0.49 (0.20)	0.18 (0.07)	2.75 (0.82)	0.3 (0.13)
I. echinospora	4.77 (5.54)	0.33 (0.30)	0.62 (0.22)	0.23 (0.10)	3.22 (1.28)	2.92 (4.42)
I. fluitans	7.90 (6.48)	1.91 (0.85)	7.16 (1.73)	1.23 (0.23)	2.69 (3.42)	11.97 (1.20)
I. kirkii	No data	No data	No data	No data	No data	No data
I. lacustris	3.37 (3.95)	0.13 (0.18)	0.98 (0.02)	0.16 (0.01)	3.05 (2.81)	2.28 (2.21)
I. macrospora	6.55 (5.55)	No data	No data	No data	No data	2.93 (2.86)
I. malinverniana	No data	No data	No data	No data	No data	0.11 (0.02)
I. maritima	No data	No data	No data	No data	No data	No data
I. philippinensis	No data	No data	No data	No data	No data	No data
I, sabatina	17.18 (2.49)	12.31 (1.38)	54.6	No data	25.67 (3.1)	49.94 (5.91)

Note: Mean data and standard deviation are reported for physicochemical variables.

[a]One outlier was deleted.

[b]Two outliers were deleted.

[c]Three outliers were deleted.

FIGURE 3 Significance of Dunn's post hoc test for pH (upper right corner) and conductivity (lower left corner). If the difference in the mean values measured between species is significant ($p < 0.001$), the box identified by the double species entry is coloured. If the difference is not significant, then the cell is white.

the only anomalous species, which is detected in environments with much higher TP concentrations (Table 2, Figure 2). There are few significant differences among species for TP, further indicating the genus' preference for low TP waters (Figure 5). Among these, we note the significant difference shown by Dunn's post hoc test between *I. lacustris* and *I. macrospora*.

Aquatic *Isoëtes* are most found in waters with low TN concentrations; specifically, most records were in waters with <0.5 mg/L

FIGURE 4 Significance of Dunn's post hoc test for chloride ion (upper right corner) and calcium ion (lower left corner). If the difference in the mean values measured between species is significant ($p < 0.001$), the box identified by the double species entry is coloured. If the difference is not significant, then the cell is white.

Ca \ Cl	asi	azo	bor	can	cre	ech	flu	lac	mac	mal	sab
asiatica					●	●				●	
azorica					●					●	
boryana									●		
cangae	●		●								
creussensis				●		●			●		●
echinospora				●			●			●	●
fluitans				●							
lacustris					●						●
macrospora					●			●		●	
malinvernia									●		
sabatina				●	●	●		●	●		

FIGURE 5 Significance of Dunn's post hoc test for total phosphorus (upper right corner) and total nitrogen (lower left corner). If the difference in the mean values measured between species is significant ($p < 0.001$), the box identified by the double species entry is coloured. If the difference is not significant, then the cell is white.

TN \ TP	alp	bor	can	cre	ech	kir	lac	mac	sab
alpina		●						●	●
boryana	●			●			●	●	
cangae									
creussensis		●						●	●
echinospora							●	●	●
kirkii									
lacustris		●			●			●	●
macrospora	●	●		●	●		●		
sabatina		●			●		●		

TN (Table 2, Figure 2). The mean, minimum, and maximum values for TN for the two most common species (*I. echinospora*, *I. lacustris*) were similar (Table 2, Figure 5). Interestingly, the difference between the mean TN values for *I. lacustris* (376 µg/L) and *I. macrospora* (655 µg/L) is significant. Most other species also had similar associations, except for *I. creussensis*, which colonised sites with average TN concentrations halved (mean = 154 µg/L).

Lastly, we explored the ecological differences for taxonomically unsettled species pairs such as *I. lacustris* and *I. macrospora* (Grigoryan et al., 2021) and *I. asiatica* and *I. echinospora* (Britton et al., 1999; Mochalova, 2006). Our results show that some of the assessed differences between *I. macrospora* and *I. lacustris* are significant, such as alkalinity, calcium ion concentration, TP, and TN (Table 2 and Figures 2–5). However, none of the differences between *I. asiatica* and *I. echinospora* are significant (Table 2 and Figures 2–5).

3.3 | Ecological space of *I. echinospora* and *I. Lacustris*

The first three axes of the PCA were used for the hypervolume implementation, which together explained 82.6% of the total variation (Figure 6). The ecological space of the two species greatly overlapped, with *I. echinospora* occupying, on average, 92.1% of the total niche volume and *I. lacustris* occupying 62.8%, based on bootstrapped estimates. Besides, *I. echinospora* showed 40.0% mean niche uniqueness and *I. lacustris* showed only 12.0%. Based on the bootstrap estimates, the differences between the two species' niche size and uniqueness were significant (Figure 6 and Appendixes *03_Hypervolume analysis*). Accordingly, we conclude that *I. lacustris* mostly occupies a subset of *I. echinospora*'s ecological space, with the latter also occupying sites with more extreme environmental conditions.

4 | DISCUSSION

The formalisation of the GaID data set is a first attempt to collect and systematise the ecological information on aquatic *Isoëtes*, which are representative of a biological form of macrophytes (isoëtids) and have been used as a bioindicator of ecosystem integrity for a long time (e.g., Schaumburg et al., 2007; Seddon, 1972). This international initiative launched for the purpose of creating the GaID is important for our understanding of this genus' habitats, which include many rare and endangered species and representative of habitats experiencing sharp declines because of eutrophication and rising temperatures (Dudgeon, 2019; IPBES, 2019; Reid et al., 2019).
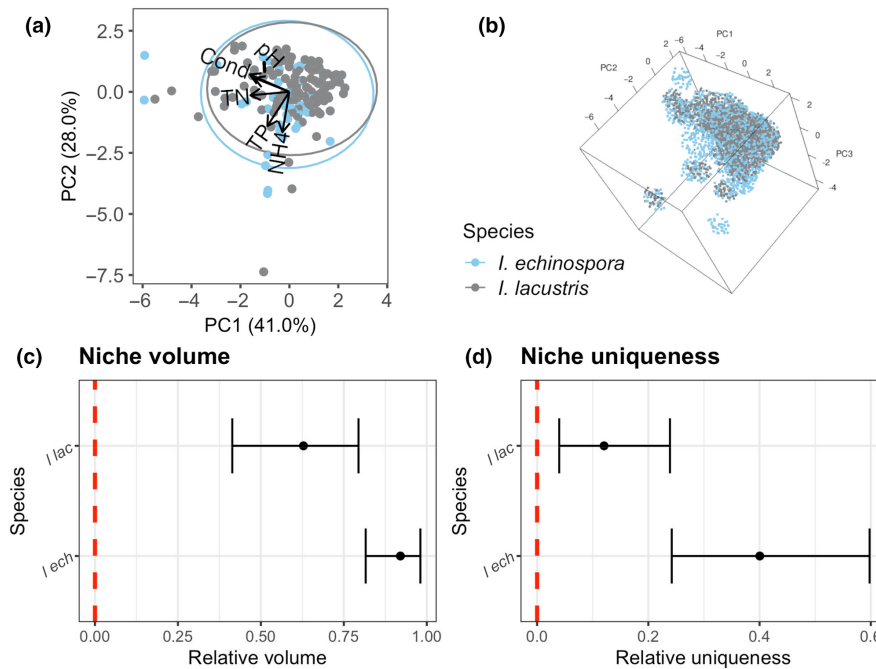
FIGURE 6 Ecological space of *Isoëtes echinospora* and *Isoëtes lacustris*. The first three axes of the principal component (PC) analysis (a axes 1 and 2) explained variance of each axis in brackets. Cond, conductivity; pH, pH of water column; TN, total nitrogen; TP, total phosphorus. Three-dimensional representation of the hypervolumes (b) computed on the three PC analysis axes. Different point colours indicate different species. (c, d) Uncertainty estimates of ecological niche size (relative to the total volume occupied by both species) and uniqueness (relative to the volume of each species) of *I. echinospora*, *I. lacustris* (relative to the total volume calculated on 199 bootstrapped hypervolumes). Difference between species is significant and each species has niche size or uniqueness significantly >0 if black bars do not overlap or cross the dashed red line, respectively.

4.1 | Ecological insights for aquatic *Isoëtes*

We are a long way from having a complete assessment of the ecological characteristics of the aquatic *Isoëtes* species, and few exceptions are motivated by urgent conservation needs (e.g., Abeli et al., 2020; Bolpagni et al., 2021; Prado et al., 2023). Many of the *aquatic* species of *Isoëtes* are known only taxonomically (e.g., *Isoëtes elatior*, *Isoëtes hypsophyla*; Garrett & Kantvilas, 1992; Liu et al., 2004). In the GaID, there are ecological data for only 16 of the 59 species identified as aquatic (see Appendixes *01_Aquatic Isoëtes*). Substantial knowledge gaps remain about the ecological requirements of many aquatic *Isoëtes* species, except for a qualitative description based on field observations of the environments in which they live. Detailed analyses of ecological growth conditions have been collected for only a few species that are either endangered (*I. boryana*) or critically endangered (*I. cangae*, *I. malinverniana*, *I. philippinensis*, *I. sabatina*) or have a narrow distribution range (*I. fluitans*, *I. creussensis*). Furthermore, we do not know if these species have greater ecological plasticity and, thus, if the ecological characteristics observed and measured today are truly representative of their ecological niche or current distribution. Although the areas with the largest number of known aquatic species are from North and South America (a total of 31 species), GaID data have been retrieved only for four species from this area: *I. cangae*, *I. bolanderi*, *I. echinospora*, and *I. macrospora*. This knowledge gap is particularly urgent to fill in for the species that grow in only one

known site (*Isoëtes cipoensis*, *Isoëtes dubsii*, *Isoëtes fuliginosa*, *Isoëtes longifolia*, *Isoëtes longpingii*, *Isoëtes viridimontana*).

Our observed significant differences between *I. macrospora* and *I. lacustris* seem supportive of the taxonomic distinction of *I. macrospora* at some level, as already evidenced from morphological (Taylor et al., 2016) and molecular (Grigoryan et al., 2021) points of view. In contrast, the absence of significant differences in the ecological context between *I. echinospora* and *I. asiatica* leaves open questions about the taxonomic differences between the two taxa. In fact, there is still a debate about the taxonomic validity of these species; as examples, recent molecular information led to uncertainty as to whether *I. lacustris* from North America is the distinct species *I. macrospora* (Grigoryan et al., 2021), and some studies indicated that *I. asiatica* may be phylogenetically very close to *I. echinospora* (Britton et al., 1999; Mochalova, 2006).

Our knowledge of the hydrologic requirements of *Isoëtes* has changed considerably over time. Fifty years ago, it was still thought that most species in the genus *Isoëtes* were predominantly aquatic (Hutchinson, 1975), and even more recent studies indicated a dominance of aquatic species (e.g., Chappuis et al., 2016 reported that 60% of *Isoëtes* species are aquatic). Based on the definition of *aquatic* quillwort adopted in this study, only one-third of *Isoëtes* are aquatic and most *Isoëtes* species are amphibious.

The almost complete absence of aquatic species in Africa and Southeast Asia is probably because of a knowledge gap, which could have implications for the framework of species biogeography

and the reconstruction of evolutionary relationships among species. In fact, the genus *Isoëtes* is monophyletic and diversified first in aquatic habitats and then, later, adapted to amphibious and terrestrial environments (Hickey, 1986). Although preferences in habitat ecology have limited taxonomic value (as has been accepted since Pfeiffer, 1922, who proposed to use megaspore morphological characteristics to distinguish species), habitat preferences have played a role in the evolution and differentiation of species (Taylor & Hickey, 1992). Thus, it is probably no coincidence that the only known aquatic species in Africa, *Isoëtes wormaldii*, is a genetically distant taxon from the other species in the genus (Larsén et al., 2022).

Contrary to the usual description of aquatic *Isoëtes* species growing in *slightly acidic* waters (e.g., Vöge, 2004), the present study indicates that currently, only one species can be confirmed as occurring only in acidic water (*I. cangae*), but some others grow in waters at higher pH values (*I. boryana*, *I. malinverniana*) or even alkaline waters rich in carbonates and calcium ions (*I. sabatina*). This does not mean that no populations of aquatic *Isoëtes* grow in acidic waters. Populations of aquatic *Isoëtes* in the Northern Hemisphere (e.g., *I. asiatica*, *I. echinospora*, *Isoëtes tuckermanii*) are commonly detected growing in acidic brownish waters and acidic sediments, even consisting partly of peat or decaying vegetable material, such as in bog pools and some northern boreal lakes and lakelets surrounded by tundra-like landscapes. Recently, Čtvrtlíková et al. (2023) reported that in strongly acidified waters, *I. lacustris* and *I. echinospora* adults can grow and survive, even for decades, but fail to recruit new sporelings. The data available in the literature or collected by environmental monitoring agencies (which constitute most of the data collected in the GaID) were collected as part of standard monitoring programmes that are not designed to assess pH or take into account the potentially large daily fluctuations because of autotrophic and heterotrophic processes. For example, high fluctuations in daily pH values were registered in a Pyrenean temporary pond that hosts *I. echinospora* (Gacia & Ballesteros, 1993). With regard to *I. boryana*, we are certain that the lakes in which it survives can be considered *acidic lakes*, with siliceous and organic-poor sediments ($0.9 \pm 0.7\%$ as loss of ignition), and that the high pH values measured are because of the activity of cyanobacteria and invasive submersed macrophytes, which in central hours of the day (when light and temperature increase and pH is routinely measured), can bring pH values above 8 units (Ribaudo et al., 2018). In those shallow lakes, the decline of *I. boryana* could be because of the increase of phosphorus loads in the past, which have favoured opportunistic primary producers and our data confirm preference for low phosphorus concentration for the majority of the assessed species. In contrast, the seasonal and diurnal pH fluctuations are likely to be much narrower in deep lakes. Alkaline lakes for edaphic and geological reasons are also rich in carbonates and calcium ions. Only *I. sabatina* seems to be able to thrive under these physical and chemical conditions, but the presence of at least one species adapted to different conditions shows us that aquatic *Isoëtes* could also colonise hard

waters. Therefore, it cannot be ruled out that there are other species adapted to conditions differing from those of most aquatic species in the genus *Isoëtes*.

Overall, we confirm that the species included in the study are all typical of oligotrophic waters, as indicated in many previous works on *I. lacustris* and *I. echinospora* (e.g., Vöge, 2004). Nutrients and organic matter content in the sediment are generally not well documented for most species, whereas these are key variables in assessing whether an ecosystem is capable of hosting aquatic *Isoëtes*. Indeed, minimal increases in organic matter in the surface sediment have been widely shown to impair the viability of root systems of isoëtids (Smolders et al., 2002).

4.2 | Conservation issues and challenges

A global assessment of the ecological characteristics of the genus was lacking prior to this study. There are assessments made exclusively on individual species and species comparisons made on pairs that grow in the same biogeographical context and thus often live in the same lakes (*I. echinospora* and *I. lacustris*, *I. echinospora* and *I. macrospora*, *I. asiatica*, and *I. maritima*). Hutchinson (1975) wrote in his treatise on limnology: "when they occur in the same lake, they presumably have different ecological requirements, though it is not clear what these requirements are." Fifty years later, we are still at this point for the analysis of the ecological space of *I. echinospora* and *I. lacustris*, as well as for the ecological differences between *I. asiatica* and *I. echinospora*. Our analysis of ecological space shows a significant difference in the niche size between *I. echinospora* and *I. lacustris*, aside from a substantial overlap between the niche volume of these species. Thus, based on the available data, *I. lacustris* has a smaller niche volume and is more sensitive to environmental factors compared to *I. echinospora* but, because of the limited ecological data collected, it is not possible to statistically analyse the niche-like peculiarities of the two species. More detailed information on within-lake conditions would guarantee a more comprehensive understanding of species-specific ecological requirements and tolerance to habitat changes. When more than one species is present in a lake, it seems that the main difference in bottom colonisation is in the depth of growth; thus, we can postulate that other factors affected by depth, such as underwater light, sediment resuspension (Bertrin et al., 2017), winter ice depth, and temperature fluctuations (Gacia & Ballesteros, 1994) could affect depth distributions of aquatic *Isoëtes* species. Unfortunately, there are few data about underwater light conditions (Keeley et al., 1983; Rattray et al., 1992), and even if experiments conducted on *I. creussensis* demonstrated the crucial importance of light (and organic matter in the sediment) for survival (Chappuis et al., 2016) and growing period (Gacia & Ballesteros, 1994), further research will be necessary to establish which ecological variables differentiate the niches among species living in the same waterbody.

More focused studies of *Isoëtes* diversity are needed to accurately assess the ecology and conservation status of this taxonomic

group. We acknowledge that *Isoëtes*' diversity and ecology are complicated by geographic variation (bioregions, geodiversity), hybridisation, and fertile allopolyploids (as reviewed by Hickey et al., 1989). Multiple species can occur in the same waterbody but are not identified because of the lack of morphological expertise or molecular tools needed for species-level identification. Assessments should include traditional field sampling campaigns coupled with microscopic morphological verification (micro- and macrospores characterisation) and molecular and environmental DNA assessments for a comprehensive understanding of environmental requirements. At the same time, our analysis shows a non-negligible ecological plasticity to some variables (e.g., conductivity and alkalinity) that will need to be further investigated to determine ecological differences among species through long-term monitoring. Long-term monitoring is also useful to identify changes in water and sediment quality that may in turn affect the conservation of *Isoëtes* species. For example, conductivity in *I. malinvernina* sites has almost doubled in the last 10 years, indicating that this species can tolerate a wide range of conductivity levels, but further investigation is needed to verify if conductivity fluctuation could affect the reproduction rate, for example, as low pH values affect other species (Čtvrtlíková et al., 2023). Other variables (e.g., water temperature), however, will need to be monitored to evaluate the possible role of climate change in driving the decline of isoëtids (Prado et al., 2023; Zandonadi et al., 2021). More systematically gathered data on temperature, which has a significant effect on the germination of *Isoëtes* spores, are needed. In addition, more accurate data on the minimum and maximum growing depth of the species coupled with underwater light conditions are required, as well as more fulsome datasets on TP, TN, sediment pH, and total organic content to determine how these variables contribute to characterising the ecological niches of *Isoëtes* taxa.

5 | CONCLUSIONS

Our work herein has started to shed light on aquatic *Isoëtes* ecology, which is not well documented worldwide. However, their ecological assessment is important given that more than 25% of the species we reported in the GaID were listed as critically endangered or endemic to a small region. We urge others with existing data to collaborate by expanding the GaID or use the existing data in novel ways. In addition, future research using traditional field surveys and newer conservation tools such as environmental DNA is needed for comprehensive understanding to guide conservation planning and aquatic plant management. Our current research shows a strong sensitivity of *Isoëtes* to eutrophication; therefore, water quality management is key to the protection of all aquatic species in this genus.

AUTHOR CONTRIBUTIONS

Mattia M. Azzella and Alice Dalla Vecchia should be considered joint first authors. Conceptualisation: Thomas Abeli, Mattia M. Azzella, Rossano Bolpagni, Simona Ceschin, Sara Magrini, Angelo Troìa. Developing methods: Mattia M. Azzella, Rossano Bolpagni.

Conducting the research and data acquisition: all authors. Data analysis: Alice Dalla Vecchia. Data interpretation: Mattia M. Azzella, Rossano Bolpagni, Danelle M. Larson, Alice Dalla Vecchia. Preparation of figures and tables: Mattia M. Azzella. Writing: all authors.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest exits in the submission of this manuscript, and the manuscript has been approved by all authors for publication. The work described was original research that has not been published previously.

DATA AVAILABILITY STATEMENT

The data, the supplementary materials, and the Global aquatic *Isoëtes* Database are freely available in the Science Base Catalogue (https://www.sciencebase.gov/catalog/). USGS. https://doi.org/10.5066/P149GQAV. (Larson et al., 2024).

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