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Transnational efforts for the conservation of reproductively diverse taxa

Early joint actions in the ProPartS project (Biodiversa+), using *Chara canescens* as a case study

Abstract

Chara canescens is a charophyte restricted to brackish waters. Most known populations of this stonewort consist entirely of female individuals reproducing through parthenogenesis, nevertheless exhibiting a remarkably high genetic diversity. In contrast, populations that include males, and thus have the potential for sexual reproduction, are rare. The ProPartS project aims to characterize the gene flow between *C. canescens* populations using population-genetic analyses. Combined with investigations of ecological niche structure and the restoration potential of former habitats, it is intended to contribute to developing effective conservation strategies, which are to be disseminated and implemented within a transnational stakeholder network. This article reports on the first joint steps taken by the ProPartS project partners in pursuit of this goal.

Keywords: Charophytes, population genetics, restoration, niche structure, stakeholder network

1 Introduction

Chara canescens LOISELEUR 1810 is a complex green alga within the Charophyceae. Closely related to land plants, this class belongs to the division Streptophyta (KRIENITZ & NOWAK 2024). Due to their specific characteristics and adaptations, members of the family Characeae play an important ecological role in aquatic habitats. When they occur in dense populations, charophytes can influence water clarity. Among other aspects, this is because they are able to reduce nutrient concentrations, for example through their uptake and the co-precipitation of phosphorus with carbonates during photosynthesis (e. g., OTSUKI & WETZEL 1972, CRAWFORD 1977, MURPHY et al. 1983), which consequently leads to a decrease in phytoplankton biomass (BLINDOW & VAN DE WEYER 2016). Charophytes also produce allelochemicals (e. g., polyphenols), which can negatively affect phytoplankton,

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contributing to improving water clarity (RODRIGO et al. 2024). In addition, the morphology of Characeae populations promotes sedimentation and reduces the resuspension of particles in the water column, which is also reflected in lower turbidity levels (VERMAAT et al. 2000). Charophytes can also be used for the remediation of water bodies. By absorbing heavy metals and toxic organic chemicals, they can improve the condition of polluted waters (SCHNEIDER & NIZZETTO 2012, MAHAJAN et al. 2019). In interaction with their biotic environment, stoneworts provide sustenance and protection from predators and currents for juvenile and herbivorous fish and invertebrates, thereby positively influencing the biodiversity of the waters they inhabit (e. g., LAKE et al. 2002, BAKER et al. 2010, SCHNEIDER et al. 2015, BLINDOW & VAN DE WEYER 2024). In addition, they provide a valuable food supply for birds by serving both as habitat for prey species and as a food source themselves (HARGEBY et al. 1994, SCHMIEDER et al. 2006). The latter is also important for the population dynamics of the algae, as it promotes the long-distance dispersal of oospores. Endoornithochory is considered to be the main mode of dispersal of characean oospores (PROCTOR 1962, BONIS & GRILLAS 2002, CLAUSEN et al. 2002), which play a key role in the propagation, persistence, and colonization of (new) habitats (HOLZHAUSEN et al. 2024).

In contrast to many other charophytes, which predominantly prefer freshwater habitats, *C. canescens* only colonizes brackish waters and can therefore not be found in freshwater or waters with marine salt content (SCHUBERT et al. 2024). Due to the low salinity of the Baltic Sea, which is caused by its distinct hydrological and bathymetric structure (LEHMANN et al. 2022), the coasts of this sea are particularly suitable for the occurrence of *C. canescens*. However, the species can also be found in brackish lagoons along the coasts of seas with fully marine conditions (SCHUBERT et al. 2024). In addition to coastal locations, *C. canescens* also grows in so-called inland brackish sites (SCHUBERT et al. 2024). They are either fed by water originating from salt deposits which have formed in the geological past as, e.g. during evaporation episodes of the Zechstein Sea or, alternatively, are the result of recent climatic conditions characterized by high evaporation in combination with pronounced seasonality of precipitation (so called “step-lakes”). These inland occurrences of *C. canescens* are assumed to be important links between the coastal habitats. Globally, this charophyte species is widely distributed in the northern hemisphere. The main occurrences are in Europe, but *C. canescens* is also known from North America, Asia, and northern Africa. South of the equator, it has only been recorded in Australia (KORSCH 2018, SCHUBERT et al. 2024).

In the context of distribution, beyond the restriction to brackish water, attention must be drawn to the true peculiarity of *C. canescens*: it is the only charophyte species capable of reproducing parthenogenetically (BRAUN 1856). Moreover, it is unique in exhibiting two different reproductive strategies. Populations consisting exclusively of female individuals and reproducing parthenogenetically account for a large part of the aforementioned wide distribution of *C. canescens* (SCHUBERT et al. 2024). These populations have the advantage of not being dependent on fertilization and of being able to colonize new habitats rapidly (SCHAIBLE & SCHUBERT 2008). However, this type of reproduction also carries the risk of genetic impoverishment, as there is no recombination of genes in the absence of meiosis and fertilization. As a result, such populations have a lower potential for acclimatization and may quickly enter an extinction vortex (BLOMQVIST et al. 2010, ROUGER et al. 2016). However, an analysis of the genetic diversity of parthenogenetic and sexual populations of the species by SCHAIBLE et al. (2009) revealed that parthenogenetic populations are more genetically diverse than would be expected based on their reproductive strategy. The ProPartS project currently assumes that this is due to the introduction of oospores from bisexually reproducing populations into parthenogenetic populations, suggesting some degree of genetic exchange. These sexual populations constitute only a very small proportion of the overall distribution. To date, only seven such occurrences have been confirmed, all located in inland Europe: in Austria (near Lake Neusiedl; KÜSTER, SCHAIBLE & SCHUBERT 2005), Spain (Bodon Blanco, Las Eras, Caballo Alba, La Iglesia; ARNAL et al. 2025), Italy (Sardinia; BECKER 2019), and Serbia (SABOVLJEVIC et al. 2021). Historical records from Kazakhstan (MIGULA 1889-1897), Romania (MIGULA 1889-1897), France (CORILLION 1957), and Hungary (MIGULA 1889-1897) have not been confirmed recently. An occurrence of bisexual *C. canescens* in Lago di Pergusa (Sicily) remains uncertain.

2 The ProPartS Project

Based on many unanswered questions regarding the distribution and population connectivity of *Chara canescens*, the Biodiversa+ project "ProPartS - Developing strategies for the protection of taxa consisting of interconnected sexual and parthenogenetic reproducing strains" was initiated. This project aims to develop strategies for the protection of species with rare sexual and common parthenogenetic populations within a network of protected areas that extends beyond national borders. To achieve this aim, knowledge about fundamental aspects of the species' biology and ecology is required and shall be gathered by the project's consortium.

To provide a comprehensive overview of the current distribution, suitable brackish water habitats for *C. canescens* in both inland and coastal regions are to be identified. To this end, literature searches on historical salt works and recent inland brackish sites are carried out. Their suitability as habitats is assessed through salinity measurements in the field, and existing populations are surveyed. Water chemistry, sediment composition, and the microbial and fungal community in the sediment are analyzed to more precisely define the charophytes' habitat requirements. Also of importance is the analysis of the species' ability to recolonize a habitat after a certain period of time. Both, number as well as viability of oospores in the sediment is to be investigated through vitality assessments and germination experiments. Finally, population genetic sequencing approaches are used to gain insights into the current and historical genetic diversity of the species and to explore potential genetic exchange between populations. The knowledge gained is expected to support the long-term conservation of *C. canescens* and serve as a basis to develop a conservation strategy that can be extrapolated to other species - including those inhabiting fragmented environments.

3 Central Project Activities

3.1 Kickoff Meeting in Sankt Andrä am Zicksee (Austria)

The start of the project was marked with a meeting of all project partners at the end of May 2023 in Sankt Andrä am Zicksee (Austria). The municipality in Burgenland is located in the immediate vicinity of the Neusiedler See - Seewinkel National Park. Its temporarily drying saline shallow waters are often vegetated by *Chara canescens*. In the Stinkergraben, for example, which is located above the upper Stinkersee, both female and male individuals of the species can be found. A parthenogenetic population grows about 5 kilometers away in the Apetlon bathing lake.

In addition to visiting and sampling the Austrian bisexual population of *Chara canescens*, this meeting aimed to introduce the scientists involved in the project (Fig. 1) and to clarify details regarding the work packages (WP) to be carried out.

Fig. 1 ProPartS participants during the meeting in Palermo (Sicily): K.-G. Bernhardt, A. Troía, M. A. Rodrigo, R. Guarino, H. Schubert, J. Weitzel, A. Arnal, and P. García-Murillo (Absent: B. Turner, K. Tremetsberger, V. Ilardi, and D. Salemi). Photo credit: E. Nauner-Bernhardt, 2024.

The main focus was initially on WP4, which primarily aims at installing a transnational network of stakeholders responsible for (sexual) *C. canescens* sites. This network shall provide a platform for an exchange of information about the recent (annual) status of the populations as well as sharing knowledge about the efficiency of conservation measures. By regular meetings and workshops awareness of stakeholders for species protection requirements shall be raised and kept alive. Although the sites where the species grows are often under protection, they are most commonly designated as bird reserves, the management of which may be counterproductive for the conservation of the stonewort. Cooperation between stakeholders in conservation efforts should also be facilitated, as protecting only habitats of parthenogenetic populations is unlikely to be effective without also securing the survival of bisexual populations. In order to reach as many different target groups as possible, the network should address not only the scientific community but also political decision-makers, local authorities, environmental organizations, and the general public. This part of the project is led by the Italian project partner, comprising four scientists from the Department of Biological, Chemical, and Pharmaceutical Sciences and Technologies at the University of Palermo. This project partner is also responsible for public outreach for ProPartS, which will initially include the creation of a logo, a website, and a flyer. While coordination lies with this partner, other project members have actively contributed – for instance, the logo was designed by A. Arnal, and the brochure, originally drafted in English, has been translated into French, Italian, German, Romanian, and Spanish by various members of the project.

Further discussions mainly concerned strategies for the collection and exchange of samples required for WP1 to WP3. WP3 focuses on the evaluation of the oospore sediment bank and interfertility experiments. These investigations will provide information on whether former charophyte sites can be recolonized under more favorable habitat conditions from diaspores still present in the sediment. The responsible project partner, based in Spain, includes scientists from the University of València (Cavanilles Institute for Biodiversity and Evolutionary Biology, Integrative Ecology Group) and the University of Seville (Department of Plant Biology and Ecology). WP2, led by three Austrian scientists from the Department of Ecosystem Management, Climate and Biodiversity at the BOKU University in Vienna, investigates the population genetics of *C. canescens* to draw conclusions about past and recent gene flow between bisexual and parthenogenetic populations. In addition to collecting fresh plant material, herbaria are screened for available *C. canescens* specimens, and suitable material from selected sites and collection years is sampled. Finally, WP1 focuses on identifying suitable inland brackish sites and describing the ecological niche of the species, serving as a foundation for conservation program development. Regarding abiotic factors, water chemistry and sediment composition are analyzed. From a biotic perspective, the accompanying vegetation is recorded while the microbial and fungal community in the bulk soil is examined by the scientists responsible at the University of Rostock (Chair of Aquatic Ecology).

For all work packages, initial ideas on sampling locations, strategies, and sample numbers were compiled. Details were summarized in protocols by the responsible project partners.

Initial test runs of sampling were conducted during the field campaign following the official part of the project meeting. Visited sites are shown in Figure 2a-b.

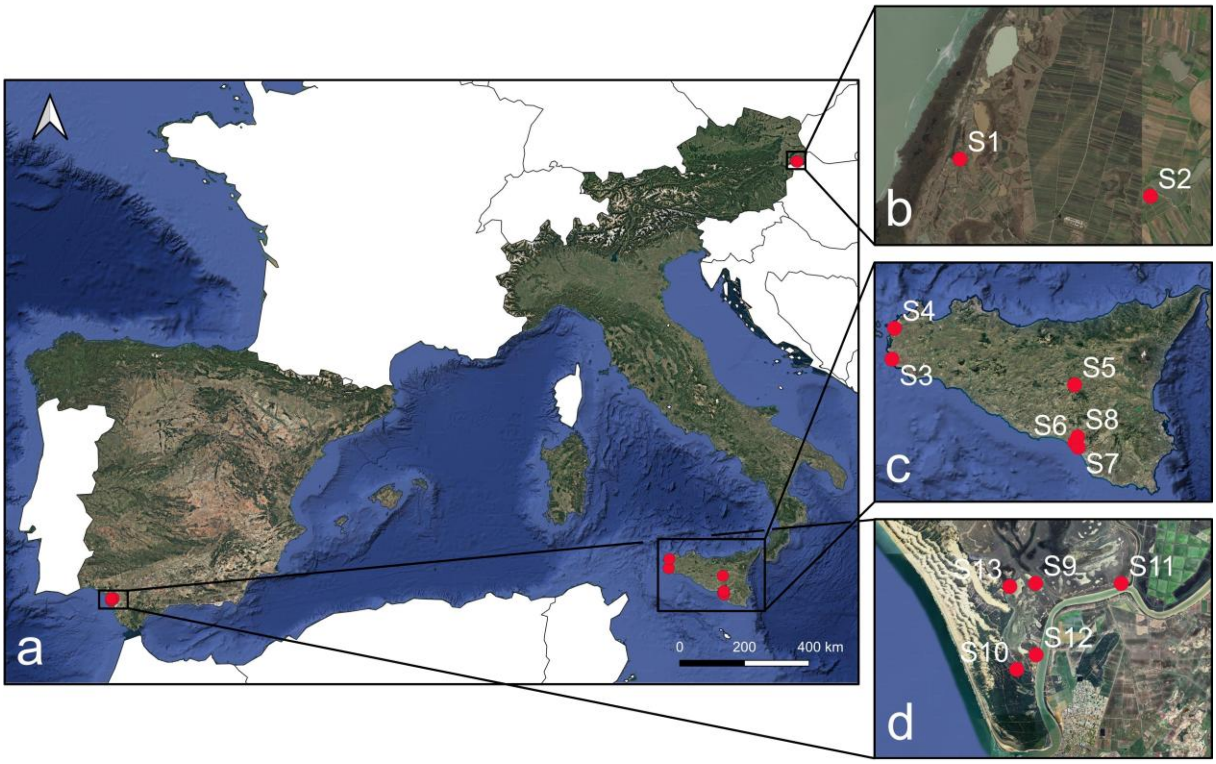

Fig. 2 (a) Overview of joint field campaigns during project meetings in: (b) Austria (S1 - Stinkergraben, S2 - Apetlon Bathing Lake), (c) Sicily, Italy (S3 - Margi Milo, S4 - Saline di Trapani, S5 - Lago di Pergusa, S6 - Contrada Spinasanta, S7 - Biviere di Gela, S8 - Geloi wetlands), (d) Spain (S9 - Lucio Largo, S10 - Laguna Larga, S11 - Lucio del Membrillo, S12 - Salinas de San Isidoro, S13 - Lucio de Vetalenguas). Created by J. Weitzel, basemap data © 2015 Google.

First, the Apetlon bathing lake was visited. This water body is a former open-cast mine with gravelly sediment and clear water, which is fed exclusively by precipitation and groundwater. The elevated salinity originates from former marine sediments in the subsoil, which are responsible for the markedly increased salt content of the groundwater throughout the entire Seewinkel region. The bathing lake covers approximately 1.5 ha and has a maximum depth of 4 m (Federal Ministry of Social Affairs, Health, Care and Consumer Protection & Office of the Burgenland Provincial Government 2023). The macrophyte vegetation of the lake was sampled by snorkeling and rake sampling from the shore. Individuals of *C. canescens* (exclusively female), *Chara globularis* Thuiller 1799/*Chara virgata* Kützing 1834, *Chara subspinosa* Ruprecht 1846, and *Tolypella* sp. were recorded.

The second site visited was Stinkergraben, located in the immediate vicinity of Lake Neusiedl. This temporary water body is characterized by sandy sediment and had a depth of only 50 cm at the time of sampling. Accordingly, the bisexual, monospecific population of *C. canescens*, shown in Figure 3, could be collected there by wading. Also, water and sediment samples were taken at both sites.

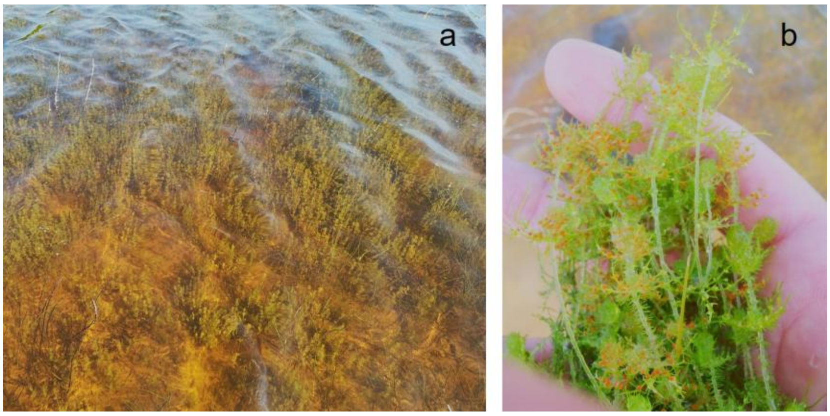

Fig. 3 (a) *Chara canescens* stands, Stinkergraben, Austria; (b) Sampled male *Chara canescens* individuals, Stinkergraben, Austria. Photo credit: J. Böhm, 2023.

Subsequently, a meeting was held with the stakeholders of the Illmitz Biological Station. They were informed about the urgent need to conserve the sexual populations of *C. canescens*, highlighting their role in supporting regional biodiversity. It was emphasized that *C. canescens* contributes to habitat structure and provides feeding grounds for many bird species occurring in Illmitz. The participants expressed strong support for the conservation initiative and acknowledged the ecological and scientific significance of protecting the sexual populations. A discussion followed on how local expertise and resources could be integrated to ensure the monitoring, sustainable management, and long-term conservation of this important component of regional biodiversity.

3.2 Project Meeting in Palermo (Sicily)

As part of the kick-off meeting, it was decided to also visit the *C. canescens* populations in Sicily as part of a joint project meeting. This took place at the end of January 2024 and began at the premises of the botanical garden in Palermo. The focus of the administrative part was on planning the 2024 field work and presenting brief status reports from all project partners. The latter included finalized sampling protocols, presentation of initial laboratory experiments, and the completion of the project website (https://proparts.unipa.it/) and brochure. Regarding the establishment of the stakeholder network, the pros and cons of online versus face-to-face meetings were discussed, along with strategies for overcoming language barriers and determining which stakeholder groups should be invited to which events. In this context, "core stakeholders" were defined as regional authorities and site managers of bisexual and key parthenogenetic sites, who should be the primary focus of initial contact. Engagement was decided to be best conducted on site, as project partners are often accompanied by these core stakeholders when sampling *C. canescens* habitats. In Palermo, an overview of the sites to be visited and sampled during the field campaigns in 2024 was compiled (see Figure 4). These sites are distributed across the countries of the project participants. In addition, Romania - due to a sexual population of *C. canescens* recorded there in the 19th century - and France were included. The latter hosts many locations of the charophyte, one of which may contain a sexual population.

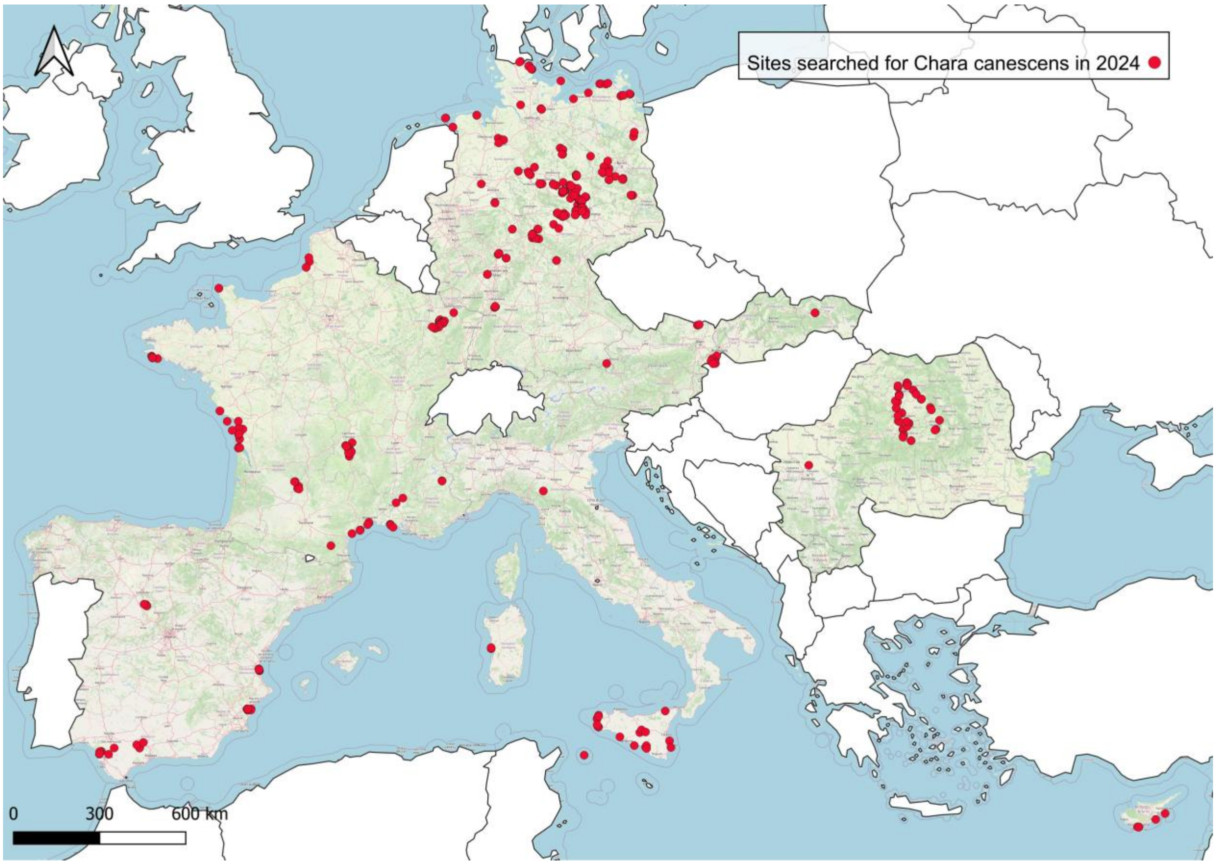

Fig. 4 Overview of sampling efforts conducted by all ProPartS partners during 2024. Created by J. Weitzel, basemap data © OpenStreetMap Contributors (ODbL).

To assess the condition of Sicilian *C. canescens* habitats, six sites were visited following the meeting in Palermo (see Fig. 2c). The first site was the wetlands behind the beach of Margi Milo. In this Natura 2000 site, depressions separated from the sea by a sandbar fill with groundwater and seawater in winter and dry out in summer. These habitats are important resting places for migrating birds and hold great floristic significance (TROIA & NAPOLITANO 2017). At the time of the visit, the sandy subsoil and parts of the terrestrial vegetation were submerged under up to 15 cm of water. While wading through the marshland, few individuals of *C. canescens* were found, all of which were female.

Saline di Trapani, which was visited next, is a Ramsar site and nature reserve (managed by WWF Italia) encompassing former and, in some parts, still active sea salt extraction areas. The wetland includes numerous salt pans and salt marshes, which serve as a habitat for resting birds and endemic plant and animal species (MESSINA et al. 2012). No *C. canescens* individuals were detected, nor was any aquatic vegetation visible, suggesting that the interval between water influx and the time of sampling was too short for algae development. Sediment was therefore collected for laboratory germination experiments. This approach was applied consistently in similar situations at all the mentioned project meetings.

The third site was Lago di Pergusa, where a sexual population of *C. canescens* was reported in the 19th and 20th centuries (TROIA 2020). This lake has no inflows or outflows and is naturally fed by precipitation and groundwater enriched with salts from Pliocene marine sediments. As a bird protection area, efforts are made to regulate strong water level fluctuations (SADORI et al. 2013). Although TROIA (2020) had reported the disappearance of charophytes in the lake, the same author found remains of *C. canescens* individuals on the dried shores during preliminary investigations within the framework of the ProPartS project in September 2023. Similar remains were also detected during the current visit. The individuals carried only female gametangia. On site, the project partners met with local stakeholders, including the manager of Lago di Pergusa reserve (Libero Consorzio Comunale di Enna) and the scientific coordinator of the Rocca di Cerere Geopark, to which the lake belongs. They expressed interest in the ProPartS project and the stakeholder network but were pessimistic about improvements in the lake management in the near future.

Piana del Signore, a retrodunal brackish marsh system surrounded by expanding industrial areas (RONSISVALLE 1971), showed no presence of charophytes, likely because the site had only recently been reflooded after a period of dryness. This condition is part of the site's natural annual cycle, as it is only temporarily flooded (SCIANDRELLO 2007). Many individuals of several characean species had recently been observed at this site by the Italian project partner and were reported by SCIANDRELLO (2007).

The next visit was to Riserva Naturale Orientata Biviere di Gela, a bird reserve and Ramsar site managed by the ONG LIPU, encompassing a 120-hectare lake with depths of up to 6 m depending on water levels. The lake is fed by rainfall, seawater, and two rivers, and was originally separated from the sea by dunes (BRULLO & SCIANDRELLO 2006, NOTI et al. 2009). Satellite images now show extensive greenhouse developments near the lake. High temperatures, low precipitation, and excessive irrigation-related water removal have led to drastic drops in water level during summer (BRULLO & SCIANDRELLO 2006). The lake was in poor ecological condition: water transparency was low, and no macrophyte vegetation was found. According to the site manager, whom the project partners met on site, the problems include high nitrogen inputs from the greenhouses and the introduction of carp. For economic and political reasons, no management activities to improve the lake's ecological condition have been implemented to date.

The final site was Geloi wetlands, a private nature reserve focused on avifaunal conservation. Surrounded by agricultural land, this reserve aims to restore wetland areas lost due to past melioration measures. In addition to temporary brackish marshes, the reserve includes artificially created lakes (ZAFARANA 2020). Two water bodies that had not dried up were visited. The first, a freshwater lake, showed no presence of charophytes. A second, brackish lake with seasonal salinity changes due to salty groundwater contained freshly germinated individuals of *C. globularis* and *Tolypella glomerata* (DESVAUX) LEONHARDI 1863 (see PAGANA et al. 2024). Again, the project partners presented the scope and goals of ProPartS to local stakeholders (Stiftung Pro Artenvielfalt).

3.3 Project Meeting in Doñana National Park (Huelva-Seville, Spain)

The last in-person project meeting, at the time, took place in mid-March 2024 in the Doñana National Park in southern Spain. As a detailed organizational discussion had taken place shortly beforehand in Palermo, this part was limited to the finalization of the 2024 field sampling plans and a brief introduction to the methodology to be used for the population genetic studies. ThreeRAD sequencing (Restriction site Associated DNA Sequencing) is used to investigate genetic variation. DNA is cut with restriction enzymes, and the resulting fragments are sequenced. This allows genetic variation to be mapped across the entire genome without having to sequence it completely (PANTE et al. 2015).

However, the main focus of this meeting was on visiting the local *C. canescens* sites in various (temporary) waterbodies of the Doñana National Park (Fig. 2d). This is a large complex of biodiverse wetlands influenced by the Atlantic Ocean and the mouth of the Guadalquivir River (GREEN et al. 2018, PAREDES et al. 2021). Based on reports of the presence of sexual individuals of *C. canescens* at these sites, the Lucio Largo (GARCÍA-MURILLO 1993) and Laguna Larga (GARCÍA-MURILLO, pers. comm.) were visited. Additional locations in the area that were surveyed during the field trip - including Lucio del Membrillo, Salinas de San Isidoro, and Lucio de Vetalenguas – had either previous sightings of the target species or were known to host commonly associated species (GARCÍA MURILLO, pers. comm.). All of these sites were either dry at the time of the visit due to the long drought the Iberian Peninsula was facing, or had been filled by rainfall only a week prior, so no aquatic vegetation was found.

The third, and equally important, part of the project meeting in southern Spain was establishing contact with key stakeholders in the area. During individual meetings with representatives of the Doñana National Park and the SEO/BirdLife organization, the importance of the region's brackish water sites was emphasized following a brief presentation of the project and the target species. As conservation efforts in these areas often focus primarily on bird protection, it was highlighted that the conservation of macrophytes can be complementary, as they represent an important food source, and distinct macrophyte populations can attract avifauna. This can also bring economic benefits: if sufficient food is available, fewer birds are likely to move to nearby fields to rest, thus reducing crop damage. Further discussion addressed the lack of rainfall and the resulting low water availability.

After an introduction to the benefits of the stakeholder network and clarification of outstanding questions, the representatives of both organizations agreed to become members of the core group of an international network of site managers of *Chara canescens*-occurences.

3.4 Online Stakeholder Meeting

In September 2024, the "First Meeting of the International Network of Wetlands with sexual *Chara canescens*" was finally held. In addition to the project partners, representatives from regions with bisexual populations of *C. canescens*, previously defined as core stakeholders, took part. Among them were local and regional authorities as well as non-governmental organizations from Austria, Italy, Spain, and

Serbia. After a brief introduction of the project and *C. canescens*, the external participant were given the opportunity to introduce themselves and raise concerns regarding the management and conservation of the species' habitats. Issues mentioned included increasing habitat pollution, the predominant focus of conservation efforts on coastal areas and birds, and the perspective of local communities whose priorities are often economic rather than conservation-oriented. Overall, there was strong interest from stakeholders in exchanging ideas and contributing to the network, for example by sharing data collected over the years. To ensure continued engagement, a structured communication channel involving all stakeholders will be established, serving also to announce the next meeting, which will be open not only to core stakeholders but to all interested parties.

4 Conclusion

ProPartS deals with conservation strategies for taxa with interconnected parthenogenetic and sexual populations, exemplified by the stonewort *Chara canescens*. Since the start of the project, responsibilities for the associated research have been clearly defined, focusing on the species' habitat requirements, the restoration potential of these habitats based on the oospore sediment bank, and the gene flow between populations. As a starting point, a comprehensive sampling campaign was planned and carried out to collect data across a wide geographical area. The most important locations of sexual populations in the project countries were visited jointly. At the same time, contact was established with key regional stakeholders to bring them together in an international network.

During the remaining project period, final sampling at sites not yet covered is planned. However, the focus will shift to analysing the collected data and disseminate the resulting recommendations for conservation strategies both within and beyond the stakeholder network, which will continue to grow and foster more intensive exchange in the future.

Zusammenfassung

Chara canescens ist eine Art der Familie Characeae, die ausschließlich brackige Gewässer besiedelt. Der Großteil der bekannten Populationen dieser Armleuchteralge besteht nur aus sich parthenogenetisch vermehrenden weiblichen Individuen, die dessen ungeachtet eine den sexuellen Vorkommen vergleichbar hohe genetische Diversität aufweisen. Sexuelle Populationen, in denen auch männliche Individuen vorkommen, sind hingegen selten.

Ziel des ProPartS Projekts ist es, mithilfe populationsgenetische Analysen den Genfluss zwischen *C. canescens*-Populationen zu charakterisieren. In Kombination mit Untersuchungen zur ökologischen Nischenstruktur und dem Wiederansiedlungspotential in früheren Habitaten soll dies zur Entwicklung effektiver Schutzstrategien beitragen. Diese sollen in einem transnationalen Stakeholder-Netzwerk verbreitet und umgesetzt werden. In diesem Beitrag wird über die Ziele und die erstes gemeinsames Schritte der ProPartS-Projektpartner berichtet.

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