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Ecological and Genetic Insights Into Antarctic Fairy Shrimp, *Branchinecta gaini* Daday, 1910 (Branchiopoda: Anostraca) Populations on King George Island, Antarctica

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ABSTRACT

1. The Antarctic fairy shrimp (*Branchinecta gaini*) is the largest inland animal in Antarctica and a key component of its freshwater ecosystems. Despite its broad distribution, little is known about its population structure, habitat tolerance, and potential vulnerability to climate change. This study investigated the occurrence, genetic structure, and cyst bank dynamics of *B. gaini* across freshwater habitats on King George Island (KGI), South Shetland Islands.
2. Field surveys were conducted at 35 freshwater sites during the summer of 2021–2022. The presence of *B. gaini* was determined through net sampling, and cyst density was measured from sediment samples using flotation and image analysis. Water chemistry and habitat characteristics were recorded. Genetic analyses were performed on mitochondrial *cox*1 and 16S rRNA genes across 13 sites to assess population structure and phylogenetic relationships.
3. *Branchinecta gaini* was detected at 17 sites, displaying wide habitat tolerance in ponds varying in size, salinity, and morphometry. Genetic analysis of 92 *cox*1 sequences revealed three haplotypes, suggesting the absence of cryptic species. However, AMOVA indicated significant genetic differentiation among regions ($\Phi = 0.453$, $p < 0.001$). Cyst densities varied markedly, with an average of 1.08 cysts cm^{-3}. A young (~20-year-old) postglacial pond near the Ecology Glacier had a notable cyst density of 0.272 cysts cm^{-3}, indicating successful colonisation and supporting the monopolisation hypothesis. The highest cyst density (10.91 cysts cm^{-3}) was recorded in a pond in the area of Ornithologists Creek.
4. *Branchinecta gaini* demonstrates ecological opportunism, inhabiting a broad range of Antarctic freshwater habitats, including postglacial and coastal water bodies. While three haplotypes were detected, their regional distribution suggests some level of spatial structuring. Shared haplotypes across regions may reflect either historical connectivity or sporadic dispersal events. Environmental factors such as pH, sediment type, and water flow were associated with the presence and cyst abundance of *B. gaini*.
5. Our findings confirm that *B. gaini* is an adaptable species capable of establishing populations in dynamic Antarctic freshwater systems. The presence of cyst banks in young ponds highlights the species' potential for rapid colonisation. Further research using higher-resolution molecular tools is recommended to clarify dispersal mechanisms and assess long-term population resilience under changing environmental conditions.

1 | Introduction

The Antarctic continent is predominantly ice-covered, with less than 1% comprising ice-free terrestrial areas accompanied by freshwater environments (Burton-Johnson et al. 2016). Within these limited ice-free zones, freshwater ecosystems such as lakes, streams, and ponds occupy an even smaller fraction. For instance, on Byers Peninsula, one of the largest ice-free areas in maritime Antarctica, freshwater bodies collectively cover only about 1.5% of its 60.6 km^2 surface area (Toro et al. 2007).

While populations of iconic marine mammals (e.g., Weddell seal *Leptonychotes weddellii*, Antarctic fur seal *Arctocephalus gazella*, orca *Orcinus orca*), seabirds (e.g., emperor penguin *Aptenodytes forsteri*, Adélie penguin *Pygoscelis adeliae*, wandering albatross *Diomedea exulans*), and fish and invertebrates are continuously monitored and well studied, Antarctic freshwater ecosystems remain inadequately investigated (Convey 2010).

Nevertheless, both terrestrial and freshwater habitats are vital components of the Antarctic landscape. Compared to marine systems, they are more directly sensitive to climate-driven changes, such as rising temperatures, sea levels, and the expansion of ice-free areas (Lee et al. 2017). Accordingly, the study of these environments is essential for understanding and conserving Antarctic biodiversity (Convey and Peck 2019).

Most ice-free areas in the Antarctic are cold deserts characterised by sparse biota and a limited number of cryptogamic, terrestrial, and aquatic invertebrate species due to the short vegetation period and lack of water and nutrients (Duffy and Lee 2019). In general, Antarctic terrestrial and freshwater ecosystems are characterised by low primary production (Rochera and Camacho 2019). However, the development of more complex inland habitats is possible in locations characterised by a favourable microclimate and supplied with organic substances and essential biogenic elements by seasonally migrating marine animals during the reproductive season (Convey et al. 2014).

Inland faunal communities comprise invertebrates, including Diptera, as well as Crustacea, Acari, Collembola, Nematoda, Rotifera and Tardigrada (Hughes and Convey 2010). Additionally, plant communities consist of mosses, liverworts, lichens, and only two vascular plant species: Antarctic hair grass (*Deschampsia antarctica*), Antarctic pearlwort (*Colobanthus quitensis*). An indispensable component, particularly in freshwater bodies, is represented by microorganisms exhibiting unquestionably large variability and constituting the foundation of trophic networks (Cavicchioli 2015; Ellis-Evans 1996).

The freshwater habitats of the Antarctic are often surrounded by ice-free, barren areas, which, in turn, are encircled by ice deserts. Such water bodies or their clusters are frequently spaced several hundred kilometres apart, particularly when situated on Antarctic islands. Populations inhabiting these environments may thus experience pronounced isolation. The combination of this isolation and the strong influence of local variables creates conditions conducive to genetic divergence (Dillon 1984; Lee and Mitchell-Olds 2011; Orsini et al. 2013; Spurgin et al. 2014). However, the case of Antarctic freshwater ecosystems is distinctive in that this region likely lacked such ecosystems entirely during the Last Glacial Maximum (LGM) (20,000–18,000 ya) (Pugh and Convey 2008). This implies that many organisms inhabiting the area are immigrants from subantarctic refuges or, possibly, rare cases of glacial refugia that survived glaciation (Cromer et al. 2006; Maturana et al. 2020).

Despite the extreme isolation of Antarctic water bodies, dispersal of organisms such as the fairy shrimp *B. gaini* appears to be facilitated primarily through passive means. The occurrence of this species across distant, unconnected freshwater habitats on the Antarctic Peninsula supports the role of passive dispersal particularly zoochory, where dormant cysts are transported by birds. These cysts are highly resistant to desiccation and freezing, enabling them to survive transit through both external attachment (epizoochory) and ingestion (endozoochory) by birds such as skuas, which frequent freshwater pools (Hawes 2009). While hydrochory and anemochory may enable limited local dispersal between adjacent pools, long-distance colonisation events almost certainly depend on zoochory, especially endozoochory, which offers a mechanism for directed dispersal over significant distances. Such passive but effective dispersal explains how *B. gaini* could have recolonised newly available habitats following glacial retreat in the Holocene, likely originating from subantarctic or South American source populations (Hawes 2009; Peck 2004). This pattern aligns with broader observations in aquatic organisms, where high dispersal potential through resistant propagules coexists with significant genetic differentiation among isolated populations a phenomenon described as the 'Monopolisation Hypothesis'.

This concept posits that early colonists of a habitat can dominate local resources through priority effects and local adaptation, thereby reducing opportunities for subsequent immigrants to establish (De Meester et al. 2002). In the context of Antarctic freshwater systems, where dispersal events are rare and isolated habitats are prevalent, this hypothesis may help explain patterns of endemism and population structure observed in Antarctic freshwater crustaceans.

These conditions render the Antarctic landscape a promising natural laboratory for studying evolutionary phenomena, particularly for testing hypotheses related to dispersal, initial stages of allopatric speciation, adaptations, and phenotypic plasticity. Thus, we focused on freshwater habitats on King George Island (KGI, South Shetlands Archipelago), inhabited by the largest Antarctic freshwater crustacean, *Branchinecta gaini* Daday, 1910. The Antarctic fairy shrimp (*B. gaini*) is in the order Anostraca. This order likely diverged from other branchiopods at 495 ± 17 Ma YBP (Sun et al. 2016). In general, these crustaceans inhabit small inland water bodies worldwide. Anostracan habitats are characterised by instability and are often seasonal, astatic, and fishless (Brendonck et al. 2008). The periodic nature of these habitats is linked to surviving dry periods or complete freezing, for which fairy shrimp have adapted by producing dormant forms known as cysts (Brendonck et al. 2008). Cyst production facilitates dispersion, as cysts can survive in the digestive systems of birds, enabling long-distance zoochory through wind or water transport (Bohonak and Whiteman 1999; Incagnone et al. 2015; Rogers 2014). These phenomena are

pivotal in the biogeography of these freshwater invertebrates and contribute greatly to their global distribution. Branchiopods are frequently studied to verify hypotheses related to ecology, biogeography, and evolutionary biology. The specifics of their distribution and dispersal capabilities prompt inquiries among researchers regarding gene flow, adaptive potential, and the role of cyst banks in evolutionary processes (Lindholm et al. 2016; Ripley et al. 2004; Rogers 2015; Sainz-Escudero et al. 2023). Moreover, Anostraca is relevant in aquaculture, with ongoing research focused on refining cultivation techniques, nutrition, and diseases (Arbeláez-Rojas and Melão 2022; Purivirojkul and Khidprasert 2009; Saejung et al. 2011). A critical area of toxicology research concerning anostracans is developing toxicity tests based on fairy shrimp and brine shrimp (Arulvasu et al. 2014; Bai et al. 2022; Bergami et al. 2022; Blinova et al. 2013; Nalecz-Jawecki and Persoone 2006).

The Antarctic fairy shrimp, *B. gaini*, is the only known anostracan species in Antarctica and belongs to the family Branchinectidae, which includes 53 species distributed across all continents except Australia (Beladjal and Mounia 2023; Rogers 2013; Rogers and Aguilar 2020). It inhabits isolated freshwater pools throughout the Antarctic Peninsula and parts of the subantarctic and southern South America, including South Georgia and the Falkland Islands (Lukić et al. 2023; Rogers and Aguilar 2020). *B. gaini* thrives in ponds, shallow pools, and glacial lakes, feeding primarily on microbial mats, small invertebrates, and detritus (Hawes 2008; Paggi 1996). More specifically, it acts as a detritivorous, herbivorous filter-feeder, consuming amorphous organic material of vegetal origin, filamentous algae such as *Spirogyra*, fungal hyphae and spores, protozoans, rotifers, and fragments of invertebrates including other *B. gaini* individuals (Paggi 1996). Feeding is concentrated near sediments using specialised scraping appendages. Adults grow up to 2.9 cm in length and reach sexual maturity around 30 days post-hatching. Females lay from a few to approximately 200 eggs every few days, though many aspects of their reproductive biology remain poorly understood (Jurasz et al. 1983; Hawes et al. 2008).

Branchinecta gaini exhibits exceptional adaptations to the extreme and fluctuating conditions of Antarctic freshwater systems. Its dormant cysts (resting eggs) can survive freezing temperatures down to −25°C and play a crucial role in persistence through winter (Peck 2004). These cysts are characterised by a smooth outer surface, in contrast to the often sculptured or ornamented cysts of many other anostracan species. Embryos develop within two membranes, with hatching initiated by rupture of the outer layer, leaving the embryo enclosed in a thin inner envelope (Jurasz et al. 1983).

While *B. gaini* has traditionally been considered a distinct species, recent phylogeographic analyses indicate it is not reciprocally monophyletic with its Patagonian relative, *B. granulosa*, raising questions about their taxonomic boundaries (Pokorný et al. 2024). This suggests possible cryptic diversity or unresolved lineage divergence within the *B. gaini–B. granulosa* complex. Notably, historical reports of *B. granulosa* from areas such as Palmer Land are now thought to represent misidentified *B. gaini*, underscoring the need for integrative taxonomic approaches to clarify the evolutionary relationships and biogeographic patterns of this lineage.

Our aim was to identify the occurrences of *B. gaini* on KGI, describe their habitats, and assess their population size and structure. Through our research, we endeavoured to identify threats to the genetic diversity of Antarctic freshwater ecosystems associated with global climate change. We formulated the following research questions: (1) Does *B. gaini* exhibit habitat opportunism? (2) Are there cryptic species within the populations on KGI? (3) Do habitat variables explain the size of the cyst bank (average cyst density in sediments)? (4) What is the distribution of cyst density at the bottom of the pond? In addition, we aimed to relate the results to the monopolisation hypothesis postulated by De Meester et al. (2002). Our work represents the first extensive study of *B. gaini* in the Antarctic.

2 | Materials and Methods

2.1 | Sites and Sampling

The study was conducted in ice-free areas along the southern coast of King George Island (KGI) (Figure 1; additional site details in Appendix S1), the largest among the South Shetland Islands. KGI spans approximately 1310 km^2, with around 90% of its terrain covered by permanent ice (Pętlicki et al. 2017; Pudełko 2003; Rückamp et al. 2011). Of the remaining ice-free areas, a relatively small but ecologically significant portion estimated between 2 and 4 km^2 is occupied by freshwater systems such as lakes, ponds, and meltwater streams, primarily located in low-elevation coastal regions like the Fildes Peninsula and Admiralty Bay (Falk and Silva-Busso 2021; Kim et al. 2020). These freshwater features represent roughly 0.2%–0.35% of the island's total area and are critical for understanding hydrological processes and microbial ecosystem dynamics in a changing polar environment (Rosa et al. 2020; Szopińska et al. 2018).

The study area included Antarctic Specially Managed Area 1 and two Antarctic Specially Protected Areas: ASPA 128 (Western shore of Admiralty Bay), ASPA 151 (Lions Rump) and two Important Bird Areas (IBAs no. 045 and 046) (Harris et al. 2015; Komarowska et al. 2025).

Glaciological changes in Admiralty Bay have been continuously monitored, with notable alterations in ice coverage and elevation being documented in recent decades (Pętlicki et al. 2017; Szopińska et al. 2018). These shifts have led to the emergence of new meltwater ponds, facilitating the formation of potential habitats for species such as *B. gaini*. Long-term hydrological, chemical, and ecological monitoring in the area (Komarowska et al. 2025; Osińska et al. 2023; Plenzler et al. 2025; Potapowicz et al. 2022; Rakusa-Suszczewski 2002; Szopińska et al. 2018; Szumińska et al. 2021) has contributed to a comprehensive understanding of these dynamic freshwater systems.

The availability of data from these disciplines for this region enables contextualisation of our results within a broader framework and provides a strong rationale for selecting the southern coast of the KGI as the sampling area.

Detailed analysis of maps (approx. 500 km^2) (Pętlicki et al. 2017; Pudełko 2003) and orthophotomaps (approx. 8.289 km^2) was employed to select the water bodies for

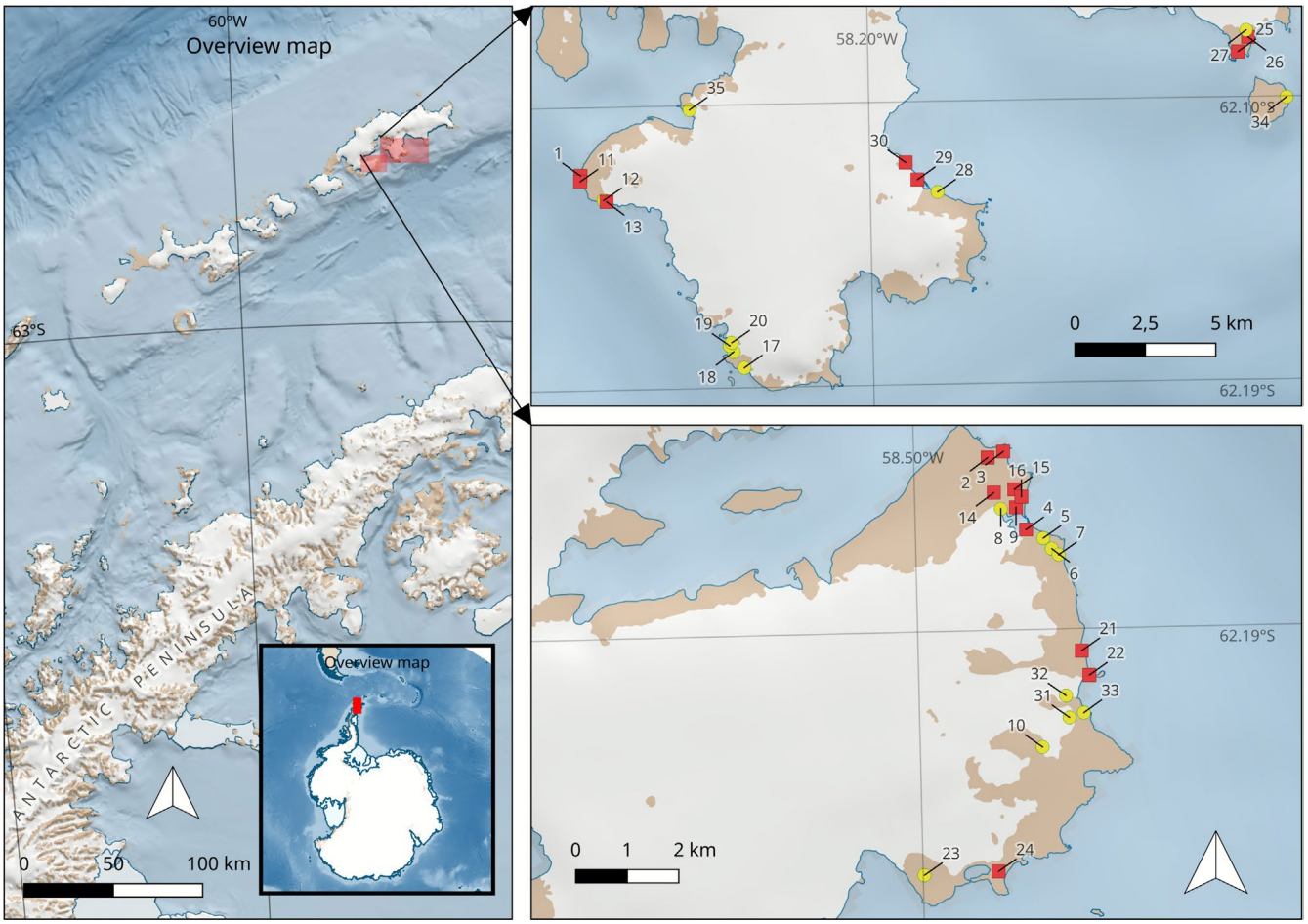

FIGURE 1 | Map showing surveyed locations on King George Island, red squares correspond to locations where *Branchinecta gaini* was detected and yellow circles are locations where *B. gaini* was undetected. Maps were created using the Quantarctica 3 QGIS package (Matsuoka et al. 2021) with coastline and rock outcrop data from the Antarctic Digital Database (ADD). Detailed maps including UAV-based imagery (orthophotomaps) are provided in Appendix S1.

investigation within the ice-free areas. The selected sites encompassed a range of habitats, including freshwater ponds, lakes and brackish pools, each offering distinct environmental conditions (Figure 6).

Fieldwork was conducted during the summer (from December 2021 to February 2022). The following activities were carried out to identify sites, perform habitat characterisation and gather fairy shrimp individuals for species identification and further genetic studies. At each surveyed site, trial catches were conducted using a hand net with a diameter of 28 cm and a mesh size of 70 μm. Whenever feasible, catches were performed at various locations within each site. If individuals of *B. gaini* were captured, further catches were discontinued, as the objective of the catches was to ascertain the presence of mature individuals.

At sites where individuals were successfully captured and were locally abundant, 20–40 individuals were immediately transferred to a 50 mL Falcon tube filled with 96% ethanol. This number of specimens was selected to ensure sufficient biological material not only for the current study but also for future analyses, including investigations into gut microbiota composition and diet, which require multiple or pooled individuals to obtain robust and representative data.

The remaining individuals were released into the environment. The preserved individuals were transported to the field laboratory at the Henryk Arctowski Polish Antarctic Station within a few hours and placed in a freezer at −20°C. Air temperature during the period of field work varied from −1.7°C to 11.7°C and the average was 2.18°C (Plenzler et al. 2025), which allowed the samples to be safely transported to the station without exposure to heating.

Sediment samples were collected from 35 pond sites using a small shovel. At each site, 10 subsamples were taken from different parts of the reservoir, including both the central and shoreline areas, depending on site morphology and accessibility. The goal was to gather representative material from across each pond, but due to heterogeneity in bottom structure including rocky substrates or limited sediment zones sampling methods were adapted accordingly. In such cases, samples were collected from the most sediment-rich area available. Each individual subsample was taken from an area of approximately 5 × 5 cm and included the upper 5 cm of sediment. These 10 subsamples

were then combined into a single composite 1000 mL sample per site for downstream analysis.

The physicochemical water variables temperature, conductivity, and pH were measured using a Pro1030 device (YSI Inc., Xylem Inc.). At each site, measurements were taken at three distinct locations, typically near the areas where sediment was collected.

Aerial photogrammetric surveys were conducted using a DJI Inspire 2 unmanned aerial vehicle equipped with a high-resolution camera. These surveys were successfully performed at 25 of the 35 pond sites.

A note was also made about each site, including information such as turbidity (visual investigation, transparent or turbid), bottom type (soft or rocky), the presence of penguins, elephant seals, and skuas in the area, and water flow, for example, a stream flowing through the pond (constant flow, temporary flow or static). During this work, a principle was established whereby a series of interconnected water bodies was treated as single sites. Wildlife presence was recorded only when individual animals were directly observed at or near the sampling site during field visits, and these observations were noted informally in field notebooks as part of general site documentation.

Table 1 shows the site coordinates acquired from the GNSS receiver (GPSMAP 78, Garmin Ltd.) during the sampling and geographical features such as pond area, distance from the coast, distance from the nearest glacier front and elevation. The points shown on the map (Figure 1) and the coordinates from Table 1 correspond to all 35 investigated sites and include information about the presence of *B. gaini*.

At sites 2 and 3, a detailed analysis of the cyst density in the sediment was carried out. For this purpose, a 5 cm layer of sediment was collected using a 3.5 cm diameter cylindrical sampler from 18 randomly selected sampling points (3 samples per sampling point) covering the study pond and the immediate surrounding area. During sampling, the depth was measured, and the coordinates were recorded using a GNSS receiver (GPSMAP 78, Garmin Ltd.), which enabled spatial analysis and evaluation of the relationship between depth and the number of cysts.

These two ponds were chosen for the analysis of cyst distribution in the ponds, as they were in close proximity to the station and it was possible to carry out sampling without time constraints. This type of dense sampling was not undertaken at other sites due to concerns about interference with these habitats. Two different sediment sampling approaches were applied due to varying research goals and logistical constraints. At all sites, composite samples were collected using a small shovel from 10 locations per pond to estimate general cyst density. At two accessible ponds (sites 2 and 3), a more detailed spatial analysis was performed using a cylindrical sampler at 18 randomly selected points (three replicates per point) to assess cyst distribution and its correlation with depth. This second method was more time-consuming and intrusive, requiring access to the entire pond surface. Due to challenging weather conditions and the remoteness of other ponds, such detailed sampling was limited to sites near the research station where weather windows allowed safe and repeated access.

2.2 | Methods for Isolating and Counting Cysts in Sediments

Each sediment sample was rehydrated in 10 L of tap water and then filtered through a sieve (100 μm) to remove heavy sand and retain light materials, including cysts, on the sieve. The collected material on the sieve was subsequently transferred to Falcon tubes (50 mL) and then covered with a 50% sucrose solution. Afterward, the samples underwent centrifugation for 5 min at 3000 rpm (Eppendorf Centrifuge 5810R), followed by draining the solution from the sediment and rinsing the separated cysts on the sieve. Then, we photographed (Lumix G DMC-G7EG, Panasonic Corporation) the separated cysts in Petri dishes, followed by preserving the cysts either by freezing or by placing them in 96% ethanol. Finally, the cysts were counted from the images using QuPath software (Bankhead 2017).

2.3 | Methods for Assessing the Genetic Structure of the Studied Populations

Ethanol-fixed specimens from sites 2, 4, 9, 11, 13, 14, 15, 16, 21, 22, 25, 26, and 30 were transported at −20°C to Poland, where further molecular analyses were carried out. Thoracopods from 100 specimens (13 sites in total) were dissected and used for DNA extraction by means of the Xpure Cell&Tissue Micro Kit (A&A Biotechnology). The concentration and purity of the isolated DNA were checked using a NanoPhotometer NP80 (Implen GmbH). The isolated DNA served as a template for the amplification of mitochondrial *cox*1 and the 16S rRNA gene region. The following primers were used for *cox*1 gene amplification: LCO1490 5′GGTCAACAAATCATAAAGATATTGG3′ and HCO2198 5′TAAACTTCAGGGTGACCAAAAAATCA3′ (Folmer et al. 1994), and for the 16S rRNA gene: 16S-FintE 5′AGGGCCGTGGTATTTTAACC3′ and 16S-RintE 5′ATCCCTGAACCAACATCGAG3′ (Deiner et al. 2017). PCR was performed using PCR Mix Plus Green (A&A Biotechnology). The thermal cycling parameters used for *cox*1 gene amplification were as follows: Initial denaturation at 94°C for 1 min; 30 cycles at 95°C for 30 s, 46°C for 90 s and 72°C for 45 s; and a final 3 min extension at 72°C. The parameters used for 16S rRNA gene amplification were as follows: Initial denaturation at 94°C for 1 min; 30 cycles at 95°C for 30 s, 46°C for 90 s and 72°C for 45 s; and a final 3 min extension at 72°C. After purification with a Clean-up concentrator (A&A Biotechnology) and quality assessment (NanoPhotometer NP80, Implen GmbH), the amplicons were sequenced with ABI3730 DNA Analyzers (Thermo Fisher Scientific). The obtained chromatograms were trimmed visually, and multiple alignments were obtained using MUSCLE (Edgar 2004) implemented in Mega11 (Tamura et al. 2021) with standard

TABLE 1 | Id—site identifiers, coordinates, region names distance from the coast (dfc), distance from the glacier front (dfg), elevation above sea level (msl), pond area for the studied localities (m^2).

Id	Date	Lon	Lat	Region	B. gaini	dfc	dfg	msl	Area
1	6.12.2021	−58.396968	−62.121477	Hennequin	1	44	695	10	5005.005
2	7.12.2021	−58.472434	−62.159937	Thomas	1	53	1135	5	1049.908
3	7.12.2021	−58.466643	−62.158947	Thomas	1	8	1372	5	5878.562
4	12.12.2021	−58.458751	−62.172596	Thomas	1	20	520	5	2631.767
5	12.12.2021	−58.452434	−62.174162	Llano	0	101	748	30	294.545
6	12.12.2021	−58.449346	−62.176017	Llano	0	222	687	30	38.418
7	12.12.2021	−58.446864	−62.17708	Llano	0	90	738	5	1586.572
8	17.12.2021	−58.46798	−62.168891	Thomas	0	66	280	10	3899.037
9	17.12.2021	−58.462328	−62.168697	Thomas	1	46	617	5	6256.875
10	18.12.2021	−58.4544	−62.21047	Demay	0	982	170	85	39691.455
11	21.12.2021	−58.397182	−62.123351	Hennequin	1	54	720	5	361.964
12	24.12.2021	−58.381491	−62.129492	Hennequin	0	28	182	10	763.308
13	24.12.2021	−58.379589	−62.129935	Hennequin	1	44	0	10	47419.574
14	27.12.2021	−58.470453	−62.166035	Thomas	1	360	512	50	115.271
15	29.12.2021	−58.462794	−62.165561	Thomas	1	214	731	30	24.53
16	29.12.2021	−58.460228	−62.166858	Thomas	1	42	779	10	12.457
17	30.12.2021	−58.287974	−62.183641	Vaureal	0	20	0	5	5530.215
18	30.12.2021	−58.295091	−62.1785	Vaureal	0	13	118	5	2790.44
19	30.12.2021	−58.297654	−62.176572	Vaureal	0	11	173	5	1661.272
20	30.12.2021	−58.296345	−62.175591	Vaureal	0	5	145	5	36184.485
21	4.01.2022	−58.43898	−62.19385	Sphinx	1	45	778	5	3139.144
22	4.01.2022	−58.436366	−62.19813	Sphinx	1	25	628	5	1143.135
23	4.01.2022	−58.499547	−62.232285	Red Hill	0	426	406	110	4014.144
24	4.01.2022	−58.471866	−62.231922	Patelnia	1	7	400	10	58.637
25	5.01.2022	−57.948461	−62.085723	Turret	1	127	1191	5	10746.507
26	5.01.2022	−57.941458	−62.081114	Turret	1	238	661	45	1002.141
27	5.01.2022	−57.942742	−62.07889	Turret	0	396	342	35	2773.998
28	5.01.2022	−58.154176	−62.128853	Lions	0	20	627	5	967.348
29	5.01.2022	−58.167736	−62.124783	Lions	1	20	283	5	1476.755
30	5.01.2022	−58.175649	−62.119297	Lions	1	9	342	5	5365.715
31	14.01.2022	−58.444117	−62.205423	Demay	0	364	0	30	401.984
32	14.01.2022	−58.445255	−62.20162	Demay	0	68	75	20	59.0127
33	14.01.2022	−58.438709	−62.204633	Demay	0	47	267	5	801.48
34	16.01.2022	−57.915821	−62.10032	Penguin Island	0	64	3022	5	27968.195
35	17.01.2022	−58.321896	−62.101272	Warkocz	0	131	126	60	8269.187

settings. Multiple sequence alignment was necessary to ensure homologous positions were correctly compared across all individuals.

The *cox*1 and 16S rRNA gene sequences of *B. gaini* were deposited in GenBank under the following accession numbers: *cox*1 (PP800340-PP800432) and 16S rRNA (PP801324-PP801330).

2.4 | Data Analysis and Visualisation

2.4.1 | Cyst Abundance–Pond Depth Relationship Analyses

The triangulated irregular network (TIN) algorithm implemented in the QGIS environment (QGIS Development Team 2024) was employed to interpolate the pond depth and cyst abundance in the bottom sediments. This method uses a series of interconnected triangles to model irregularly spaced survey points, thereby creating a continuous surface representation of reservoir depth and cyst abundance across site 2 and site 3. Statistical evaluation of the correlation between depth and the number of cysts was carried out in the R4.3.2 environment (R Core Team 2024), using the ggscatter package (Kassambara 2025; Wickham 2016) for data visualisation and the stat_cor function for correlation analysis. The relationship between pond depth and cyst abundance was examined through scatter plots, and Pearson correlation coefficients were calculated to assess the strength and direction of the relationship.

To explore the relationships between the collected variables and the cyst bank size, principal component analysis (PCA) was employed. PCA is a dimensionality reduction technique that identifies orthogonal axes (principal components) that capture the maximum variance in the dataset, thereby revealing underlying structures and patterns (Jolliffe 2002). Prior to conducting PCA, data preprocessing was performed to ensure compatibility and reliability. Numerical data were standardised to a mean of 0 and a standard deviation of 1 to mitigate the influence of scale discrepancies. PCA was carried out using the 'prcomp' function available in the 'FactoMineR' package (Lê et al. 2008) in R software. This function computes the principal components and their corresponding loadings, facilitating the interpretation of variable contributions to each component. A correlation circle was used to visualise the relationships among the variables. To examine potential associations between cyst abundance and environmental variables, we first assessed the distribution of all variables using the Shapiro–Wilk test. Where variables deviated from normality, we applied Yeo–Johnson power transformations to improve normality. Pearson's correlation coefficients were then calculated using the transformed data. In parallel, non-parametric Spearman's rank correlations were performed on the raw data to provide a distribution-free comparison. To account for testing multiple environmental variables, *p*-values were adjusted using the Holm and Benjamini–Hochberg procedures. All analyses were conducted in R (R Core Team 2024) with the packages *car* (Fox and Weisberg 2019), psych (Revelle 2025), and *rstatix (*Kassambara 2023).

To increase the scope of the analysis, factor analysis of mixed data (FAMD) was used, which enables the use of a combination of quantitative and descriptive variables. The descriptive variables used included data regarding the presence of large animals such as birds and mammals near the study site, the presence of accumulated organic matter on the pond bottom, the occurrence of turbidity of the water and signs of water flow through the pond. The same variables used for PCA were used as numerical variables except for cyst density. The FAMD was implemented using the 'FactoMineR' package (Lê et al. 2008) in R software (R Core Team 2024). This package offers comprehensive functionalities for factor analysis, including support for mixed data types. FAMD was executed by fitting a factorial model to the mixed dataset, followed by dimensionality reduction and factor extraction. The results were visualised in a 2-dimensional plot where individual observations were marked as the presence/absence of *B. gaini*. This enabled the relationship between the descriptive data and the occurrence of *B. gaini* to be illustrated.

2.5 | Phylogeny and Haplotype Analyses

Cox1 sequences (92) were sorted by region of origin according to Table 1. These sequences and that obtained from the GenBank database (MZ265218) were used to construct a haplotype network using PopART software (Leigh and Bryant 2015). Additionally, a haplotype map was generated using QGIS (QGIS Development Team 2024). Phylogenetic analyses based on 16S rRNA gene sequences obtained from specimens from KGI and species from the Branchinectidae family from Rogers and Aguilar (2020) were conducted using the maximum likelihood method (ML) in MEGA11 (Tamura et al. 2021). The tree was rooted with the *Thamnocephalus platyurus* 16S rRNA gene sequence obtained from GenBank (DQ470611.1). Final dataset consisted of 94 *cox1* sequences for haplotype network and 32 *16S* rRNA sequences for phylogeny.

Genetic differentiation among subpopulations was assessed using Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992) based on *cox1* haplotypes. A per-individual dataset was generated from haplotype frequencies across seven regions. Pairwise genetic distances were calculated using the Kimura 2-parameter model in the ape package (Paradis et al. 2004), and AMOVA was performed with the amova() function in pegas (Paradis 2010), using the formula dna_dist—region. The Phi statistic was used to quantify genetic structure among regions.

3 | Results

During this study, we identified 17 *B. gaini* occurrence sites in the surveyed area. Alignment of 7 sequences of the 16S rRNA coding region did not reveal variation in these populations but was used in combination with sequences from GenBank for phylogenetic analysis, which confirmed the species affiliation of *B. gaini* (Figure 2). A total of 93 *cox*1 sequences were obtained, and their alignment (with a length of 333 bp) revealed variations among individuals at two polymorphic sites, resulting in three haplotypes. The frequencies of haplotypes across seven regions are depicted on the map (Figure 3) and presented in Table 2.

In addition, Figure 4 shows the haplotype network including the Barton Peninsula sequence extracted from the GenBank database (MZ265218). The presence of only three haplotypes for the *cox1* gene and the lack of variation within the 16S rRNA gene region did not support the presence of cryptic species. However, analysis of molecular variance (AMOVA) revealed significant genetic structuring among subpopulations on King George Island, with 45.3% of the genetic variation attributed to differences among regions ($\Phi = 0.453$, $p < 0.001$). This differentiation was driven by marked differences in haplotype frequencies: individuals from the Thomas region exclusively carried haplotype

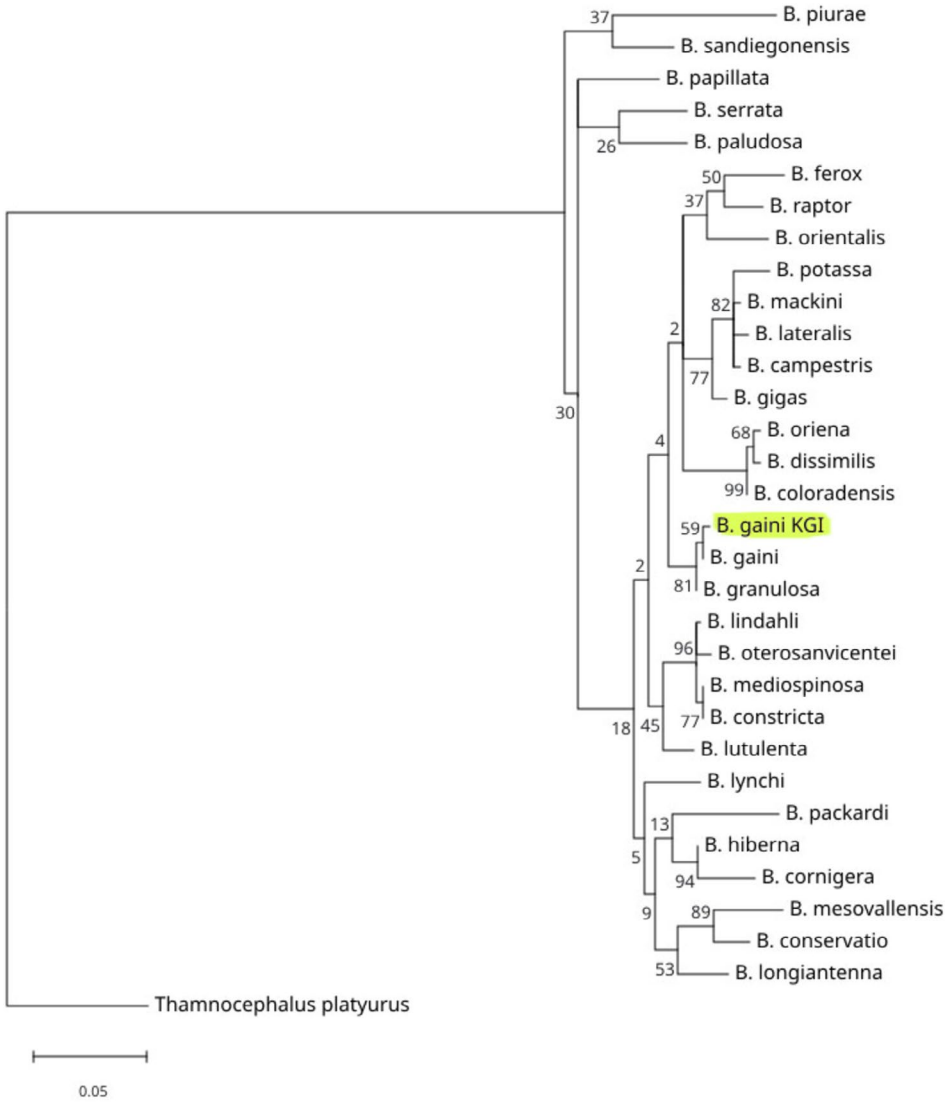

FIGURE 2 | Phylogenetic tree constructed based on 16S rRNA mitochondrial sequences in the family Branchinectidae. Sequence from one individual of *B. gaini* collected on King George Island is highlighted in yellow. The phylogeny was inferred using the Maximum Likelihood (ML) method under the Tamura–Nei (TN93) substitution model. The tree with the highest log-likelihood score (−2007.84) is shown. Initial starting trees for the heuristic search were generated automatically using Neighbor-Joining and BioNJ algorithms based on pairwise distances estimated with the Tamura–Nei model, selecting the topology with the best log-likelihood value. Branch lengths are drawn to scale and represent the number of substitutions per site. The final alignment contained 32 nucleotide sequences with 334 conserved positions in the dataset. A total of 334 sites were retained in the final ML analysis. All evolutionary analyses were performed in MEGA11.

2, while those from the Turret and Lions Rump regions carried haplotypes 1 and 3. The remaining sites exhibited a mix of haplotypes 1 and 2. Despite these regional patterns, the occurrence of shared haplotypes across sites and the widespread distribution of haplotypes 1 and 2 suggest ongoing gene flow and connectivity among subpopulations.

Thirteen of the 17 *B. gaini* occurrence sites are near the sea (< 50 m), between 0 and 5 m above sea level. Higher salinity, likely caused by seawater intrusion during a storm, resulting in the death of mature *B. gaini* individuals, was recorded at two sites (no. 3 and 35). Additionally, two surveyed sites (no. 11 and 16) dried out during the study period, leading to the death of fairy shrimp. Moreover, there was a large increase in pH at site 2 between 24.12.2021, when the value was 7.58, and 14.01.2022, when the value reached 9.63. During this time, dead specimens of *B. gaini* were observed. At all the sites surveyed, mature specimens were observed at sites with pH values ranging from 6.44 to 8.83 and conductivities ranging from 149.17 to 776.33 ($\mu S\ cm^{-1}$). Figure 5 shows the ranges of conductivity and pH values in which mature individuals of *B. gaini* were found compared with the data from all the sites. The surface area of the basins where *B. gaini* was detected ranged from 12.46 to 47419.57 m^2. At the remaining sites where *B. gaini* was not observed, pH ranged from 5.54 to 8.76, conductivity from 70.06 to 41,519.33 $\mu S\ cm^{-1}$, and surface area from 38.418 to 39,691.45 m^2.

Table 2 presents a comparison of physicochemical variables with cyst density. At three investigated sites (no. 1, 3, 29) where mature individuals were absent, cysts were isolated from the sediment at densities of 0.0200, 0.5650, 0.0100 cysts cm^{-3}

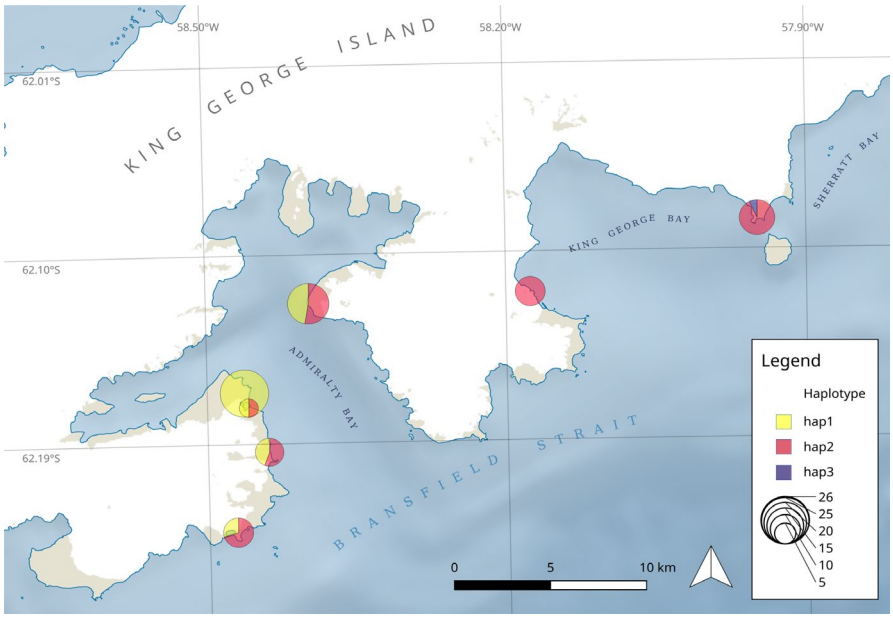

FIGURE 3 | Map of King George Island with graphical representation of the distribution of individual haplotypes of the COX1 gene in Branchinecta gaini populations across seven regions. The map was created using the Quantarctica 3 QGIS package (Matsuoka et al. 2021) with coastline and rock outcrop data from the Antarctic Digital Database (ADD).

respectively. Conversely, at site 13 in the Hennequin region (Figure 6e), mature individuals were observed, but cysts were not found in the sediment. The highest recorded cyst density, reaching 10.910 cyst cm^{-3} of sediment, was documented at site 14 (Figure 6b). The average density across all sites was 1.0797 cysts cm^{-3} (SD = 2.599). The PCA results illustrated patterns suggesting some association between cyst abundance and pH, pond area, and distance from the glacier terminus (Figure 7). However, subsequent correlation analyses indicated relationships were not statistically significant ($p > 0.05$). Only distance from the glacier terminus showed a positive correlation with cyst abundance (Pearson $r = 0.36$, $p = 0.04$; Spearman $\rho = 0.45$, $p = 0.012$), but this association did not remain significant after correction for multiple comparisons (Bonferroni, Holm, Benjamini-Hochberg). Meanwhile, the FAMD results suggested that the soft bottom of the reservoir, turbidity and lack of water flow were key characteristics associated with the occurrence of *B. gaini* (Figure 8).

Detailed analysis of cyst density in the sediments and reservoir depth at sites 2 and 3 revealed a highly significant ($p < 0.01$) correlation for site 2 and a significant ($p < 0.05$) correlation for site 3 (Figure 9), indicating the accumulation of cysts in the deeper parts of the ponds (Table 3).

4 | Discussion

4.1 | *Branchinecta gaini* on KGI

The first report of *B. gaini* on KGI dates back to 1977 on the Fildes Peninsula (Campos et al. 1978 as cited in Jurasz et al. 1983). But first from Point Thomas Oasis were made by Jurasz et al. (1983), who described the life cycle of fairy shrimp found in Wujka Lake (location no. 3) near the Arctowski Polish Antarctic Station. Moreover, two additional sites were identified. One was situated adjacent to the main station facilities, in an area that has undergone significant anthropogenic transformations, including terrain levelling, construction of service infrastructure, and increased foot and vehicle traffic. These changes have altered the native soil structure and local microhabitats, making it unclear whether a comparable undisturbed site currently exists (Rakusa-Suszczewski and Krzyszowska 1991). The third site, located near the Ecology Glacier between terminal and lateral moraines, is also uncertain due to glacial retreat and sediment displacement observed in recent seasons.

Notably, Pond 9, which was beneath the surface of the Ecology Glacier in the 1980s (Pasik et al. 2021) and likely had a direct connection to the saline waters of the Suszczewski Cove in the 1990s (Pudełko 2003), deserves special attention. Our analyses of cyst bank presence indicated the presence of *B. gaini* and the formation of a substantial cyst bank in the sediments of this very young reservoir (approximately 20-year-old pond). The case of site 9 confirmed a crucial assumption of the monopolisation hypothesis (De Meester et al. 2002), namely, the potential for new population founders to create a cyst bank in a very short time, enabling resource monopolisation and rendering subsequent migration events of marginal importance in shaping the genetic population structure. Near the Ecology Glacier, there is another, likely younger, reservoir (no. 8) where we did not detect *B. gaini*. Further monitoring and research is needed to enable tracking of colonisation processes in postglacial freshwater lakes. In addition, site 13 was presumably a younger postglacial lake, which could explain the absence of detectable cysts.

Sites where *B. gaini* was not detected displayed a range of environmental conditions that may collectively explain its absence. Some reservoirs, such as site 8, are likely too young to have accumulated sufficient organic matter or microbial mats to support a

TABLE 2 | Environmental characteristics and genetic sampling results from surveyed freshwater sites on King George Island. The table summarises visual turbidity, animal presence (penguins, skuas), water flow, bottom type, and observed haplotypes of *Branchinecta gaini* (hap1, hap2, hap3), along with the total number of individuals (*N*) successfully genotyped at each site. Empty fields indicate no individuals were collected or sequenced from those sites.

Id	Turbidity	Animals	Flow presence	Bottom type	hap1	hap2	hap3	*N*
1	Turbid	Skua	No flow	Rocky				
2	Turbid	Skua	No flow	Soft	2	0	0	2
3	Turbid	Skua	No flow	Soft				
4	Turbid	Skua	No flow	Soft	2	3	0	5
5	Transparent	Penguin	Flow	Rocky				
6	Transparent	Penguin	Probable flow	Soft				
7	Turbid	Penguin	No flow	Soft				
8	Transparent	No	Probable flow	Rocky				
9	Turbid	Skua	No flow	Soft	6	0	0	6
10	Turbid	Skua	Flow	Rocky				
11	Transparent	Skua	No flow	Soft	8	1	0	9
12	Transparent	Skua	Flow	Rocky				
13	Transparent	Skua	Probable flow	Rocky	1	9	0	10
14	Transparent	Skua	Probable flow	Soft	9	0	0	9
15	Transparent	Skua	No flow	Soft				
16	Turbid	Penguin	No flow	Soft	8	0	0	8
17	Transparent	Elephant seal	No flow	Rocky				
18	Transparent	Elephant seal	Probable flow	Rocky				
19	Transparent	Elephant seal	Probable flow	Rocky				
20	Transparent	Elephant seal	No flow	Soft				
21	Turbid	No	No flow	Soft	4	0	0	4
22	Turbid	Skua	No flow	Soft	0	5	0	5
23	Transparent	Skua	No flow	Soft				
24	Turbid	Elephant seal	No flow	Soft	3	7	0	10
25	Turbid	Skua	No flow	Soft	0	9	1	10
26	Transparent	Skua	No flow	Rocky	0	4	0	4
27	Turbid	Skua	Flow	Soft				
28	Transparent	Elephant seal	No flow	Soft				
29	Turbid	Elephant seal	No flow	Soft				
30	Turbid	Elephant seal	Probable flow	Soft	0	10	0	10
31	Turbid	No	Flow	Rocky				
32	Transparent	No	Flow	Rocky				
33	Transparent	No	Flow	Rocky				
34	Transparent	Penguin	No flow	Rocky				
35	Transparent	No	No flow	Rocky				

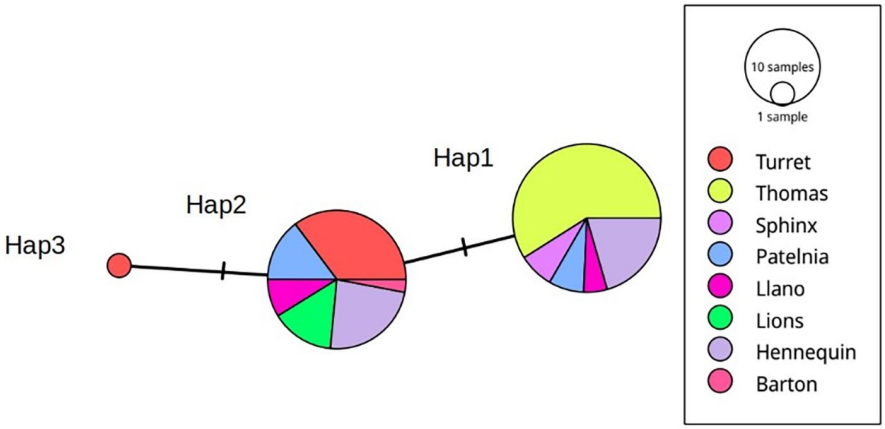

FIGURE 4 | Haplotype network of the COX1 gene in Branchinecta gaini populations across 8 regions.

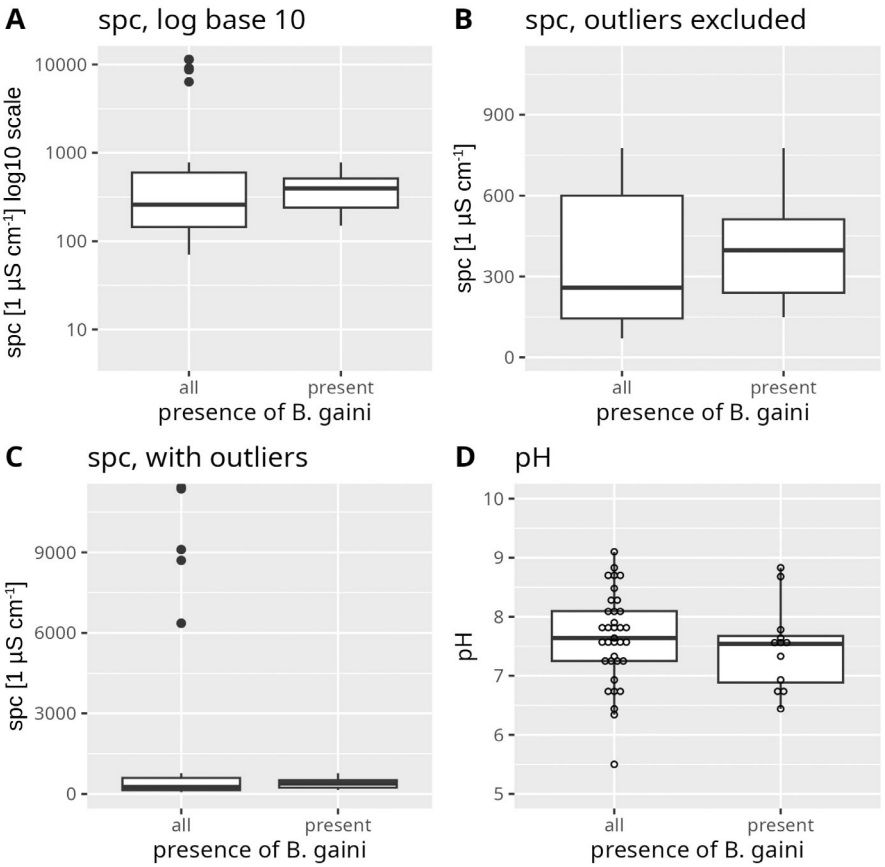

FIGURE 5 | Box-plots of conductivity and pH values for sites where mature *Branchinecta gaini* individuals were observed compared to all sites: (A) specific conductivity—spc in log base 10 scale (B) spc without outliers (C) spc with outliers (D) pH.

viable food base. Similarly, sites 10, 27, 31, 32, and 33 are recently formed or intermittent-flow systems that likely lack a stable benthic layer and may be subject to periodic flushing of both cysts and active individuals. Site 5 is directly connected to a flowing watercourse, making it hydrologically unstable for cyst deposition and retention. Several locations, including sites 6 and 7, may contain elevated nitrogen compounds and are situated near large gentoo penguin (*Pygoscelis papua*) colonies, which can increase nutrient loading but also create harsh biological and chemical conditions (e.g., high ammonia or organic pollution). Reservoir 23, located on Red Hill, remains snow-covered for much of the year, and the persistence of liquid water may be too brief to allow *B. gaini* to complete its life cycle. Site 35 is a lake with a gravelly, rocky bottom and no visible detrital layer, suggesting a poor food base. Most critically, sites 17, 18, 19, 20, 28, and 34 are located near the coast and are likely subject to frequent saltwater intrusion due to storm surges or overwash events. These saline disturbances likely exceed the known tolerance thresholds for *B. gaini* adults (Peck 2004; Hawes et al. 2008), preventing long-term persistence.

In 1984, parasitic tapeworms (*Branchiopodataenia* sp.) were detected in *B. gaini* near Arctowski Station. Jarecka (1984) proposed

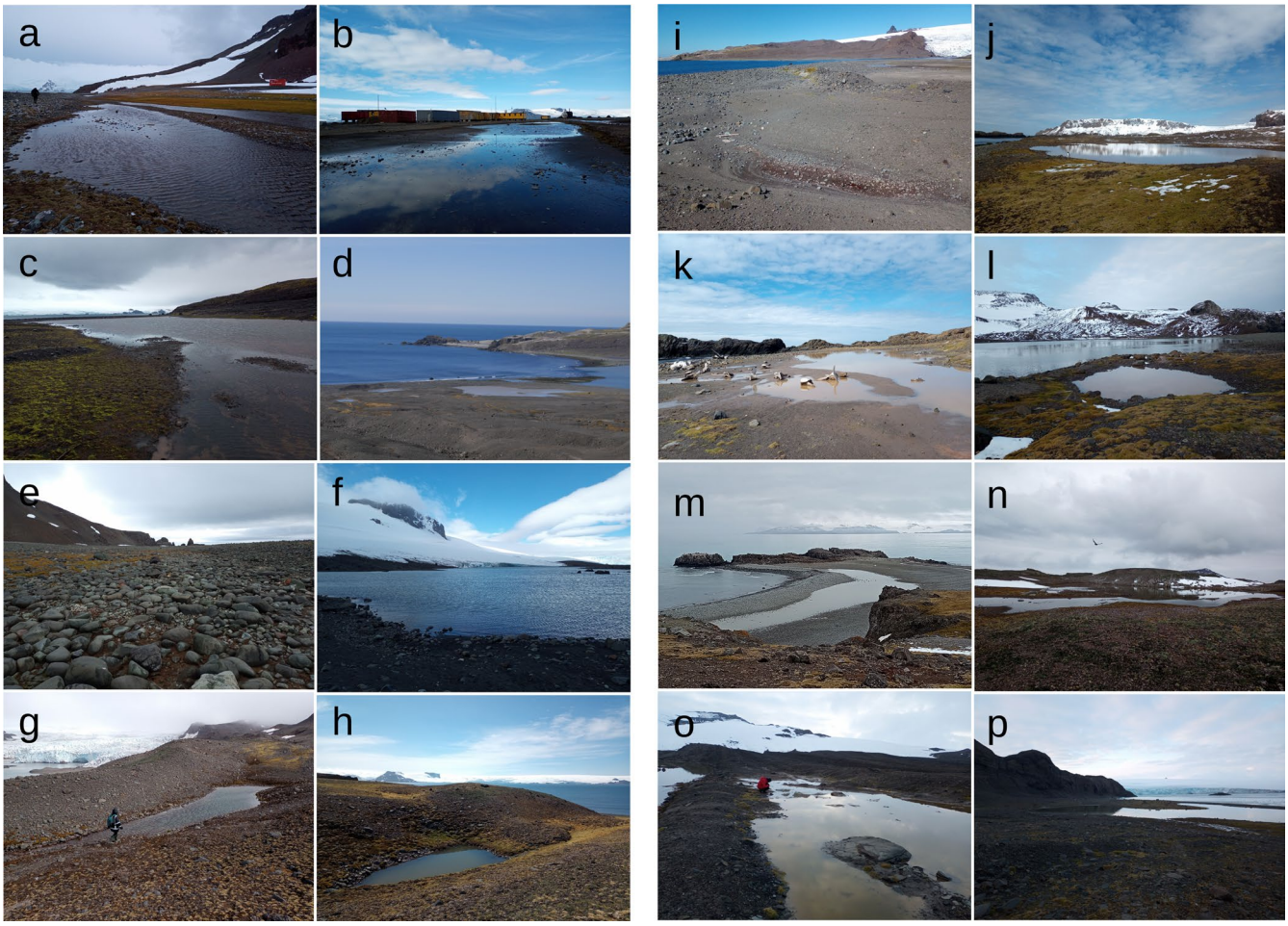

FIGURE 6 | Photographs showing ponds in which *Branchinecta gaini* is found: (a) location no. 1, pond located at Point Hennequin near the refuge of Ecuador, visible as an orange container; (b) location no. 2 and 3 ponds located in the direct vicinity of the Arctowski Polish Antarctic Station, called Lake Wujka; (c) location no. 4, pond located at Llano Point near Suszczewski Bay; (d) location no. 9, pond located on the opposite side of Suszczewski Bay from location no. 4, closer to Rakusa Point; (e) location no. 11, completely dried out pond located at Point Hennequin near the refuge *of Ecuador*; (f) location no. 13, glacial lake at the head of the Vieville glacier; (g) location no. 14, pond located between moraines near the Ecology Glacier; (h) location no. 15, pond located between moraines near the Ecology Glacier; (i) location no. 16, completely dried up very small body of water near the beach at Rakusa Point; (j) location no. 21, pond located at the foot of Sphinx Hill; (k) location no. 22, pond located on the beach at Agat Point; (l) location no. 24, small pond at the Patelnia Point; (m) location no. 25, pond at the Turret Point; (n) location no. 26, pond between moraines near Turret Point; (o) location no. 29, brackish pond located near Lions Rump; (p) location no. 30, brackish pond located near Lions Rump.

the possibility of parasite transfer between *B. gaini* and kelp gulls (*Larus dominicanus)*. This constitutes a clue in attempting to reconstruct potential dispersal scenarios for this fairy shrimp species. Our unpublished data indicated the presence of *B. gaini* cysts in the faeces of South polar skua (*Stercorarius maccormicki*) found in the Turret Point region. This finding may support the hypothesis raised by Hawes (2009) regarding the function of endozoochory in the dispersion of Antarctic fairy shrimp.

Other studies addressing the occurrence of *B. gaini* on KGI focused on Lake Wujka (site no. 3), investigated its phenology and the impact of salinity changes resulting from periodic inflows of saltwater from Admiralty Bay into the pond (Pociecha 2007; Pociecha and Dumont 2008). There are also two publications from 2022 on the use of *B. gaini* individuals from the western coast of KGI in ecotoxicological studies (Bergami et al. 2022).

Finally, Cukier et al. (2023) pointed out the potential for human-mediated cyst dispersal, including inadvertent transport on footwear or equipment. Their study specifically assessed this by analysing dry sediment (mud) adhering to the shoes of scientists working in the field. The reported cyst density of 2.83 cysts g^{-1} of dry sediment at site 9 reflects sediment that was highly viscous and adhesive conditions that likely favoured the accumulation and retention of cysts on footwear. In contrast, our study was designed to assess the natural size of the cyst bank within aquatic habitats and involved sediment collection directly from the pond bottom, without regard to viscosity or adhesiveness. As such, our result of 0.272 cysts cm^{-3} represents a more ecologically integrated estimate of cyst abundance in situ.

4.2 | Habitat Tolerance

Antarctic marine ecosystems are characterised by high biodiversity. There are numerous highly specialised and adapted animals living in cold but relatively stable environments (Convey et al. 2014). However, freshwater ecosystems are exposed to

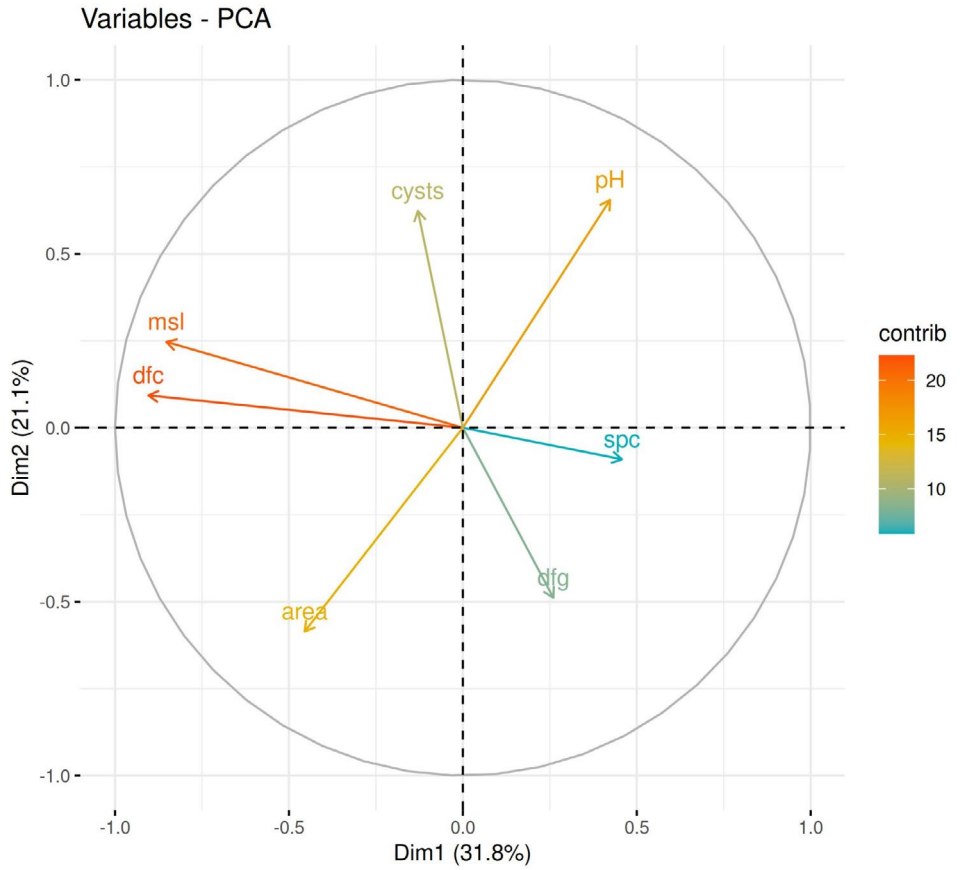

FIGURE 7 | Principal component analysis (PCA) quantitative variable correlation and contribution. The analyzed variables include: cyst density cm^{-3} (cysts), pH; distance from coast (dfc), distance from glacier front (dfg) elevation above mean sea level, (msl), specific conductivity (spc).

FIGURE 8 | Factor analysis of mixed data (FAMD) graph of individuals with qualitative variables.

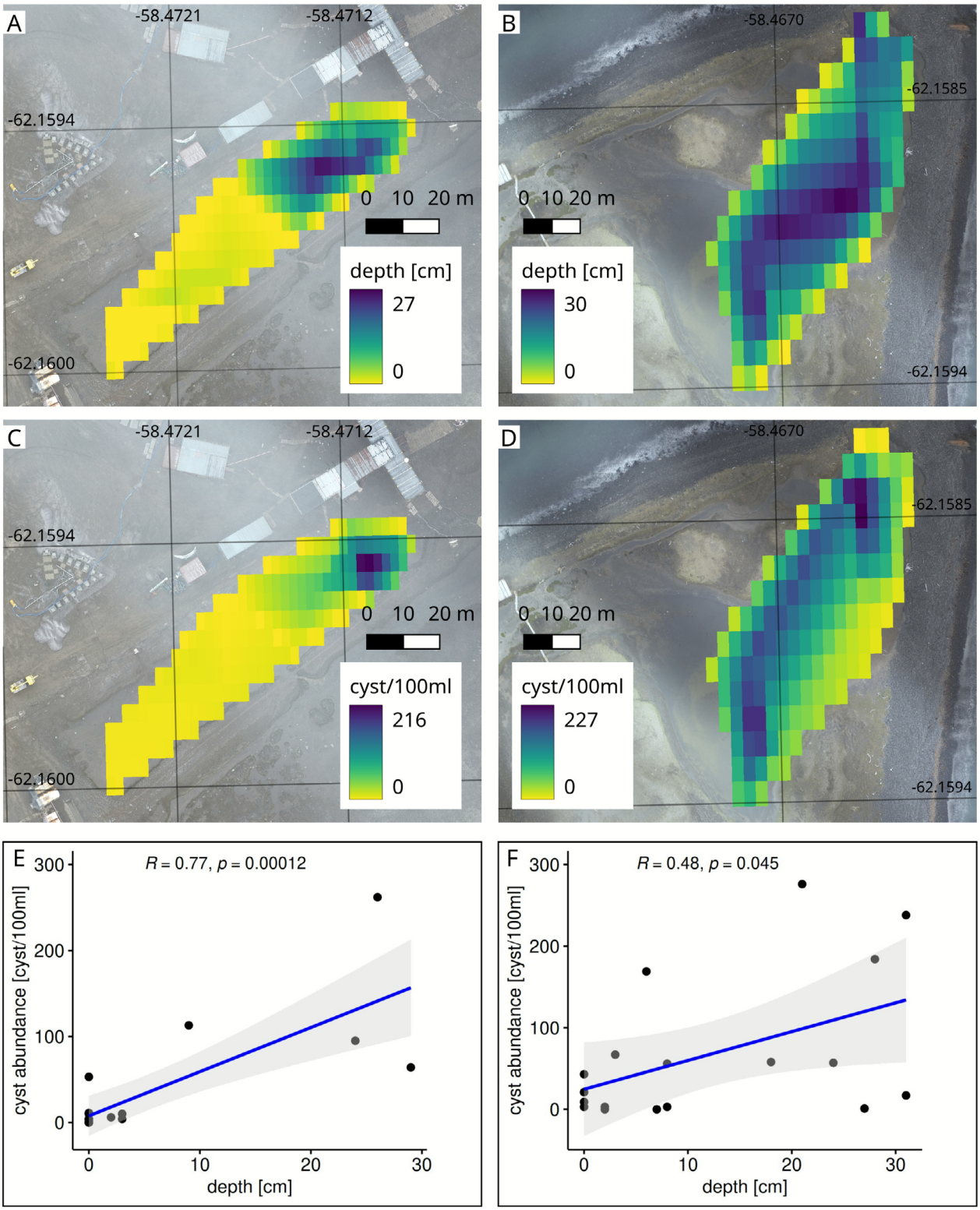

FIGURE 9 | Relationship between cyst abundance and pond depth at sites 2 and 3. (A) TIN (Triangular Irregular Interpolation) of pond depth at site 2, (B) TIN interpolation of pond depth at site 3, (C) TIN cyst abundance at site 2 (D) TIN cyst abundance at site 3, (E) scatter plot of the relationship between depth and cyst abundance at site 2 (F) scatter plot of the relationship between depth and cyst abundance at site 3.

large daily and annual temperature fluctuations, desiccation, freezing and salinity fluctuations, among others. Such conditions enable only species with a wide habitat tolerance to survive (Convey et al. 2008). Furthermore, *B. gaini* is an example of a distinctly freshwater Anostraca with a short life cycle, using the brief weather window of the summer (Hawes et al. 2008). The success of these fairy shrimps in colonising freshwater ponds in Antarctica is attributed not to specific adaptations to the extreme conditions prevailing in the region but to their ability to acclimate to reservoirs and the occurrence of dormant forms,

TABLE 3 | Summary of environmental variables and cyst abundance across study sites. The table includes site identifiers (Id), pH (mean, maximum, and minimum), specific conductivity (μS cm^{-1}) (mean, maximum, and minimum), temperature (°C) (mean, maximum, and minimum), and cyst abundance in surface sediment (cysts cm^{-3}).

Id	Data	pH	pH max	pH min	Cond	Cond max	Cond min	Temp	Tempmax	Tempmin	Cystscm
1	6.12.2021	7.213	7.27	7.11	172.366	211.9	93.6	5.8	8	4.7	0.02
2	7.12.2021	7.53	7.55	7.51	507.566	512.6	499	6.166	6.2	6.1	0.565
3	7.12.2021	7.25	7.29	7.23	10425.2	11,363	9419	4.666	4.7	4.6	0.68
4	12.12.2021	8.826	8.9	8.68	461.666	465	459	5.633	5.7	5.6	0.061
5	12.12.2021	8.266	8.46	8.1	119.433	122.7	117.2	1.866	2	1.8	0
6	12.12.2021	5.5	5.54	5.46	536.033	555	516	4.3	4.3	4.3	0
7	12.12.2021	7.29	7.35	7.23	443.4	444.5	441.4	2.933	3	2.9	0
8	17.12.2021	7.903	7.96	7.85	237.433	247.3	232.3	3.2	3.2	3.2	0
9	17.12.2021	7.776	7.86	7.63	776.33	784	763	4.033	4.2	3.9	0.272
10	18.12.2021	6.73	6.75	6.7	122.466	122.7	122.3	3.5	3.5	3.5	0
11	21.12.2021	6.7133	6.81	6.62	253.066	271.5	229	NA	NA	NA	0.212
12	24.12.2021	6.336	6.41	6.28	85.166	88.9	80.6	3.933	4	3.9	0
13	24.12.2021	6.44	6.61	6.31	149.166	151.4	147.3	3.366	3.5	3.3	0
14	27.12.2021	8.683	8.89	8.56	246.266	313	113.3	8.2	8.4	8.1	10.91
15	29.12.2021	6.76	6.78	6.74	425.233	426.2	424	5.866	6	5.8	0.022
16	29.12.2021	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.029
17	30.12.2021	7.853	7.87	7.84	6359.333	6451	6310	1.533	1.8	1.4	0
18	30.12.2021	7.816	8.06	7.62	70.066	73.2	68.2	1.233	1.7	0.9	0
19	30.12.2021	7.6	7.72	7.53	95.666	111.6	65.5	4.1	4.7	3.7	0
20	30.12.2021	8.733	8.76	8.71	27,724	27,823	27,560	5.433	5.5	5.4	0
21	4.01.2022	7.583	7.59	7.57	369.766	376.6	359.7	6.833	7	6.7	0.488
22	4.01.2022	7.643	7.69	7.6	649.866	652.6	648	11.766	12.1	11.5	1.782
23	4.01.2022	7.27	7.3	7.24	242.966	264.6	212.9	2.733	3.1	2.5	0
24	4.01.2022	7.333	7.35	7.32	552.033	672	324.1	7.4	7.6	7.3	1.837
25	5.01.2022	6.933	7.02	6.87	175.333	179.8	170.6	9.1	10.3	7.9	0.349
26	5.01.2022	7.54	7.56	7.53	219.133	220.8	218.3	7.033	7.1	7	1.026
27	5.01.2022	7.586	7.61	7.57	164.033	165.4	163.1	5.866	5.9	5.8	0
28	5.01.2022	8.08	8.12	8.03	41519.333	42,178	40,684	9.566	9.8	9.2	0
29	5.01.2022	9.1	9.1	9.1	9102.333	9152	9026	9.6	9.6	9.6	0.01
30	5.01.2022	8.47	8.48	8.45	11432.333	11,608	11,168	9.466	9.6	9.4	0.0905
31	14.01.2022	8.293	8.32	8.27	110.8	117	104.8	2.1	2.1	2.1	0
32	14.01.2022	8.103	8.19	8	125.233	125.4	125	10.2	10.3	10	0
33	14.01.2022	7.823	7.88	7.77	139.4	140.8	138.5	6.533	6.6	6.5	0
34	16.01.2022	7.853	7.92	7.8	8699.666	8702	8698	6.266	6.3	6.2	0
35	17.01.2022	8.083	8.09	8.07	130.1	130.1	130.1	7.1	7.3	6.8	0

which enable them to withstand the most unfavourable periods of the year and engage in long-distance dispersal. Analysing instances of *B. gaini* occurrence revealed that *B. gaini* thrives in diverse freshwater habitats, displaying variation in its productivity and morphometric characteristics. These lake habitats exhibit contrasting features, including highly developed photoautotrophic growth (Nedbalová et al. 2017) and a predominant phytoplankton community (Heywood et al. 1980). The adaptability of *B. gaini* is remarkable, with populations thriving in water bodies as small as ~1 m^2 and ±50 cm in depth, even under climatically severe conditions, such as those in the Marguerite Bay area (Hawes et al. 2008).

Ecological opportunism does have limitations, and local populations of Antarctic fairy shrimp may be susceptible to population decline for various reasons. Our results showed that an increase in pH (9.63) in pond no. 2 likely led to the death of part of the cohort at that time, suggesting an exceedance of tolerance limits. The most frequently cited variable regarding the habitat tolerance of *B. gaini* is electrical conductivity. On James Ross Island, fairy shrimp persist in lakes with a conductivity of approximately 1100 μS cm^{-1} (Nedbalová et al. 2013). Additionally, observations in Lake Wujka (no. 3) on KGI revealed the presence of nauplii and metanauplii even at approximately 2950 μS cm^{-1} (Pociecha and Dumont 2008). Conversely, in the more favourable southern Patagonia region (45°–53° S), a detailed assessment showed *B. gaini* preferred shallow waters with lower conductivity (550 ± 40 μS cm^{-1}) and was absent from lakes with considerably higher values (de los Ríos et al. 2008). While *B. gaini* demonstrates notable physiological flexibility and can exploit highly variable conditions (Peck 2004), projected climate-driven changes to Antarctic hydrology such as increased evaporation, prolonged ice-free periods, and altered snowmelt patterns may progressively reduce the availability of suitable habitats (Hawes 2009). If unfavourable conditions persist across multiple seasons, the resilience of local cyst banks could be compromised. Therefore, although extinction risk at the species level appears low, declines or local extirpation of populations remain plausible under future environmental scenarios.

Interpreting the impact of conductivity on *B. gaini* survival requires caution, as evidenced by paleolimnological research on James Ross Island by Björck et al. (1996). The core data from Boulder (Monolith) Lake spanning over 2000 years revealed the persistent presence of *B. gaini*, primarily when the conductivity was approximately 2000 μS cm^{-1} and spiked to nearly 9000 μS cm^{-1}. These values were strongly influenced by sulfate anions and divalent metal cations. Björck et al. (1996) reported that increasing soluble $CaSO_4$ concentrations had a minimal effect on adult *B. gaini* survival unless gypsum precipitation occurred, obstructing filtration appendages and reducing filtration efficiency.

During our investigation, the water in Lake Wujka (no. 3) reached a conductivity of approximately 1100 μS cm^{-1}, which was likely responsible for the absence of adult individuals in the 2021–2022 season. In another lake in the Lions Rump region, we observed the mass death of a fairy shrimp cohort when conductivity surged to 11,400 μS cm^{-1}. This extreme increase in salinity was likely caused by seawater intrusion during a storm event, a known mechanism in coastal Antarctic systems. Laboratory experiments by Hawes et al. (2008) showed that adult *B. gaini* exposed to seawater-like conductivities (~9400 μS cm^{-1}) experienced 100% mortality within 6 days. These observations suggest that sudden increases in salinity beyond physiological thresholds, especially from marine overwash events, pose a substantial (although temporarily) risk to local populations.

Our findings support evidence in the literature that *B. gaini* tolerates a wide range of environmental conditions, enabling it to live in very different types of ponds. We found Antarctic fairy shrimp in very small and evaporating puddles, small and deep ponds, shallow reservoirs near the seashore and a typical glacial lake. The small reservoirs in which *B. gaini* is often found are especially characterised by instability. A small volume of water can heat quickly during a sunny day and cool or freeze at night or on colder days. The occurrence of these fairy shrimp in these cold habitats indicates a tolerance for short-term temperature changes, which is supported by the findings of Hawes et al. (2008). Notably, *B. gaini* is found in nutrient-rich habitats fed, for example, by windborne marine algae and in relatively poor glacial lakes or those between moraines far from the sea. Undoubtedly, the ability to survive in such poorer habitats may be due to the particular feeding strategy described by Paggi (1996). Unlike most nonselective filter-feeding anostracans, *B. gaini* procures food from the bottom or scrapes microbial mats from the rock surface. This strategy in reservoirs with little plankton, such as those common in the Antarctic, has enabled *B. gaini* to colonise these areas.

Our results and those of Björck et al. (1996) indicate that the presence of organic matter such as detritus or microbial mats is a critical habitat feature for *B. gaini*. This confirms the importance of detritus as a food source for this species. Our work also indicates the importance of the absence of water flow through a pond for *B. gaini* presence. The presence of flowing water even temporarily can result in the flushing out of mature individuals, cysts and detritus that forms the basis of the food source. Recognising the function of water flow for the presence of *B. gaini* therefore appears reasonable. The FAMD results also indicated a link between turbidity and *B. gaini* presence. This turbidity could be of various origins, possibly linked to the presence of organic matter and planktonic organisms. However, it is difficult to interpret this result without more detailed analyses. In summary, *B. gaini* is an opportunistic species that lives in bodies of water where liquid water is present for at least 2.5 months, and it requires fresh or temporarily brackish standing water with the presence of detritus or microbial mats.

4.3 | Cyst Abundance

Large branchiopod survey methods are commonly based on the isolation and identification of dormant cysts from collected bottom sediments, which is particularly valuable in ephemeral aquatic habitats where adults are only present for a short period (Martin et al. 2018). In our study, we employed a mixed-methods approach that combined (1) sediment analysis for the presence and abundance of cysts and (2) direct sampling of live individuals from the water column and benthic zones. This dual approach enabled a more comprehensive assessment of

B. gaini distribution. For example, we detected *B. gaini* cysts at several sites where mature individuals were not observed, indicating potential reproductive or colonisation events that would have gone unnoticed by adult sampling alone. Conversely, at site 13, we observed mature individuals but failed to isolate cysts, highlighting that cyst-based methods alone can yield false negatives. Therefore, integrating both cyst and adult sampling provides a more reliable and complete picture of species presence and reproductive dynamics in Antarctic freshwater systems.

The analysis of the relationship between cyst density and pond depth at the 2 sites indicated uneven cyst deposition in the sediments. We observed that the highest cyst abundance occurred in the deepest parts of the studied ponds. This was likely due to the physical properties of the cysts and possibly the preferences of females for depositing cysts in a certain manner. Due to the astatic nature of these reservoirs, it can be expected that at low water levels, the pond area includes only the deepest zones, which limits the area where individuals deposit cysts.

Considering the periodic inflow of seawater and increasing salinity at site 3, questions arise regarding changes in water buoyancy relative to cysts, which could contribute to the accumulation of drifting cysts on the shores of the pond. In the case of *Artemia* spp., a saltwater representative of Anostraca, the phenomenon of cyst buoyancy and accumulation on lake shores is known to occur (Gajardo and Beardmore 2012; Manaffar et al. 2018). The possibility of using the salt flotation method to separate cysts of large branchiopods also confirms this possibility (Rogers 2014). However, our observations did not indicate that this phenomenon occurs for *B. gaini*.

The formation of cyst banks by aquatic organisms has wide implications related to the survival of populations under unfavourable conditions, the possibility of passive dispersion, and the preservation of unique genes that have been displaced during periodic environmental changes (Hairston Jr. and Kearns 2002; Ripley et al. 2004).

4.4 | Phylogeny and Genetic Structure

In the literature, there is a clear deficit of genetic data enabling the formulation or verification of hypotheses about phylogeographic or population patterns in *B. gaini*. On the Barton Peninsula near the King Sejong Korean Antarctic Station, mitochondrial genome analysis and nuclear genome sequencing have been conducted for only a single individual (Jo et al. 2022). These data remain the only available molecular information for *B. gaini* from King George Island (KGI) prior to our study. Our findings show a pattern of partial haplotype segregation: haplotype 1 was found exclusively in the Point Thomas Oasis region, while haplotype 2 occurred only in the Turret and Lions Rump regions. This distribution may suggest some degree of spatial genetic structuring and limited gene flow across regions. However, both haplotypes were present in the remaining four regions, which tend to support a scenario of gene flow between populations.

Interestingly, the regions where both haplotypes co-occur are located on opposite sides of Admiralty Bay. This may indicate that bird-mediated dispersal of cysts (Hawes 2009) is more frequent within or across this bay, enhancing connectivity between these populations. An alternative explanation is that the founding populations were already genetically mixed, leading to the current pattern. Nevertheless, it is puzzling that only a single haplotype (haplotype 1) was detected in the Thomas region, despite its geographic proximity to other regions where both haplotypes are present. This may reflect stochastic founder effects, restricted dispersal events, or ecological differences influencing local establishment success. Further genetic sampling and higher-resolution molecular markers will be necessary to disentangle these potential scenarios and to assess whether the observed patterns reflect recent colonisation history, limited dispersal, or longer-term population structure.

5 | Conclusion

Our results demonstrated that *B. gaini* populations on KGI are numerous and inhabit various types of lakes, indicating broad ecological tolerance. The presence of a large cyst bank in a relatively young (20-year-old) pond (no. 9) confirms the assumption of the homogenisation hypothesis. Molecular analyses do not indicate the presence of cryptic species in the area studied but suggest the existence of homogeneity dependent on geographic distribution. To draw further conclusions about the population genetics of *B. gaini* on KGI, we believe that research using more sensitive molecular methods, such as microsatellites or single nucleotide polymorphisms, is necessary. The large increase in salinity in ponds near the seashore led us to speculate that the rising sea levels associated with global warming may impact the survival of *B. gaini* populations and, consequently, the biodiversity of Antarctic ecosystems.

Author Contributions

S.C., J.G. and R.B.: conceptualisation, developing methods, conducting the research, data interpretation, writing. S.C.: data analysis, preparation of figures and tables.

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The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The obtained sequences will be available in the genbank database (https://www.ncbi.nlm.nih.gov/genbank/) with the following accession numbers: PP800340–PP800432 and PP801324–PP801330. Other data are available from the authors upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Maps showing the locations of study sites on King George Island, Antarctica. Detailed site groups include: (A) Point Thomas Oasis (sites 2, 3, 14, 15, 16, 9, 8); (B) Llano Point (sites 4, 5, 6, 7); (C) Sphinx and Demay areas (sites 21, 22); (D) Patelnia Point (site 24); (E) Hennequin Point (sites 1, 11, 13, 12); (F) Vaureal (sites 20, 17); (G) Lions Rump (sites 29, 30); (H) Turret Point (sites 25, 26, 27). The overview maps were created using the Quantarctica 3 QGIS package (Matsuoka et al. 2021) with coastline and rock outcrop data from the Antarctic Digital Database (ADD). The detailed site maps were generated using high-resolution orthophotos acquired via UAV flights (DJI Inspire 2) conducted during the 2021–2022 summer at 25 of the 35 pond locations.