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A first glimpse into the biogeographic affinities of the shallow benthic communities from the sub-Antarctic Crozet archipelago

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Sub-Antarctic islands are expected to show a high degree of endemism due to their remoteness. However, biogeographic affinities in the sub-Antarctic remain poorly understood, especially in the marine realm. Sub-Antarctic islands being at the crossroads between Antarctic and cold temperate regions, biodiversity characterization and biogeographic analyses are a priority for monitoring and rapidly assessing variations associated with environmental changes. One underexplored sub-Antarctic area is Crozet, a protected archipelago located halfway between Antarctica and South Africa. In this study, we investigated the shallow-water Crozet macrofaunal diversity, distribution patterns and biogeographic affinities based on the examination of fieldwork specimens via a thorough morphological identification and a genetic characterisation. The resulting dataset provides an important baseline for further studies and conservation strategies, compiling the first genetic and taxonomic database for the Crozet archipelago. In total, 100 morphotypes were found, belonging to nine different phyla, among which arthropods (32), molluscs (18) and echinoderms (17) were the richest. Forty-seven morphotypes were identified to the species level, among which 20 were reported in Crozet for the first time. This confirms that Crozet is a poorly known region, even compared to other sub-Antarctic areas. A large proportion of species (62%) had circum Southern Ocean or circum sub-Antarctic distributions. These species were mostly shared with Kerguelen (72%),

the Magellan Province (64%), and Prince Edward Islands (64%), confirming the patterns found in macroalgae and specific macrofaunal groups. However, this large-distribution statement needs to be counterbalanced by the detection (genetic data) of more restricted distributions than expected in four study cases (the tanaid *Apseudes spectabilis*, the nudibranch *Doris kerguelenensis*, the polychaete *Neanthes kerguelensis* and the chiton *Hemiarthrum setulosum*). Considering that most morphotypes had no genetic data available from other regions, the proportion of morphotypes with restricted distribution is likely to increase alongside future investigations. In addition, we also found a few cases of unrecognized diversity that might lead to the descriptions of new species, some likely to be endemic to Crozet (e.g., within the polychaete genus *Harmothoe* and the bryozoan genus *Antarctothoa*). Altogether, this stresses the need to maintain conservation efforts in Crozet and pursue integrative investigations in order to highlight and protect its unusual diversity.

KEYWORDS

biodiversity, Southern Ocean, taxonomy, barcoding, marine

Introduction

In the Southern Ocean, the sub-Antarctic region is located between the subtropical and the Antarctic polar marine fronts, comprising a series of remote archipelagos and small islands (De Broyer et al., 2014). Due to the geographic remoteness of sub-Antarctic islands, a high degree of endemism can be expected in both terrestrial and marine taxa (Frenot et al., 2001). Several studies from the last decades have highlighted contrasting patterns of distribution in various taxonomic groups, from single island endemism to species exhibiting broad distributions (e.g., Poulin et al., 2014; Figuerola et al., 2017, Figuerola et al., 2018; Féral et al., 2021; Vantomme et al., 2023). Overall, biodiversity and biogeographic affinities of the different sub-Antarctic areas remain poorly understood, with a contrast of knowledge existing among sub-Antarctic islands (Griffiths and Waller, 2016; Féral et al., 2021). It is thus particularly important to better characterize their biodiversity and biogeographical affinities to improve our understanding of marine life dynamics, especially considering their key location at the crossroads between high Antarctic and cold temperate areas (Hemmings and Stephens, 2010; De Broyer et al., 2014). It is also central to conservation considerations, given that high endemism can mean increased sensitivity to global change and risk of biodiversity loss (Griffiths, 2010; Park et al., 2014; Jossart et al., 2019; Féral et al., 2021). In particular, an improved biogeographic baseline would thus detect distribution shifts that are expected in response to current and future environmental changes (Saucède et al., 2017; Guillaumot et al., 2018). In response to these conservation concerns, some sub-Antarctic islands have already been given the status of Marine Protected Areas (Crozet MPA, Heard Island and McDonald Islands MPA, Kerguelen MPA, Prince Edward Islands MPA; Brooks et al., 2020)

or have received a significant extension of their marine parks (e.g., Macquarie Island Marine Park in 2023, parksaustralia.gov.au).

Crozet's benthic marine biodiversity is notably underexplored. This five-island archipelago is located in the Indian Ocean sector of the Southern Ocean (45°48'S–46°26'S; 50°14'E–52°15'E) and is about 2,300 km away from both Antarctica and South Africa. Crozet originated from three main cycles of volcanism, among which the first one initiated nine million years ago (Chevallier and Nougier, 1981). The archipelago is strongly influenced by the Antarctic Circumpolar Current (ACC), which is expected to increase its connectivity with other sub-Antarctic areas (Pollard et al., 2007; De Broyer et al., 2014). Previous terrestrial studies have been undertaken on Crozet terrestrial biodiversity (e.g., Frenot et al., 2001; Convey, 2007; Hullé and Vernon, 2021), however, marine benthic ecosystems have received far less attention (Canteras and Arnaud, 1985; Sicinski and Gillet, 2002; Griffiths and Waller, 2016; Lelièvre et al., 2023). The waters around Crozet are part of the National Nature Reserve of the French Southern Territories MPA and recognized as a UNESCO World Heritage site (whc.unesco.org/en/list/1603). Marine coastal areas have also additionally received an IUCN “enhanced protection” status (Féral et al., 2021). In this context of conservation initiatives, the French polar institute project #1044 Proteker was implemented to monitor nearshore marine benthic communities of the French Southern Territories (Kerguelen, Saint-Paul & Amsterdam, and Crozet). The main goal of this ongoing project is to establish a baseline for monitoring biodiversity dynamics and assess the impact of climate change on coastal marine ecosystems (Féral et al., 2016). Since its initiation, samples collected from Crozet during the Proteker campaigns have been invaluable for the investigation of biogeographic patterns in selected taxa (e.g., González-Wevar et al.,

2021; Rosenfeld et al., 2023). At the community level, using underwater video-imagery, Lelièvre et al. (2023, 2024) investigated the taxonomic and functional diversities of shallow benthic communities associated with hard substrates. The authors highlighted high taxonomic diversity and low functional richness, evenness, and redundancy. This suggests a potential vulnerability to current and future environmental changes, which, again, stresses the need for further investigations based on additional biological samples (Mason et al., 2005; Lelièvre et al., 2023).

In our study, we investigated the diversity and biogeography of macrofauna (>1 mm) at two sites (*Baie du Marin* and *Crique du Sphinx*) around Possession island (“*Ile de la Possession*”, 46°25’S; 51°45’E), the largest island of the Crozet archipelago. Based on the examination of specimens collected in a recent fieldwork campaign, we combined a thorough morphological identification by taxonomic experts with a genetic characterisation based on the COI barcode region. The joint use of morphological and genetic data has been demonstrated to be effective in species identification of various taxonomic groups (e.g., Gostel and Kress, 2022; Jossart et al., 2023). In addition, comparing recorded biogeographic patterns (relying on morphology-based taxonomy) with genetic data was also important in the revision and clarification of species distributions (Christiansen et al., 2018; Jossart et al., 2019; Vantomme et al., 2023). In Lelièvre et al. (2023), Crozet faunal communities exhibited a high-level diversity, notably in echinoderms. Regarding its geographic situation and oceanic features (strong influence by the ACC), we expect benthic marine communities of Crozet to be composed of both endemic and circum sub-Antarctic species, with high-level faunal affinities to both Kerguelen and Prince Edward Islands. In fact, while remoteness can explain endemism after rare colonisation and settlement events, a certain homogenisation can also be expected due to the role of the ACC in larval or even adult (kelp rafting) transport (Moore et al., 2018; Fraser et al., 2022). By compiling the first genetic and taxonomic database including specimen images and occurrence records for Crozet, this study’s newly generated dataset provides an important identification tool and baseline for further studies and conservation purposes.

Materials and methods

Fieldwork

Specimen sampling occurred during November 2021 as part of the Proteker 9 campaign onboard the R/V *Marion Dufresne II*. Two geographically close sites (< 2 km), *Baie du Marin* and *Crique du Sphinx*, were investigated on the east coast of Possession Island (“*Ile de la Possession*”, Crozet archipelago). This investigation was part of an impact study on benthic communities after the recent installation of the International Monitoring System (IMS) hydroacoustic station HA04 in the area (Lelièvre et al., 2023). Biological samples were opportunistically collected by hand during six dives at depths ranging from 4 to 20 m. Specimens were then preserved in 96% ethanol for subsequent analyses.

Morphological identification

Each specimen was examined under a stereomicroscope (Leica LK300 LED), assigned to a morphotype (or putative species) and a preliminary identification was obtained using available taxonomic resources (e.g., Féral et al., 2019; Lelièvre et al., 2023). Each morphotype was also photographed using the same stereomicroscope and an Olympus OMD-EM1 camera with a 60 mm macro lens. Preliminary identifications of morphotypes were then confirmed or refined by taxonomic experts (Supplementary Material 1). Macro photographs were sent to taxonomists as well as voucher specimens when required in the identification process.

Genetic characterization

The barcode region of the cytochrome c oxidase subunit I (COI; 658 base pairs) was obtained for each morphotype (one to twenty individuals sequenced per morphotype). DNA extractions were undertaken on a small piece of tissue, following the salting-out protocol of Sunnucks and Hales (1996). For COI amplification, we used a PCR mix of 12.5 μL Accustart Toughmix (QuantaBio), 10 μL ultrapure water, 0.5 μL each primer (10 μM) and 1.5 μL DNA extract. PCR conditions consisted of 40 cycles for the following temperature steps: 30 s at 94°C (denaturation), 30 s at 45–49°C (annealing, see details below) and 45 s at 72°C (elongation). These cycles were preceded by 3 min at 94°C and followed by 3 min at 72°C. Amplifications occurred using either universal or taxon-specific primers. Bryozoans, chordates, cnidarians, nemerteans and sponges were amplified using the universal primer pair “F-LCO1490 + R-HCO2198” at an annealing temperature of 45°C (Folmer et al., 1994). Amphipods and pycnogonids were amplified with the same primers at an annealing temperature of 49°C. Isopods and tanaids were amplified using the primer pair “F-CrustCOIF + R-HCO2198” at an annealing temperature of 47°C (Teske et al., 2006). Echinoderms were amplified using the primer pair “F-LCOech1aF1 + R-HCO2198” at an annealing temperature of 45°C (Folmer et al., 1994; Layton et al., 2016). Annelids were amplified using either the primer pair “F-LCO1490 + R-HCO2198” and the primer pair “F-polyHCO + R-polyLCO” at an annealing temperature of 47°C (Carr et al., 2011). Molluscs were amplified using either the primer pair “F-LCO1490 + R-HCO2198” (annealing temperature: 45°C) or the primer pair “F-COI-mol & R-COI-mol” (annealing temperature: 49°C; Owada et al., 2013). The sequences of each of the aforementioned primer were F-LCO1490: GGTCAACAAATCATAAAGATATTGG; R-HCO2198: TAAACTTCAGGGTGACCAAAAAATCA; F-CrustCOIF: TCAACAAATCAYAAAGAYATTGG; F-LCOech1aF1: TTTTCTACTAAACACAAGGATATTGG; F-polyHCO: TAMACTTCWGGGTGACCAARAATCA; R-polyLCO: GAYTATWTTCAACAAATCATAAAGATATTGG; F-COI-mol: TCWACAAATCAYAAAGATATTGG; R-COI-mol: ACYTCMGGRTGMCCAAAAATCA. The purification step and Sanger sequencing were carried out at the sequencing centre of

Azenta/Genewiz Germany. Sequences were edited and checked for stop codons in Geneious Prime 2023.2.1 (Kearse et al., 2012). Barcodes were then aligned (Muscle algorithm), compared among them within classes/orders (e.g., amphipods) and to the ones available on GenBank and Barcode of Life (BOLD) databases (Ratnasingham and Hebert, 2007). Combining morphological and genetic data, a final identification was then determined at the most accurate taxonomic level possible. In addition to the COI sequences aforementioned, additional genetic barcodes from non-Crozet areas were obtained from either scientific publications or public BOLD records. Combining all the barcodes for each taxon, alignments were then realized in Geneious Prime 2023.2.1. Templeton Crandall Singh (TCS) haplotype networks were then constructed in PopART 1.7 (Leigh and Bryant, 2015) and mean pairwise genetic distances (uncorrected p-distances) were calculated between clades in the software MEGA X (Kumar et al., 2018). We then used a genetic distance threshold of 2% (among clades of the haplotype network) to consider that an initial morphotype represents more than one putative species. This threshold is based on the most common interspecific genetic distances observed in the groups of interest [crustaceans: Vieira et al., 2016, molluscs: Layton et al., 2014, polychaetes: Carr et al., 2011, sea stars: Ward et al., 2008 and Moreau et al., 2021]. Species delimitation methods, such as ASAP and ABGD, were not used considering their low suitability for datasets with limited number of sequences (Puillandre et al., 2012, Puillandre et al., 2021). A public Barcode of Life (BOLD) project (HAOIV: Shallow benthic communities of Crozet archipelago) was created on the platform boldsystems.org. Macro pictures were uploaded for each specimen as well as COI barcodes, when successfully obtained (Table 1).

Biogeographic and phylogeographic analyses

The biogeographic distribution of taxa identified up to the species level was analysed based on the following bioregionalization of the Southern Ocean: Magellan Province, South Georgia/South Sandwich Islands, Bouvet (= Atlantic Sector of the Southern Ocean); Prince Edward Islands (Prince Edward and Marion islands), Crozet, Kerguelen, Heard Island (= Indian Ocean Sector of the Southern Ocean); Macquarie/sub-Antarctic New Zealand region (= Pacific sector of the Southern Ocean); West Antarctica; East Antarctica). Species occurrence data were either based on: 1) occurrences mentioned in scientific publications; 2) GBIF occurrences (Global Biodiversity Information Facility; <https://www.gbif.org>); 3) additional unpublished occurrences obtained from direct contacts with taxonomic experts. If a contradiction arose, occurrences obtained from direct contacts with taxonomists or dedicated publications had precedence over GBIF records. COI barcodes were also used to refine the species distribution of four species (e.g., the expected presence of *Hemiarthrum setulosum* P. P. Carpenter, 1876 in Antarctica was not considered as it appeared that Antarctic specimens were highly divergent from the ones of Crozet, see results). Four different distribution patterns were then considered: 1) restricted sub-Antarctic distribution (i.e., recorded in a single

sector of the sub-Antarctic); 2) broad sub-Antarctic distribution (i.e., recorded in two sectors of the sub-Antarctic); 3) circum sub-Antarctic (i.e., recorded in three sector of the sub-Antarctic); 4) circum Southern Ocean (recorded both in the Antarctic and in either two or three sub-Antarctic sectors). Based on identified species occurrence data, a similarity network was produced using Gephi 0.10.1 (Bastian et al., 2009). In the network, nodes (circles; $n = 56$) represent both geographic regions and species that are connected by edges (lines; $n = 241$). The graph type was undirected, and no edge merging strategy was used. The layout algorithm "ForceAtlas2" (Jacomy et al., 2014) was then used to spatialize the network. The following input settings were used: tolerance: 0.05, approximate repulsion: checked, approximation: 1.2, scaling: 50, stronger gravity: checked, gravity: 0.01. Considering the low number of occurrences at Bouvet, this bioregion was not taken into account in the similarity network analysis.

Results

Overall taxonomic diversity

In total, 1050 specimens representing 100 morphotypes were collected in the study area (Table 1), and 173 individual genetic barcodes were obtained from 67 distinct morphotypes, indicating a barcoding success rate of 67%. The 100 morphotypes belonged to nine different phyla, among which Arthropoda (32 morphotypes) was the richest, followed by Mollusca (18), Echinodermata (17), Annelida (8), Chordata (8), Bryozoa (6), Cnidaria (5), Porifera (4) and Nemertea (2) (Figure 1). In Arthropoda, the diversity was split between amphipods (19 morphotypes), isopods (8 morphotypes), pycnogonids (3 morphotypes) and tanaids (2 morphotypes) (Figure 1). The phylum Mollusca was composed of gastropods (13 morphotypes), bivalves (4 morphotypes) and chitons (1 morphotype) (Figure 1). Within the Echinodermata, asteroids were the most speciose class (9 morphotypes), followed by holothuroids (6 morphotypes), echinoids (1 morphotype), and ophiuroids (1 morphotype) (Figure 1). From the 100 different morphotypes, 47 were identified to the species level (Table 1) among which 20 species were reported at Crozet for the first time (Table 1): three holothuroid species (*Cladodactyla crocea* var. *croceoides* (Vaney, 1908), *Echinopsolus splendidus* (Gutt, 1990), *Scoliorhapis massini* O'Loughlin & VandenSpiegel, 2010); two polychaete species (*Neoleprea streptochoeta* (Ehlers, 1897), *Platynereis australis* (Schmarda, 1861)); two bryozoan species (*Antarctothoa* cf. *bougainvillei* (d'Orbigny, 1842), *Beania magellanica* (Busk, 1852)); two gastropod species (*Doris kerguelensis* (Bergh, 1884); *Fusinella jucunda* (Thiele, 1912)); one chiton species (*Hemiarthrum setulosum*); ten amphipod species (*Acontistoma marionis* Stebbing, 1888, *Atyloella* cf. *magellanica* (Stebbing, 1888), *Haplocheira barbimana* (Thomson, 1879), *Iphimediella paracuticoxa* Andres, 1988, *Jassa* cf. *alonsoae* Conlan, 1990, *Jassa* cf. *hartmannae* Conlan, 1990, *Jassa* cf. *justi* Conlan, 1990, *Oradarea* cf. *unidentata* Thurston, 1974, *Pagetina monodi* (Nicholls, 1938), *Podocerus capillimanus* Nicholls, 1938); and one isopod species (*Cryosignum lunatum* (Hale, 1937)).

TABLE 1 List of the 100 taxa found in the studied area, ranked by alphabetical order of phyla (then by class or order).

Taxa	Code	BOLD	Distribution
Annelida – Clitellata			
<i>Piscicolidae</i> sp. Johnston, 1865	ANE_Q	HAOIV064-24 *	-
Annelida – Polychaeta			
<i>Eulalia</i> sp. Savigny, 1822	ANE_R	HAOIV065-24 *	-
<i>Harmothoe</i> spp. Kinberg, 1856	ANE_B-X-Y-Z	HAOIV029-24 *	-
<i>Neanthes kerguelensis</i> (McIntosh, 1885)	1 - ANE_D	HAOIV033-24 *	Circum SUB
<i>Neoleprea streptochoeta</i> (Ehlers, 1897) #	2 - ANE_A	HAOIV028-24 *	Circum SO
<i>Platynereis australis</i> (Schmarda, 1861) #	3 - ANE_E	HAOIV046-24 *	Circum SUB
<i>Thelepus spectabilis</i> Ehlers, 1897	4 - ANE_O	HAOIV174-24	Circum SO
<i>Serpulidae</i> sp. Rafinesque, 1815	UND_A	HAOIV175-24	-
Arthropoda – Amphipoda			
<i>Acontiostoma marionis</i> Stebbing, 1888 #	5 - AMP_V	HAOIV176-24	Circum SO
<i>Atyoella</i> cf. <i>magellanica</i> (Stebbing, 1888) #	6 - AMP_27	HAOIV002-24 *	Circum SO
<i>Corophioidea</i> sp. Leach, 1814	AMP_Q	HAOIV024-24 *	-
<i>Haplocheira barbimana</i> (Thomson, 1879) #	7 - AMP_G	HAOIV011-24 *	Circum SO
<i>Haplocheira</i> sp. Haswell, 1879	AMP_K	HAOIV023-24 *	-
<i>Iphimediella paracuticoxa</i> Andres, 1988 #	8 - AMP_21	HAOIV177-24	HANT+Rest.SUB
<i>Ischyrocerus</i> sp. Krøyer, 1838	AMP_H	HAOIV178-24	-
<i>Jassa</i> cf. <i>alonsoae</i> Conlan, 1990 #	9 - AMP_ZZ	HAOIV179-24	Circum SUB
<i>Jassa</i> cf. <i>hartmannae</i> Conlan, 1990 #	10 - AMP_A	HAOIV004-24 *	Broad SUB
<i>Jassa</i> cf. <i>justi</i> Conlan, 1990 #	11 - AMP_E	HAOIV180-24	Circum SUB
<i>Oedicerotidae</i> sp. Lilljeborg, 1865	AMP_P	HAOIV181-24	-
<i>Oradarea</i> cf. <i>unidentata</i> Thurston, 1974 #	12 - AMP_B	HAOIV007-24 *	Circum SO
<i>Pagetina monodi</i> (Nicholls, 1938) #	13 - AMP_12	HAOIV182-24	Broad SUB
<i>Paramoera fissicauda</i> (Dana, 1852)	14 - AMP_X	HAOIV025-24 *	Circum SO
<i>Parawaldeckia kidderi</i> (S.I. Smith, 1876)	15 - AMP_18	HAOIV001-24 *	Circum SO
<i>Podocerus capillimanus</i> Nicholls, 1938 #	16 - AMP_I	HAOIV012-24 *	Circum SO
<i>Proboloides</i> sp. Della Valle, 1893	AMP_ZF	HAOIV183-24	-
<i>Prostebbingia</i> sp. Schellenberg, 1926	AMP_J	HAOIV013-24 *	-
<i>Tryphosella</i> sp. Bonnier, 1893	AMP_ZC	HAOIV027-24 *	-
Arthropoda – Isopoda			
<i>Cassinidopsis emarginata</i> (Guérin-Méneville, 1843)	17 - ISO_C	HAOIV142-24 *	Circum SUB
<i>Cryosignum lunatum</i> (Hale, 1937) #	18 - ISO_8	HAOIV137-24 *	Circum SO
<i>Iathrippa</i> sp. Bovallius, 1886	ISO_G	HAOIV147-24 *	-
Limnoriidea sp. Brandt & Poore in Poore, 2002	ISO_I	HAOIV149-24 *	-
<i>Septemserolis septemcarinata</i> (Miers, 1875)	19 - ISO_J	HAOIV185-24	Circum SO
<i>Sphaeromatidae</i> sp. 1 Latreille, 1825	ISO_A	HAOIV184-24	-
<i>Sphaeromatidae</i> sp. 2 Latreille, 1825	ISO_D	HAOIV145-24 *	-
<i>Spinoserolis latifrons</i> (White, 1847)	20 - ISO_B	HAOIV139-24 *	Circum SO

(Continued)

TABLE 1 Continued

Taxa	Code	BOLD	Distribution
Arthropoda – Pycnogonida			
<i>Endeis viridis</i> Pushkin, 1976	21 - PYC_B	HAOIV166-24 *	Rest. SUB
<i>Nymphon</i> cf. <i>brevicaudatum</i> Miers, 1875	22 - PYC_A	HAOIV165-24 *	Circum SO
<i>Tanystylum neorhetum</i> Marcus, 1940	23 - PYC_D	HAOIV186-24	Circum SO
Arthropoda – Tanaidacea			
<i>Apseudes spectabilis</i> Studer, 1884	24 - TAN_2	HAOIV167-24 *	Circum SUB
<i>Pancoloides litoralis</i> (Vanhöffen, 1914)	25 - TAN_A	HAOIV168-24 *	Circum SO
Bryozoa – Gymnolaemata			
<i>Antarctothoa</i> cf. <i>bougainvillei</i> (d'Orbigny, 1842) #	26 - BRY_F	HAOIV187-24	Circum SO
<i>Antarctothoa</i> sp. Moyano, 1987	BRY_B	HAOIV188-24	-
<i>Beania magellanica</i> (Busk, 1852) #	27 - BRY_E	HAOIV189-24	Broad SUB
<i>Chaperiopsis</i> sp. Uttley, 1949	BRY_D	HAOIV190-24	-
<i>Fenestrulina</i> sp. 1 Jullien, 1888	BRY_1	HAOIV104-24*	-
<i>Fenestrulina</i> sp. 2 Jullien, 1888	BRY_C	HAOIV191-24	-
Chordata – Actinopterygii			
<i>Gobionotothen marionensis</i> (Günther, 1880)	28 - VER_B	HAOIV173-24 *	Broad SUB
<i>Harpagifer</i> sp. Richardson, 1844	VER_A	HAOIV192-24	-
Chordata – Ascidiacea			
Ascidiacea sp. 1 Blainville, 1824	TUN_B	HAOIV193-24	-
Ascidiacea sp. 2 Blainville, 1824	TUN_D	HAOIV194-24	-
Polyclinidae sp. 1 Milne Edwards, 1841	TUN_A	HAOIV195-24	-
Polyclinidae sp. 2 Milne Edwards, 1841	TUN_C	HAOIV196-24	-
Polyclinidae sp. 3 Milne Edwards, 1841	TUN_E	HAOIV171-24 *	-
<i>Sycozoa</i> sp. Lesson, 1832	TUN_F	HAOIV197-24	-
Cnidaria			
Actiniaria sp. 1 Hertwig, 1882	CNI_B	HAOIV198-24	-
Actiniaria sp. 2 Hertwig, 1882	CNI_E	HAOIV115-24 *	-
Alcyoniidae sp. Lamouroux, 1812	CNI_C	HAOIV112-24 *	-
<i>Staurocladia</i> sp. Hartlaub, 1917	CNI_A	HAOIV111-24 *	-
Tubulariidae sp. Goldfuss, 1818	CNI_D	HAOIV113-24 *	-
Echinodermata – Asteroidea			
<i>Anasterias rupicola</i> (Verrill, 1876)	29 - AST_K	HAOIV098-24 *	Broad SUB
Asteriidae sp. Gray, 1840	AST_H	HAOIV090-24 *	-
<i>Diplasterias meridionalis</i> (Perrier, 1875)	30 - AST_G	HAOIV088-24 *	Broad SUB
<i>Glabraster antarctica</i> (E. A. Smith, 1876)	31 - AST_C	HAOIV199-24	Circum SO
<i>Henricia obesa</i> (Sladen, 1889)	32 - AST_D	HAOIV084-24 *	Circum SO
<i>Henricia</i> cf. <i>spinulifera</i> (E. A. Smith, 1876)	33 - AST_1	HAOIV078-24 *	Rest. SUB
<i>Leptychaster kerguelenensis</i> E. A. Smith, 1876	34 - AST_J	HAOIV095-24 *	Broad SUB
<i>Pteraster affinis</i> Smith, 1876	35 - AST_F	HAOIV087-24 *	Circum SO

(Continued)

TABLE 1 Continued

Taxa	Code	BOLD	Distribution
Echinodermata – Asteroidea			
<i>Smilasterias triremis</i> (Sladen, 1889)	36 - AST_A	HAOIV081-24 *	Circum SO
Echinodermata – Echinoidea			
<i>Pseudechinus</i> sp. Mortensen, 1903	ECH_A	HAOIV117-24 *	-
Echinodermata – Holothuroidea			
<i>Cladodactyla crocea</i> var. <i>croceoides</i> (Vaney, 1908) #	37 - HOL_A	HAOIV129-24 *	Broad SUB
Cucumariidae sp. Ludwig, 1894	HOL_C	HAOIV134-24 *	-
<i>Echinopsolus splendidus</i> (Gutt, 1990)	38 - HOL_B	HAOIV130-24 *	HANT+Rest.SUB
<i>Pentactella laevigata</i> Verrill, 1876	39 - HOL_E	HAOIV135-24 *	Rest. SUB
<i>Pentactella</i> sp. Verrill, 1876	HOL_F	HAOIV136-24 *	-
<i>Scoliorhapis massini</i> O'Loughlin & VandenSpiegel, 2010 #	40 - UND_23	HAOIV172-24 *	Broad SUB
Echinodermata – Ophiuroidea			
<i>Ophiosabine vivipara</i> (Ljungman, 1871)	41 - OPH_A	HAOIV158-24 *	Broad SUB
Mollusca – Bivalvia			
<i>Bivalvia</i> sp. Linnaeus, 1758	BIV_D	HAOIV200-24	-
<i>Gaimardia</i> sp. A. Gould, 1852	BIV_B	HAOIV201-24	-
<i>Imparidentia</i> sp. Bieler, P. M. Mikkelsen & Giribet, 2014	BIV_A	HAOIV202-24	-
<i>Lissarca</i> sp. E. A. Smith, 1877	BIV_E	HAOIV103-24 *	-
Mollusca – Gastropoda			
Aeolidiidae sp. Gray, 1827	NUD_A	HAOIV153-24 *	-
<i>Chlanidota</i> sp. E. von Martens, 1878	GAS_C	HAOIV120-24 *	-
<i>Doris kerguelenensis</i> (Bergh, 1884) #	42 - NUD_B	HAOIV154-24 *	Broad SUB
<i>Eatoniella</i> sp. Dall, 1876	GAS_I	HAOIV124-24 *	-
<i>Falsimohnia</i> sp. A. W. B. Powell, 1951	GAS_L	HAOIV128-24 *	-
<i>Fusinella jucunda</i> (Thiele, 1912) #	43 - GAS_12	HAOIV118-24 *	Rest. SUB
<i>Laevilacunaria pumilio</i> (E. A. Smith, 1877)	44 - GAS_G	HAOIV122-24 *	Rest. SUB
<i>Margarella</i> sp. Thiele, 1893	GAS_D	HAOIV203-24	-
<i>Marseniopsis</i> sp. Bergh, 1886	GAS_J	HAOIV126-24 *	-
<i>Nacella delesserti</i> (R. A. Philippi, 1849)	45 - GAS_A	HAOIV204-24	Rest. SUB
<i>Nudibranchia</i> sp. Cuvier, 1817	NUD_C	HAOIV155-24 *	-
<i>Pellilitorina setosa</i> (E. A. Smith, 1875)	46 - GAS_H	HAOIV123-24 *	Circum SO
<i>Xanthodaphne</i> sp. A. W. B. Powell, 1942	GAS_M	HAOIV205-24	-
Mollusca – Polyplacophora			
<i>Hemiarthrum setulosum</i> P. P. Carpenter, 1876 #	47 - CHI_A	HAOIV108-24 *	Circum SUB
Nemertea – Hoplonemertea			
<i>Antarctonemertes</i> sp. Friedrich, 1955	UND_C	HAOIV206-24	-
<i>Monostilifera</i> sp. Brinkmann, 1917	NEM_U	HAOIV150-24 *	-

(Continued)

TABLE 1 Continued

Taxa	Code	BOLD	Distribution
Porifera			
<i>Hemigellius</i> sp. Burton, 1932	POR_C	HAOIV162-24 *	-
Chalinidae sp. Gray, 1867	POR_A	HAOIV207-24	-
Myxillidae sp. Dendy, 1922	POR_B	HAOIV208-24	-
<i>Rossella</i> sp. Carter, 1872	POR_D	HAOIV209-24	-

after the taxon name indicates a species reported for the first time in Crozet by the current study. BOLD: Public accession number of one specimen from each species, * indicates that a genetic barcode is available. Distribution: Circum SO (circum Southern Ocean, presence in both Antarctica and the sub-Antarctic), Circum SUB (circum sub-Antarctic, presence in three sub-Antarctic sectors), Broad SUB (broad sub-Antarctic, presence in two sub-Antarctic sectors), Rest. SUB (restricted sub-Antarctic, presence in a single sub-Antarctic sector), HANT + Rest. SUB (presence in the high Antarctic and in a single sub-Antarctic sector). Numbers prior to code of taxa identified to the species levels correspond to the unique numbers reported in Figure 4.

Biogeographic and phylogeographic patterns

Out of the 47 taxa identified to the species level, 35 were successfully barcoded in our study. Previous reference databases with COI sequence data were available for only 14 species (Miya et al., 2016; Moreau et al., 2021; Sands et al., 2021). The four following phylogeographic patterns were observed (Figure 2): (1) a circum Southern Ocean distribution such as in the sea stars *Pteraster affinis* Smith, 1876 and *Smilasterias triremis* (Sladen, 1889), and the pycnogonid *Nymphon brevicaudatum* Miers, 1875; (2) a circum sub-Antarctic distribution such as in the amphipod *Parawaldeckia kidderi* (S.I. Smith, 1876) and the brittle star

Ophiosabine vivipara (Ljungman, 1871); (3) a broad sub-Antarctic distribution such as for the fish species *Gobionotothen marionensis* (Günther, 1880), and the sea stars *Anasterias rupicola* (Verrill, 1876) and *Diplasterias meridionalis* (Perrier, 1875); and (4) a restricted sub-Antarctic distribution such as in *Henricia* cf. *spinulifera* (E. A. Smith, 1876).

Based on COI data, some unrecognized species diversity was found in six taxa (genetic distance among clades > 2%, cf. Materials and Methods; Figure 3; Supplementary Material 2). In the chiton *Hemiarthrum setulosum* P. P. Carpenter, 1876, specimens showed an important genetic distance from sequences known from the Antarctic Peninsula (11.19%). Analysed specimens of the nudibranch *Doris kerguelensis* are found within a clade with

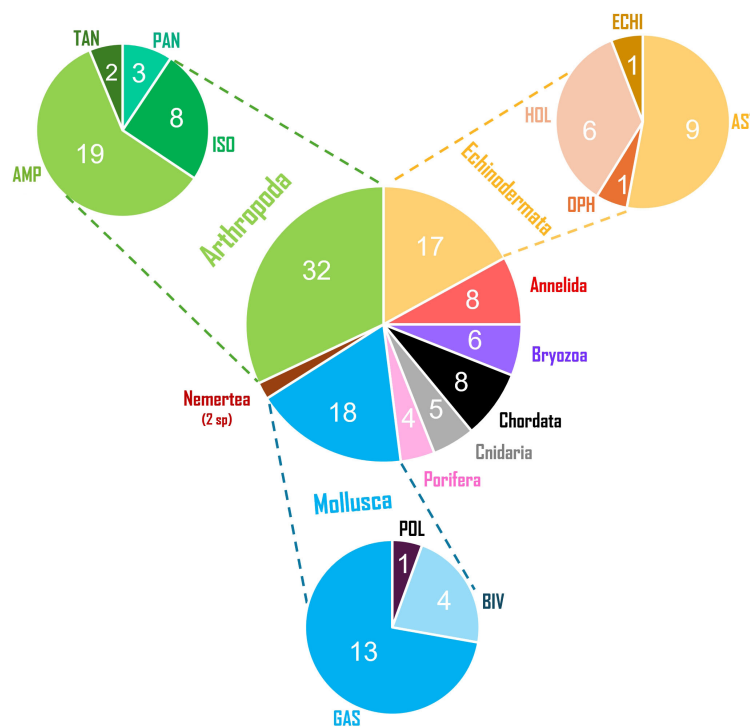
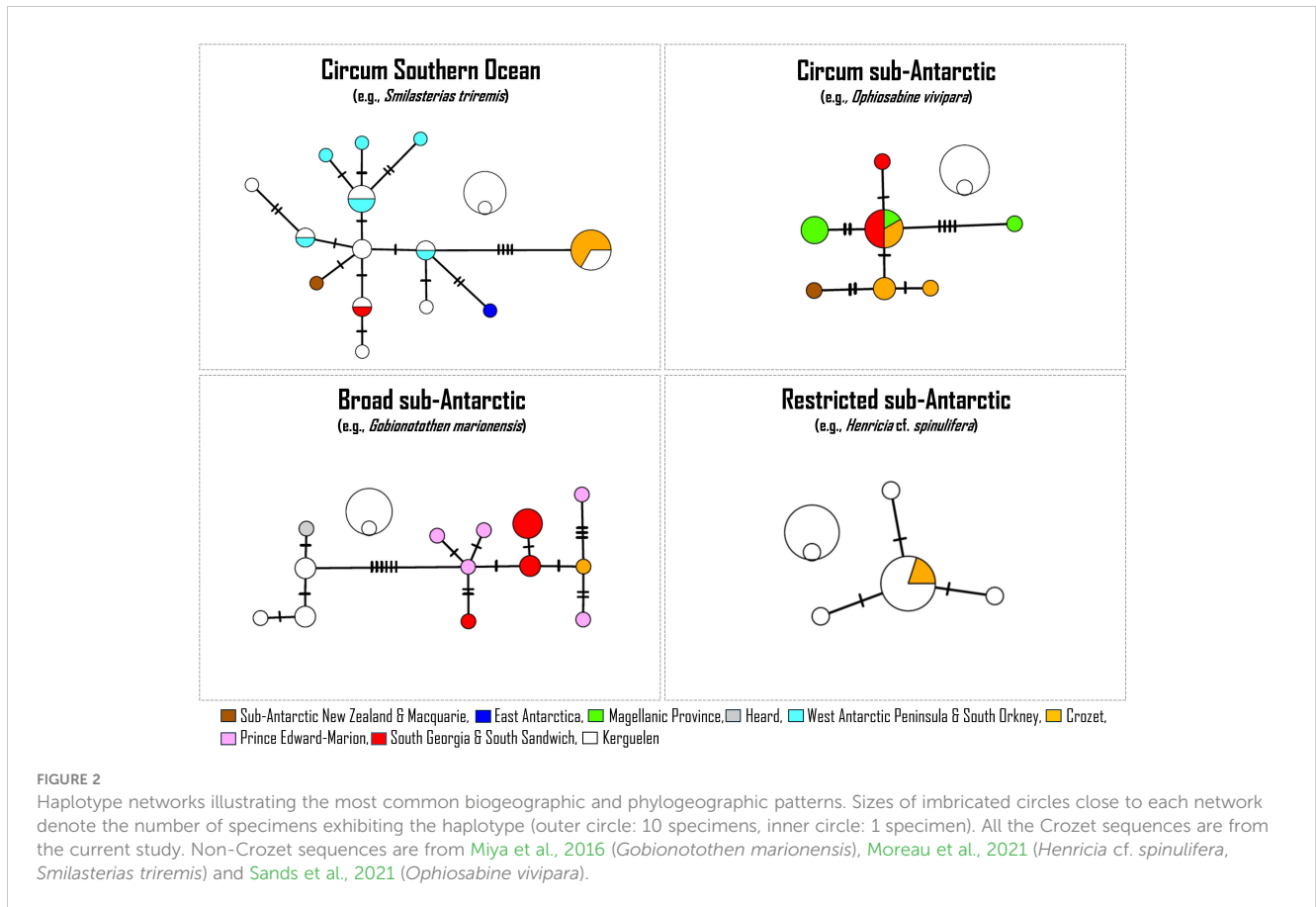


FIGURE 1 Overall taxonomic diversity for the 100 morphotypes found in the current Crozet investigation. Centre chart depicts phyla, top-left chart depicts arthropod orders (AMP, Amphipoda; ISO, Isopoda; TAN, Tanaidacea; PAN, Pantopoda), top-right chart depicts echinoderm classes (AST, Asterozoa; ECHI, Echinozoa; HOL, Holothurozoa; OPH, Ophiurozoa) and bottom chart depicts mollusc classes (BIV, Bivalvia; GAS, Gastropoda; POL, Polyplacophora). Numbers in each slice indicate the number of morphotypes for the specific group.



specimens from South Georgia (genetic distance of 2.82% with the closest related clade; Figure 3). In the tanaid *Apeudes spectabilis* Studer, 1884, the single Crozet specimen showed a p-distance of 2.32% with a specimen from Kerguelen. For the polychaete *Neanthes kerguelensis* (McIntosh, 1885), Crozet specimens clustered with specimens from South Georgia and Kerguelen and are well-differentiated from two distinct Antarctic groups (3.93% et 4.82%, respectively; Figure 3). In addition, potential species complexes were found in the polychaete *Harmothoe* Kinberg, 1856 and the pycnogonid *Nymphon brevicaudatum* (Supplementary Material 2). Finally, while the level of genetic divergence was lower than the defined threshold (1.65%), the Crozet specimens of the sea star *Leptychaster kerguelensis* interestingly clustered into a clade sharing no haplotype with other sub-Antarctic and Antarctic specimens.

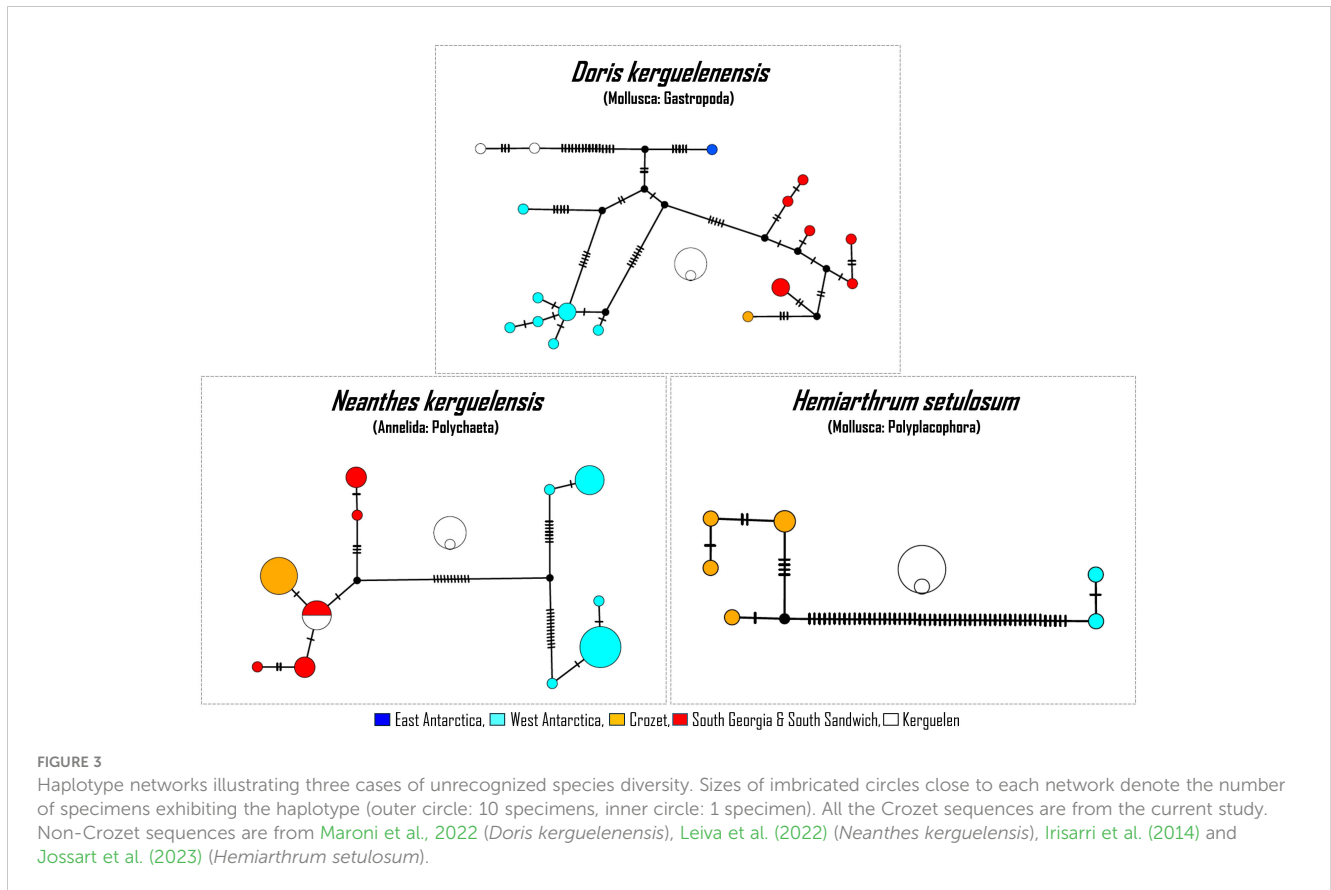
After updating distribution information for four species based on available genetic barcodes (cf. results above), we found that nearly half of the 47 morphotypes identified to the species level were reported to have a circum Southern Ocean distribution (47%), 15% to have a circum sub-Antarctic distribution, 21% to have a broad sub-Antarctic distribution, and 13% to have a distribution restricted to one sector of the Southern Ocean (Table 1; Supplementary Material 3). Two species (the holothurian *Echinopsolus splendidus* (Gutt, 1990) and the amphipod *Iphimediella paracuticoxa* Andres, 1988) also showed a peculiar distribution across the polar front, being present in the high Antarctic and a single sub-Antarctic sector (4%). As highlighted in the similarity network (Figure 4), 34 of these

47 taxa were also found to occur in Kerguelen, 30 in the Magellan Province, 30 in Prince Edward (Marion) Islands, 25 in Macquarie/sub-Antarctic New Zealand region, 22 in South Georgia/South Sandwich Islands, 23 in West Antarctica, 16 in Heard Island, and 13 in East Antarctica. The faunal similarity with Kerguelen was notably marked in most echinoderms (10/13), molluscs (4/6), arthropods (14/21) and in all annelids (4/4) (Supplementary Material 3). This faunal similarity pattern was also observed when comparing with Prince Edwards Islands and the Magellan province (Figure 4; Supplementary Material 3). The similarity of Crozet with Macquarie and sub-Antarctic New Zealand was associated to different shared taxa: it was very high in amphipods (11/12) and in arthropods in general (16/21), but far less marked in echinoderms (5/13) and molluscs (1/6) (Supplementary Material 3).

Discussion

High levels of overlooked diversity

The current study has highlighted the presence of one hundred macrofaunal species in the shallow waters of Crozet. Among these 100 species, we reported 20 species for the first time in Crozet. In addition, 17 taxa identified to the family level (2), genus level (5) or species level (10) were already inventoried by Lelièvre et al. (2023), based on imagery transects of the same area. This included two annelids: Serpulidae Rafinesque, 1815 and *Thelepus spectabilis* Ehlers, 1897;



the isopod *Spinoserolis latifrons* (White, 1847); six sea stars: *Anasterias rupicola*, *Diplasterias meridionalis*, *Glabraster antarctica* (E. A. Smith, 1876), *Henricia* sp. Gray, 1840, *Leptychaster kerguelensis* E. A. Smith, 1876 and *Smilasterias triremis*; the sea urchin *Pseudechinus* sp. Mortensen, 1903, two holothuroids: *Echinopsolus splendidus* (Gutt, 1990) and *Pentactella* sp. Verrill, 1876; the brittle star *Ophiosabine vivipara*; the bivalve *Gaimardia* sp. Gould, 1852; the nudibranch Aeolidiidae sp. Gray, 1827; the gastropods *Margarella* sp. Thiele, 1893 and *Nacella delesserti*; and the sponge *Hemigellius* sp. Burton, 1932. The 100 taxa belonged to nine different phyla, with 75% belonging to either arthropods (32 species), molluscs (18), echinoderms (17) or annelids (8; Figure 1). At lower taxonomic levels, amphipods and gastropods were particularly represented (31% of the species; Figure 1). The high-level species richness found in echinoderms is similar to the value previously reported by Lelièvre et al. (2023) (17 versus 14 species). However, our results contrast with the former study regarding the diversity of amphipods (19 versus 0) and gastropods (13 versus 3 species). Such contrast among sampling and imagery, are related to the higher detection of smaller specimens in physical inventory, which is not surprising and already highlighted in other studies (e.g., Hanafi-Portier et al., 2021).

Interestingly, several taxa found during our investigation might be species that are new to science. First, some specimens could not be assigned to known species by taxonomic experts based on morphology. This was notably the case in amphipods (*Prostebbingia* sp. Schellenberg, 1926), bryozoans (*Antarctothoa*

sp. Moyano, 1987, *Fenestulina* sp. Jullien, 1888) and echinoids (*Pseudechinus* sp). Second, the genetic analysis revealed an unexpected diversity in some taxa. This included species that exhibited more restricted distribution than initially expected (see details below) as well as taxa for which we found more putative species than formerly inventoried in Crozet. For instance, three species of the polychaete genus *Harmothoe* were previously reported around Crozet (*Harmothoe crosetensis* (McIntosh, 1885), *Harmothoe magellanica* (McIntosh, 1885), and *Harmothoe spinosa* Kinberg, 1856). However, our genetic analyses indicated that none of the studied specimens belong to either *Harmothoe crosetensis* or *Harmothoe magellanica* (based on specimens from Cowart et al., 2022) alongside a pattern of species complex (Supplementary Material 2). The exact number of species within this complex remains to be determined, but it is likely that more species than initially expected occur in Crozet. Altogether, our results have significant taxonomic implications for our knowledge of the diversity of various taxa, and definitively call for additional investigations, which are out of the scope of the current study.

Biogeography of Crozet

Among the 47 taxa identified to species level, we found that nearly half of the species (47%) were reported to have a circum Southern Ocean distribution. The rest had a circum sub-Antarctic

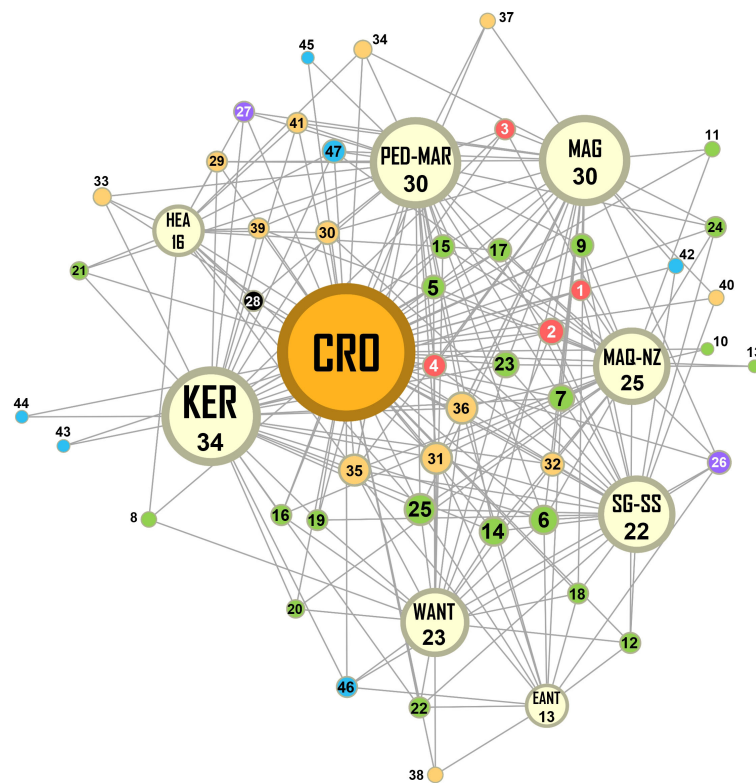


FIGURE 4

Similarity network produced with Gephi 0.10.1. Bioregions (except Crozet in orange) are represented by pale-yellow circles (CRO, Crozet; EANT, East Antarctica; HEA, Heard Island; KER, Kerguelen; MAG, Magellan Province; MAQ-NZ, Macquarie/sub-Antarctic New Zealand region; PED-MAR, Prince Edward (Marion) Islands; SG-SG, South Georgia/South Sandwich Islands; WANT, West Antarctica). The size of each circle is proportional to the total number of species shared by this bioregion with Crozet (indicated under the bioregion acronym). The smaller coloured circles indicate the 47 taxa identified up to the species level: their colours denoting their phyla (black: Chordata; blue: Mollusca; green: Arthropoda; purple: Bryozoa; red: Annelida; yellow: Echinodermata), their sizes denoting the number of bioregions where the species is found; their unique numbers indicating the species referenced in Table 1 (e.g., "45" refers to the gastropod *Nacella delesserti* (R. A. Philippi, 1849) that occurs in two bioregions).

(15%), broad sub-Antarctic (21%), restricted sub-Antarctic (13%) distributions or a peculiar distribution across the polar front (4%, see results). These 47 species were mostly found in three other bioregions: Kerguelen (72%), the Magellan Province (64%), and Prince Edward Islands (64%), and notably associated with high-level sharing of echinoderms (9 to 10 species shared). Such biogeographic affinity of Crozet with other sub-Antarctic areas has been highlighted from historical and recent studies (e.g., Hedgepeth, 1969; Griffiths et al., 2009). More specifically, the high affinity with Kerguelen was recently highlighted for marine macroalgae (Féral et al., 2021) while not found for deeper water polychaetes (Sicinski and Gillet, 2002). The high-level similarity with Prince Edward Islands was already found in sea stars at both species and genus levels (Moreau et al., 2017). Interestingly, some species are also reported a few latitudinal degrees above the subtropical front, and therefore outside the Southern Ocean (Supplementary Material 3). For example, several species occur in Tristan da Cunha archipelago (37°S; e.g., the pycnogonid *Tanystylum neorhetum* Marcus, 1940; the amphipod *Parawaldeckia kidderi*) or around Australia and New Zealand (e.g., the polychaete *Platynereis australis*, the amphipod *Jassa* cf.

justi) (Conlan, 1990; Hutchings and Reid, 1991). Some other species are also expected to occur in both hemispheres such as the sea star *Pteraster affinis* (confirmed by genetic data) or the tanaid *Apseudes spectabilis* (Larsen and Shimomura, 2006; Jossart et al., 2021). Overall, the investigated species therefore include a high proportion of widely distributed species, for which the known biogeographic distribution was confirmed by our genetic results for eight species in the current study (the sea stars *A. rupicola*, *D. meridionalis*, *P. affinis* and *S. triremis*, the pycnogonid *N. brevicaudatum*, the amphipod *P. kidderi*, the brittle star *O. vivipara*, and the fish *G. marionensis*). In contrast, it is very likely that more species with restricted distribution will be found as additional specimens and genetic data are obtained. This situation is illustrated in the present study by four study cases. The tanaid *Apseudes spectabilis*, the nudibranch *Doris kerguelenensis*, the polychaete *Neanthes kerguelensis*, and the chiton *Hemiarthrum setulosum* showed restricted distributions. The tanaid *A. spectabilis*, while expected to have a circum sub-Antarctic distribution (Schmidt and Brandt, 2001), also showed a significant genetic divergence (2.5%) between specimens from Crozet and those from Kerguelen. The nudibranch *D.*

keruelensis was shown to be composed of 60 putative species exhibiting contrasting distribution ranges (Maroni et al., 2022; Maroni and Wilson, 2022). The Crozet specimen belonged to a clade that might be distributed in two sub-Antarctic sectors (Atlantic and Indian oceans; Figure 3). The Crozet specimen of polychaete *N. kerguelensis* belongs to a sub-Antarctic clade and our results are in line with Leiva et al. (2022), highlighting an important divergence from an Antarctic clade (Figure 3). Finally, the chiton *H. setulosum*, the single species of the genus *Hemiarthrum*, was expected to have a circum Southern Ocean distribution (type locality: Kerguelen). Present genetic results indicate a high genetic distance between the specimens from Crozet and those from Antarctica (11.19%; Irisarri et al., 2014; Jossart et al., 2023; Figure 3). From now, it would be relevant to further explore whether this species is largely distributed in the sub-Antarctic, notably in the Macquarie-New Zealand region where another *Hemiarthrum* species was previously recognized (*H. hamiltonorum* Iredale & Hull, 1932; Srenko, 2006).

Conclusions and perspectives

Our study shows the importance of integrative inventories to fill the gap of diversity knowledge existing in remote ecosystems that are facing important environmental changes. We found one hundred taxa, amongst which 20 were reported for the first time in the Crozet archipelago. This emphasizes the fact that shallow coastal waters around Crozet are an under-investigated area, even compared to most other sub-Antarctic areas. Regarding biogeographic patterns, a greater proportion of broadly distributed species was found but this statement needs to be counterbalanced by the detection (based on genetic data) of more restricted distributions than expected for several species. It is likely that future sampling will increase the proportion of species with restricted distribution. We also found a few cases of unrecognized diversity that might lead to the future descriptions of new species, some likely to have a restricted distribution or even be endemic to Crozet. Combined with the high diversity observed, this stresses the need to protect the biologically unusual Crozet archipelago. Additional sampling and barcoding efforts are necessary to better unravel marine faunal diversity and affinities with other sub-Antarctic islands and the overall Southern Ocean in the future. While this was not possible in the current study (due to the limited data available), bathymetry should notably be taken into account in such further investigations. In fact, for several taxonomic groups, species initially reported to occur over broad depth ranges has been showed to rather be distinct species with more restricted depth ranges (Barnes and Kuklinski, 2010; Neal et al., 2018; Moreau et al., 2019). Finally, another perspective includes the sharing of our data for conservation purposes. While the publicly available data will benefit to large scale projects related to conservation (genetic or occurrence data), they will also be directly shared with nature reserve managers of the French Southern Territories. In addition, the creation of a field guide of the most common species investigated is an ongoing work that would help in the monitoring of this area in the future.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The animal study was approved by TAAF-UB convention #2258 – French Polar Institute project #1044 Proteker – Project #2021-0882 “Nearshore Cable Inspection and Environmental Survey at IMS Hydroacoustic Station HA04 Crozet, France”. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

QJ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. YL: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. AK: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. BF: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. CM: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. DD: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. JM: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. MV: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. MM: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. RD: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. SR: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. SH: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. TS: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2024.1455329/full#supplementary-material>

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