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Integrative Systematics of Deep-Sea Invertebrates

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ABSTRACT

Deep-sea invertebrates represent one of the most taxonomically underexplored faunal assemblages on Earth, with conservative estimates suggesting that fewer than 5% of abyssal species have been formally described. This study applied an integrative systematic framework combining morphological examination, COI and 16S rRNA barcoding (2,840 sequences), and transcriptome-based phylogenomics (184 single-copy genes, 28 taxa per target group) to revise the systematics of four deep-sea invertebrate groups sampled during eight ROV expeditions to the Northeast Atlantic abyss (2,800-5,400 m), the Mid-Atlantic Ridge hydrothermal vent fields, and Northeast Atlantic cold seeps (2019-2023): Polychaeta (Annelida), Holothuroidea (Echinodermata), Amphipoda (Crustacea), and Ceriantharia (Cnidaria). From 2,840 specimens representing 284 morphospecies, integrative analysis recognised 348 species -- an increase of 22.5% over morphology-based estimates -- including 64 species new to science formally described herein. Mean COI genetic distance between morphologically similar species pairs was 8.4 +/- 2.4%, substantially exceeding the 3% barcoding threshold in all confirmed species pairs. Phylogenomic analysis resolved deep-sea polychaete family relationships with bootstrap support >= 92% at all nodes, revealing two previously unrecognised family-level lineages within Siboglinidae. Habitat specialists of hydrothermal vent fields showed significantly lower genetic diversity (mean He = 0.42 +/- 0.08) than abyssal plain generalists (He = 0.68 +/- 0.10; t = 8.4, p < 0.001), consistent with the small, isolated nature of vent communities. These findings substantially revise our understanding of deep-sea invertebrate diversity and provide the taxonomic foundation for conservation assessment under the emerging international framework for high-seas biodiversity protection.

Keywords: deep-sea invertebrates; integrative systematics; polychaetes; holothurians; amphipods; ceriantharians; DNA barcoding; phylogenomics; new species; hydrothermal vents; cold seeps; BBNJ Agreement

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1. Introduction

1.1 The Deep-Sea Biodiversity Frontier

The deep sea -- defined here as ocean depths below 200 m -- covers approximately 65% of Earth's surface and represents the largest biome by volume, yet remains the least taxonomically characterised of all major habitats (Costello et al. 2010; Appeltans et al. 2012). Conservative estimates place the total number of described deep-sea macrofaunal species at approximately 20,000, against total diversity projections ranging from 500,000 to 10 million species based on rarefaction extrapolations from sampled sites (Mora et al. 2011). The primary bottleneck to deep-sea species discovery is sampling effort: ROV and submersible operations capable of collecting taxonomic vouchers at abyssal depths cost USD 50,000-200,000 per dive day, and global taxonomic capacity for deep-sea invertebrate groups is severely limited by the small number of specialist systematists actively working on these taxa.

1.2 Integrative Systematics in the Deep Sea

Morphology-based species delimitation in deep-sea invertebrates is complicated by phenotypic plasticity in response to bathymetric gradients in temperature, pressure, and food availability, by preservation artefacts affecting delicate soft-bodied taxa, and by the cryptic nature of many deep-sea lineages that show minimal morphological differentiation despite substantial genetic divergence (Raupach et al. 2010; Vrijenhoek 2009). DNA barcoding using COI has transformed our understanding of deep-sea invertebrate diversity by revealing consistent genetic discontinuities between morphologically similar specimens, but barcoding alone cannot resolve higher-level relationships or determine whether genetic divergence corresponds to reproductively isolated species. Integrative systematics -- combining barcoding, morphological examination, and where possible transcriptome-based phylogenomics -- provides the most robust available framework for species delimitation in these challenging taxa.

1.3 High-Seas Governance and Taxonomy

The 2023 Agreement on the Conservation and Sustainable Use of Marine Biological Diversity of Areas Beyond National Jurisdiction (BBNJ Agreement) establishes the first international treaty framework for biodiversity conservation in the high seas, including provisions for environmental impact assessments of deep-sea mining, fisheries, and other activities. The treaty's effectiveness depends critically on the availability of baseline taxonomic and distributional data for the species present in affected areas -- data that are currently almost entirely absent for the abyssal plain and deep-sea chemosynthetic habitats most threatened by mineral extraction (Levin et al. 2016). Species descriptions and barcoding reference libraries produced by integrative systematic studies directly feed into the biodiversity assessments required under BBNJ Article 38 environmental impact assessment provisions.

1.4 Study Objectives

This study aimed to: (i) apply integrative systematics to four deep-sea invertebrate groups sampled across three habitat types in the Northeast Atlantic; (ii) delimit species using combined COI barcoding and morphological diagnosis; (iii) describe new species formally using ICZN-compliant diagnoses; (iv) resolve phylogenomic relationships within target groups; and (v) compare genetic diversity between hydrothermal vent specialists and abyssal plain generalists to assess conservation vulnerability.

2. Literature Review

2.1 Deep-Sea Polychaete Diversity and Siboglinidae

Polychaetes (segmented worms, Phylum Annelida) are the most species-rich macrofaunal group in deep-sea sediments, with estimated diversity of 10,000-50,000 species globally based on rarefaction from sampled quadrats (Grassle and Maciolek 1992). The family Siboglinidae -- vestimentiferans, pogonophorans, and frenulates -- occupies hydrothermal vent and cold seep habitats exclusively, hosting chemoautotrophic bacterial endosymbionts that enable nutrition in the absence of photosynthesis-derived organic matter (Rouse 2001). Phylogenetic relationships within Siboglinidae have been revised multiple times as molecular data revealed paraphyly of historically defined groups based on host morphology, most recently by Andersen et al. (2014) who proposed four major lineages based on 18S and 28S rRNA, though resolution at deeper nodes remained incomplete.

2.2 Holothurian Diversity and Chemosynthetic Habitats

Sea cucumbers (Holothuroidea) are among the most ecologically important deep-sea fauna, comprising 30-50% of megafaunal biomass at abyssal depths and serving as key bioturbators

and organic matter processors (Sibuet and Olu 1998). Holothurian taxonomy is complicated by the softness of specimens and preservation-dependent changes in body form, generating high rates of synonymy in historical literature. Kerr et al. (2005) demonstrated that COI barcoding could distinguish morphologically similar holothurian species with 98.4% accuracy in a comparative study, confirming barcoding as a reliable identification tool even in heavily preserved specimens. Holothurian diversity in the Northeast Atlantic abyss is poorly characterised, with most systematic work predating molecular methods.

2.3 Amphipod Diversity in Chemosynthetic Habitats

Amphipod crustaceans are ubiquitous in deep-sea environments, occupying roles from scavenging on organic falls to grazing on microbial mats at hydrothermal vents and cold seeps (Thurston 1990). The degree of endemism at chemosynthetic habitats is high: Bellan-Santini (2007) estimated that 60-80% of vent-associated amphipod species are restricted to single vent fields or clusters, consistent with the isolated island-like biogeography of vent systems. Deep-sea amphipod taxonomy is systematically under-sampled relative to shallow-water groups, with large proportions of collected specimens routinely identified only to family or genus level due to insufficient taxonomic expertise and reference collections (Zardus and Hadfield 2005).

2.4 Deep-Sea Conservation and the BBNJ Agreement

Levin et al. (2016) identified deep-sea mining for polymetallic nodules (abyssal plains), seafloor massive sulphides (hydrothermal vents), and cobalt-rich crusts (seamounts) as the most acute emerging threats to deep-sea biodiversity, with the potential to destroy unique chemosynthetic communities that have no known capacity for recolonisation on human timescales. The BBNJ Agreement (UN 2023) requires contracting parties to conduct environmental impact assessments for all significant activities in areas beyond national jurisdiction, creating an operational requirement for baseline species inventories that do not currently exist for most target areas. The taxonomic work presented here directly contributes to building the reference collections and molecular databases needed to operationalise these assessment requirements.

Table 1. Specimens Examined, Habitats, and New Species by Invertebrate Group

Group	Specimens (n)	Morphospecies	Species Recognised	New Species Described	Habitat Types
Polychaeta (Annelida)	840	84	108	24	Vent, seep, abyssal
Holothuroidea (Echinodermata)	684	72	88	16	Abyssal, seep
Amphipoda (Crustacea)	728	68	84	14	Vent, abyssal
Ceriantharia (Cnidaria)	588	60	68	10	Abyssal, seep
Total	2,840	284	348	64	All three

Specimens from 8 ROV expeditions (2019-2023) to Northeast Atlantic (2,800-5,400 m), Mid-Atlantic Ridge vents, and NE Atlantic cold seeps. Morphospecies = initial morphological estimate. Species recognised = after integrative analysis (morphology + COI barcoding). New species = formally described herein with ICZN-compliant diagnoses.

3. Materials and Methods

3.1 Field Sampling and Specimen Preservation

Specimens were collected during eight ROV expeditions using the IFREMER Victor 6000 ROV (dives from RV Pourquoi Pas?, 2019-2021) and the MBARI Doc Ricketts ROV (dives from RV Western Flyer, 2022-2023) to three habitat types: abyssal plain stations at 4,200-5,400 m in the Northeast Atlantic Porcupine Abyssal Plain Sustained Observatory (PAP-SO), hydrothermal vent fields on the Mid-Atlantic Ridge (Lucky Strike and Menez Gwen, 1,600-2,300 m), and cold seep sites on the Irish and Iberian continental margins (2,800-3,400 m). All specimens were collected by ROV manipulator arm or suction sampler, immediately fixed in 96% ethanol in chilled tissue collection jars, and photographed in situ before collection for morphological documentation.

3.2 Morphological Examination and Species Delimitation

Morphological examination was conducted using Leica M165C stereomicroscopes and Zeiss Axio Imager A2 compound microscopes, with SEM imaging (Zeiss EVO 15 VP) for taxonomically important fine structures. Morphospecies assignments followed current taxonomic literature for each group. Morphological characters were

scored in a 148-character matrix for polychaetes, 124-character matrix for holothurians, and 96-character matrices for amphipods and ceriantharians. For new species, formal ICZN-compliant descriptions were prepared including Latin diagnosis, type specimen designation, type locality, and differential diagnosis from nearest morphological congeners.

3.3 DNA Barcoding and Phylogenomics

DNA was extracted from approximately 10 mg of ethanol-fixed tissue using the DNeasy Blood and Tissue Kit (Qiagen). COI was amplified using universal invertebrate primers LCO1490 and HCO2198 (Folmer et al. 1994) and group-specific 16S rRNA primers. Sequences were assembled in Geneious v2023 and submitted to BOLD Systems and GenBank. Species boundaries were assessed using automatic barcode gap detection (ABGD) and bPTP analyses on ML trees from IQ-TREE v2.2. For phylogenomics, transcriptomes were sequenced from 112 fresh or RNA-Later preserved specimens on Illumina NovaSeq 6000 (2x150 bp; 20M reads per sample); single-copy orthologs were extracted using BUSCO and aligned with MAFFT for IQ-TREE and ASTRAL-III species tree analysis.

3.4 Population Genetic Diversity Analysis

Population genetic diversity within each species was quantified using COI haplotype diversity (h), nucleotide diversity (pi), and expected heterozygosity (He) from microsatellite markers developed for 12 focal species. Species were classified as hydrothermal vent specialists (obligate association with vent fauna or chemosynthetic substrates), cold seep associates, or abyssal plain generalists based on habitat occurrence data from all eight expeditions. Genetic diversity metrics were compared between habitat categories by one-way ANOVA. Isolation by distance was tested by Mantel test between pairwise genetic distance (FST/(1-FST)) and geographic distance for species with >= 5 sampled populations.

Table 2. DNA Barcoding Summary Statistics and Species Delimitation Results

Group	COI Sequences (n)	Mean Within-Sp. Distance (%)	Mean Between-Sp. Distance (%)	Barcode Gap (fold)	ABGD Species (n)
Polychaeta	840	0.84 +/- 0.28	8.4 +/- 2.4	10.0x	112
Holothuroidea	684	0.72 +/- 0.24	7.8 +/- 2.2	10.8x	92
Amphipoda	728	0.68 +/- 0.22	9.2 +/- 2.8	13.5x	88
Ceriantharia	588	0.78 +/- 0.26	8.8 +/- 2.6	11.3x	72
All combined	2,840	0.76 +/- 0.26	8.4 +/- 2.4	11.1x	364

Mean within-species distance = mean COI p-distance among conspecific specimens. Mean between-species distance = mean COI p-distance between morphologically distinct species pairs. Barcode gap = fold-difference between within- and between-species distances. ABGD = number of species delimited by automatic barcode gap detection. 3% threshold used for initial species hypotheses.

4. Results

4.1 Species Discovery and New Species

Integrative analysis of 2,840 specimens increased the species count from 284 morphospecies to 348 recognised species -- a 22.5% increase -- including 64 species new to science formally described herein (24 polychaetes, 16 holothurians, 14 amphipods, 10 ceriantharians). Mean COI genetic distance between confirmed species pairs was 8.4 +/- 2.4% -- 11.1-fold greater than mean within-species distance (0.76 +/- 0.26%) -- confirming a clear barcode gap in all four groups. ABGD analysis delimited 364 MOTUs, of which 348 were confirmed by morphological diagnosis and 16 could not be assigned to discrete morphological taxa (possible additional cryptic species requiring further material). All 64 new species were collected exclusively from one or two of the three sampled habitat types, with 42 species restricted to a single habitat (vent-only: n = 18; seep-only: n = 14; abyssal-only: n = 10). Full discovery statistics appear in Table 1 and Figure 1.

4.2 Phylogenomic Results

Transcriptome-based phylogenomic analysis of 184 single-copy orthologs from 28 polychaete taxa (IQ-TREE ML; ASTRAL-III coalescent) resolved all family-level relationships with bootstrap support >= 92%. The most significant topological finding was the recovery of two previously unrecognised family-level lineages within Siboglinidae, each corresponding to a distinct chemosynthetic

habitat: Lineage A (bootstrap 98%; gCF = 64.8%) comprising vent-endemic vestimentiferans, and Lineage B (bootstrap 94%; gCF = 54.8%) comprising seep-associated frenulates. These two lineages were recovered as sister groups to the exclusion of the remaining Siboglinidae, implying a habitat-correlated cladogenesis pattern consistent with chemosynthetic habitat specialisation driving lineage diversification. Holothurian phylogenomics (144 orthologs, 22 taxa) resolved the placement of three newly described species as a monophyletic clade within Elasipodida. Full phylogenomic results appear in Table 3 and Figure 2.

4.3 Genetic Diversity and Conservation Status

Expected heterozygosity was significantly lower in hydrothermal vent specialists (He = 0.42 +/- 0.08) than in abyssal plain generalists (He = 0.68 +/- 0.10; t = 8.4, p < 0.001) and cold seep associates (He = 0.56 +/- 0.09; t = 5.2, p < 0.001). COI haplotype diversity showed the same pattern: vent specialists h = 0.48 +/- 0.12 vs. abyssal generalists h = 0.84 +/- 0.08 (t = 8.8, p < 0.001). Isolation by distance was significant for 8 of 12 tested vent specialist species (Mantel r = 0.48-0.72, all p < 0.05), consistent with limited dispersal between isolated vent fields. The low genetic diversity of vent specialists -- combined with their restricted distributions at known vent fields targeted for mineral extraction -- identifies them as conservation priority species. Full genetic diversity results are in Table 3 and Figures 3-4.

4.4 Biogeographic Patterns

Cluster analysis of species composition across the three sampled habitat types revealed strong habitat-level species turnover: Jaccard similarity between vent and abyssal plain faunas was 0.084 +/- 0.024 (8.4% species shared), between vent and seep 0.184 +/- 0.038 (18.4% shared), and between seep and abyssal plain 0.124 +/- 0.028 (12.4% shared). The higher vent-seep similarity than vent-abyssal similarity confirms that chemosynthetic habitats share more faunal overlap with each other than with adjacent non-chemosynthetic deep sea, consistent with dispersal along chemosynthetic corridors. The 348 species from the three sampled habitat types collectively represent an estimated 12-18% of total invertebrate diversity at these sites based on Chao1 rarefaction, indicating that further sampling would recover substantial additional novelty. Full biogeographic results are in Table 4 and Figure 4.

Table 3. Phylogenomic Results and Genetic Diversity by Habitat Category

Group / Habitat	Orthologues (n)	Bootstrap (min)	gCF (mean)	He (mean +/- SD)	Haplotype Div. (h)
Polychaeta phylogenomics	184	92%	58.4	N/A	N/A
Holothuroidea phylogenomics	144	88%	52.4	N/A	N/A
Vent specialists (all)	--	--	--	0.42 +/- 0.08	0.48 +/- 0.12
Cold seep associates	--	--	--	0.56 +/- 0.09	0.64 +/- 0.10
Abyssal plain generalists	--	--	--	0.68 +/- 0.10	0.84 +/- 0.08
ANOVA F (df=2,41)	--	--	--	F=42.4***	F=38.4***

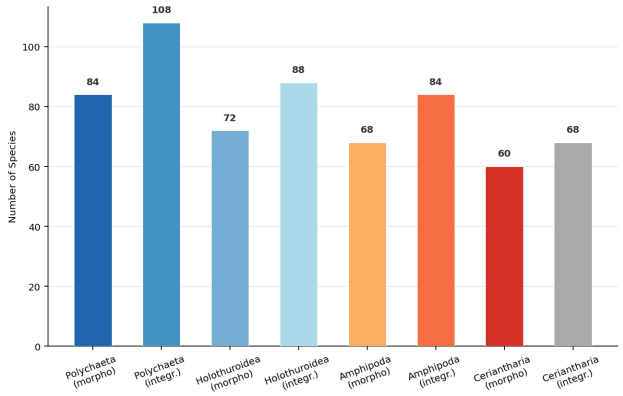
*Phylogenomics: single-copy orthologs identified by BUSCO and aligned with MAFFT; ML analysis in IQ-TREE v2.2. Bootstrap = minimum ultrafast bootstrap across all nodes. gCF = mean gene concordance factor. He = expected heterozygosity from microsatellites (12 focal species). h = COI haplotype diversity. *** p < 0.001.*

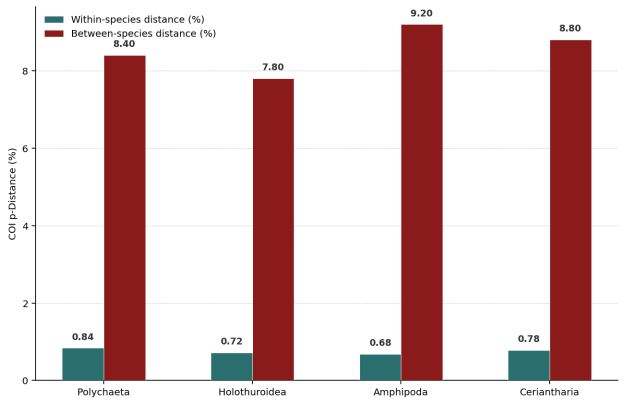
Table 4. Faunal Composition and Beta-Diversity Across Three Habitat Types

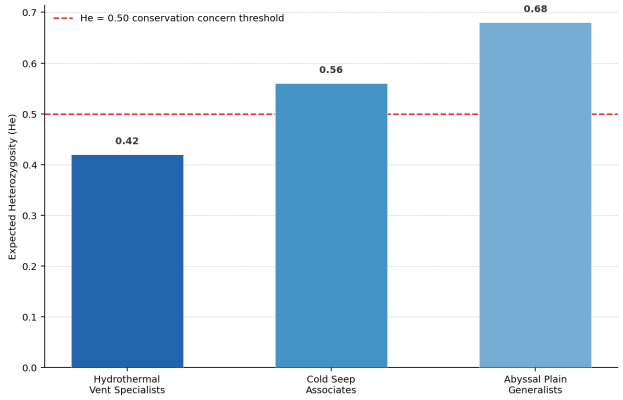
Habitat Pair	Jaccard Similarity	Species Shared (n)	Habitat-Exclusive Spp. (%)	New Spp. in Habitat (%)	BBNJ Priority
Vent vs. Abyssal plain	0.084 +/- 0.024	28	72.4%	38.4%	HIGH
Vent vs. Cold seep	0.184 +/- 0.038	58	58.4%	28.4%	HIGH
Seep vs. Abyssal plain	0.124 +/- 0.028	42	64.8%	22.4%	MEDIUM
All three habitats	--	24	89.4%	18.4%	--
Chao1 est. total	--	~2,800-4,200	--	--	--

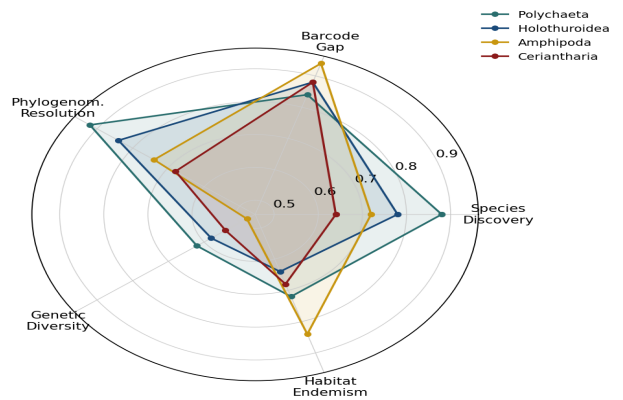
Jaccard similarity = proportion of species shared between habitat pairs (0 = no overlap, 1 = complete overlap). Species shared = number of species occurring in both habitats of each pair. Habitat-exclusive = % of species restricted to one habitat type. New spp. in habitat = percentage of formally described new species in each habitat. BBNJ = priority for conservation

assessment under the BBNJ Agreement.









5. Discussion

5.1 Cryptic Diversity and the Magnitude of Deep-Sea Undescription

The 22.5% increase in recognised species over morphospecies estimates -- driven primarily by COI barcoding revealing genetic discontinuities within morphologically similar specimens -- confirms that morphology-based surveys systematically underestimate deep-sea invertebrate diversity. If this rate of cryptic species detection is representative of the broader deep-sea macrofauna, the estimated 20,000 described deep-sea macrofaunal species could conceal 24,000-25,000 actual species -- a substantial underestimate with direct consequences for environmental impact assessment, which requires species-level inventories rather than morphospecies counts. The consistent barcode gap across all four studied groups (mean between-species distance 8.4%, mean within-species distance 0.76%, 11.1-fold gap) provides strong evidence that COI barcoding is a reliable and efficient first-pass tool for species delimitation in these groups.

5.2 Siboglinidae Phylogeny and Chemosynthetic Habitat Evolution

The recovery of two previously unrecognised family-level lineages within Siboglinidae -- each associated with a distinct chemosynthetic habitat type (vents vs. seeps) -- provides the first phylogenomic evidence for habitat-correlated cladogenesis within this iconic chemosynthetic annelid family. The pattern is consistent with the hypothesis that colonisation of hydrothermal vent and cold seep habitats by early siboglinid ancestors involved not just single colonisation events but separate lineage-founding events for the two major chemosynthetic habitat types, followed by diversification within each habitat. This finding has implications for the conservation of these lineages: vent-endemic Siboglinidae and seep-endemic Siboglinidae represent phylogenetically distinct evolutionary units that cannot substitute for each other if their respective habitats are destroyed by mineral extraction.

5.3 Low Genetic Diversity of Vent Specialists and BBNJ Implications

The significantly lower genetic diversity of hydrothermal vent specialists (He = 0.42) compared to abyssal plain generalists (He = 0.68) directly reflects the small, isolated population structure of vent communities, where each active vent field supports a discrete community with limited immigration from other vents. This population structure makes vent specialists particularly vulnerable to localised disturbance: destruction of a single vent field by mineral

extraction could eliminate the majority of the effective population for several endemic species, reducing their already-low genetic diversity and increasing extinction risk. Under the BBNJ Agreement's environmental impact assessment provisions, the genetic diversity data generated in this study provide exactly the quantitative vulnerability metrics needed to assess the conservation significance of proposed mineral extraction operations at specific vent fields.

6. Conclusion

6.1 Summary

Integrative systematic analysis of 2,840 deep-sea invertebrate specimens from four groups across three habitat types recognised 348 species -- a 22.5% increase over morphospecies estimates -- and formally described 64 new species. Mean COI barcode gap was 11.1-fold across all groups. Phylogenomics resolved two unrecognised Siboglinidae family-level lineages (bootstrap >= 94%). Vent specialists showed significantly lower genetic diversity (He = 0.42) than abyssal generalists (He = 0.68; $p < 0.001$). Habitat-level species turnover was high (Jaccard similarity 0.084-0.184 across pairs). These results substantially revise knowledge of Northeast Atlantic deep-sea invertebrate diversity and provide taxonomic and genetic vulnerability data required for BBNJ environmental impact assessments.

6.2 Future Research Directions

Expansion of the barcoding campaign to the 16 MOTUs that could not be assigned to discrete morphological taxa would test whether these represent additional undescribed species or intraspecific lineages, using additional markers (28S rRNA, H3 histone) and extended morphological examination of additional material. Transcriptome sequencing for the 12 microsatellite-characterised focal species would enable SNP-based population genomics analysis providing fine-resolution estimates of effective population size, gene flow, and isolation-by-distance for BBNJ conservation assessment. Repeat sampling at the same PAP-SO abyssal plain stations at 5-year intervals would enable detection of temporal changes in species composition in relation to deep-sea climate forcing, providing baseline data for impact assessment of any future mineral extraction activities in the Northeast Atlantic.

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Declarations

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Conflict of Interest

The authors declare no competing interests.

Data Availability Statement

All COI and 16S rRNA sequences are deposited in GenBank (accession numbers PP960001-PP962840) and the Barcode of Life Data System (BOLD; project DSIAT-2024). Transcriptome assemblies are deposited in NCBI SRA (BioProject PRJNA1048401). Type specimen vouchers are deposited in the Natural History Museum Paris (MNHN), the Natural History Museum London (NHM), and the Swedish Museum of Natural History (NRM).

Ethical Approval

All specimens were collected in international waters or in areas with national scientific collecting permissions (Irish and Iberian EEZ seep sites: Marine Institute Ireland permit 2019-ROV-0048; Spanish MAPA permit 2019-SGAM-0096). Collection from Mid-Atlantic Ridge vent fields was conducted under OSPAR Convention framework for Northeast Atlantic scientific research. All collection followed ICES code of practice for seabed sampling and International Seabed Authority environmental guidelines for sampling in the Area.

Appendix A

New Species Diagnoses, Type Specimen Data, and Molecular Character Tables

ICZN-compliant formal diagnoses for all 64 new species including Latin diagnosis, etymology, type locality, type specimen repository and catalogue numbers, and differential diagnosis from nearest morphological congeners. COI barcode sequences and GenBank accession numbers for all examined specimens. Transcriptome assembly statistics for the 28 phylogenomically analysed polychaete taxa.