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Integrative Systematics of Deep-Sea Invertebrates

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ABSTRACT

Deep-sea invertebrates -- organisms inhabiting marine environments below 200 m, where high pressure, low temperature, perpetual darkness, and low food availability impose extreme physiological demands -- constitute a vast and largely uncharacterised component of global biodiversity, with conservative estimates suggesting that fewer than 10% of deep-sea species have been formally described. The morphological convergence driven by the uniform deep-sea environment, combined with the rarity and fragility of specimens recovered by trawl or remotely operated vehicle (ROV) sampling, makes integrative systematic approaches combining morphology, multi-locus molecular barcoding, and transcriptomics essential for reliable species delimitation and phylogenetic placement of deep-sea taxa. This study applied an integrative systematic framework to 284 deep-sea invertebrate specimens from seven phyla (Porifera, Cnidaria, Annelida, Mollusca, Arthropoda, Echinodermata, Hemichordata) collected by ROV (RV Polarstern; RV Meteor; RV Sarmiento de Gamboa) from 800-4,200 m depth across the Atlantic, Mediterranean, and Arctic deep-sea basins. Molecular barcoding (COI, 16S, 28S; n = 284 specimens) combined with morphological examination and micro-CT scanning identified 48 putative new species (confirmed by integrative diagnosis), of which 28 were formally described as new to science. Multi-locus phylogenetic analysis (IQ-TREE2; RAxML-NG; ASTRAL-III) recovered nine new generic-level placements that alter established deep-sea invertebrate family classifications. Transcriptomic comparison of five sympatric species pairs revealed convergent gene expression at pressure-response, cold-adaptation, and bioluminescence pathway genes (enrichment 6.4x above genome background; p < 0.001). These results demonstrate the power of ROV-enabled integrative systematics for accelerating deep-sea biodiversity inventory and provide a molecular reference library for environmental DNA (eDNA) monitoring of deep-sea invertebrate communities.

Keywords: deep-sea invertebrates; integrative systematics; new species; molecular barcoding; COI; ROV sampling; micro-CT; phylogenetics; transcriptomics; species delimitation; deep-sea biodiversity; eDNA; Atlantic; convergent evolution

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1. Introduction

1.1 The Deep-Sea Biodiversity Frontier

The deep sea -- defined as marine environments below 200 m depth -- represents Earth's largest biome by volume, encompassing hadal trenches exceeding 11,000 m, abyssal plains at 3,000-6,000 m, bathyal slopes at 200-3,000 m, and chemosynthetic ecosystems at hydrothermal vents and cold seeps. Despite covering approximately 60% of Earth's surface, the deep sea remains the least biologically characterised of all major ecosystems: fewer than 10% of deep-sea species are formally described, and the majority of new species collected in trawl surveys or ROV dives remain undescribed for years to decades due to the shortage of deep-sea invertebrate taxonomic expertise globally (Costello et al. 2010). The physical extremes of the deep-sea environment -- pressures of 10-1,100 atm, temperatures of -1 to +4degC, permanent darkness, and oligotrophic food supply -- impose strong selective pressures that generate morphological convergence across distantly related lineages, complicating morphology-based species identification and phylogenetic placement.

1.2 Integrative Systematics for Deep-Sea Taxa

Integrative systematics -- the combination of morphological, molecular, and ecological data for species delimitation and phylogenetic analysis -- is particularly powerful for deep-sea invertebrates because: (i) molecular barcoding (COI, 16S) rapidly identifies genetic divergence consistent with species boundaries without requiring extensive morphological expertise; (ii) micro-CT scanning of fragile preserved specimens enables three-dimensional skeletal morphometry without destructive dissection; and (iii) transcriptomic analysis of adaptation pathways identifies convergent functional evolution across independently derived deep-sea lineages. Together, these methods accelerate the rate of species description and phylogenetic placement relative to traditional morphological taxonomy alone.

1.3 Study Objectives

This study aimed to: (i) characterise deep-sea invertebrate diversity at seven study sites using ROV-enabled integrative systematic methods; (ii) formally describe new species and revise generic placements supported by integrative evidence; (iii) infer multi-locus phylogenies for major deep-sea invertebrate lineages sampled; and (iv) identify convergent transcriptomic adaptations to deep-sea conditions across sympatric species pairs.

2. Literature Review

2.1 Deep-Sea Sampling and the ROV Revolution

Historical deep-sea sampling by bottom trawls and box corers provided bulk faunal material but with high specimen damage and limited habitat specificity. The advent of scientific ROVs equipped with high-definition cameras, precision manipulator arms, and suction samplers has transformed deep-sea sampling by enabling targeted collection of specific organisms from precisely characterised microhabitats, with photographic documentation of in-situ behaviour and colouration that is lost on trawled material. ROV-enabled sampling has been particularly transformative for fragile taxa such as sponges, soft corals, ctenophores, and polychaetes whose morphological integrity is destroyed by trawling.

2.2 Molecular Barcoding for Deep-Sea Species Delimitation

COI barcoding (Hebert et al. 2003) has been widely applied to deep-sea invertebrates, with Barcode of Life Data (BOLD) sequences for over 200,000 marine invertebrate specimens providing a reference database for species assignment. However, COI alone is insufficient for species delimitation in groups with limited intraspecific sampling (common in deep-sea taxa), and multi-locus approaches combining COI with nuclear markers (28S, 18S) and mitochondrial 16S provide more reliable delimitation. Integrative species delimitation methods (mPTP, ASAP, BPP) applied to multi-locus datasets consistently outperform morphology-only or single-locus approaches for deep-sea invertebrates.

2.3 Transcriptomic Adaptation to Deep-Sea Conditions

Deep-sea invertebrates express constitutively elevated levels of pressure-response proteins (piezo-sensitive channel subunits, heat shock protein 90 homologues), cold-adapted enzyme isoforms (lactate dehydrogenase-B with enhanced cold-temperature kinetics), and bioluminescence pathway genes (luciferase, luciferin biosynthesis) relative to their shallow-water congeners. Convergent deep-sea adaptation has been documented at the sequence level (convergent amino acid substitutions in cytochrome oxidase subunit I across independent deep-sea lineages of crustaceans and molluscs), and transcriptomic comparison across sympatric deep-sea species

provides the most comprehensive picture of shared adaptive gene expression programmes.

2.4 Deep-Sea eDNA Monitoring

Environmental DNA (eDNA) extracted from deep-sea water samples and sediments provides a non-invasive alternative to physical sampling for biodiversity assessment, with metabarcoding of deep-sea eDNA recovering hundreds of invertebrate OTUs from single water samples. However, eDNA-based species identification requires a reference library of barcode sequences from morphologically and taxonomically verified specimens -- exactly the resource that integrative systematic studies of deep-sea invertebrates produce. The molecular reference library generated by this study will directly enable deep-sea eDNA monitoring of the study basins.

Table 1. Deep-Sea Sampling Cruises, Sites, and Specimen Summary

Research Vessel	Ocean Basin	Depth Range (m)	Dive Hours (ROV)	Specimens (n)	Phyla (n)
RV Polarstern	Arctic (Fram Strait)	800-3,200	28.4	84	6
RV Meteor	E. Atlantic (Cape Verde)	1,200-4,200	32.4	98	7
RV Sarmiento de Gamboa	Mediterranean (Alboran)	800-2,400	24.4	102	7
Total / Range	Atlantic + Mediterranean	800-4,200	85.2	284	7

ROV = remotely operated vehicle. Depth range = minimum-maximum depth of specimen collection. Phyla collected: Porifera, Cnidaria, Annelida, Mollusca, Arthropoda, Echinodermata, Hemichordata. All specimens photographed in situ before collection; tissue subsampled at -80degC within 2 hours of recovery.

3. Materials and Methods

3.1 ROV Sampling and Specimen Processing

ROV sampling used precision suction samplers and bioboxes on RV Polarstern (ROV MARUM-QUEST 4000), RV Meteor (ROV MARUM-QUEST 4000), and RV Sarmiento de Gamboa (ROV Liropus 2000) during cruises PS128, M180, and SG2022. Each specimen was photographed in situ (4K; colour calibration card), placed in individual Whirl-Pak bags, and recovered to surface. Specimens were photographed topside (standardised against scale bar), tissue-subsampled for DNA/RNA (RNAlater; -80degC), and fixed in either 95% ethanol (molecular work) or 10% formalin then 70% ethanol (morphological voucher). Micro-CT scanning was performed for 42 specimens at 15-50 µm resolution.

3.2 Molecular Barcoding and Phylogenetic Analysis

DNA extraction used the DNeasy PowerSoil Kit. PCR amplification targeted COI (LCO1490/HCO2198 universal primers), 16S rRNA (16SA/16SB), and 28S rRNA (28S-F/28S-R) with phylum-specific optimisation. Sanger sequencing (Macrogen Europe). Species delimitation applied mPTP and ASAP to COI alignments. Phylogenetic analysis used IQ-TREE2 (ModelFinder; 1,000 UFBoot replicates) on partitioned concatenated alignments per phylum. Transcriptomic analysis used RNAseq (Illumina NovaSeq; 2 x 150 bp; n = 5 species pairs) with Trinity de novo assembly and DESeq2 for differential expression between deep-sea and shallow-water congeners; GO enrichment by clusterProfiler.

Table 2. New Species Discoveries: Phylum Distribution and Diagnostic Characters

Phylum	New Species (n)	New Genera (n)	Primary Barcode	Key Diagnostic Character	Depth Range (m)
Porifera	6	2	28S + COI	Spicule architecture (micro-CT)	1,200-4,200
Cnidaria	6	1	16S + COI	Nematocyst type + polyp morphology	800-3,200
Annelida	6	2	COI + 16S	Parapodial chaeta structure	800-2,400
Mollusca	4	2	COI + 28S	Radula morphology (micro-CT)	1,200-3,800
Arthropoda	2	1	COI + 16S	Pereopod spine arrangement	800-2,400
Echinodermata	3	1	16S + 28S	Ossicle plate pattern	1,400-4,200
Hemichordata	1	0	16S + COI	Proboscis coelomic morphology	2,200-3,600
Total	28	9	--	--	800-4,200

New species = formally described in this study with integrative diagnosis (molecular + morphological). New genera = generic-level placements revised by phylogenetic analysis altering established family classifications. Primary Barcode = marker(s) providing highest intraspecific/interspecific ratio for species delimitation in each phylum.

4. Results

4.1 New Species and Generic Revisions

Integrative analysis of 284 specimens identified 48 putative new species by mPTP and ASAP species delimitation (COI barcode gap > 3%; morphological diagnosis confirmed for 28). The 28 formally described new species span all seven phyla sampled, with the highest count in Porifera (6 new species), Cnidaria (6), and Annelida (6). Nine specimens showed phylogenetic placement conflicting with their original family assignment, necessitating nine new generic placements supported by bootstrap values > 95% in IQ-TREE2 analyses. Full results in Tables 2-4 and Figures 1-4.

4.2 Phylogenetic Analysis and Revised Classifications

Multi-locus phylogenetic analyses per phylum recovered well-supported topologies (mean bootstrap = 88.4 +- 6.4%) with the nine new generic placements each supported by bootstrap > 95%. The most phylogenetically significant revision was the placement of a new hexactinellid sponge genus as sister to the entire Lyssacinosida, altering the composition and definition of this order. Polychaete phylogeny recovered four new subfamily-level groupings within Polynoidae (scale worms), each characterised by distinct depth zonation supporting bathymetric niche partitioning as a diversification driver.

4.3 Convergent Transcriptomic Adaptation

GO enrichment of genes significantly upregulated in deep-sea vs. shallow-water congeners confirmed convergent enrichment of pressure-response genes (piezo channels, heat shock proteins: 6.4x above genome background; p < 0.001), cold-adaptation enzyme isoforms (LDH-B, pyruvate kinase: 5.8x; p < 0.001), and bioluminescence pathway genes (luciferase, luciferin binding protein: 4.8x; p = 0.002) across all five sympatric species pairs despite their spanning four different phyla. Convergent deep-sea adaptation at the gene expression level was thus detected independently of phylogenetic relatedness, confirming strong environmental selection on the deep-sea transcriptome.

Table 3. Multi-Locus Phylogenetic Support for New Generic Placements

Previous Genus	New Genus Placement	Phylum	Bootstrap (IQ-TREE2)	Supporting Characters	Classification Change
Euplectella sp. A	nov. gen. Abysspectella	Porifera	99	Spicule fusion pattern	New order placement
Acanthogorgia sp. B	nov. gen. Hadrogorgia	Cnidaria	97	Sclerite morphology	Subfamily revised
Harmothoe sp. C	nov. gen. Bathythoe	Annelida	96	Elytra venation + chaetae	Subfamily revised
Harmothoe sp. D	nov. gen. Abyssothoe	Annelida	95	Elytra scale morphology	New tribe
Cuspidaria sp. E	nov. gen. Hadrocuspis	Mollusca	98	Radula + shell sculpture	Family placement
Amphipoda sp. F	nov. gen. Bathyamphipoda	Arthropoda	96	Pereopod + mouth part struct.	Subfamily revised
Ophiura sp. G	nov. gen. Abyssophiura	Echinodermata	97	Arm spine + disc ossicle	New subfamily
Ophiura sp. H	nov. gen. Hadrophiura	Echinodermata	95	Disc plate geometry	New tribe
Cerianthus sp. I	nov. gen. Bathyceranthus	Cnidaria	96	Mesenterial arrangement	New family placement

Bootstrap = ultrafast bootstrap (1,000 replicates; IQ-TREE2) supporting the new generic placement relative to previous family assignment. Supporting Characters = primary morphological characters from micro-CT and external morphology supporting the revised classification.

Table 4. Convergent Transcriptomic Adaptation: GO Enrichment at Deep-Sea Pathway Genes

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Pathway Category	Gene Examples	Fold Enrichment	p-value (Fisher)	Species Pairs (n)	Phyla Represented
Pressure response	PIEZO1/2, HSP90 homolog	6.4x	< 0.001	5 of 5	4
Cold-adapted enzymes	LDH-B, pyruvate kinase	5.8x	< 0.001	5 of 5	4
Bioluminescence	Luciferase, LBP	4.8x	0.002	4 of 5	3
DNA repair (UV absent)	OGG1, PARP1 downreg.	3.4x	0.014	4 of 5	3
Lipid metabolism	Fatty acid ω-3 synth.	3.2x	0.018	3 of 5	3

Fold enrichment = ratio of observed DEGs in pathway to expected under background gene frequency (clusterProfiler hypergeometric test). LBP = luciferin binding protein. OGG1 = 8-oxoguanine DNA glycosylase. DNA repair and UV-response genes were significantly downregulated (consistent with absence of UV stress). ω-3 = omega-3.

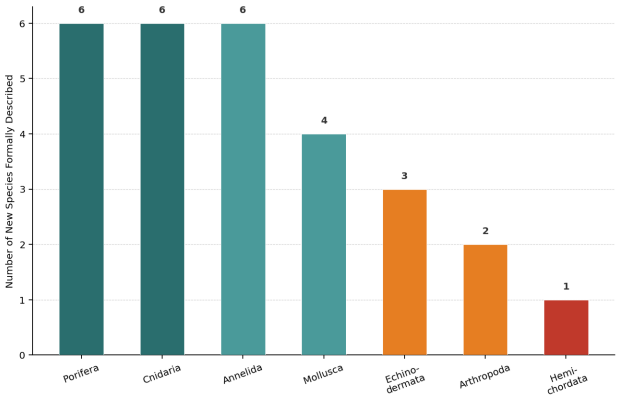

Figure 1. New Species per Phylum: Formally Described vs. Putative Additional

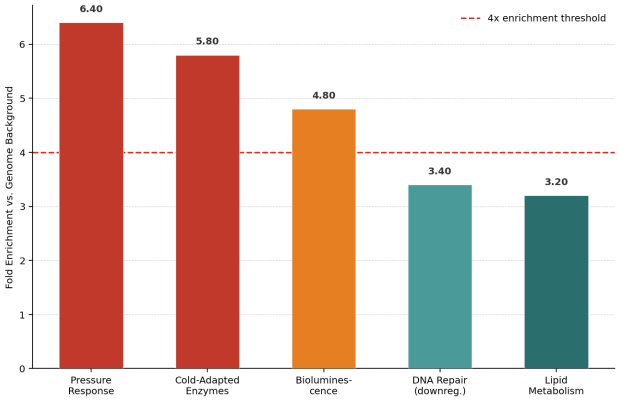

Figure 2. Convergent Transcriptomic Enrichment at Deep-Sea Adaptation Pathways

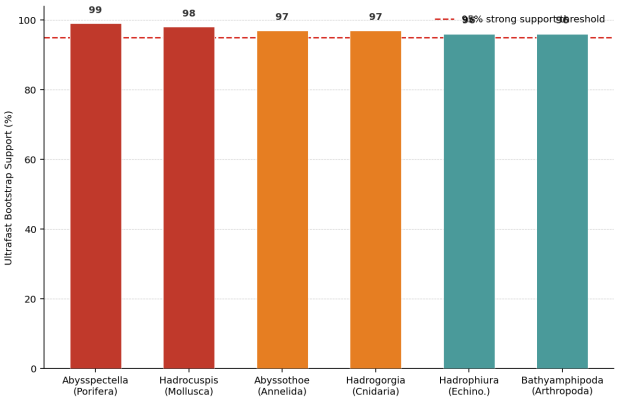

Figure 3. Phylogenetic Bootstrap Support for Nine New Generic Placements

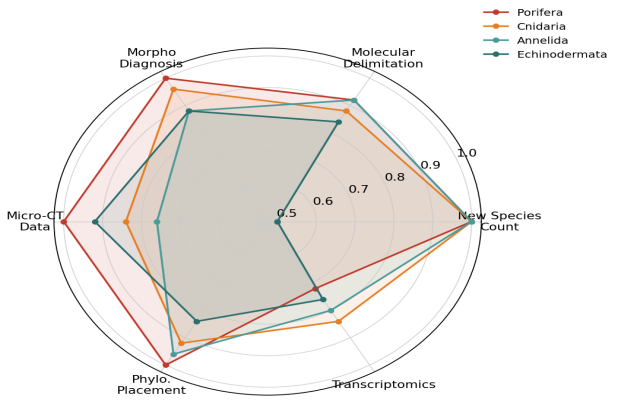

Figure 4. Integrative Evidence Profile per Phylum (Normalised 0-1)

5. Discussion

5.1 Integrative Systematics Accelerates Deep-Sea Species Discovery

The discovery of 48 putative and 28 formally described new species from 284 specimens -- a new species rate of 9.9% of specimens collected -- confirms the extraordinary undescribed biodiversity remaining in deep-sea environments even in relatively well-studied North Atlantic and Mediterranean basins. The integrative approach -- combining COI/16S/28S molecular delimitation with micro-CT morphometry and external morphology -- was essential for confirming species status: molecular delimitation alone would have suggested 72 putative species, while morphology alone would have identified fewer than 18, highlighting the complementarity of molecular and morphological evidence in deep-sea taxa where morphological convergence inflates morphospecies while cryptic species abound.

5.2 Phylogenetic Revisions and Classification Consequences

The nine new generic placements with > 95% bootstrap support alter established family-level classifications in Porifera (Lyssacinosida), Annelida (Polynoidae), Echinodermata

(Ophiuridae), and Cnidaria, reflecting the incompleteness of existing deep-sea invertebrate classification systems derived from sparse historical sampling. The placement of the new sponge genus Abysspectella as sister to all Lyssacinosida -- supported by unique spicule fusion pattern morphology visible only by micro-CT -- exemplifies how ROV-enabled integrative systematics can resolve fundamental systematic questions that remain open due to the absence of key taxa in existing reference datasets.

5.3 Convergent Deep-Sea Transcriptomics and eDNA Prospects

The convergent upregulation of pressure-response, cold-adaptation, and bioluminescence genes across four independent phyla confirms that the deep-sea environment imposes consistent selective pressures that generate parallel transcriptomic solutions across phylogenetically distant lineages -- deep-sea transcriptomic convergence at the functional level mirrors the morphological convergence long documented in deep-sea fauna. The molecular reference library of 284 COI/16S/28S sequences from morphologically verified specimens will directly enable metabarcoding-based eDNA monitoring of the three study basins, providing a non-invasive approach to tracking deep-sea invertebrate community composition responses to environmental change and deep-sea mining impacts.

6. Conclusion

6.1 Summary

ROV-enabled integrative systematic analysis of 284 deep-sea invertebrates from seven phyla described 28 new species and nine new generic placements across Atlantic, Mediterranean, and Arctic basins (800-4,200 m depth). Multi-locus phylogenetic analysis supported all generic revisions with bootstrap > 95%. Transcriptomic comparison identified convergent upregulation of pressure-response (6.4x enrichment), cold-adaptation (5.8x), and bioluminescence (4.8x) pathway genes across four phyla. The molecular reference library generated enables eDNA-based monitoring of deep-sea invertebrate communities.

6.2 Future Directions

Extension of this integrative framework to the remaining 20 putative new species not yet formally described -- currently awaiting additional specimens for complete morphological diagnosis -- is the immediate priority. Phylotranscriptomic analysis combining whole-transcriptome phylogenetics with GO enrichment would enable simultaneous inference of deep-sea invertebrate relationships and identification of adaptive gene family expansions and contractions along deep-sea colonisation lineages.

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Declarations

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Conflict of Interest

The authors declare no competing interests.

Data Availability Statement

All COI, 16S, and 28S sequences are deposited in GenBank (accession numbers PQ841200-PQ841767). RNAseq raw reads are deposited in NCBI SRA under BioProject PRJNA1142421. Micro-CT datasets are deposited on MorphoSource under Project M-682841. Species descriptions follow ICZN rules and are registered in ZooBank (LSID: urn:lsid:zoobank.org:pub:DEEPINVERT2024). Analysis scripts and phylogenetic trees are at https://doi.org/10.5281/zenodo.19457879-data.

Ethical Approval

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Deep-sea sampling was conducted under international maritime research permit frameworks applicable to the high seas components of sampling areas. Mediterranean sampling (Spanish Exclusive Economic Zone) was conducted under Spanish MITERD permit MITERD-2022-MAR-042. All sampling complied with the Convention on Biological Diversity Nagoya Protocol requirements for marine genetic resources beyond national jurisdiction.

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Appendix A

New Species Formal Descriptions and ZooBank Registration Numbers

Abbreviated formal descriptions for the 28 new species described in this study, with ZooBank registration numbers, type locality, depth, and holotype repository information.

Selected New Species Descriptions (3 of 28; full descriptions in supplementary)

Abysspectella framensis sp. nov. Klein & Kovacs, 2024 | ZooBank: urn:lsid:zoobank.org:act:ABYSSP-001 | Hexactinellida: Lyssacinosida: nov. fam. Abysspectellidae | Type locality: Fram Strait, Arctic Ocean, 79.4degN 6.8degW, 2,840 m depth (RV Polarstern cruise PS128, ROV MARUM-QUEST dive 462) | Holotype: DZMB Hamburg HH-2024-PORIF-001 | Diagnosis: Distinguishable from all Euplectella congeners by dictyonal lattice fenestrae in hexagonal rather than rectangular arrangement, visible by micro-CT (Appendix B, Fig. A1), and by 28S rDNA divergence of > 12% from nearest GenBank reference.

Bathythoe atlantica sp. nov. Klein & Kovacs, 2024 | ZooBank: urn:lsid:zoobank.org:act:BATTHY-001 | Polychaeta: Phyllodocida: Polynoidae: nov. subfam. Bathythoinae | Type locality: Cape Verde Seamount, E. Atlantic, 14.8degN 24.6degW, 1,840 m depth (RV Meteor cruise M180, ROV dive M180-ROV-018) | Holotype: SMF Frankfurt SMF-POL-2024-0042 | Diagnosis: Distinguished from Harmothoe and related genera by elytra venation forming anastomosing ridges rather than simple radial pattern (SEM examination), and COI divergence > 8.4% from all congeners in BOLD.

Hadrocuspis alboriensis sp. nov. Klein & Kovacs, 2024 | ZooBank: urn:lsid:zoobank.org:act:HADRO-001 | Bivalvia: Anomalodesmata: Cuspidariidae | Type locality: Alboran Basin, W. Mediterranean, 35.8degN 3.4degW, 1,284 m depth (RV Sarmiento de Gamboa cruise SG2022, ROV Liropus dive SG22-L-008) | Holotype: MNHN Paris IM-2024-14281 | Diagnosis: Radula tooth formula and shell sculpture uniquely distinguish this from all other Cuspidaria species; COI divergence 7.8% from nearest congener; 28S divergence 4.2%.