

How many metazoan species live in the world's largest mineral exploration region?

Highlights

- We provide the first checklist for the Clarion Clipperton Zone (CCZ) metazoan fauna
- 5,142 unnamed species (informal names) are recorded from the CCZ
- Total estimates of species richness range from >6,000–>8,000
- An estimated 88%–92% of species in the CCZ region in total are undescribed

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In brief

Species-level biodiversity information is key to understanding ecosystems and tracking environmental impacts. Rabone et al. provide the first checklist (436 species) and total species estimates (>6,000–>8,000) for the world's largest mineral exploration region, the CCZ. Estimates provide a baseline to build biodiversity knowledge at a regional scale.



Article

How many metazoan species live in the world's largest mineral exploration region?

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SUMMARY

The global surge in demand for metals such as cobalt and nickel has created unprecedented interest in deep-sea habitats with mineral resources. The largest area of activity is a 6 million km² region known as the Clarion-Clipperton Zone (CCZ) in the central and eastern Pacific, regulated by the International Seabed Authority (ISA). Baseline biodiversity knowledge of the region is crucial to effective management of environmental impact from potential deep-sea mining activities, but until recently this has been almost completely lacking. The rapid growth in taxonomic outputs and data availability for the region over the last decade has allowed us to conduct the first comprehensive synthesis of CCZ benthic metazoan biodiversity for all faunal size classes. Here we present the CCZ Checklist, a biodiversity inventory of benthic metazoa vital to future assessments of environmental impacts. An estimated 92% of species identified from the CCZ are new to science (436 named species from a total of 5,578 recorded). This is likely to be an overestimate owing to synonyms in the data but is supported by analysis of recent taxonomic studies suggesting that 88% of species sampled in the region are undescribed. Species richness estimators place total CCZ metazoan benthic diversity at 6,233 (+/–82 SE) species for Chao1, and 7,620 (+/–132 SE) species for Chao2, most likely representing lower bounds of diversity in the region. Although uncertainty in estimates is high, regional syntheses become increasingly possible as comparable datasets accumulate. These will be vital to understanding ecological processes and risks of biodiversity loss.

INTRODUCTION

The Clarion-Clipperton Zone (CCZ) is an area of seabed roughly twice the size of India (approx. 6 million km²), spanning 5°–20° North between the Clarion and Clipperton oceanic fracture zones, and 115°–160° West. This vast region, between Hawaii, Kiribati, and Mexico, lies entirely within areas beyond national jurisdiction (ABNJ), legally designated under the United Nations Convention on the Law of the Sea (UNCLOS). The region is composed of abyssal seafloor at depths of 4,000–6,000 m, characterized by muddy sediments overlain by potato-sized polymetallic nodules, rich in minerals. Despite the darkness and low food availability, nodule field habitats contain diverse communities of benthic invertebrate fauna, albeit at low densities compared with coastal and shelf ecosystems.¹

Mineral exploration began in the CCZ in the 1960s, later formalized under the International Seabed Authority (ISA).² Currently, there are 17 contracts for mineral exploration covering 1.2 million km². Despite decades of intensive exploration, there

has been a historical lack of taxonomic work in the region. Large-scale CCZ environmental surveys conducted in the late 1970s to early 1990s produced lists of informal species names,³ but few species were formally described. Informal names refer to species differentiated by morphology and/or molecular data and recorded with temporary names before formal description^{4,5} (hereafter “unnamed species”). These names present challenges to taxonomic standardization and regional-scale synthesis of biological data. Molecular work provides an arbiter for compatibility across identifications,^{6–8} but is not without challenges. Adding to this complexity, cryptic species, or those with similar or identical morphology but separate molecular lineages are numerous in deep-sea environments,^{9,10} including the CCZ.^{11,12}

Other factors contributing to the lack of comparability across datasets are variable sampling methods,¹³ and more fundamentally, a lack of data.¹⁴ As a result, CCZ synthetic works are rare and primarily focus on particular taxa, size classes, and/or regions.^{15–19} Information gaps span all size classes, from



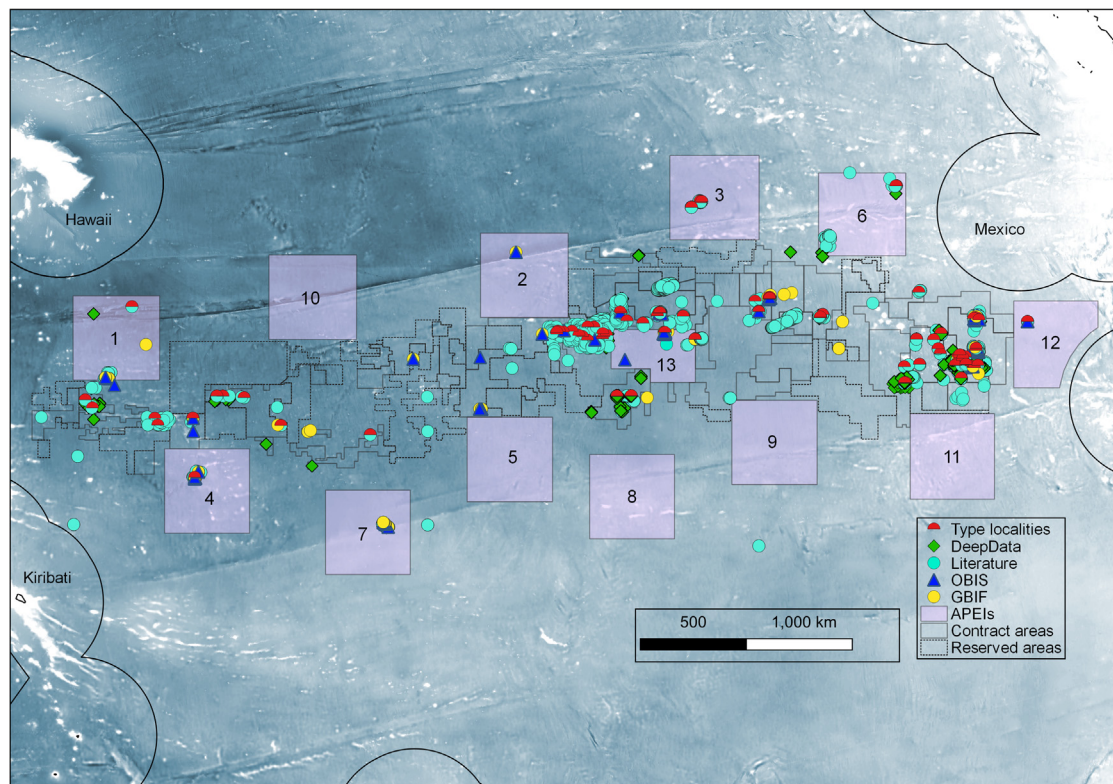


Figure 1. All geolocated published records of benthic metazoa from the literature and databases

Areas of Particular Environmental Interest (APEIs) and exploration mining contract areas, both active and reserved, are shown in outline. The type localities of all species described from the CCZ to date are also shown (185 in total). Background layer: the GEBCO Grid, 2022. See also [Figures S4–S6](#), the [key resources table](#), and [supplemental information](#).

small meiofauna (typically $>150\ \mu\text{m}$) and macrofauna ($>300\ \mu\text{m}$), to large megafauna (typically $>10\ \text{mm}$).²⁰ The data deficiency is particularly notable for the network of Areas of Particular Environmental Interest (APEIs), regions protected from mining²¹ (but see Bonifácio et al.¹⁵, Brix et al.¹⁷, Błażewicz et al.²², and Hauquier et al.²³). This has hampered assessment of their representativeness, with clear implications for environmental management.²⁰ Biodiversity knowledge is essential to robust assessments of species ranges and rarity over time and space, and therefore to evidence-based Regional Environmental Management Plans (REMPs) and future environmental impact assessments (EIAs) in the event of mining operations.^{24,25} The need for regional-scale environmental management has been increasingly recognized by policymakers and the ISA,²¹ supporting a recent resurgence of comparative taxonomic work, including incorporation of DNA methods that allow for a more comparable methodology.^{13,26} Critical to the development of CCZ biodiversity knowledge is the creation of a curated checklist of known taxa and estimates of total undescribed species. Building on recent regional syntheses,²⁰ we present the first comprehensive synthesis of benthic metazoan biodiversity and checklist for this vast region on the eve of possible large-scale mining operations. We make these data and interpretations open to all stakeholders to inform the ongoing debate on deep-sea mineral extraction and to grow our knowledge of the largest ecosystem on our planet.

RESULTS

How many animal species are known to live in the CCZ?

The synthesis produced $>100,000$ records compiled from seven data sources ([Figure 1](#), [key resources table](#)). Recent growth in taxonomic efforts for the CCZ is evident, particularly over the past 5 years ([Figure 2A](#)). To date, 219 taxa new to science (families, genera, and species) have been described from the CCZ. Most of these new taxa have been described in recent years, with only seven descriptions prior to the year 2000. The CCZ Checklist presented here comprises 436 named benthic metazoan species of all size classes ([Table 1](#); [Data S1](#)). These include 185 species, three families, and 31 genera described from the CCZ (see [Figures 2 and 3](#)). Only six of the 185 CCZ new species have also been recorded elsewhere, namely the sea cucumbers *Psychronaetes hanseni* (Pawson, 1983)⁴¹ and *Psychropotes dyscrita* (Clark, 1920)³²; the nematode, *Erebussau tenebricosus* (Bussau, 1993) Bezerra, Pape, Hauquier & Vanreusel, 2021³⁶; the carnivorous sponge, *Axoniderma longipinna* (Ridley & Dendy, 1886)⁴²; the crinoid *Hyocrinus foelli* Roux & Pawson, 1999⁴³; and the antipatharian coral, *Abyssopathes anomala* Molodtsova & Opresko, 2017³¹ (latter in [Figure 3](#)).

The CCZ Checklist records 27 phyla, 49 classes, 163 orders, 501 families, and 1,119 genera in total ([Table 1](#)). For all species-level identifications in the Checklist, 42% are based on morphology and molecular data (185/436), 50%, morphology only (217/436),

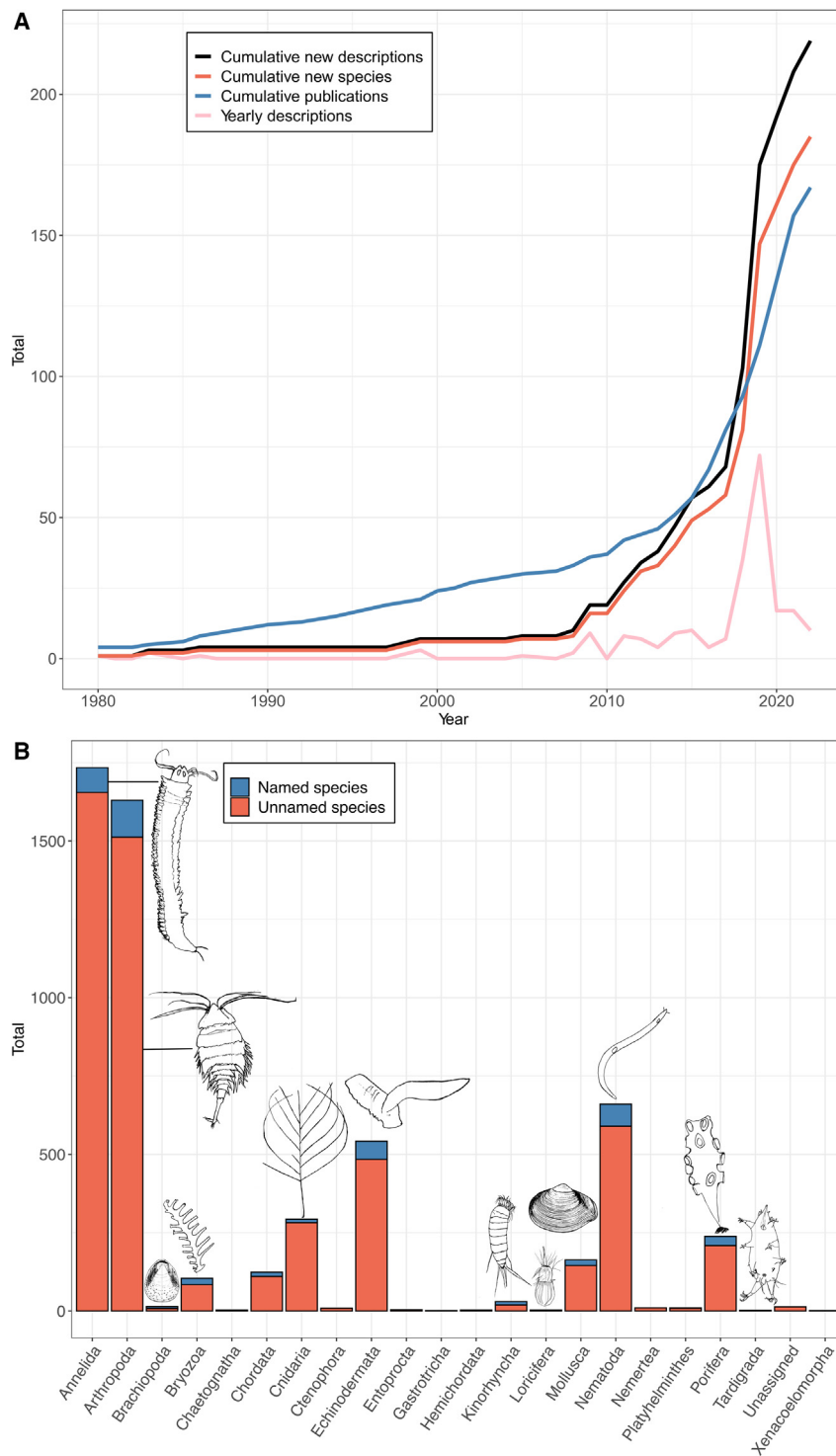


Figure 2. Rates of species descriptions in the CCZ; proportion of species diversity in the CCZ that is undescribed

(A) Rates of new descriptions and publications in the CCZ. Cumulative totals of new taxa (families, genera, and species combined) and new species described from the CCZ and taxonomic publications per year, over the period 1980–2022. Yearly totals of new descriptions also shown.

(B) Proportion of recorded benthic metazoan diversity from the CCZ that is undescribed: named species recorded in blue (both those described from the CCZ and elsewhere), unnamed species shown in red (“unassigned” are records not identified to phyla). Depictions of some of the new CCZ species by phyla: Annelida, *Neanthes goodayi* Drennan, Wiklund, Rabone, Georgieva, Dahlgren & Glover, 2021²⁷; Arthropoda, *Siphonis aureus* Mercado-Salas, Khodami & Martínez Arbizu, 2019²⁸; Brachiopoda, *Oceanithyris juveniformis* Bitner & Zezina, 2013²⁹; Bryozoa, *Pandanipora helix* Grischenko, Gordon & Melnik, 2018³⁰; Cnidaria, *Abyssopathes anomala* Molodtsova & Opresko, 2017³¹; Echinodermata, *Psychropotes dyscrita* (Clark, 1920)³²; Kinorhyncha, *Meristoderes taro* Sánchez, Pardos & Martínez Arbizu, 2019³³; Loricifera, *Fafnirioricus polymetallicus* Fujimoto, 2020³⁴; Mollusca, *Ledella knudseni* J. D. Taylor & Wiklund, 2017³⁵; Nematoda, *Odetenema gesarae* Bezerra, Pape, Hauquier & Vanreusel, 2021³⁶; Porifera, *Chaunoplectella megapora* Wang, Zhang, Lu & Wang, 2018³⁷; and Tardigrada, *Moebjergarctus clarionclippertonensis* Bai, Wang, Zhou, Lin, Meng & Fontoura, 2020.³⁸ See also [Data S1](#) and [S2](#) and [Table S1](#).

species, different names for the same species), resulting in a corrected total of 5,142 ([Table 1](#); [Data S2](#)).

What types of animals live in the CCZ?

The CCZ Checklist illustrates the overall composition of the CCZ fauna identified to date. The five most speciose phyla are the Arthropoda (27%), Annelida (18%), Nematoda (16%), Echinodermata (13%), and Porifera (7%) ([Figures 2B](#) and [4](#); all size fractions, named species only). Proportions for unnamed species are similar, apart from a predominance of Annelida (class Polychaeta, [Figures 2B](#) and [4](#)). In the World Register of Deep-Sea Species (WoRDSS, 2023),⁴⁴ a thematic node of the World Register of Marine Species

(WoRMS, 2023),⁴⁵ there are currently 36,579 named metazoan deep-sea species found globally at depths >500 m. Within WoRDSS, the most speciose phyla are Arthropoda, 31%, Mollusca, 17%, Chordata, 15%, and Annelida and Echinodermata, 10%. Key differences include relatively more annelids, nematodes, and echinoderms in the CCZ (and to a lesser degree, sponges and bryozoans), and conversely more molluscs (class

the remainder, data not available ([Data S1](#)), 51% of the new species are described solely by morphology, and for meiofauna, 86% are described on morphology alone. For the key macrofaunal groups (tanaids, isopods, and polychaetes), 23% of species in the Checklist have type localities outside the CCZ, including other ocean basins (33/145). In total, 5,367 unnamed species are recorded, an estimated 3.9% of which are synonyms (sensu named

Table 1. Summary of benthic metazoan biodiversity in the Clarion-Clipperton Zone based on CCZ Taxonomic Knowledge (published taxonomic and ecological works); the CCZ Checklist (summary of all named benthic metazoans from all published data sources) and CCZ Biodiversity Estimators (based on the analysis in this study)

CCZ Taxonomic knowledge		CCZ Checklist		CCZ Biodiversity Estimators	
New species:	185	Phyla:	27	Unnamed species:	5,142
New genera:	31	Classes:	49	Total species: ^a	5,578
New families:	3	Orders:	163	Chao1 species richness:	6,233 (+/–82 SE)
Total CCZ Descriptions:	219	Families:	501	Chao2 species richness:	7,620 (+/–132 SE)
Taxonomy/ecology papers:	168	Genera:	1,119	ACE estimator:	6,109 (+/45 SE)
Papers with descriptions:	64	Species:	436	Jackknife2 estimator:	8,514 (+/–438 SE)
N/A		Species inc. qualifiers: ^b	654	N/A	

^aTotal species from the CCZ: combined total of named species and unnamed species. The overall proportion of unnamed species diversity in the CCZ is estimated at 92% (5,142/5,578). Sample sizes for CCZ species Biodiversity Estimators: Chao1 and ACE, N = 112,428 ind., S(obs) = 4716; Chao2 and 2nd order Jackknife, N = 1,668 samples, S(obs) = 4,779. Extrapolation max. N: Chao1: 224,858 ind.; Chao2: 3,336 samples. See also [Table S1](#), [Data S1–S5](#).

^bThe CCZ Checklist contains 436 named species in total without identification qualifiers (i.e., cf. aff.) and 654 species including those recorded with identification qualifiers e.g. c.f. of aff (i.e., in open nomenclature) or identified solely from imagery.

Gastropoda) and chordates (class Teleostei) in WoRDSS ([Figure 4](#)). Another notable difference at class level is that Holothuroidea (named and unnamed) are relatively more speciose in the CCZ than other key echinoderm classes (Asterozoa, Ophiurozoa) compared to WoRDSS. Though many faunal gaps are evident in the CCZ Checklist across phyla (e.g., no Pycnogonida in Arthropoda), these groups are recorded from the CCZ in the unnamed species list ([Data S2](#)).

Examining common faunal groupings, 50% of the species in the Checklist are macrofauna (220), with similar proportions for megafauna, 28% (122), and meiofauna, 22% (96). Similarly, most studies primarily assess macrofauna (46%), followed by megafauna (30%) and meiofauna (22%). Descriptions by size class (families, genera, and species combined) are 153 for macrofauna, 24 for megafauna, and 42 for meiofauna. A dominant feature of the CCZ is the unusual combination of mud and hard substrate/nodule fauna. Overall, 14% of named species and 13% of unnamed species in the CCZ are estimated to be primarily nodule dwellers ([Data S1](#) and [S2](#)). Several nodule megafauna descriptions (cnidarians and sponges) have recently been published^{37,39,40,46,47}; but only two quantitative studies for metazoan nodule fauna.^{48,49} The majority of CCZ macrofaunal nodule dwellers (primarily bryozoans and sponges) are undescribed ([Figure 2](#), [Data S2](#)), a rare exception being a recent monograph on Bryozoa describing 16 species, nine genera, and two families new to science.³⁰

How many species might live in the CCZ?

The Chao1 estimator (abundance-based) for total species richness in the CCZ is 6233 (+/– 82 SE, N = 112,429 ind., S(obs) = 4,716) and Chao2 (sample-based), 7,620 (+/–132 SE; N = 1,668 samples, S(obs) = 4,779; [Table 1](#)). Species rarefaction and accumulation curves are far from reaching an asymptote ([Figures 5](#) and [S1](#)). Other species estimates range from 6,109 (+/42 SE), ACE to 8,514 (+/–438 SE), Jackknife2 ([Table 1](#)). At lower taxonomic levels, the family accumulation curve approaches asymptote, with an estimated total family richness of 469 total (+/–18 SE, N = 70,597 ind., F(obs) = 406) for Chao1 and 544 total (+/–24 SE; N = 2,179 samples, F(obs) = 423) for Chao2 ([Figures 5](#) and [S1](#)). These estimates are based on a

subset of the data where abundance and site information are available. In comparison, the CCZ Checklist incorporating all records includes 501 families in total. Estimates of total genera range from 947 (+/–26 SE) for Chao1 to 1,034 (+/–32 SE) for Chao2, with relatively more flattening of rarefaction curves than for species but still far from asymptote ([Figures 5](#) and [S2](#)). This compares to 1,119 genera in the Checklist ([Table 1](#)). Sampling completeness curves show higher completeness for family-level estimates than species, and higher completeness for Chao1 than Chao2 estimates ([Figure S3](#)).

The proportion of undescribed species in the CCZ overall is estimated at 92% (5,142 unnamed species/5,578 named and unnamed species combined; [Table 1](#)). The subset-analysis of 18 publications provides an average figure of 88% undescribed species ([Table S1](#)). Proportions undescribed within the major macrofaunal groups range from 99.4% for tanaids^{3,22} to 96.8% for isopods^{3,17} and 87% for polychaetes^{11,15,50–56} ([Table S1](#)). The figure of 92% undescribed CCZ species is similar to the proportion of known global marine (eukaryotic) species currently in WoRMS⁴⁵ versus the global estimate of Mora et al.⁵⁷ at 89% (241,129 described versus 2,200,000 estimated). It is notably higher than the ranges of Appeltans et al.⁵⁸ from 59%–70% (241,129 described compared to 704,000–972,000 estimated).

Distribution of sampling effort

Sampling effort, as density of unique sampling sites, shows a highly uneven distribution across the region. Samples are concentrated in central and eastern CCZ contract areas, and large regions with very few samples are evident ([Figure S4](#)). APEIs have very low density of sampling or no samples at all. Large regions, particularly between the west and central CCZ, are close to unsampled ([Figures 1](#) and [S4](#)). The density of sampling is highest at certain depths (e.g., ~4,200m, ~5,000m; [Figure S5](#)). These densities correlate with depths of the contract areas in the eastern and central CCZ ([Figure S6](#)). Where abundance data are available, 37% of species occur as singletons, i.e., represented by a single specimen across all sampling deployments (1,586/4,409), indicating extensive under-sampling. Of these singletons, 91% are in mining/reserved areas/the vicinity (1,441), the remainder (145) are found only in

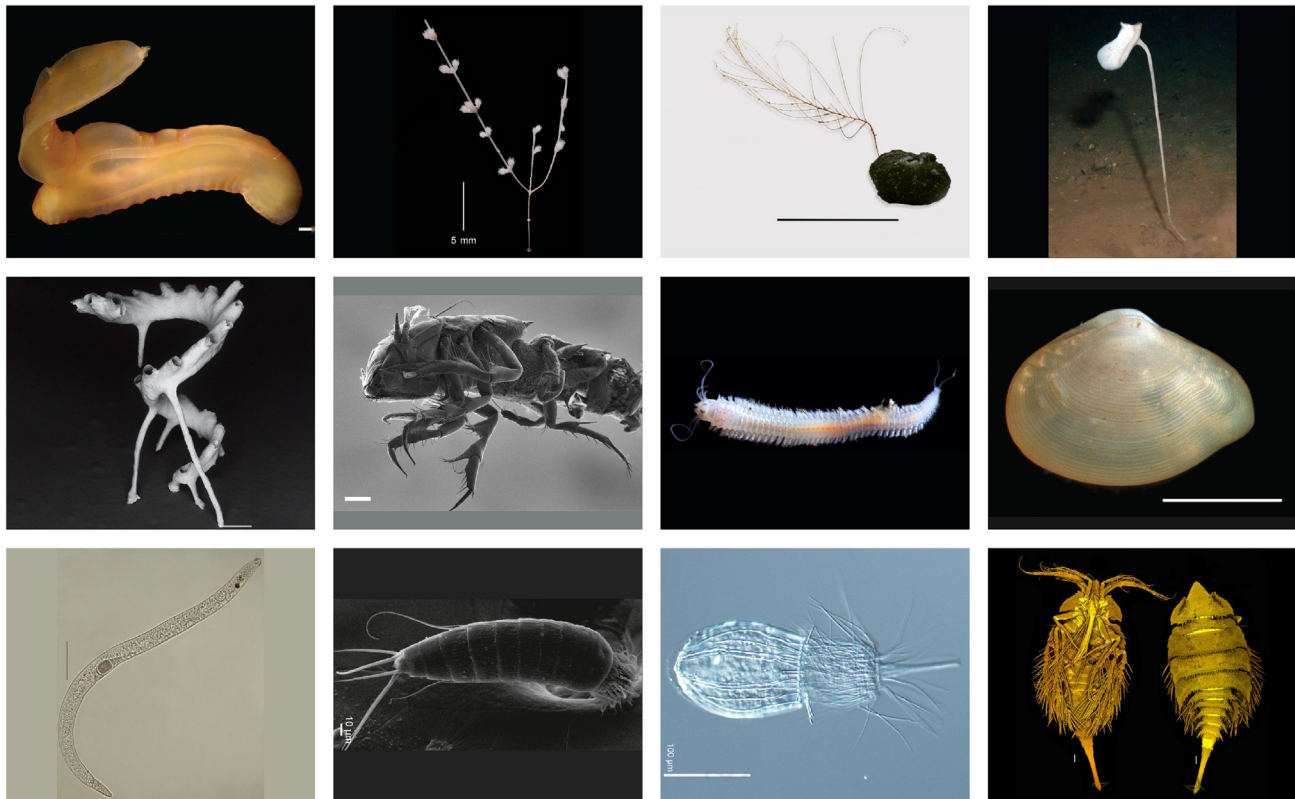


Figure 3. Fauna from the CCZ

(A–J) All fauna are species described from the region and illustrating a range of phyla and size classes, (A) the sea cucumber, *Psychropotes dyscrita* (Clark, 1920),³² commonly known as the “gummy squirrel!” (scale bar: 5 cm); (B) the primnoid coral *Abyssoprinnia gemina* Cairns, 2015³⁹ (scale bar: 5 mm, note the rights to this image are owned by Springer Nature who have granted permission for reuse); (C) the antipatharian coral, *Abyssopathes anomala* Molodtsova & Opresko, 2017³¹ (scale bar: 2 cm); and (D) the hexactinellid sponge, *Sympagella clippertonae* Herzog, Amon, Smith & Janussen, 2018.⁴⁰ (scale bar: 1 cm).

Row 2, (E) the cyclostomatid bryozoan, *Pandanipora helix* Grischenko, Gordon & Melnik, 2018³⁰ (scale bar: 500 μ m); (F) the isopod, *Macrostylis metallicola* Riehl & De Smet, 2020⁷ (scale bar: 0.2 mm); (G) the polychaete, *Neanthes goodayi* Drennan, Wiklund, Rabone, Georgieva, Dahlgren & Glover, 2021²⁷; and (H) the mollusc, *Ledella knudseni* J. D. Taylor & Wiklund, 2017³⁵ (scale bar: 0.5 mm).

Row 3, (I) the nematode, *Odetenema gesarae* Bezerra, Pape, Hauquier & Vanreusel, 2021³⁶ (scale bar: 100 μ m); (J) the kinorhynch, *Meristoderes taro* Sánchez, Pardos & Martínez Arbizu, 2019³³ (scale bar: 10 μ m); the loriciferan, *Fafnirloricus polymetallicus* Fujimoto, 2020³⁴ (scale bar: 100 μ m), and the copepod, *Siphonis aureus* Mercado-Salas, Khodami & Martínez Arbizu, 2019²⁸ (scale bar: 100 μ m).

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APEIs ([Data S3](#) and [S4](#)). Most species are recorded in the eastern CCZ, closely followed by the central CCZ, with few in the west ([Figure 6](#)). The majority of all species are recorded from contract or reserved areas, with few in APEIs. Overall, 95% of named/unnamed species have not been recorded in the APEIs.

DISCUSSION

Species richness estimates are likely to increase as the data improve

This synthesis of all published biodiversity from the CCZ has allowed the first estimates of both the known and unknown species richness across the region. This is important as it sets a baseline for the current state of knowledge while placing the CCZ in a global context.

At species level, it is clear that sampling of the CCZ is very far from complete. Species are accumulating rapidly with increasing samples, with rarefaction and accumulation curves far from asymptote ([Figures 5](#) and [S1](#)). Estimates at family level may be

more robust given the lower likelihood of synonyms and misidentifications than for species.⁵⁹ The Chao1 total family estimate of 469 (+/18 SE) falls short of the current total in the Checklist at 501, but Chao2 at 544 (+/–24 SE) exceeds it. Family-level diversity is expected to be higher than is currently recorded in the Checklist given evidence of extensive under-sampling and the observation that curves have not reached asymptote ([Figures 5](#), [S1](#), and [S2](#)). Chao2 (sample-based) estimates exceeding the Checklist appear more robust, which may partly stem from Chao2 accounting more for missed data in surveys. However, few species records in the dataset represent whole-sample analyses (i.e., only select taxa are identified), likely contributing to underestimation of diversity in these estimates also.^{60,61}

Data duplication can contribute to underestimates of diversity, as relative proportions of rare species, including singletons, will be affected.^{15,62–66} Extensive record duplication is evident in the ISA database DeepData, estimated to be at least a quarter of the total. Although removed for final analysis, further duplication is suspected but cannot be definitively identified owing to

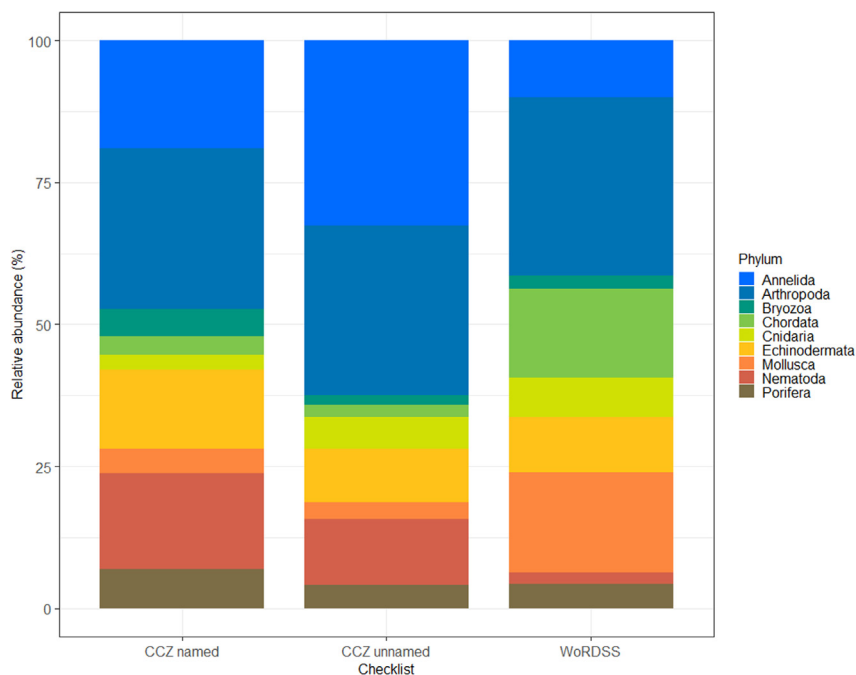


Figure 4. Phylum-level composition of CCZ and global benthic metazoan checklists

Relative abundance of phyla in the CCZ Checklist—named/known species (Data S1); the CCZ unnamed species list (Data S2); and all global deep-sea metazoan species recorded in WoRDSS (World Register of Deep-Sea Species)⁴⁴ on 1st January, 2023.

prevalent in the deep sea^{9,10,82,83} and previously recorded from the CCZ.^{11,12} Resolving these identifications requires genetic data both from the CCZ specimen and the type locality of the species it most closely resembles. Diversity based solely on morphological assessment can underestimate biodiversity by 20%–25%.^{82,84} Although most of the CCZ new species have been described since the advent of DNA taxonomy methods (Figure 2A), 51% are described by morphology only. This figure rises to 86% for meiofauna, partly reflecting challenges of molecular sub-sampling from small-sized specimens.⁸⁵ Un-

underlying limitations of the database.⁶⁷ Including the known duplicates, species estimates are >1,000 lower.

Perhaps most importantly for these estimates, some regions and habitats of the CCZ have barely been sampled at all. For example, there are only six published studies of rocky seamounts and outcrops, which appear to host very different communities.^{68–73} The CCZ, with abundant nodules and rocky outcrops, exhibits high habitat heterogeneity^{16,74} compared with sedimented abyssal plains^{75,76} (although a recent study suggests rocky outcrops may be more common than widely assumed⁷⁷). This unusual “mosaic” habitat of nodule and sediment at local scales supports relatively higher benthic biodiversity.^{16,74,78,79} Overall, many regions of the CCZ are almost unsampled (Figures 1 and S4) and this data deficiency will contribute to underestimation of diversity for the region.

Estimates of species richness are subject to other biases which can either inflate or reduce projections. Synonyms for unnamed species appear rare at 4%, but additional synonyms yet to be identified are inevitable, which would inflate the species estimate. Inflation of informal names can also accrue over time as designations change, and names proliferate. Misidentifications could increase or reduce the diversity estimates, but similarly contribute to overall uncertainty. An unknown proportion of the named species in the CCZ Checklist will be misidentified, owing in part to the lack of regional field guides. Conversely some of the unnamed species may be known species yet to be correctly identified. The lack of field guides can also contribute to range-inflation of cosmopolitan species.^{80,81}

For the key macrofaunal groups in the CCZ Checklist (polychaetes, tanais, and isopods), 23% of species have type localities outside the region, including other ocean basins (33/145). Although wide-ranging benthic species have been confirmed,^{80,81} including in the CCZ²⁶, the 23% may be undescribed cryptic species (or species complexes), particularly

unknown cryptic speciation may be high in this size fraction for the CCZ³³ but this may be quite taxon-specific.⁸⁶ The figure of 92% of species undescribed is likely to be overestimating undescribed species owing to synonyms, but underestimating given known levels of cryptic species¹¹ and under-sampling in the CCZ (Figure S4). In the subset-analysis of taxonomic studies, the potential for misidentification is greatly reduced as groups are examined by their specialists (Table S1). This provides an additional line of evidence to support ~90% of CCZ species being undescribed.

Where does CCZ biodiversity fit in a global context?

Species composition of the CCZ Checklist differs from WoRDSS, even at phylum level (Figure 4). Though some trends (such as relatively high diversity of holothurians) may be real, they will be heavily influenced by taxonomic trends, size fractions assessed, sampling bias, and availability of specialists. The majority of species (named and unnamed) are macrofauna, reflecting numerous studies on this size class. Megafauna, comprising the largest and thereby least abundant species⁸⁷ are rarely collected, compromising species-level identification. Aside from descriptions, there are only two synthetic taxonomic checklist studies with archived vouchers that cover multiple megafaunal taxa,^{88,89} and three covering specific taxa.^{90–92} This reflects the challenges of collecting larger animals, typically involving remotely operated vehicles (ROVs)—which are expensive and require specialists to operate—or trawls, which are inherently destructive of animals.⁹³ Meiofauna, often regarded as the dominant component of deep-sea ecosystems, at least in terms of biomass if not diversity,^{94,95} are also likely to have considerable undocumented species richness given significant sampling challenges.⁹⁶ Biases may also be present in WoRDSS (Figure 4) given chronic under-sampling in the deep sea^{97,98} and taxon-specific factors, e.g., Nematoda being highly speciose but notoriously difficult to identify to species level.^{36,96}

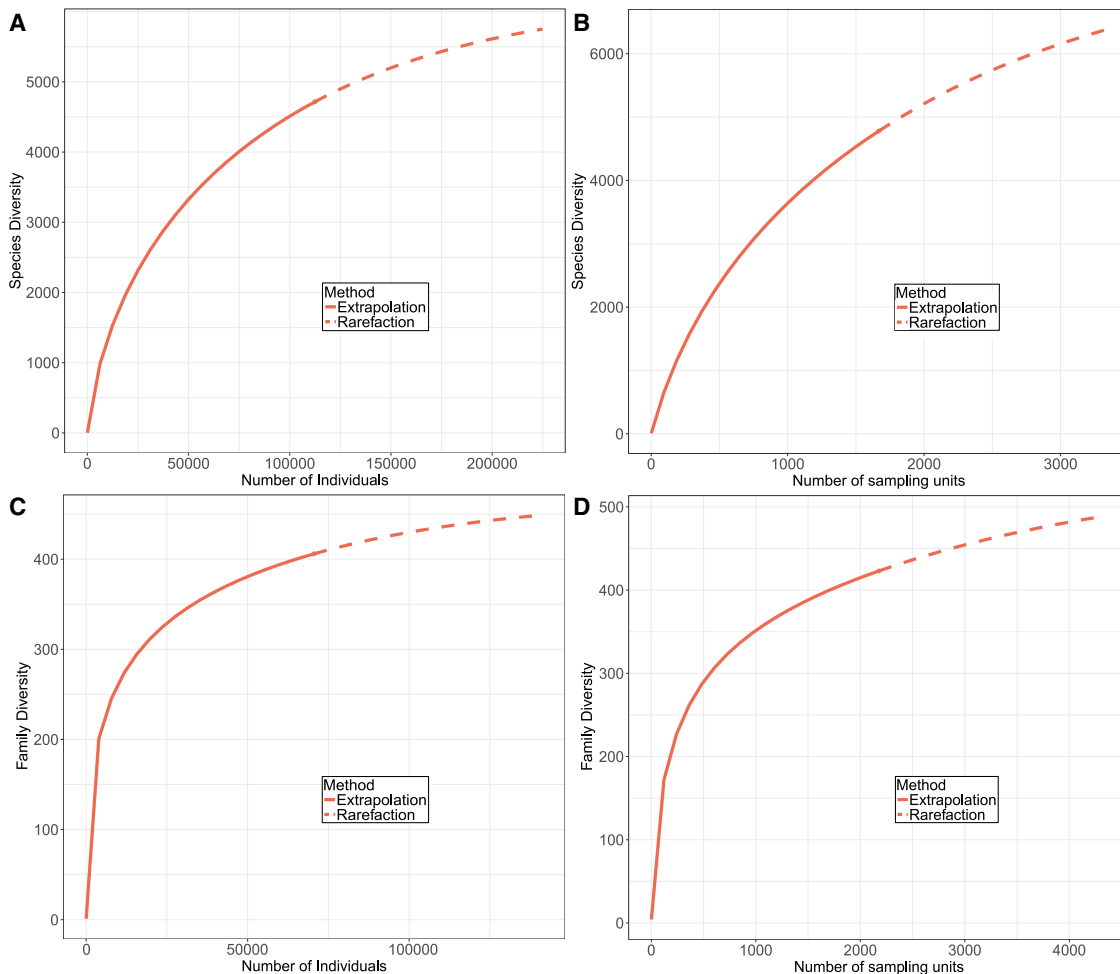


Figure 5. Species and family diversity in the Clarion-Clipperton

(A–D) Diversity estimators (solid line, rarefaction; dashed line, extrapolation): (A) Chao1 species diversity 6,233 (+/–82 SE); N = 112,428 ind., S(obs) = 4,716; extrapolation maximum sample size: 224,858 ind.; (B) Chao2 species diversity 7,620 (+/–132 SE); N = 1,668 samples; S(obs) = 4,779, extrapolation maximum sample size, 3,336 samples. Family diversity estimators: (C) Chao1 family diversity 469 (+/–18 SE); N = 70,597 ind., F(obs) = 406; extrapolation maximum. N: 141,194 ind.; (D) Chao2 family diversity 544 (+/–24 SE); N = 2,179 samples; F(obs) = 423; extrapolation maximum N: 4,358 samples.

See also [Table 1](#), [Figures S1–S3](#), and [Data S3](#) and [S4](#).

There are few comparable estimators of biodiversity in other broad-scale regions of the deep sea. One study of the Southern Ocean deep sea reported 674 isopod species of which a high proportion (87%) were new to science.⁸³ Undescribed CCZ isopods are higher with an estimated 96% being new species^{3,17} (23 named species, 474 unnamed). Total marine species richness estimates reviewed in Appeltans et al.⁵⁸ range from 300,000⁹⁹ to 10 million,¹⁰⁰ the latter regarded as a significant overestimate, and the former a significant underestimate.^{97,101} Our figure of 92% is similar to the proportion of currently known marine species in WoRMS (241,129)⁴⁵ to the Mora et al.⁵⁷ global estimate at 89%. The current CCZ Checklist represents just 1% of currently recorded deep-sea species in WoRDSS (36,579).⁴⁴ Including unnamed species, this would rise to 15%, or species estimators, 17%–24%.

Clearly the CCZ represents significant undescribed biodiversity. With 31 new genera and three new families, (and several additional new genera and at least one additional new family known to the

authors), the Checklist illustrates the novelty of the region at deep taxonomic levels. Evolutionary novelty has been previously recorded in the CCZ for echinoderms,¹² but it is noteworthy that this extends across further taxa. Diversity of life-history strategies are beginning to be recorded in the CCZ¹⁰² as elsewhere in the deep sea, such as association with sponge stalks.^{89,103} Characteristic sediment-dwelling infauna such as nematodes, isopods, and polychaetes are now being found living in and on nodules, illustrating the interconnectivity of nodule-sediment dwelling life-styles.^{27,104,105} Beyond nodule-dwellers, many suspension-feeding forms depend on nodules. Spatial ecology studies report 60%–80% of the megafauna (largely dominated by suspension feeders in the CCZ) to be found growing attached to nodules.^{78,79} Pertinent questions remain on the relative vulnerability of nodule and sediment fauna to mining impacts.¹⁰⁶ Remarkably little is known of life-history traits of these species and answering these questions is an immense challenge in a region where most species are rare and a third appear to have been found only once.

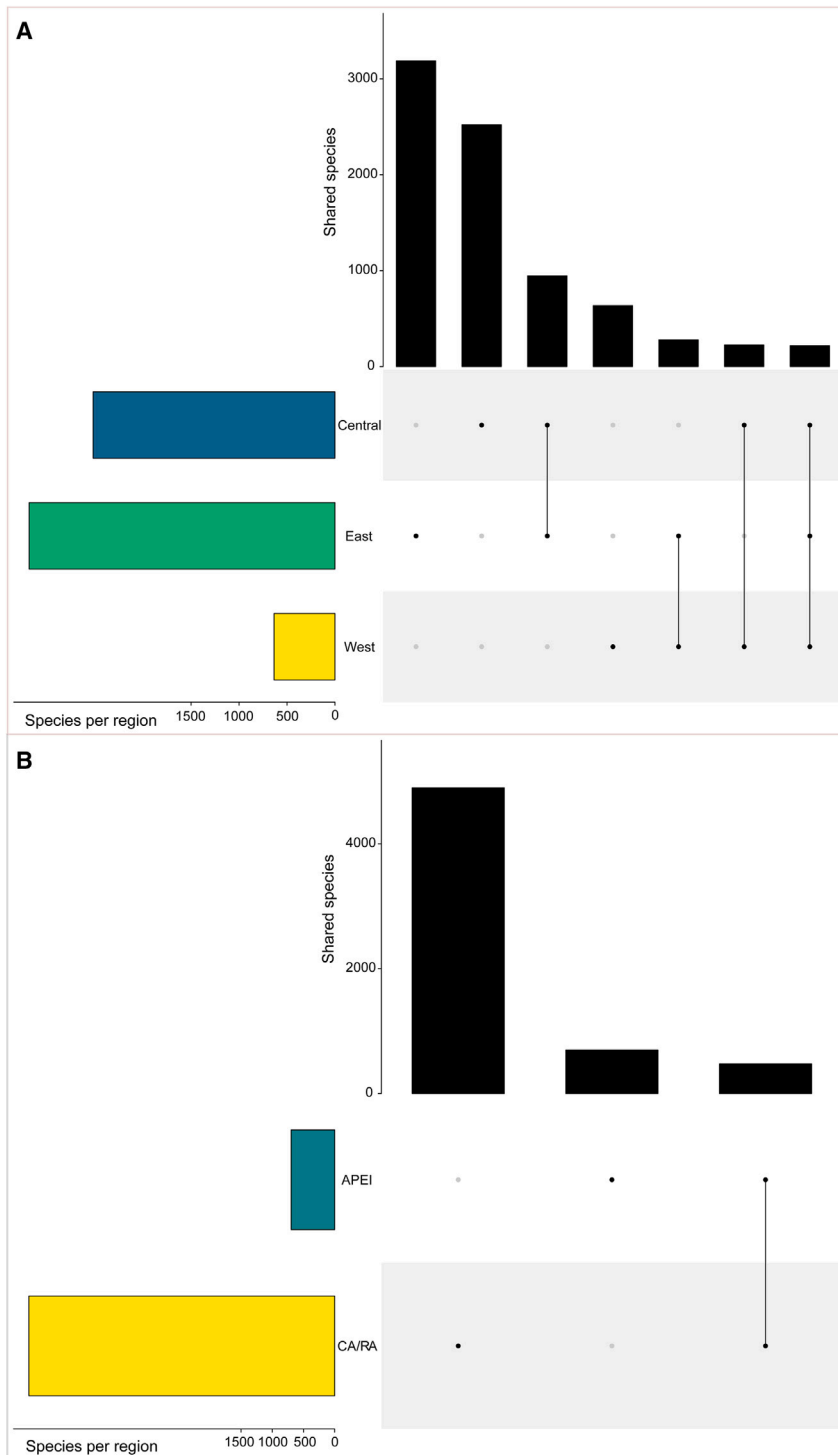


Figure 6. UpSet plot of all CCZ species (named and unnamed species combined) at regional scales

(A and B) Top bars show total species shared or independent, intersecting with region in the lower panel (species independent per region correlate to a “dot” or shared between region to a “dash” connecting the regions). Side bars show total species per region. (A): all species by region, (B), all species in contract areas and reserved areas pooled versus those in APEIs pooled.

See also [Data S3](#) and [S4](#).

CCZ taxonomy will require extensive collaboration between stakeholders supported by regulatory bodies/governments and appropriate and sustained funding.^{17,67,85,107,108} Programs such as the new ISA Sustainable Seabed Knowledge Initiative (SsKI)¹⁰⁹ recognized under the UN Ocean Decade should be leveraged to fund descriptions in all taxonomic groups. As the new species will take years to be formally described, a robust approach to open nomenclature in the medium term is also important to ensure that species-level taxa can be referenced and that datasets are comparable and linked to open data and specimen vouchers.^{5,85,90,91} The CCZ Checklist is a key step forward in an iterative process towards field guides for the region, which will dramatically improve identifications and reduce uncertainty. Our study provides the first regional estimates of species diversity for all size classes. Although uncertainty is high, these estimates provide a starting point to be developed as additional data and approaches become available. Development in statistical methods for estimating species richness will be critical to future assessments of diversity in such poorly sampled environments.^{110,111} Given mining operations may be imminent, a key consideration for the CCZ is the application of biodiversity data for environmental management, in particular assessing species extinction risk. Often assumed to be lower in marine environments, this appears largely an artefact of lower taxonomic knowledge compared to terrestrial ecosystems.¹¹² The UNCLOS states that “no

Conclusions

The proportion of undescribed species in the CCZ has been reported as being over 80% within taxa.^{11,17,22} Our study provides the first quantitative support for that figure across multiple taxonomic groups, with two estimators (88% and 92%) clearly illustrating the remaining taxonomic impediment to an understanding of CCZ biodiversity. Addressing the “lost decades” of

serious harm” can occur from any mining activities and that necessary measures must be taken to protect the environment from any harmful effects. Although sometimes equated with no loss of biodiversity, the definition of the term “serious harm” (and that of “lower environmental thresholds”) remains to be clarified.^{24,113} Accurately quantifying species ranges and rarity, key components of extinction risk, requires a comprehensive

approach to taxonomy,¹¹⁴ extensive molecular studies,¹¹⁵ and standardized quantitative methods²⁰ enabling regional analyses. This is particularly important given that the CCZ remains one of the few remaining areas of the global ocean with high intactness of wilderness.¹¹⁶ Sound data and understanding are essential to shed light on this unique region and secure its future protection from human impacts.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
 - Lead contact
 - Materials availability
 - Data and code availability
- **METHOD DETAILS**
 - Data collection, processing and exploratory analysis: Databases
 - Published literature
 - The CCZ Checklist: Known/named species
 - Unnamed species/informal name species list
- **QUANTIFICATION AND STATISTICAL ANALYSIS**
 - Diversity estimates
 - Assessment of regional-scale sampling effort
 - Comparison of CCZ Checklist with global checklists
- **ADDITIONAL RESOURCES**

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.cub.2023.04.052>.

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AUTHOR CONTRIBUTIONS

M.R. and A.G.G. conceptualized the study. J.H.W., T.H., A.M.E., D.O.B.J., and E.S.-L. provided additional input into general approach. M.R. collected and curated data, conducted investigation and analysis, created figures, and wrote the first draft. J.H.W. provided additional input into figures and data/code archiving. T.H., T.G.D., H.W., and A.G.G. undertook QA/QC on the CCZ Checklist. All authors edited and reviewed manuscript drafts and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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REFERENCES

1. Hessler, R.R., and Sanders, H.L. (1967). Faunal diversity in the deep-sea. *Deep-Sea Res. Oceanogr. Abstr.* *14*, 65–78.
2. Jones, D.O.B., Kaiser, S., Sweetman, A.K., Smith, C.R., Menot, L., Vink, A., Trueblood, D., Greinert, J., Billett, D.S.M., Arbizu, P.M., et al. (2017). Biological responses to disturbance from simulated deep-sea polymetallic nodule mining. *PLoS One* *12*, 0171750. <https://doi.org/10.1371/journal.pone.0171750>.
3. Wilson, G.D.F. (2017). Macrofauna abundance, species diversity and turnover at three sites in the Clipperton-Clarion Fracture Zone. *Mar. Biodivers.* *47*, 323–347. <https://doi.org/10.1007/s12526-016-0609-8>.
4. Horton, T., Marsh, L., Bett, B.J., Gates, A.R., Jones, D.O.B., Benoist, N.M.A., Pfeifer, S., Simon-Lledó, E., Durden, J.M., Vandepitte, L., and Appeltans, W. (2021). Recommendations for the standardisation of open taxonomic nomenclature for image-based identifications. *Front. Mar. Sci.* *8*, <https://doi.org/10.3389/fmars.2021.620702>.
5. Sigovini, M., Keppel, E., and Tagliapietra, D. (2016). Open Nomenclature in the biodiversity era. *Methods Ecol. Evol.* *7*, 1217–1225. <https://doi.org/10.1111/2041-210X.12594>.
6. Kaiser, S., Brix, S., Kihara, T.C., Janssen, A., and Jennings, R.M. (2018). Integrative species delimitation in the deep-sea genus *Thaumastosoma* Hessler, 1970 (Isopoda, Asellota, Nannoniscidae) reveals a new genus and species from the Atlantic and central Pacific abyss. *Deep Sea Res. 2 Top. Stud. Oceanogr.* *148*, 151–179. <https://doi.org/10.1016/j.dsr2.2017.05.006>.
7. Riehl, T., and De Smet, B. (2020). *Macrostylis metallica* spec. nov.—an isopod with geographically clustered genetic variability from a polymetallic-nodule area in the Clarion-Clipperton Fracture Zone. *PeerJ* *8*, 8621. <https://doi.org/10.7717/peerj.8621>.

8. Brix, S., Bober, S., Tschesche, C., Kihara, T.C., Driskell, A., and Jennings, R.M. (2018). Molecular species delimitation and its implications for species descriptions using desmosomatid and nannoniscid isopods from the VEMA fracture zone as example taxa. *Deep Sea Res. 2 Top. Stud. Oceanogr.* *148*, 180–207. <https://doi.org/10.1016/j.dsr2.2018.02.004>.
9. Knowlton, N. (1993). Sibling species in the sea. *Annu. Rev. Ecol. Syst.* *24*, 189–216. <https://doi.org/10.1146/annurev.es.24.110193.001201>.
10. Brandt, A., Griffiths, H., Gutt, J., Linse, K., Schiaparelli, S., Ballerini, T., Danis, B., and Pfannkuche, O. (2014). Challenges of deep-sea biodiversity assessments in the Southern Ocean. *Adv. Polar Sci.* *25*, 204–212. <https://doi.org/10.13679/j.advps.2014.3.00204>.
11. Bonifácio, P., and Menot, L. (2019). New genera and species from the Equatorial Pacific provide phylogenetic insights into deep-sea Polynoidae (Annelida). *Zool. J. Linn. Soc. Zool. J. Linn. Soc.* *185*, 555–635. <https://doi.org/10.1093/zoolinnean/zly063>.
12. Christodoulou, M., O'Hara, T.D., Hugall, A.F., and Arbizu, P.M. (2019). Dark ophiroid biodiversity in a prospective abyssal mine field. *Curr. Biol.* *29*, 3909–3912.e3. <https://doi.org/10.1016/j.cub.2019.09.012>.
13. Glover, A., Dahlgren, T., Wiklund, H., Mohrbeck, I., and Smith, C. (2015). An end-to-end DNA taxonomy methodology for benthic biodiversity survey in the Clarion–Clipperton Zone, central Pacific abyss. *J. Mar. Sci. Eng.* *4*, 2. <https://doi.org/10.3390/jmse4010002>.
14. Mincks, S., and Smith, C.R. (2006). *Critical Review of Benthic Biological Data from the Clarion–Clipperton Zone (CCZ) and Adjoining Areas Project: Development of Benthic Biological Parameters for Input into the Geological Model (Geological Model Phase 2)*. ISA report.
15. Bonifácio, P., Martínez Arbizu, P., and Menot, L. (2020). Alpha and beta diversity patterns of polychaete assemblages across the nodule province of the eastern Clarion–Clipperton Fracture Zone (equatorial Pacific). *Biogeosciences* *17*, 865–886. <https://doi.org/10.5194/bg-17-865-2020>.
16. Simon-Lledó, E., Pomee, C., Ahokava, A., Drazen, J.C., Leitner, A.B., Flynn, A., Parianos, J., and Jones, D.O. (2020). Multi-scale variations in invertebrate and fish megafauna in the mid-eastern Clarion Clipperton Zone. *Prog. Oceanogr.* *187*, 102405. <https://doi.org/10.1016/j.pocean.2020.102405>.
17. Brix, S., Osborn, K.J., Kaiser, S., Truskey, S.B., Schnurr, S.M., Brenke, N., Malyutina, M., and Martínez Arbizu, P. (2020). Adult life strategy affects distribution patterns in abyssal isopods—implications for conservation in Pacific nodule areas. *Biogeosciences* *17*, 6163–6184. <https://doi.org/10.5194/bg-17-6163-2020>.
18. Drazen, J.C., Leitner, A.B., Jones, D.O.B., and Simon-Lledó, E. (2021). Regional variation in communities of demersal fishes and scavengers across the Clarion Clipperton Zone and Pacific ocean. *Front. Mar. Sci.* *8*, 1110. <https://doi.org/10.3389/fmars.2021.630616/full>.
19. Lejzerowicz, F., Gooday, A.J., Barrenechea Angeles, I., Cordier, T., Morard, R., Apothéoz-Perret-Gentil, L., Lins, L., Menot, L., Brandt, A., Levin, L.A., et al. (2021). Eukaryotic biodiversity and spatial patterns in the Clarion–Clipperton Zone and other abyssal regions: insights from sediment DNA and RNA metabarcoding. *Front. Mar. Sci.* *8*, 671033. <https://doi.org/10.3389/fmars.2021.671033>.
20. Smith, C.R., Clark, M.R., Goetze, E., Glover, A.G., and Howell, K.L. (2021). Editorial: Biodiversity, Connectivity and Ecosystem Function Across the Clarion–Clipperton Zone: A Regional Synthesis for an Area Targeted for Nodule Mining. *Front. Mar. Sci.* *8*, 797516. <https://doi.org/10.3389/fmars.2021.797516>.
21. Wedding, L.M., Friedlander, A.M., Kittinger, J.N., Watling, L., Gaines, S.D., Bennett, M., Hardy, S.M., and Smith, C.R. (2013). From principles to practice: a spatial approach to systematic conservation planning in the deep sea. *Proc. Biol. Sci.* *280*, 20131684. <https://doi.org/10.1098/rspb.2013.1684>.
22. Błażewicz, M., Józwiak, P., Menot, L., and Pabis, K. (2019). High species richness and unique composition of the tanaidacean communities associated with five areas in the Pacific polymetallic nodule fields. *Prog. Oceanogr.* *176*, 102141. <https://doi.org/10.1016/j.pocean.2019.102141>.
23. Hauquier, F., Macheriotou, L., Bezerra, T.N., Egho, G., Martínez Arbizu, P., and Vanreusel, A. (2019). Distribution of free-living marine nematodes in the Clarion–Clipperton Zone: implications for future deep-sea mining scenarios. *Biogeosciences* *16*, 3475–3489. <https://doi.org/10.5194/bg-16-3475-2019>.
24. Amon, D.J., Gollner, S., Morato, T., Smith, C.R., Chen, C., Christiansen, S., Currie, B., Drazen, J.C., Fukushima, T., Gianni, M., et al. (2022). Assessment of scientific gaps related to the effective environmental management of deep-seabed mining. *Mar. Policy* *138*, 105006. <https://doi.org/10.1016/j.marpol.2022.105006>.
25. Durden, J.M., Murphy, K., Jaeckel, A., Van Dover, C.L., Christiansen, S., Gjerde, K., Ortega, A., and Jones, D.O. (2017). A procedural framework for robust environmental management of deep-sea mining projects using a conceptual model. *Mar. Policy* *84*, 193–201. <https://doi.org/10.1016/j.marpol.2017.07.002>.
26. Janssen, A., Kaiser, S., Meißner, K., Brenke, N., Menot, L., and Martínez Arbizu, P. (2015). A reverse taxonomic approach to assess macrofaunal distribution patterns in abyssal Pacific polymetallic nodule fields. *PLoS One* *10*, 0117790. <https://doi.org/10.1371/journal.pone.0117790>.
27. Drennan, R., Wiklund, H., Rabone, M., Georgieva, M.N., Dahlgren, T.G., and Glover, A.G. (2021). *Neanthes goodayi* sp. nov. (Annelida, Nereididae), a remarkable new annelid species living inside deep-sea polymetallic nodules. *Eur. J. Taxon.* *760*, 160–185. <https://doi.org/10.5852/ejt.2021.760.1447>.
28. Mercado-Salas, N.F., Khodami, S., and Martínez Arbizu, P. (2019). Convergent evolution of mouthparts morphology between Siphonostomatoida and a new genus of deep-sea Aegisthidae Giesbrecht, 1893 (Copepoda: Harpacticoida). *Mar. Biodivers.* *49*, 1635–1655. <https://doi.org/10.1007/s12526-018-0932-3>.
29. Bitner, M.A., Melnik, V.P., and Zezina, O.N. (2013). New paedomorphic brachiopods from the abyssal zone of the North-eastern Pacific Ocean. *Zootaxa* *3613*, 281–288. <https://doi.org/10.11646/zootaxa.3613.3.6>.
30. Grischenko, A.V., Gordon, D.P., and Melnik, V.P. (2018). Bryozoa (Cyclostomata and Ctenostomata) from polymetallic nodules in the Russian exploration area, Clarion–Clipperton Fracture Zone, eastern Pacific Ocean—taxon novelty and implications of mining. *Zootaxa* *4484*, 1–91. <https://doi.org/10.11646/zootaxa.4484.1.1>.
31. Molodtsova, T.N., and Opresko, D.M. (2017). Black corals (Anthozoa: Antipatharia) of the Clarion–Clipperton Fracture Zone. *Mar. Biodivers.* *47*, 349–365. <https://doi.org/10.1007/s12526-017-0659-6>.
32. Gebruk, A.V., Kremenetskaia, A., and Rouse, G.W. (2020). A group of species “*Psychropotes longicauda*” (Psychropotidae, Elsipodida, Holothuroidea) from the Kuril–Kamchatka Trench area (North–West Pacific). *Prog. Oceanogr.* *180*, 102222. <https://doi.org/10.1016/j.pocean.2019.102222>.
33. Sánchez, N., Pardos, F., and Martínez Arbizu, P. (2019). Deep-sea Kinorhyncha diversity of the polymetallic nodule fields at the Clarion–Clipperton Fracture Zone (CCZ). *Zool. Anz.* *282*, 88–105. <https://doi.org/10.1016/j.jcz.2019.05.007>.
34. Fujimoto, S., and Murakami, C. (2020). A new genus and species of Nanaloricidae (Loricifera: Nanaloricida) from the Clarion–Clipperton Fracture Zone. *Zool. Anz.* *289*, 177–188.
35. Wiklund, H., Taylor, J.D., Dahlgren, T.G., Todt, C., Ikebe, C., Rabone, M., and Glover, A.G. (2017). Abyssal fauna of the UK-1 polymetallic nodule exploration area, Clarion–Clipperton Zone, central Pacific Ocean: Mollusca. *ZooKeys* *707*, 1–46. <https://doi.org/10.3897/zookeys.707.13042>.
36. Bezerra, T.N., Pape, E., Hauquier, F., and Vanreusel, A. (2021). Description and distribution of *Erebussau* nom. nov. pro *Erebus* Bussau, 1993 nec *Erebus* Latreille, 1810 with description of a new species, and of *Odetenema gesaræ* gen. nov., sp. nov. (Nematoda: Desmoscolecida) from nodule-bearing abyssal sediments in the Pacific. *Zootaxa* *4903*. zootaxa.4903.4.4. <https://doi.org/10.11646/zootaxa.4903.4.4>.

37. Wang, C., Zhang, Y., Lu, B., and Wang, D. (2018). New Hexactinellid Sponge *Chaunoplectella megapora* sp. nov. (Lyssacosida: Leucopsacidae) from Clarion-Clipperton Fracture Zone, Eastern Pacific Ocean. *Zootaxa* 4375, 136–142. <https://doi.org/10.11646/zootaxa.4375.1.8>.
38. Bai, L., Wang, X., Zhou, Y., Lin, S., Meng, F., and Fontoura, P. (2020). *Moebjergarctus clarionclippertonensis*, a new abyssal tardigrade (Arthrotardigrada, Halechiniscidae, Euclavartinae) from the Clarion-Clipperton Fracture Zone, North-East Pacific. *Zootaxa* 4755, 561–575. <https://doi.org/10.11646/ZOOTAXA.4755.3.8>.
39. Cairns, S.D. (2016). New abyssal Primnoidea (Anthozoa: Octocorallia) from the Clarion-Clipperton fracture zone, equatorial northeastern Pacific. *Mar. Biodivers.* 46, 141–150. <https://doi.org/10.1007/s12526-015-0340-x>.
40. Herzog, S., Amon, D.J., Smith, C.R., and Janussen, D. (2018). Two new species of *Sympagella* (Porifera: Hexactinellida: Rossellidae) collected from the Clarion-Clipperton Zone, East Pacific. *Zootaxa* 4466, 152–163. <https://doi.org/10.11646/zootaxa.4466.1.12>.
41. Pawson, D.L. (1983). *Psychronaetes hanseni*, a new genus and species of elaspipodan sea cucumber from the eastern central Pacific (Echinodermata: Holothuroidea). *Proc. Biol. Soc. Wash.* 96, 154–159.
42. Ridley, S.O., and Dendy, A. (1887). Report on the Monaxonida collected by H.M.S. 'Challenger' during the years 1873–76. Report on the Scientific Results of the Voyage of H.M.S. Challenger during the years 1873–76. *Zoology* 20, 1–275.
43. Roux, M., and Pawson, D.L. (1999). Two new Pacific Ocean species of hyocrinid crinoids (Echinodermata), with comments on presumed giant-dwarf gradients related to seamounts and abyssal plains. *Pac. Sci.* 53, 289–298.
44. Glover, A.G., Higgs, N., and Horton, T. (2023). World Register of Deep-Sea species (WoRDS). Accessed at <https://doi.org/10.14284/352>. <https://www.marinespecies.org/deepsea>.
45. WoRMS Editorial Board (2023). World Register of Marine Species (VLIZ). <https://doi.org/10.14284/170>. <https://www.marinespecies.org>.
46. Kersken, D., Janussen, D., and Martínez Arbizu, P. (2018). Deep-sea glass sponges (Hexactinellida) from polymetallic nodule fields in the Clarion-Clipperton Fracture Zone (CCFZ), northeastern Pacific: Part I—Amphidiscophora. *Mar. Biodivers.* 48, 545–573. <https://doi.org/10.1007/s12526-017-0727-y>.
47. Kersken, D., Janussen, D., and Arbizu, P.M. (2019). Deep-sea glass sponges (Hexactinellida) from polymetallic nodule fields in the Clarion-Clipperton Fracture Zone (CCFZ), northeastern Pacific: Part II—Hexasterophora. *Mar. Biodivers.* 49, 947–987. <https://doi.org/10.1007/s12526-018-0880-y>.
48. Veillette, J., Sarrazin, J., Gooday, A.J., Galéron, J., Caprais, J.C., Vangriesheim, A., Étoubleau, J., Christian, J.R., and Kim Juniper, S. (2007). Ferromanganese nodule fauna in the Tropical North Pacific Ocean: Species richness, faunal cover and spatial distribution. *Deep Sea Res. 1 Oceanogr. Res. Pap.* 54, 1912–1935. <https://doi.org/10.1016/j.dsr.2007.06.011>.
49. Mullineux, L.S. (1987). Organisms living on manganese nodules and crusts: distribution and abundance at three North Pacific sites. *Deep Sea Res.* 34, 165–184. [https://doi.org/10.1016/0198-0149\(87\)90080-X](https://doi.org/10.1016/0198-0149(87)90080-X).
50. Bonifácio, P., Neal, L., and Menot, L. (2021). Diversity of deep-sea scale-worms (Polynoidae) in the Clarion-Clipperton Fracture Zone. *Front. Mar. Sci.* 8, 656899. <https://doi.org/10.3389/fmars.2021.656899>.
51. Neal, L., Wiklund, H., Rabone, M., Dahlgren, T.G., and Glover, A.G. (2022). Abyssal fauna of polymetallic nodule exploration areas, eastern Clarion-Clipperton Zone, central Pacific Ocean: Annelida: Spionidae and Poecilochaetidae. *Mar. Biodivers.* 52, 51–48. <https://doi.org/10.1007/s12526-022-01277-1>.
52. Neal, L., Wiklund, H., Gunton, L.M., Rabone, M., Bribiesca-Contreras, G., Dahlgren, T.G., and Glover, A.G. (2022). Abyssal fauna of polymetallic nodule exploration areas, eastern Clarion-Clipperton Zone, central Pacific Ocean: Annelida: Amphinomidae and Euphosinidae. *ZooKeys* 1137, 33–74. <https://doi.org/10.3897/zookeys.1137.86150>.
53. Glover, A.G., Smith, C.R., Paterson, G.L.J., Wilson, G.D.F., Hawkins, L., and Shearer, M. (2002). Polychaete species diversity in the central Pacific abyss: local and regional patterns, and relationships with productivity. *Mar. Ecol. Prog. Ser.* 240, 157–170.
54. Guggolz, T., Meißner, K., Schwentner, M., Dahlgren, T.G., Wiklund, H., Bonifácio, P., and Brandt, A. (2020). High diversity and pan-oceanic distribution of deep-sea polychaetes: Prionospio and Aurospio (Annelida: Spionidae) in the Atlantic and Pacific Ocean. *Org. Divers. Evol.* 1–17. <https://doi.org/10.1007/s13127-020-00430-7>.
55. Wiklund, H., Neal, L., Glover, A.G., Drennan, R., Rabone, M., and Dahlgren, T.G. (2019). Abyssal fauna of polymetallic nodule exploration areas, eastern Clarion-Clipperton Zone, central Pacific Ocean: Annelida: Capitellidae, Opheliidae, Scalibregmatidae, and Traviidae. *ZooKeys* 883, 1–82. <https://doi.org/10.3897/zookeys.883.36193>.
56. Smith, C.R., Paterson, G., Lamshead, J., Glover, A., Rogers, A., Gooday, A., Kitazato, H., Sibuet, M., Galéron, J., and Menot, L. (2008). Biodiversity, species ranges, and gene flow in the abyssal Pacific nodule province: predicting and managing the impacts of deep seabed mining. ISA report.
57. Mora, C., Tittensor, D.P., Adl, S., Simpson, A.G.B., and Worm, B. (2011). How many species are there on Earth and in the ocean? *PLoS Biol.* 9, 1001127. <https://doi.org/10.1371/journal.pbio.1001127>.
58. Appeltans, W., Ahyong, S.T., Anderson, G., Angel, M.V., Artois, T., Bailly, N., Bamber, R., Barber, A., Bartsch, I., Berta, A., et al. (2012). The magnitude of global marine species diversity. *Curr. Biol.* 22, 2189–2202. <https://doi.org/10.1016/j.cub.2012.09.036>.
59. Costello, M.J., Bouchet, P., Boxshall, G., Fauchald, K., Gordon, D., Hoeksema, B.W., Poore, G.C.B., van Soest, R.W.M., Stöhr, S., Walter, T.C., et al. (2013). Global coordination and standardisation in marine biodiversity through the World Register of Marine Species (WoRMS) and related databases. *PLoS One* 8, 51629. <https://doi.org/10.1371/journal.pone.0051629>.
60. Gotelli, N.J., and Colwell, R.K. (2001). Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecol. Lett.* 4, 379–391.
61. Longino, J.T., Coddington, J., and Colwell, R.K. (2002). The ant fauna of a tropical rain forest: estimating species richness three different ways. *Ecology* 83, 689–702.
62. Coddington, J.A., Agnarsson, I., Miller, J.A., Kuntner, M., and Hormiga, G. (2009). Undersampling bias: the null hypothesis for singleton species in tropical arthropod surveys. *J. Anim. Ecol.* 78, 573–584. <https://doi.org/10.1111/j.1365-2656.2009.01525.x>.
63. Colwell, R.K., Chao, A., Gotelli, N.J., Lin, S.Y., Mao, C.X., Chazdon, R.L., and Longino, J.T. (2012). Models and estimators linking individual-based and sample-based rarefaction, extrapolation and comparison of assemblages. *J. Plant Ecol.* 5, 3–21. <https://doi.org/10.1093/jpe/rtr044>.
64. O'Hara, R.B. (2005). Species richness estimators: how many species can dance on the head of a pin? *J. Anim. Ecol.* 74, 375–386. <https://doi.org/10.1111/j.1365-2656.2005.00940.x>.
65. Ugland, K.I., and Gray, J.S. (2004). Estimation of species richness: analysis of the methods developed by Chao and Karakassis. *Mar. Ecol. Prog. Ser.* 284, 1–8. <https://doi.org/10.3354/meps284001>.
66. Chao, A., and Chiu, C.H. (2016). Nonparametric estimation and comparison of species richness. *Els.* 1–11. <https://doi.org/10.1002/9780470015902.a0026329>.
67. Rabone, M., Horton, T., Jones, D.O.B., Simon-Lledó, E., and Glover, A.G. (2023). A review of the International Seabed Authority database DeepData from a biological perspective: challenges and opportunities in the UN Ocean Decade. *Database* 2023, baad013. <https://doi.org/10.1093/database/baad013>.
68. Cuvelier, D., Ribeiro, P.A., Ramalho, S.P., Kersken, D., Martínez Arbizu, P., and Colaço, A. (2020). Are seamounts refuge areas for fauna from

- polymetallic nodule fields? *Biogeosciences* 17, 2657–2680. <https://doi.org/10.5194/bg-17-2657-2020>.
69. Jones, D.O., Simon-Lledó, E., Amon, D.J., Bett, B.J., Cauille, C., Clément, L., Connelly, D.P., Dahlgren, T.G., Durden, J.M., Drazen, J.C., et al. (2021). Environment, ecology, and potential effectiveness of an area protected from deep-sea mining (Clarion Clipperton Zone, abyssal Pacific). *Prog. Oceanogr.* 197, 102653. <https://doi.org/10.1016/j.pocean.2021.102653>.
 70. Leitner, A.B., Drazen, J.C., and Smith, C.R. (2021). Testing the Seamount Refuge Hypothesis for Predators and Scavengers in the Western Clarion-Clipperton Zone. *Front. Mar. Sci.* 8, 1146. <https://doi.org/10.3389/fmars.2021.636305>.
 71. Durden, J.M., Putts, M., Bingo, S., Leitner, A.B., Drazen, J.C., Gooday, A.J., Jones, D.O.B., Sweetman, A.K., Washburn, T.W., and Smith, C.R. (2021). Megafaunal ecology of the western Clarion Clipperton Zone. *Front. Mar. Sci.* 8, 722. <https://doi.org/10.3389/fmars.2021.671062>.
 72. Bribiesca-Contreras, G., Dahlgren, T.G., Horton, T., Drazen, J.C., Drennan, R., Jones, D.O., Leitner, A.B., McQuaid, K.A., Smith, C.R., Taboada, S., et al. (2021). Biogeography and connectivity across habitat types and geographical scales in Pacific abyssal scavenging amphipods. *Front. Mar. Sci.* 8, 705237. <https://doi.org/10.3389/fmars.2021.705237>.
 73. Mejía-Saenz, A., Simon-Lledó, E., Partridge, L.S., Xavier, J.R. and Jones, D.O.B. (2023). Rock outcrops enhance abyssal benthic biodiversity. *Deep Sea Research Part I: Oceanographic Research Papers*, 195, p.103999. <https://doi.org/10.1016/j.dsr.2023.103999>.
 74. Simon-Lledó, E., Bett, B.J., Huvenne, V.A.I., Schoening, T., Benoist, N.M.A., Jeffreys, R.M., Durden, J.M., and Jones, D.O.B. (2019). Megafaunal variation in the abyssal landscape of the Clarion Clipperton Zone. *Prog. Oceanogr.* 170, 119–133. <https://doi.org/10.1016/j.pocean.2018.11.003>.
 75. Durden, J.M., Bett, B.J., Jones, D.O., Huvenne, V.A., and Ruhl, H.A. (2015). Abyssal hills – hidden source of increased habitat heterogeneity, benthic megafaunal biomass and diversity in the deep sea. *Prog. Oceanogr.* 137, 209–218. <https://doi.org/10.1016/j.pocean.2015.06.006>.
 76. Ruhl, H.A. (2008). Community change in the variable resource habitat of the abyssal northeast Pacific. *Ecology* 89, 991–1000. <https://doi.org/10.1890/06-2025.1>.
 77. Riehl, T., Wöfl, A.C., Augustin, N., Devey, C.W., and Brandt, A. (2020). Discovery of widely available abyssal rock patches reveals overlooked habitat type and prompts rethinking deep-sea biodiversity. *Proc. Natl. Acad. Sci. USA* 117, 15450–15459. <https://doi.org/10.1073/pnas.1920706117>.
 78. Simon-Lledó, E., Bett, B.J., Huvenne, V.A.I., Schoening, T., Benoist, N.M.A., and Jones, D.O.B. (2019). Ecology of a polymetallic nodule occurrence gradient: Implications for deep-sea mining. *Limnol. Oceanogr.* 64, 1883–1894. <https://doi.org/10.1002/lno.11157>.
 79. Amon, D.J., Ziegler, A.F., Dahlgren, T.G., Glover, A.G., Goineau, A., Gooday, A.J., Wiklund, H., and Smith, C.R. (2016). Insights into the abundance and diversity of abyssal megafauna in a polymetallic-nodule region in the eastern Clarion-Clipperton Zone. *Sci. Rep.* 6, 1–12. <https://doi.org/10.1038/srep30492>.
 80. Bik, H.M., Thomas, W.K., Lunt, D.H., and Lamshead, P.J.D. (2010). Low endemism, continued deep-shallow interchanges, and evidence for cosmopolitan distributions in free-living marine nematodes (order Enoplida). *BMC Evol. Biol.* 10, 1–10. <https://doi.org/10.1186/1471-2148-10-389>.
 81. Brandt, A., Błażewicz-Paszkowycz, M., Bamber, R.N., Mühlenhardt-Siegel, U., Maljutina, M.V., Kaiser, S., De Broyer, C., and Havermans, C. (2012). Are there widespread peracarid species in the deep sea (Crustacea: Malacostraca)? *Pol. Polar Res.* 33, 139–162. <https://doi.org/10.2478/v10183-012-0012-5>.
 82. Brasier, M.J., Wiklund, H., Neal, L., Jeffreys, R., Linse, K., Ruhl, H., and Glover, A.G. (2016). DNA barcoding uncovers cryptic diversity in 50% of deep-sea Antarctic polychaetes. *R. Soc. Open Sci.* 3, 160432. <https://doi.org/10.1098/rsos.160432>.
 83. Brandt, A., Gooday, A.J., Brandão, S.N., Brix, S., Brökeland, W., Cedhagen, T., Choudhury, M., Cornelius, N., Danis, B., De Mesel, I., et al. (2007). First insights into the biodiversity and biogeography of the Southern Ocean deep sea. *Nature* 447, 307–311. <https://doi.org/10.1038/nature05827>.
 84. Smith, C.R., Washburn, T., Menot, L., Bonifácio, P., Pape, E., and Ju, S.J. (2019). Deep-sea biodiversity synthesis workshop-Macrofaunal report. In *Proceedings of the Deep CCZ Biodiversity Synthesis Workshop (University of Hawaii at Manoa & International Seabed Authority)*.
 85. Rabone, M., Harden-Davies, H., Collins, J.E., Zajderman, S., Appeltans, W., Droege, G., Brandt, A., Pardo-Lopez, L., Dahlgren, T.G., Glover, A.G., and Horton, T. (2019). Access to Marine Genetic Resources (MGR): raising awareness of best-practice through a new agreement for Biodiversity Beyond National Jurisdiction (BBNJ). *Front. Mar. Sci.* 6, 520. <https://doi.org/10.3389/fmars.2019.00520>.
 86. Miljutin, D.M., and Miljutina, M.A. (2016). Intraspecific variability of morphological characters in the species-rich deep-sea genus *Acantholaimus* Allgén, 1933 (Nematoda: Chromadoridae). *Nematology* 18, 455–473. <https://doi.org/10.1163/15685411-00002970>.
 87. White, E.P., Ernest, S.K.M., Kerkhoff, A.J., and Enquist, B.J. (2007). Relationships between body size and abundance in ecology. *Trends Ecol. Evol.* 22, 323–330. <https://doi.org/10.1016/j.tree.2007.03.007>.
 88. Amon, D.J., Ziegler, A.F., Drazen, J., Grischenko, A., Leitner, A., Lindsay, D., Voight, J., Wicksten, M., Young, C., and Smith, C.R. (2017). Megafauna of the UKSRL exploration contract area and eastern Clarion-Clipperton Zone in the Pacific Ocean: Annelida, Arthropoda, Bryozoa, Chordata, Ctenophora, Mollusca. *Biodivers. Data J.* 5, e14598. <https://doi.org/10.3897/BDJ.5.e14598>.
 89. Bribiesca-Contreras, G., Dahlgren, T.G., Amon, D.J., Cairns, S., Drennan, R., Durden, J.M., Eléaume, M.P., Hosie, A.M., Kremenetskaia, A., McQuaid, K., et al. (2022). Benthic megafauna of the western Clarion-Clipperton Zone, Pacific Ocean. *ZooKeys* 1113, 1–110. <https://doi.org/10.3897/zookeys.1113.82172>.
 90. Amon, D.J., Ziegler, A.F., Kremenetskaia, A., Mah, C.L., Mooi, R., O'Hara, T., Pawson, D.L., Roux, M., and Smith, C.R. (2017). Megafauna of the UKSRL exploration contract area and eastern Clarion-Clipperton Zone in the Pacific Ocean: Echinodermata. *Biodivers. Data J.* 5, e11794. <https://doi.org/10.3897/BDJ.5.e11794>.
 91. Christodoulou, M., O'Hara, T., Hugall, A.F., Khodami, S., Rodrigues, C.F., Hilario, A., Vink, A., and Martinez Arbizu, P. (2020). Unexpected high abyssal ophiuroid diversity in polymetallic nodule fields of the northeast Pacific Ocean and implications for conservation. *Biogeosciences* 17, 1845–1876. <https://doi.org/10.5194/bg-17-1845-2020>.
 92. Christodoulou, M., De Grave, S., Vink, A., and Martinez Arbizu, P. (2022). Taxonomic assessment of deep-sea decapod crustaceans collected from polymetallic nodule fields of the East Pacific Ocean using an integrative approach. *Mar. Biodivers.* 52, 61. <https://doi.org/10.1007/s12526-022-01284-2>.
 93. Pawson, D.L. (1988). *Abyssal plain megafauna in the Clarion Clipperton Fracture Zone, Eastern Central Pacific Ocean: Photographic survey of a manganese nodule field*. NOAA report, pp. 1–80.
 94. Sinniger, F., Pawlowski, J., Harii, S., Gooday, A.J., Yamamoto, H., Chevaldonné, P., Cedhagen, T., Carvalho, G., and Creer, S. (2016). Worldwide analysis of sedimentary DNA reveals major gaps in taxonomic knowledge of deep-sea benthos. *Front. Mar. Sci.* 3, 92. <https://doi.org/10.3389/fmars.2016.00092>.
 95. Le, J.T., Levin, L.A., Lejzerowicz, F., Cordier, T., Gooday, A.J., and Pawlowski, J. (2022). Scientific and budgetary trade-offs between morphological and molecular methods for deep-sea biodiversity assessment. *Integr. Environ. Assess. Manag.* 18, 655–663. <https://doi.org/10.1002/ieam.4466>.
 96. Lins, L., Zeppilli, D., Menot, L., Michel, L.N., Bonifácio, P., Brandt, M., Pape, E., Rossel, S., Uhlenkott, K., Macheriotou, L., et al. (2021). Toward a reliable assessment of potential ecological impacts of

- deep-sea polymetallic nodule mining on abyssal infauna. *Limnol Oceanogr. Methods* 19, 626–650. <https://doi.org/10.1002/lom3.10448>.
97. Higgs, N.D., and Attrill, M.J. (2015). Biases in biodiversity: wide-ranging species are discovered first in the deep sea. *Front. Mar. Sci.* 2, 61. <https://doi.org/10.3389/fmars.2015.00061>.
 98. Gage, J.D., and May, R.M. (1993). A dip into the deep seas. *Nature* 365, 609–610. <https://doi.org/10.1038/365609a0>.
 99. Costello, M.J., Wilson, S., and Houlding, B. (2012). Predicting total global species richness using rates of species description and estimates of taxonomic effort. *Syst. Biol.* 61, 871–883. <https://doi.org/10.1093/sysbio/syr080>.
 100. Grassle, J.F., and Maciolek, N.J. (1992). Deep-sea species richness: regional and local diversity estimates from quantitative bottom samples. *Am. Nat.* 139, 313–341. <https://doi.org/10.1086/285329>.
 101. Poore, G.C.B., Avery, L., Błażewicz-Paszkwowycz, M., Browne, J., Bruce, N.L., Gerken, S., Glasby, C., Greaves, E., McCallum, A.W., Staples, D., et al. (2015). Invertebrate diversity of the unexplored marine western margin of Australia: taxonomy and implications for global biodiversity. *Mar. Biodivers.* 45, 271–286. <https://doi.org/10.1007/s12526-014-0255-y>.
 102. Kristensen, R.M., Gooday, A.J., and Goineau, A. (2019). Loricifera inhabiting spherical agglutinated structures in the abyssal eastern equatorial Pacific nodule fields. *Mar. Biodivers.* 49, 2455–2466. <https://doi.org/10.1007/s12526-019-00962-y>.
 103. Lörz, A.N., and Horton, T. (2021). Investigation of the Amathillopsidae (Amphipoda, Crustacea), including the description of a new species, reveals a clinging lifestyle in the deep sea worldwide. *ZooKeys* 1031, 19–39. <https://doi.org/10.3897/zookeys.1031.62391>.
 104. Malyutina, M.V. (2011). Description of two new species of munnopsid isopods (Crustacea: Isopoda: Asellota) from manganese nodules area of the Clarion-Clipperton Fracture Zone, Pacific Ocean. *Zootaxa* 2783, 1–20. <https://doi.org/10.11646/zootaxa.2783.1.1>.
 105. Pape, E., Bezerra, T.N., Gheerardyn, H., Buydens, M., Kieswetter, A., and Vanreusel, A. (2021). Potential impacts of polymetallic nodule removal on deep-sea meiofauna. *Sci. Rep.* 11, 19996–15. <https://doi.org/10.1038/s41598-021-99441-3>.
 106. Ardron, J.A., Simon-Lledó, E., Jones, D.O.B., and Ruhl, H.A. (2019). Detecting the effects of deep-seabed nodule mining: simulations using megafaunal data from the Clarion-Clipperton Zone. *Front. Mar. Sci.* 6, <https://doi.org/10.3389/fmars.2019.00604>.
 107. Engel, M.S., Ceriaco, L.M.P., Daniel, G.M., Dellapé, P.M., Löbl, I., Marinov, M., Reis, R.E., Young, M.T., Dubois, A., Agarwal, I., et al. (2021). The taxonomic impediment: a shortage of taxonomists, not the lack of technical approaches. *Zool. J. Linn. Soc.* 193, 381–387. <https://doi.org/10.1093/zoolinnean/zlab072>.
 108. Mammola, S., Fukushima, C.S., Biondo, G., Bongiorno, L., Cianferoni, F., Domenici, P., Fruciano, C., Lo Giudice, A., Macías-Hernández, N., Malumbres-Olarte, J., et al. (2023). How much biodiversity is concealed in the word ‘biodiversity’? *Curr. Biol.* 33, R59–R60. <https://doi.org/10.1016/j.cub.2022.12.003>.
 109. ISA. Sustainable Seabed Knowledge Initiative. <https://www.isa.org/jm/sski/>.
 110. Caley, M.J., Fisher, R., and Mengersen, K. (2014). Global species richness estimates have not converged. *Trends Ecol. Evol.* 29, 187–188. <https://doi.org/10.1016/j.tree.2014.02.002>.
 111. Fisher, R., O’Leary, R.A., Low-Choy, S., Mengersen, K., Knowlton, N., Brainard, R.E., and Caley, M.J. (2015). Species richness on coral reefs and the pursuit of convergent global estimates. *Curr. Biol.* 25, 500–505. <https://doi.org/10.1016/j.cub.2014.12.022>.
 112. Webb, T.J., and Mindel, B.L. (2015). Global patterns of extinction risk in marine and non-marine systems. *Curr. Biol.* 25, 506–511. <https://doi.org/10.1016/j.cub.2014.12.023>.
 113. Levin, L.A., Mengerink, K., Gjerde, K.M., Rowden, A.A., Van Dover, C.L., Clark, M.R., Ramirez-Llodra, E., Currie, B., Smith, C.R., Sato, K.N., et al. (2016). Defining “serious harm” to the marine environment in the context of deep-seabed mining. *Mar. Policy* 74, 245–259. <https://doi.org/10.1016/j.marpol.2016.09.032>.
 114. Kaiser, S., Kihara, T.C., Brix, S., Mohrbeck, I., Janssen, A., and Jennings, R.M. (2021). Species boundaries and phylogeographic patterns in new species of *Nannoniscus* (Janiroidea: Nannoniscidae) from the equatorial Pacific nodule province inferred from mtDNA and morphology. *Zool. J. Linn. Soc.* 193, 1020–1071. <https://doi.org/10.1093/zoolinnean/zlaa174>.
 115. Macheriotou, L., Rigaux, A., Derycke, S., and Vanreusel, A. (2020). Phylogenetic clustering and rarity imply risk of local species extinction in prospective deep-sea mining areas of the Clarion-Clipperton Fracture Zone. *Proc. Biol. Sci.* 287, 20192666. <https://doi.org/10.1098/rspb.2019.2666>.
 116. Jones, K.R., Klein, C.J., Halpern, B.S., Venter, O., Grantham, H., Kuempel, C.D., Shumway, N., Friedlander, A.M., Possingham, H.P., and Watson, J.E.M. (2018). The location and protection status of Earth’s diminishing marine wilderness. *Curr. Biol.* 28, 2506–2512.e3. <https://doi.org/10.1016/j.cub.2018.06.010>.
 117. R Core Team (2023). R: A language and environment for statistical computing (R Foundation for Statistical Computing).
 118. QGIS Development Team (2023). QGIS Geographic Information System (Open Source Geospatial Foundation Project). <http://qgis.osgeo.org/>.
 119. Provoost, P., and Bosch, S. (2017). robis: R Client to access data from the OBIS API. In Ocean Biogeographic Information System (Intergovernmental Oceanographic Commission of UNESCO). R package version 1.0.0. <https://cran.r-project.org/package=robis>.
 120. Ocean Biodiversity Information System (OBIS) ISA Node. <https://obis.org/node/9d2d95be-32eb-4d81-8911-32cb8bc641c8>.
 121. GADMTTools. GitHub lamKDO - GADMTTools. <https://github.com/lamKDO/GADMTTools>.
 122. Pebesma, E.J., and Bivand, R.S. (2005). Classes and methods for spatial data in R. *R. News* 5, 9–13. <https://CRAN.R-project.org/doc/Rnews/>.
 123. Evans, J.S. (2021). spatialEco. R package version 1.3-6. <https://github.com/jeffrejevans/spatialEco>.
 124. maptools. Tools For Handling Spatial objects. 1.1-7. <http://maptools.r-forge.r-project.org/>.
 125. rgdal. Bindings for the Geospatial Data Abstraction Library. 1.6-4. <http://rgdal.r-forge.r-project.org/>.
 126. R Forge. R Interface to GEOS: Project Home. <https://r-forge.r-project.org/projects/rgeos/>.
 127. Wilkinson, M.D., Dumontier, M., Aalbersberg, I.J.J., Appleton, G., Axton, M., Baak, A., Blomberg, N., Boiten, J.W., da Silva Santos, L.B., Bourne, P.E., et al. (2016). The FAIR Guiding Principles for scientific data management and stewardship. *Sci. Data* 3, 160018–160019. <https://doi.org/10.1038/sdata.2016.18>.
 128. Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., Blomberg, S.P., and Webb, C.O. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26, 1463–1464. <https://doi.org/10.1093/bioinformatics/btq166>.
 129. Barbosa, A.M. (2015). fuzzySim: applying fuzzy logic to binary similarity indices in ecology. *Methods Ecol. Evol.* 6, 853–858. <https://doi.org/10.1111/2041-210X.12372>.
 130. Conway, J.R., Lex, A., and Gehlenborg, N. (2017). UpSetR: an R package for the visualization of intersecting sets and their properties. *Bioinformatics* 33, 2938–2940. <https://doi.org/10.1093/bioinformatics/btx364>.
 131. Hill, M.O. (1973). Diversity and Evenness: A Unifying Notation and Its Consequences. *Ecology* 54, 427–432.
 132. Chao, A., and Jost, L. (2012). Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size. *Ecology* 93, 2533–2547. <https://doi.org/10.1890/11-1952.1>.
 133. Chao, A., Gotelli, N.J., Hsieh, T.C., Sander, E.L., Ma, K.H., Colwell, R.K., and Ellison, A.M. (2014). Rarefaction and extrapolation with Hill numbers:

- a framework for sampling and estimation in species diversity studies. *Ecol. Monogr.* 84, 45–67. <https://doi.org/10.1890/13-0133.1>.
134. Chao, A. (1987). Estimating the population size for capture-recapture data with unequal catchability. *Biometrics* 43, 783–791. <https://doi.org/10.2307/2531532>.
135. Hsieh, T.C., Ma, K.H., and Chao, A. (2016). iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods Ecol. Evol.* 7, 1451–1456. <https://doi.org/10.1111/2041-210X.12613>.
136. Colwell, R.K., and Coddington, J.A. (1994). Estimating terrestrial biodiversity through extrapolation. *Phil. Trans. R. Soc. Lond. B* 345, 101–118. <https://doi.org/10.1098/rstb.1994.0091>.
137. Burnham, K.P., and Overton, W.S. (1979). Robust estimation of population size when capture probabilities vary among animals. *Ecology* 60, 927–936. <https://doi.org/10.2307/1936861>.
138. Oksanen, J., Blanchet, G., Friendly, M., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., et al. (2020). *vegan: Community Ecology Package*. R package Version 2.5-7. <http://CRAN.Rproject.org/package=vegan>.
139. National Security Agency (2022). Density Analysis plugin for QGIS 3. Originally written by C Hamilton. Version 2022.9.14. <https://github.com/NationalSecurityAgency/qgis-densityanalysis-plugin>.
140. OBIS (2023). Ocean Biodiversity Information System (Intergovernmental Oceanographic Commission of UNESCO). www.obis.org.
141. GBIF: The Global Biodiversity Information Facility (2023). What is GBIF? <https://www.gbif.org/what-is-gbif>.
142. GenBank (2023). Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1982] - [cited 2023-03-28]. <https://www.ncbi.nlm.nih.gov/genbank/>.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Raw and Processed data compiled from secondary sources (see 'Other'); analysis script	This paper	https://github.com/howlerMoonkey/CCZ_BIODIVERSITY/ ; Data S5
CCZ Checklist	This paper	https://www.marinespecies.org/deepsea/ccz_checklist.php/ ; Data S1
Software and algorithms		
WoRMS (World Register of Deep-Sea Species)	WoRMS ⁷	https://www.marinespecies.org/ ; https://github.com/howlerMoonkey/CCZ_BIODIVERSITY/tree/main/Data-fin
R version 4.0.2 (2020-06-22) "Taking Off Again"	R Development Core Team ¹¹⁷	https://www.r-project.org/
QGIS (Quantum GIS) version 3.10, Coruña (QGIS.org, 2020).	QGIS Development Team ¹¹⁸	https://www.qgis.org/
Other		
DeepData (database of the International Seabed Authority)	N/A	https://data.isa.org.jm/isa/map/
OBIS (Ocean Biodiversity Information System)	OBIS ¹⁴⁰	http://www.obis.org/
GBIF (Global Biodiversity Information Facility)	GBIF ¹⁴¹	https://www.gbif.org/
Published literature	N/A	https://github.com/howlerMoonkey/CCZ_BIODIVERSITY/tree/main/Data-fin
GenBank	GenBank ¹⁴²	https://www.ncbi.nlm.nih.gov/genbank/
BOLD (Barcode of Life)	N/A	https://ibol.org/
PANGAEA	N/A	https://www.pangaea.de/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Muriel Rabone (m.rabone@nhm.ac.uk).

Materials availability

This study did not generate new reagents, sequences or eventuate in the archiving of specimens.

Data and code availability

- This paper primarily analyses existing, publicly available data. The sources are listed in the [key resources table](#) and the datasets are also available in this paper's [supplemental information \(Data S1–S4\)](#). All data generated/utilised in this study are also deposited at GitHub and publicly available as of the date of publication. DOIs are listed in the [key resources table](#).
- All code has been deposited at GitHub and is publicly available as of the date of publication. DOIs are listed in the [key resources table](#). All code is also available in this paper's [supplemental information \(Data S5\)](#).
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

METHOD DETAILS

Data collection, processing and exploratory analysis: Databases

All data were analyzed and processed in R, version 4.0.2 (2020-06-22) "Taking Off Again", R Core Team,¹¹⁷ and Microsoft Excel 365. The R script is included in supplementary ([Data S5](#) and in GitHub (https://github.com/howlerMoonkey/CCZ_BIODIVERSITY/tree/main/R)). All mapping was done in R and in Quantum GIS (QGIS), version 3.10, Coruña (QGIS.org, 2020).¹¹⁸

Biological data were downloaded from the DeepData database web portal (<https://data.isa.org.jm/isa/map/>) on the 12th of July, 2021. The data selection was as follows: 'Layers' tab: 'Mineral Type': 'Polymetallic Nodules', 'Location': 'Clarion Clipperton Fracture Zone', Search tab, 'Biological data', 'Point', and to export the data, 'export query'. The same search procedure was run for 'Trawl

line'. The separate 'Point' and 'Trawl Line' data downloads were combined into the same dataset. Data and column headings varied between the two datasets, e.g. 'actual latitude' in the 'Point' data, and 'start latitude' and 'end latitude' in the 'Trawl Line' data. Data were harmonized, e.g. for coordinates and depth the end-point was used and additional columns added to the 'Point' data to allow the datasets to be combined. Initial data exploration found that the database export did not contain a record identifier. To examine the data, first it was necessary to establish a unique key or record identifier for every individual record (or row of data) in the dataset. A composite key was created to ensure a unique key or identifier for every record by combining the following DeepData identifier fields: 'ContractorID' + 'StationID' + 'SampleID'. The composite key was checked for any duplicates, and none were found. Data columns were checked and edited where necessary, e.g. for depth, missing values were listed as -9, these were replaced with 'NA'. Where possible this was scripted in R, but where multiple entries for character variables were present, this was done in Microsoft Excel 365. Any data point needing cleaning or editing was copied so the original data column and the processed data column were in the same dataset, with the latter renamed with a suffix '_ed'.

Initial examination of taxonomic information found variable recording of data. Taxonomic information was cleaned with the 'taxon-match' tool in WoRMS, a QA/QC function on the web portal where scientific names can be validated against the database. As above, data columns were copied and edits made on the copied column, with spelling and formatting mistakes removed. Taxonomy was mapped to the correct column, e.g. class names in the order column were moved to the class column. No column for scientific name was present, i.e. the actual identification of the specimen referenced in a given record, here a column was added, populated with the lowest taxonomic level reported (i.e. species name if recorded rather than genus name only). If a name was noted with question mark, recorded with a qualifier indicating uncertainty in identification (e.g. *Incerta*) or written as two names, then the next highest taxonomic level was recorded, e.g. if two family names were recorded, the order name was recorded instead. For informal names or open nomenclature designations, scientific name was also recorded, mapped to the lowest scientific level recorded above species level. If a species name was present, e.g. *Paralicella cf. caperesca* no 5, the genus name was recorded for the scientific name. This resulted in a final dataset of 40,518 records for DeepData (https://github.com/howlerMoonkey/CCZ_BIODIVERSITY/blob/main/Data-fin/Data_S6_DeepData.csv).

For contextual spatial data, all mining exploration contract areas, both active and reserved, and Areas of Particular Environmental Interest (APEI) shapefiles were downloaded from the ISA website (<https://www.isa.org.jm/exploration-contracts/maps/>); combined into one shapefile in QGIS version 3.10, Coruña (QGIS.org, 2020). Bathymetric data were sourced from GEBCO (General Bathymetric Chart of the Oceans; <https://www.gebco.net/>). A search area was created covering the entire CCZ region. Coordinates for a polygon covering the CCZ including the combined CCZ shapefile were established with the following coordinates (in decimal degrees): northwest -164.01462, 15.70629; southwest -155.04998-5.51238; southeast -101.9181 6.05623; northeast -117.66088 23.72549 (see R script, [Data S5](#)).

Data were collected from the Ocean Biodiversity Information System (OBIS) and the Global Biodiversity Information Facility (GBIF). OBIS occurrence data were downloaded as a Darwin Core file also on the 12th of July, 2021 using the 'occurrence' function in the *robis* package,¹¹⁹ with the CCZ polygon as delineated above, for all depths. DeepData records have been harvested by OBIS since June 2021 and published on the OBIS ISA node.¹²⁰ These records were analyzed separately in the parallel study, Rabone et al.⁶⁷ to examine ISA data mapping procedures. To avoid duplication of DeepData records across the databases, they were not included in the dataset for analysis (identified as records tagged as owned by the ISA in the Darwin Core 'accessRights' field). GBIF occurrence data were downloaded from the web portal also on the 12th of July, 2021; from all depths, using the polygon search function, with the CCZ polygon coordinates.

All records from GBIF and OBIS were mapped together with the CCZ shapefile, using the following R packages: 'GADMTools'¹²¹; 'sp'¹²²; 'spatialEco'¹²³; 'maptools',¹²⁴ 'rgdal'¹²⁵ and 'rgeos'.¹²⁶ All dataset records were sub-selected by depth, with depths of 3000m and greater included. Some records without depth values were present, those falling within or near the CCZ shapefile were reviewed and included if valid, for example if a benthic species/taxa associated with a publication and a benthic collection method e.g. a box core sample; and/or a relevant reference in 'datasetName' or 'associatedReferences' column. As an additional check to ensure all relevant benthic records were selected and pelagic records removed, the scientific names recorded were cross-referenced to habitat information recorded in WoRMS (the World Register of Marine Species).⁴⁵ Following record selection by depth, datasets were remapped. The data selection by depth resulted in a significant reduction in records, with all records at depth falling within contract areas/APEIs or close by. The latter records falling outside the CCZ shapefile were reviewed to check all relevant records were captured. In the final data selection, all non-metazoan and fossil records were excluded from datasets. This resulted in a final dataset of 2185 records for OBIS (https://github.com/howlerMoonkey/CCZ_BIODIVERSITY/blob/main/Data-fin/Data_S7_OBIS.csv) and 2405 records for GBIF (https://github.com/howlerMoonkey/CCZ_BIODIVERSITY/blob/main/Data-fin/Data_S8_GBIF.csv).

Published literature

A systematic review of the literature was undertaken; the *a priori* research question being: *what taxonomic information is available for benthic metazoans in the CCZ?* Online databases were searched for publications through the Natural History Museum (NHM) London library, including Google Scholar, Scopus, ScienceDirect, and Web of Science. Citations were checked, and any additional relevant publications identified were included. References of all papers were systematically checked and added if they also had not previously been identified through database searches. The literature review was carried out primarily from 2nd February to 20th August 2021, with the date of last search the 1st of January, 2023. Search terms included: "Clarion Clipperton (Fracture) Zone"; "Central"/"East"/"Pacific"; new"/"species"/"genera"/"genus"/"family"/"description"; "biodiversity"; "megafauna"; "macrofauna"; "meiofauna";

“community”/“composition”; “species”/“assemblages”; “taxonomic studies”; “ecology”; “genetic”; “genomic”; “polymetallic”/“manganese”/“nodule”. Within-journal searches were also conducted for key journals (e.g. Zootaxa) using the same search terms. Criteria for inclusion were publications with records of benthic metazoan taxa.

Publications in all languages were included in the review. The search was not confined to lower taxonomic ranks, with all records included regardless of the level of taxonomic resolution. Publications without any taxonomic records were excluded. Any publications solely examining pelagic taxa, microbes, or foraminifera (e.g. xenophyophores) were similarly excluded (https://github.com/howlerMoonkey/CCZ_BIODIVERSITY/blob/main/Data-fin/Data_S9A_lit_papers.csv). Records identified from imagery, e.g. ROV or AUV (Autonomous Underwater Vehicle) seabed surveys were included, i.e. the selection was not restricted to specimen-based studies. All size classes of metazoans were included. Definition of size fractions were as follows: meiofauna: animals being retained on a 63µm sieve, macrofauna, on a 250–300µm sieve, and megafauna, as 2cm and larger. Relevant information, for example taxonomic and sampling information was captured and compiled into a table (https://github.com/howlerMoonkey/CCZ_BIODIVERSITY/blob/main/Data-fin/Data_S9B_lit_taxa.csv). Type localities for species described from the CCZ were collated from the literature, with coordinates recorded verbatim and converted to decimal format. Datasets associated with publications were also sourced from PANGAEA, incorporated into the literature dataset. The final literature dataset with georeferenced records included 57,858 records (https://github.com/howlerMoonkey/CCZ_BIODIVERSITY/blob/main/Data-fin/Data_S9C_lit_site%2Btaxa.csv). Taxonomic information was cleaned with the ‘taxon-match’ tool in WoRMS. Identification qualifiers were standardized according to guidelines in the literature, e.g. n sp. recorded as sp. nov.^{4,5} For records held on International Nucleotide Sequence Database Collaboration (INSDC) databases (primarily GenBank) and BOLD, accession numbers were collated from publications, either direct from publications or supplementary files, and where key information was missing, e.g. marker, the databases themselves were cross-referenced. This resulted in a data file of 4738 records from GenBank and 1674 records from BOLD (https://github.com/howlerMoonkey/CCZ_BIODIVERSITY/blob/main/Data-fin/Data_S10_INSDC.csv).

The CCZ Checklist: Known/named species

All scientific names were collated from the different data sources to create a checklist of known benthic metazoan taxa recorded from the region, here referenced as the ‘CCZ Checklist’. For unnamed/undescribed species, scientific name was also added, mapped to the lowest scientific level recorded above species. Names were recorded at their taxonomic level, and post taxon-match to WoRMS, any higher taxonomic names not present were inferred, e.g. for species records present, the genus name was added if it was not already separately recorded. Any unaccepted names were replaced with accepted names, and where names were not found in WoRMS, this was recorded and the original name retained (seven in total including four species, not included in final totals). Where names were only present in one data source, the origin of the records were reviewed. A taxon match to WoRDSS,⁴⁴ the deep-sea node of WoRMS, was also performed to ascertain if taxa were already logged as deep-sea using the Flanders Marine Institute VLIZ web-services (<https://www.lifewatch.be/data-services/>).

Suspected potential pelagic taxa records e.g. Calanoida and Cyclopoida copepods; ostracods; hyperiid amphipods and Tomopteridae polychaetes were present. These groups were assessed by specialists, Geoff Boxshall and Pedro Martinez for Copepoda; Simone Brandao for Ostracoda; author TH for Amphipoda and authors TD, HW and AGG for Polychaeta. Other potential pelagic taxa were identified by cross-referencing ‘attribute’ information in WoRMS where available. After these assessments, any pelagic names identified were removed from the final total, and all records of pelagic species/taxa were removed for the diversity analysis. These names were retained in the main dataset for wider reference (tagged as pelagic). Any name with a known degree of taxonomic uncertainty was noted and removed from the CCZ Checklist *sensu stricto*, e.g. a name interpolated from an imagery record, from a morphospecies/temporary/informal name or with any of the following qualifiers: aff.; cf.; indet; *Incerta* (or *incertae sedis* used as proxy for *Incerta*); and/or sp. inc. (any records with the qualifier sp. nov were included at species level, or gen. nov at genus level). Species identified to be likely misidentifications (e.g. *Eurythenes gryllus*, *Valettieta gracilis*) were also tagged and as for pelagic taxa, removed for analysis and overall totals. In total, 656 names were tagged to be excluded, but were retained in the whole dataset (tab 2 of [Data S1](#)).

For the two major faunal groups, the arthropods (tanaids and isopods) and the annelids (polychaetes), the names were assessed by authors TH and TD respectively to gauge the level of potential misidentification in key groups. Here the type locality (including depth) was determined from the original description where available, otherwise the record was checked on WoRMS. For the main macrofaunal groups, any species described using specimens from the CCZ but a holotype originating from elsewhere were recorded as such in the Checklist. Life-history and functional traits such as key habitat association (e.g. sediment-dwelling) and feeding type were recorded from the literature where present. The checklist was prepared for publication as a webpage in WoRDSS to allow data to be FAIR.¹²⁷ A landing page was prepared by the WoRMS Secretariat in consultation with authors TH and MER in February–April 2023, available at (https://www.marinespecies.org/deepsea/ccz_checklist.php).

Unnamed species/informal name species list

A separate list of ‘open nomenclature’ informal names for the unnamed species was collated for analysis ([Data S2](#)). Open nomenclature is a system of signs to describe uncertainty around identifications, or designate taxa as undescribed.^{4,5} Temporary names are also termed ‘morphospecies’, ‘informal names’, ‘undescribed species’, ‘working species’, morphotypes’ or ‘molecular/operational taxonomic units’ (M/OTUs).^{4,5} These terms differ slightly conceptually, for example morphotype generally refers to a temporary name given to megafauna identified solely from imagery (e.g. ROV footage), and MOTUs to taxa have been discriminated to species

level by comparison of genetic sequence data (also see List of Abbreviations and Terms in [supplemental information](#)). For consistency, here we use the term ‘unnamed species’ a term to delineate undescribed species but which allows for the inclusion of uncertainty in identification, or the possibility some of these unnamed species may be known species yet to be correctly identified. This term is equivalent to ‘taxonConceptID’ in Darwin Core⁴ (<https://dwc.tdwg.org/terms/#dwc:taxonConceptID>). Names recorded specifically as ‘undescribed new species/genera’ were distinguished as such in the unnamed species list ([Data S2](#)). Species recorded with open nomenclature identification qualifiers, i.e. cf. and aff. were included. These records were recorded at genus level only for scientific name and tagged as ‘open nomenclature’, with the verbatim species name recorded in the Darwin Core term ‘taxonConceptID’. Any duplicates of names across data sources e.g. identical names were identified by cross referencing and removed. Also, text string analysis showed very similar names published at different times and identified to have originated from the same dataset but with slightly different formatting (e.g. underscores added or removed). These were also removed as duplicates (all retained in the full dataset). Synonyms within the unnamed species list (sensu synonyms in scientific names i.e. multiple names for the same species) were also identified through cross-referencing publications and databases. These were tagged as synonyms in the main dataset and removed from totals ([Data S2](#)).

QUANTIFICATION AND STATISTICAL ANALYSIS

Diversity estimates

Records at all taxonomic levels from all data sources, DeepData, the literature (including those harvested from PANGAEA), OBIS and GBIF that included either abundance and/or sampling information were compiled into a dataset for diversity analysis ([Data S3](#) and [S4](#)). Any duplicates identified within and across data sources were removed, for example records from the same dataset present both in the literature and in GBIF, or duplication of datasets present within the DeepData database.⁶⁷ The species-level data included named and unnamed species. In total, 27 of the informal names were specified as higher taxa, e.g. an undescribed genus name. These names were included in the species-level dataset given that any unnamed higher taxa record would also represent a new unnamed species. All synonyms in the informal names identified as above via cross-referencing were removed for analysis to avoid inflation of estimates. This resulted in a final dataset for diversity analysis of 91,996 records at all taxonomic levels and 66,679 at species level only ([Data S4](#)).

For alpha diversity, simple metrics of diversity, total number of new species, by region, size class and contract area/APEI were estimated. Abundance species matrix tables were generated with the R package *picante*¹²⁸ ([Data S3](#)) and presence/absence species matrix tables with *FuzzySim*.¹²⁹ Singletons, or species represented by a sole specimen for a given deployment were calculated from the abundance dataset. A broad regional classification of east, central and west was added to the final datasets, with breakpoints at -128 , and -140 degrees longitude for east to central and central to west respectively. Broad patterns in species diversity and distribution at regional level were visualized with UpSet plots.¹³⁰ These plots allow visualization of multiple sets in the data, such as species richness intersections with region.¹³³

Non-parametric estimators were used to estimate total species diversity for all size classes at a regional scale, i.e. for the entire CCZ region. Hill numbers for rarefied taxa richness ($q = 0$)¹³¹ were estimated in *iNEXT*.¹³² Rarefaction/extrapolation both for Chao2^{133,134} incidence-based by sample and Chao1 abundance-based was performed in *iNEXT*^{135,136} and plotted using *ggiNEXT* function ([Figure 5](#)). Sample/incidence-based species richness estimates, Chao2, and first and second order jackknife¹³⁷ were also conducted using the *specpool* function in *vegan*.¹³⁸ Abundance-based richness estimates, Chao1 and the Abundance-based Coverage Estimator ACE^{65,66} were also conducted in *vegan*, using the *estimateR* function. Rarefaction for all species CCZ-wide were estimated in *vegan* using the *rarefy* and *rarecurve* functions. Species accumulation curves were plotted (with sampling effort defined as number of sampling events), with 1000 randomizations, using the *specaccum* function in *vegan* ([Figure S1](#)). Plots of sampling completeness (type = 2) were also performed in *iNEXT* ([Figure S3](#)).

Since taxonomic uncertainty is lower at higher taxonomic ranks, with less likelihood of synonyms and misidentifications (and likely higher proportional sampling completeness) than at species level, richness estimates (Chao1 and Chao2) at genus and family level were performed ([Figures 5](#), [S1](#), and [S2](#)). This also allowed diversity estimates at higher taxonomic levels to be compared to the known totals of taxa in the CCZ Checklist (i.e. total families/genera recorded). Estimates were based on subsets of the data where abundance and/or site-sampling information was recorded, while the Checklist was based on all records deemed valid post qa/qc as above.

To account for potential synonymies and misidentifications in the unnamed species, an analysis of recent molecular and morphology-based taxonomic studies that provided estimates of the numbers of species taxa was carried out. For the analysis assessing proportion of named to unnamed/undescribed species in the literature (the ‘subset’ analysis), publications were selected where a taxonomic group (or groups) were assessed in totality, rather than a taxonomic description of a new species, and where all taxa were identified to species level (named and unnamed); based on morphology and/or molecular approaches (publications where identifications were based on imagery were therefore excluded, [Table S1](#)).

Assessment of regional-scale sampling effort

A broad assessment of CCZ-wide sampling effort was also conducted to visualize sampling coverage and particular data gaps. Sampling effort was visualized as a heatmap of unique sampling sites using the Density Analysis plugin in QGIS, based on the combined all-taxa dataset¹³⁹ ([Figure S4](#)). To visually assess sampling coverage and sampling gaps or under-sampling by depth, density of

records by depth was computed in R to visualize sampling effort by depth, by subdividing data into 10 sample quantiles (Figure S5). Total sample records by contract area/APEI and depth were also plotted to visualize differences by contract area, given the known depth gradient in the CCZ (Figure S6).⁸⁴

Comparison of CCZ Checklist with global checklists

To provide a degree of global context to CCZ biodiversity as currently recorded, the proportion of named to unnamed species was compared to published estimates of global marine species diversity versus the current recorded total of known global marine (eukaryotic) species currently in WoRMS (241,129).⁴⁴ Relevant literature was searched to identify estimates and any assessments of their accuracy. No global estimates of deep-sea species richness published to date were identified in the search, therefore global marine species richness estimates were examined. Estimates from Mora et al.⁵⁷ and Appeltans et al.⁵⁸ were primarily used on basis of analysis by Poore et al.¹⁰¹ To examine taxonomic composition of the Checklist in relation to global datasets, data were requested from WoRMS and a database copy of WoRDSS⁴⁴ was provided from the 1st of January, 2023 and archived on GitHub (https://github.com/howlerMoonkey/CCZ_BIODIVERSITY/blob/main/Data-fin/Data_S11_WoRDSS.csv). Non-metazoans were removed from the dataset and relative proportions of species by phyla were calculated and plotted to compare the CCZ to all deep-sea metazoan species recorded to date.

ADDITIONAL RESOURCES

The CCZ Checklist created in this study is published as a webpage, available via the World Register of Deep-Sea Species WoRDSS,⁴⁴ subregister of the World Register of Marine Species (WoRMS)⁴⁵ at https://www.marinespecies.org/deepsea/ccz_checklist.php.