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BCBC2020-26: Octocoral bycatch diversity on the Chatham Rise

Draft Final Report

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Executive summary

The deep seas of Aotearoa/New Zealand harbour diverse and abundant communities of branched gorgonian corals, which are associated with demersal fish and invertebrates, including commercially significant fisheries that are targeted by bottom trawling. The incidental contact of fishing gear with arborescent gorgonian colonies causes damage and entanglement, potentially resulting in corals being brought to the surface when trawls are retrieved, as bycatch. The extent and severity of gear interactions with coral communities resulted in their protection under a 2010 amendment to the Wildlife Act, but encounters have continued, prompting further studies on the spatial correspondence of corals and fishing activity, plus coral recoverability following such disturbance. Although impacts on coral ecosystems and coral biomass have been documented, the extent of species-level diversity affected by bottom trawling is less understood, especially for gorgonian corals. This is in part due to their diverse and highly variable growth forms, which makes visual identification difficult and prone to error.

This study expanded upon a previous examination of gorgonian coral bycatch across the NZ EEZ by focusing on a single family – the Primnoidae (‘bottlebrush’ corals) – in a more spatially explicit region with relevance to commercial fisheries – the Chatham Rise. Samples of primnoid corals were obtained from the NIWA Invertebrate Collection, with their origins in a mixture of commercial fisheries bycatch sampled by fisheries observers, NIWA fisheries research trawl bycatch (incidental catch during fisheries assessment cruises for QMS target species), and samples collected during NIWA biodiversity research cruises using tows of epibenthic sleds. Genetic barcoding was used to objectively identify the number of unique taxa present and a subset of samples were subjected to high-resolution genomic sequencing, to examine the benefits of recent technological advancements and to determine if cryptic species might be overlooked by more traditional barcoding approaches.

One-hundred-and-fifty primnoid samples were chosen for DNA sequencing and 122 of these produced viable results for at least one of three genetic markers. A genealogical analysis indicated that 13 distinct primnoid taxa were present, along with representatives of two other cryptic gorgonian families that were originally misidentified as Primnoidae. One of these two was tentatively identified as the Pleurogorgiidae and, if correct, would represent a first record for this family in New Zealand and may require addition to protection measures under the Wildlife Act. The 13 primnoid taxa were comparable to 15 previous published records for the Chatham Rise and in combination suggests that the full extent of primnoid diversity on the Chatham Rise is at least 17 species in total.

Samples originating from commercial and research bycatch were too few and broadly dispersed among target fishery species to perform numerical comparisons of diversity by sampling method or target fishery. However, it is noteworthy that among the updated list of species of Primnoidae documented on the Chatham Rise, 65% are documented as fisheries bycatch despite these samples representing less than 30% of the total sample size for this study. These results provide a baseline for bottom-trawling impacts on the diversity of a widespread and ecologically important family of protected gorgonian corals in New Zealand.

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1 Background

The deep seas of Aotearoa/New Zealand harbour diverse and abundant communities of cnidarian corals, which include hydrocorals (Class Hydrozoa: Family Stylasteridae), stony corals (Class Anthozoa: Order Scleractinia), black corals (Class Anthozoa: Order Antipatharia) and both soft and gorgonian octocorals (Class Anthozoa: Subclass Octocorallia). The upright, branching growth form of many of these species increases rugosity and topographic relief of their epibenthic habitat, which provides refuge for demersal fish and invertebrate communities (Husebø et al. 2002; Buhl-Mortenson & Mortensen 2005; Milligan et al. 2016). This association of commercially important fish and shellfish with coral communities has resulted in disturbance from contact with fishing gear – particularly deep-sea trawling on seamounts and slopes (Clark et al. 2016, Yoklavich et al. 2018). The extent and severity of these interactions are ecologically significant (Clark et al. 2016) and can result in long-term reductions of coral biomass and impacts to coral-associated fauna (Clark et al. 2019). These effects prompted the New Zealand Department of Conservation to list arborescent coral groups (black and stony corals, hydrocorals and gorgonian corals) in a 2010 amendment to Schedule 7A of the Wildlife Act 1953.

The impacts and outcomes of gear interactions with coral communities have been well documented in terms of spatial overlap (Tracey et al. 2011), spatial extents (Anderson et al. 2020), impacts on community biodiversity (Anderson & Clark 2003; Anderson et al. 2017; Bowden & Leduc 2017), and long-term recoverability (Clark et al. 2019). However, examinations of the impacts on particular coral species are less common, as they require reliable identification of bycatch photographs and infrequent sampling of damaged, fragmentary specimens – both of which are collected and recorded by Government Fisheries Observers (hereafter 'observers') aboard deep-sea fishing vessels. Observer records of coral bycatch taxa are known to be prone to error due to the high difficulty associated with non-expert identification of highly variable and similar-looking coral species, which is typically conducted in challenging and dynamic operational situations aboard commercial vessels underway at sea. However, although identification reliability is improved by examination of images and specimens by taxonomic specialists and trained para-taxonomists (Tracey et al. 2019), errors can still persist for cryptic and highly plastic groups of corals, especially black corals (Bilewitch & Tracey 2020a) and gorgonian octocorals (Bilewitch & Tracey 2020b).

A previous report by NIWA for the Conservation Services Programme (Bilewitch & Tracey 2020b) examined the extent of bottom-trawling impacts on species-level diversity of gorgonian corals within the New Zealand EEZ, using bycatch samples that had been collected by observers and submitted to the NIWA Invertebrate Collection (NIC) for identification and archiving under project INT2019-04 (Tracey et al. 2019). Due to the highly cryptic and diverse nature of gorgonian octocorals, plus a lack of taxonomic descriptions for many of the species found in New Zealand, genetic barcoding at three markers (loci) was used as an efficient and objective means to identify and delineate a broad range of species. Among the 62 specimens that produced DNA sequence data, 34 different species were delineated among five octocoral families. Specimens of each family were widely distributed across the NZ EEZ and the majority of specimens originated from the Orange Roughy trawl fishery. Taxon discovery curves for the 62 analysed specimens indicated that the full extent of diversity in the bycatch community had not been sampled. Additionally, stakeholder feedback suggested that knowledge of the extent of natural octocoral diversity would be beneficial, for a baseline context in which trawling-related bycatch diversity can be placed.

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In consultation with the Marine Species Team at the Department of Conservation, the current project was designed to expand on previous efforts to document octocoral bycatch by attempting to delineate the full extent of natural diversity present within a spatially explicit area. The protected octocoral family Primnoidae (bottlebrush gorgonian corals) was chosen since it has the highest number of specimens available in the NIC from bycatch collections and is well-represented in research expedition collections. The Primnoidae in the NIC have also been extensively studied and identified by an expert taxonomist, which has resulted in three successive monographs (Cairns 2012, Cairns 2016, Cairns 2021). The Chatham Rise was chosen as a study area due to its relevance to New Zealand fishing activities and because it has the highest number of primnoid samples available for study. The Rise and associated seamounts have also been the subject of several research expeditions and trawl surveys by NIWA, which have also significantly contributed to available NIC specimens.

A collection of Primnoidae from the Chatham Rise was identified from the NIC and genetic barcoding at three loci was used to delineate taxa, as an attempt to avoid subjectivity of morphological identification, to identify cryptic species, and to contribute to the development of a reference genetic dataset describing the extent of diversity among New Zealand octocorals. In addition to using the genetic data to estimate the total number of primnoid species on the Chatham Rise, the dataset was also partitioned according to sample 'collection' method, to examine differences in catchability or diversity coverage of different collection 'methods. Differences in taxonomic coverage were compared for samples from commercial bycatch (predominantly bottom trawling), NIWA fisheries research trawls, and targeted NIWA surveys using epibenthic sleds. Although the fishery target species for bycaught and fisheries research trawl specimens was obtained, an analysis of diversity by fishery was not undertaken due to low and uneven sample sizes.

Although traditional DNA sequencing (Sanger sequencing) methods have been used to identify and describe octocoral species for over 20 years, there is still a lack of suitable markers for the consistent and precise delineation of closely related species. This was observed in the previous study of bycatch diversity (Bilewitch & Tracey 2020b), where for some groups it was difficult to distinguish genus-level variation from species-level variation (*e.g.* among the Isididae), and variation within a species from variation between cryptic or mis-identified species (*e.g. Paragorgia arborea*). Advances in phylogenomic methods (using genome-scale data to determine genetic relationships of species) combined with decreasing costs of genomic sequencing have resulted in new methods that are capable of producing millions of bytes of sequencing data at a cost that is significantly less than Sanger sequencing on a dollar-per-base-pair basis. For octocorals, this has been demonstrated with the application of genomic enrichment and sequencing of Ultra-Conserved Elements (UCEs) – thousands of targeted sections of the genome that are relatively conserved among a group of organisms, but which possess informative variation in adjacent regions that is collectively capable of determining genealogical relationships (Quattrini et al. 2017). Genomic DNA is enriched for these UCE loci, which results in a higher density of comparative genomic data for genealogical reconstructions when samples are subjected to shotgun genome sequencing (Faircloth et al. 2012). Whereas traditional DNA sequencing produces hundreds of basepairs of informative genetic variation, UCE sequencing produces thousands or tens-of-thousands of variable loci, producing phylogenetic trees at a much higher resolution (Quattrini et al. 2019). In the current study, UCE sequencing of a subset of primnoid samples explored the usefulness of this new technique for documenting and delineating coral diversity and as a means to test if traditional sequencing of three loci was sufficient to detect cryptic species.

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2 Methods

2.1 Selection of study material

Specimens of the Primnoidae from the Chatham Rise that are archived within the NIC were selected for DNA sequencing using a restrictive query of the *niwainvert* collections database. Specimens were chosen wherever they were identified as Primnoidae, collected since 1990, and preserved in ethanol (or alcohol). These query results were mapped onto benthic topographic layers in QGIS v3.10.4 (QGIS Development Team 2020), based on their reported GPS coordinates for collection locality, and specimens originating from the Chatham Rise were selected for further study. Specimens were then broadly categorised according to their collection method: commercial fishery trawl bycatch, research trawl bycatch, or targeted research collection via epibenthic or hyperbenthic sleds (Figure 2-1). The taxonomic distribution of this resulting list was examined and 150 specimens representing the breadth of diversity (based on pre-existing identifications) were selected for sampling and genetic analysis.

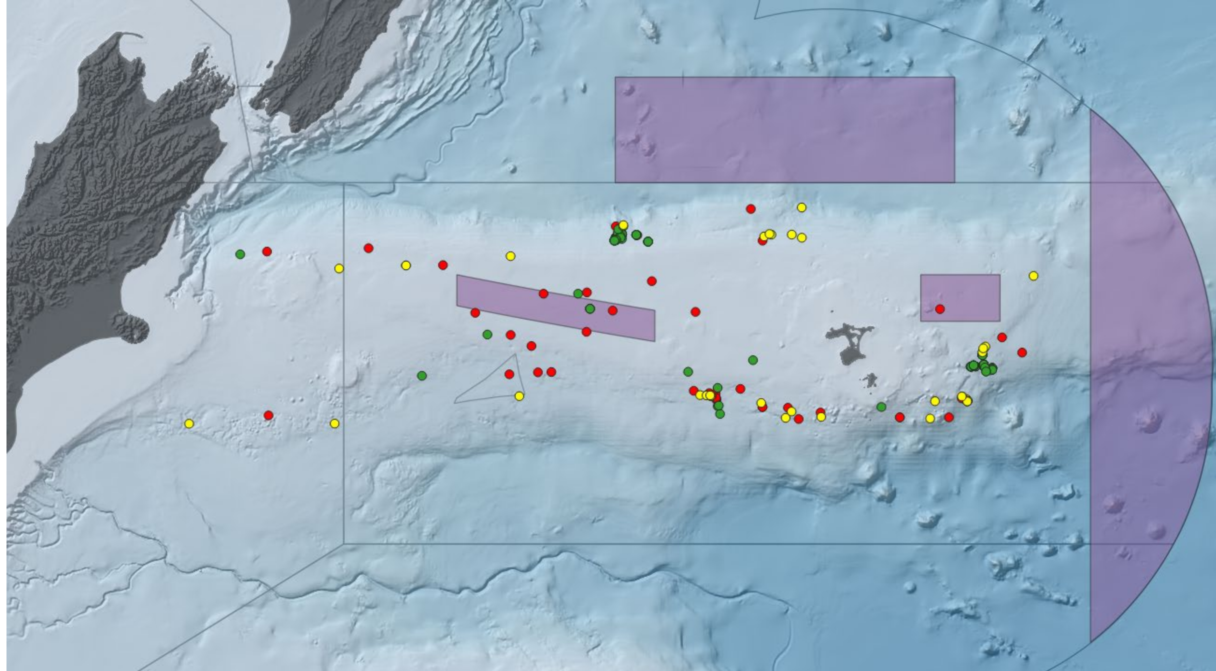

Figure 2-1: Sample availability across Chatham Rise. Samples identified as being suitable for genetic analysis were grouped according the method by which they were collected: commercial fisheries bycatch (yellow), NIWA fisheries research trawl (red), or NIWA biodiversity research sled (green).

2.2 Genetic barcoding

The chosen primnoid specimens were located within the NIC and approximately 10mg of tissue was removed for DNA extraction. Tissue samples were soaked in sterile water to remove trace ethanol prior to genomic DNA (gDNA) extraction with a DNeasy Blood & Tissue kit (Qiagen Inc.). DNA extractions followed the manufacturer's recommended protocol except that incubations in proteinase K were conducted overnight and two volumes of 40ul of AE buffer were used for a final elution, to increase gDNA concentration.

Three loci were chosen for PCR-amplification based on their efficacy in delineating octocoral taxa in a previous study (Bilewitch & Tracey 2020b), as well as their use in other studies of primnoid diversity

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(Cairns & Wirshing 2018). Two regions of the mitochondrial *mtMutS* gene (the 5'-end and a section of the domain III region near the 3'-end) and a portion of the *28S* ribosomal DNA unit were amplified in 25ul total reaction volumes using 1X MyTaq RedMix (Bioline Inc.), 0.5uM of each primer pair (Table 2-1) and 3-9ul of gDNA extract. Conditions for all three loci used a thermocycling profile of 95°C for 3 min, followed by 35 cycles of 95°C for 15 s, 51°C for 20 s and 72°C for 25s, with a final extension of 72°C for 2 min. Amplification products were visualised on a 1% agarose gel and successful reactions were purified using 1 unit of ExoSAP-IT (ThermoFisher Sci. Inc.) following the manufacturer's recommendations, prior to submission to a commercial facility for Sanger DNA sequencing (Macrogen Inc.) in both forward and reverse directions.

Table 2-1: Loci targeted for DNA sequencing. For each locus sequenced in the current study, the corresponding primer pair and their origin are provided.

Locus	Primers	Reference
5'-mtMutS mtDNA	AnthoCorMSH: AGGAGAATTYTAAGTATGG	Modified from Herrera et al. 2010
	Mut-3458R: TGRAGCAAAAGCCACTCC	Modified from Sánchez et al. 2003
3'-mtMutS mtDNA	mtMutS-DIII_IntF: TCTTTACATCGTCAATGGGCAAT	CITE BYCATCH PAPER
	mtMutS-DV_R: AAACTAATATYATGAGCTACACATTCT	Bilewitch et al. 2014
28S rDNA	28S_F: CACGAGACCGATAGCGAA	McFadden & van Ofwegen 2012
	28S_R: TCGCTACGAGCTTCCACCAGTGTTT	McFadden & van Ofwegen 2012

The resulting DNA sequences were visually inspected for quality and were trimmed and assembled in Geneious Prime v2021.1.1 (Biomatters Ltd.). Sequences were submitted to the BLASTn server of GenBank (https://blast.ncbi.nlm.nih.gov/), to ensure they did not represent contaminant organisms, and were then aligned by locus using MAFFT v7.450 (Katoh & Standley 2013). The resulting alignments were manually inspected and adjusted where necessary and were then submitted for phylogenetic tree building using MrBayes v3.2.6 (Huelsenbeck & Ronquist 2001). Bayesian model parameters used a GTR+G model of distance correction, 10^7 MCMC generations sampled at 10^3 intervals, with 10^5 generations discarded as burn-in. Resulting trees were outgroup-rooted and examined for concordance between each locus, prior to concatenating all loci in a repeated Bayesian phylogenetic analysis, using identical parameters but in a locus-partitioned model. The posterior output of all model runs was examined for parameter convergence and effective sample size of parameter estimates.

2.3 Sequencing of Ultra-Conserved Elements (UCEs)

Preliminary phylogenetic results from the genetic barcoding of three loci were used to select twelve samples that represented the breadth of observed primnoid diversity, as well as groups of multiple specimens that lacked any genetic difference but could contain cryptic variation. Specimens 65546 - *Calyptrophora*, 53305 - *Primnoa*, 54329 - *Narella*, 102463 - *Thouarella*, 25426 – *Tokoprymno*, 53275 – *Tokoprymno*, 28746 – *Dasystenella*, 102402 – *Thouarella*, 128287 – *Thouarella*, 91997 – *Thouarella*, 102298 – *Metafannyella*, and 66289 – *Metafannyella* were selected for UCE sequencing. The concentration of gDNA in these samples was quantified using a Quant-iT Picogreen dsDNA kit (Invitrogen Inc.) and extracts were dried down for shipping to Daicel Arbor Biosciences (USA) for further quality control, target bait enrichment and sequencing via their *myReads* and *myBaits-Custom* service. A Calcaxonia-specific bait-set was used (developed in Untiedt et al. 2021) to focus target enrichment on intra-familial relationships of the Primnoidae as much as possible. Sample

D
R
A
F
T

libraries were dual-indexed and sequenced on a partial flowcell of an Illumina NovaSeq 6000 in S4 PE150 mode using v1.5 chemistry.

<<<The resulting UCE sequence data is still undergoing analysis due to delays in delivery of the sequencing results by a commercial subcontractor, thus methodology on UCE data processing and analysis is pending and will be presented in the final report.>>>

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3 Results

3.1 Sanger DNA sequencing of three target loci

A query of *niwainvert* for ethanol-preserved primnoids from Chatham Rise that were collected since 1990 yielded 209 records. Of these, 14 specimens could not be located or were not suitable for sampling due to their small size or lack of tissue. An additional 46 specimens were not sampled as they were taxa that were represented by other sampled specimens, and 27 specimens produced no viable results, despite repeated attempts at DNA extraction and PCR amplification. The remaining 122 specimens produced DNA sequence data at one (n=25), two (n=63) or three (n=34) loci. After trimming and alignment, the 5'-end of *mtMutS* consisted of 786bp, the Domain-III region of *mtMutS* consisted of 792bp and the *28S*-rDNA locus had 716bp. The Domain-III region of *mtMutS* produced the highest number of successful sequences (114 specimens), followed by the 5'-end of *mtMutS* (104) and then *28S*-rDNA (54). However, BLASTn-queries of sequences from the *28S* locus indicated that its amplification was plagued by contamination from both non-coral sources (fungi, sponges) and non-primnoid octocorals (often soft coral or stoloniferan sequences; data not shown). Similar results were not seen in BLAST results for the two *mtMutS* loci, indicating that the *28S* primers had low fidelity for octocoral amplification. This cross-amplification resulted in only 35 sequences of *28S* being reliably ascribed to the specimens from which they were amplified, which rendered the nuclear *28S* dataset less useful for phylogenetic analysis of primnoid diversity, as compared to the more-replete mitochondrial loci.

Phylogenetic analyses of the individual *mtMutS* loci produced results that were largely congruent, except for minor discrepancies in closely related taxa. A combined phylogenetic analysis of the 122 primnoid specimens from the Chatham Rise is given in Figure 3-1, which resolved the specimens into 15 distinct taxa in total. Thirteen specimens were determined to be non-primnoid taxa (*i.e.* specimens originally misidentified as primnoids), which was confirmed through visual inspection of physical specimens. Six of these belonged to the Plexauridae and seven to the Pleurogorgiidae (a family which previously has not been recorded from New Zealand). The identities of the remaining 89 primnoid specimens were ascribed to 13 OTUs through a comparison of their expert taxonomic identification to their phylogenetic relationship to reference primnoid sequences obtained from GenBank. The resulting taxonomic list is presented in Table 3-1 as a comparison of original morphology-based identity to a revised identity based on genetic evidence.

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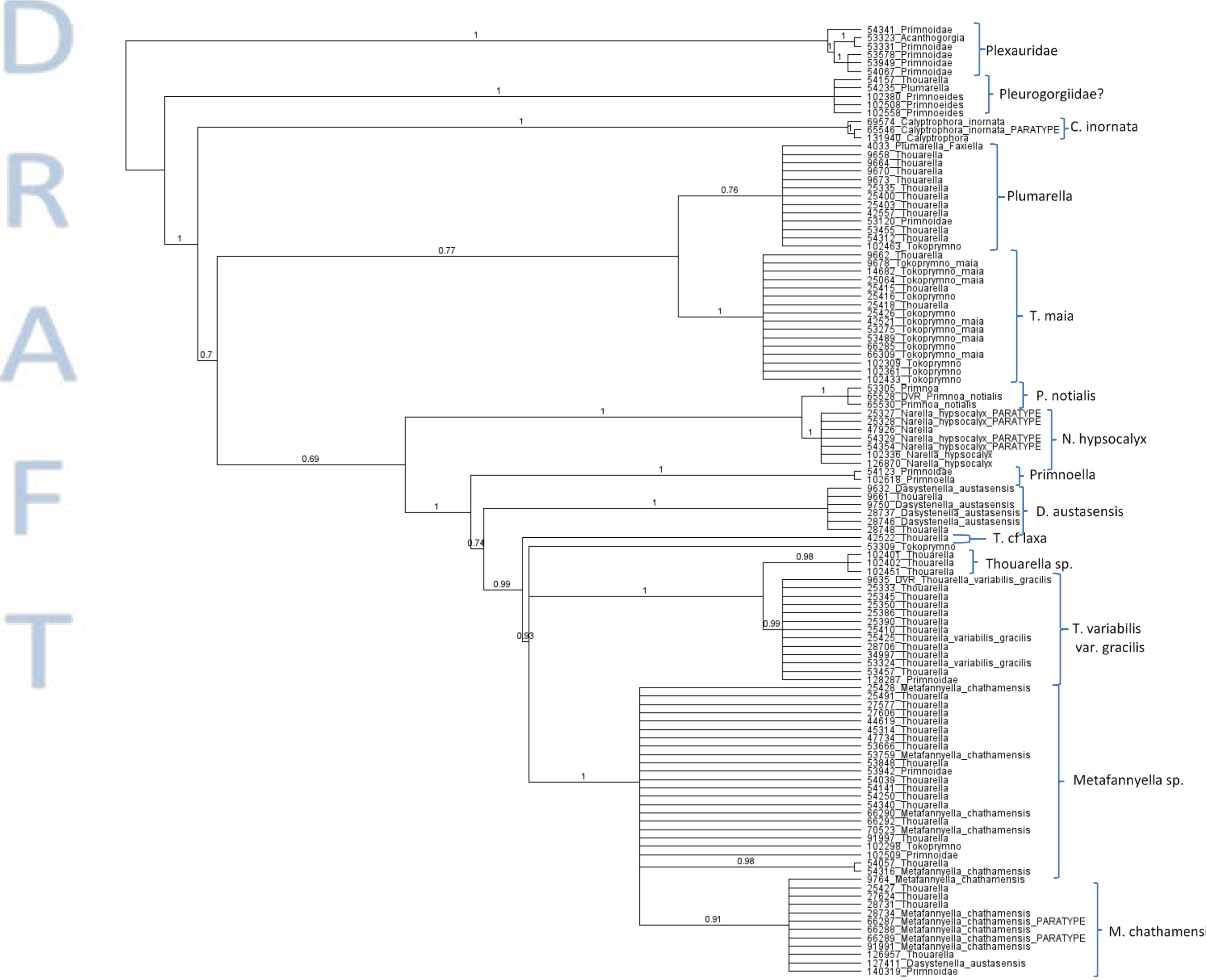

Figure 3-1: Phylogenetic relationships of sampled Primnoidae from the Chatham Rise. Bayesian phylogeny of sequenced specimens at two *mtMutS* loci produced using 10^7 MCMC generations, sampled at 10^3 intervals with 10% discarded as burn-in. Branch labels are posterior probability support values. Putative taxon names next to bracketed taxa were derived from comparisons to reference sequences from Cairns & Wirshing (2018) and Taylor & Rogers (2015). Names for individual sequences are the original morphological identifications given in the *niwainvert* database.

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Table 3-1: Primnoid taxa confirmed by DNA sequencing. A list of putative taxa from the Chatham Rise, identified via molecular systematic comparison to reference sequences. The methods from which sequenced specimens originated and the original specimen identifications (via morphological examination) are also given.

Molecular Systematic Identification	Comm. Bycatch	Res. Bycatch	Res. Sled	Original Identifications
Calyptrophora inornata	Y	-	-	*C. inornata*
Dasystenella austasensis	-	Y	Y	*D. austasensis, Thouarella* sp.
Metafannyella sp.	Y	Y	Y	*Metafannyella* sp., *M. chathamensis, Primnoidae, Thouarella* sp., *Tokoprymno* sp.
Metafannyella chathamensis	Y	Y	Y	*M. chathamensis, Thouarella* sp.
Narella hypsocalyx	Y	Y	Y	*Narella* sp., *N. hypsocalyx*
Plumarella (Faxiella) sp.	Y	-	Y	*Plumarella (Faxiella)* sp., *Primnoidae, Thouarella* sp., *Tokoprymno* sp.
Primnoa notialis	Y	-	Y	*Primnoa* sp., *P. notialis*
Primnoella sp.	-	-	Y	*Primnoella* sp.
Primnoella insularis	-	-	Y	*Primnoidae*
Thouarella sp.	-	-	Y	*D. austasensis, Thouarella* sp.
Thouarella cf. *laxa*	Y	-	Y	*Thouarella* sp., *Tokoprymno* sp.
Thouarella variabilis var. *gracilis*	Y	Y	Y	*M. chathamensis, Primnoidae, Thouarella, T. variabilis* var. *gracilis*
Tokoprymno maia	Y	-	Y	*Thouarella* sp., *Tokoprymno* sp., *T. maia*

3.2 UCE-sequencing of selected specimens

<<< This section is pending completion of UCE analysis and will be presented in the final report >>>

3.3 Diversity patterns

Molecular systematic analysis of the Sanger-DNA sequencing dataset indicated a minimum of thirteen primnoid species were present across the Chatham Rise (Figure 3-1, Table 3-1). The distribution of sequenced samples covered a depth range from 243m to 1436m, with a mean depth of 848m. Nineteen of the DNA-sequenced specimens originated as bycatch from commercial fisheries, 17 originated as bycatch from NIWA fisheries research trawls, and 86 were collected by benthic sled samplers during NIWA research voyages. Among the commercial bycatch specimens, eight originated from orange roughy bottom trawling events, one from scampi, and the remainder were not confidently assigned to a target fishery species. For the research trawl specimens, eight originated from trawls targeting hoki, five from oreo, two from orange roughy, and two were from trawls targeting multiple finfish species.

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Unequal sample sizes were produced for the three different categories of sampling methods, making it difficult to compare the proportion of primnoid diversity that was represented by each. Samples originating from epibenthic sled sampling produced the highest number of distinct taxa (92% of total diversity), but also represented 70% of the sequenced samples. In comparison, commercial bycatch represented 69% of the total diversity recovered from 16% of the samples, and research trawls represented 38% of the diversity among 14% of the samples.

Overall similarities in the presence and absence of taxa recovered by each sampling method indicated that commercial bycatch was most similar to epibenthic sled sampling, sharing 62% similarity in the presence and absence of recovered taxa. Commercial bycatch and research trawl bycatch had 54% similarity while research trawl bycatch and sled samples shared 46% similarity in the taxa they recovered. These patterns of similarity broadly corresponded to overlap in spatial distribution of samples from each sampling method. Commercial bycatch samples were distributed along the northern and southern slope margins of the Rise whereas samples from epibenthic sled tows were mostly confined to seamount complexes along the slope margins, including the Graveyard Complex to the north and Andes Seamounts to the east. Samples from research trawl bycatch were broadly distributed across the top of the central Rise from the northern to southern slope margins (Figure 3-2).

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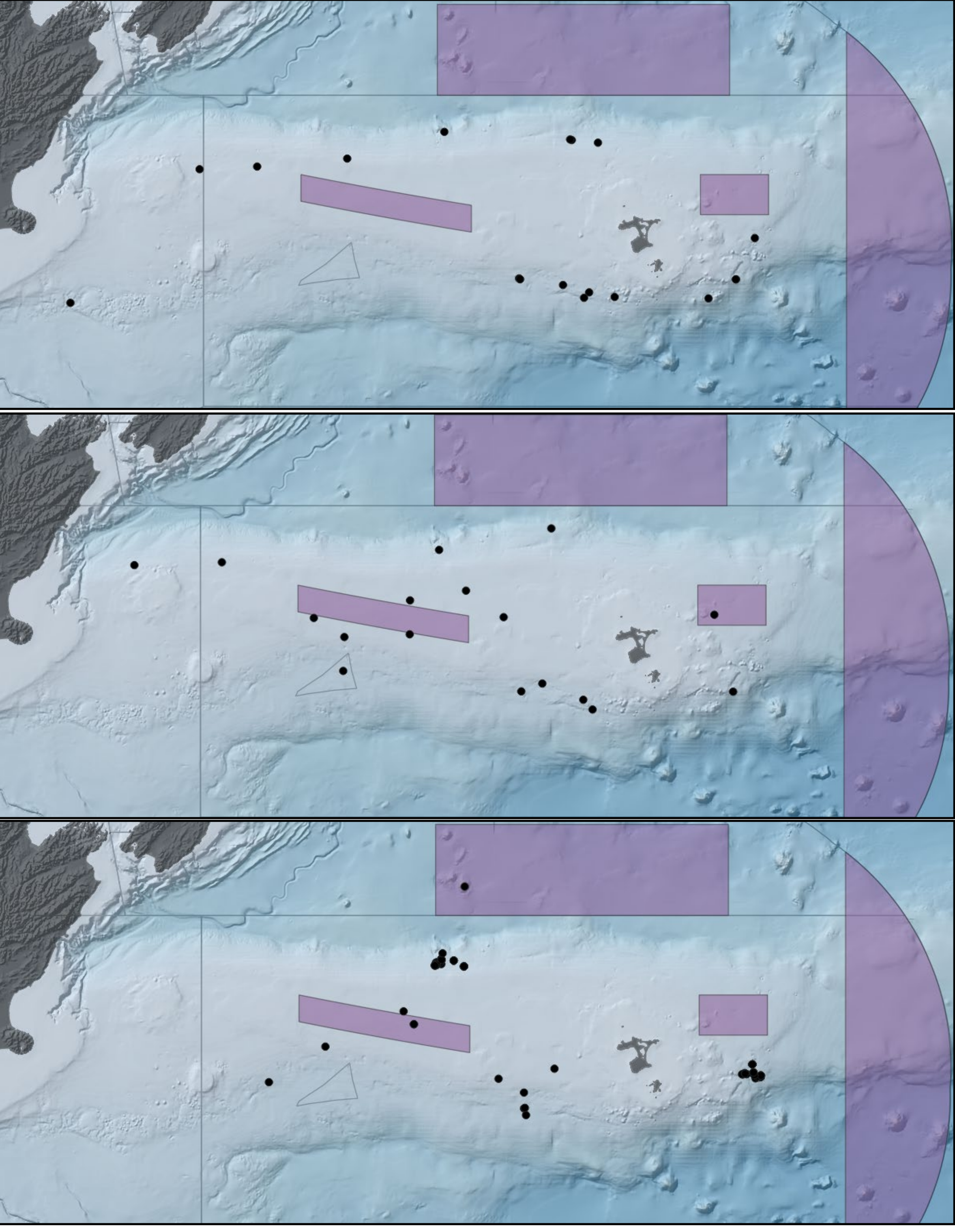

Figure 3-2: Distribution of Sequenced Specimens. Dots indicate collection location of Primnoidae specimens, as collected by commercial fishing vessels (top), NIWA fisheries research trawls (middle) or benthic sleds during NIWA research voyages (bottom).

Species discovery curves were produced for the aggregated sample set, as well as for samples originating from sled sampling (Figure 3-3). The taxon discovery rate was not analysed for fisheries bycatch nor research trawls due to their low sample sizes. Discovery rates for the sled samples had

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an average of one unique taxon discovered for every six samples sequenced and displayed a linear increase with no indication of an asymptote (= a plateau limit to species discovery). However, the discovery curve for the aggregated sample set had an irregular profile with periods of rapid increase in unique taxa (1 new taxon per 3 sequenced samples) punctuated by stretches with no further discovery (zero new taxa from 24 successive sequences). As such, it was difficult to ascertain whether the limits of species discovery were being approached for the combined set of samples, since the data fit neither linear nor exponential patterns of progression.

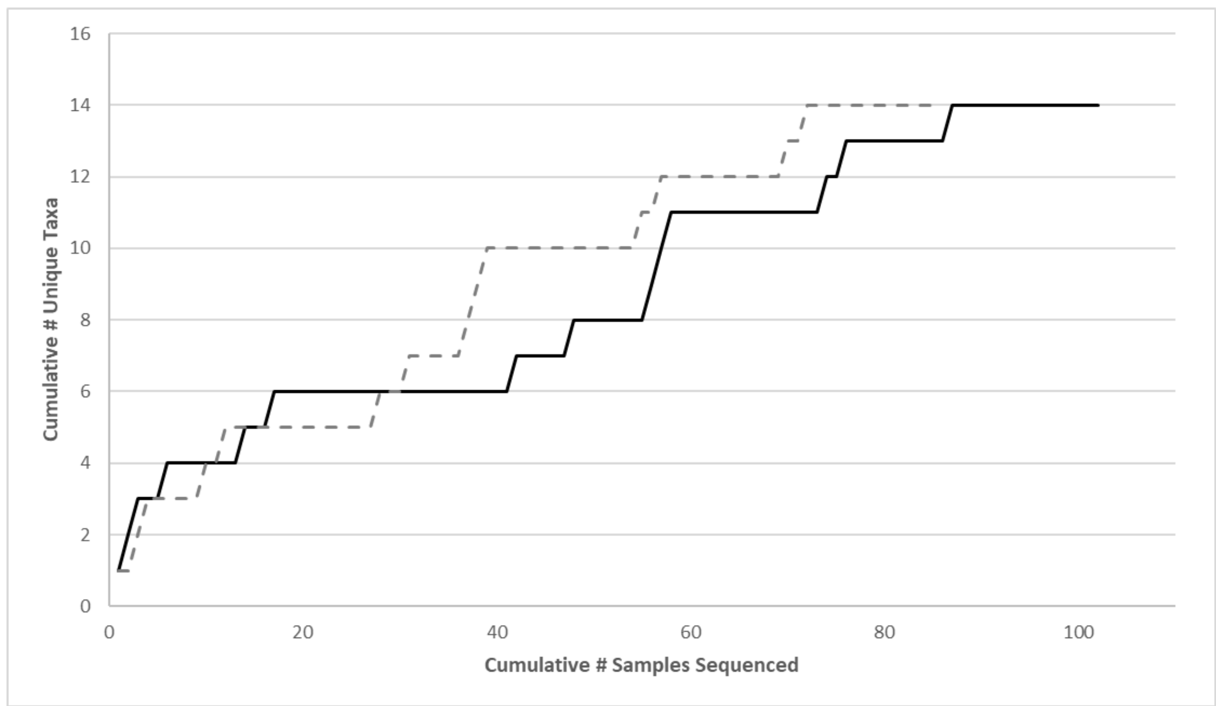

Figure 3-3: Species discovery curves. The cumulative number of unique taxa discovered by DNA sequencing of successive samples is displayed for the combined dataset (black line: all sampling sources) and for samples originating from epibenthic sled tows (grey dashed line). Note that non-primnoid taxa are also included since they nonetheless represent novel diversity and make realistic allowances for misidentification of sample sets in the calculation of discovery rates.

Although species discovery curves did not clearly indicate an asymptote was being approached, the number of unique taxa delineated by DNA sequencing in this study (13) is close to the total number of primnoid taxa previously recorded from the Chatham Rise (15), which was reported in a series of morphological assessments of taxonomy and distribution (Table 3-2, compiled from Cairns 2012, Cairns 2016, Cairns 2021). Specimens of *Callozostron acanthodes* and *Calyptrophora niwa* that were suitable for DNA sequencing were not available in the NIC and were not detected in any of the included samples. Figure 3-1 also indicated support for two species each of *Metafannyella* and *Primnoella* among included material, but it could not be determined which of *M. moseleyi* or *M. rigida* was present in addition to *M. chathamensis*, and a lack of reference sequences for *Primnoella distans* prevented confirmation of its presence, in addition to *Primnoella insularis*. The phylogenetic results presented here also indicated that three species of *Thouarella* were found, whereas previous records only include two species. Aside from confirming the presence of *Thouarella variabilis* var. *gracilis*, it could not be determined which (if either) of the additional two species of *Thouarella* belonged to *Thouarella hilgendorfi* or to other species that have not previously been recorded from the Chatham Rise (*e.g. Thouarella laxa*). It is also difficult to assess whether the several records of *Tokoprymno* presented here for the Chatham Rise are noteworthy since this genus has not been

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included in past monographs of the New Zealand Primnoidae. It would appear to occur on the Rise with some frequency since 15 samples of it were included here and only two of these were from the same sampling location.

Table 3-2: List of Primnoidae previously recorded from the Chatham Rise. Species that have been recorded in a series of New Zealand monographs are listed, along with the reference for their record. * = species that were not sampled nor observed in the current study; 1 = species that may have been observed in the current study, but a lack of reference sequence data prevents assignment below genus-level.

Primnoidae recorded from the Chatham Rise, New Zealand	Reference
*Callozostron acanthodes**	Cairns 2021
Calyptrophora inornata	Cairns 2012
*Calyptrophora niwa**	Cairns 2012
Dasystenella austasensis	Cairns 2021
Metafannyella chathamensis	Cairns 2016
Metafannyella moseleyi[1]	Cairns 2021
Metafannyella rigida[1]	Cairns 2021
Narella hypsocalyx	Cairns 2012
*Parastenella pacifica**	Cairns 2016
Plumarella (Faxiella) deliculata	Cairns 2016
Primnoa notialis	Cairns 2016
Primnoella distans[1]	Cairns 2016
Primnoella insularis	Cairns 2016
Thouarella variabilis var. *gracilis*	Cairns 2021
Thouarella hilgendorfi[1]	Cairns 2021

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4 Conclusions

Genetic barcoding represents an objective and relatively inexpensive means to delineate species that are otherwise difficult to identify and distinguish. This was demonstrated in this study where 122 specimens of a highly variable family of octocorals were partitioned into 13 distinct taxa – a task which would have otherwise required extensive microscopic examination by a taxonomic expert, which for the Primnoidae does not exist in New Zealand. The use of parataxonomists (non-specialists that are trained in the identification of particular region-specific groups) for identification is often necessary in such cases but carries increased risk of misidentification (Tracey et al. 2019). Misidentifications of the primnoid specimens included here were frequent and occurred in eight of the 13 taxa (Table 3-1). In particular, specimens were most often incorrectly identified as *Thouarella* – a genus that is diverse and problematic (Cairns 2021, Cairns & Wirshing 2018). Misidentifications have likely occurred due to unfamiliarity with the breadth of diversity of forms in the Primnoidae, where frequent lumping of bottlebrush-shaped octocorals into *Thouarella* occurs without recognition that this growth form can occur in any of four other genera present in New Zealand (*Metafannyella, Fannyella, Dasystenella* and sometimes *Plumarella*). Furthermore, misidentification of six specimens of *Acanthogorgia* as primnoids also highlights the difficulty in identifying primnoids and distinguishing them from other highly variable gorgonian groups such as the speciose Plexauridae and Acanthogorgiidae – families that also displayed high levels of cryptic diversity in a prior study (Bilewitch & Tracey 2020b). As in that previous report, the use of routine genetic barcoding for octocoral identification is supported by these new observations and is thus recommended for future specimen collections.

In addition to resolving and correcting identifications of primnoid specimens, the genetic barcoding methods applied here were also successful in uncovering new records of taxa for the Chatham Rise, and possibly for New Zealand. *Tokoprymno maia* and a third, unidentified species of *Thouarella* were recorded from the Rise, bringing the total number of recorded Primnoidae from the region to 17. Even more noteworthy was the discovery of members of the family Pleurogorgiidae among specimens misidentified as primnoids, which would represent a new record for New Zealand. Species of this esoteric family are outwardly similar to the Primnoidae, but also share features with the Chrysogorgiidae. Although these seven records require confirmation through taxonomic examination of their morphology, this would significantly expand the breadth of known octocoral diversity in New Zealand and necessitate the inclusion of another gorgonian family within the protection of the Wildlife Act.

This study sequenced 122 specimens but it remains to be determined whether the resulting 13 taxa delineated by molecular systematics represents the entire breadth of primnoid diversity on the Chatham Rise, a significant portion of it, or a smaller fraction of the actual total number of species. Comparisons with the records contained within the taxonomic monographs of Cairns (2012, 2016, 2021) suggest that the majority of diversity may have been documented here. However, species descriptions and diversity estimates based solely on morphology-based can be misleading, since they may overlook cryptic diversity and infer relationships that are not reflective of genetic similarity or evolutionary relationships (*e.g.* Kessel 2021). The Primnoidae is known to harbour many problematic and incorrect taxa and is in need of a large-scale taxonomic revision (Cairns & Wirshing 2018), thus the 15 taxa previously reported for the Chatham Rise may actually be a complex of both cryptic and plastic species, which confounds and reduces the confidence of the number of species present. Regardless, the species discovery curve presented in Figure 3-3 suggests that the methods employed

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in this study may be approaching their limits of detection, although further sequencing effort would be needed to confirm this.

Although attempts were made to include as many samples from fisheries bycatch and research trawls, the sequence dataset was heavily skewed in favour of material collected by epibenthic sleds, which represented 133 of the 209 NIC specimens originally identified as suitable for genetic analysis, and 86 of 122 samples that produced sequence data for this study. Only five samples of commercial fishery bycatch and 16 samples of research trawl remain unsampled in the NIC, thus even if the DNA sequencing results were supplemented with the remaining NIC bycatch specimens, the result would still be a dataset where the majority of samples originate from targeted research sampling, rather than incidental bycatch. Furthermore, the origins of the included bycatch material was roughly distributed evenly between orange roughy, oreo and hoki target fisheries, without a representative sample size for any single target species. As with the previous study of broadscale patterns of gorgonian bycatch diversity (Bilewitch & Tracey 2020b), restrictive sample sizes prevented a numerical comparison of primnoid bycatch by target fishery. Instead, Table 4-1 presents an updated list of Primnoidae recorded from the Chatham Rise by both previous records and this study, annotated with known occurrences within bottom trawling bycatch. This diversity can be incorporated into estimates of bottom trawling impacts on protected corals within New Zealand and complements previous fishery impact measurements that focus on coral biomass (Anderson & Clark 2003; Anderson et al. 2017).

Table 4-1: Updated list of Primnoidae recorded from the Chatham Rise. This list incorporates previous records of Cairns (2012, 2016, 2021) with the records observed in the current study. For each taxon, its known occurrence is given in trawl bycatch from the Chatham Rise ('Y') or from multiple records elsewhere in New Zealand ('*') that are held by the NIC. '?' indicates uncertainty as to which of two species of *Metafannyella* pertain to bycatch specimens included in this study, in addition to *Metafannyella chathamensis*. The reference for the occurrence on the Chatham Rise is given, as well as references for occurrence as bycatch.

Taxon	Bycatch Occurrence	Reference for Record
Callozostron acanthodes	-	Cairns 2021
Calyptrophora inornata	Y	Cairns 2012; this study
Calyptrophora niwa	-	Cairns 2012
Dasystenella austasensis	Y	Cairns 2021; this study
Metafannyella chathamensis	Y	Cairns 2016; this study
Metafannyella moseleyi	?	Cairns 2021
Metafannyella rigida	?	Cairns 2021
Narella hypsocalyx	Y	Cairns 2012; this study
Parastenella pacifica	*	Cairns 2016
Plumarella (Faxiella) deliculata	Y	Cairns 2016; this study
Primnoa notialis	Y	Cairns 2016; this study
Primnoella distans	-	Cairns 2016

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Taxon	Bycatch Occurrence	Reference for Record
Primnoella insularis	-	Cairns 2016; this study
Thouarella variabilis var. *gracilis*	Y	Cairns 2021; this study
Thouarella hilgendorfi	*	Cairns 2021
Thouarella cf. *laxa*	Y	This study
Tokoprymno maia	Y	This study

<<< The final report will also include discussion concerning the results and benefits of UCE sequencing and its application for discovery of cryptic diversity among protected corals in New Zealand >>>

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