BCBC2020-26: Octocoral bycatch diversity on the Chatham Rise

Final Report

Prepared for the Marine Species Team, Department of Conservation

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Climate, Freshwater & Ocean Science

Prepared by:
Dr. Jaret P. Bilewitch

For any information regarding this report please contact:

Jaret Bilewitch
Environmental Isotopes & Molecular Biology
+64-4-386 0502
jaret.bilewitch@niwa.co.nz

National Institute of Water & Atmospheric Research Ltd (NIWA)

301 Evans Bay Parade
Wellington 6021
Private Bag 14901
Kilbirnie
Wellington 6241
Phone +64 4 386 0300

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Cover image: Primnoid octocoral collected on Tangaroa voyage TAN0803. [Atlas Library, NIWA]

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Executive summary

The deep seas of Aotearoa/New Zealand harbour diverse and abundant communities of branched gorgonian corals that are found in areas where bottom trawling and long-lining activities target commercially important fisheries species. The incidental contact of fishing gear with gorgonian colonies, particularly those with branched or bushy growth forms, causes damage and entanglement, potentially resulting in corals being brought to the surface as bycatch when trawls are retrieved. Although fishing damage to coral communities resulted in their protection under a 2010 amendment to the Wildlife Act, bycatch has continued to occur, 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 well understood, especially for gorgonian corals. This is in part due to their highly diverse and 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 New Zealand Exclusive Economic Zone by focusing on a single family (the Primnoidae: 'sea-fan' and 'bottlebrush' corals) across the Chatham Rise – a region where multiple commercial trawl fisheries occur. Samples of primnoid corals were obtained from the NIWA Invertebrate Collection, with their origins from a mixture of fisheries bycatch sampled by fisheries observers, NIWA research trawl bycatch (incidental catch during fisheries assessment cruises for Quota Management System target species), and samples collected during NIWA biodiversity research cruises using tows of benthic sleds. Genetic barcoding was used to identify the number of unique taxa present and a subset of samples were subjected to high-resolution genomic sequencing, to test the utility of recent technological advancements for biodiversity discovery and to determine whether 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 phylogenetic analysis based upon two genetic markers 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, with protection under the Wildlife Act. The 13 primnoid taxa were comparable in diversity and identity 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. Genomic sequencing of a subset of samples resolved fine-scale relationships among representative taxa at high levels of confidence using over 2700 genetic markers, and provided hundreds to thousands of variable positions that were capable of distinguishing closely related species and potentially delineating population-level differences for future genetic connectivity studies of protected corals.

Samples originating from commercial and research bycatch were too few and too broadly dispersed among target fishery species to perform numerical comparisons of diversity by sampling method or by 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.

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 and overlap 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; Goode et al. 2020). These effects prompted the New Zealand Department of Conservation to list arborescent coral groups (black and stony corals, hydrocorals and gorgonian octocorals) 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 for New Zealand 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; Goode et al. 2020). However, examinations of the impacts on certain coral groups or specific taxa are less common, as they require reliable identification of bycatch photographs and are dependent upon 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 time constraints and the high difficulty associated with non-expert identification of highly variable and similar-looking coral species. However, although identification reliability is improved by examination of images and specimens by trained experts (Tracey et al. 2019), errors can 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 (CSP project INT2019-05, Bilewitch & Tracey 2020b) examined the extent of bottom-trawling impacts on species-level diversity of gorgonian corals within the New Zealand Exclusive Economic Zone (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, DNA barcoding at three markers (gene regions or loci) was used as an efficient and objective means to inform identification 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 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 increased knowledge of the extent of natural octocoral diversity would be beneficial, for a baseline context in which trawling-related octocoral bycatch diversity can be placed.

In consultation with the Conservation Services Programme 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 octocorals) 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.

All genetically viable samples of Primnoidae from the Chatham Rise that are available within the NIC were DNA-sequenced at three barcode markers (loci) to delineate taxa. Barcoding was used to avoid subjectivity of morphological identification, to identify cryptic species, and to contribute to the development of a reference genetic dataset documenting 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 bycatch diversity recovered by different collection methods. 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.

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 capable of producing millions of base pairs of sequencing data that, on a dollar-per-base-pair basis, are significantly less expensive than traditional DNA sequencing (Sanger sequencing of usually five or less markers). 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 before sequencing, which results in an increased 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 base-pairs 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 over 3000 loci for a subset of 12 primnoid samples explored the usefulness of this new technique for documenting and delineating coral diversity and to test whether traditional sequencing of three loci was sufficient to detect cryptic species.

In summary, the four specific objectives of this project were to:

1. Document the breadth of regional diversity using both bycatch and non-bycatch octocorals.
2. Obtain an in-depth estimate of diversity for a single gorgonian group: the Primnoidae.
3. Focus on the Chatham Rise, which produced the highest amounts of historical bycatch and non-bycatch samples and is relevant to commercial trawling operations.
4. Pilot the utility and effectiveness of UCE sequencing for a subset of samples, for recognition of species boundaries and for potential application in future studies of genetic connectivity.

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 region. Specimens were then broadly categorised according to their collection method: commercial fishery trawl bycatch, research trawl bycatch, or targeted research collection via epibenthic or benthic sleds (Figure 2-1). The taxonomic distribution of this resulting list (Appendix A) was examined and 150 specimens representing the breadth of diversity (based on pre-existing identifications) were selected for genetic analysis.

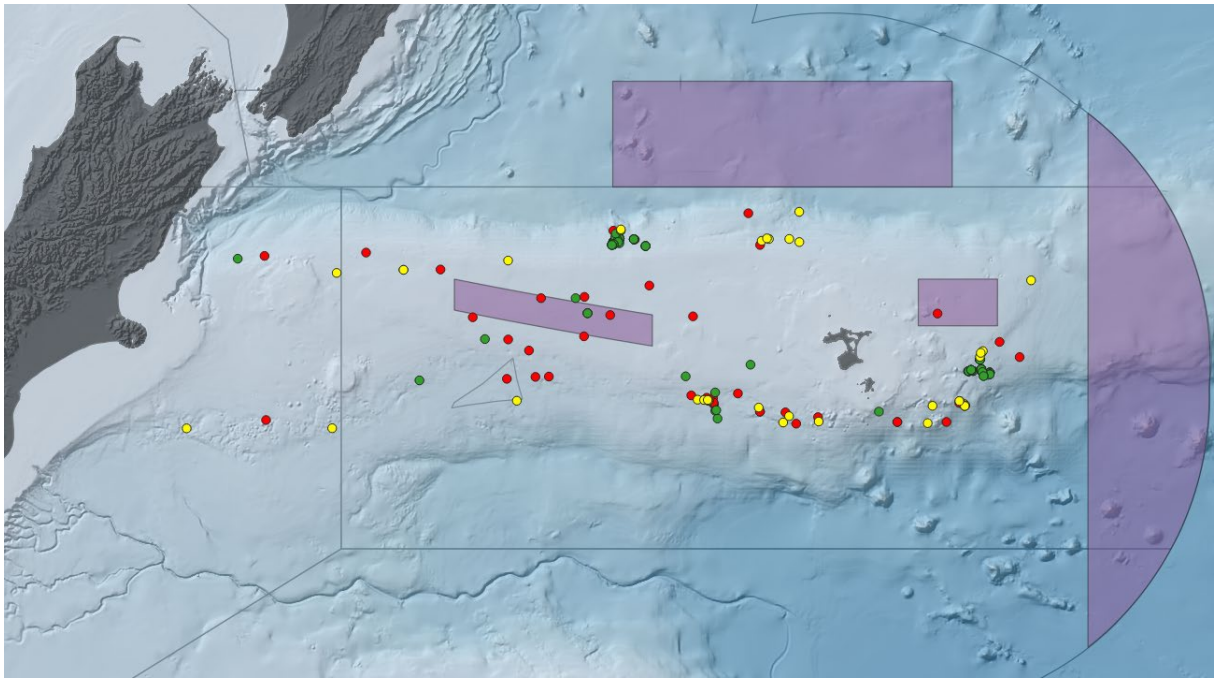

Figure 2-1: Sample availability across Chatham Rise. Samples identified as being suitable for genetic analysis were grouped by the method by which they were collected: commercial fisheries bycatch from observers (yellow), NIWA fisheries research trawl (red), or NIWA biodiversity research benthic sled (green). Benthic Protection Areas are shown in purple shading.

2.2 Genetic barcoding

Approximately 10mg of tissue was removed from each specimen 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 40µl 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 (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. These gene regions were amplified in 25µl total reaction volumes using 1X MyTaq RedMix (Bioline Inc.), 0.5µM of each primer pair (Table 2-1) and 3-9µl 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	Bilewitch & Tracey 2020b
	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 using sequences of Plexauridae and were examined for concordance between each locus, prior to concatenating loci into a single dataset that was partitioned by locus for a repeated Bayesian phylogenetic analysis using identical parameters. The posterior output of all model runs was examined to verify parameter convergence and effective sample size of parameter estimates, and to ensure an appropriate burn-in value was used.

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 observable genetic variation among traditional barcode sequences, to test for potential cryptic genetic variation. Specimens (NIC catalogue numbers) 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 bait-set (biotinylated RNA probes) specific to the Suborder Calcaxonia (including the Primnoidae) was used (developed in Untiedt et al. 2021) to focus target enrichment on relationships within the Primnoidae.

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

UCE sequence data was processed using the *phyluce* bioinformatic package (Faircloth 2016). Sequencing reads were cleaned and trimmed using the *illumiprocessor* module then assembled using SPAdes v.3.15.3 (Bankevich et al. 2012). Resulting contigs (contiguous assemblies of individual DNA sequences) were matched to a list of 18,783 bait probes and extracted according to UCE loci. UCE-specific assemblies were concatenated and aligned using MAFFT (Katoh et al. 2013) and were trimmed using the *phyluce_align_seqcap_align* and *phyluce_align_get_gblocks_trimmed_alignments_from_untrimmed* modules in 'phyluce'. Two alignments were produced: one including all UCE loci that had data from at least 75% of the 12 included samples (*i.e.,* 9 or more) and one that had data from at least 91% (11 or 12) of included samples. Bayesian phylogenetic analyses of each alignment were performed using ExaBayes (Aberer et al. 2014), with $1x10^6$ generations sampled every 500 generations and 25% of samples discarded as burn-in. Alignments were partitioned according to UCE loci and the resulting output was examined for evidence of convergence using Tracer (Rambaut et al. 2018). Phylogenetic trees were examined for correspondence to those produced by traditional barcode sequencing methods and alignments from each were used to assess and compare the informative content of sequence data produced by each method, using Geneious Prime.

3 Results

3.1 Sanger DNA sequencing of three target loci

A query of *niwainvert* for ethanol-preserved primnoids from the 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 27 specimens produced no viable results despite repeated attempts at DNA extraction and PCR amplification and 46 specimens were not sampled since they were previously identified as taxa that were already represented by other sampled specimens. The remaining 122 specimens produced DNA sequence data at one (n=25), two (n=63) or three (n=34) loci (Appendix A). After trimming and alignment, the 5'-end of *mtMutS* was 786 base-pairs (bp) long with 215 informative (variable) sites, the Domain-III region of *mtMutS* consisted of 792bp with 340 informative sites and the *28S*-rDNA locus had 745bp with 433 informative sites. 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 datasets.

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 using the two *mtMutS* markers (1578 bp) from 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 misidentified as primnoids, which was confirmed through visual inspection of physical specimens. Six of these belonged to the octocoral family Plexauridae and seven to the octocoral family 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.

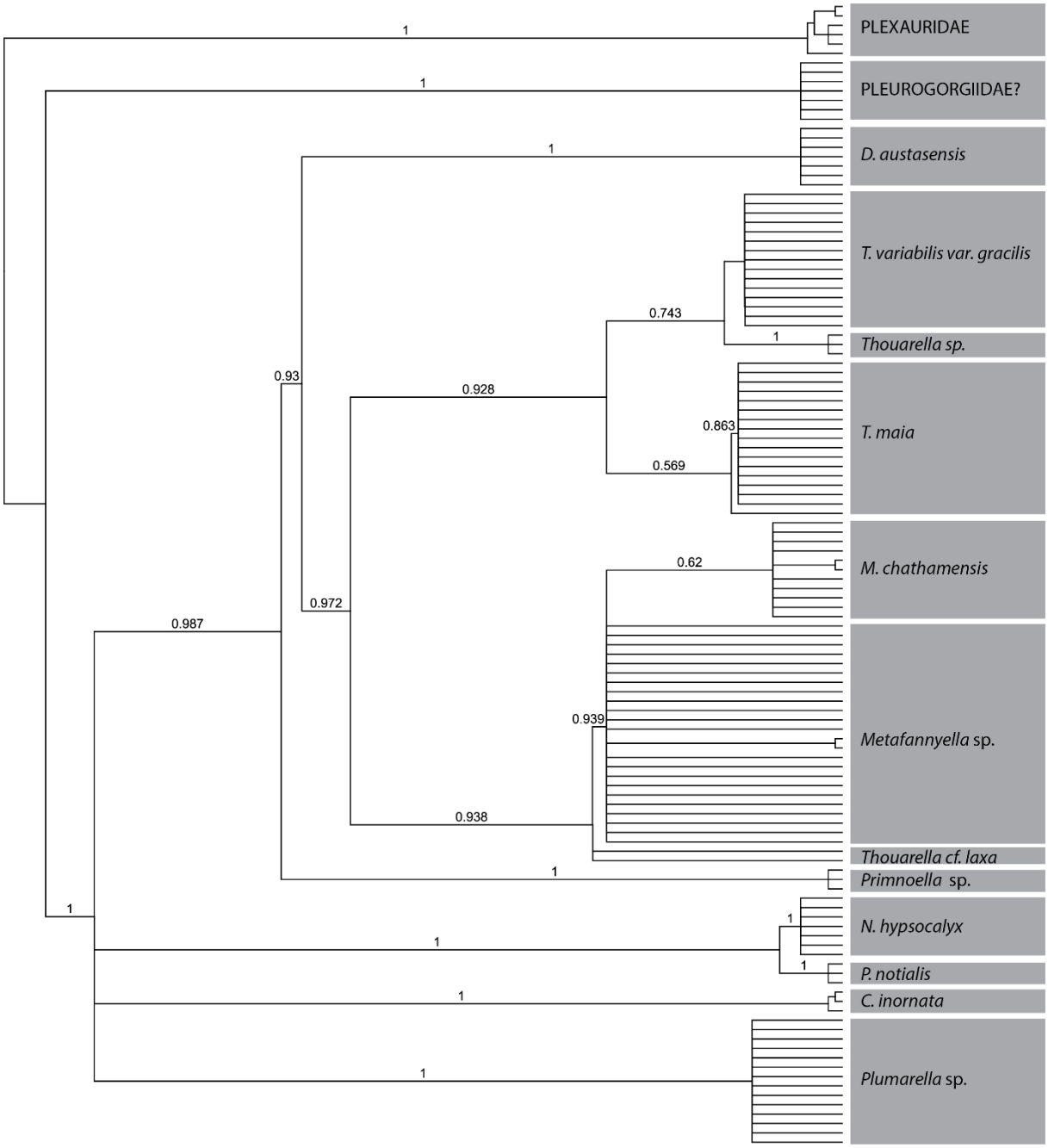

Figure 3-1: Phylogenetic relationships of sampled Primnoidae from the Chatham Rise. Bayesian phylogeny of sequenced specimens at two *mtMutS* loci produced using 10^7 MCMC generations, sampled at 10^3 intervals with 10% discarded as burn-in. Branch labels are posterior probability support values. Each branch 'tip' represents a single sequenced specimen. Taxon names were derived from phylogenetic comparisons to reference sequences from Cairns & Wirshing (2018) and Taylor & Rogers (2015). Full taxon names are listed in Table 3-1. The original phylogeny with individual specimen designations is shown in Appendix B.

Table 3-1: Primnoid taxa confirmed by DNA sequencing. A list of putative primnoid taxa from the Chatham Rise, identified via molecular systematic comparison to reference sequences. The methods by which sequenced specimens were collected and the original specimen identifications (via morphological examination) are also given. * = taxa not previously recorded from the Chatham Rise by Cairns (2012, 2016, 2021). Results exclude specimens that were originally identified as Primnoidae but which sequencing confirmed as belonging to the Plexauridae (n=6) or Pleurogorgiidae (n=7) – all of which originated from research sled sampling.

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

UCE-enriched genomic sequencing of 12 samples produced over 240 million reads, resulting in over 33 billion base-pairs of sequencing data after trimming and quality control (see Appendix C for detailed data). Each sample had an average of 20 million reads (SE=1.9 million) and read assembly produced an average of 180,273 contigs per sample (SE=37,234), ranging from 70147 (sample NIWA102298) to 513,967 (sample NIWA25426) with a maximum contig length of 249,035bp (sample NIWA53275). Matching to UCE loci resulted in an average of 1998 contigs (SE=59), with a range of 1749-2250. The average length of UCE contigs was 937bp (SE=76), with a maximum length ranging from 1166 to 8447bp.

Concatenation and alignment of UCE loci resulted in 2785 loci with a total length of 1,136,131bp, of which 194,824 positions showed informative variation. Restricting the alignment to loci that were sequenced for 75% or 91% of all 12 samples resulted in alignments with 1583 and 1140 loci, which were 664,003 bp and 508,878bp in length, respectively.

Independent Bayesian phylogenetic analyses of the 75% and 91% concatenated and partitioned datasets produced trees that were identical in topology and support values (Figure 3-2). Trees from both subsets had completely resolved relationships for all 12 included samples, which represented ten out of twelve resolved taxonomic lineages produced by the two-barcode analysis (see Section 3.1 and Figure 3-1). Only *Primnoella* and *Thouarella cf. laxa* were not included in the UCE results, since no samples of these taxa were sequenced. The UCE phylogenies showed higher resolution (100% posterior probability of branch splits) in the basal relationships of the ten taxa, compared to barcoding results where the four most ancestral relationships of the Primnoidae were not resolved (*i.e.,* a polytomy) (Figure 3-1). The UCE analyses also resolved relationships of closely related taxa that previously had low support, such as the relationships between *Metafannyella* sp. and *M. chathamensis* and between *Tokoprymno maia* and the clades containing *Thouarella* and *Metafannyella*. Within a single taxon (tentatively equivalent to a species), UCE sequence data were also capable of distinguishing between individual specimens that were otherwise identical in outward morphology and their barcode sequences. The two included specimens of *T. maia* differed from each other by 2435 to 2980bp (unambiguous differences not due to missing or low-quality data) for the 91% and 75% datasets, respectively. Likewise, UCE data discriminated two specimens of *Metafannyella chathamensis*, which differed by 5646 to 6749bp (91% and 75% datasets, respectively) and distinguished both from *Metafannyella* sp., whereas *Metafannyella* sp. and *M. chathamensis* were not confidently resolved using barcode sequencing (62% posterior probability).

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 octocorals 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 during NIWA research voyages. Among the commercial bycatch specimens, 11 originated from orange roughy-targeted trawling, five from smooth oreo, two from scampi and one from hoki. For the research trawl specimens, eight originated from trawls targeting hoki, five from oreo (smooth and rough oreos combined), two from orange roughy, and two were from trawls targeting multiple finfish species.

Unequal sample sizes were produced for the three different collection 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 (Table 3-1). 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 Chatham Rise whereas samples from benthic sled tows were mostly confined to seamount complexes along the slope margins, including the Graveyard Complex to the northwest and Andes Seamounts to the east. Samples from

research trawl bycatch were broadly distributed across the top of the central Chatham Rise from the northern to southern slope margins (Figure 3-3).

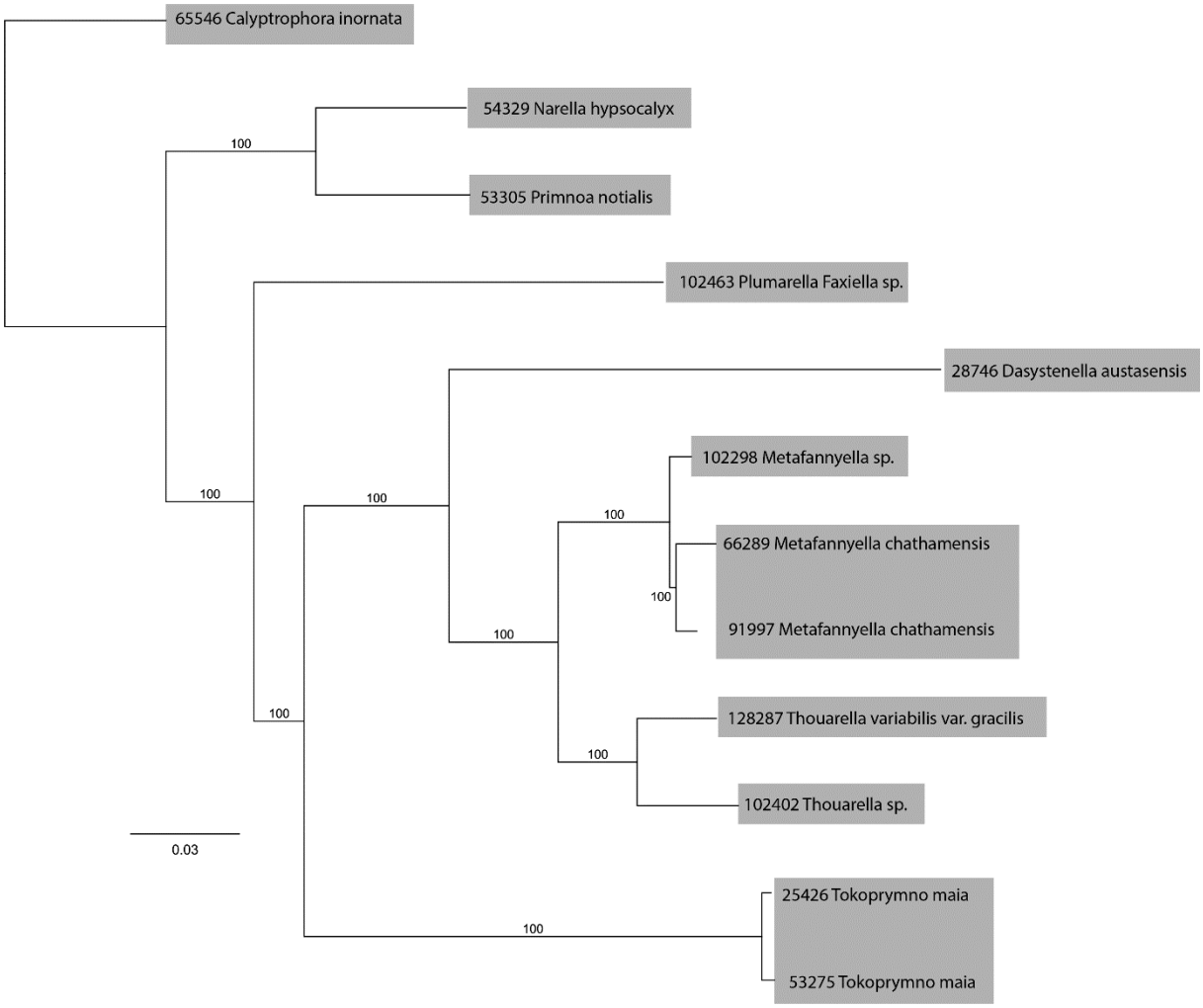

Figure 3-2: Phylogenetic results of UCE sequencing analysis. The results of 10^6 generations of Bayesian phylogenetic analysis performed on UCE datasets, with posterior probability support values displayed for each branch. The resulting trees for both ≥75% and ≥91% of taxa were identical. Labels inside grey boxes give NIWA specimen numbers and the corresponding taxon to which they were assigned in the 2-locus barcoding results. Trees were arbitrarily rooted with specimen 65546, following the results of Figure 3-1 where no resolved basal ingroup taxon could be resolved among *Narella, Primnoa, Plumarella* and *Calyptrophora.*

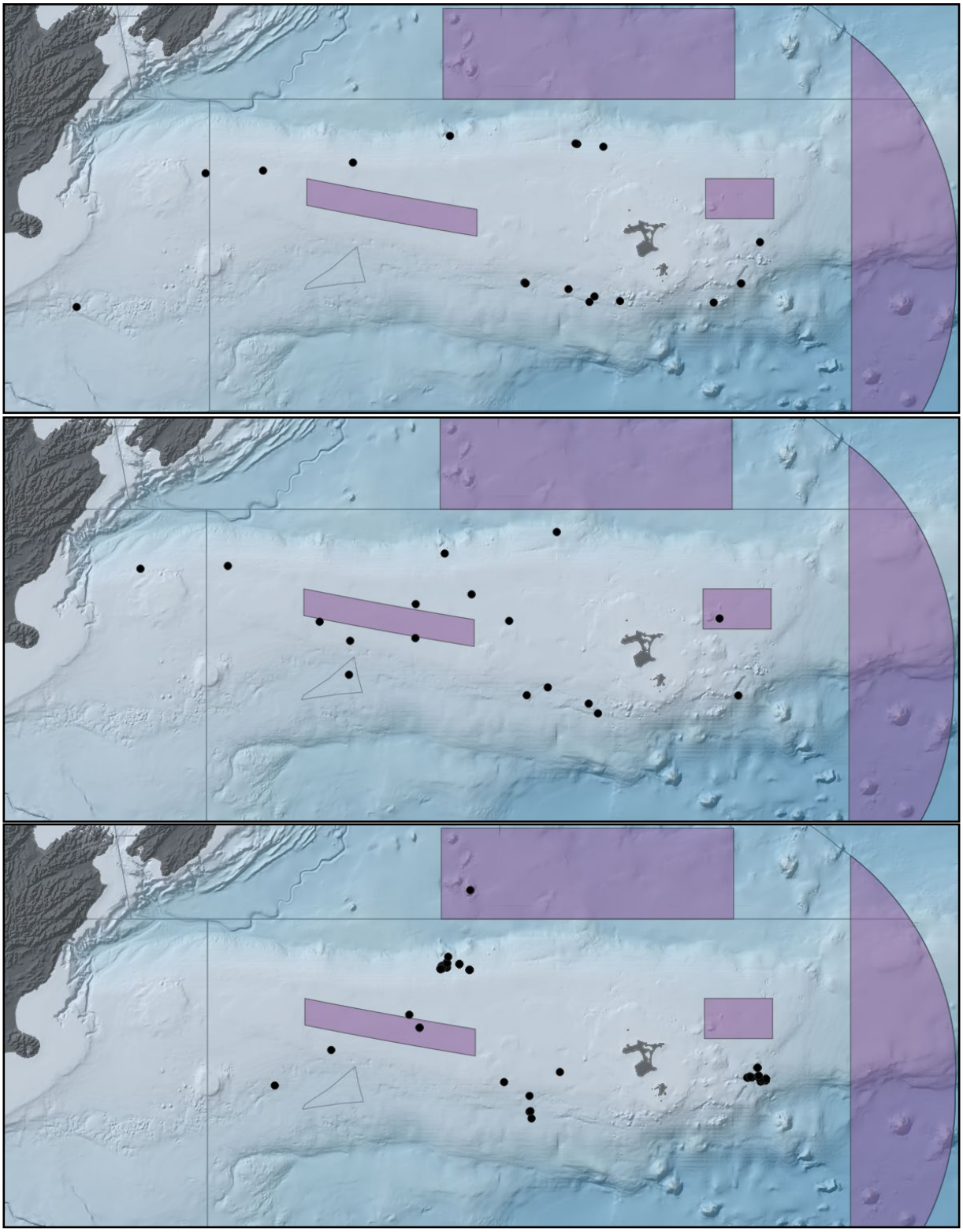

Figure 3-3: Distribution of Sequenced Specimens. Dots indicate collection location of Primnoidae specimens, as collected by commercial fishing vessels (top, n=19), NIWA fisheries research trawls (middle, n=17) or benthic sleds during NIWA research voyages (bottom, n=86). Benthic Protection Areas are shown in purple shading.

Species discovery curves were produced for the aggregated sample set, as well as for samples originating from sled sampling (Figure 3-4). The taxon discovery rate was not analysed for fisheries

bycatch nor research trawls due to their low sample sizes. Discovery rates for the benthic sled samples had 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.

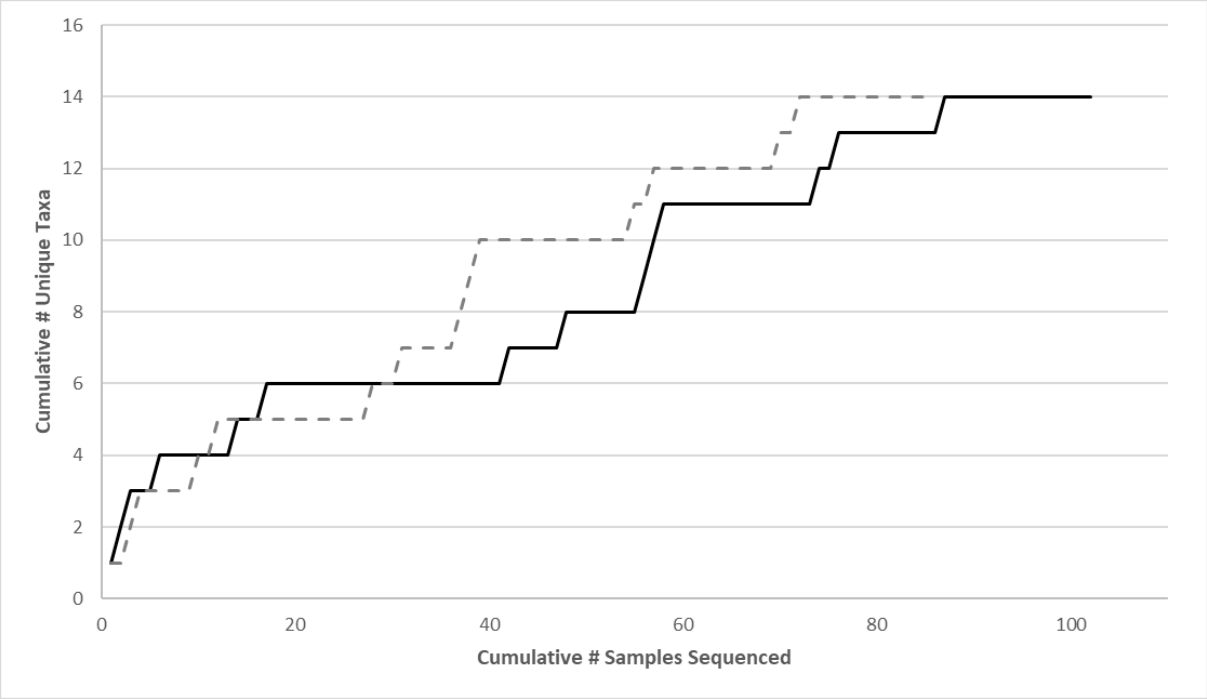

Figure 3-4: Species discovery curves. The cumulative number of unique taxa discovered by DNA sequencing of successive samples is displayed for the combined dataset (black line: all sampling sources) and for samples originating from epibenthic sled tows (grey dashed line). Note that non-primnoid taxa 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 was not possible to determine 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 was not possible to determine 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 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

4 Conclusions

4.1 Genetic assessments of primnoid diversity

Genetic barcoding represents an objective and relatively inexpensive means to delineate species that are otherwise difficult or time-consuming 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 still carries 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 difficult to identify (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 of primnoid octocorals 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 the New Zealand region. *Tokoprymno maia* and (tentatively) *Thouarella* cf. *laxa* were recorded from the Chatham 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 ('gold' octocorals). 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 add another gorgonian family to the protection measures of the Wildlife Act. Taken as a whole, these new records of Primnoidae and other octocorals significantly expand our estimates of protected gorgonian diversity on the Chatham Rise and contributes to distribution records across the New Zealand region.

This study sequenced 122 specimens across broad geographical and depth ranges of the Chatham Rise, but it remains to be determined whether the resulting 13 taxa delineated by molecular systematics represents the entire breadth of primnoid diversity for the region, 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 most diversity may have been documented here. However, species descriptions and diversity estimates based solely on morphology 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 taxonomically challenging and incorrect taxa and

needs large-scale taxonomic revision (Cairns & Wirshing 2018), thus the 15 taxa previously reported for the Chatham Rise may be a complex of both cryptic (multiple species that look the same) and plastic (high morphological variation within a species) taxa, which confounds and reduces the confidence of the number of species present. Although the species discovery curve presented in Figure 3-4 lacks signs of approaching an asymptote, a comparison of taxa identified here to previous taxonomic records (Table 3-1 *vs*. 3-2) suggests that the methods employed in this study may be approaching their limits of detection. However, further sequencing effort would be needed to confirm this.

Although traditional 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 bamboo corals, the Keratoisididae and Mopseidae), and variation within a species from variation between cryptic or mis-identified species (*e.g.,* the bubblegum coral *Paragorgia arborea*). Likewise, the phylogenetic results of the current study showed low resolution for closely related taxa, such as *Metafannyella* sp. compared to *M. chathamensis*, as well as ancestral relationships between the clades containing *Narella, Primnoa, Calyptrophora* and *Plumarella* (represented as a polytomy in Figure 3-1). Low support was also observed for the relationship of *Thouarella* sp., *T. variabilis* and *Tokoprymno maia*. These issues were overcome in the results of the UCE analysis, where the order and placement of both ancestral and recent divergences were unequivocal and well-supported, through the application of thousands of informative loci. The variation amongst UCE loci was even sufficient to distinguish between individuals of the same species, showing promise for future applications in population genetic analyses where hundreds to thousands of informative genetic variations are required to determine population connectivity, parentage and identify geographic hotspots of genetic variation. In studies where medium to large sample sizes are required, the use of enriched genomic sequencing methods such as UCEs would represent an attractive high-resolution and cost-effective prospect for addressing conservation-related questions for protected corals in New Zealand.

4.2 Diversity of primnoid bycatch

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. For primnoid octocorals, 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 most samples originate from targeted research sampling, rather than incidental bycatch.

As with the previous study of broadscale patterns of gorgonian octocoral bycatch diversity (Bilewitch & Tracey 2020b), restrictive sample sizes prevented a numerical comparison of primnoid bycatch by target fishery. However, the origins of the included bycatch material (both commercial and research trawl) were roughly evenly distributed between orange roughy, oreo and hoki target fisheries, suggesting that the impacts of bottom trawl fisheries on protected primnoid corals are not unique to any single target fishery. 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 among 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). The overall range of taxa represented by bycatch in the current study demonstrates that bottom trawling is encountering the majority of primnoid octocoral species that are found on the Chatham Rise. Future efforts should attempt to marry these metrics of diversity to coral abundance (via quantitative species distribution modelling: Stephenson et al. 2021; also see ongoing DOC-CSP project POP2021-02) or coral bycatch biomass (Anderson et al. 2017; Tracey et al. 2019), to provide information on the frequency with which trawl gear impacts particular taxa within the breadth of this documented diversity.

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, 'Y' indicates records of NIC specimens that are recorded as trawl bycatch from the Chatham Rise and '*' indicates NIC bycatch specimens from elsewhere in New Zealand. '?' 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. Results exclude misidentified Primnoidae taxa that sequencing indicated were Plexauridae (n=6) or Pleurogorgiidae (n=7) – none of which occurred 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
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

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Appendix A

Table A-1: List of NIC specimens of Primnoidae from the Chatham Rise that were identified as being suitable for DNA sequencing attempts. Samples were collected since 1990, were preserved in ethanol and were located within the current NIC collections (*ie*. not on loan). Of the total list, a selection was chosen to represent the different sampling methods and the breadth of taxonomic diversity (Sampled = Y). Selected specimens were then sequenced at three loci, with an indication of success (Y), partial success (~) or failure (N) specified. Target fishery was only determined for those trawl specimens that produced useable sequence data.

Sampled	5'-MutS	3'-MutS	28S	NIWA Number	Sampling Method	Target Fishery	Original Identification	Station ID	Collection Date	Latitude	Longitude	Depth
Y	Y	Y	N	4033	Sled	N/A	*Plumarella Faxiella*	TAN0104/44	16/04/2001	-42.76	-179.99	977
				9615	Res Trawl		*Dasystenella austasensis*	TAN9812/31	9/10/1998	-44.58	-177.83	978
Y	N	N	N	9616	Res Trawl		*Dasystenella austasensis*	TAN9812/31	9/10/1998	-44.58	-177.83	978
Y	N	N	N	9623	Bycatch		*Metafannyella chathamensis*	TRIP1054/25	24/11/1997	-43.07	176.92	368
Y	Y	Y	N	9632	Res Trawl	OEO	*Dasystenella austasensis*	TAN9812/85	25/10/1998	-44.48	-178.52	940
				9634	Res Trawl		*Dasystenella austasensis*	TAN9812/15	2/10/1998	-44.21	179.06	959
Y	Y	Y	N	9635	Res Trawl	OEO	*Thouarella (Thouarella) variabilis var. gracilis*	TAN9812/03	29/09/1998	-44.23	178.44	1090
				9636	Res Trawl		*Metafannyella*	TAN9812/72	20/10/1998	-44.63	-176.98	848
				9637	Res Trawl		*Metafannyella chathamensis*	TAN9812/86	25/10/1998	-44.43	-178.52	868
Y	N	N	N	9640	Res Trawl		*Metafannyella chathamensis*	TAN9812/85	25/10/1998	-44.48	-178.52	940
Y	N	N	N	9651	Sled		*Thouarella*	TAN0104/48	16/04/2001	-42.79	-179.99	993
Y	Y	Y	Y	9658	Sled	N/A	*Thouarella*	TAN0104/394	21/04/2001	-42.76	-179.99	920
Y	N	N	N	9659	Sled		*Thouarella*	TAN0104/391	21/04/2001	-42.79	180.00	1044
Y	Y	Y	Y	9661	Sled	N/A	*Thouarella*	TAN0104/197	18/04/2001	-42.77	-179.93	987
Y	N	Y	N	9662	Sled	N/A	*Thouarella*	TAN0104/389	21/04/2001	-42.78	179.99	1000
				9663	Sled		*Thouarella*	TAN0104/337	20/04/2001	-42.77	-179.92	970
Y	Y	Y	Y	9664	Sled	N/A	*Thouarella*	TAN0104/288	19/04/2001	-42.76	-179.99	972
Y	N	N	N	9667	Sled		*Thouarella*	TAN0104/82	17/04/2001	-42.72	-179.96	1051
Y	Y	Y	N	9670	Sled	N/A	*Thouarella*	TAN0104/194	18/04/2001	-42.79	-180.00	1042
Y	Y	Y	Y	9673	Sled	N/A	*Thouarella*	TAN0104/150	18/04/2001	-42.72	-179.91	1181
				9675	Res Trawl		*Metafannyella chathamensis*	TAN9812/87	26/10/1998	-44.43	-178.62	843
Y	N	Y	N	9678	Sled	N/A	*Tokoprymno maia*	TAN0104/194	18/04/2001	-42.79	-180.00	1042
				9683	Sled		*Tokoprymno maia*	TAN0104/44	16/04/2001	-42.76	-179.99	977
Y	Y	N	N	9684	Bycatch	SCI	*Metafannyella chathamensis*	TRIP1054/25	24/11/1997	-43.07	176.92	368
				9685	Sled		*Tokoprymno maia*	TAN0104/48	16/04/2001	-42.79	-179.99	993
				9686	Sled		*Tokoprymno*	TAN0104/48	16/04/2001	-42.79	-179.99	993
				9697	Sled		*Narella hypsocalyx*	TAN0104/153	18/04/2001	-42.73	-179.90	1076
				9699	Sled		*Narella hypsocalyx*	TAN0104/391	21/04/2001	-42.79	180.00	1044
Y	N	N	N	9701	Sled		*Primnoella distans*	TAN0104/195	18/04/2001	-42.78	180.00	973
Y	Y	Y	N	9750	Res Trawl	OEO	*Dasystenella austasensis*	TAN9812/25	7/10/1998	-44.39	-178.16	805
Y	Y	N	N	9751	Res Trawl	OEO	*Thouarella (Thouarella) variabilis var. gracilis*	TAN9812/39	11/10/1998	-44.70	-177.30	1100
Y	Y	Y	N	9764	Res Trawl	OEO	*Metafannyella chathamensis*	TAN9812/47	12/10/1998	-44.58	-177.46	850
				11188	Sled		*Narella hypsocalyx*	TAN0104/336	20/04/2001	-42.77	-179.92	955
				11222	Res Trawl		*Narella hypsocalyx*	X495	7/07/1994	-44.00	-174.01	1436
				11223	Res Trawl		*Narella hypsocalyx*	X494	6/07/1994	-43.84	-174.30	885
				11758	Res Trawl		*Metafannyella chathamensis*	TAN9511/44	12/10/1995	-44.21	178.86	1004
Y	N	N	N	14677	Res Trawl		*Plumarella (Verticillata)*	TRIP1184/73	19/01/1999	-44.68	-175.08	1050
Y	Y	Y	N	14682	Res Trawl	ORH	*Tokoprymno maia*	AEX9901/69	4/07/1999	-42.73	-179.92	1055
				19330	Sled		*Thouarella*	TAN0104/391	21/04/2001	-42.79	180.00	1044
Y	N	Y	Y	25064	Sled	N/A	*Tokoprymno maia*	TAN0604/8	28/05/2006	-42.79	180.00	898
Y	Y	Y	N	25327	Sled	N/A	*Narella hypsocalyx*	TAN0604/111	7/06/2006	-42.80	179.99	970
Y	Y	Y	Y	25328	Sled	N/A	*Narella hypsocalyx*	TAN0604/111	7/06/2006	-42.80	179.99	970
Y	Y	Y	Y	25333	Sled	N/A	*Thouarella*	TAN0604/8	28/05/2006	-42.79	180.00	898
Y	Y	Y	N	25335	Sled	N/A	*Thouarella*	TAN0604/9	28/05/2006	-42.76	-179.93	1019
Y	Y	Y	Y	25345	Sled	N/A	*Thouarella*	TAN0604/25	29/05/2006	-42.76	-179.98	1017
Y	N	N	N	25347	Sled		*Primnoella distans*	TAN0604/31	30/05/2006	-42.79	180.00	1020
Y	Y	Y	N	25350	Sled	N/A	*Thouarella*	TAN0604/38	30/05/2006	-42.77	-179.93	930
				25359	Sled		*Primnoella distans*	TAN0604/105	4/06/2006	-42.73	-179.90	992
Too small				25367	Sled		*Thouarella*	TAN0604/116	7/06/2006	-42.80	179.99	950
Y	N	N	N	25367	Sled			TAN0604/116	07/06/2006	-42.80	179.99	950
				25381	Sled		*Thouarella (Euthouarella)*	TAN0604/7	28/05/2006	-42.78	180.00	917

Sampled	5'-MutS	3'-MutS	28S	NIWA Number	Sampling Method	Target Fishery	Original Identification	Station ID	Collection Date	Latitude	Longitude	Depth
Y	Y	N	N	25382	Sled	N/A	*Thouarella*	TAN0604/9	28/05/2006	-42.76	-179.93	1019
Y	Y	Y	N	25386	Sled	N/A	*Thouarella*	TAN0604/16	29/05/2006	-42.77	-179.99	993
Y	Y	Y	N	25390	Sled	N/A	*Thouarella*	TAN0604/16	29/05/2006	-42.77	-179.99	993
Y	Y	Y	Y	25400	Sled	N/A	*Thouarella*	TAN0604/31	30/05/2006	-42.79	180.00	1020
Y	Y	Y	N	25403	Sled	N/A	*Thouarella*	TAN0604/31	30/05/2006	-42.79	180.00	1020
Y	Y	Y	N	25410	Sled	N/A	*Thouarella*	TAN0604/39	30/05/2006	-42.79	-180.00	1021
Y	N	Y	N	25415	Sled	N/A	*Thouarella (Euthouarella)*	TAN0604/97	4/06/2006	-42.79	-179.99	882
Y	N	Y	N	25416	Sled	N/A	*Tokoprymno*	TAN0604/97	4/06/2006	-42.79	-179.99	882
Y	N	Y	N	25418	Sled	N/A	*Thouarella (Euthouarella)*	TAN0604/98	4/06/2006	-42.79	-179.99	960
				25422	Sled		*Thouarella (Euthouarella)*	TAN0604/99	4/06/2006	-42.79	-179.99	890
Y	Y	Y	N	25425	Sled	N/A	*Thouarella (Thouarella) variabilis var. gracilis*	TAN0604/105	4/06/2006	-42.73	-179.90	992
Y	N	Y	N	25426	Sled	N/A	*Tokoprymno*	TAN0604/106	5/06/2006	-42.73	-179.90	1030
Y	Y	Y	N	25427	Sled	N/A	*Thouarella*	TAN0604/108	6/06/2006	-43.53	179.63	375
Y	Y	Y	N	25428	Sled	N/A	*Metafannyella chathamensis*	TAN0604/110	7/06/2006	-43.53	179.63	378
Y	Y	Y	N	25491	Res Trawl	HOK	*Thouarella*	TAN0501/58	6/01/2005	-43.57	-178.82	447
				25504	Res Trawl		*Metafannyella chathamensis*	TAN0301/9	30/12/2003	-43.06	177.46	320
				25508	Res Trawl		*Metafannyella chathamensis*	TAN0401/67	12/01/2004	-43.93	178.77	613
				25683	Sled		*Thouarella*	TAN0604/11	28/05/2006	-42.72	-179.96	935
Y	Y	Y	~	27577	Res Trawl	HOK	*Thouarella*	TAN0701/56		-43.78	179.58	446
Y	Y	Y	Y	27606	Res Trawl	HOK	*Thouarella*	TAN0701/9	30/12/2006	-43.58	177.94	355
Y	Y	Y	Y	27624	Res Trawl	HOK	*Thouarella*	TAN0701/14	31/12/2006	-43.36	179.58	409
Y	Y	Y	N	28706	Sled	N/A	*Thouarella*	TAN0705/58	7/04/2007	-43.81	178.12	497
Y	Y	Y	N	28731	Sled	N/A	*Thouarella*	TAN0705/88	9/04/2007	-44.20	-178.93	470
Y	Y	Y	Y	28734	Sled	N/A	*Metafannyella chathamensis*	TAN0705/92	10/04/2007	-44.37	-178.49	804
Y	Y	Y	Y	28737	Sled	N/A	*Dasystenella austasensis*	TAN0705/94	10/04/2007	-44.57	-178.49	1110
Y	Y	Y	N	28746	Sled	N/A	*Dasystenella austasensis*	TAN0705/101	10/04/2007	-44.65	-178.46	1230
Y	Y	Y	Y	28748	Sled	N/A	*Thouarella*	TAN0705/103	11/04/2007	-44.08	-177.97	474
				28749	Sled		*Thouarella*	TAN0705/111	11/04/2007	-44.58	-176.08	415
				28816	Sled		*Thouarella (Thouarella) variabilis var. gracilis*	TAN0705/291	28/04/2007	-42.95	174.48	950
Y	Y	Y	N	34997	Res Trawl	ORH	*Thouarella*	TAN0709/116	22/07/2007	-44.48	-174.90	1199
Y	N	N	N	35284	Sled		*Thouarella*	TAN0604/108	6/06/2006	-43.53	179.63	375
				42413	Bycatch		*Thouarella*	TRIP2714/85	13/11/2008	-44.52	-175.29	709
Y	N	Y	N	42521	Bycatch	SSO	*Tokoprymno maia*	TRIP2710/7	23/10/2008	-44.75	173.72	940
Y	Y	Y	N	42522	Bycatch	ORH	*Thouarella*	TRIP2699/132	19/10/2008	-42.73	-177.70	1129
Y	N	N	N	42554	Bycatch		*Metafannyella chathamensis*	TRIP2101/128	14/06/2005	-42.73	-177.40	1107
Y	Y	Y	N	42557	Bycatch	ORH	*Thouarella*	TRIP2626/184	21/06/2008	-42.63	-179.88	1049
Y	Y	Y	N	44619	Bycatch	SSO	*Thouarella*	TRIP2520/38	13/11/2007	-44.69	-177.49	1209
				44623	Bycatch		*Thouarella*	TRIP2520/101	21/11/2007	-42.75	-177.81	932
				45311	Res Trawl		*Thouarella*	TAN0801/13	30/12/2007	-43.55	179.96	402
Y	Y	Y	Y	45314	Res Trawl	HOK	*Thouarella*	TAN0801/21	31/12/2007	-43.24	-179.46	508
Y	Y	Y	N	47734	Bycatch	SCI	*Thouarella*	TRIP1707/75	22/10/2002	-43.10	175.93	400
Y	N	Y	N	47926	Bycatch	ORH	*Narella*	TRIP2699/17	2/10/2008	-44.46	-174.89	1008
Y	Y	Y	N	53120	Sled	N/A	*Primnoidae*	TAN0905/40	17/06/2009	-42.78	-179.90	921
Y	N	N	N	53267	Sled		*Primnoella distans*	TAN0905/46	18/06/2009	-42.67	-179.96	1020
Y	N	Y	N	53275	Sled	N/A	*Tokoprymno maia*	TAN0905/48	18/06/2009	-42.64	-179.88	1052
Y	Y	Y	N	53305	Sled	N/A	*Primnoa*	TAN0905/60	20/06/2009	-42.81	-179.52	1251
Y	Y	Y	N	53309	Sled	N/A	*Tokoprymno*	TAN0905/60	20/06/2009	-42.81	-179.52	1251
				53321	Sled		Primnoidae	TAN0905/60	20/06/2009	-42.81	-179.52	1251
Y	Y	Y	Y	53323	Sled	N/A		TAN0905/60	20/06/2009	-42.81	-179.52	1251
Y	Y	Y	Y	53324	Sled	N/A	Thouarella (Thouarella) variabilis var. gracilis	TAN0905/60	20/06/2009	-42.81	-179.52	1251
Y	Y	Y	Y	53331	Sled	N/A		TAN0905/61	20/06/2009	-41.80	-179.50	1219
Y	Y	Y	N	53455	Sled	N/A	*Thouarella*	TAN0905/70	22/06/2009	-42.74	-179.69	840
				53456	Sled		*Narella hypsocalyx*	TAN0905/70	22/06/2009	-42.74	-179.69	840
Y	Y	Y	Y	53457	Sled	N/A	*Thouarella*	TAN0905/70	22/06/2009	-42.74	-179.69	840
Y	Y	N	N	53468	Sled	N/A	*Primnoidae*	TAN0905/70	22/06/2009	-42.74	-179.69	840
Too small				53482	Sled		*Thouarella*	TAN0905/70	22/06/2009	-42.74	-179.69	840
Y				53482	Sled			TAN0905/70	22/06/2009	-42.74	-179.69	840

Sampled	5'-MutS	3'-MutS	28S	NIWA Number	Sampling Method	Target Fishery	Original Identification	Station ID	Collection Date	Latitude	Longitude	Depth
Y	N	Y	N	53489	Sled	N/A	*Tokoprymno maia*	TAN0905/71	22/06/2009	-42.74	-179.69	820
No tissue				53549	Sled		*Primnoidae*	TAN0905/95	25/06/2009	-44.14	-174.72	613
Y	Y	Y	Y	53578	Sled	N/A	*Primnoidae*	TAN0905/97	26/06/2009	-44.15	-174.69	440
				53638	Sled		*Metafannyella chathamensis*	TAN0905/98	26/06/2009	-44.15	-174.70	720
Dried sample				53662	Sled		*Primnoella insularis*	TAN0905/99	26/06/2009	-44.14	-174.72	641
Y	Y	Y	N	53666	Sled	N/A	*Thouarella*	TAN0905/99	26/06/2009	-44.14	-174.72	641
				53733	Sled		*Metafannyella chathamensis*	TAN0905/101	26/06/2009	-44.13	-174.57	645
Y	Y	Y	N	53759	Sled	N/A	*Metafannyella chathamensis*	TAN0905/103	26/06/2009	-44.16	-174.56	520
				53830	Sled		*Thouarella*	TAN0905/105	26/06/2009	-44.16	-174.55	485
Y	N	Y	N	53848	Sled	N/A	*Thouarella*	TAN0905/105	26/06/2009	-44.16	-174.55	485
Too small				53851	Sled		*Thouarella*	TAN0905/105	26/06/2009	-44.16	-174.55	485
Y	N	Y	N	53942	Sled	N/A	*Primnoidae*	TAN0905/110	27/06/2009	-44.13	-174.57	650
Y	Y	Y	~	53949	Sled	N/A	*Primnoidae*	TAN0905/111	27/06/2009	-44.15	-174.69	458
				54038	Sled		*Metafannyella chathamensis*	TAN0905/112	27/06/2009	-44.14	-174.72	760
Y	Y	Y	Y	54039	Sled	N/A	*Thouarella*	TAN0905/112	27/06/2009	-44.14	-174.72	760
Y	Y	Y	~	54057	Sled	N/A	*Thouarella*	TAN0905/113	27/06/2009	-44.15	-174.76	519
Y	Y	Y	Y	54067	Sled	N/A	*Primnoidae*	TAN0905/113	27/06/2009	-44.15	-174.76	519
Dried sample				54069	Sled		*Primnoella insularis*	TAN0905/113	27/06/2009	-44.15	-174.76	519
Y	Y	Y	N	54123	Sled	N/A	*Primnoidae*	TAN0905/114	27/06/2009	-44.15	-174.77	830
Y	Y	Y	N	54141	Sled	N/A	*Thouarella*	TAN0905/115	27/06/2009	-44.14	-174.72	610
Y	Y	Y	Y	54157	Sled	N/A	*Thouarella*	TAN0905/115	27/06/2009	-44.14	-174.72	610
Y	Y	Y	Y	54235	Sled	N/A	*Plumarella*	TAN0905/118	27/06/2009	-44.16	-174.45	1040
Y	Y	Y	N	54250	Sled	N/A	*Thouarella*	TAN0905/119	28/06/2009	-44.16	-174.56	487
Y	Y	Y	Y	54312	Sled	N/A	*Thouarella*	TAN0905/120	28/06/2009	-44.03	-174.59	796
Y	Y	Y	N	54316	Sled	N/A	*Metafannyella chathamensis*	TAN0905/120	28/06/2009	-44.03	-174.59	796
Y	N	N	N	54318	Sled		*Primnoidae*	TAN0905/120	28/06/2009	-44.03	-174.59	796
Y	N	N	N	54326	Sled		*Primnoella distans*	TAN0905/120	28/06/2009	-44.03	-174.59	796
Y	Y	Y	Y	54329	Sled	N/A	*Narella hypsocalyx*	TAN0905/121	28/06/2009	-44.03	-174.59	801
Y	Y	Y	N	54340	Sled	N/A	*Thouarella*	TAN0905/121	28/06/2009	-44.03	-174.59	801
Y	Y	Y	N	54341	Sled	N/A	*Primnoidae*	TAN0905/121	28/06/2009	-44.03	-174.59	801
Y	Y	Y	N	54354	Sled	N/A	*Narella hypsocalyx*	TAN0905/121	28/06/2009	-44.03	-174.59	801
Y	N	N	N	65527	Bycatch		*Tokoprymno maia*	TRIP3028/133	10/01/2010	-44.46	-178.59	730
Y	Y	Y	Y	66292	Bycatch	ORH	*Thouarella*	TRIP2862/113	7/06/2009	-42.77	-177.25	965
Y	N	N	N	65529	Bycatch		*Primnoa notialis*	TRIP3028/127	9/01/2010	-44.45	-178.66	690
Y	Y	Y	N	65528	Bycatch	ORH	*Primnoa notialis*	TRIP3028/136	10/01/2010	-44.45	-178.60	735
				65534	Bycatch		*Calyptrophora inornata*	TRIP2744/75	30/12/2008	-43.18	-173.84	987
Y	N	N	N	65535	Bycatch		*Calyptrophora inornata*	TRIP2744/252	23/01/2009	-44.00	-174.59	810
Y	N	N		65536	Bycatch		*Calyptrophora inornata*	TRIP2744/253	23/01/2009	-43.96	-174.58	659
				65545	Bycatch		*Primnoa notialis*	TRIP2807/241	9/03/2009	-44.51	-174.81	1270
Y	Y	Y	N	65530	Bycatch	ORH	*Primnoa notialis*	TRIP3028/131	10/01/2010	-44.46	-178.59	
Y	Y	Y	Y	66289	Bycatch	ORH	*Metafannyella chathamensis*	TRIP3004/33	24/11/2009	-44.46	-178.59	710
Y	Y	Y	N	65546	Bycatch	ORH	*Calyptrophora inornata*	TRIP2744/162	12/01/2009	-43.96	-174.57	669
Y	N	Y	N	66285	Bycatch	ORH	*Tokoprymno*	TRIP2955/26	7/10/2009	-44.70	-175.36	1085
Y	Y	Y	N	66287	Bycatch	SSO	*Metafannyella chathamensis*	TRIP3004/43	25/11/2009	-44.68	-176.97	947
Y	Y	Y	N	66288	Bycatch	SSO	*Metafannyella chathamensis*	TRIP3004/80	1/12/2009	-44.62	-177.40	891
Y	Y	Y	N	66290	Bycatch	SSO	*Metafannyella chathamensis*	TRIP3004/35	24/11/2009	-44.53	-177.85	911
Y	N	N	N	66298	Bycatch		*hawaiiensis*	TRIP2894/80	12/07/2009	-35.25	165.23	915.0
Y	N		N	66306	Bycatch		*Primnoidae*	TRIP2955/165	26/10/2009	-44.46	178.59	730
Y	N	Y	N	66309	Bycatch	ORH	*Tokoprymno maia*	TRIP2955/33	8/10/2009	-44.47	-174.90	1006
				66313	Bycatch		*Thouarella*	TRIP2970/105	3/12/2009	-44.45	-178.75	977
Y	N	N	N	68214	Res Trawl		*Tokoprymno maia*	TAN9406/254	4/07/1994	-42.74	-179.67	817
Y	Y	Y	N	69574	Bycatch	HOK	*Calyptrophora inornata*	TRIP3235/14	1/12/2010	-42.97	178.46	447
Y	Y	Y	N	70523	Res Trawl	HOK	*Metafannyella chathamensis*	TAN1101/98	22/01/2011	-43.81	178.46	450
Too small				70700	Bycatch		*Calyptrophora inornata*	TRIP2744/189	15/01/2009	-43.94	-174.55	754
Y	Y	Y	N	91991	Res Trawl	HOK	*Metafannyella chathamensis*	TAN1401/5	3/01/2014	-42.88	176.37	524
Y	Y	Y	Y	91997	Res Trawl	HOK	*Thouarella*	TAN1401/106	21/01/2014	-42.92	174.87	744
Dried sample				99701	Sled		*Primnoella insularis*	TAN0905/113	27/06/2009	-44.15	-174.76	519

Sampled	5'-MutS	3'-MutS	28S	NIWA Number	Sampling Method	Target Fishery	Original Identification	Station ID	Collection Date	Latitude	Longitude	Depth
Y	N	N	N	102180	Bycatch		*Narella*	TRIP1307/53	28/01/2000	-44.75	175.87	1020
Y	Y	Y	Y	102298	Sled	N/A	*Tokoprymno*	TAN1503/44	2/04/2015	-42.77	-179.92	990
			N	102308	Sled		*Tokoprymno*	TAN1503/56	3/04/2015	-42.79	-179.99	918
Y	N	Y	N	102309	Sled	N/A	*Tokoprymno*	TAN1503/56	3/04/2015	-42.79	-179.99	918
Y	Y	Y	Y	102336	Sled	N/A	*Narella hypsocalyx*	TAN1503/67	4/04/2015	-42.80	179.99	936
Y	N	Y	N	102361	Sled	N/A	*Tokoprymno*	TAN1503/67	4/04/2015	-42.80	179.99	936
Y	Y	N	N	102378	Sled	N/A	*Primnoella*	TAN1503/101	9/04/2015	-44.18	-174.51	1005
Y	Y	Y	Y	102380	Sled	N/A	*Primnoeides*	TAN1503/101	9/04/2015	-44.18	-174.51	1005
Y	Y	Y	N	102401	Sled	N/A	*Thouarella*	TAN1503/101	9/04/2015	-44.18	-174.51	1005
Y	Y	Y	Y	102402	Sled	N/A	*Thouarella*	TAN1503/102	9/04/2015	-44.17	-174.45	963
Y	Y	N	N	102403	Sled	N/A	*Primnoeides*	TAN1503/102	9/04/2015	-44.17	-174.45	963
Y	Y	Y	N	102433	Sled	N/A	*Tokoprymno*	TAN1503/102	9/04/2015	-44.17	-174.45	963
Y	Y	Y	N	102443	Sled	N/A	*Primnoeides*	TAN1503/103	9/04/2015	-44.18	-174.45	1099
Y	Y	Y	N	102451	Sled	N/A	*Thouarella*	TAN1503/103	9/04/2015	-44.18	-174.45	1099
Y	Y	Y	Y	102463	Sled	N/A	*Tokoprymno*	TAN1503/103	9/04/2015	-44.18	-174.45	1099
Y	Y	N	N	102471	Sled	N/A	*Primnoidae*	TAN1503/116	11/04/2015	-44.16	-174.55	497
Y	Y	Y	N	102508	Sled	N/A	*Primnoeides*	TAN1503/117	11/05/2014	-44.13	-174.57	740
Y	N	Y	N	102509	Sled	N/A	*Primnoidae*	TAN1503/117	11/05/2014	-44.13	-174.57	740
Y	Y	Y	N	102558	Sled	N/A	*Primnoeides*	TAN1503/119	11/04/2015	-44.20	-174.54	846
Y	Y	Y	N	102618	Sled	N/A	*Primnoella*	TAN1503/121	11/04/2015	-44.14	-174.71	724
				102632	Sled		*Primnoidae*	TAN1503/121	11/04/2015	-44.14	-174.71	724
				106208	Res Trawl		*Primnoidae*	TAN1511/134	20/08/2015	-43.37	178.94	394
				113820	Res Trawl		*Metafannyella chathamensis*	AEX1601/OP14	21/06/2016	-42.80	-177.83	806
				113999	Res Trawl		*Dasystenella austasensis*	TAN9511/18	8/10/1995	-44.66	174.89	818
Y	Y	Y	N	126870	Res Trawl	Multi-spp.	*Narella hypsocalyx*	TAN1801/25	11/01/2018	-42.45	-178.00	865
Y	Y	Y	N	126957	Res Trawl	Multi-spp.	*Thouarella*	TAN1801/48	15/01/2018	-43.54	-175.22	243
Y	Y	N	N	127411	Sled	N/A	*Dasystenella austasensis*	TAN0705/99	10/04/2007	-44.56	-178.48	1076
Y	N	N	N	127416	Res Trawl		*Tokoprymno maia*	AEX9901/11	24/06/1999	-42.64	-179.99	1270
Y	N	N		127502	Res Trawl		*Tokoprymno*	X484	4/07/1994	-42.77	-179.91	899
Y	Y	Y	Y	128287	Sled	N/A	*Primnoidae*	TAN0705/53	7/04/2007	-44.25	177.15	955
Y	Y	Y	N	131940	Bycatch	ORH	*Calyptrophora*	TRIP5844/32	3/12/2019	-42.73	-177.73	1156
Y	Y	Y	N	140319	Sled	N/A	*Primnoidae*	TAN1903/107	21/06/2019	-43.37	179.45	391
				141784	Res Trawl		*Primnoidae*	TAN2001/63	19/01/2020	-44.50	-178.51	991
				141795	Res Trawl		*Primnoidae*	TAN2001/64	19/01/2020	-44.41	-178.84	865
				148120	Sled		*Thouarella*	TAN2009/57	16/08/2020	-44.16	-174.55	486
				148133	Sled		*Thouarella*	TAN2009/58	16/08/2020	-44.20	-174.54	782
				148162	Sled		*Thouarella*	TAN2009/80	19/08/2020	-44.14	-174.72	640
				154698	Sled		*Thouarella*	TAN2009/80	19/08/2020	-44.14	-174.72	640

Appendix B

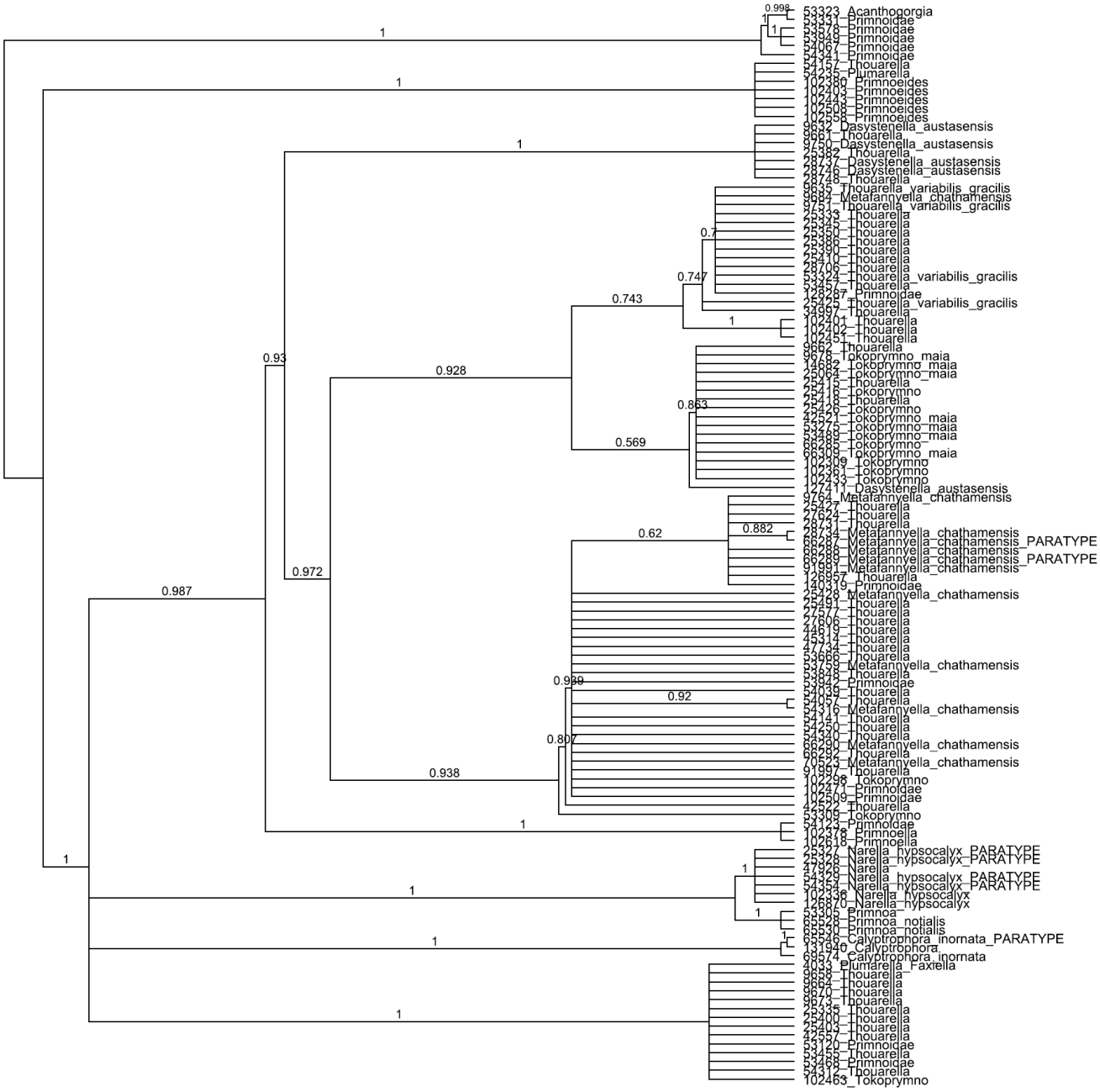

Figure B-1: Phylogeny of primnoids sequenced using two mtMutS loci. Original output of the Bayesian phylogenetic analysis of all sequenced specimens, showing individual NIWA accession numbers and their original identifications. Numbers next to branches indicate posterior probability values. 'PARATYPE' refers to sequenced specimens that represent paratypes included in the original taxonomic description for that species. Refer to Figure 3-1 for updated identifications of clades, based on comparison to reference sequences from previous studies (Cairns & Wirshing 2018; Taylor & Rogers 2015).

Appendix C

Table C-1: UCE sequencing results summary. 'Concentration' = concentration of gDNA extracted from specimen tissues; 'Post-trimming statistics' = summary of sequence data after implementing *trimmomatic* quality control; 'Post-assembly statistics' = summary of contigs assembled by *SPAdes*; 'Post-UCE matching statistics' = summary of assembled contigs that were matched to UCE loci.

		Post-trimming statistics							Post-assembly statistics								Post-UCE/exon matching statistics							
Sample name	*Concentration (ng/µl)*	*# reads*	*sum of read lengths*	*average read length*	*SE of read length*	*min read length*	*max read length*	*median read length*	*# contigs*	*total bp*	*mean length*	*95 CI length*	*min length*	*max length*	*median length*	*# contigs >1kb length*	*# contigs*	*total bp*	*mean length*	*95 CI length*	*min length*	*max length*	*median length*	*# contigs >1kb length*
102298	2.9	17785970	2332835438	131	0.01	40	151	151	70147	20307513	289	1.19	71	7164	197	3036	1749	1847572	1056	10.61	92	2926	1053	947
102402	34.4	24254854	3331368348	137	0.01	40	151	151	121576	33803976	278	0.85	51	10438	220	3919	2177	2067554	950	8.23	64	4296	927	917
102463	10.3	15658114	2052382203	131	0.01	40	151	151	334610	73848214	221	0.48	53	14805	147	5231	2209	1486487	673	5.96	93	5866	651	157
128287	44.8	24603905	3420054326	139	0.01	40	151	151	55615	25127337	452	1.81	67	7182	296	5297	1787	2169362	1214	12.69	149	6504	1185	1190
25426	8.5	27192052	3798472709	140	0.00	40	151	151	513967	117152183	228	0.47	47	18511	124	11611	2250	2356162	1047	10.08	208	5416	1013	1159
28746	6.9	8601085	1038013342	121	0.01	40	151	130	80203	17199393	214	0.72	51	3757	149	734	2131	1308197	614	5.17	79	3339	594	108
53275	16.1	22072809	3099086133	140	0.01	40	151	151	164292	73764127	449	2.34	74	249035	287	11296	1889	2440380	1292	20.16	148	8447	1122	1158
53305	54.2	23849448	3260341629	137	0.01	40	151	151	177428	73353379	413	1.05	76	22389	298	8761	2050	2372120	1157	15.53	159	7767	1037	1120
54329	44.1	24744145	3365499655	136	0.01	40	151	151	196699	76598192	389	0.85	73	18948	290	8215	1667	1578075	947	14.39	97	7000	853	538
65546	3.5	24228142	3304092488	136	0.01	40	151	151	195887	81295514	415	2.04	76	260033	284	8852	1813	1742157	961	13.58	132	8319	886	620
66289	0.6	6775878	778401136	115	0.01	40	151	119	118382	18080966	153	0.45	53	19000	84	285	2168	811802	374	2.44	76	1166	358	4
91997	27	23711095	3242302235	137	0.01	40	151	151	134466	35308398	263	0.70	51	5645	222	3341	2080	2004011	963	7.91	113	3167	970	967