DRAFT

Coral biodiversity in deep-water fisheries bycatch INT2019-05

Draft Final Report

Prepared for Conservation Services Programme Technical Working Group, Department of Conservation

July 2020

Prepared by:
Jaret Bilewitch
Di Tracey

For any information regarding this report please contact:
Jaret Bilewitch
Molecular Biologist
Environmental Isotopes & Molecular Biology
+64-4-386 0502
jaret.bilewitch@niwa.co.nz

National Institute of Water & Atmospheric Research Ltd
Private Bag 14901
Kilbirnie
Wellington 6241

Phone +64 4 386 0300

NIWA CLIENT REPORT No: 2020223WN
Report date: July 2020
NIWA Project: DOC19304

Quality Assurance Statement		
	Reviewed by:	Rachael Peart
	Formatting checked by:	Alex Quigley
	Approved for release by:	Rosie Hurst

Cover photo: Bamboo coral and stony coral samples in fisheries bycatch. [Observer photo, MPI Fisheries Observer Programme]

Bilewitch, J.P., Tracey, D. (2020) Coral biodiversity in deep-water fisheries bycatch. Prepared by NIWA for the Conservation Services Programme, Department of Conservation. *NIWA Client Report* 2020223WN: 29.

Contents

Tables

Figures

Executive summary

The overlap in habitat between deep-sea corals and commercial fish species results in unintentional bycatch, particularly for bottom trawling fisheries, such as Tier 1 deep-water stocks. The impact of fisheries on coral communities, which are protected under New Zealand law, has typically been measured as affected biomass and visual estimates of biodiversity. Among protected gorgonian corals, the identification of species by *in situ* observations and morphological study of specimens are known to underestimate species diversity, however. Using archived specimens collected by Government Fisheries Observers, we examined the genetic diversity of bottom-trawled bycatch gorgonian corals to determine the accuracy and precision of observer and taxonomist identifications, and to re-examine the effects of bottom trawling on protected coral diversity.

A pool of 129 bycatch specimens of gorgonian corals was identified and 91 of these were sampled for genetic analysis, producing viable DNA sequence data for 62 specimens at three genetic markers. Among these, we found a minimum of 34 different species that were distributed among seven protected families of octocorals. Our rate of discovery of new species indicates that many more species remain unsampled within the bycatch community. In addition, our results present the first broad-scale examination of octocoral diversity in New Zealand and demonstrate that many species remain to be discovered and described.

Comparisons of bycatch identification methods indicated an increasing level of precision and accuracy with increased technicality as specimens were progressed from visual identifications by observers, to morphological identifications by taxonomists, to genetic barcoding in this study. Overall, genetic barcoding and morphological study showed similarly high levels of identification accuracy, but barcoding provided increased resolution of identification, to finer taxonomic scales. Our 8% estimate of undiscovered diversity among bycatch specimens identified with traditional methods is also consistent with previous studies of New Zealand bycatch diversity.

The genetic and taxonomic diversity uncovered here was spread across the New Zealand Exclusive Economic Zone (EEZ) and adjacent South Pacific Regional Management Organisation (SPRFMO) zones. Within the EEZ, bycatch samples were examined from seven Fisheries Management Areas (FMAs) and ten target fisheries, with most specimens and most diversity recovered from the orange roughy bottom-trawl fishery. Although our sampling design did not allow for quantitative analyses of interactions for each fishery, we observed linear relationships between new species discovery and the number of bycatch-containing bottom trawls, indicating that we have not yet documented the limits of gorgonian coral diversity within the sampled bycatch community.

The high diversity of gorgonian octocorals uncovered within bycatch supports a role for genetic barcoding in routine identification and assessment of fisheries impacts. We recommend a focused genetic assessment and comparison of coral diversity within each trawl fishery and a consideration for evolutionary and genetic diversity in impact assessments and management decisions. Better understanding of the evolutionary processes that underpin the diversity of affected corals can improve our predictions of how they may be impacted by commercial fisheries, as well as their ability to recover from these impacts. Improved baseline information on genetic diversity will also improve conservation efforts through improved consideration of the evolutionary and genetic mechanisms that have produced New Zealand's protected coral species.

1 Background

New Zealand's deep-sea corals represent a diverse and wide-spread assemblage of animals that are often found in association with hard-bottom regions, including seamounts, ridges and continental shelf edges (Rowden et al. 2002; Tracey et al. 2011; reviewed in Tracey & Hjorvarsdottir 2019). Their three-dimensional growth forms supplement the topography and rugosity of the benthic environment, providing relief and refuge for demersal fish and invertebrate species (Husebø et al. 2002; Buhl-Mortenson & Mortensen 2005; Milligan et al. 2016). The association of coral ecosystems with commercially important fish species has resulted in anthropogenic disturbance as a result of gear interactions with the benthic environment (Clark et al. 2016, Yoklavich et al. 2018). The severity of such interactions may be extensive (Clark et al. 2016) and long-lasting (Clark et al. 2019), prompting the Department of Conservation to list several families of corals as protected in a 2010 amendment to Schedule 7A of the Wildlife Act 1953: the Order Antipatharia (black corals), the Order Scleractinia (hard- or stony corals), the Family Stylasteridae (hydrocorals), and the 'gorgonian' octocorals (previously species belonging to Order Gorgonacea, which was subsumed into Order Alcyonacea by Bayer (1981); see also McFadden et al. (2006)).

Past examinations and predictions of the impacts of commercial fishing activities (particularly bottom trawling) on deep-sea corals have focused on, for example, the correspondence of trawling paths (trawl footprint) to observed (Tracey et al. 2011) and predicted coral habitat (Anderson et al. 2020), direct observations of the extent and recovery of damaged habitat (Clark et al. 2019; Yoklavitch et al. 2018), and assessments of life-history traits and distributional characteristics in the form of a pilot risk assessment (Clark et al. 2014). For over a decade, Government Fisheries Observers (referred to as observers throughout) placed aboard fishing vessels have also been documenting fishery impacts as the occurrence of non-target species ('bycatch') in commercial catch. Observer documentation includes sampling protected coral bycatch and depositing voucher specimens within the NIWA Invertebrate Collection (NIC), although historically some samples were deposited in Te Papa (Blom et al. 2009; Tracey & Sanders 2010). Observer photographs and voucher material are examined by taxonomists and other expert identifiers. This identified bycatch component has been used as an estimate of fisheries impacts, both in terms of biomass (e.g., Anderson & Clark 2003) and biodiversity (e.g., Probert et al. 1997, Blom et al. 2009). However, estimates of coral bycatch biodiversity have relied on morphological identification, which often underestimates the 'true' species diversity present (e.g., McFadden et al. 2014; Quattrini et al. 2019). To our knowledge, there has not been a genetic assessment of the diversity of coral species impacted by commercial fisheries within New Zealand.

The goal of this project was to use recent observer collections of coral voucher specimens to genetically quantify the species-level diversity contained within deep-water fisheries bycatch, to improve our understanding of fishery impacts on biodiversity. Morphology-based identification of voucher specimens and observer photographs is often incapable of relating them to species and genus names, particularly for protected gorgonian octocorals (Anthozoa: Octocorallia: Alcyonacea (=Gorgonacea)) that display a range of similar forms between different genera and families (Sánchez 2004; Bilewitch et al. 2014), and excessive plasticity in form within a given species (Sánchez et al. 2007; Bilewitch et al. 2010). As such, current measures of octocoral biodiversity within the bycatch 'community' may underestimate actual species diversity, by overlooking genetically distinct, but visually similar species. We therefore used genetic barcoding to establish how many distinct and

potentially undocumented (cryptic) species are present among recent Observer collections in NIC from Tier-1 deep-water trawl fishery bycatch (e.g., hoki, hake, oreos, orange roughy). This study also contributes data on the taxonomy, genetic diversity and relationships of the breadth of New Zealand's gorgonian octocorals, which has not previously been attempted except on a per-family basis (e.g., Herrera et al. 2010, Dueñas et al. 2014).

2 Methods

2.1 Specimen selection, sampling and DNA extraction

Samples of protected octocorals were selected from the NIWA Invertebrate Collection in Wellington, using a filtered query of the Specify *niwainvert* database. Specimens were chosen that qualified as:

1. Subclass Octocorallia.
2. Not a member of unprotected soft coral families (e.g., Alcyoniidae, Anthothelidae, Clavulariidae).
3. Preserved in alcohol or were frozen vouchers (i.e., not dried specimens).
4. Sampled by government observers as commercial fisheries bycatch and not from a research cruise (assigned a TRIP number).
5. Originally collected since November 2009 and had a TRIP number ≥3000.
6. Obtained as bycatch from bottom trawls.
7. Not from outside the EEZ, particularly specimens collected from Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) Antarctic regions. However, some SPRFMO samples were included since they occurred in neighbouring regions that may be representative of- or contiguous with- habitats and ecosystems contained within EEZ boundaries.

Also included were additional reference specimens for increased taxonomic representation, including some bycatch specimens from bottom longline fisheries (n=8), a specimen of *Swiftia* collected in 2008, some non-bycatch specimens from research voyages (n=4) and representatives of *Corallium* from Antarctic (CCAMLR) regions (n=3).

Specimens were photographed and approximately 10mg (0.5cm^3) was dissected using sterile forceps. The tissue sample was soaked in distilled water to remove trace ethanol prior to DNA extraction using a combined salting-out/commercial kit method. Briefly, tissues were digested in cell lysis buffer (100mM Tris-HCl, 100mM EDTA, 1%SDS, 55mM DTT) with 200-500mg of proteinase K at 56°C overnight (as per TL Jenkins – Univ. of Exeter, pers. comm.). Protein was precipitated by addition of 7.5M ammonium acetate, followed by purification using the AL, AW1 and AW2 steps of a DNeasy Blood & Tissue kit as per the manufacturer's protocol (Qiagen Inc.), with final DNA elution into 50ul of AE buffer.

2.2 Selection of loci for octocoral genotyping

Since protected octocoral taxa ('gorgonian' growth forms) span the breadth of the octocoral tree-of-life, we identified known genetic markers with conserved regions potentially capable of acting as a 'universal' octocoral barcode at genus- or species-level. The 5'-end of the *mtMutS* mitochondrial gene was chosen as it has been extensively used for higher-level (families, genera, some species) octocoral systematics (McFadden et al. 2010), as well as a region of the *28S* rDNA gene that has previously been used to elucidate finer scale relationships (genera and species) (McFadden & van Ofwegen 2012). Although a large pre-existing dataset is available for the 5'-end of *mtMutS* in GenBank, it was previously noted that a downstream region in 'Domain III' of the gene has higher levels of information content (Bilewitch et al. 2014), although it is deficient for many octocoral taxa.

We thus explored the individual and combined performance of these three separate loci in their ability to discriminate a broad range of taxonomic scales for the Alcyonacea, as expected from a diverse bycatch assemblage.

2.3 PCR amplification & DNA sequencing

Amplifications of target loci were conducted using primers AnthoCorMSH (AGG AGA ATT YTA AGT ATG G; modified from Herrera et al. 2010) plus Mut-3458R (TGR AGC AAA AGC CAC TCC; modified from Sánchez et al. 2003) for the 5'-end of *mtMutS* and mtMutS-DIII_IntF (TCT TTA CAT CGT CAA TGG GCA AT; this study) plus mtMutS-DV_R (AAA CTA ATA TYA TGA GCT ACA CAT TCT; modified from Bilewitch et al. 2014) for the 3'-end of *mtMutS*. Initially we used 28S_F (CAC GAG ACC GAT AGC GAA CAA GTA) plus 28S_R (TCG CTA CGA GCT TCC ACC AGT GTT T) for the *28S* rDNA region (McFadden & van Ofwegen 2012), but found amplification success to be variable, so we designed new primers 28S_Univ_F (GCG AAC AAG TAC YTG GAG) and 28S_Univ_R (AGT GTT TCC KCT GGC TTC) based on preliminary sequence data. All PCR reactions were conducted in 25ul total volumes containing 1X MyTaq RedMix (Bioline Inc.), 0.5uM of each primer and 2-4ul of DNA extract. PCR thermocycling conditions for each locus are given in Table 1. Amplification products were visualised on 1% agarose gels via electrophoresis and successful reactions were purified using 0.5 units of ExoSAP-IT (ThermoFisher Sci. Inc.) following the manufacturer's recommendations and were submitted to a commercial facility for DNA sequencing (Macrogen Inc.).

Table 1: Genetic loci used for PCR amplification and DNA sequencing. 'Size' = estimated size range of the resulting amplicon; 'PCR profile' = optimised PCR thermocycling conditions.

Locus Name	Size (bp)	PCR profile
5'-*mtMutS* mtDNA	850-1000bp	95°C/3min, (95°C/15s, 50°C/20s, 72°C/25s)^{35}, 72°C/2min
3'-*mtMutS* mtDNA	850-1000bp	95°C/3min, (95°C/15s, 51°C/20s, 72°C/25s)^{35}, 72°C/2min
28S rDNA	700-900bp	95°C/3min, (95°C/15s, 54°C/15s, 72°C/20s)^{35}, 72°C/2min

2.4 Data analysis

Chromatograms of DNA sequences were visually inspected for quality and errors and were trimmed and assembled using Geneious Prime software v2019.0.3 (Biomatters Ltd.). Sequences were submitted to GenBank-BLASTn, to ensure they did not result from contaminating organisms. For each locus, sequences were aligned using MAFFT v7.388 (Katoh & Standley 2013) and were manually adjusted wherever necessary. Additional sequences of octocorals were obtained from GenBank and included in alignments for reference purposes.

Bayesian phylogenetic analysis of aligned DNA matrix was conducted using MrBayes v3.2.6 (Huelsenbeck & Ronquist 2001). Each locus was analysed separately and as a combined (concatenated) dataset using a GTR+G distance correction model, with four chains of 10^6 MCMC steps sampled at 10^3 intervals and 10^5 steps discarded as burn-in. Analysis of the combined multi-locus dataset used partitioned model parameters as independent estimates for each locus. The posterior output was examined for evidence of convergence in all model parameters. Resulting trees were rooted based on known higher-level relationships of the Octocorallia (McFadden et al. 2006; McFadden & van Ofwegen 2012).

The genetic diversity of trawl bycatch octocorals was used to examine the efficacy of identification. Genetic identities were compared to tentative specimen identifications made by vessel-based government fisheries observers, and to expert identifications made by taxonomists and parataxonomists. Both misidentification and differences in taxonomic resolution of identification (e.g., PRI – Family Primnoidae *vs.* THO – the primnoid genus *Thouarella*) were considered.

We also examined patterns of bycatch genetic diversity in relation to target fishery. The Centralised Observer Database (*COD;* MPI-FNZ, administered by NIWA) was used to obtain a list of observed bycatch records for protected octocorals from bottom trawling fisheries since 10/2009. Query conditions are given in Appendix A. *COD* records for target fishery were matched to sampled specimens by matching trip and station number.

3 Results

3.1 DNA sequencing coverage

A total of 129 specimens within the NIWA Invertebrate Collection were identified as meeting the criteria for inclusion in this study (see section 2.1 for details). Of these, 87 were sampled for genetic analysis, but three specimen containers were found to include multiple specimens, which were treated as separate samples. This increased the total number of extracted bottom-trawl-bycatch samples to 91. Of these, 41 specimens did not produce PCR products for any of the three target loci. Sequence data for an additional eight specimens in our list were obtained from previous studies, via GenBank (seven for 5'-*mtMutS*, one for *28S*) (Appendix B). We also obtained new sequence data for an additional 16 reference NIC specimens (see section 2.1), which were used to expand the taxonomic completeness of the phylogenetic analyses. In total, we were able to obtain new DNA sequence data for seven protected octocoral families: Acanthogorgiidae, Chrysogorgiidae, Coralliidae, Isididae, Paragorgiidae, Plexauridae and Primnoidae. The numbers of qualifying bycatch specimens sequenced for each family are given in Table 2.

Table 2: Sequencing and depth coverage of protected octocoral families. '# Sequenced' = number of specimens from which sequence data was recovered. 'Sampled Depth Range' only includes sequenced specimens of bottom-trawl bycatch, not reference material. [a] = additional reference samples in parentheses; [b] = seven additional specimen sequences obtained from past studies via GenBank; [c] = one additional specimen sequence via GenBank.

Protected Families	# Sequenced	Sampled Depth Range (m)
Acanthogorgiidae	3 (+1) [a]	137 - 967
Chrysogorgiidae	8	437 - 1200
Coralliidae	(+3)	-
Isididae	15 [b]	431 - 1208
Paragorgiidae	9 (+1)	541 - 1228
Plexauridae	7 (+7)	263 - 1182
Primnoidae	9 (+4) [b]	447 - 1100

The newly generated DNA sequence dataset contained 65 sequences of 5'-*mtMutS* (828bp), 54 sequences of 3'-*mtMutS* (844bp) and 38 sequences of 28S (716bp). A total of 34 specimens were sequenced at all three target loci, 19 were sequenced for two loci and 17 produced sequence data for only a single locus. The highest pairwise genetic distances were observed for the 28S dataset (54%), followed by 3'-*mtMutS* (31%), then 5'-*mtMutS* (30%). The 5'-*mtMutS* region contained three highly variable insertion/deletion (indel) regions, with ambiguous alignment positions in each. We thus compared phylogenetic results with and without inclusion of these three variable indel regions. The 3'-*mtMutS* alignment had one large indel but alignment was unambiguous thus no modifications of this locus were considered. High levels of variability in the *28S* locus meant that DNA alignments of highly divergent taxa could not be accomplished without ambiguity. To avoid this issue, the *28S* locus was considered on a per-family basis, rather than across the breadth of all sampled octocorals.

3.2 Taxonomic diversity of sequenced bycatch community

Results of the phylogenetic analyses indicated a diverse assemblage of octocoral species are present among the sampled bycatch community (Figure 1; Appendix C). Of the 62 bottom-trawled specimens included in our analyses (54 sequenced here plus eight from previous studies), there are (at least) 34 unique genotypes indicative of distinct octocoral species. Each locus included a different proportion of the total specimen pool, and thus recovered a slightly different complement of distinct taxa. 5'-*mtMutS* displayed 28 distinct taxa among 61 specimens (46%), 3'-*mtMutS* recovered 26 species among 45 specimen sequences (58%), and a combined analysis with both markers plus *28S* recovered 34 species among 63 specimen sequences (54%).

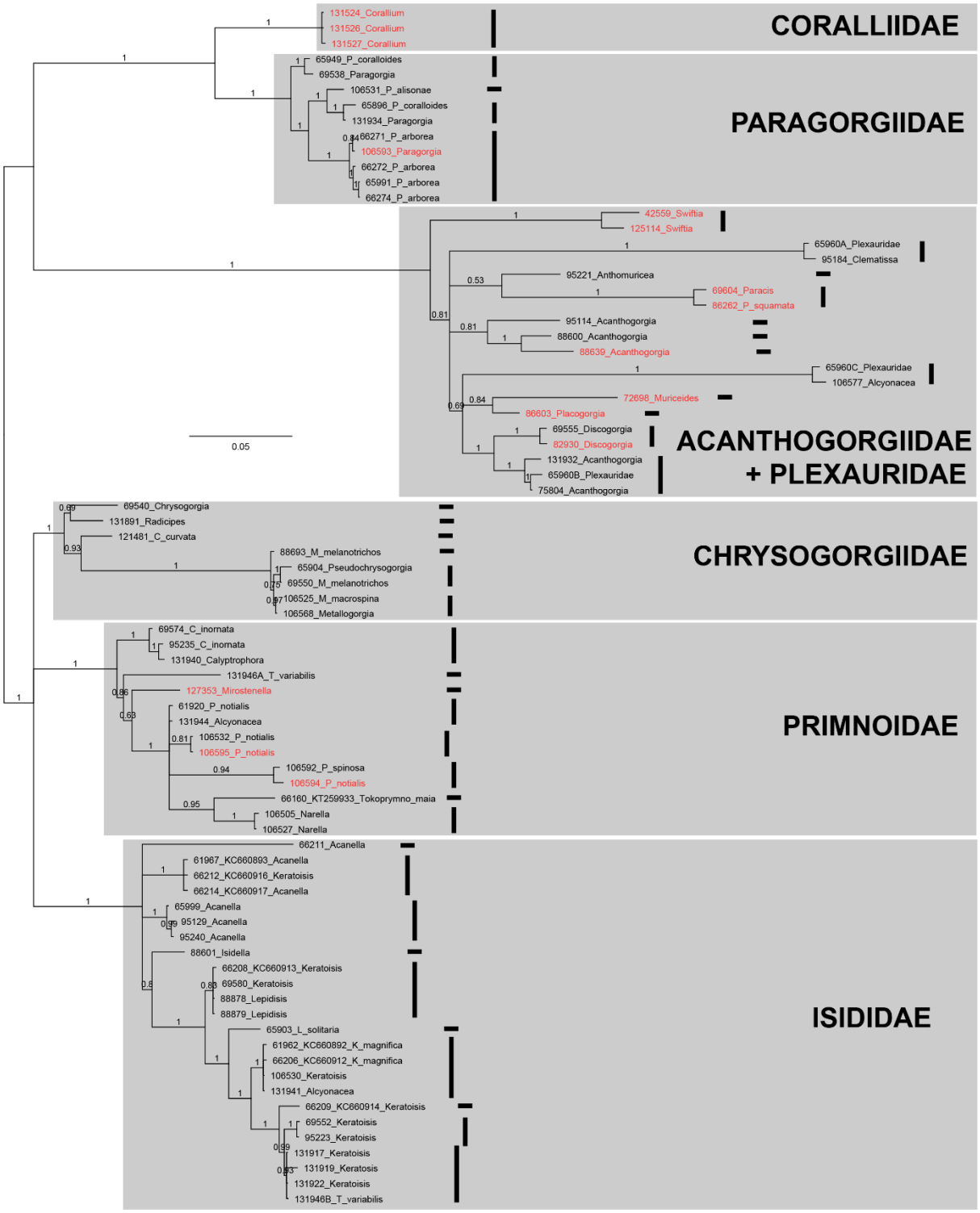


Figure 1: Bayesian phylogenetic tree of sampled genetic diversity among bycatch octocorals. This tree represents the combined (concatenated) and partitioned analysis including DNA sequence data for 5'-*mtMutS*, 3'-*mtMutS* and *28S* regions. Taxon labels give the most recent morphological identification. Grey boxes correspond to protected octocoral families (note: Acanthogorgiidae and Plexauridae are mixed); black bars indicate unique genotypes (equivalent to species); specimens in red are reference samples (not trawl bycatch). Values at each branch node indicate posterior probability support levels.

The rate of discovery of unique taxa (roughly equivalent to species) as DNA sequencing of specimens progressed was found to follow a roughly linear increase for the first 40 sequences, with indications of a more logarithmic relationship in the last 50% of sampled specimens (Figure 2). No indication was seen that our rate of new taxon discovery was approaching stasis and forecasting the logarithmic model out over 100 additional samples did not produce an obvious horizontal asymptote (data not shown). This suggests that our sample size is not yet adequate to estimate the total genetic diversity of the octocoral bycatch community, and that the number of octocoral species likely to be impacted by bottom trawling exceeds that recorded here.

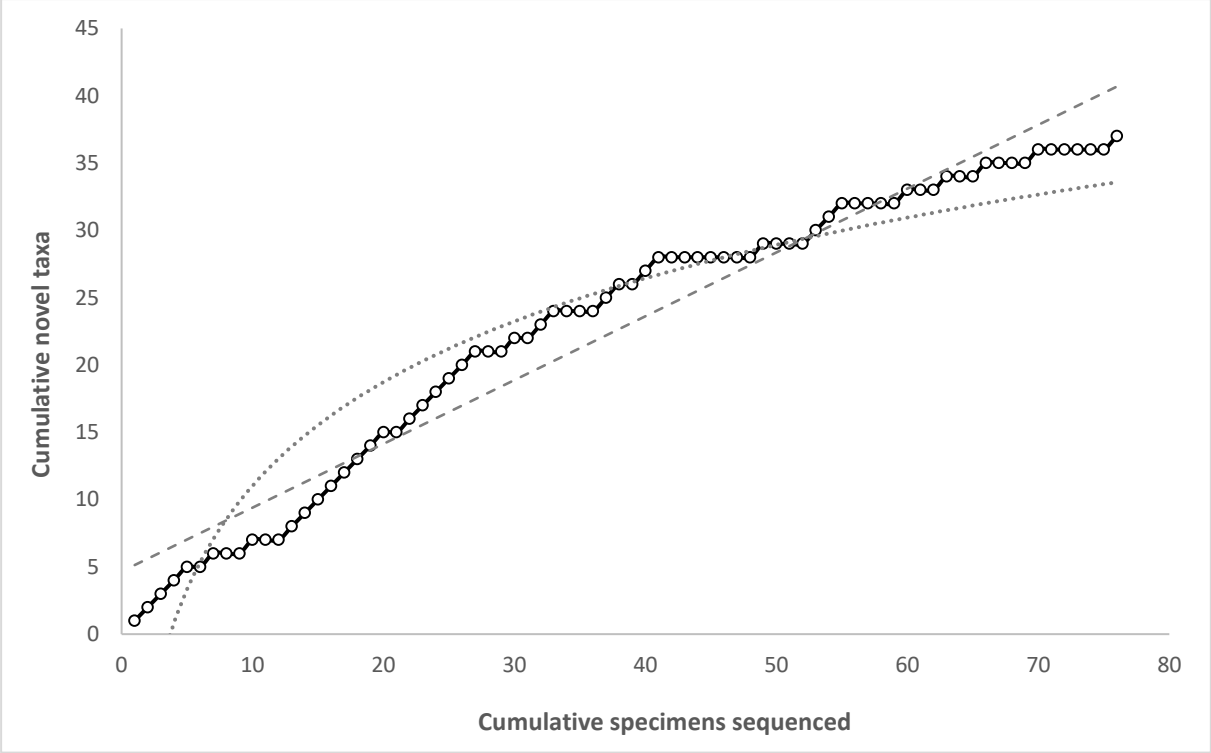


Figure 2: Species accumulation curve for genetic dataset. The cumulative number of DNA-sequenced specimens are compared to the cumulative number of unique genotypes discovered, for all included specimen sequences (trawl bycatch + reference specimens). Dotted grey line = logarithmic trendline (y=11.119ln(x) - 14.578, r^2 = 0.8978); dashed grey line = linear trendline for observed data (y=0.5654x, r^2=0.9049).

3.3 Comparisons of coral identification accuracy

Comparisons of specimen identities according to vessel observers, morphological analysis by taxonomic experts, and the genetic data presented here are given in Table 3. The highest rates of discordance were seen between original observer identifications and genetic identifications, regardless of whether strict or relaxed criteria were used as qualifiers. However, the highest agreement between identification stages was dependent on criteria, with genetics and morphology showing the most similarity in accuracy (lowest discordance under relaxed criteria), but observers and morphology showing the most similarity in precision (lowest under strict criteria). Overall, the much higher discordance values for a strict criterion are consistent with a pattern of increasing resolving power as specimen identification progresses from ship-based gross identification, to detailed morphological study, to genetic barcoding.

Table 3: Discordance between stages of identification for studied specimens. For each specimen, a comparison of the accuracy ('Relaxed' criteria) and precision ('Strict' criteria) of its identification was by pairwise comparisons of each of three stages of the specimen collection process: initial observer records on commercial fishing vessels, expert morphological study at NIWA, and genetic barcoding. For 'Relaxed' criteria, identifications did not have to match for a given taxonomic level but had to be accurate (e.g., both 'Gorgonian Octocoral' *vs*. Genus *Primnoa* and Family Primnoidae *vs*. Genus *Primnoa* would be considered as matches). Under 'Strict' criteria, identifications had to be consistent to at least family level for comparisons with observer data (e.g., 'Gorgonian Octocoral' vs. Family Primnoidae would be a mismatch but Family Primnoidae *vs*. Genus *Primnoa* would be considered a match). For strict comparisons of genetic and morpho-taxonomy, identifications had to match to the same taxonomic level (e.g., *Primnoa vs*. *Primnoa notialis* would be a mismatch).

Relaxed \ Strict	Observer	Morpho-taxonomy	Phylogenetics
Observer	-	19% (12/64)	33% (15/45)
Morpho-taxonomy	11% (7/64)	-	21% (16/76)
Phylogenetics	22% (10/45)	8% (6/76)	-

3.4 Geographic distributions and fishery interactions

Our dataset included bycatch samples from a broad geographical range of sites within the New Zealand EEZ and neighbouring regions (Figure 3). Since most of the genetically distinct taxa reported here have not been identified to species-level and were represented by few replicate samples, we did not assess and compare their distributional patterns with previous records (e.g., www.OBIS.org or www.GBIF.org) within- or outside of the New Zealand EEZ, but instead focused on family-level patterns. Most specimens came from the Chatham Rise, the Lord Howe Rise and, to a lesser extent, the Campbell and Bounty Plateaus. Representatives of specific families were also obtained in lesser numbers from the Louisville Seamount Chain (Acanthogorgiidae and Plexauridae), Macquarie Ridge (Isididae and Primnoidae) and the West Norfolk Ridge (Chrysogorgiidae and Isididae). The overall distributional patterns of bycatch origins closely matched those reported in Tracey et al. (2011) for the Paragorgiidae, Isididae and all other gorgonian families.

Within the EEZ, DNA sequences were obtained from samples from all FMAs except for FMA7 (off the west coast of the South Island), FMA8 (off the west coast of the southern North Island) and FMA10 (the Kermadec/Rangitāhua Arc). Most sequences originated in FMA4 (the Chatham Rise) and FMA6 (Campbell and Bounty Plateaus). As most gorgonian families studied here included some representative bycatch specimens from international regions, we note that the diversity uncovered here will also likely be significant to impacts of Australian and SPRFMO trawl fisheries, although they were not the focus of the current study.

An examination of sequenced specimens by target fishery is give in Table 4. The highest number of both bycaught octocoral specimens and unique genotypes were sampled from the orange roughy fishery, followed by smooth oreo. Overall, 32 unique genotypes were obtained from 62 specimens obtained as bycatch during 54 bottom trawling events. The relationship of cumulative tows to the number of bycaught specimens and the number of unique genotypes (Figure 4) indicates that 1) the discrepancy between the quantity of bycatch and bycatch diversity increases with sample size and 2) there is again no sign of an asymptote in the relationship of the sampled number of trawls versus the detected genetic diversity, indicating the total octocoral biodiversity within the bycatch community

has not yet been achieved by this study. We also note that the relationship of trawling events to bycatch specimen quantity presented here should be interpreted with caution when viewed on its own, since trawls that yielded no bycatch were not included in our analysis. However, an overall synthesis and estimation of bycatch catch rates in orange roughy and oreo fisheries, including for protected gorgonian corals, has been presented by Anderson et al. (2017).

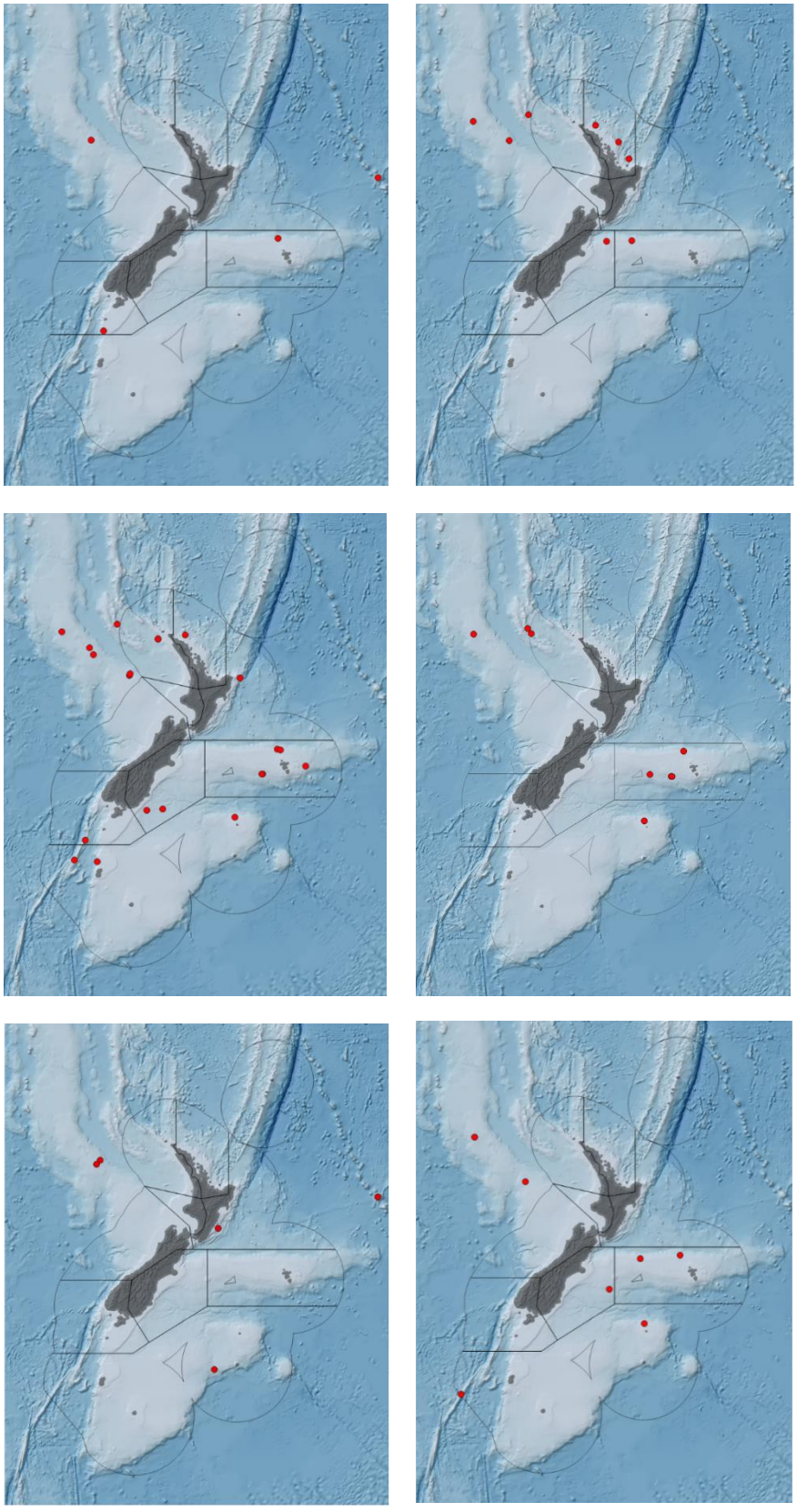


Figure 3: Distribution of trawl bycatch samples from the five sampled families of protected octocorals: Left to right, top to bottom: Acanthogorgiidae, Chrysogorgiidae, Isididae, Paragorgiidae, Plexauridae, Primnoidae.

Table 4: Distribution of DNA-sequenced bycatch specimens and unique genotypes according to the target fishery from which they originated. For each entry, the left number is the # of unique genotypes and the right number is the total # bycaught specimens. The total number of trawls represented by each target fishery are given in the bottom row. BOE = black oreo; BYS = alfonsino; BYX = alfonsino & long-finned beryx; HOK = hoki; HPB = hapuku & bass; LIN = ling; ORH = orange roughy; SSO = smooth oreo; TAR = tarakihi; WWA = white warehou.

Row Labels	BOE	BYS	BYX	HOK	HPB	LIN	ORH	SSO	TAR	WWA	Total
Acanthogorgiidae	-	-	-	-	-	1/1	2/3	-	1/1	-	**2/5**
Chrysogorgiidae	-	1/1	-	2/2	-	-	4/5	-	-	-	**7/8**
Isididae	1/2	1/1	-	-	-	-	7/16	3/3	-	1/1	**10/23**
Paragorgiidae	1/2	-	1/1	-	-	-	3/4	2/2	-	-	**4/9**
Plexauridae	-	-	1/1	-	1/1	-	4/4	1/1	-	-	**5/7**
Primnoidae	-	1/2	-	1/1	-	-	4/5	1/2	-	-	**5/10**
Total	**2/4**	**3/4**	**2/2**	**3/3**	**1/1**	**1/1**	**23/37**	**7/8**	**1/1**	**1/1**	**32/62**
# Trawls	**4**	**4**	**2**	**3**	**1**	**1**	**29**	**8**	**1**	**1**	**54**

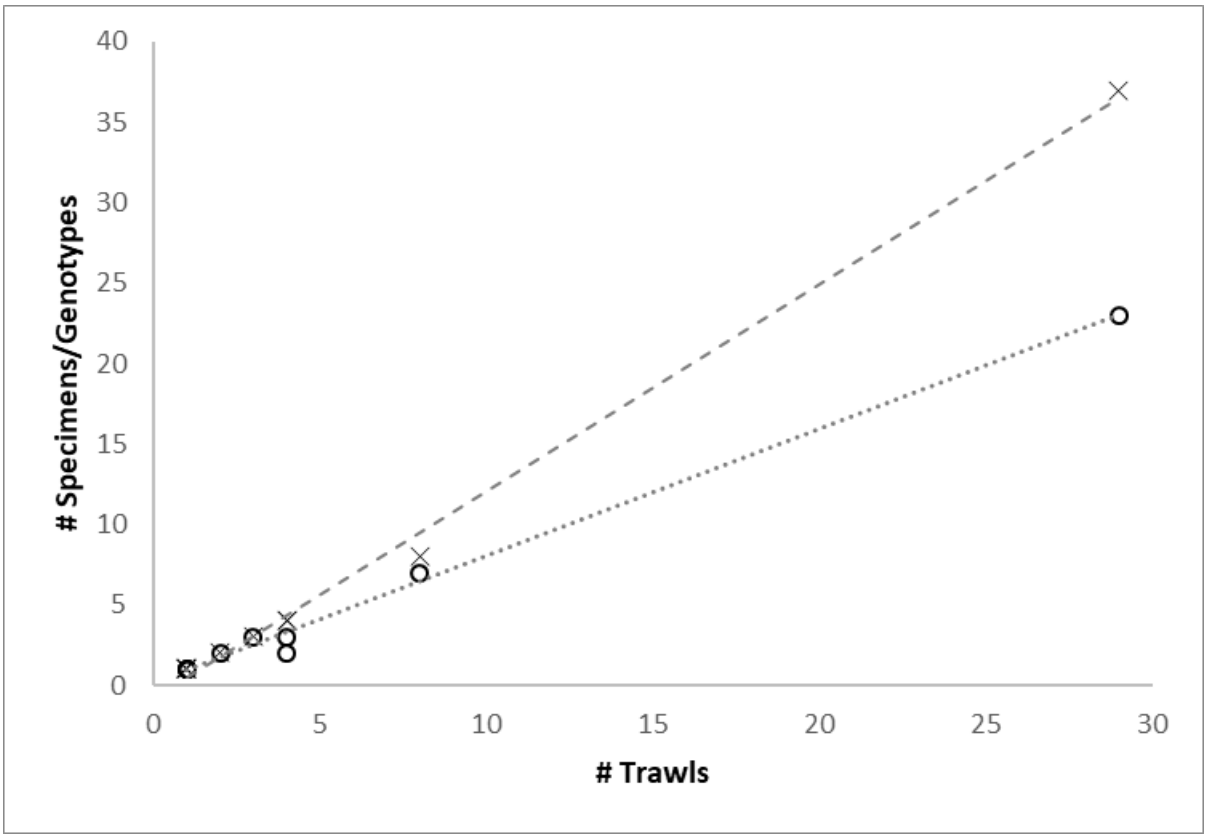


Figure 4: Relationship of total number of bottom trawls to # bycatch specimens and # unique bycatch taxa. 'x' = # specimens; 'o' = # genotypes. Trendlines are given for # specimens (dashed) and # unique genotypes (dotted).

4 Summary and Discussion

4.1 Estimating coral biodiversity

Our current concept of coral bycatch biodiversity in New Zealand relies on visual observations of deep-sea species recorded *in situ* (e.g., Clark et al. 2010) and on collected material that was identified using morphological characters (e.g., Macpherson et al. 2018; Mills et al. 2019; Tracey et al. 2019). Three prior studies have employed genetics to resolve fine-scale diversity among New Zealand octocorals (Herrera et al. 2010; Dueñas et al. 2014; Moore et al. 2016), whereas octocoral diversity is otherwise based on morphological analysis (e.g., Sanchez 2005; Cairns 2012). However, our assessment of diversity has instances for every protected family where genetically distinct specimens were morphologically identified by taxonomists as the same species or genus (Figure 2: *Paragorgia coralloides*, *Acanthogorgia*, *Primnoa notialis*, *Metallogorgia melanotrichos*, *Acanella* and *Keratoisis*). Given that a relatively large number of specimens can be routinely extracted, amplified and sequenced on a small budget (as seen here), we recommend the use of genetic barcoding when estimates of octocoral diversity are needed, whether it is for bycatch characterisation or documenting natural diversity. In this way, the findings and methods developed here would promote an improved understanding of coral taxonomy, as recommended in the Medium-Term Research Plan for Protected Corals (Department of Conservation 2019).

Factors such as growth form, size, density and rigidity can affect the likelihood of gorgonian octocorals being recovered by commercial fishing gear (Clark et al. 2014), and thus bycatch (sampled bycatch in the case of this study) is not likely to be representative of the total or undisturbed coral community for a given benthic region. It can, however, serve as a proxy for the minimum level of community diversity (that portion that is likely to be 'sampled' by trawling). Our study found (at least) 32 species among 54 trawls, but our per-trawl rate of species discovery is likely to be inflated by only considering trawls that have produced bycatch samples, and not those for which no bycatch was present or sampled (as in Anderson et al. 2017). Previous historical observations of octocoral diversity among fisheries bycatch were made by Probert et al. (1997), who recorded 11 species from 73 tows for orange roughy along the Chatham Rise, and Blom et al. (2009), who recorded 16 gorgonian species from 35 trawls across the New Zealand EEZ and adjacent SPRFMO regions. More recently, the NIWA annual bycatch identification programme (INT2015-03) has identified 67 protected gorgonian species among observer photographs taken from 96 trawls (Tracey et al. 2019), and 24 taxa among 70 sampled specimens of protected gorgonians from 61 tows (Macpherson et al. 2020).

It is difficult to compare our rates of identification error to past studies, since the latter have relied on morphological identifications. Tracey et al. (2011) specifically examined observer identification error in comparison to morphological identifications by expert researchers and found 56% error for gorgonian corals and 15% for bamboo corals, compared to 11% reported here overall, whereas their estimates for bubblegum corals (2.6%) were lower than ours. Our sample size for each family was too small and uneven to conduct a similar break-down and these differences may be reflective of varying identification difficulties between each family. On the other hand, Blom et al. (2009) estimated the number of undescribed species within their bycatch sample at around 10% of the total, which matches closely our estimate of 8% disagreement in identification by morphology versus genetics (Table 3), supporting our proposition that previous bycatch diversity measures have significantly underestimated actual species diversity.

Our recovered phylogenetic tree indicates that a vast amount of octocoral diversity is present within trawl bycatch (Figure 1) and a nearly linear rate of unique species discovery (Figure 2) suggests we have not yet approached the maximum number of species detectable using these genetic markers, even though 74 specimens were sequenced in total. In particular, high numbers of species were seen among the bamboo corals (Isididae), which are numerically dominant for octocoral bycatch (37% of bycatch specimens), but the largest levels of genetic diversity were seen among the Acanthogorgiidae and Plexauridae, which are less common in the sampled bycatch community (19% of bycatch specimens). The overall genetic diversity of the Isididae within New Zealand has previously been documented as high (Dueñas et al. 2014), and our study includes a significant proportion of this reported total (10 taxa here, compared to 22 supported taxa in their study). There has been no detailed morphological nor genetic examination of diversity within the Acanthogorgiidae nor the Plexauridae for New Zealand. Our results indicate that these families are likely to yield a vast number of distinct species, many of which will likely be new to science.

4.2 Fishery interactions and coral diversity

Although the presence (and quantity) of biomass observed within fisheries bycatch itself indicates an impact on protected coral communities, we sought to improve our understanding of the extent of such impacts and their nature. The use of genetic discrimination for estimating taxonomic diversity provides a highly sensitive and objective means to revise community-scale effects of bottom trawling. For example, there are at least 23 species of protected gorgonian coral impacted by the orange roughy fishery alone, and based on the high diversity within a small sample size (7 species in 8 specimens) we would expect similarly high numbers within the smooth oreo fishery as well, if additional bycatch was sampled to the same extent as for orange roughy. On a per-family basis, we saw the most diversity represented within the Isididae bycatch (ten species), with representation in five of the ten sampled target fisheries. The chrysogorgiid gold corals were also diverse (seven species) across three target fisheries, whereas the Paragorgiidae, Plexauridae and Primnoidae were represented in four fisheries, but at lower total diversity (four, five and five species, respectively). The Acanthogorgiidae was found in three fisheries and had the lowest observed diversity (two species). These observations are likely to be affected by our sample size, however, and more comprehensive bycatch testing of non-orange roughy fisheries is needed before patterns of shared bycatch diversity across fisheries can be adequately examined.

Although we have summarised coral groups by target fishery for reference, we do not have sufficient sample size to examine bycatch diversity within each target fishery at the same level of coverage, but a linear increase in bycatch diversity with increasing numbers of trawls is seen across all sampled fisheries (Figure 4). It must be noted, however, that each trawling event is not equal in effort (e.g., tow length and trawl-door spread) and our selection of bycatch specimens was made haphazardly, without reference to each specific fishery. Therefore, while our absolute measures of diversity are quantitative, any relationships to numbers of trawls or between fisheries must be treated in a qualitative sense until such time as a more structured examination may be accomplished. For example, with increased bycatch sampling across different target fisheries, differences in the species composition of bycatch may be apparent, according to differences in targeted depth, habitat type, or region. Although this study cannot yet address such patterns, the bycatch diversity uncovered here clearly indicates that such studies would have merit.

Anthropogenic effects on biodiversity are commonly framed within an ecological perspective (counts of species impacted), since management decisions are typically made based on contemporary (or recent history) states of the impacted ecosystem. However, there have been recent calls for increased consideration of the role of evolutionary processes in generating ecosystem health through biodiversity (reviewed in Swenson 2019). Since all species are the product of evolution, the concept of phylogenetic ecology suggests that timeframes and diversity on an evolutionary scale are important in shaping the current ecological niche of any species and its capacity to adapt and be resilient to biotic and abiotic factors within its environment. With reference to the current study, the impact of bottom trawling on gorgonian corals can be viewed in terms of length (time) as well as breadth (diversity). The assemblage of octocoral species contained within bottom trawling bycatch are genetically distinct from each other in the contemporary sense, but the evolutionary processes that generated this current breadth of diversity span a period of at least 140 million years (Park et al. 2012). Evolutionary distinctiveness and rates of evolutionary change, for instance, could be incorporated into management decisions for particular taxa since they relate to their uniqueness and capacity for adaptation (Weber & Agrawal 2012), and phylogenetic approaches can be used to improve predictions of niche partitioning, and therefore species distributions (Godoy et al. 2018), particularly how they relate to fishing effort (Anderson et al. 2020).

Genetic data on the (otherwise) cryptic diversity of corals can also contribute to other management applications, including an ecological risk assessment (ERA) (*sensu* Clark et al. 2014). For instance, cryptic diversity may lower estimates of susceptibility to impact by diluting the incidence of contact among a greater number of species (e.g., mortality of five colonies of one species → one colony of each of five species). On the other hand, it may increase estimates of selectivity impacts as the community biomass is subdivided into more species that more infrequent (e.g., impacting one common species → impacting each of several uncommon ones). Aside from these diversity-based considerations, there is no immediately available means to incorporate evolutionary features into the pilot ERA model and accounting for phylogenetic processes and evolutionary distinctiveness would require an expansion of faunal diversity and disturbance criteria for selectivity and productivity categories, respectively (see Clark et al. 2014 for pilot ERA structure).

5 Recommendations

- The sampling and submission for archiving of coral specimens by Government Observers produces a valuable resource, as available voucher material is produced for more regions than could be feasibly achieved through targeted research cruises. Continued support for observer collections of protected corals for the purposes of genetic investigation of species diversity is warranted.
- Genetic barcoding should be employed for routine identification of octocoral bycatch (and as part of routine deep-sea monitoring), to avoid underestimates of biodiversity-related impacts and to improve baseline understanding of New Zealand's coral communities. This could be integrated into ongoing bycatch sampling-related CSP projects (INT2019-04: Tracey et al. 2019).
- Managers and policy makers could reconsider in what ways biodiversity can be used as a measure of community health, and the limitations of how it is/was estimated. An evolutionary perspective could be incorporated, or hierarchical taxonomic diversity could be used as a proxy.
- This research can be used to address or supplement critical gaps in our understanding of New Zealand protected corals (see Department of Conservation 2017), including:
  - Improved Understanding of Taxonomy.
  - Identification of Biodiversity Hotspots.
  - Further Genetic Collections from Sources Not Already Explored.
  - Further Investigation into the Impacts of Trawling.
  - Identification of Areas of Highest Protection Value for Deep Sea Corals.

6 Acknowledgements

This project could not have been accomplished without the efforts of many Government Observers who diligently recorded, collected and submitted bycatch corals to the NIWA Invertebrate Collections. Their efforts have created and are growing an invaluable repository of specimens that contain a wealth of undocumented diversity for New Zealand. We are also grateful to Sadie Mills and Diana Macpherson (NIWA Invertebrate Collection), who are responsible for the careful curation, development and identification of bycatch collections. Thanks to the generous assistance of Amalia Calle (NIWA Intern), we were able to sample and analyse many more specimens than would otherwise have been possible after the project was seriously delayed due to Covid-19. We also acknowledge the timely assistance of Jade Maggs and Aiden Liu from the NIWA Fisheries Data Service and thank Chris Dick of FNZ Data Management services at MPI for providing permissions for the use of Observer fisheries data. The analyses and production of this report were greatly enhanced by advice and discussion with Lyndsey Holland (FNZ-MPI) and administrative support for project management was provided by Megan Wintringham (NIWA) and Shannon Weaver (DOC-CSP). The advice of Rachael Peart (NIWA) in reviewing and improving this manuscript is also greatly appreciated. Finally, we thank the Conservation Services Program, DOC for providing essential funding for the study of New Zealand protected corals.

7 References

Anderson, O., Clark, M.R. (2003) Analysis of bycatch in the fishery for orange roughy, *Hoplostethus atlanticus*, on the South Tasman Rise. *Marine and Freshwater Research*, 54: 643–652.

Anderson, O., Stevenson, F., Behrens, E. (2020) Updated habitat suitability modelling for protected corals in New Zealand waters. *NIWA Client Report* 2020174WN: 108.

Anderson, O.F., Ballara, S.L., Edwards, C.T.T. (2017) Fish and invertebrate bycatch and discards in New Zealand orange roughy and oreo trawl fisheries from 2001–02 until 2014–15. *New Zealand Aquatic Environment and Biodiversity Report*, No. 190: 216.

Bayer, F.M. (1981) Key to the genera of Octocorallia exclusive of Pennatulacea (Coelenterata: Anthozoa), with diagnoses of new taxa. *Proceedings of the Biological Society of Washington*, 94: 902-947.

Bilewitch, J.P., Coates, K.A., Currie, D.C., Trapido-Rosenthal, H.G. (2010) Molecular and morphological variation supports monotypy of the octocoral *Briareum* Blainville, 1830 (Octocorallia: Alcyonacea) in the Western Atlantic. *Proceedings of the Biological Society of Washington*, 123(2): 93-112.

Bilewitch, J.P., Ekins, M., Hooper, J., Degnan, S.M. (2014) Molecular and morphological systematics of the Ellisellidae (Coelenterata: Octocorallia): Parallel evolution in a globally distributed family of octocorals. *Molecular Phylogenetics and Evolution*, 73(1): 106-118.

Blom, W., Webber, R., Schultz, T. (2009) Invertebrate bycatch from bottom trawls in the New Zealand EEZ. *Tuhinga,* 20: 33-40.

Buhl-Mortensen, L., Mortensen, P.B. (2005) Distribution and diversity of species associated with deep-sea gorgonian corals off Atlantic Canada. In: A. Freiwald & J.M. Roberts (Eds). *Cold-water Corals and Ecosystems*. Springer Berlin Heidelberg: 849-879.

Cairns, S.D. (2012) New Zealand Primnoidae (Anthozoa:Alcyonacea). Part 1: Genera *Narella*, *Narelloides*, *Metanarella*, *Calyptrophora*, and *Helicoprimnoa*. NIWA, Wellington, N.Z.: 72.

Clark, M.R., Althaus, F., Schlacher, T.A., Williams, A., Bowden, D.A., Rowden, A.A. (2016) The impacts of deep-sea fisheries on benthic communities: a review. *ICES Journal of Marine Science*, 73: 51-69. 10.1093/icesjms/fsv123

Clark, M.R., Bowden, D.A., Rowden, A.A., Stewart, R. (2019) Little Evidence of Benthic Community Resilience to Bottom Trawling on Seamounts After 15 Years. *Frontiers in Marine Science*, 6. 10.3389/fmars.2019.00063

Clark, M.R., Rowden, A.A., Schlacher, T., Williams, A., Consalvey, M., Stocks, K.I., Rogers, A.D., O'Hara, T.D., White, M., Shank, T.M., Hall-Spencer, J.M. (2010) The Ecology of Seamounts: Structure, Function, and Human Impacts. *Annual Review of Marine Science,* 2(1): 253-278. 10.1146/annurev-marine-120308-081109

Clark, M.R., Tracey, D.M., Anderson, O., Parker, S. (2014) Pilot ecological risk assessment for protected corals. Prepared for Marine Species and Threats, Department of Conservation, *NIWA Client Report* WLG2014-70.

Department of Conservation (2019) Draft Coral Medium-Term Research Plan. Prepared by *Conservation Services Programme.* https://www.doc.govt.nz/globalassets/documents/conservation/marine-and-coastal/marine-conservation-services/plans/draft-coral-medium-term-research-plan-2019.pdf

Department of Conservation (2017) Workshop on research needs for protected corals in New Zealand waters. 12 October 2017. https://www.doc.govt.nz/our-work/conservation-services-programme/other-publications/research-needs-for-protected-corals-in-new-zealand-waters/

Dueñas, L.F., Alderslade, P., Sánchez, J.A. (2014) Molecular systematics of the deep-sea bamboo corals (Octocorallia: Isididae: Keratoisidinae) from New Zealand with descriptions of two new species of *Keratoisis*. *Molecular Phylogenetics and Evolution,* 74: 15-28. 10.1016/j.ympev.2014.01.031

Godoy, B.S., Camargos, L.M., Lodi, S. (2018) When phylogeny and ecology meet: Modelling the occurrence of Trichoptera with environmental and phylogenetic data. *Ecology & Evolution*, 8(11): 5313-5322. 10.1002/ece3.4031

Herrera, S., Baco, A., Sánchez, J.A. (2010) Molecular systematics of the bubblegum coral genera (Paragorgiidae, Octocorallia) and description of a new deep-sea species. *Molecular Phylogenetics & Evolution*, 55: 123-135.

Huelsenbeck, J.P., Ronquist, F. (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17: 754-755.

Husebø, Å., Nøttestad, L., Fosså, J.H., Furevik, D.M., Jørgensen, S.B. (2002) Distribution and abundance of fish in deep-sea coral habitats. *Hydrobiologia*, 471(1): 91-99. 10.1023/A:1016549203368

Katoh, K., Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology & Evolution*, 30(4): 772-780. 10.1093/molbev/mst010

Macpherson, D., Tracey, D., Mills S., Thomas, H. (2018) Identification of Protected Corals. Final Report prepared by NIWA for the Conservation Services Programme, Department of Conservation. INT2015-03. *NIWA Client Report* 201850WN: 49.

Macpherson, D., Tracey, D., Mills, S. (2020) INT2019-04 Identification and storage of cold-water coral bycatch specimens 1 July 2019 – 31 December 2019. *Milestone 1. Six monthly progress update prepared by NIWA for the Conservation Services Programme, Department of Conservation.* DOC20303 - INT2019-04: 22.

McFadden, C.S., Benayahu, Y., Pante, E., Thoma, J.N., Nevarez, P.A., France, S.C. (2010) Limitations of mitochondrial gene barcoding in Octocorallia. *Molecular Ecology Resources*, 11: 19-31.

McFadden, C.S., Brown, A.S., Brayton, C., Hunt, C.B., Ofwegen, L.P. (2014) Application of DNA barcoding in biodiversity studies of shallow-water octocorals: molecular proxies agree with morphological estimates of species richness in Palau. *Coral Reefs,* 33: 275-286. 10.1007/s00338-013-1123-0

McFadden, C.S., France, S.C., Sánchez, J.A., Alderslade, P. (2006) A molecular phylogenetic analysis of the Octocorallia (Cnidaria: Anthozoa) based on mitochondrial protein-coding sequences. *Molecular Phylogenetics & Evolution*, 41: 513-527.

McFadden, C.S., Van Ofwegen, L.P. (2012) Stoloniferous octocorals (Anthozoa, Octocorallia) from South Africa, with descriptions of a new family of Alcyonacea, a new genus of Clavulariidae, and a new species of *Cornularia* (Cornulariidae). *Invertebrate Systematics,* 26(4): 331-356. 10.1071/IS12035

Milligan, R.J., Spence, G., Roberts, J.M., Bailey, D.M. (2016) Fish communities associated with cold-water corals vary with depth and substratum type. *Deep Sea Research Part I: Oceanographic Research Papers,* 114: 43-54. https://doi.org/10.1016/j.dsr.2016.04.011

Mills, S., Tracey, D., Schnabel, K. (2019) Contributing Authors: Thomas, H., Yeoman, J., Kelly, M., Stevens, D., Forman, J., Macpherson, D. Identification of benthic invertebrate samples from research trawls and observer trips 2018-2019. *Final Research Report prepared for Fisheries New Zealand*. DAE2017-02: 36.

Moore, K., Alderslade, P., Miller, K. (2016) A taxonomic revision of the genus *Primnoisis* Studer [& Wright], 1887 (Coelenterata: Octocorallia: Isididae) using morphological and molecular data. *Zootaxa,* 4075 (1): 001–141.

Park, E., Hwang, D.S., Lee, J.S., Song, J.I., Seo, T.K., Won, Y.J. (2012) Estimation of divergence times in cnidarian evolution based on mitochondrial protein-coding genes and the fossil record. *Molecular Phylogenetics and Evolution*, 62: 329-345. 10.1016/j.ympev.2011.10.008

Probert, P.K., McKnight, D.G., Grove, S.L. (1997) Benthic invertebrate bycatch from a deep-water trawl fishery, Chatham Rise, New Zealand. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 7: 27-40.

Quattrini, A.M., Wu, T., Soong, K., Jeng, M.S., Benayahu, Y., McFadden, C.S. (2019) A next generation approach to species delimitation reveals the role of hybridization in a cryptic species complex of corals. *BMC Evolutionary Biology*, 19(1): 116. 10.1186/s12862-019-1427-y

Rowden, A.A., O'Shea, S., Clark, M.R. (2002) Benthic biodiversity of seamounts on the northwest Chatham Rise. *Marine Biodiversity Biosecurity Report,* No. 2: 21.

Sánchez, J.A. (2004) Evolution and dynamics of branching colonial form in marine modular cnidarians: gorgonian octocorals. *Hydrobiologia*, 530/531: 283-290. 10.1007/s10750-004-2684-2

Sánchez, J.A. (2005) Systematics of the bubblegum corals (Cnidaria: Octocorallia: Paragorgiidae) with description of new species from New Zealand and the Eastern Pacific. *Zootaxa*, 1014: 1-72.

Sánchez, J.A., Aguilar, C., Dorado, D., Manrique, N. (2007) Phenotypic plasticity and morphological integration in a marine modular invertebrate. *BMC Evolutionary Biology,* 7: 1-9.

Sánchez, J.A., McFadden, C.S., France, S.C., Lasker, H.R. (2003) Molecular phylogenetic analyses of shallow-water Caribbean octocorals. *Marine Biology,* 142(5): 975-987. 10.1007/s00227-003-1018-7

Swenson, N.G. (2019) *Phylogenetic Ecology: A History, Critique, and Remodeling*. University of Chicago Press: 240.

Tracey, D., Hjorvarsdottir, F. (2019) The State of Knowledge of Deep-Sea Corals in the New Zealand Region: 140.

Tracey, D., Macpherson, D., Mills, S. (2019) Identification and storage of cold-water coral bycatch specimens - Final Annual Report. Prepared for the Conservation Services Programme, Department of Conservation. *NIWA Client Report* 2019362WN: 39.

Tracey, D., Sanders, B. (2010) Updated coral identifications and subsequent loading into COD, – supplement report to (Tracey, 2010) prepared for Marine Conservation Services, Department of Conservation: 4.

Tracey, D.M., Baird, S.J., Sanders, B., Smith, M.H. (2011) Distribution of protected corals in relation to fishing effort and assessment of accuracy of observer identification. *NIWA Client Report* WLG2011-33: 70.

Weber, M.G., Agrawal, A.A. (2012) Phylogeny, ecology, and the coupling of comparative and experimental approaches. *Trends in Ecology & Evolution,* 27(7): 394-403. 10.1016/j.tree.2012.04.010

Yoklavich, M.M., Laidig, T.E., Graiff, K., Elizabeth Clarke, M., Whitmire, C.E. (2018) Incidence of disturbance and damage to deep-sea corals and sponges in areas of high trawl bycatch near the California and Oregon border. *Deep Sea Research Part II: Topical Studies in Oceanography*, 150: 156-163. 10.1016/j.dsr2.2017.08.005

Appendix A SQL Query of Centralised Observer Database (COD) for protected gorgonian bycatch

```
-- SQL written by Aiden Liu and Jade Maggs 2020-07-10

\o data_NI2002.txt

select v.trip_number,

 v.station_number,

 v.fishing_year,

 v.target_species,

 v.event_start_date,

 v.start_obs_fma,

 v.start_stats_area,

 xe.start_latitude, --(full precision, decimal degrees, permission granted by MPI)

 xe.start_longitude, --(full precision, decimal degrees, permission granted by MPI)

 y.species_true,

 y.species_obs,

 y.catch_weight,

 xs.common_name,

 y.phylum

from x_event xe left join v_station v on xe.event_key = v.event_key

 left join y_benthic y on v.trip_key = y.trip_key and v.station_number = y.station_number

 left join x_species_codes xs on y.species_true = xs.species_code

where (coalesce(y.species_true, y.species_obs) in ('PAN','BOO','LLE','ISI','LIL','PTP','THO',
'PAB','ACN','CLG','CTP','CHR','GOC','TRH', 'IRI','MTL','PLL','CLL','PMN','PML','PRI','NAR','PLE','MIN'))

and v.target_species in ('HOK','HAK','LIN','ORH','SCI','JMA','SSO','BEO','SBW')

and v.fishing_year in
('2009/10','2010/11','2011/12','2012/13','2013/14','2014/15','2015/16','2016/17','2017/18','2018/19
','2019/20')

and v.fishing_method in ('TWL', 'BT', 'MW');

\o
```

Appendix B Sample metadata for sequenced gorgonian specimens

Catalog number = NIWA Invertebrate Collection catalog number; Family & ID = identifications at the outset of this project; 28S, 5'-MutS, 3'-MutS = loci which have viable sequence data are indicated by 'X', '!' = sequence from GenBank; Molec ID = revised identification according to molecular systematics; Observed ID = original ID by vessel-based observers; Target Fishery = MPI-FNZ code for target fishery for which the specimen was bycatch; Station = Trip & tow number for fishery vessel; Gear = fishing method; Date = date specimen was collected; Latitude & Longitude = GPS coordinates for start of fishing event; Depth = depth or depth range of tow.

Catalog Number	Family	ID	28S	5'-MutS	3'-MutS	MOLEC ID	Observer ID	Target Fishery	Station	Gear	Date	Latitude	Longitude	Depth
42559	Plexauridae	*Swiftia*			X	*Swiftia*		SSO	TRIP2571/122	Trawl, Fish, Bottom	13/03/2008	-50.0	176.7	839-912
61920	Primnoidae	*Primnoa notialis*		X		*Primnoa notialis*	PMN	SSO	TRIP3065/214	Trawl, fish, bottom	09/03/2010	-45.0	175.5	1070-1100
61962	Isididae	*Keratoisis magnifica*		!		*Keratoisis magnifica*	BOO	ORH	TRIP3077/43	Trawl, fish, bottom	27/02/2010	-50.0	166.0	850
61967	Isididae	*Acanella*		!		*Acanella* sp1		BOE	TRIP3077/19	Trawl, fish, bottom	23/02/2010	-49.9	163.8	835
65896	Paragorgiidae	*Paragorgia coralloides*	X	X	X	*Paragorgia* sp2	PAB	ORH	TRIP3140/45	Trawl, fish, bottom	29/06/2010	-34.0	168.2	836-955
65903	Isididae	*Lepidisis solitaria*	X	X	X	*Lepidisis solitaria*	BOO	ORH	TRIP3155/11	Trawl, fish, bottom	10/07/2010	-34.4	174.2	918-1077
65904	Chrysogorgiidae	*Pseudochrysogorgia*	X	X	X	*Pseudochrysogorgia*	CHR	ORH	TRIP3155/11	Trawl, fish, bottom	10/07/2010	-34.4	174.2	918-1077
65949	Paragorgiidae	*Paragorgia coralloides*		X	X	*Paragorgia* sp1		BYX	TRIP3177/37	Trawl, fish, bottom	01/09/2010	-34.1	162.7	541-596
65991	Paragorgiidae	*Paragorgia arborea*	X	X	X	*Paragorgia arborea*	PAB	SSO	TRIP3223/74	Trawl, fish, bottom	18/11/2010	-44.3	179.3	1036
65999	Isididae	*Acanella*		X	X	*Acanella* sp2		ORH	TRIP3238/62	Trawl, fish, bottom	30/11/2010	-37.7	179.3	1047-1208
66160	Primnoidae	*Tokoprymno maia*	!			*Tokoprymno maia*			TRIP3112/37	Trawl, fish, bottom				
66206	Isididae	*Keratoisis magnifica*		!		*Keratoisis magnifica*		ORH	TRIP3004/33	Trawl, fish, bottom	24/11/2009	-44.5	-178.6	710
66208	Isididae	*Keratoisis*		!		*Lepidisis*		ORH	TRIP3028/169	Trawl, fish, bottom	14/01/2010	-43.9	-174.6	660
66209	Isididae	*Keratoisis*		!		*Keratoisis* sp1		SSO	TRIP3028/9	Trawl, fish, bottom	22/12/2009	-46.8	172.1	1035-1396
66211	Isididae	*Isidella*		X	X	*Isidella* sp1	ACN	SSO	TRIP3028/11	Trawl, fish, bottom	23/12/2009	-46.8	170.6	
66212	Isididae	*Keratoisis*		!		*Acanella* sp1		WWA	TRIP3028/18	Trawl, fish, bottom	24/12/2009	-48.7	164.8	363-431
66214	Isididae	*Isidella*		!		*Acanella* sp1		BOE	TRIP3028/128	Trawl, fish, bottom	09/01/2010	-44.5	-178.7	670-920

Catalog Number	Family	ID	28S	5'-MutS	3'-MutS	MOLEC ID	Observer ID	Target Fishery	Station	Gear	Date	Latitude	Longitude	Depth
66271	Paragorgiidae	*Paragorgia arborea*	X	X	X	*Paragorgia arborea*		BOE	TRIP3028/140	Trawl, fish, bottom	10/01/2010	-44.5	-178.7	660-940
66272	Paragorgiidae	*Paragorgia arborea*	X	X	X	*Paragorgia arborea*		BOE	TRIP3028/144	Trawl, fish, bottom	11/01/2010	-44.5	-178.6	740-954
66274	Paragorgiidae	*Paragorgia arborea*	X	X	X	*Paragorgia arborea*	PAB	ORH	TRIP3028/136	Trawl, fish, bottom	10/01/2010	-44.5	-178.6	735
69538	Paragorgiidae	*Paragorgia*	X	X	X	*Paragorgia* sp1	PAB	ORH	TRIP3252/10	Trawl, fish, bottom	30/12/2010	-33.6	167.8	776-998
69540	Chrysogorgiidae	*Chrysogorgia*	X	X	X	*Chrysogorgia* sp1	UNI	HOK	TRIP3235/23	Trawl, fish, bottom	05/12/2010	-42.9	177.6	437-465
69550	Chrysogorgiidae	*Metallogorgia melanotrichos*	X	X	X	*Pseudochrysogorgia*	MTL	ORH	TRIP3246/22	Trawl, fish, bottom	31/12/2010	-35.6	166.0	851-1141
69552	Isididae	*Keratoisis*		X		*Keratoisis* sp2		BYS	TRIP3246/11	Trawl, fish, bottom	27/12/2010	-34.2	162.6	431-645
69555	Plexauridae	*Discogorgia*	X	X	X	*Discogorgia*	GOC	ORH	TRIP3246/23	Trawl, fish, bottom	31/12/2010	-35.6	165.9	747-1078
69574	Primnoidae	*Calyptrophora inornata*		X		*Calyptrophora inornata*		HOK	TRIP3235/14	Trawl, fish, bottom	01/12/2010	-43.0	178.5	447-447
69580	Isididae	*Keratoisis*		X		*Lepidisis*	BOO	ORH	TRIP3252/9	Trawl, fish, bottom	30/12/2010	-33.6	167.8	841-1049
69604	Plexauridae	*Paracis*		X		*Paracis squamata*		BNS	TRIP3248/17	Bottom longline	15/12/2010	-32.5	166.8	356-367
72698	Plexauridae	*Muriceides*		X		*Muriceides*			TAN1104/102	Sled, epibenthic	17/03/2011	-35.7	178.5	440-605
75804	Acanthogorgiidae	*Acanthogorgia*	X	X	X	*Acanthogorgia* sp2	PLE	LIN	TRIP3426/57	Trawl, fish, bottom	14/01/2012	-48.8	166.4	575-608
82930	Plexauridae	*Discogorgia*		X		*Discogorgia*			TAN1206/99	Sled, epibenthic	24/04/2012	-36.4	177.8	850-927
86262	Plexauridae	*Paracis squamata*		X		*Paracis squamata*			TAN1213/22	Sled, epibenthic	18/10/2012	-30.1	179.8	483-530
86603	Plexauridae	*Placogorgia*		X		*Placogorgia*			TAN1105/42	Sled, epibenthic	28/03/2011	-34.0	171.8	92-96
88600	Acanthogorgiidae	*Acanthogorgia*	X	X	X	*Acanthogorgia* sp1	GOC	ORH	TRIP3812/20	Trawl, fish, bottom	12/07/2013	-35.6	165.2	928-967
88601	Isididae	*Isidella*	X	X	X	*Isidella* sp2		ORH	TRIP3812/12	Trawl, fish, bottom	10/07/2013	-35.4	165.2	844-971
88639	Acanthogorgiidae	*Acanthogorgia*		X		*Acanthogorgia* sp1		BAS	TRIP3933/8	Bottom longline	09/11/2013	-32.5	167.5	94-104
88693	Chrysogorgiidae	*Metallogorgia melanotrichos*	X	X	X	*Metallogorgia melanotrichos*	BOO	ORH	TRIP4038/5	Trawl, fish, bottom	17/02/2014	-37.0	177.4	1000-1200
88878	Isididae	*Lepidisis*	X	X	X	*Lepidisis*	BOO	ORH	TRIP4161/20	Trawl, Fish, Bottom	22/07/2014	-42.8	-177.2	982-988
88879	Isididae	*Lepidisis*	X	X	X	*Lepidisis*	BOO	ORH	TRIP4161/22	Trawl, Fish, Bottom	22/07/2014	-42.8	-176.9	853-880

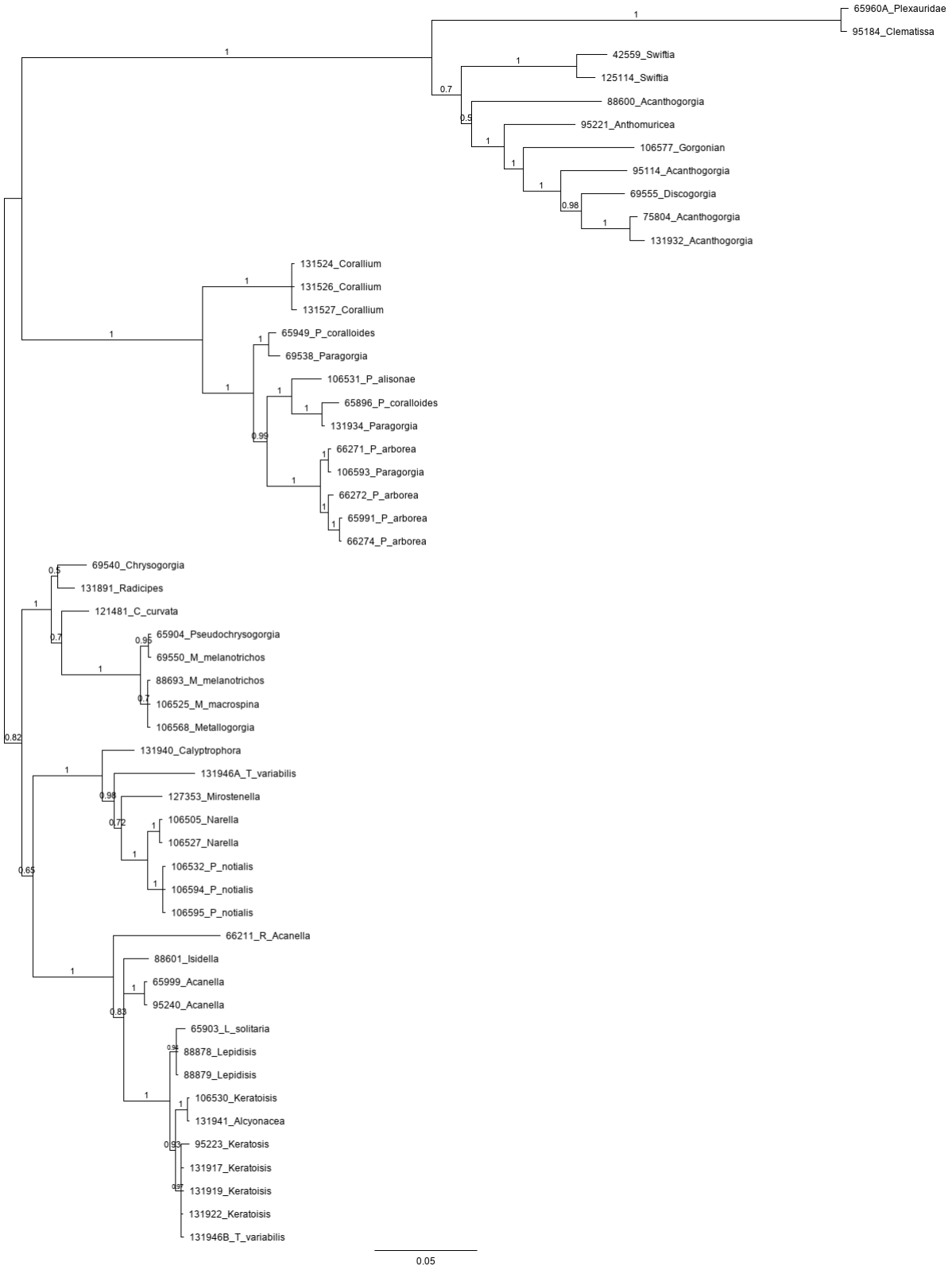
Catalog Number	Family	ID	28S	5'-MutS	3'-MutS	MOLEC ID	Observer ID	Target Fishery	Station	Gear	Date	Latitude	Longitude	Depth
95114	Acanthogorgiidae	*Acanthogorgia*		X	X	*Acanthogorgia* sp1	PLE	TAR	TRIP4255/43	Trawl, fish, bottom	02/12/2014	-34.3	173.0	137-151
95129	Isididae	*Acanella*		X		Acanella sp2	ISI	ORH	TRIP4364/36	Trawl, fish, bottom	09/04/2015	-37.5	169.0	980
95184	Plexauridae	*Clematissa*		X	X	*Clematissa*	PLE	HPB	TRIP4448/41	Trawl, fish, bottom	16/07/2015	-38.4	-168.1	263-298
95221	Plexauridae	*Anthomuricea*			X	*Anthomuricea*	GOC	ORH	TRIP4546/101	Trawl, fish, bottom	14/12/2015	-35.9	165.6	686-1090
95223	Isididae	*Keratoisis*		X	X	*Keratoisis* sp2	BOO	ORH	TRIP4546/101	Trawl, fish, bottom	14/12/2015	-35.9	165.6	686-1090
95235	Primnoidae	*Calyptrophora inornata*	X	X		*Calyptrophora inornata*	PRI	ORH	TRIP4546/17	Trawl, fish, bottom	27/11/2015	-37.4	167.5	730-946
95240	Isididae	*Acanella*	X	X	X	*Acanella* sp2	ACN	ORH	TRIP4546/4	Trawl, fish, bottom	16/11/2015	-37.4	169.0	1039-1046
106505	Primnoidae	*Narella*	X	X	X	*Narella*		BYS	TRIP4823/57	Trawl, fish, bottom	21/10/2016	-34.0	162.6	504-703
106525	Chrysogorgiidae	*Metallogorgia macrospina*	X	X	X	*Metallogorgia macrospina*		BYS	TRIP4823/56	Trawl, fish, bottom	21/10/2016	-34.1	162.5	493-864
106527	Primnoidae	*Narella*		X	X	*Narella*		BYS	TRIP4823/39	Trawl, fish, bottom	19/10/2016	-34.0	162.6	505-743
106530	Isididae	*Keratoisis*	X	X	X	*Keratoisis magnifica*	BOO	ORH	TRIP4815/10	Trawl, Fish, Bottom	10/10/2016	-47.3	178.8	787
106531	Paragorgiidae	*Paragorgia alisonae*	X	X	X	*Paragorgia alisonae*	PAB	SSO	TRIP4815/38	Trawl, Fish, Bottom	15/10/2016	-47.4	178.8	918-930
106532	Primnoidae	*Primnoa notialis*	X	X	X	*Primnoa notialis*	COU	SSO	TRIP4815/20	Trawl, Fish, Bottom	11/10/2016	-47.3	178.9	911
106568	Chrysogorgiidae	*Metallogorgia*	X	X	X	*Metallogorgia*	MTL	ORH	TRIP5058/39	Trawl, fish, bottom	16/07/2017	-35.7	176.4	787
106577	(Alcyonacea)		X	X	X	*Plexauridae* sp1		BYX	TRIP5117/16	Trawl, fish, bottom	02/09/2017	-40.7	177.0	787
106592	Primnoidae	*Parastenella spinosa*	X			*Primnoa* sp1	PRI	PTO	TRIP4837/31	Bottom longline	29/01/2017	-51.6	161.4	1062-1132
106593	Paragorgiidae	*Paragorgia*	X	X	X	*Paragorgia arborea*	PAB	PTO	TRIP4837/14	Bottom longline	21/01/2017	-51.7	161.4	1001-1381
106594	Primnoidae	*Primnoa notialis*	X	X	X	*Primnoa* sp1		PTO	TRIP4837/5	Bottom longline	18/01/2017	-51.6	161.3	1364-1650
106595	Primnoidae	*Primnoa notialis*	X	X	X	*Primnoa notialis*		PTO	TRIP4837/5	Bottom longline	18/01/2017	-51.6	161.3	1364-1650
121481	Chrysogorgiidae	*Chrysogorgia curvata*		X	X	*Chrysogorgia curvata*	CHR	ORH	TRIP3246/5	Trawl, fish, bottom	24/12/2010	-33.6	167.8	959-1104
125114	Plexauridae	*Swiftia*		X	X	*Swiftia*		ATO	TRIP5072/9	Bottom longline	18/09/2017	-59.7	-143.8	1518-1675
127353	Primnoidae	*Mirostenella*			X	*Mirostenella*		BAS	TRIP3933/21	Bottom longline	11/11/2013	-33.4	167.6	312-383
131524	Coralliidae	*Corallium*		X	X	*Corallium*			TRIP3412/53					

Catalog Number	Family	ID	28S	5'-MutS	3'-MutS	MOLEC ID	Observer ID	Target Fishery	Station	Gear	Date	Latitude	Longitude	Depth
131526	Coralliidae	*Corallium*		X	X	*Corallium*			TRIP3412/47					
131527	Coralliidae	*Corallium*		X	X	*Corallium*			TRIP3412/47					
131891	Chrysogorgiidae	*Radicipes*		X	X	*Radicipes*	CHR	HOK	TRIP5613/96	Trawl, fish, bottom	01/05/2019	-42.9	175.2	522-560
131917	Isididae	*Keratoisis*	X	X	X	*Keratoisis* sp3	ISI	ORH	TRIP5854/24	Trawl, fish, bottom	05/12/2019	-34.8	171.6	1050-1166
131919	Isididae	*Keratoisis*		X	X	*Keratoisis* sp3	ISI	ORH	TRIP5854/24	Trawl, fish, bottom	05/12/2019	-34.8	171.6	1050-1166
131922	Isididae	*Keratoisis*	X	X	X	*Keratoisis* sp3	ISI	ORH	TRIP5854/24	Trawl, fish, bottom	05/12/2019	-34.8	171.6	1050-1166
131932	Plexauridae	*Acanthogorgia*	X	X	X	*Acanthogorgia* sp2	PRI	ORH	TRIP5844/37	Trawl, fish, bottom	04/12/2019	-42.7	-177.3	1182-1182
131934	Paragorgiidae	*Paragorgia*	X	X	X	*Paragorgia* sp2	PAB	ORH	TRIP5844/136	Trawl, fish, bottom	25/12/2019	-42.7	-177.5	1210-1228
131940	Primnoidae	*Calyptrophora*	X	X	X	*Calyptrophora inornata*	PRI	ORH	TRIP5844/32	Trawl, fish, bottom	03/12/2019	-42.7	-177.7	1156-1165
131941	(Alcyonacea)		X	X	X	*Keratoisis magnifica*	BOO	SSO	TRIP5851/89	Trawl, fish, bottom				
131944	(Alcyonacea)			X		*Primnoa notialis*	BOO	ORH	TRIP5851/92	Trawl, fish, bottom				
131946-A	Primnoidae	*Thouarella variabilis*		X	X	*Thouarella variabilis*	THO	ORH	TRIP5851/92	Trawl, fish, bottom				
131946-B	Primnoidae	*Thouarella variabilis*		X	X	*Keratoisis* sp3	THO	ORH	TRIP5851/92	Trawl, fish, bottom				
65960-A	Plexauridae		X		X	*Clematissa*	CHR	ORH	TRIP3142/56	Trawl, fish, bottom	24/06/2010	-38.4	-168.0	280-280
65960-B	Plexauridae		X	X		*Acanthogorgia* sp2	CHR	ORH	TRIP3142/56	Trawl, fish, bottom	24/06/2010	-38.4	-168.0	280-280
65960-C	Plexauridae			X		*Plexauridae* sp1	CHR	ORH	TRIP3142/56	Trawl, fish, bottom	24/06/2010	-38.4	-168.0	280-280

Appendix C Phylogenetic results by individual locus

28S results are not shown since they were only included on a per-family basis in the concatenated analysis (Figure 1) and were not examined independently due to small sample sizes.

5'-mtMutS Bayesian tree



3’-mtMutS Bayesian tree