



# Phylogenetic relationships among octocoral species at bathyal depths in the Phoenix Islands Protected Area

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## Background

- Remote areas of the equatorial central Pacific remain under-characterized with respect to deep-sea benthic fauna and species inventories remain incomplete for areas under protection.
- The Phoenix Islands Protected Area (Republic of Kiribati) is one the largest no-take reserves in the Pacific Ocean whose deep waters are virtually unexplored (Fig. 1).
- Recent explorations throughout PIPA by the NOAA Ship Okeanos Explorer and R/V Falkor since 2017 have yielded a substantial number of deep-water coral collections with many still in the process of being identified.
- The objective of this project was to present a phylogenetic overview of bathyal octocorals in the Phoenix Islands. This effort grew out of a need for better ways to differentiate and identify species of morphologically complex groups of octocorals observed in seafloor ROV video transects.

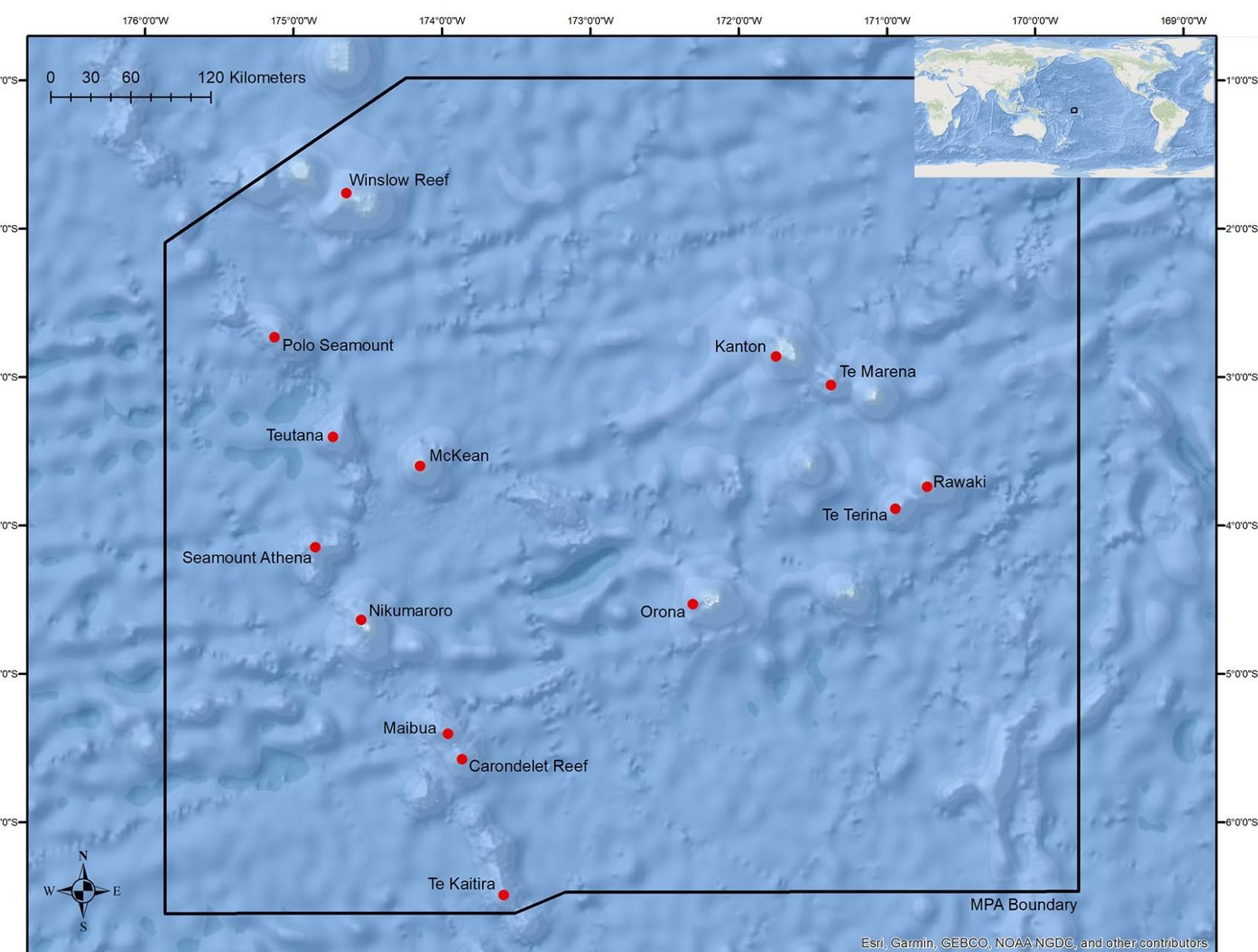


Figure 1: Map of the Phoenix Islands Protected Area and major seamounts, islands, and atolls sampled during 2017 exploration cruises onboard the NOAA Ship Okeanos Explorer and R/V Falkor.

## Collections summary

- Samples used in this analysis were collected across 17 dives in October-November, 2017 on the R/V Falkor ROV Subastian (Table 1).

Table 1: Summary of octocoral collections by family or order from seamounts, islands, and atolls in PIPA during FK171005.

Taxon	# of Specimens	Depth Range (m)
Chrysogorgiidae	21	788 - 2210
Primnoidae	22	378 - 2181
Isididae	6	842 - 2215
Plexauridae	12	374 - 1939
Acanthogorgiidae	5	541 - 1329
Victorgorgiidae	5	574 - 1471
Coralliidae	10	485 - 2215
Paragorgiidae	3	770 - 2069
Alcyoniidae	3	602 - 1369
Pennatulacea	2	1585 - 2319
<b>Total</b>	<b>84</b>	<b>374 - 2319</b>

## Methods

- Tissue was preserved in 95% ethanol and stored at -80°C. Template DNA was extracted using either a Qiagen DNeasy Blood and Tissue Kit or Plant Mini Kit for use in PCR amplification.
- Both *MutS* and *COI+igr1* gene regions were amplified with PCR following the methodology described in Quattrini et al 2014 and McFadden et al 2011. Forward strands were sequenced resulting in ~900 bp length sequences of the *COI+igr1* region and ~725 bp length sequences of the *MutS* region for each sample.
- Alignments were initially conducted within each region using MUSCLE (MEGA v.7), trimmed, and concatenated to form the extended *COI+igr1+MutS* barcode.
- Bayesian inferences tests of the phylogenies were performed to evaluate statistical support for tree structures.

## Results

- In all, 74 haplotypes were identified in this phylogenetic inference representing some 57 species or morphospecies and 19 genera (Fig. 2). The 7 families present in this analysis included the Coralliidae (Fig. 3), Plexauridae (Fig. 4), Acanthogorgiidae (Fig. 4), Chrysogorgiidae (Fig. 5), Primnoidae (Fig. 6), Isididae (Fig. 7), and Victorgorgiidae (Fig. 8).
- The most diverse taxa by number of unique haplotypes were the Chrysogorgiids (15 spp.), followed by the Primnoidae (13 spp.), and Plexauridae (12 spp.).
- Species relationships within the Plexauridae, and specifically the *Paramuricea-Placogorgia* clade, were among the most poorly resolved based on posterior probabilities. Other poorly resolved clades included the relatively speciose Primnoid genus *Calyptrophora* (Fig. 5).
- The *COI+igr1* region could not be amplified from 12 total specimens in the Paragorgiidae, Coralliidae, Alcyoniidae, and Pennatulacea.

## Coralliidae

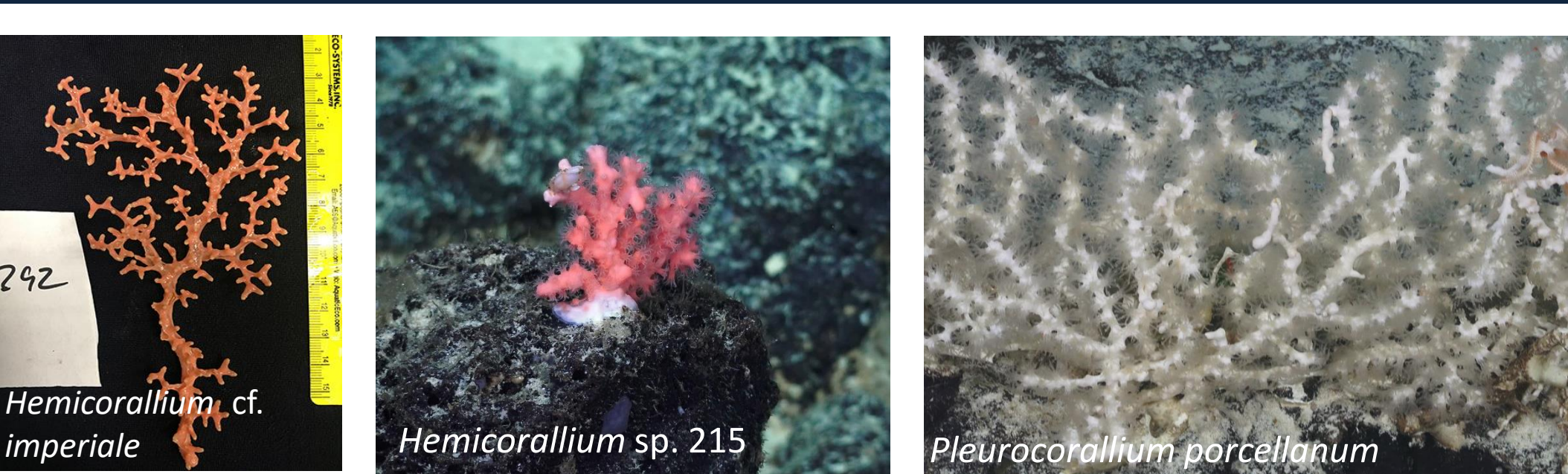


Figure 3: Selected precious corals sampled from the Phoenix Islands Protected Area during FK171005.

## Depth of collection (m)

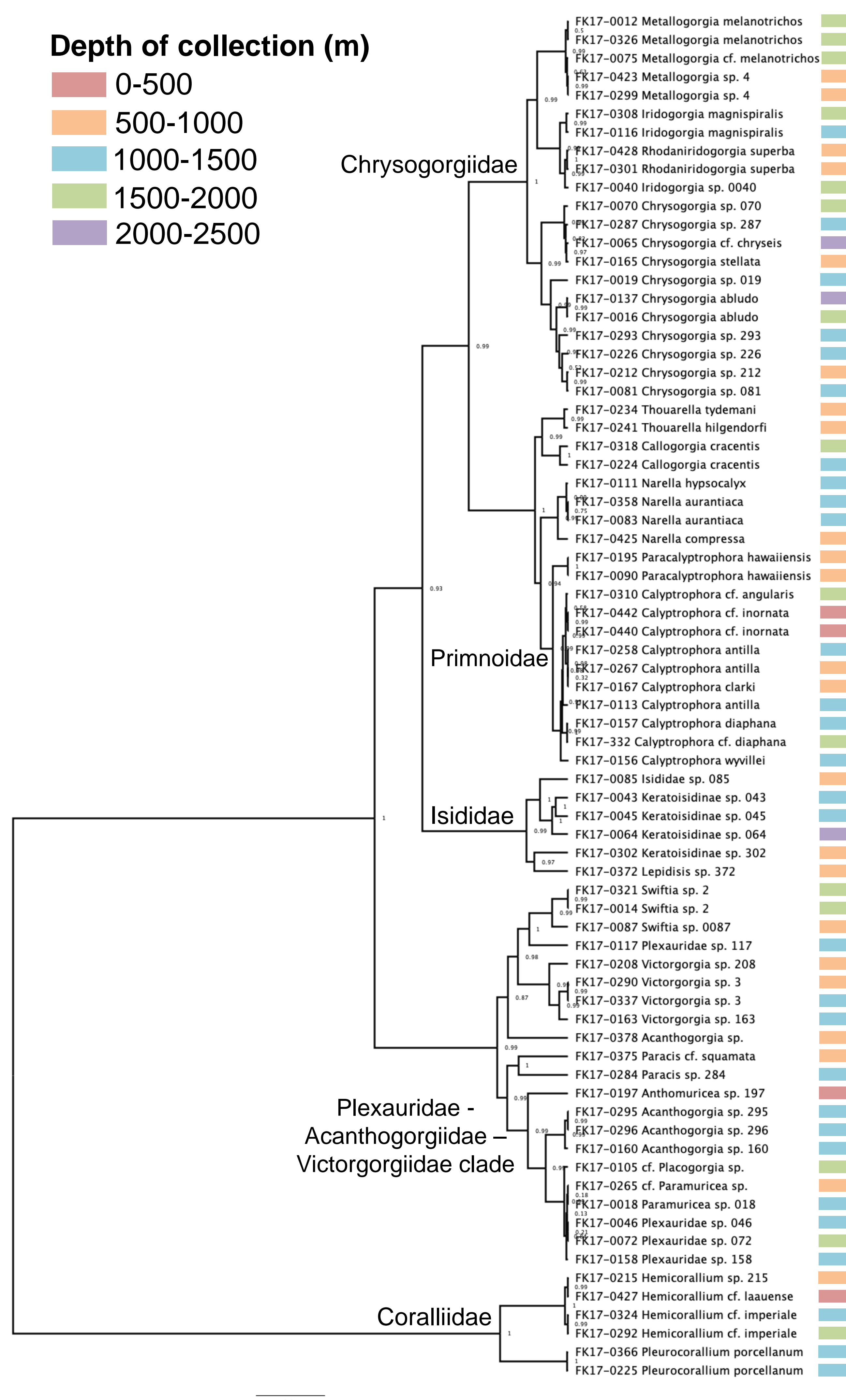
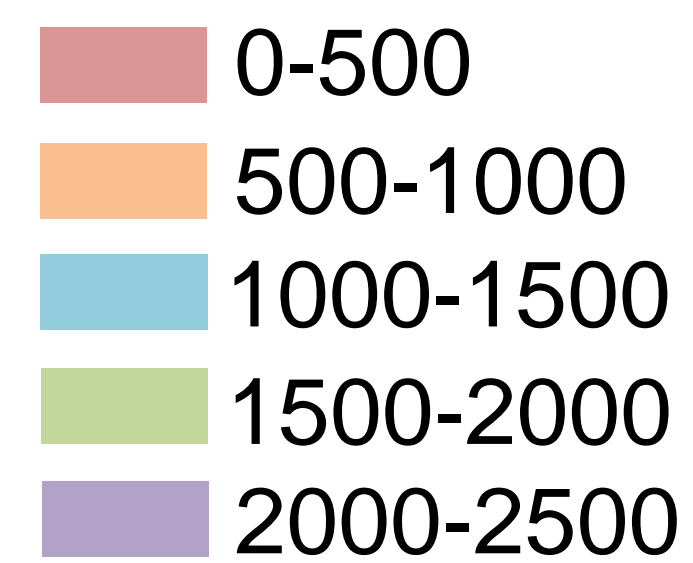


Figure 2: Phylogenetic inference of 74 octocoral haplotypes collected in the Phoenix Islands Protected Area collected during FK171005. This phylogeny is based on an extended mitochondrial barcode of the *mtMutS* + *mtCOI+igr1* genes. Initial alignments were made using the MUSCLE algorithm in MEGA v.7. A consensus tree was constructed using Bayesian inference in BEAST v.2.4.7. Node values indicate Bayesian posterior probability values. Tree was rooted at the midpoint. Color bars to the right of each individual haplotype refer to the depth of the collection. Tree was edited using FigTree v.1.4.4.

## Plexauridae & Acanthogorgiidae

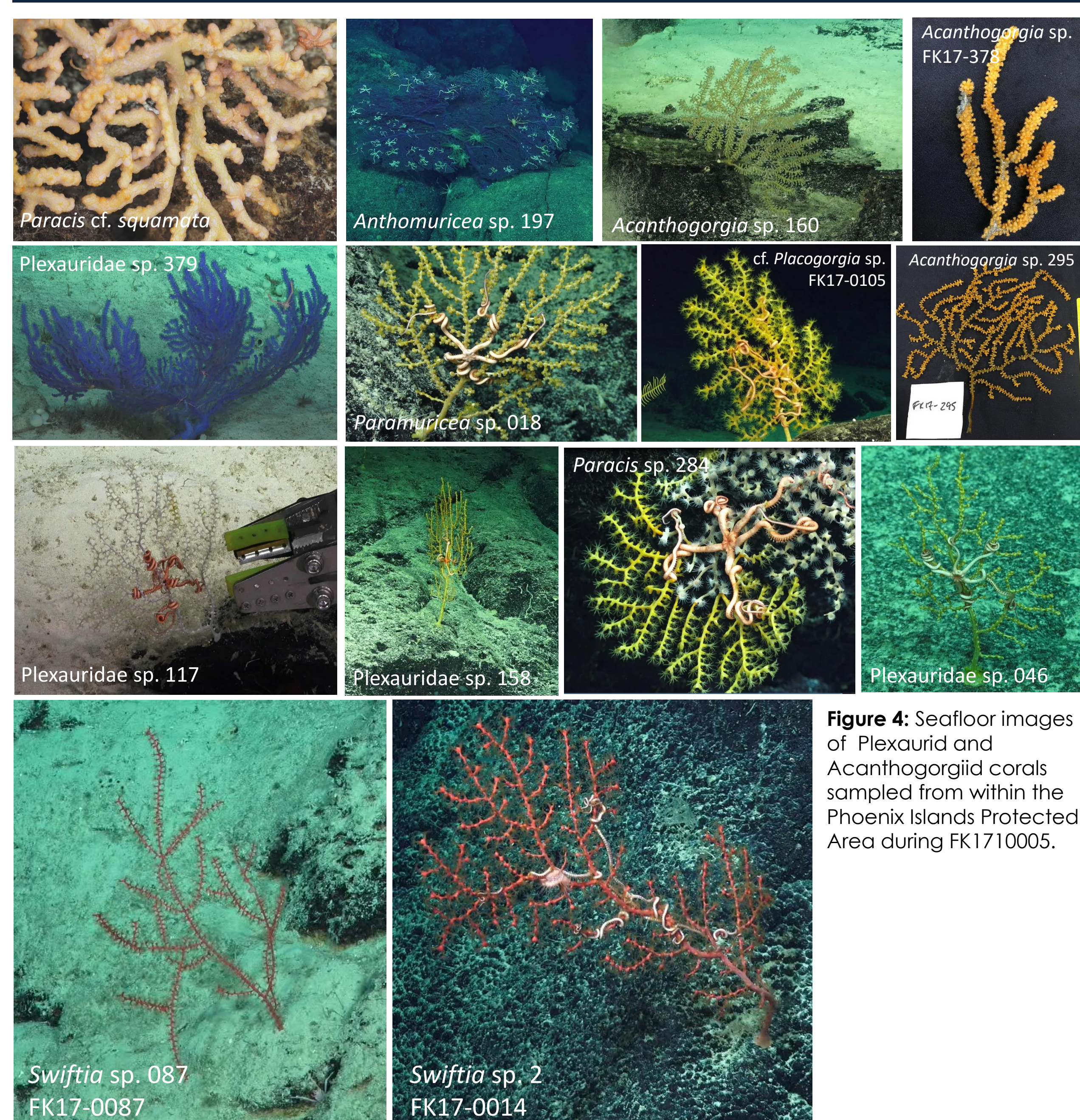


Figure 4: Seafloor images of Plexauridae and Acanthogorgiidae corals sampled from within the Phoenix Islands Protected Area during FK171005.

## Chrysogorgiidae

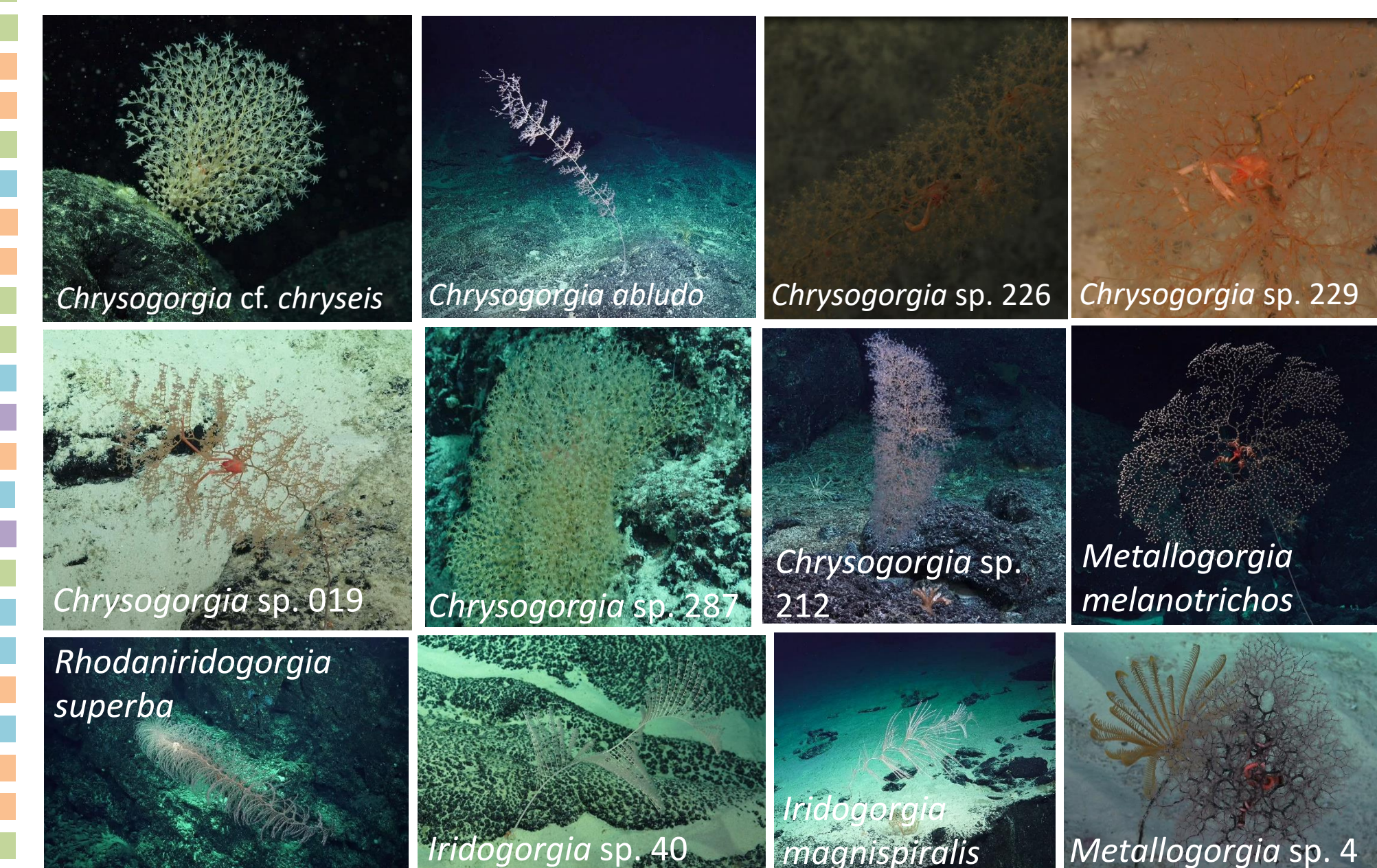


Figure 5: Selected seafloor imagery of Chrysogorgiidae corals from the Phoenix Islands Protected Area collected during FK171005.

## Primnoidae

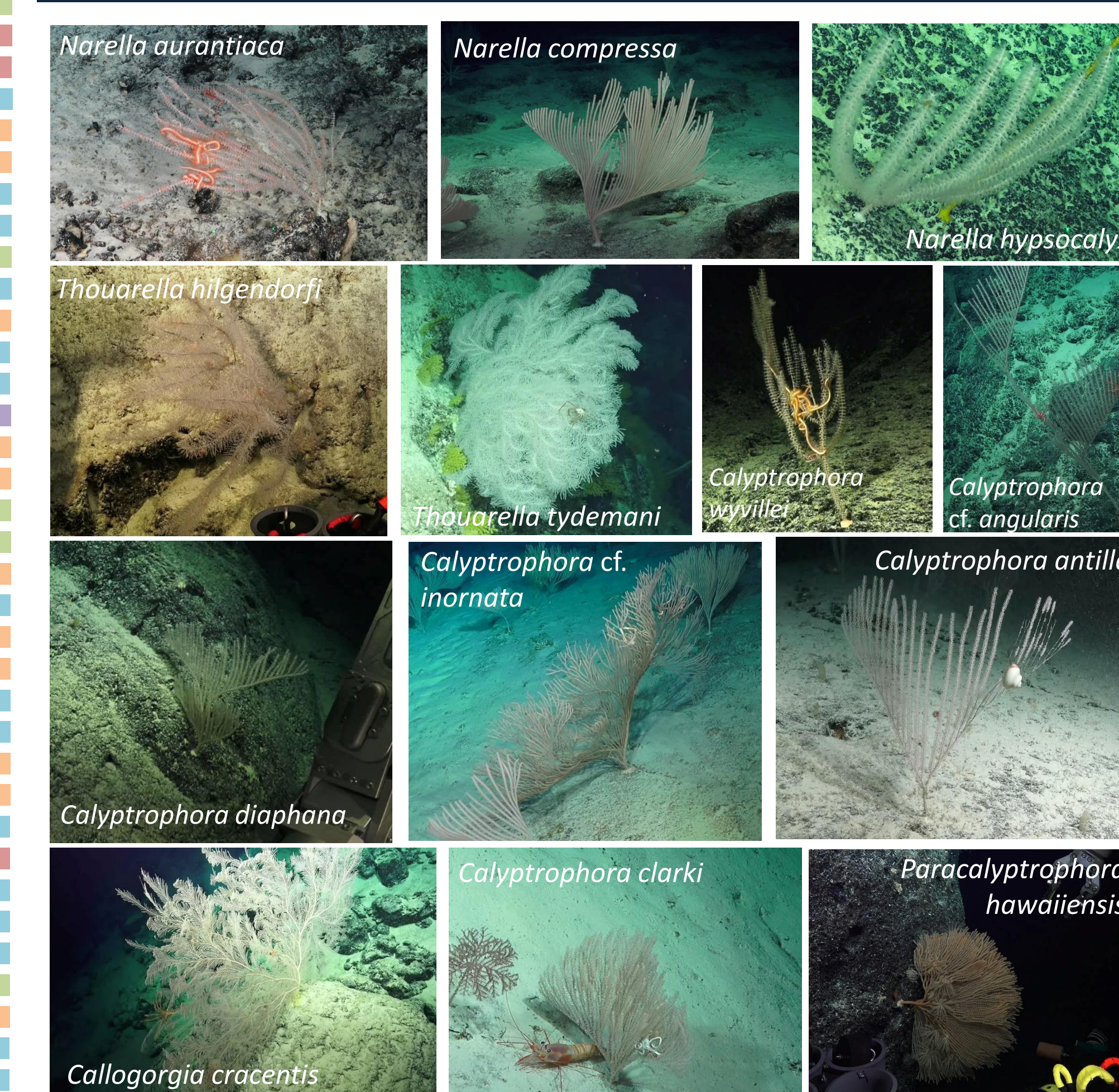


Figure 6: Selected seafloor imagery of Primnoid corals from the Phoenix Islands Protected Area during FK171005.

## Isididae

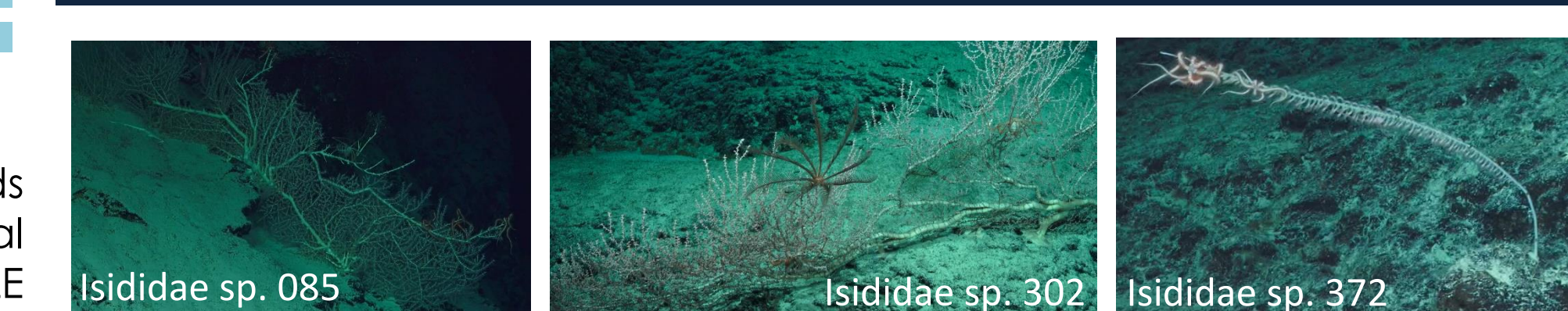


Figure 7: Selected bamboo corals sampled during FK171005 from the Phoenix Islands Protected Area.

## Victorgorgiidae

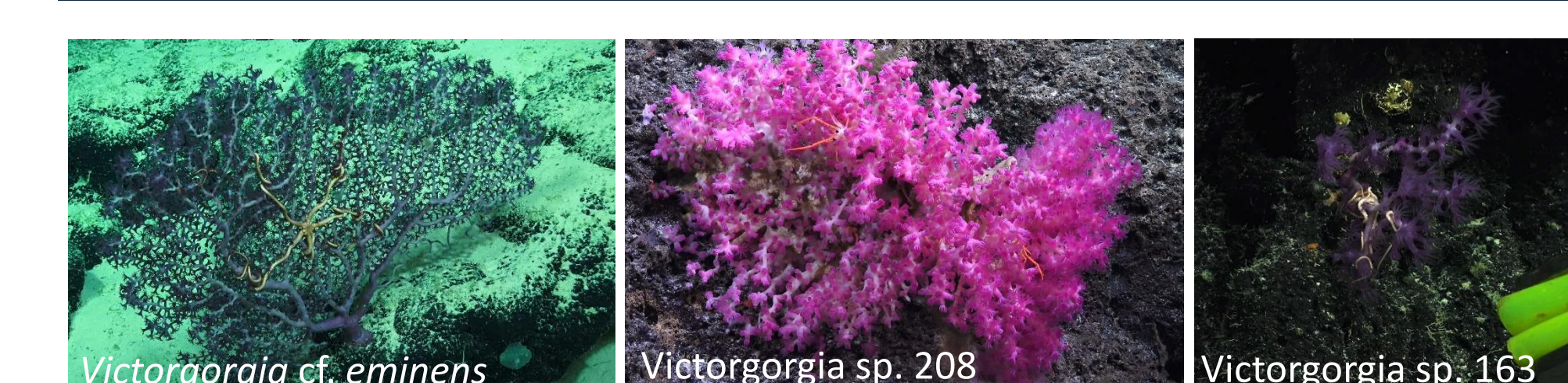


Figure 8: Selected Victorgorgia sp. morphotypes observed and sampled within the Phoenix Islands Protected Area sampled during FK171005.

## Conclusions

- Partial mitochondrial gene sequences were generated for the *mtMutS* and *mtCOI+igr1* genes resulting in 74 haplotypes.
- Of the 74 haplotypes, sequences were obtained from 6 recently described species or rarely observed species, primarily from the Primnoidae (Cairns 2018).
- Poorly resolved portions of the Plexauridae and Primnoidae require further attention to delineate species boundaries using both molecular and morphological tools.
- Complex genetic relationships were also observed within the Primnoid genus *Calyptrophora* which has been found to be relatively speciose within PIPA and the central Pacific.
- Bathymetric patterns within and between octocoral clades were not clearly delineated and require further evaluation to understand the influence of depth on phylogenetic community structure.
- Video analyses have identified as many as 159 visually distinct octocoral morphospecies within PIPA (Auscavitch et al, in review) suggesting a substantial diversity remains to be sampled to complete species inventories for the protected area.
- Upon identification and submission to the NCBI GenBank, these gene sequences for octocorals within PIPA will represent a substantial increase in the available deep-sea phylogenetic resources for the central Pacific Ocean.

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**References:** Auscavitch, S. R., Deere, M. C., Keller, A. G., Rotjan, R. D., Timothy, M., & Cordes, E. E. (In Review). Oceanographic drivers of deep-sea coral species distribution and community assembly on seamounts, islands, atolls, and reefs within the Phoenix Islands Protected Area. *Frontiers in Marine Science*. Cairns, S. D. (2018). Primnoidae (Cnidaria: Octocorallia: Calcaxonia) of the Okeanos Explorer expeditions (CAPSTONE) to the central Pacific. *Zootaxa*, 4532(1), 1-43. McFadden, C. S. et al. (2011). Limitations of mitochondrial gene barcoding in Octocorallia. *Mol Ecol Resour*, 11(1), 19-31. Quattrini, A. M et al. (2014). A phylogenetic approach to octocoral community structure in the deep Gulf of Mexico. *Deep Sea Res. II*, 99, 92-102.