# Phylogenomics and Species Delimitation of Primnoidae (class: Octocorallia) Utilising Ultra-Conserved Elements Sequencing for Evolutionary Analysis

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## Abstract

Previous studies of the deep-sea coral family Primnoidae have relied on mitochondrial and a small number of nuclear genetic markers to reconstruct phylogenies. Here we apply a phylogenomic approach using ultra-conserved element (UCE) loci sequenced from more than 100 Primnoid specimens, with the dual goals of reconstructing a robust phylogeny and testing species boundaries through Bayesian species delimitation. The UCE-based phylogeny produced highly supported topologies, resolving deep divergences and clarifying relationships across the family. Several genera, including *Primnoa* and *Narella*, were recovered as strongly monophyletic, while others, such as *Plumarella* and *Primnoella*, were shown to be polyphyletic, indicating that some diagnostic morphological characters do not reflect evolutionary history. Previously unsampled genera, including *Microprimnoa* and *Narelloides*, were sequenced for the first time and placed into well-supported clades, filling key gaps in Primnoid systematics. To evaluate species boundaries, we conducted coalescent-based species delimitation in BPP on multiple subsets of taxa. In the genus *Thouarella*, distinct genetic lineages were recovered corresponding to several morphospecies, including *T. aureolabyrinthea sp. nov., T. koellikeri, T. viridis, T. brucei,* and *T. crenelata*. By contrast, *T. pendulina, T. variabilis, T. diadema,* and *T. andeep* clustered as a single lineage, suggesting these nominal taxa represent morphological variants within one species. Within the *Primnoella* clade, *P. antarctica* and *O. kuekenthali* were genetically indistinguishable, although they are not proposed as synonyms given their distinct morphological traits. A broader BPP analysis across Primnoidae genera supported all tested morphospecies as independent lineages, underscoring general agreement between morphology and genome-scale data. These

findings demonstrate the capacity of UCEs to resolve both deep and shallow evolutionary relationships in a family where traditional markers have failed, and they highlight the importance of integrating molecular and morphological evidence when delineating species. This work provides the most comprehensive phylogenomic framework yet produced for Primnoidae, expands molecular sampling to include underrepresented genera, and emphasizes the value of genome-scale data for accurate taxonomy and evolutionary inference in cold-water corals.

# Chapter 1: Introduction: Deep-Sea, Phylogenomics, Species Delimitation, and Primnoidae (Class: Octocorallia)

The term deep-sea refers to depths exceeding 200 meters, where sunlight cannot reach (Paulus, 2021). Surprisingly, 90% of the Earth's oceans constitute the deep-sea, making it the largest yet least-explored environment on our planet, with extensive range of animals and ecosystems (Robison, 2009; Taylor and Rogers, 2017). Organisms in this environment navigate extremes such as near-freezing or extremely high temperatures, high pressure, low nutrient availability and the absence of light (Feng *et al.*, 2022; Robison, 2009). Despite these challenges, life in the deep-sea has not only endured but thrived, evidenced by the abundance of species. Notably, two-thirds of the described Primnoidae species come from the depths greater than 50m (Cairns, 2007).

## Anthropogenic Pressures and Conservation Frameworks in Deep-Sea Ecosystems

The deep-sea environment, once considered a remote and insulated, is increasingly recognised as vulnerable to multifaceted anthropogenic pressures ranging from resource exploration to global cimate shifts (Danovaro *et al.*, 2004; White *et al.*, 2012). Fishing, particularly bottom trawling, represents and the most immediate physical threat.  By dragging heavy nets across the seabed, these operations cause significant habitat destruction and sediment resuspension (Clark *et al.*, 2016; De Madron *et al.*, 2005). This process, often compared to the clear-cutting of terrestrial forests, removes essential habitat-forming species like cold-water corals (CWCs) and fundamentally alters benthic ecosystem functions (Watling, 2005) Furthermore,

sediment plumes can smother organisms far beyond the immediate fishing zone. Controlled laboratory experiments by Mobilia *et al*. (2023) demonstrated that elevated sedimentation leads to significant tissue damage and decreased survival in species such as *Goniocorella dumosa*. Because deep-sea species possess slow growth rates, these ecosystems show little to no signs of recovery even decades after disturbance ceases (Clark *et al.*, 2019).

Danovaro *et al*. (2004) showed that small temperature changes can significantly affect deep-sea biodiversity, suggesting that further warming may pose risk in environments where species are already close to their thermal limits.

Pollution and the influx of microplastics also pose significant risks to deep-ocean health. The 2010 Deepwater Horizon disaster demonstrated the catastrophic reach of oil spills, with hydrocarbons settling on the seafloor and causing long-term stress to coral communities over 20 kilometres away (White *et al*., 2012; Fisher *et al.,* 2014). Additionally, the widespread presence of microplastics in deep-sea sediments and organisms suggests persistent ecological consequences. These particles can cause physical blockages in feeding structures and introduce toxic chemicals into the deep-sea food web (Kane *et al*., 2020; Taylor *et al*., 2016). To mitigate these compounding impacts, Marine Protected Areas (MPAs) and Vulnerable Marine Ecosystems (VMEs) serve as indispensable tools for deep-sea conservation. These frameworks range from multiple-use areas to strict no-take zones where all extractive activities are prohibited (Costello and Ballantine, 2015; Halpern, 2003). By restricting bottom trawling and deep-sea mining, MPAs maintain the physical integrity of fragile CWC reefs and the vast biodiversity they support (Freiwald *et al*., 2004; Auster, 2005). Beyond habitat protection, these areas facilitate the recovery of degraded ecosystems and ensure the long-term survival of commercially and ecologically significant species

(Girard *et al*., 2023; Paulus, 2021). Furthermore, protecting these reefs enhances their capacity for carbon sequestration, thereby contributing to global climate change mitigation efforts (Thresher *et al*., 2011). Case studies such as the OSPAR Convention in the North-East Atlantic and the Ross Sea Region MPA, which is one of the world's largest protected areas, highlight the effectiveness of these designations in safeguarding large-scale ecological processes and preserving critical deep-water habitats against increasing industrial encroachment (Johnson *et al*., 2014; Brooks *et al*., 2020).

## Cold-Water Corals and their ecosystem services

Cold-water corals (CWCs) are vital components of deep-sea ecosystems, providing habitat, breeding grounds, and feeding areas for a diverse range of marine species. These corals are found in all the world's oceans, typically at depths ranging from 50 meters to over 2000 meters (Roberts *et al.*, 2006). They form complex reef structures that are hotspots of biodiversity and are critical for the health of marine ecosystems (Davies *et al.*, 2007).

CWCs play a crucial role in deep-sea ecosystems, serving as vital habitats for a variety of marine organisms. These corals provide essential ecosystem services, including serving as nesting grounds for fishes and offering shelter from strong currents and predation (Auster, 2005; Paulus, 2021). The structural complexity and diversity of CWC reefs create a variety of microhabitats that support a high diversity of species, including commercially valuable fish, molluscs, echinoderms, and crustaceans (Girard *et al.*, 2023; Paulus, 2021).

### Role of CWC as Habitat and Shelter

Cold-water corals, such as those found in the North Atlantic and Pacific Oceans, offer critical habitat for many deep-sea species (Henry and Roberts, 2017). For example, *Primnoa* spp., a genus of gorgonian corals, provides habitat for various megafaunal groups, including fish, crabs, and shrimp (Krieger and Wing, 2002). These organisms utilize coral branches for protection against predators, as well as for feeding, with species like sea stars preying on coral polyps and suspension feeders attaching to the coral structure (Henry and Roberts, 2017). The structural complexity of CWCs, particularly the large branching colonies, offers essential protection and feeding grounds that are vital for the survival of many species in the deep sea (Henry and Roberts, 2017).

**Role of CWC as Nursery Grounds**

Cold-water corals also serve as nursery grounds for various fish species, playing a critical role in their early life stages. For instance, Baillon *et al.* (2012) observed the close association of redfish larvae (*Sebastes* spp.) with sea pens in deep waters off the east coast of Canada. This study highlighted that CWCs provide a protective habitat for these larvae during their vulnerable developmental stages, underscoring the ecological importance of CWCs as essential fish habitats (Baillon *et al.*, 2012).

**CWC are Biodiversity Hotspots**

The biodiversity supported by CWCs is comparable to that of tropical coral reefs, with these ecosystems hosting a wide variety of species, including those that are commercially valuable (Freiwald *et al.*, 2004). The presence of economically important species within these reefs increases the risk of habitat destruction due to fishing practices such as trawling (Roberts, 2009). Trawling has been shown to cause

significant physical damage to coral habitats, leading to long-term changes in the associated megafaunal communities and potentially resulting in irreversible losses in biodiversity (Girard *et al.*, 2023; Paulus, 2021).

### Ecological and Economic Importance of CWC

The reliance of marine organisms on CWCs highlights the dual ecological and economic value of these ecosystems. CWCs are not only crucial for maintaining biodiversity and ecosystem health, but they also support species that are of commercial interest (Girard *et al.*, 2023). This dual role increases the vulnerability of CWCs to human activities, making it essential to balance conservation efforts with economic interests to prevent the degradation of these vital deep-sea habitats (Girard *et al.*, 2023).

## Ultra-Conserved Elements (UCEs)

Ultra-Conserved Elements (UCEs) are highly conserved genomic regions found across diverse taxa. These elements are invaluable for molecular studies because they enable researchers to obtain genetic data from specimens with highly degraded DNA, such as those found in museum collections. UCEs are particularly useful because they include conserved core regions, facilitating the capture of genetic material even from fragmented DNA, while the flanking regions provide variable sequences suitable for phylogenetic and population genetic analyses (Faircloth *et al.*, 2012; McCormack *et al.*, 2016). UCEs are a powerful tool facilitating the targeted enrichment and sequencing of numerous orthologous loci, even among species that have undergone evolutionary divergence spanning hundreds of millions of years (Faircloth *et al.*, 2012). UCEs are typically short segments dispersed across the genome, with considerable genomic gaps between them. They preserve an imprint of

evolutionary history, the temporal depth of which depends on their proximity to the central UCE region and the substantial genomic spans separating them (Faircloth *et al.*, 2012).

## Primnoidae Family: Diversity and Morphology

The family Primnoidae is a relatively common and well-distributed group of sessile, suspension-feeding octocorals, found in all the world's oceans at depths ranging from 8 to 6400 meters (Madsen, 1956; Taylor and Rogers, 2015). As the fourth largest family by number of species and the third largest in terms of genera within the class Octocorallia, Primnoidae plays a crucial role in deep-sea ecosystems. These corals are particularly abundant in regions such as the North Atlantic and the Southern Ocean, where they form significant cold-water coral (CWC) habitats on continental shelves, slopes, seamounts, and deep oceanic trenches (Cairns, 2007; Freiwald *et al.*, 2004; Roberts *et al.*, 2006).

Primnoidae corals are characterized by unique scale structures that contribute to their ecological importance as habitat providers for various marine species, including fish and other sea life (Cairns and Bayer, 2009). Large colonies, such as those of *Primnoa*, can reach up to 2 meters in height and 7 meters in width, offering critical refugia on seamounts and other deep-sea features (Cairns and Bayer, 2009). The solid, layered axes of these corals not only provide structural support but also enable researchers to conduct isotope analyses of their skeletal material, a method also widely applied to scleractinian corals (Little *et al*., 2021), as well as gorgonians, offering valuable insights into past ocean temperatures and paleoclimatic conditions (Risk *et al.*, 2002; Sherwood *et al.*, 2005; Sinclair *et al.*, 2005).

Primnoidae exhibits a remarkable range of morphological diversity, making taxonomic classification within this family both complex and intriguing (Cairns, 2011). Traditional morphological characters used to delineate genera and species within the family include colony shape, polyp arrangement, the presence of specific body wall scales, and the number of rows of these scales (Núñez-Flores *et al.*, 2020; Pérez *et al.*, 2016; Taylor *et al.*, 2013). However, many of these traits are highly labile, often exhibiting significant homoplasy due to convergent evolution (Cairns and Wirshing, 2018). For instance, branching morphology, a key trait in differentiating genera, can vary significantly even within species, complicating taxonomic efforts (Cairns and Wirshing, 2018).

## Classical Taxonomy and Historical Systematics

Primnoid taxonomy has a long history spanning over 150 years. Its origins trace back to 1857, when the group was implicitly established by Milne Edwards under the name "Primnoacées"; and formally named Primnoidae by Gray, 1858. During the late 19th and early 20th centuries, extensive deep-sea expeditions produced many primnoid specimens. Researchers such as Kükenthal, Versluys, Nutting, Thomson and Kinoshita described hundreds of species. A landmark was Kükenthal's monographs which included keys to all known primnoid genera and species and refined higher classification (Cairns and Bayer, 2009).

In the mid-20th century, F. M. Bayer further advanced primnoid systematics. His 1956 revision in the Treatise on Invertebrate Paleontology diagnosed every genus and organised them into four subfamilies based on operculum development and scale arrangements (Cairns and Bayer 2009). Bayer *et al*. (1983) also produced illustrated dichotomous keys to genera.

The traditional taxonomy of primnoids relies on morphological characters (colony shape, polyp arrangement, and especially details of the external scales and opercula). However, many of these features are evolutionarily labile or convergent in octocorals. Comparative mapping of characters on genetic trees has shown extensive homoplasy: for example, colonial branching form often evolves repeatedly in separate lineages (Cairns and Wirshing, 2018).

The understanding of Primnoidae phylogeny has undergone a significant transformation over the last two decades, driven primarily by an evolution from traditional morphology to sophisticated molecular analysis. Early molecular studies, notably the large-scale phylogenies conducted by Taylor and Rogers between 2012 and 2017, established that the Primnoidae form a monophyletic clade. Utilizing a comprehensive five-loci approach involving cox1, mtMutS, 16S, 18S, and 28S, these investigations expanded from an initial 64 species to a broader scope of 81 species across 29 genera. A major finding from this period was that the primnoid tree splits into clades that largely reflect biogeographic patterns, such as the distinction between Antarctic and non-Antarctic lineages. These results supported the hypothesis of an Antarctic origin for the family, suggesting that a major radiation of species occurred following the opening of the Drake Passage.

This genetic framework also revealed that many traditional taxonomic groupings did not align with evolutionary history. While genera like Narella and Parastenella were confirmed as monophyletic, others such as *Callogorgia, Fanellia, Primnoella, Plumarella, and Thouarella* were found to be polyphyletic or paraphyletic. These discrepancies led to several necessary taxonomic revisions during this era, including the synonymization of *Digitogorgia* with *Primnoeides* and the elevation of subgenera like *Faxiella* and *Verticillata* to full genus rank (Taylor and Rogers, 2017).

By identifying these "unclean" genetic clades, researchers began to realize that outward appearance often masked the true evolutionary relationships within the family.

In 2018, Cairns and Wirshing built upon this foundation by providing a highly detailed analysis of character evolution. They integrated a four-gene molecular set with an intensive examination of morphological traits. By sequencing 78 species across 33 genera and mapping nine key physical characters onto the resulting tree, they were able to distinguish between reliable phylogenetic markers and traits resulting from morphological convergence. Their analysis included parameters such as polyp whorl arrangement, branching morphology, and the structure of ascus, marginal, and opercular scales. They discovered that many traditional characters, such as branching patterns and calyx shape, had evolved repeatedly and were therefore poor indicators of ancestry. Conversely, features like the presence of ascus body wall scales and the number of longitudinal scale rows proved to be more reliable for taxonomic classification.

The integration of these morphological insights with genetic data led to further systematic refinements, such as the merging of Fanellia into Callogorgia and the consolidation of Dicholaphis into Plumarella. This comprehensive approach has paved the way for a more nuanced comprehension of the intricate interplay between genetic lineages and physical features. While some genus and species level relationships remain the subject of ongoing research, the combined efforts of these investigators have substantially reshaped primnoid systematics. This has moved the field toward a more stable and biologically accurate classification system that accounts for both genetic data and evolutionary character traits.

## UCEs for Poorly Preserved Specimens

The robustness of UCEs in dealing with degraded DNA has been demonstrated in various studies. McCormack *et al.* (2016) successfully used UCEs to extract genetic data from bird museum specimens, some of which were over a century old. This study showed that UCEs could provide reliable genetic information despite the significant age and degradation of the samples. Similarly, Derkarabetian *et al.* (2019) extended the application of UCEs to historical ethanol-preserved specimens, proving that UCE capture is efficient even with considerable DNA fragmentation due to preservation methods. These findings underscore the broad applicability of UCEs in museum-based molecular studies, highlighting their potential to unlock genetic information from specimens that would otherwise be challenging to analyse (Derkarabetian *et al.*, 2019; McCormack *et al.* (2016). UCEs are capable of being utilized on relatively small DNA fragments, around 800 base pairs (bp), making them suitable for studies involving degraded or limited genetic material. This capability is crucial for incorporating valuable but often degraded historical specimens into phylogenetic studies, thereby broadening the scope and depth of genetic research (Faircloth *et al.*, 2012).

Derkarabetian *et al.* (2019) demonstrated the effectiveness of UCEs in extracting meaningful data from historical specimens, providing enough data for comprehensive phylogenomic analyses. Erickson *et al.* (2021) further highlighted the application of UCEs in poorly preserved samples, emphasizing their robustness in expanding the range of usable genetic material.

### Phylogenomic Studies utilising UCEs

In phylogenomic studies, UCEs have proven to be powerful tools for resolving evolutionary relationships. Gueuning *et al.* (2020) investigated the phylogenetic utility

of UCEs in pinned insect specimens, such as large carpenter bees, demonstrating that UCEs can be effectively used to construct robust phylogenies from specimens with varying degrees of DNA degradation. Their study identified a moderate but significant negative correlation between specimen age and DNA yield, yet they were able to obtain reliable phylogenetic data, confirming the utility of UCEs for historical insect collections (Blaimer *et al.*, 2016). The study by Blaimer *et al.* (2015) on formicine ants further supported the superior phylogenetic resolution of UCEs compared to traditional multi-locus approaches, highlighting their effectiveness in resolving both ancient and shallow evolutionary relationships (Blaimer *et al.*, 2015; Faircloth *et al.*, 2012).

Quattrini *et al.* (2023) conducted phylogenomic analysis on octocorals, including 11 primnoid specimens. Utilizing UCEs as a powerful genomic tool, their methodology demonstrated notable success; in creation of phylogenomic tree. The resulting phylogenetic tree exhibited a high degree of confidence, as evidenced by the substantial support values. This study underscores the robustness and reliability of UCEs in resolving phylogenetic relationships even among complex and morphologically diverse groups such as octocorals (Quattrini *et al.*, 2023).

## Species Delimitation

### Species Differences and Delimitation

The concept of species has been a central focus in evolutionary biology for over a century. Traditionally, species were defined based on morphological traits, with the assumption that distinct morphological features signified reproductive isolation and, therefore, separate species (Coyne and Orr, 2004; Mayr, 1999). However, advancements in molecular biology have shifted the focus towards genetic

differentiation as a more reliable criterion for species identification (De Queiroz, 2007; Sites and Marshall, 2003). De Queiroz (2007) proposed the unified species concept, which posits that species are independently evolving metapopulation lineages. This concept emphasizes that species should not be defined by any single trait, such as reproductive isolation or ecological niche, but rather by their status as distinct evolutionary lineages within the broader tree of life (Carstens *et al.*, 2013; De Queiroz, 2007).

The recognition that species represent unique evolutionary trajectories aligns with the phylogenetic species concept, which defines species as the smallest diagnosable clusters of organisms that share a common ancestor, as indicated by synapomorphies (Cracraft, 1983; Nixon and Wheeler, 1990). This concept is supported by the growing body of evidence demonstrating that genetic differences are often the most reliable indicators of species boundaries, particularly in cases where morphological data are ambiguous or incomplete (Sites and Marshall, 2003; Wiens, 2007).

## Species Hypotheses

Formulating species hypotheses is a fundamental step in biodiversity studies, with these hypotheses often generated based on observed differences in morphology, behavior, or genetic data (Sites and Marshall, 2003). The phylogenetic species concept, which has gained prominence in recent years, suggests that species should be defined as the smallest monophyletic groups distinguishable by unique combinations of character states (Cracraft, 1983; Nixon and Wheeler, 1990). This approach is grounded in the idea that species can be diagnosed based on their genetic

makeup, which is best revealed through phylogenetic analyses (Leaché and Fujita, 2010; Wiens and Penkrot, 2002).

**Species Delimitation Using SNPs and NGS**

The advent of next-generation sequencing (NGS) technologies has revolutionized species delimitation by providing the ability to generate large genomic datasets quickly and cost-effectively (Davey *et al.*, 2011; McCormack *et al.*, 2013). Among the most commonly used markers in NGS-based species delimitation are Single Nucleotide Polymorphisms (SNPs), which are abundant across genomes and provide fine-scale resolution of genetic differences (Davey *et al.*, 2011; Hohenlohe *et al.*, 2010). SNPs are particularly valuable in species delimitation because they allow for the detection of subtle genetic differences that may not be evident from morphological or ecological data alone (Leaché *et al.*, 2014; Shaffer and Thomson, 2007).

SNP-based species delimitation is often conducted within a coalescent framework, a probabilistic model that describes the genealogical history of sampled alleles by tracing lineages backward in time to their most recent common ancestor.This framework provides the statistica; basis for distinguishing between gene flow within populations and the genetic isolation associated with lineage divergence (Carstens *et al.*, 2013; Yang and Rannala, 2010). O'Meara (2010) highlights the importance of SNPs in generating accurate species delimitations, noting that SNP data can reveal cryptic species and clarify relationships in cases of recent divergence (Fujita *et al.*, 2012; Leaché and Fujita, 2010). The use of SNPs also enhances the ability to delimit species in taxa with complex histories of hybridization or incomplete lineage sorting, where traditional markers might fail (Pinho and Hey, 2010)

In practical terms, SNP datasets generated with NGS can be analysed under unified statistical frameworks that jointly estimate species delimitation and phylogenetic relationships (McCormack *et al.*, 2013; Shaffer and Thomson, 2007). This integrated approach offers a comprehensive understanding of biodiversity, particularly in groups where species boundaries are not well-defined. For example, genomic studies using SNPs have successfully resolved species boundaries in a wide range of taxa, from plants to animals, demonstrating the versatility of this approach (Eaton and Ree, 2013; Leaché *et al.*, 2014).

Moreover, NGS and SNP-based methods have been particularly effective in cases where species exhibit high levels of gene flow or where divergence is recent, scenarios that are traditionally challenging for species delimitation (Carstens *et al.*, 2013). By leveraging the power of large genomic datasets, researchers can achieve greater resolution in species delimitation, leading to more accurate biodiversity assessments and better-informed conservation strategies (Fujita *et al.*, 2012; Shaffer and Thomson, 2007).

**Species Delimitation using UCEs**

Ultraconserved elements (UCEs) have been instrumental in resolving species boundaries, particularly in taxa where morphological traits alone are insufficient for accurate delimitation. For instance, Blaimer *et al.* (2016) demonstrated the utility of UCEs in species delimitation within formicine ants. Despite the historical challenges in distinguishing species due to morphological similarities and limited genetic divergence using traditional markers, UCEs provided the necessary resolution to clearly delineate species boundaries. Similarly, Smith *et al.* (2014) applied UCEs to delineate species in birds, where traditional methods failed to provide clear boundaries. Their work

exemplified how UCEs can reveal cryptic species that are genetically distinct but morphologically similar, offering a more accurate representation of biodiversity.

Building on the success observed in insect studies, UCEs have also proven highly effective in marine organisms, particularly in corals. Erickson *et al.* (2021) utilised UCEs to explore species boundaries and population structures in anthozoans, class that includes corals. Their research demonstrated that UCEs could resolve species boundaries that are challenging to define using morphological traits or traditional molecular markers. This high-resolution genetic data was crucial for accurately understanding the diversity and evolutionary relationships within this group, providing new insights into the population structure of anthozoans (Erickson *et al.*, 2021).

These studies collectively demonstrate the remarkable potential of UCEs in species delimitation and population genetic research, paving the way for more comprehensive and accurate analyses of biodiversity across a wide range of taxa, from ants to corals (Blaimer *et al.*, 2016; Erickson *et al.*, 2021; Smith *et al.*, 2014)

The application of UCEs in the family Primnoidae (Octocorallia) can similarly enhance phylogenomic analyses and species delimitation. UCEs offer a promising approach for resolving complex phylogenetic relationships and accurately identifying species boundaries. This is particularly relevant for the description of new species within Primnoidae, where traditional morphological methods may fall short (Faircloth *et al.*, 2012; McCormack *et al.*, 2016).

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# Chapter 2: Resolving Deep Phylogenomic Relationships in Primnoidae (class: Octocorallia) Using Ultra-Conserved Elements (UCEs)

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## Abstract

Primnoidae, a highly diverse family of octocorals, plays a critical ecological role in deep-sea environments by providing structural habitats for a wide range of marine species. While previous studies have utilized genetic markers to investigate phylogenetic relationships within this family, some deeper relationships within clades have remained difficult to resolve. In this study, we apply ultra-conserved elements (UCEs) to enhance the phylogenomic resolution and clarify these deeper relationships. Recovered maximum likelihood tree shows well supported relationships within the family. The results reveal clades within the Primnoidae, confirm the monophyly of key genera such as *Primnoa* and *Narella*, and includes two genera, *Microprimnoa* and *Narelloides* that were not included in previous phylogenies. Additionally, taxonomic inconsistencies with the previous phylogenetic studies on Primnoidae were observed, particularly in the placement of *Plumarella*, *Fannyella* and *Primnoella*, suggesting the need for further investigation. These findings enhance the understanding Primnoidae evolutionary history and provide valuable insights for future taxonomic revisions and conservation efforts.

## Introduction

Primnoidae, a prominent family within the subclass Octocorallia, is distinguished by its extensive species diversity and wide geographical range. With a currently recognised total of 318 species and 46 genera according to World Register of Marine Species (WoRMS, April 2026), Primnoidae is not only one of the most diverse families but also ranks first in generic diversity among octocorals (Cairns and Wirshing, 2018). This family has extensive distribution from the Arctic to the Antarctic, predominantly inhabiting deep-sea environments (Cairns and Bayer, 2009). Notably, 96% of Primnoidae species are found at depths exceeding 100 meters, with some reaching as deep as 6400 meters (Cairns, 2011; Cairns, 2016). More than half of the octocoral species living below 3000 meters are members of Primnoidae family (Cairns, 2016).

Understanding the phylogenomic relationships within the deep-sea coral communities, especially the family Primnoidae, is vital for both ecological studies and conservation efforts. Primnoidae plays a key role in deep-sea ecosystems by providing structural habitats that support a variety of marine life (Cairns and Bayer, 2009). However, the evolutionary relationships within this family have been challenging to resolve due to the morphological plasticity of the species and the limitations of traditional genetic markers (Cairns and Wirshing, 2018; McFadden *et al.*, 2017).

Historically, the classification of Primnoidae relied heavily on morphological characteristics, such as colony branching patterns, sclerite shapes, and polyp arrangements (Cairns and Bayer, 2009; Taylor and Rogers, 2017). The modern foundation was laid by Cairns and Bayer (2009) in a comprehensive revision: they reviewed all known genera (36, seven subgenera, 233 species) with diagnoses

illustrated by scanning electron micrographs. They constructed an indented dichotomous key and performed a 27-character cladistic analysis. Key results were that many traditional subfamilies are not monophyletic and that primnoids likely originated in the Antarctic.

While these morphological traits have been useful, they often result in taxonomic ambiguity due to their plasticity and convergent evolution (Taylor and Rogers, 2015). Consequently, the advent of molecular techniques has been crucial in improving our understanding of Primnoidae phylogeny. Early molecular studies focused on mitochondrial DNA (mtDNA) markers like COI and nuclear markers such as 28S rDNA, but these often lacked the resolution needed to resolve closely related species or deeper phylogenomic relationships (Cairns and Wirshing, 2018; Taylor and Rogers, 2015; Taylor and Rogers, 2017).

Recent investigations have significantly enhanced our understanding of Primnoidae phylogeny, with notable contributions from studies by Taylor and Rogers (2015) Taylor and Rogers (2017) and Cairns and Wirshing (2018). Taylor and Rogers (2015) and Taylor and Rogers (2017) employed a comprehensive methodological approach, utilizing five distinct genetic loci: cox1, mtMutS, 16S, 18S, and 28S. Their 2015 study analysed 64 species across 25 genera, which was further expanded in their 2017 study to include 81 species spanning 29 genera.

They recovered Primnoidae as monophyletic and found the family's base to be Pacific in origin, contrary to the Antarctic origin hypothesis. They also reported that species-rich Antarctic lineages represent a secondary radiation However, they observed several genera as non-monophyletic: *Callogorgia, Fanellia, Primnoella,*

*Plumarella, and Thouarella* did not form single clades (Taylor and Rogers, 2015; Taylor and Rogers, 2017).

Cairns and Wirshing (2018), while maintaining a phylogenetic perspective, utilized a slightly reduced set of loci, focusing on cox1, mtMutS, 18S, and 28S. This study encompassed 78 species across 33 genera. The research placed a significant emphasis on the morphological characteristics critical to primnoid taxonomy. They meticulously examined various parameters, including the presence and number of ascus body wall scales, the coordination and number of polyps, the number of marginal scales, the correspondence of opercular and marginal scales, the number of longitudinal rows of body wall scales, the number of scales on the abaxial face, and the branching and colony morphology (Cairns and Wirshing, 2018).

To address ongoing challenges in resolving phylogenetic relationships, recent studies have turned to phylogenomic approaches, particularly the use of Ultra-Conserved Elements (UCEs). UCEs are highly conserved regions of the genome that are flanked by more variable sequences, making them ideal for resolving phylogenomic relationships across different taxonomic levels (Erickson *et al.*, 2021; Faircloth *et al.*, 2012; McCormack *et al.*, 2013). The application of UCEs in marine invertebrates, including octocorals, has proven to be a powerful tool for clarifying evolutionary relationships that were previously unresolved using traditional genetic markers (Erickson *et al.*, 2021; McFadden *et al.*, 2017; McFadden *et al.*, 2021). However, within Octocorallia, published UCE-based phylogenomic studies have not yet specifically focused on Primnoidae.

The adoption of UCE-based phylogenomics in octocoral studies, particularly in Primnoidae, not only improves phylogenomic resolution but also allows for the

integration of genetic data with morphological and ecological information. This integrative approach is crucial for accurately delineating species boundaries, especially in taxa with high morphological variability like Primnoidae (Cairns and Wirshing, 2018; Erickson *et al.*, 2021; McFadden *et al.*, 2017). UCEs can resolve discrepancies in previous phylogenetic analyses involving genera such as *Narella* and *Candidella*, where traditional markers have provided conflicting signals (Cairns and Wirshing, 2018; Erickson *et al.*, 2021; McFadden *et al.*, 2017).

Moreover, UCEs facilitate the examination of deep and shallow phylogenomic relationships simultaneously, making them particularly useful for studies aimed at understanding the evolutionary processes that shape deep-sea biodiversity. This is especially important in the current context of increasing anthropogenic pressures on deep-sea ecosystems, where a clear understanding of species relationships and distributions is critical for effective conservation efforts (Girard *et al.*, 2023; Roberts *et al.*, 2006; Watling, 2005). The ability of UCEs to provide insights into the historical biogeography and species diversification within Primnoidae makes them a valuable tool for informing conservation strategies aimed at preserving these vulnerable deep-sea habitats (Erickson *et al.*, 2021; McFadden *et al.*, 2017; Roberts, 2009).

In summary, this study builds on the foundational work of Cairns and Wirshing (2018) by applying UCE-based phylogenomics to aid understanding of Primnoidae species evolution. The aim is to construct a highly resolved phylogenomic tree of the Primnoidae using UCE loci, thereby clarifying deep relationships and informing taxonomy and conservation efforts within Primnoidae family (Erickson *et al.*, 2021; McFadden *et al.*, 2017; Taylor and Rogers, 2017).

## Methods

**DNA Extraction and library preparation**

DNA was extracted using the Qiagen DNeasy Blood & Tissue Kit, following the manufacturer's protocol. After the initial lysis step, either 2 µL of RiboShredder was added and incubated at 30°C for 10 minutes, or 4 µL of RNase A was added and incubated at room temperature for 10 minutes. This step was performed to remove RNA contaminants and ensure high-purity DNA. The elution step was carefully conducted by passing 60 µL of elution buffer through the spin column filter twice, maximizing DNA yield. Genomic DNA libraries were constructed using the Kapa Hyper Prep protocol (Kapa Biosystems). For samples requiring enzymatic fragmentation, we employed the Kapa Hyper Plus kit to achieve a target insert size of approximately 400–600 bp Quattrini *et al.* (2018). Target enrichment was conducted using the octo-v2 probe set (Erickson *et al*., 2021). Following the optimization results of Quattrini *et al*. (2018), which demonstrated that reduced bait concentrations do not significantly impact locus recovery, we utilized a 1/2 bait strength (250 ng per reaction) (Quattrini *et al*., 2018). This modification allowed for a more cost-effective enrichment process without compromising the capture of ultraconserved elements (UCEs) or exon loci. Enriched libraries were pooled and sequenced on an Illumina HiSeq 3000.

**Post sequencing analysis**

Post-sequencing analyses were conducted following the protocols outlined in PHYLUCE, based on the online tutorial provided by Faircloth (2016), with modifications to accommodate specific research needs. The steps are detailed below, from raw data processing to the final phylogenomic inference.

Raw sequencing reads were processed with Illumiprocessor to remove adapter contamination and low-quality bases. This step ensures that downstream de novo

assemblies are not biased by sequencing artifacts. Cleaned reads were assembled into contigs using SPAdes v. 3.12 (Bankevich *et al*., 2012). To diagnose the quality of the assembly, we assessed the distribution of contig lengths. This metric served as a primary filter for sample viability; samples yielding fewer than 1,000 total contigs (FM59, AV13, JCR3220, S158, Y6, and Z57) were excluded from further analysis. This strict cutoff was implemented to prevent the inclusion of poorly enriched samples that could introduce significant missing data into the final matrices. Targeted loci were identified by matching the assembled contigs against the octo-v2 bait set using a 70/70 stringency threshold (70% identity and 70% coverage). This specific threshold was selected to accommodate the natural sequence divergence between octocoral clades while remaining stringent enough to exclude non-target or paralogous sequences.

Loci were aligned using MAFFT, which is well-suited for capturing both the highly conserved UCE cores and the more variable flanking regions. To ensure the final phylogeny was based on a dense data matrix, we filtered the alignments to maintain a 75% taxon occupancy. By excluding loci present in fewer than 75% of the taxa, we mitigated potential phylogenetic artifacts, such as long-branch attraction, which are often exacerbated by high levels of missing data (Quattrini *et al*., 2018).Phylogenomic Analysis: The final data matrix, representing loci with at least 75% taxon occupancy, was concatenated into a single alignment file using phyluce_align_concatenate_alignments. Phylogenomic inference was then conducted using IQ-TREE (Nguyen *et al.*, 2015) with the command iqtree -s 75p-iq.phylip -m MFP -nt 40 -keep-ident -pers 0.1 -bb 1000. This analysis involved ModelFinder integrated within IQ-TREE (Kalyaanamoorthy *et al.*, 2017) to select the best-fit substitution model, which was identified as TVM+F+R4 according to the Bayesian Information

Criterion (BIC). Node support in the resulting phylogenomic tree was assessed using 1000 ultrafast bootstrap replicates.

Outgroup Selection and Rooting: The phylogenomic tree was rooted using an outgroup composed of several genera some of which are from the taxa which have previously fallen as sister group to Primnodiae, Keratoisididae: *Acanella,* and others, *Trichogorgia, Anthomastus,Corallium, Paragorgia, Radicipes, Iridogorgia* and *Scleraxis*.

## Results and Discussion

A total of 140 taxa were analysed, with 2,988 UCE loci recovered from the 3,040 loci in the probe set. Trimmed reads were assembled into a mean of 1,572 ± 159.21 SD contigs per sample, with a mean contig length of 1,052 ± 269.38 bp, using SPAdes (Bankevich *et al.*, 2012). UCE loci retrieval per sample ranged from 68 to 78.

After alignment and trimming, 2,959 UCE loci remained, with a total alignment length of 826,072 bp and a mean locus length of 279 bp (range: 36-3,274 bp). Informative sites totalled 341,602, averaging 115 per locus. Taxon representation per locus ranged from 3 to 139 taxa (mean: 75.3).

The phylogenomic analysis conducted in this study using ultra-conserved elements (UCEs) represents the first application of this method to the Primnoidae family as a main study group. The resulting tree (Figure 1) provides a detailed and well-supported phylogeny, revealing several clades that correspond to those identified in previous studies, including Cairns and Wirshing (2018). However, the UCE-based tree also introduces novel insights, particularly regarding the placement of certain genera and includes two genera, *Microprimnoa* and *Narelloides,* not previously

included in published phylogenies. To facilitate direct comparison and maintain consistency with previous research, the clade designations initially established by Taylor and Rogers (2015) and subsequently adopted by Cairns and Wirshing (2018), have been followed in this analysis. Comparison with previous studies shows enhanced resolution, supporting the use of UCEs.

The overall topology recovered in this study is highly congruent with that of Cairns and Wirshing (2018), with major clades (e.g. Clades 1A, 1B, and 5) consistently resolved across both analyses. This agreement reinforces the stability of higher-level phylogenetic structure within Primnoidae and suggests that these clades likely represent true evolutionary lineages. However, as also observed by Cairns and Wirshing (2018), several genera, including *Candidella* and *Primnoella*, exhibit polyphyletic patterns, indicating that current taxonomic classifications do not accurately reflect evolutionary relationships.

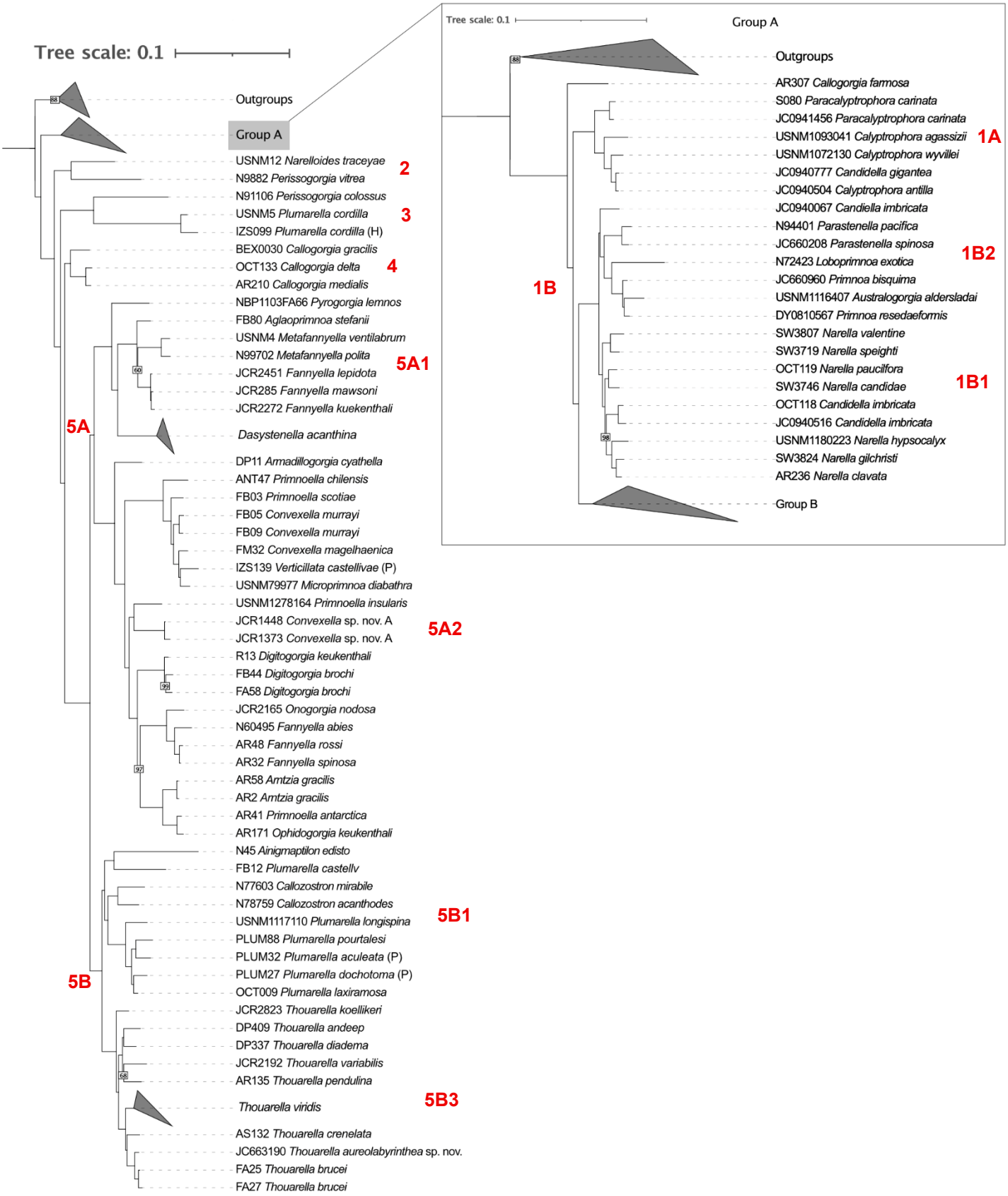


Figure 1. Maximum Likelihood phylogenomic tree of Primnoidae inferred from ultra-conserved elements (UCEs) using IQ-TREE. The tree was generated from a concatenated alignment under the best-fit substitution model selected by ModelFinder Plus. Node support values were estimated with 1,000 ultrafast bootstrap replicates, and bootstrap percentages <99 are displayed on the tree. Holotypes and paratypes are indicated with (H) and (P), respectively. The tree is rooted with outgroup taxa, and

major clades are labeled according to the previous phylogenetic study of Primnoidae. A portion of the tree has been magnified as an inset to improve readability within the A4 format. Clade structure

Clade 1A

Clade 1A in the UCE-based phylogeny includes *Paracalyptrophora*, *Calyptrophora*, and *Candidella*, mirroring the structure found in Cairns and Wirshing (2018). The consistency in the placement of these genera across studies supports the robustness of their phylogenomic relationships. However, this study does not include *Paranarella*, a genus that Cairns placed within this clade. The absence of *this genus* limits direct comparisons, but the strong support for the remaining genera in this clade reinforces the stability of this clade.

*In contrast to Cairns and Wirshing (2018), where Candidella was represented by a single species (Candidella helminthophora) and therefore appeared restricted to a single clade (1B2), the inclusion of multiple Candidella species in this study reveals clear polyphyly. Specifically, Candidella gigantea is placed within Clade 1A, embedded within Calyptrophora, whereas Candidella imbricata is recovered in Clade 1B2. This discordance demonstrates that the apparent stability of Candidella in previous analyses likely reflects limited taxon sampling rather than true evolutionary relationships. The polyphyletic distribution observed here suggests that current genus-level classification does not accurately represent evolutionary history and highlights the need for broader taxon sampling and taxonomic re-evaluation of the genus.*

Clade 1B

Clade 1B in the current study retains the genera Narella, Loboprimnoa, and Primnoa, similar to Cairns and Wirshing (2018). The placement of Australogorgia

within this clade suggests a close evolutionary relationship with Primnoa. However due to the lack of replicates for this genus further analysis is recommended to confirm the relationship.

Clade 2

In Clade 2, this study introduces the genus *Narelloides*. The absence of *Pachyprimnoa* in this study limits direct comparisons, but the strong support for *Narelloides* in this clade emphasizes the utility of UCEs in uncovering new phylogenomic relationships helping us further understand Primnoidae family.

Clade 3

The presence of *Plumarella* in Clade 3 does not align with Cairns and Wirshing (2018) findings. The presence of *Plumarella cordilla* within this clade was not expected as it does not align with the rest of the genus which is placed within the 5B1 clade supporting the previous findings. This suggests the need for taxonomic re-evaluation of *Plumarella cordilla* placement within the genus.

Clade 4

Clade 4 in the UCE-based tree includes *Callogorgia*, consistent with Cairns and Wirshing (2018).

Clade 5

Clade 5A1

Clade 5A1 includes *Dasystenella, Metafannyella, Aglaoprimnoa*, and *Pyrogorgia*, mirroring the structure found in Cairns' study. However, *Heptaprimnoa*, a genus included by Cairns, is absent in this study. The inclusion of *Pyrogorgia* in Clade

5A1 suggests it shares a closer evolutionary relationship with these genera separating earlier from the rest of the clade with *Dasystenella* being the sister group to *Pyrogorgia*.

Clade 5A2

This UCE-based analysis introduces *Microprimnoa* for the first time, which was placed in Clade 5A2. Inclusion of *Microprimnoa* sheds light on the evolutionary pathway within the Primnoidae family, highlighting the potential for taxonomic revision. The consistent placement of genera such as *Armadillogorgia*, *Primnoella*, and *Convexella* across studies reinforces the clade's stability. However, the polyphyletic genus *Primnoella*, consisting of *Primnoella antarctica*, *Primnoella chilensis*, and *Primnoella insularis*, is found within the 5A2 clade, consistent with Cairns and Wirshing 2018. Notably, each of these three Primnoella species occurs in a different subclade. *Primnoella antarctica* is grouped with *Ophidiogorgia* as the sister group; *P. insularis* and *P. chilensis* both are grouped with *Convexella* as the sister group.

Clade 5B1

In Clade 5B1, the genera *Ainigmaptilon*, *Callozostron*, and *Plumarella* are consistently placed within this clade compared to (Cairns and Bayer, 2009), reflecting their stable phylogenomic positions. This placement is consistent with previous study and suggests that these species form a cohesive evolutionary group within the Primnoidae family. The absence of *Tokoprymno* and *Mirostenella* from this study results in the omission of Clade 5B2, which was identified by Cairns and Wirshing (2018). This highlights the need for further research to better understand the clade's composition and relationships.

Clade 5B3

*Thouarella* has consistent placement within Clade 5B2 in comparison to Cairns and Bayer (2009), reaffirming its monophyly and strong support within the Primnoidae family.

Cairns and Wirshing (2018) highlighted the limitations of morphology-based taxonomy in Primnoidae, noting that many diagnostic characters, such as colony branching structure and sclerite arrangement, are prone to convergence and phenotypic plasticity. The results of this study strongly support this conclusion. The repeated occurrence of polyphyly in genera such as Candidella and Primnoella suggests that these morphological traits are not reliable indicators of phylogenetic relationships. Instead, these patterns indicate that similar morphological features may have evolved independently in response to comparable environmental pressures.

Overall, the UCE-based phylogeny provides strong support for major relationships within Primnoidae, particularly at deeper nodes, demonstrating improved resolution compared to previous multi-locus studies such as Taylor and Rogers (2015) and Cairns and Wirshing (2018). The recovery of consistent clade structure across studies suggests that many higher-level relationships within the family are robust. However, conflicting placements observed in genera such as *Plumarella, Candidella, and Primnoella* indicate that some taxonomic groupings remain unstable and may reflect either incomplete lineage sorting, convergent morphological evolution, or limitations in earlier marker-based approaches.

The application of ultra-conserved elements (UCEs) in this study proved highly effective for resolving deep phylogenetic relationships within Primnoidae. Compared to traditional markers such as mitochondrial and ribosomal genes, UCEs provided a substantially larger dataset and improved statistical support. Several previously

unresolved basal relationships within Primnoidae were clarified with strong bootstrap support, and the overall backbone of the phylogeny was more robustly resolved through the use of UCEs.

Despite the strengths of this study, several limitations should be acknowledged. Taxon sampling remains incomplete, with several genera absent that were included in previous studies, limiting direct comparisons across all clades. Additionally, some genera are represented by a small number of specimens, which may affect the robustness of their inferred placement. Furthermore, geographic sampling gaps may obscure biogeographic patterns and evolutionary history within the family.

Future research should aim to expand taxon sampling, particularly for underrepresented and taxonomically uncertain genera such as Candidella and Plumarella. Integrating genomic approaches with detailed morphological re-examination will be essential for resolving taxonomic inconsistencies. Additionally, incorporating ecological and biogeographic data may provide further insight into the evolutionary drivers shaping Primnoidae diversity.

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# Chapter 3 Integrative Species Delimitation in the Primnoidae (class: Octocorallia) Using Ultra-Conserved Elements and Bayesian Coalescent Analysis

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## Abstract

The family Primnoidae (Octocorallia) represents one of the most diverse and widely distributed deep-sea coral groups, yet species delimitation remains challenging due to morphological plasticity, homoplasy, and limited resolution of mitochondrial markers. To address these issues, we applied a phylogenomic framework using ultra-conserved elements (UCEs) and Bayesian species delimitation in BPP to test whether morphologically defined Primnoidae species boundaries correspond to distinct genetic lineages across multiple genera. DNA was extracted and sequenced, with bioinformatic processing conducted via the PHYLUCE pipeline. Species delimitation analyses were performed on three subsets (the Thouarella clade, Primnoella, and a dataset spanning the entire family Primnoidae), with BPP evaluating between 10 and 30 alternative species delimitation models per dataset.Across all analyses, BPP consistently recovered a single best-supported model with strong posterior probabilities, confirming the distinctiveness of most taxa. In *Thouarella, T. koellikeri, T. crenelata, T. viridis, T. brucei, and T. aureolabyrinthea sp. nov.*, were supported as independent lineages, whereas *T. pendulina, T. variabilis, T. diadema, and T. andeep* clustered as a single genetic lineage despite morphological differentiation. Similarly, BPP grouped *Primnoella antarctica* with *Ophidiogorgia kuekenthali*, reflecting potential

over-splitting or incomplete lineage divergence. By contrast, all species in the third subset, representing multiple genera, were strongly supported as distinct lineages. These results demonstrate the effectiveness of UCE-based approaches for resolving cryptic diversity and highlight discordances between morphology and genomic data in Primnoidae. Our findings emphasize the need for integrative taxonomy and provide novel genomic evidence that advances the understanding of species boundaries within this ecologically important deep-sea coral family.

## Introduction

The deep sea is one of Earth's most extreme and least explored environments with around 8% of the ocean sea floor being mapped with high-resolution sonar data and visually observed less than 0.001% of the sea floor (Bell *et al.*, 2025; Mayer *et al.*, 2018), characterized by high pressure, low temperatures, and complete darkness below 200 meters (Paulus, 2021; Roberts *et al.*, 2006).Over 90% of the ocean’s volume lies in the deep-sea regions and it hosts remarkable biodiversity(Robison, 2009). This vast and remote ecosystem supports an array of life forms that have developed unique adaptations to survive, ranging from bioluminescent fish to structurally intricate cold-water corals (Feng *et al.*, 2022; Roberts *et al.*, 2006; Robison, 2009).

Corals are among the most important organisms in the deep sea, as they create essential habitats for numerous marine species. Two-thirds of all described coral species are found in deep-sea environments, reflecting their ecological importance (Cairns, 2007; Roberts *et al.*, 2006). Cold-water corals (CWCs) are particularly vital, forming extensive reef structures that serve as shelters and spawning grounds for

diverse marine life, including commercially valuable fish species (Auster, 2005; Roberts, 2009; Roberts *et al.*, 2006). These reefs, much like their shallow-water counterparts, contribute to the structural complexity of the seafloor, creating microhabitats that support enhanced biodiversity (Girard *et al.*, 2023; Paulus, 2021; Roberts, 2009).

Within the subclass Octocorallia, the Primnoidae family is notable for its extraordinary diversity and widespread distribution. Comprising of 318 species and 46 genera, it is the most diverse octocoral family in terms of genera (Cairns and Wirshing, 2018). The family is found from the Arctic to the Antarctic, primarily inhabiting deep-sea regions (Cairns and Bayer, 2009). An impressive 96% of Primnoidae species occur at depths greater than 100 meters, with some reaching depths of up to 6400 meters (Cairns, 2011; Cairns, 2016). Furthermore, over half of the octocoral species living below 3000 meters belong to the Primnoidae family, highlighting its ecological prominence in the deep sea (Cairns, 2016).

Species delimitation in the deep-sea coral family Primnoidae is particularly challenging due to pronounced morphological plasticity and homoplasy (Cairns and Wirshing, 2018; Waller *et al.*, 2019). Traditional taxonomy, which is mainly based on colony morphology, polyp arrangement, and sclerite shape, frequently fails to distinguish closely related or cryptic species (Cairns and Wirshing, 2018). In addition, mitochondrial DNA markers commonly used in species delimitation are limited in Anthozoa due to their slow evolutionary rate (McFadden *et al.*, 2011; Shearer *et al.*, 2002), which provides low resolution for recently diverged species. (McFadden *et al.*, 2011; Shearer *et al.*, 2002) They are also prone to incomplete lineage sorting and introgression, often resulting in conflicts with nuclear data (Erickson *et al.*, 2021). These issues are well documented in Octocorallia, where genetically distinct species

can difficult to differentiate morphologically (Erickson *et al.*, 2021; Quattrini *et al.*, 2019).

Ultra-conserved Elements (UCEs) are highly conserved regions of DNA found across a wide range of organisms. Each UCE comprises a conserved central region with adjacent variable sequences, which together provide informative genetic variation useful for phylogenetic analysis (Faircloth *et al.*, 2012). While UCEs were initially developed for reconstructing deep evolutionary relationships, they have since proven effective at resolving species-level relationships in many invertebrate groups, including insects and birds (Rohde *et al.*, 2025; Smith *et al.*, 2014). Because UCEs capture data from hundreds to thousands of unlinked loci, they are well suited to overcoming the limitations of single-locus markers and allow for robust multi-locus coalescent-based species delimitation, which infers species boundaries by modelling the shared evolutionary history of allrlrs across multiple gene loci while accounting for gene tree discordance (Erickson *et al.*, 2021; Smith *et al.*, 2014). In octocorals, UCE-based approaches have been successfully used to uncover cryptic species, infer gene flow, and reconstruct well-supported phylogenies (Morrissey *et al.*, 2023; Quattrini *et al.*, 2019; Untiedt *et al.*, 2021). These methods are also highly effective when working with degraded or museum-quality DNA, which is especially important in studies of deep-sea taxa (Erickson *et al.*, 2021; Quattrini *et al.*, 2023).

To test species boundaries using UCE data, coalescent-based approaches such as BPP (Bayesian Phylogenetics and Phylogeography) are ideal. BPP models the evolutionary process of lineage sorting under the multispecies coalescent, allowing species delimitation while accounting for gene tree discordance across loci (Flouri *et al.*, 2018; Yang, 2015). This is especially valuable in groups where incomplete lineage sorting, hybridization, or recent divergence make species identification challenging, as

is the case in Primnoidae (Erickson *et al.*, 2021). The incorporation of multilocus data generated from UCEs into BPP enables evaluation of species delimitation hypotheses, facilitating the identification of distinct evolutionary lineages despite limited or absent morphological differentiation (Rohde *et al.*, 2025; Smith *et al.*, 2014). BPP achieves this by modelling lineage sorting under multispecies coalescent, which describes how gene copies sampled from different populations/species trace back to common ancestors (Yang and Rannala, 2010). While UCEs have been applied in octocoral systematics, there are currently no published studies using BPP to delimit species in this group, highlighting a significant gap and the need for coalescent-based frameworks in octocoral taxonomy (Erickson *et al.*, 2021). The aim of this study is to test species boundaries in the family Primnoidae using phylogenomic data. Specifically, we employ UCE sequences in combination with coalescent-based species delimitation in BPP to test weather morphologically defined species correspond to distinct evolutionary lineages. This approach allows assessment of convergence between morphological taxonomy and genomic evidence.

## Methods

### DNA Extraction and library preparation

DNA was isolated using the DNeasy Blood & Tissue Kit (Qiagen), following standard manufacturer instructions with the following modifications: after the initial lysis phase, RNA was enzymatically removed to ensure DNA purity by incorporating either 2 µL of RiboShredder with incubation at 30°C for 10 minutes, or alternatively, 4 µL of RNase A with a 10-minute incubation at 37°C. To maximize the final DNA yield, the elution process was optimized by passing 60 µL of elution buffer through the spin column twice.Library preparation followed the UCE-specific protocols established for

octocorals. For samples requiring enzymatic fragmentation, we targeted an insert size of 400–600 bp to ensure consistency across the dataset. Targeted enrichment was performed using the custom octo-v2 probe set. Following the efficiency standards set by Quattrini *et al.* (2018).

**Post-sequencing analysis**

All bioinformatic steps were carried out using the PHYLUCE pipeline, guided by the workflow outlined in Faircloth (2016), and customized to fit the needs of this study. The key stages of analysis are as follows:

The bioinformatic processing was designed to transform raw genomic reads into a refined dataset suitable for species delimitation, following the PHYLUCE pipeline and the methodological framework of Faircloth (2016). In the initial stage of analysis, raw sequencing reads were processed through Illumiprocessor to remove adapter sequences and filter out low-quality bases. This cleaning step ensures that downstream de novo assemblies are not biased or fragmented by sequencing artifacts. Cleaned reads were then assembled into contiguous sequences (contigs) using SPAdes v3.12. To diagnose the success of the assembly and the viability of each sample, we assessed the distribution of contig lengths. This evaluation served as a primary quality filter, and samples with poor enrichment, such as those yielding fewer than 1,000 total contigs, were excluded from the study to prevent the introduction of excessive missing data into the final matricesOnce assemblies were verified, we identified targeted loci by matching the contigs against the octo-v2 probe set. We applied a 70% identity and 70% coverage threshold to this matching process to accommodate natural genetic divergence within octocoral clades while remaining strict enough to exclude non-target or paralogous sequences. Confirmed loci were

stored in an SQLite database, allowing for the organized retrieval of FASTA sequences for alignment. Sequence alignments were generated using MAFFT through the phyluce_align_seqcap_align command. This tool was selected for its ability to accurately align both the highly conserved UCE cores and their more variable flanking regions. To ensure that the final species delimitation was based on a robust and dense data matrix, we filtered these alignments to maintain specified taxon occupancy thresholds. This step helps mitigate potential phylogenetic artifacts, such as long-branch attraction, which are often exacerbated by data gaps. Because BPP is computationally intensive and cannot efficiently process the full genomic dataset in a single analysis, the data were divided into three distinct subsets. Subset one was established specifically to test species boundaries within the *Thouarella* group. Subset two focused on the *Primnoella* group, which was a necessary focus as prior phylogenomic analysis indicated that this genus is polyphyletic. The third subset consisted of a general sampling of taxa across the entire Primnoidae tree to provide a broader evolutionary context.

### Format Conversion for Downstream Analysis

To prepare alignments for BPP analyses, nexus-formatted alignment files were batch-converted into FASTA format using a custom Python 3.6 script incorporating the Biopython package (Cock *et al.*, 2009).

### File Conversion and Partitioning for BPP Input

Following the FASTA conversion, alignments were further processed using the fasta-phylip-partitions script (Álvarez-Carretero, 2025). This tool was used to generate BPP-compatible PHYLIP files along with the corresponding partition file required for multispecies coalescent analyses.

### Species Delimitation with BPP

BPP v4.7.0 was used to perform Bayesian species delimitation under the multispecies coalescent (MSC) model, which accounts for incomplete lineage sorting across loci (Yang, 2015). We implemented analysis A10, which delimits species using a fixed species tree as a guide. This tree was generated in advance using IQ-TREE (Nguyen *et al.*, 2015) analyses. Each model in BPP is represented by a string of 1s and 0s, where each digit indicates whether a given population split is supported (1) or not supported (0). Posterior probabilities (PP) reflect the probability of each model given the data.

## Results

Bayesian species delimitation analyses in BPP were performed in BPP v4.7.0 on three independent subsets. For each subset BPP assessed species delimitation models under multispecies coalescent framework. Across all analyses, the number of models ranged from 10 to 30 per subset. In each case BPP strongly favoured single delimitation model,

Subset 1

BPP evaluated 26 potential models of species delimitation (Table 1). Single model (model 10: 111000111) was highly supported (posterior probability (PP) = 1.0), while all other models received no support. BPP strongly supported delimitation of *T. koellikeri, T. crenelata, T. aureolabyrinthea sp. nov., T. brucei,* and *T. viridis* (PP = 1.0) as distinct species. However, split between *T. variabilis* and *T. pendulina*, and between *T. andeep* and *T. diadema*, were unsupported (PP = 0.0) (Figure 1).

**Table 1.** BPP species delimitation results showing model (where 1 are where the model supported split between the species and 0 where the split is not supported), prior probabilities, and posterior probabilities. In bold is the model that BPP found to have highest probability.for Subset 1.

| Model | Code | Prior | Posterior |
|---|---|---|---|
| 1 | 000000000 | 0.038462 | 0.000000 |
| 2 | 100000000 | 0.038462 | 0.000000 |
| 3 | 110000000 | 0.038462 | 0.000000 |
| 4 | 110000100 | 0.038462 | 0.000000 |
| 5 | 110000110 | 0.038462 | 0.000000 |
| 6 | 110000111 | 0.038462 | 0.000000 |
| 7 | 111000000 | 0.038462 | 0.000000 |
| 8 | 111000100 | 0.038462 | 0.000000 |
| 9 | 111000110 | 0.038462 | 0.000000 |
| **10** | **111000111** | **0.038462** | **1.000000** |
| 11 | 111100000 | 0.038462 | 0.000000 |
| 12 | 111100100 | 0.038462 | 0.000000 |
| 13 | 111100110 | 0.038462 | 0.000000 |
| 14 | 111100111 | 0.038462 | 0.000000 |
| 15 | 111101000 | 0.038462 | 0.000000 |
| 16 | 111101100 | 0.038462 | 0.000000 |
| 17 | 111101110 | 0.038462 | 0.000000 |
| 18 | 111101111 | 0.038462 | 0.000000 |
| 19 | 111110000 | 0.038462 | 0.000000 |
| 20 | 111110100 | 0.038462 | 0.000000 |
| 21 | 111110110 | 0.038462 | 0.000000 |

| | | | |
|---|---|---|---|
| 22 | 111110111 | 0.038462 | 0.000000 |
| 23 | 111111000 | 0.038462 | 0.000000 |
| 24 | 111111100 | 0.038462 | 0.000000 |
| 25 | 111111110 | 0.038462 | 0.000000 |
| 26 | 111111111 | 0.038462 | 0.000000 |

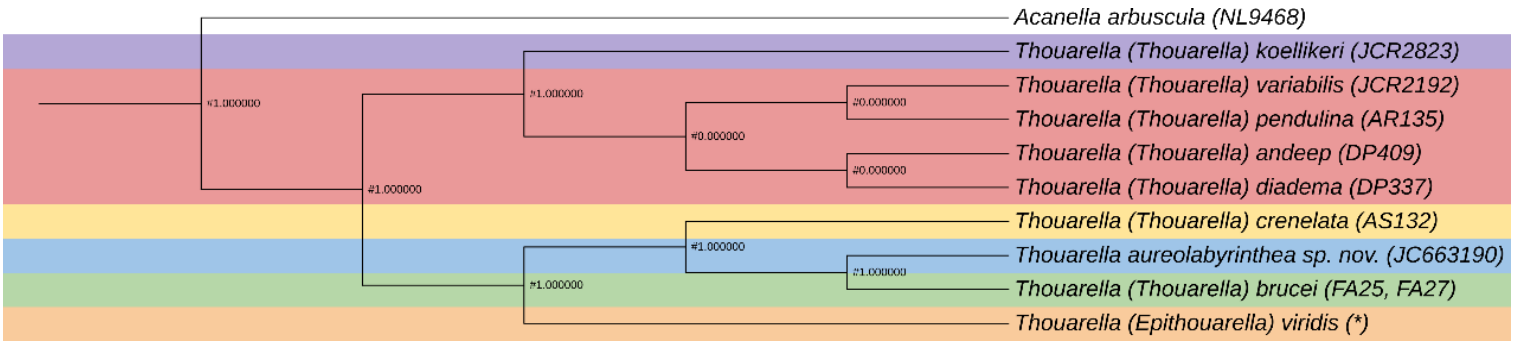


Figure 1. Species delimitation results for *Thouarella* subset inferred with BPP. The posterior probabilities of speciation events, with node support values shown on branches. Colours correspond to BPP species delimitation results. Lineages shown in the same colour were grouped into a single species, while those in different colours were supported as separate species.

Subset 2

BPP analysed 30 species delimitation models for this subset (Table 2). The preferred model was model 29 (1111111110) with strong support (PP = 0.914). Splits between following species were strongly supported *F. kuekenthali, P. chilensis, C. murrayi, P. scotiae, P. insularis, Convexella sp. nov., P. brochi,* and *F. rossii* (PP=0.999). On the other hand, *P. antarctica* and *O. kuekenthali* showed low support for the split with PP = 0.086) (Figure 2).

**Table 2.** BPP species delimitation results for Subset 2, showing each model (where 1 are where the model supported split between the species and 0 where the split is not supported), prior probabilities, and posterior probabilities. In bold is the model that BPP found to have highest probability.

| Model | Code | Prior | Posterior |
|---|---|---|---|
| 1 | 0000000000 | 0.033333 | 0.000037 |
| 2 | 1000000000 | 0.033333 | 0.000000 |
| 3 | 1100000000 | 0.033333 | 0.000000 |
| 4 | 1110000000 | 0.033333 | 0.000000 |
| 5 | 1110010000 | 0.033333 | 0.000000 |
| 6 | 1110010100 | 0.033333 | 0.000000 |
| 7 | 1110010110 | 0.033333 | 0.000000 |
| 8 | 1110010111 | 0.033333 | 0.000000 |
| 9 | 1110011000 | 0.033333 | 0.000000 |
| 10 | 1110011100 | 0.033333 | 0.000000 |
| 11 | 1110011110 | 0.033333 | 0.000000 |
| 12 | 1110011111 | 0.033333 | 0.000000 |
| 13 | 1111000000 | 0.033333 | 0.000000 |
| 14 | 1111010000 | 0.033333 | 0.000000 |
| 15 | 1111010100 | 0.033333 | 0.000000 |
| 16 | 1111010110 | 0.033333 | 0.000000 |
| 17 | 1111010111 | 0.033333 | 0.000000 |
| 18 | 1111011000 | 0.033333 | 0.000000 |
| 19 | 1111011100 | 0.033333 | 0.000000 |
| 20 | 1111011110 | 0.033333 | 0.000000 |
| 21 | 1111011111 | 0.033333 | 0.000000 |
| 22 | 1111100000 | 0.033333 | 0.000000 |
| 23 | 1111110000 | 0.033333 | 0.000000 |

| | | | |
|---|---|---|---|
| 24 | 1111110100 | 0.033333 | 0.000000 |
| 25 | 1111110110 | 0.033333 | 0.000000 |
| 26 | 1111110111 | 0.033333 | 0.000000 |
| 27 | 1111111000 | 0.033333 | 0.000000 |
| 28 | 1111111100 | 0.033333 | 0.000000 |
| **29** | **1111111110** | **0.033333** | **0.914236** |
| 30 | 1111111111 | 0.033333 | 0.085727 |

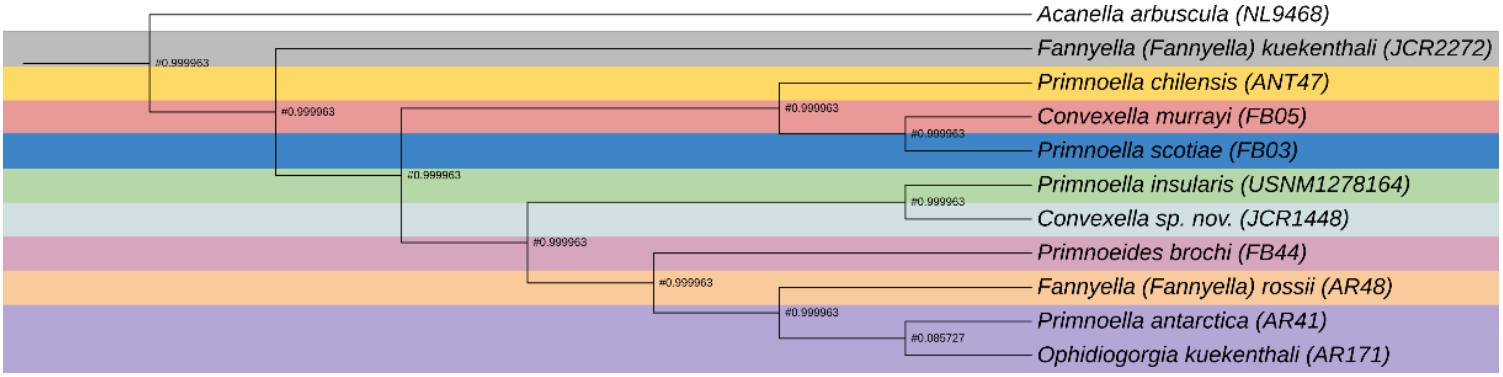


Figure 2. Species delimitation results for *Primnoella* subset inferred with BPP. The posterior probabilities of speciation events, with node support values shown on branches. Colours indicate species assignments inferred by BPP. Each colour represents a delimited species: taxa sharing the same colour were not supported as distinct

Subset 3

BPP analysed 20 species delimitation models for this subset (Table 3). Model 20 (111111111) received the highest support with a PP of 0.982. Minor support was observed for Model 18 (PP = 0.017) and Model 17 (PP = 0.001), while all other models were rejected. The preferred model supports the delimitation of all species in this subset (Figure 3), with each split receiving strong support (PP ≥ 0.982).

**Table 3.** BPP species delimitation results for Subset 3, showing model codes (where 1 are where the model supported split between the species and 0 where the split is not supported), prior probabilities, and posterior probabilities. In bold is the model that BPP found to have highest probability.

| Model | Code | Prior | Posterior |
|---|---|---|---|
| 1 | 000000000 | 0.050000 | 0.000000 |
| 2 | 100000000 | 0.050000 | 0.000000 |
| 3 | 110000000 | 0.050000 | 0.000000 |
| 4 | 110010000 | 0.050000 | 0.000000 |
| 5 | 110011000 | 0.050000 | 0.000000 |
| 6 | 110011100 | 0.050000 | 0.000000 |
| 7 | 110011110 | 0.050000 | 0.000000 |
| 8 | 110011111 | 0.050000 | 0.000000 |
| 9 | 111000000 | 0.050000 | 0.000000 |
| 10 | 111010000 | 0.050000 | 0.000000 |
| 11 | 111011000 | 0.050000 | 0.000000 |
| 12 | 111011100 | 0.050000 | 0.000000 |
| 13 | 111011110 | 0.050000 | 0.000000 |
| 14 | 111011111 | 0.050000 | 0.000000 |
| 15 | 111100000 | 0.050000 | 0.000000 |
| 16 | 111110000 | 0.050000 | 0.000000 |
| 17 | 111111000 | 0.050000 | 0.001117 |
| 18 | 111111100 | 0.050000 | 0.017210 |
| 19 | 111111110 | 0.050000 | 0.000060 |
| **20** | **111111111** | **0.050000** | **0.981614** |

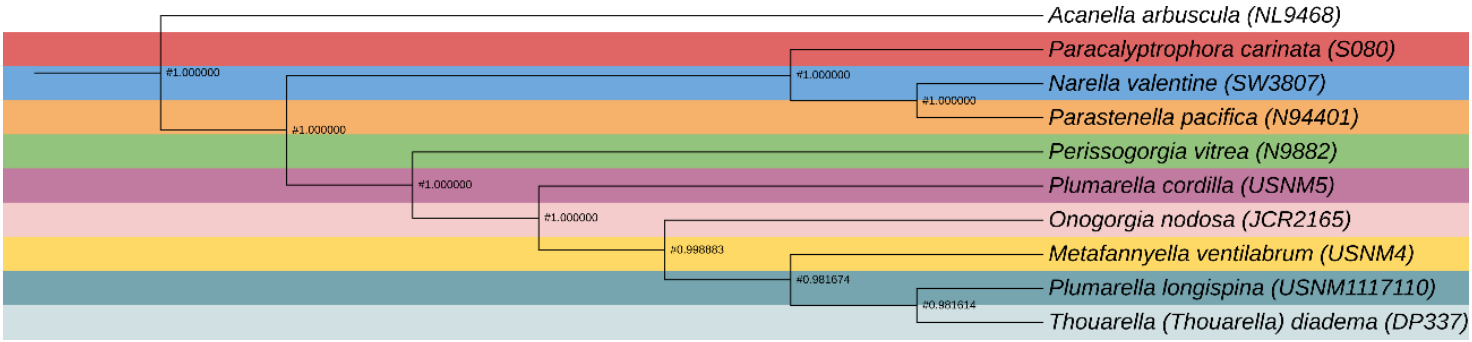


Figure 3. Species delimitation results for Primnoidae family inferred with BPP. The posterior probabilities of speciation events, with node support values shown on branches. Colours represent species assignments inferred by BPP, with each colour corresponding to a distinct species.

## Discussion

In this study we used UCEs to perform species delimitation using BPP to test whether morphologically defined Primnoidae species correspond to distinct evolutionary lineages. We found that most morphological species were supported genetically distinct, but several formed unexpected clusters. For example, four Thouarella species (*T. pendulina, T. variabilis, T.andeep, and T diadema*) merged into a single genetic group, while other Thouarella taxa (*T. viridis, T. brucei, T. crenelata, T. koellikeri,* and *T. aureolabyrinthea sp. nov*) remained distinct. Similarly, in the *Primnoella* clade *O. kuekenthali* and *P. antarctica* were not supported by genetic data, suggesting recent divergence or gene flow, whereas all taxa from subset which contained genera from across the Primnoidae tree were clearly distinct. These results indicate that traditional morphological species boundaries are largely aligned with genomic data in Primnoidae, but that closely related taxa may exhibit weak genetic divergence, highlighting the complexity of species delimitation in this family.

**Subset 1**

BPP analysed 26 potential species delimitation models for subset 1 (Table 1). Model 10 (111000111) was strongly supported (PP = 1.0), while all other models received no support. This extreme posterior probability suggests a strong and consistent genetic signal for the supported model across the loci included under the assumption of the analysis.

*Thouarella pendulina, T. variabilis, T. diadema*, and *T. andeep* do not form distinct genetic lineages (Figure 1), but instead cluster together as a single group. In the tree, there is essentially no statistical support for splitting these taxa into distinct species, indicating they represent one cohesive lineage under the chosen delimitation criteria. This result implies a lack of sufficient genetic divergence among these four *Thouarella* species to reliably delimit them using the loci sampled, despite each being described as separate based on morphology. Notably, a historic taxonomic revision of *Thouarella* already synonymized *T. sardana* with *T. diadema* after finding overlapping characters (Taylor *et al.*, 2013). The clustering of *T. diadema*, *T. andeep, T. pendulina*, and *T. variabilis* in our analysis further highlights how traditional morphological boundaries within this genus may not always correspond to clear genetic separations.

One potential explanation for the ambiguous species delimitation results is the very recent divergence of these lineages. If the putative species separated only in the recent past, there may have been insufficient time for distinct genetic differences to accumulate (Tan *et al.*, 2023). Gene trees from different loci may be inconsistently sorted among the recently diverged taxa, leading to a lack of clear species-level monophyly or diagnostic differences. This makes it difficult for coalescent-based methods like BPP to confidently distinguish the species, since the genetic signal of

divergence is weak and blended. Another important factor to consider is gene flow (hybridization) between the lineages (Quattrini *et al.*, 2019). The multispecies coalescent model implemented in BPP assumes no gene flow between the predefined species. If there has been ongoing or historical interbreeding among some of these coral lineages, they would exchange genes, causing alleles to be shared across species boundaries. Beyond biological processes, aspects of our sampling design and data set can also influence the delimitation results. One notable factor is the uneven sampling of individuals per lineage. In our analysis, *Thouarella viridis* is represented by 25 individuals, whereas most other candidate species are singletons (only one individual each, except *T. brucei* with 2 individuals). This extreme disparity in sample size per group can bias coalescent analyses (Zhang *et al.*, 2011).

There are known instances in octocorals where slow mitochondrial evolution and incomplete lineage sorting make it difficult to distinguish closely related species (Bilewitch and Degnan, 2011). Despite the genetic clustering of those four species, it is important to note that each of them does exhibit diagnostic morphological features that historically justified their distinction. *Thouarella pendulina* and *T. variabilis*, for example, have been described as very similar in colony form, however *T. variabilis* has noticeably longer, less-clustered polyps with elongated marginal scales, traits absent in *T. pendulina* (Taylor *et al*., 2013). Likewise, *T. andeep* can be told apart from *T. pendulina* and *T. variabilis* by its much larger, more flared polyps and less clustered polyp arrangement (Taylor *et al.*, 2013). *Thouarella variabilis* has smaller spines on marginals where as *T. diadema* has multi-keeled inner marginal (Cairns and Wirshing, 2018; Taylor *et al.*, 2013). Our genetic analysis does not resolve *T. pendulina*, *T. variabilis, T. diadema,* and *T. andeep* as separate units suggesting that these morphological disparities evolved recently. The four species cluster might likewise

represent a single genetic lineage that encompasses multiple described forms. This does not necessarily invalidate the morphological distinctions, but it raises the possibility of gene flow among these forms or an incomplete lineage separation in the evolutionary recent past. Future work with additional genetic markers or genomic data could clarify whether these four represent a single polytypic species or several young, incipient species within a species complex.

In contrast to the above cluster, the other *Thouarella* species in this analysis were clearly resolved as distinct lineages with strong support (Table 1). *Thouarella (Epithouarella) viridis*, for example, stands apart from the rest, consistent with its unique taxonomic status and morphology. *Thouarella viridis* (subgenus *Epithouarella*) is known for its tall opercular cones and protruding, toothed submarginal scales, features not seen in related species of subgenus *Thouarella*, strengthening the support of *T. viridis* as distinct species. Likewise, *T. brucei* remains a well-supported independent clade in our results. This aligns with classical morphology: although *T. brucei* shares the clavate polyp form with some other species, it has fewer abaxial body-wall scales and a distinct opercular keel structure compared to its closest relatives. Its genetic distinctness in the tree corroborates these differences. *Thouarella koellikeri* is another species that our analysis confirmed as distinct. *Thouarella koellikeri* has been characterized by bottlebrush, bushy to bilateral branching pattern and marginals with high single-channelled keels, setting it apart from similar bottlebrush forms. *Thouarella crenelata* which in our analysis remains isolated is morphologically distinguishable by its densely serrated marginal scale edges and high polyp density at branchlet tips. The new species *Thouarella aureolabyrinthea* sp. nov. also formed its own well-supported cluster, indicating it is genetically distinct from all analysed *Thouarella* species. This is an important validation for the putative new

species: the delimitation analysis provides independent support that *T. aureolabyrinthea* is not just a morphological variant of an existing taxon, but a separate lineage, although species delimitation was conducted without representatives from the entire genus, the results provide strong support for its taxonomic distinctiveness. While formal description of *T. aureolabyrinthea* is pending, we note that it has unique colony and sclerite features.

**Subset 2**

The model strongly supported the distinctiveness of *F. kuekenthali, P. chilensis, C. murrayi, P. scotiae, P. insularis, Convexella sp. nov., P. brochi,* and *F. rossii* (PP = 0.914). These results align well with their known morphological differentiation and provide genomic support for their recognition as separate species. However, the split between *Primnoella antarctica* and *Ophidiogorgia kuekenthali*, which has a very low support (PP = 0.086) suggesting these two nominal species form a single genetic cluster (Figure 2).

BPP grouped *P. antarctica* with *O. kuekenthali* is their lack of genetic divergence relative to within-species variation, if they split only recently in evolutionary time, there may not have been enough time for mutations to accumulate or for lineage sorting to complete (Erickson *et al.*, 2021; Quattrini *et al.*, 2019; Soler-Hurtado *et al.*, 2017). Incomplete lineage sorting can cause two morphologically different taxa to appear genetically intermixed with minimal to no molecular differentiation (Soler-Hurtado *et al.*, 2017). Another possibility is ongoing or historical gene flow (introgression) between the two, which would blur genetic boundaries. *Primnoella antarctica* and *O. kuekenthali* inhabit overlapping ranges in the Southern Ocean and are not completely reproductively isolated, interbreeding could maintain genetic similarity. The BPP result

is particularly intriguing because *P. antarctica* and *O. kuekenthali* have been treated as different genera with notable morphological differences. *Ophidiogorgia* can be distinguished from *Primnoella* by its fused basal calyces, the presence of more than eight marginal scales, granular sculpturing on the body wall scales, and having multiple layers of sclerites in both the body wall and coenenchyma (Cairns and Bayer, 2009). The BPP analysis did not split *P. antarctica* and *O. kuekenthali*, indicating very low genetic divergence with the loci used, however, given their clear morphological distinctions (Cairns and Bayer, 2009), this genetic clustering alone is not conclusive for synonymy. It suggests that, genetically, these two may belong to one lineage despite being placed in different genera. This is not entirely unexpected as recent molecular phylogenetic studies have found *Primnoella* to be polyphyletic, with some *Primnoella* species clustering tightly with *Ophidiogorgia* and other genera (Cairns and Wirshing, 2018). From an evolutionary standpoint, the low split support implies that whatever isolating mechanisms separate *P. antarctica* and *O. kuekenthali* are either recently acquired or incomplete. This might reflect a recent speciation event in the Antarctic Primnoidae where morphological differentiation (perhaps due to niche specialization) happened faster than genome-wide differentiation (Soler-Hurtado *et al.*, 2017). It underlines the importance of integrative taxonomy: relying on morphology alone could be misleading in this group, and molecular evidence is critical to correctly separating species (Yuan *et al.*, 2025).

In contrast, the other species in the analysis each maintained high support for their splits, meaning BPP found strong genetic evidence of separation. Each of those species likely has had a longer independent history or strong reproductive isolation, leading to distinct allele lineages. This suggests that their morphological differences are underpinned by deep genetic divergence. In those cases, BPP's coalescent

analysis saw little gene flow or allele sharing across the species, consistent with them being true biological species. The *P. antarctica* + *O. kuekenthali* case stands out as an anomaly where morphology-based taxonomy and genetic evidence disagree.

**Subset 3**

In the BPP species delimitation analysis, all ten sampled taxa were strongly supported as distinct species, with each lineage receiving high posterior probability (PP = 1.0) for its species status. This included *Thouarella (Thouarella) diadema, Plumarella longispina, Metafannyella ventilabrum, Onogorgia nodosa, Plumarella cordilla, Perissogorgia vitrea, Parastenella pacifica, Narella valentine,* and *Paracalyptrophora carinata*. Posterior probabilities for each species were essentially all equal (Figure 3), indicating high support for the hypothesis that every sampled specimen represents a distinct evolutionary lineage. Bayesian multi-species coalescent model found negligible uncertainty or ambiguity in delineating species boundaries among these samples. Notably, all specimens belong to different genera and are morphologically distinct, and this clear phenotypic and taxonomic separation is in line with the genomic delimitation results. The near-1.0 posterior support for each taxon's distinctiveness underscores that there is no evidence of cryptic conspecific relationships among the samples. Each taxon is confirmed as a separate species with overwhelming statistical confidence. This outcome provides robust confirmation that traditional morphological identifications are accurately reflected in the genetic data, as BPP's species delimitation yielded strong, unanimous support for recognizing each of these taxa as a distinct species in the analysis.

In summary, our delimitation analyses largely confirmed that many traditional Primnoidae species represend distinct genetic lineages, yet some exceptions

underscore the challenges of delineating species. Most taxa were strongly supported as separate species, consistent with their diagnostic morphology. However, the genetic clustering of the *Thouarella pendulina-variabiis-diadema-andeep* and the *P. antarctica/O. kuekenthali* suggest recent divergence or gene flow. These findings underscore the evolutionary complexity of Primnoidae: morphological differences can develop before complete genetic separation or be blurred by hybridization. Overall. Integrating genomic data has proven valuable for refining Primnoidae taxonomy: While our results validate many classic species, they also highlight lineages that require further investigation with comprehensive genetic sampling.

A major limitation of this study is that the majority of species are represented by singleton samples. This is primarily due to the logistical challenges associated with deep-sea sampling, where specimen collection is often limited by accessibility, cost, and low encounter rates. However, the use of singleton samples has important implications for coalescent-based species delimitation. In cases where only a single individual per morphospecies is available, the parameter θ (mutation rate scaled by effective population size) cannot be estimated from the data and must instead be inferred from the prior distribution. This limitation may help explain the observed lumping of *P. antarctica* and *O. kuekenthali* into a single species, as the model may lack sufficient information to distinguish recent divergence from intraspecific variation. Future work should prioritise increased sampling per morphospecies to enable more reliable estimation of θ and improve the robustness of species delimitation. Additionally, sensitivity analyses exploring different prior settings would help assess the stability of the results.

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## Chapter 4: Summary

This thesis set out to resolve the phylogenomic relationships and species boundaries within the deep-sea coral family Primnoidae using ultra-conserved elements (UCEs). Primnoidae are a diverse and widespread group of octocorals, forming critical components of deep-sea ecosystems from the Arctic to Antarctic oceans (Cairns and Bayer, 2009; Cairns, 2011). However, their taxonomy has long been challenging due to pronounced morphological plasticity and convergent evolution in characters such as colony branching and sclerite form (Cairns and Wirshing, 2018). Mitochondrial DNA markers evolve slowly in Anthozoa (McFadden *et al.*, 2011; Shearer *et al.*, 2002), limiting their utility for distinguishing recently diverged species. Prior molecular studies, using a handful of loci, provided valuable but incomplete insights into Primnoidae phylogeny (Cairns and Wirshing, 2018; Taylor and Rogers, 2015; Taylor and Rogers, 2017). Building on this foundation, the present work employed a high-throughput UCE approach to overcome previous resolution limits. The key objectives were to reconstruct a robust Primnoidae phylogeny with genome-scale data and to apply coalescent-based species delimitation, thereby addressing both deep evolutionary relationships and shallow species-level questions in an integrative framework.

Methodologically, the use of UCE target capture proved to be a significant advancement for deep-sea coral phylogenomics. Hundreds of UCE loci were successfully recovered from Primnoidae samples, including museum specimens and others with potentially degraded DNA, demonstrating the method's resilience in challenging sample conditions (Faircloth *et al.*, 2012). By generating thousands of orthologous loci across the genome, UCE sequencing provided a very large multi-

locus dataset for resolving the Primnoidae tree of life. This multi-locus dataset was analysed with rigorous pipelines (PHYLUCE) and phylogenetic inference tools, yielding a well-resolved phylogeny for the family. Compared to earlier multi-gene studies, the UCE-based phylogeny achieved stronger support at many nodes. For instance, several basal relationships within Primnoidae that were previously unclear were clarified with high bootstrap support, and the backbone of the family tree was more robustly defined. The analysis confirmed the monophyly of key genera such as *Primnoa* and *Narella*, consistent with traditional taxonomy, and for the first time incorporated previously unsampled genera (*Microprimnoa* and *Narelloides*), thereby filling important taxonomic gaps in the Primnoidae tree. The inclusion of these genera revealed their evolutionary placements and affirmed that the UCE approach can integrate new taxa seamlessly into the phylogeny. Overall, the phylogenomic results substantially improved the resolution of Primnoidae relationships, providing a more confident framework for understanding the family's evolutionary history.

Crucially, the high resolution afforded by UCE data also brought to light taxonomic inconsistencies and novel insights into Primnoidae systematics. The results uncovered or confirmed instances of polyphyletic genera, cases where morphologically defined genera do not correspond to monophyletic genetic lineages. Notably, the genus *Primnoella* was recovered as polyphyletic: species assigned to *Primnoella* were scattered across multiple clades rather than grouping together, echoing previous indications that *Primnoella* is not a natural lineage (Cairns and Wirshing, 2018; Taylor and Rogers, 2015). Similarly, *Plumarella* and related genera like *Fannyella* showed unexpected placements; for example, some species of *Plumarella* clustered more closely with other genera than with each other, suggesting that the current genus definitions based on morphology do not reflect true evolutionary

relationships. These findings highlight that certain diagnostic characters have likely evolved multiple times (homoplasy) or are too variable, leading to artificial groupings in the traditional classification. The discovery of polyphyly in *Primnoella*, *Plumarella*, and other groups calls for taxonomic revision: genera may need to be redefined or split so that they correspond to clades supported by molecular evidence. In contrast, other genera were confirmed to be genetically coherent. For example, *Primnoa* and *Narella* each formed a single clade with all included species, reinforcing confidence in those genera's current delineation. By illuminating both the congruence and discord between molecular phylogeny and classical taxonomy, this work underscores the significance of genomic data in reevaluating deep-sea coral systematics.

The thesis also applied an integrative species delimitation approach, leveraging the rich genomic dataset to address species-level questions that are especially difficult in deep-sea corals. Using a Bayesian coalescent framework (BPP) (Flouri *et al.*, 2018; Yang, 2015), we tested species boundaries within select Primnoidae lineages. This analysis represents one of the first applications of multi-locus coalescent-based species delimitation in Primnoidae, filling a notable gap in methodology for the group. The results demonstrated the power of UCE data to distinguish even closely related or cryptic species. Most species were strongly supported as distinct evolutionary lineages. For example, within the genus *Thouarella*, BPP consistently identified separate species clusters corresponding to morphologically recognized species such as *T. koellikeri, T. brucei, T. viridis, T. crenelata*, and others, each with near-maximum posterior probabilities of species status. Importantly, the analysis provided genomic confirmation for a newly discovered species, *Thouarella aureolabyrinthea* sp. nov. this candidate species formed its own well-supported clade, indicating it is not simply a morphological variant of an existing taxon but a genuinely separate lineage. The

agreement of molecular delimitation with the hypothesized new species gives strong validation for its formal description. These outcomes illustrate that UCEs can reveal cryptic diversity in Primnoidae, identifying new species that might have been overlooked by morphology alone.

At the same time, the species delimitation results highlighted instances of ambiguous or unresolved boundaries, shedding light on evolutionary processes in Primnoidae. In a few cases, nominal species could not be genetically distinguished from one another, implying either very recent divergence or taxonomic over-splitting. For instance, within *Thouarella*, the analyses found *T. variabilis* and *T. pendulina* to be genetically undifferentiated, with BPP failing to support a split between them. A similar result emerged for *T. andeep* and *T. diadema* despite some morphological differences reported for these species (Cairns and Bayer, 2009; Cairns and Wirshing, 2018; Taylor *et al.*, 2013), the genomic evidence did not confirm their separation. These pairs also form a four-species cluster in the case of *T. pendulina/variabilis/diadema/andeep* likely represent cases of very recent speciation or ongoing gene flow. Their distinct morphologies may have evolved rapidly or under different environmental pressures without sufficient time for genome-wide divergence, or perhaps these forms interbreed where their distributions overlap. An even more striking finding was the relationship between *Primnoella antarctica* and *Ophidiogorgia kuekenthali*. Despite belonging to different genera and exhibiting notable morphological distinctions (Cairns and Bayer, 2009), these two deep-sea corals showed almost no genetic divergence at UCE loci. BPP placed them in a single cluster with very low support for separation (PP ≈ 0.09), suggesting that they constitute a single lineage or a case of extremely recent divergence. This result aligns with earlier phylogenetic evidence that some *Primnoella* species cluster closely with *Ophidiogorgia*, rendering *Primnoella* polyphyletic (Cairns

and Wirshing, 2018). The *P. antarctica* + *O. kuekenthali* anomaly underscores the possibility of taxonomic over-splitting based on morphology alone, it hints that reproductive isolation between these forms may be incomplete, or that speciation occurred so recently that lineage sorting is unfinished. Such cases reinforce the need for integrative taxonomy, where molecular data are used in conjunction with morphology to accurately delineate species. In summary, the species delimitation component of this thesis provided a nuanced view: while most deep-sea octocoral species examined are valid evolutionary units, a few are questionable and merit further investigation with additional data.

Overall, the findings of this study have significant implications for Primnoidae systematics and deep-sea phylogenomics. The successful application of UCEs demonstrated that even in groups with challenging DNA quality and complex histories, high-throughput genomic approaches can achieve deep phylogenetic resolution and reliable species identification. By overcoming the limitations of single-marker and few-marker studies, UCE phylogenomics has resolved longstanding questions; for example, clarifying the evolutionary branching order of major primnoid lineages and exposing incongruences in the current classification. The improved Primnoidae phylogeny serves as a robust framework for future evolutionary and biogeographic analyses, and it provides a reference point for comparing other octocoral families. Likewise, the coalescent-based species delimitation showcased a methodological advancement for biodiversity assessment in the deep sea, moving beyond classical DNA barcoding toward genome-scale evidence of species boundaries. This approach was particularly valuable in a fauna where morphological divergence can be decoupled from genetic divergence. The thesis outcomes from the identification of polyphyletic genera to the confirmation of cryptic species highlight both the progress

made and the remaining challenges. Key among these challenges is incomplete sampling: not all known Primnoidae taxa could be included, and some regions or subgroups remain unrepresented, which means certain clades could not be evaluated and may harbour additional diversity or different relationships. The discovery of polyphyly in several genera and the ambiguous species boundaries indicate that a thorough taxonomic revision is needed, one that will likely require examining many more specimens and perhaps discovering new morphological characters that better align with genetic lineages. Cryptic diversity in the deep sea is another open question: our results validated one new species and flagged a few suspicious cases of synonymy, but with hundreds of Primnoidae species described, it is probable that additional cryptic species or overlooked lineages exist.

Looking ahead, several avenues of research emerge from this work that would further advance our understanding of Primnoidae and similar deep-sea coral lineages:

Future studies should employ UCE data in a Bayesian framework (e.g. BEAST or the STACEY package in BEAST2) to infer a species tree that accounts for gene tree discordance under the multispecies coalescent. Such analyses can jointly estimate the species relationships and divergence times while explicitly modeling incomplete lineage sorting (Bouckaert *et al.*, 2019). By incorporating fossil calibrations or molecular clock constraints, a time-calibrated phylogeny of Primnoidae can be obtained alongside the species tree. This will provide an evolutionary timeline for the family, revealing when major clades diverged and how these events correlate with geological or climatic changes.

A clear priority for future work is to broaden both the taxonomic and population sampling of Primnoidae. This entails targeting under-sampled genera and regions for

example, deep-sea areas or taxa not included in the current study to ensure that the phylogeny and delimitation analyses encompass the full breadth of the family's diversity. With more extensive sampling, especially obtaining multiple individuals per species across their geographic ranges, researchers can apply additional species delimitation methods in parallel to BPP. Methods such as generalized mixed Yule-coalescent (GMYC), or other coalescent-based approaches (STACEY)

The molecular revelations from this study necessitate a thorough re-examination of Primnoidae morphology and taxonomy. Future research should take an integrative taxonomy approach, revisiting museum collections and type specimens of Primnoidae species including those flagged as polyphyletic or problematic to identify morphological characters that corroborate the molecular phylogeny. Moreover, documenting the range of morphological variation within genetically cohesive groups will improve species diagnoses, especially for cryptic species that may differ in minor characters. Ultimately, integrating morphological data with molecular clades will lead to more robust species descriptions and keys for Primnoidae, facilitating identification and ecological studies.

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# Appendix

Table 1. list of species containing extraction ID, specimen ID, and Locations

| Species name | Extraction ID | Specimen ID | Location |
|---|---|---|---|
| *Ainigmaptilon edisto* | N44 | NA | NA |
| *Algaoprimnoa stefanii* | FB79 | FA21 | Drake Passage |
| *Armadillogorgia cyathella* | DP10 | DP10 | Drake Passage |
| *Arntzia gracilis* | AR2 | AR2 | Terre Adelie |
| *Arntzia gracilis* | AR58 | AR58 | Terre Adelie |
| *Australogorgia aldersladai* | USNM1116406 | USNM1116406 | SW Indian Ocean |
| *Callogorgia medialis* | AR209 | AR209 | SW Indian Ocean |
| *Callogorgia delta* | OCT133 | USNM? | NA |
| *Callogorgia farmosa* | AR306 | AR306 | SW Indian Ocean |
| *Callogorgia gracilis* | BEX0029 | BEX0029 | Bermuda |
| *Callozostron acanthodes* | N78759 | N78759 | NA |
| *Callozostron mirabile* | N77603 | N77603 | NA |
| *Calyptrophora agassizii* | USNM1093041ex2 | USNM1093041 | N Pacific - Ecuador |
| *Calyptrophora antilla* | JC0940504 | JC094_0504 | mid Atlantic |
| *Calyptrophora wyvillei* | USNM1072130 | USNM1072130 | Hawaii |
| *Candidella imbricata* | JC0940516 | JC0940516 | mid Atlantic |
| *Candidella imbricata* | OCT118 | USNM? | NA |
| *Candiella imbricata* | JC0940067 | JC0940067 | mid Atlantic |
| *Candidella gigantea* | JC0940777 | JC0940777 | mid Atlantic |
| *Convexella magelhaenica* | FM32 | NA | NA |
| *Convexella murrayi* | FB05 | NBP11-03 | Drake Passage |
| *Convexella murrayi* | FB09 | NBP11-03 | Drake Passage |
| *Convexella sp nov A* | JCR1373 | JCR1373 | South Orkneys |
| *Convexella sp nov A* | JCR1448 | JCR1448 | South Orkneys |
| *Dasystenella acanthina* | AA49 | AA49 | Sub-Antarctic |
| *Dasystenella acanthina* | AC43 | AC43 | South Georgia |
| *Dasystenella acanthina* | AE21 | AE21 | South Georgia |
| *Dasystenella acanthina* | AE37 | AE37 | South Georgia |
| *Dasystenella acanthina* | AR182 | AR182 | South Georgia |
| *Dasystenella acanthina* | AX2 | AX2 | NA |
| *Dasystenella acanthina* | FA56 | FA56 | Drake Passage |
| *Dasystenella acanthina* | FC43 | FC43 | Drake Passage |
| *Dasystenella acanthina* | FD30 | FD30 | Drake Passage |
| *Dasystenella acanthina* | FL77 | FL77 | Drake Passage |
| *Dasystenella acanthina* | FO63 | FO63 | Drake Passage |
| *Dasystenella acanthina* | JCR0922 | JCR0922 | South Orkneys |
| *Dasystenella acanthina* | JCR1015 | JCR1015 | South Orkneys |
| *Dasystenella acanthina* | JCR1283 | JCR1283 | South Orkneys |
| *Dasystenella acanthina* | JCR3100 | JCR3100 | South Orkneys |
| *Dasystenella acanthina* | JCR3116 | JCR3116 | South Orkneys |

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| *Dasystenella acanthina* | JCR3134 | JCR3134 | South Orkneys |
| *Dasystenella acanthina* | JCR31371 | JCR31371 | South Orkneys |
| *Dasystenella acanthina* | JCR3169 | JCR3169 | South Orkneys |
| *Dasystenella acanthina* | JCR3175 | JCR3175 | South Orkneys |
| *Dasystenella acanthina* | JCR3179 | JCR3179 | South Orkneys |
| *Dasystenella acanthina* | JCR3180 | JCR3180 | South Orkneys |
| *Dasystenella acanthina* | JCR3221 | JCR3221 | South Orkneys |
| *Dasystenella acanthina* | PRIM5 | PRIM5 | South Georgia |
| *Dasystenella acanthina* | S164 | S164 | South Georgia |
| *Dasystenella acanthina* | S171 | S171 | South Georgia |
| *Dasystenella acanthina* | T63 | T63 | South Georgia |
| *Dasystenella acanthina* | V6 | V6 | South Georgia |
| *Digitogorgia brochi* | FB44 | NA | NA |
| *Digitogorgia brochi* | FA58 | NA | NA |
| *Digitogorgia keukenthali* | R13 | NA | NA |
| *Fannyella abies* | N60495 | N60495 | NA |
| *Fannyella kuekenthali* | JCR2272 | JCR2272 | South Orkneys |
| *Fannyella lepidota* | JCR2451 | JCR2451 | South Orkneys |
| *Fannyella mawsoni* | JCR285 | JCR285 | South Orkneys |
| *Fannyella rossi* | AR48 | AR48 | Terre Adelie |
| *Fannyella spinosa* | AR32 | AR32 | Terre Adelie |
| *Loboprimnoa exotica* | N72423 | N72423 | NA |
| *Metafannyella polita* | N99702 | N99702 | NA |
| *Metafannyella ventilabrum* | USNM4 | USNM? | NA |
| *Microprimnoa diabathra* | USNM79977 | USNM79977 | New Caledonia |
| *Narella clavata* | AR236 | AR236 | New Caledonia |
| *Narella gilchristi* | SW3824 | SW3824 | SW Indian Ocean |
| *Narella hypsocalyx* | USNM1180223ex2 | USNM1180223 | New Zealand |
| *Narella paucilfora* | OCT119 | USNM? | NA |
| *Narella speighti* | SW3719 | SW3719 | SW Indian Ocean |
| *Narella candidae* | SW3746 | SW3746 | SW Indian Ocean |
| *Narella valentine* | SW3807 | SW3807 | SW Indian Ocean |
| *Narelloides traceyae* | USNM12 | USNM? | NA |
| *Onogorgia nodosa* | JCR2165 | JCR2165 | South Orkneys |
| *Ophidogorgia keukenthali* | AR171 | AR171 | South Georgia |
| *Paracalyptrophora carinata* | JC0941456 | JC0941456 | New Caledonia |
| *Paracalyptrophora carinata* | S080 | NA | NA |
| *Parastenella spinosa* | JC660208 | JC660208 | SW Indian Ocean |
| *Parastenella pacifica* | N94401 | N94401 | NA |
| *Perissogorgia colossus* | N91106 | N91106 | NA |
| *Perissogorgia vitrea* | N9882 | N9882 | NA |
| *Plumarella castellv* | FB12 | FB12 | Drake Passage |
| *Plumarella pourtalesi* | PLUM88 | USNM? | NA |
| *Plumarella aculeata (P)* | PLUM32 | USNM? | NA |
| *Plumarella cordilla* | USNM5 | USNM? | NA |
| *Plumarella cordilla (H)* | IZS099 | USNM? | NA |
| *Plumarella dochotoma (P)* | PLUM27 | USNM? | NA |
| *Plumarella laxiramosa* | OCT009 | USNM? | NA |

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| *Plumarella longispina* | USNM1117110 | USNM1117110 | N Pacific - Washington |
| *Primnoa bisquima* | JC660960 | JC660960 | SW Indian Ocean |
| *Primnoa resedeformis* | DY0810567 | DY081_0567 | Greenland |
| *Primnoella antarctica* | AR41 | AR41 | Terre Adelie |
| *Primnoella chilensis* | ANT47 | USNM? | NA |
| *Primnoella insularis* | USNM1278164 | USNM1278164 | New Zealand |
| *Primnoella scotiae* | FB03 | DP55 | Drake Passage |
| *Pyrogorgia lemnos* | NBP1103FA66 | DP449 | Drake Passage |
| *Thouarella andeep* | DP409 | DP409 | Drake Passage |
| *Thouarella aureolabyrinthea sp nov* | JC663190 | JC663190 | SW Indian Ocean |
| *Thouarella brucei* | FA25 | FA25 | Drake Passage |
| *Thouarella brucei* | FA27 | NA | Drake Passage |
| *Thouarella crenelata* | AS132 | AS132 | South Orkneys |
| *Thouarella diadema* | DP337 | DP337 | Drake Passage |
| *Thouarella koellikeri* | JCR2823 | JCR2823 | South Orkneys |
| *Thouarella pendulina* | AR135 | AR135 | Terre Adelie |
| *Thouarella variabilis* | JCR2192 | JCR2192 | South Orkneys |
| *Thouarella viridis* | AX1 | AX1 | Drake Passage |
| *Thouarella viridis* | AX10 | AX10 | Drake Passage |
| *Thouarella viridis* | AX11 | AX11 | Drake Passage |
| *Thouarella viridis* | AX12 | AX12 | Drake Passage |
| *Thouarella viridis* | AX13 | AX13 | Drake Passage |
| *Thouarella viridis* | AX3 | AX3 | Drake Passage |
| *Thouarella viridis* | AX37 | AX37 | Drake Passage |
| *Thouarella viridis* | AX4 | AX4 | Drake Passage |
| *Thouarella viridis* | AX5 | AX5 | Drake Passage |
| *Thouarella viridis* | AX6 | AX6 | Drake Passage |
| *Thouarella viridis* | AX7 | AX7 | Drake Passage |
| *Thouarella viridis* | AX8 | AX8 | Drake Passage |
| *Thouarella viridis* | AX9 | AX9 | Drake Passage |
| *Thouarella viridis* | DP117 | DP117 | Drake Passage |
| *Thouarella viridis* | FA27 | DP117 | Drake Passage |
| *Thouarella viridis* | FA52 | FA62 | Drake Passage |
| *Thouarella viridis* | FC80 | FC05 | Drake Passage |
| *Thouarella viridis* | FD62 | FD62 | Drake Passage |
| *Thouarella viridis* | FE40 | FE40 | Drake Passage |
| *Thouarella viridis* | FK54 | FK54 | Drake Passage |
| *Thouarella viridis* | FN78 | FN78 | Drake Passage |
| *Thouarella viridis* | JCR3220 | JCR3220 | South Orkneys |
| *Thouarella viridis* | S75 | X29 | South Georgia |
| *Thouarella viridis* | T62 | T62 | South Georgia |
| *Thouarella viridis* | U22 | U22 | South Georgia |
| *Thouarella viridis* | U3 | NA | NA |
| *Thouarella viridis* | W39 | W39 | South Georgia |
| *Verticillata castellivae* | IZS139 | USNM? | NA |