Deep-Sea Origin and In-Situ Diversification of Chrysogorgiidae Octocorals

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Abstract

The diversity, ubiquity and prevalence in deep waters of the octocoral family Chrysogorgiidae Verrill, 1883 make it noteworthy as a model system to study radiation and diversification in the deep sea. Here we provide the first comprehensive phylogenetic analysis of the Chrysogorgiidae, and compare phylogeny and depth distribution. Phylogenetic relationships among 10 of 14 currently-described Chrysogorgiidae genera were inferred based on mitochondrial (mtMutS, cox1) and nuclear (18S) markers. Bathymetric distribution was estimated from multiple sources, including museum records, a literature review, and our own sampling records (985 stations, 2345 specimens). Genetic analyses suggest that the Chrysogorgiidae as currently described is a polyphyletic family. Shallow-water genera, and two of eight deep-water genera, appear more closely related to other octocoral families than to the remainder of the monophyletic, deep-water chrysogorgiids. Monophyletic chrysogorgiids are composed of strictly (Iridogorgia Verrill, 1883, Metallogorgia Verslyus, 1902, Radicipes Stearns, 1883, Pseudychrsogorgia Pante & France, 2010) and predominantly (Chrysogorgia Duchassaing & Michelotti, 1864) deep-sea genera that diversified in situ. This group is sister to gold corals (Primnoidae Milne Edwards, 1857) and deep-sea bamboo corals (Keratoisidinae Gray, 1870), whose diversity also peaks in the deep sea. Nine species of Chrysogorgia that were described from depths shallower than 200 m, and mtMutS haplotypes sequenced from specimens sampled as shallow as 101 m, suggest a shallow-water emergence of some Chrysogorgia species.

Introduction

Corals of the family Chrysogorgiidae Verrill, 1883 are conspicuous members of benthic communities. They are found in all major oceans, as far north as Iceland [1] and as far south as Antarctica [2]. They have been described from a variety of habitats, including shallow-water reefs [3], soft sediments, and hard bottoms [e.g., [4]]. They were recently described as predominant members of benthic communities on NW Atlantic seamounts [5,6]. The Chrysogorgiidae are particularly diverse, with about one hundred described species. In his original description of the family, Verrill [7] presented the Chrysogorgiidae as including “some of the most beautiful and interesting of all the known Gorgonians.”

The family ranges between approximately 100 and 3375 m depth [8], most species (>75%) inhabiting deep waters. The family is an assemblage of deep-water specialists (e.g., Metallogorgia Verslyus, 1902 and Iridogorgia Verrill, 1883), a shallow-water specialist (Stephanogorgia Bayer & Muzik 1976), and eurybathic genera (Chrysogorgia Duchassaing & Michelotti, 1864 and Radicipes Stearns, 1883). The variety and gradualism in the bathymetric...
range of the family makes it a noteworthy model system for the study of diversification and radiation in the deep sea. However, these advantages as a model system have little value if the family Chrysogorgiidae is not monophyletic. McFadden et al. [9], in their genus-level phylogenetic reconstruction of the subclass Octocorallia Haekel, 1866, included four of the 12 genera described at the time, and retrieved a monophyletic group. However, the specimens were all from deep waters and did not cover the morphological, ecological and biogeographic variation observed in the family.

In fact, most octocoral families – as they are currently known – are likely not monophyletic. McFadden et al. [9] included 28 of the 44 octocoral families in their analysis, 14 of which were represented by multiple genera. Only five of these 14 were monophyletic, but even these were based on limited taxonomic data: only a third or less of the genera described in each of these five families were analyzed. Under-representation was particularly important for the Isididae Lamouroux, 1812, for which only two out of 38 described genera (5%) were included, and these two both from only 1 of the 4 subfamilies.

In light of the prevalence of polyphyly among currently-described octocoral families, there is little guarantee that a phylogenetic analysis of the Chrysogorgiidae based on broader taxonomic sampling will recover a monophyletic group. In addition, none of the molecular phylogenies including chryso-rgids produced to date [9–14] have assessed the monophyly of the genus Chrysogorgia, in particular, which is the most speciose (60+ species) and geographically the most wide-ranging of the Chrysogorgiidae [8]. As Chrysogorgia makes about 60% of the species diversity in the family, assessing its monophyly is of particular importance.

Our first goal was to test the monophyly of the family Chrysogorgiidae. Second, we compared depth distributions and evolutionary relationships among genera to evaluate the hypothesis that the two are correlated (i.e., shallow-water taxa are derived from deep-water ones, or vice-versa). We inferred phylogenetic relationships based on taxa from 10 of 14 currently-recognized genera, using mitochondrial (mtMutS and cox1) and nuclear (18S) markers. Bathymetric ranges were estimated based on our collections, museum records, and a literature review. The family Chrysogorgiidae is put in a broad evolutionary context by the inclusion of DNA sequences and distributional information from all other families of the suborder Calcaxonia (the Isididae, Primnooidae Milne Edwards, 1857, Ellisellidae Gray, 1859, and Iulkeilidae Bayer, 1953).

### Results

#### Informativeness and Congruence among Genetic Markers

The information contents of mtMutS, cox1 and 18S were assessed by looking at 42 calcaxonian colonies (Table 1). The 5’ end of mtMutS was the most variable region, with 31% of sites being variable. The gene as a whole is slightly less variable (28%). 18S and cox1 were 50% less variable than mtMutS (Table 1). The same pattern was observed for parsimony-informative sites. A total of 34 haplotypes were differentiated by the first 781 bp of the mtMutS alignment. The entire gene sequence differentiated 37 haplotypes, while cox1 and 18S differentiated 28 and 36 haplotypes, respectively. All cox1 haplotypes were also differentiated by mtMutS. The additional richness observed at 18S is attributed to ambiguous positions. The molecular variation found at mtMutS and cox1, and the diagnostic potential of these markers for barcoding is further detailed in [15].

The phylogenetic signal contained in the three markers was congruent overall, and recovered the same clades at the genus and family levels (see below and Figure 1). Concatenating markers significantly improved clade support, with the notable exception of the relationship between Chrysogorgia, Radicipes, and the clade composed of Iridogorgia, Rhodaniridogorgia Watling, 2007, Metallogorgia, and Pseudochrysogorgia Pante & France, 2010 (see below). Removing complex indels of doubtful homology at mtMutS did not significantly improve taxonomic resolution. Indel removal, however, had the effect of influencing branch lengths (Figures 1 and S1).

| Table 1. Length and information content of mtMutS, cox1 and 18S alignments, alone and concatenated. |
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| **Alignment** | **N. taxa** | **N. nt** | **N. nt-min-max** | **N. var** | **N. pars** | **Model (AIC)** | **Model (BIC)** |
| 1 | mtMutS 5’ | 105 | 829 | 691–799 | 384 (46%) | 259 (31%) | TVM+G | TVM+G |
| 2 | mtMutS 5’ (Gblocks) | 105 | 688 | 679–688 | 316 (46%) | 227 (33%) | TVM+G | TVM+G |
| 3 | mtMutS | 46 | 3150 | 2889–2997 | 896 (28%) | 594 (19%) | TVM+H+G | TVM+G |
| 4 | mtMutS (Gblocks) | 46 | 2871 | 2832–2871 | 837 (29%) | 571 (20%) | TVM+H+G | TVM+G |
| 5 | cox1 | 64 | 786 | 786–786 | 133 (17%) | 92 (12%) | TVM+H+G | TP1uf+G |
| 6 | 18S | 64 | 1315 | 1293–1307 | 215 (16%) | 181 (14%) | GTR+H+G | TIM2ef+H+G |
| 7 | mtMutS (5’), cox1, 18S | 64 | 2924 | 2773–2880 | 645 (22%) | 478 (16%) | GTR+H+G | GTR+H+G |
| 8 | mtMutS, cox1, 18S | 42 | 5236 | 4969–5078 | 1192 (23%) | 819 (16%) | GTR+H+G | TVM+H+G |
| 9 | mtMutS (5’) | 42 | 781 | 691–769 | 245 (31%) | 168 (22%) | |
| 10 | 18S | 42 | 1300 | 1293–1297 | 189 (15%) | 160 (12%) | |
| 11 | cox1 | 42 | 786 | 786–786 | 108 (14%) | 72 (9%) | |
| 12 | mtMutS | 42 | 3150 | 2889–2997 | 895 (28%) | 587 (19%) | | |
Figure 1. Bayesian 50% majority rule consensus trees based on different markers (mtMutS, cox1 and 18S) and marker combinations. For mtMutS, the effect of indels on phylogeny inference was tested by removing them with Gblocks. Chrysogorgiidae taxa are either color coded (MCC) or have bolded branches (nonMCC). The MCC clade is evidenced by a gray circle. All trees are rooted to the Pennatulacea, except trees using the entire mtMutS gene (rooted to the Ellisellidae). For sake of clarity, tip labels and node support values were removed, but can be consulted on Figure S1.

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Family Monophyly

Genetic data strongly suggest that the family as currently described is not monophyletic. The following genera formed a strongly-supported phylogenetic clade: Iridogorgia, Rhodaniridogorgia, Metallogorgia, Pseudochrysogorgia, Radicipes and Chrysogorgia (Figures 2 and 3). We refer to this group as the “monophyletic Chrysogorgia clade” (MCC), as it is the only monophyletic group retrieved for genera within the family. In addition, the type species for the family, Chrysogorgia desbonni (Duchassaing & Michelotti, 1864), is from a genus in this clade. While the phylogenetic relationship between Radicipes and Chrysogorgia was difficult to retrieve with strong support, Iridogorgia, Metallogorgia and Pseudochrysogorgia consistently formed a very well-supported monophyletic clade.

All other genera for which we were able to retrieve DNA sequences fell outside of that clade. Stephanogorgia, Pleurogorgia Versluys, 1902 and Trichogorgia Hickson, 1904 formed a strongly-supported clade with the monophyletic ifalukellid genera Ifalukaella Bayer, 1955 and Plunagorgia Nutting, 1910. Helicogorgia Bayer, 1981 systematically clustered outside the MCC, either sister to that clade (cox1 phylogeny), sister to the Primnoidea Milne Edwards, 1857/MCC clade (phylogenies based on mtMutS 5’ and concatenation of mtMutS 3’, cox1 and 18S) or sister to the clade composed of Stephanogorgia, Pleurogorgia and Trichogorgia (18S phylogeny). The entire mtMutS gene from Helicogorgia specimens was not sequenced. The genus Isidoides Nutting, 1910 was sister to the monophyletic sub-family Keratoisidinae (Isididae Lamouroux, 1812). This position is conserved among markers and combination of markers with strong statistical support. Only limited genetic data could be extracted from specimens of Challogorgia Bayer, 1949 (first 187 nt of mtMutS). This short sequence grouped with Helicogorgia with poor statistical support (56% node support on the ML phylogeny based on the 5’ end of mtMutS). The large amount of missing data negatively affected node support throughout the phylogeny; data from Challogorgia were therefore removed from all other analyses. Despite numerous attempts, no DNA could be amplified from specimens of Xenogorgia Bayer & Muzik, 1976, Specimens of Distichogorgia Bayer, 1979 could not be obtained.

Genus Monophyly

Within the MCC, Metallogorgia, Pseudochrysogorgia and Radicipes always formed monophyletic clades with strong statistical support. While Chrysogorgia formed two clades in the MCC based on the 5’ end of mtMutS (Figure 2), the genus formed a well-supported monophyletic clade based on the multiple-gene phylogeny (Figure 3). Genetic data cannot distinguish the genera Iridogorgia and Rhodaniridogorgia from each other, and there is very little divergence among haplotypes (uncorrected p distances at the 5’ end of mtMutS range from 0.1 to 0.7%: Figure 2 and 3). Outside the MCC, genera Stephanogorgia, Helicogorgia and Trichogorgia (n = 3, 5, 5 nominal species, respectively) are represented by multiple specimens (n = 3, 2, 2, respectively), but single haplotypes. Genus monophyly could therefore not be assessed. The two haplotypes sampled for the monotypic genus Isidoides formed a monophyletic clade.

Richness and Geographic Distribution of mtMutS Haplotypes

Chrysogorgia is the richest genus in terms of haplotype diversity at mtMutS. We obtained 41 haplotypes, 31 of which were sampled in the Pacific and 11 from the Atlantic (Table 2). Only one out of 41 haplotypes was common between the Atlantic and the Pacific. Diversity was significantly lower (1–6 haplotypes) for the other genera of the MCC. For all other genera within the Chrysogorgia clade except *Rhodaniridogorgia*, more haplotypes were found in the Indo-Pacific compared to the Atlantic. Metallogorgia, Iridogorgia and Radicipes each had one haplotype in common between the Atlantic and the Pacific (Table 2). Relative to the number of colonies sampled, Metallogorgia was the least variable within the MCC. Among 64 colonies, two mtMutS haplotypes were sampled. The first, corresponding to Metallogorgia melanotrichos Versluys, 1902, was found 62 times across the N. Atlantic, SW and NE Pacific. Although *M. melanotrichos* was previously thought to be restricted to seamounts, we sampled colonies on a continental slope (Bahama Escarpment). The other haplotype, corresponding to *Metallogorgia macrurina* Kükenthal, 1919, was found only twice, on the Norfolk and the Kermadec ridges (vicinity of New Caledonia and New Zealand, respectively). The type of this species was originally described from West Sumatra, consistent with our observations of a SW Pacific distribution.

Bathymetric and Geographic Distribution

A total of 985 biogeographic records (2945 coral colonies) were gathered from the literature, museum collections, and our own collecting. Of these, 24 records had a depth range considered too large relative to the average depth, and were removed from the dataset (see Methods). In addition, two records were removed as being extremely shallow, and most probably errors (Metallogorgia sp. USNM 56792 and Iridogorgia pouetaleisi Verrill, 1883 Blake-station 259; see [16] and [5]). The final database used in the analysis contains 959 records representing 2302 colonies.

The genera Metallogorgia and Iridogorgia, relatively well sampled (n = 120 and 40, respectively), are found exclusively in deep waters between 567 and 2311 m. The genera Radicipes and Chrysogorgia, well sampled as well (n = 78 and 615), are more wide ranging, and Chrysogorgia in particular appears as a depth generalist, ranging from 31 to 4327 m (both extremes correspond to unidentified Chrysogorgia specimens held at the NMNH, Smithsonian Institution). Stephanogorgia is the only shallow-water specialist (n = 12, range 7–37 m, with one outlier, USNM 79628, sampled at 90 m and identified by Frederick Bayer), and Helicogorgia and Trichogorgia (n = 23 and 43), while being found predominantly in waters shallower than 200 m, were occasionally reported from about 1000 m deep (Figure 4). Many genera are only known from a few specimens and estimating their depth distribution is therefore inherently biased. Pseudochrysogorgia, Pleurogorgia, Isidoides, Xenogorgia, Distichogorgia and Challogorgia are all known from eight specimens or less. All are found in waters below 200 m (n = 24, 250–2509 m), and most have a narrow depth distribution (Figure 4). The depth distributions of haplotypes and nominal species were very similar (Tables 2 and S1).

Among the genera belonging to the MCC, nine out of 89 species extend shallower than 200 m, all of them belonging to Chrysogorgia. Specimens reported in the taxonomic literature were collected from the NW Atlantic (C. desbonni Duchassaing & Michelotti, 1864, C. thyrsiformis Deichmann, 1936, C. feckesi Verrill, 1883; [7,8,16,17]), the NW Pacific (C. sphaerica Aurivillius, 1931, C. dichotoma Thomson & Henderson, 1906, C. axillaris (Wright & Studer, 1889), C. genculata (Wright & Studer, 1889), C. cuprea (Wright & Studer, 1889; [18–21]), and the NW Indian Ocean (C. dichotoma Thomson & Henderson, 1906; [22]). With the exception of one specimen (C. cuprea, from the Banda Sea), all of these colonies were sampled in the northern hemisphere, south of 34°N (Figure 5). An additional species, held at the NMNH (USNM 91906) and identified by Charles Nutting as *C. curvata* Versluys, 1902 (but not included in his 1908 monograph [23]), was collected in the NE Pacific.
Figure 2. Maximum likelihood reconstruction of the suborder Calcaxonia (rooted to five sea pens; Order Pennatulacea) based on the 5' end of mtMutS (102 taxa, 272 colonies, 829 bp; TVM+G model; 1000 bootstrap replicates). All five families of the Calcaxonia are represented. Node support values from the ML analysis (bold, only values >70% shown) are indicated under each node, and node support values from the Bayesian analysis (≥0.90) are above each node. Chrysogorgiidae taxa are either color coded (MCC) or have bolded branches (non-MCC). The collection depth of the shallowest Chrysogorgia specimens (≤200 m) for which a DNA sequence could be produced is indicated next to the tip label (bolded).

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between 37–55 m depth. This specimen was confirmed by Dr. Stephen Cairns (Smithsonian Institution) as *Chrysogorgia*; this very shallow occurrence is therefore either real, or reflects a recording error of station data, or is the result of remnant specimens being retained in the net between dredges (Dr. Cairns notes that the preceding station for this collection was dredged from

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**Figure 3. Maximum likelihood reconstruction of the suborder Calcaxonia (rooted to *Funiculina*, a sea pen) based on concatenated sequences of the 5’ end of *mtMutS*, *cox1* and 18S (64 taxa, 2924 bp; GTR+I+G model; 500 bootstrap replicates).** All five families of the Calcaxonia are represented. Node support values from the ML analysis (>70%, bold) are indicated under each node, and node support values from the Bayesian analysis (>0.90) are above each node. Chrysogorgiidae taxa are either color coded (MCC) or have bolded branches (nonMCC).

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1384–1829 m). One of these nine species, *C. dichotoma*, was exclusively found between 165 and 188 m, but is known from only three specimens collected in Japan and the Bay of Bengal [20,22]. Other species extend much deeper, five of them being found below 1000 m.

Among the genera belonging to the MCC, a diversity peak was observed at 600 m (36 species). Species diversity was maximum between 600 and 1000 m (29–36 species). All genera of the MCC were sampled within this depth interval. Diversity decreased progressively from 1000 m to 3860 m, the depth at which the last specimen identified to the species level was collected (Figure 6). This colony is a *Chrysogorgia* that was recently described by Pante and Watling [24], and was collected on Retriever Seamount in the NW Atlantic. Observations of a diversity gradient relative to depth are, of course, intimately linked to sampling effort in octocorals [25].

**Table 2.** Bathymetric range and biodiversity within the monophyletic, deep-sea Chrysogorgiidae (MCC).

| Genus            | Data source | N.   | Depth (m)   | Total | Atl. | Ind. | Pac. | Ant. |
|------------------|-------------|------|-------------|-------|------|------|------|------|
| Chrysogorgia     | morphology  | 615  | 31–4327     | 63    | 13   | 5    | 51   | 1    |
|                  | mtMutS gene | 101  | 101–3860    | 41    | 11   | 0    | 31   | 0    |
| Metallogorgia    | morphology  | 120  | 570–2262    | 4     | 2    | 0    | 3    | 0    |
|                  | mtMutS gene | 64   | 810–2262    | 2     | 1    | 0    | 2    | 0    |
| Iridogorgia      | morphology  | 40   | 567–2311    | 5     | 4    | 0    | 1    | 0    |
|                  | mtMutS gene | 25   | 752–2311    | 4     | 2    | 0    | 3    | 0    |
| Rhodaniridogorgia| morphology  | 5    | 568–2229    | 2     | 1    | 0    | 1    | 0    |
|                  | mtMutS gene | 2    | 663–2229    | 2     | 1    | 0    | 1    | 0    |
| Radicipes        | morphology  | 78   | 196–3580    | 7     | 4    | 2    | 3    | 0    |
|                  | mtMutS gene | 16   | 308–3000    | 6     | 3    | 0    | 4    | 0    |
| Pseudochrysogorgia| morphology | 3    | 861–1429    | 1     | 0    | 0    | 1    | 0    |
|                  | mtMutS gene | 5    | 861–1429    | 1     | 0    | 0    | 1    | 0    |

Diversity: morphology estimate based on number of nominal species; genetic estimate based on number of mtMutS haplotypes. N: sample size (morphology: number of biogeographic records, and minimum number of colonies in parentheses), Atl: Atlantic Ocean, Ind: Indian Ocean, Pac: Pacific Ocean, Ant: Antarctic Ocean. Some depth estimates are based on depth ranges from trawling stations, in which case minimum and maximum depths were averaged (see notes in the Material and Methods section).

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![Figure 4](https://www.plosone.org/figure/Figure4.jpg)
Discussion

Family Monophyly and the Diagnosis of the Chrysogorgiidae

Genetic data strongly suggest that the Chrysogorgiidae is polyphyletic as currently described. In our analysis of mtMutS, chrysogorgiid genera appeared in three different clades: the “monophyletic Chrysogorgiidae clade” (MCC), the Pleurogorgia/Stephanogorgia/Trichogorgia/Iialukellidae clade, and the Isidoides/Keratoisidinae clade. The position of Helicogorgia was labile among phylogenetic trees, being either sister to the MCC, or sister to the clade composed of the Primnoidae and the MCC. The very limited data available for Chalcogorgia suggests a close affinity with Helicogorgia.

The polyphyly of the family calls for a re-assessment of the diagnostic characters used to differentiate the Chrysogorgiidae. The family is currently diagnosed as (e.g., [8]): The axis is unjointed, solid (non-spicular), and made of concentrically-layered...
scleroproteinaceous material; these layers are not undulated; the axis surface is smooth and has a metallic or iridescent sheen; colonies are branched or unbranched; the holdfast is strongly calcified, and discoidal or rhizoidal; polyps are contractile, not retractile, and are arranged in rows, sometimes bi- or multiserially, never in whorls or on opposite sides of the branch; sclerites are mostly flat scales, plates, rods and needles; when exposed to polarized light, scales show a distinct circular (not cruciform) extinction pattern (for an example see p. F221 in [26]).

_Trichogorgia_ and _Stephanogorgia_ have in common biserially-arranged polyps, which contrasts with the organization in rows found in genera of the MCC. This suggests that polyp arrangement is a diagnostic character of the Chrysogorgiidae. If the MCC, then we can predict that these three genera should not belong to the MCC. Similarly, two genera (_Pleuragorgia_ and _Helicogorgia_) have ornamented sclerites that are uncharacteristic of the Chrysogorgiidae. As _Pleuragorgia_ does not belong to the MCC, it can be predicted that _Helicogorgia_ is probably not a true chrysogorgiid. Our Pleuragorgia specimens are small whips with polyp and sclerite morphology, and biogeography, consistent with published records for this genus [19,23]. Both described species of _Pleuragorgia_ are branching, however, and our specimens might belong to undescribed species (Alderslade, pers. com) or colonies at an early pre-branching life stage.

On the other hand, some characters currently used in the diagnosis might not be good indicators of the uniqueness of chrysogorgiids. Nutting [27] initially placed the genus _Isidoides_ in the Gorgonellidae (now Ellisellidae). He noted its unusual morphology and the resemblance of sclerites to those found in some isidids. Bayer and Stefani [28] later noted that the fine, smooth scales of _Isidoides_ are typical of the Chrysogorgiidae. Although exactly when the genus _Isidoides_ was placed in the family Chrysogorgiidae is unclear, the genus appears in a key of the family as early as 1979 [29], but without justification. Later, Bayer and Grasshoff [30] stated that _Isidoides_ should belong to the Chrysogorgiidae rather than the Ellisellidae. Our phylogenetic data clearly show that _Isidoides_ does not belong to the Chrysogorgiidae, and supports Nutting’s initial suggestion that _Isidoides_ might be related to bamboo corals.

There is no published record that all nine characters diagnostic of the family have been scored for all extant genera. For example, _Chrysogorgia_ is the only genus for which clear evidence was provided that, when exposed to polarized light, scales show a distinct circular extinction pattern [26]. For this study, we confirmed that specimens from all genera of the MCC show a circular extinction pattern. However, it is also the case for _Pleurogorgia_, _Stephanogorgia_, _Helicogorgia_, _Xenogorgia_, _Isidoides_, and even bamboo corals. Circular light extinction pattern is therefore clearly not suited as a diagnostic feature of the MCC. Similarly, the composition and arrangement of axial layers are rarely reported. These results imply that characters diagnostic of the Chrysogorgiidae need to be re-evaluated for these genera; the diagnosis of the family will have to be revised.

**Trichogorgia, Stephanogorgia, Pleurogorgia, and the Ifalukellidae**

Three of the 14 chrysogorgiid genera ( _Trichogorgia_, _Stephanogorgia_, _Pleurogorgia_, _Pleuragorgia_ ) formed a well-supported clade with the Ifalukellid genera _Plumigorgia_ and _Ifalukella_. The short genetic distance (mtMutS, uncorrected p: 0.72%) between the latter two genera supports the validity of the Ifalukellidae as a family (at _mtMutS_ _Plumigorgia_ and _Ifalukella_ differ by only one amino acid, and have an identical indel structure, which is often quite variable among calcaxoneans). Based on the taxa (_Calcaxonia_: Chrysogorgiidae, Primnoiidae, Isididae) used in the study of McFadden et al. [15], the maximum intra-familial uncorrected p (at _mtMutS_) distance is 4.9%, while the minimum inter-familial distance is 3.8%. The distance between _Trichogorgia_ and _Ifalukella_ is <3.4%. Based on this criterion, we can legitimately suggest that _Trichogorgia_ be considered as an ifalukellid. On the other hand, the genetic distances between _Stephanogorgia_, _Pleurogorgia_, and the _Trichogorgia_/ _Ifalukellidae_ group are all >6.7%, suggesting that two new families may need to be erected.

The phylogenetic grouping of _Trichogorgia_, _Stephanogorgia_, _Pleuragorgia_, and the Ifalukellidae is only preliminary, and more comprehensive genetic and morphological analyses will have to be conducted. Only a single _mtMutS_ haplotype could be used to represent each genus. More extensive taxon sampling will be required to comment further on the status of these genera, confirm their placement on the Calcaxonia tree, and rule-out spurious effects of long-branch attraction (e.g., [31]). There is nevertheless congruence among morphology, ecology and phylogeny. Morphologically, _Trichogorgia_, _Stephanogorgia_, and the Ifalukellidae share small, smooth sclerites in the form of scales and plates. In addition, both _Trichogorgia_ and _Ifalukella_ have relatively few sclerites in their tissues, to an extent for _T. capensis_ Ku¨kenthal, 1919, which is completely lacking them. Also, the Chrysogorgiidae are defined as having an axis made of concentric layers that are not undulating. These layers are slightly undulating in the Ifalukellidae [26,32]. However, the descriptions of _Stephanogorgia_, _Trichogorgia_ and _Pleurogorgia_ give no indication of how the layers are arranged. Examination of this character may inform us on the relationship of these taxa to ifalukellids. Ecologically, _Trichogorgia_ and _Stephanogorgia_ are two of the shallowest chrysogorgiid genera, and _Stephanogorgia_ is a tropical lagoon-dwelling taxa. These observations are consistent with the fact that ifalukellids are strictly found above 50 m on coral reefs (e.g., [32,33] and collection records from the Smithsonian Institution).

**Genus Monophyly and Genetic Diversity**

With the exception of _Iridogorgia_ and _Rhodaniridogorgia_, all chrysogorgiid genera were monophyletic. Specimens of _Iridogorgia_ and _Rhodaniridogorgia_ have been separated by the morphology of their spiral (_Iridogorgia_: main stem coiled; _Rhodaniridogorgia_: main stem wavy) and the morphology and placement of sclerites (sclerites are larger in _Rhodaniridogorgia_, and consistently present in the branch coenenchyme) [5]. In contrast, genetic distances separating these genera were consistent with intra-generic variation. These genera might have diverged too recently for our markers to have detected their reciprocal monophyly. Therefore, more specimens and a fine-scale genetic analysis will be needed to decide whether these genera should be synonymized.

Both _Radicipes_ and _Chrysogorgia_ were supported as monophyletic genera, although the relationship between them and the clade composed of _Metallogorgia_, _Pseudochrysogorgia_, _Iridogorgia_ and _Rhodaniridogorgia_, was difficult to recover. As predicted in the taxonomic literature [in particular [19]], _Chrysogorgia_ appeared as a highly diversified genus: 73% of haplotypes within the MCC (41 out of 56) belong to _Chrysogorgia_. Pante and France [14] showed that the distribution of intra-generic and inter-generic genetic distances between _Chrysogorgia_ and _Radicipes_ are greatly overlapping, with some _Chrysogorgia_ haplotypes being more divergent than _Chrysogorgia_ _Radicipes_ pairs. In contrast to the relatively high level of divergence within _Chrysogorgia_, the very short genetic distances
between most Chrysogorgia clades suggest a rapid diversification of the genus.

The available evidence does not allow us to determine whether the diversification of Chrysogorgia is the result of an adaptive radiation. Theory predicts that an adaptive radiation would be associated with a high diversity of filled ecological niches. Four main hypotheses should be evaluated to test for adaptive radiation: (1) all taxa share a common ancestor, (2) there is a correlation between the environmental conditions and phenotypes, (3) new traits are adaptive, and (4) speciation is rapid (reviewed in [34]). Although the first requirement is met, other hypotheses remain to be tested.

First, ecological information associated with individual Chrysogorgia haplotypes is scarce; most haplotypes are singletons (i.e., sampled only once), and correspond to colonies sampled using trawls. In the case of trawled specimens, ecological information can be inferred from the whole catch (residual substrate and associated species). Specimens collected using underwater vehicles have the advantage of being associated with more extensive ecological data, but are a minority at the moment. While ecological data is scarce, some observations are congruent with an adaptive radiation. Chrysogorgia is the most widely-distributed chrysogorgiid genus, both geographically and bathymetrically (Table 2). Specimens are found from soft and hard substrates (e.g., [35]), and in continental and oceanic environments (e.g., [8,36]). Chrysogorgia colonies are characterized by rhizoidal and discoidal holdfasts, consistent with life in soft and hard substrates.

Second, phylogenetic relationships among Chrysogorgia haplotypes require more than sequencing of the 5′ end of mtMutS. Our comparative analysis shows that the relationships of some Chrysogorgia haplotypes could be resolved using mtMutS, cox1 and 18S together. Future research efforts will include a more comprehensive molecular analysis of Chrysogorgia, and the mapping of ecological and morphological characters on a better-resolved phylogeny. This exercise, however, might be complicated by the fact that morphological characters are notoriously plastic (e.g., [37–40]) and convergent [41,42] in the Octocorallia. In addition, determining whether morphological traits confer an adaptive advantage will be technically very challenging.

Finally, studying the pace of speciation in the Octocorallia remains a daunting task, as informative markers that are variable (1) all taxa share a common ancestor, (2) there is a correlation between the environmental conditions and phenotypes, (3) new traits are adaptive, and (4) speciation is rapid (reviewed in [34]). Although the first requirement is met, other hypotheses remain to be tested.

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National Marine Sanctuaries and fishery-closed areas focused on coral conservation; no permits were required. Specimens from Alaska were collected under State of Alaska Fish Resource Permits CF-04-009 and CF-06-013. Specimens from Hawaii were either collected outside of state waters, or in the Marine National Monument under permit PNNM-2009-053. Collecting in the Bahamas was done with permission from the Department of Marine Resources, Ministry of Agriculture and Marine Resources of The Bahamas, as assigned to the University of Miami/RSMAS. Collecting in New Caledonia was done within the French EEZ, by a French boat, and no permit was required. Whole colonies or portions thereof were sampled using remotely-operated vehicles (ROV), human-occupied vehicles or scientific trawls. Fragments for genetic analyses were preserved in 80–100% ethanol, RNAlater (Ambion), or frozen at −80°C. DNA was extracted using a modified CTAB protocol [10] or using the MasterPure DNA purification kit (Epicenter).

DNA Amplification and Sequencing

Three gene regions were targeted. First, we attempted to PCR-amplify the 5′ end of the mitochondrial, protein-coding mtMutS for all available specimens. Although this gene has been most frequently referred to as msh1 in the octocoral systematics literature, we will refer to it as mtMutS throughout this paper as this term makes fewer assumptions about its evolutionary origins [52]. mtMutS was chosen because it is one of the most variable and informative markers available for octocorals to date (16S: [10]; mtMutS, nd3, nd4l: [53]; cox1: [54]; [55]; nd2, nd3, nd6: [56]; mtMutS, cox2-IGR-cox1: [15]; mtMutS, cox1, nd2, nd3, 16S, 28S, ITS2: [57]). All chrysogorgiids and outgroup taxa, except the Keratoisidinae, were amplified by priming in the flanking gene msh1 with upstream primer ND4L2475F [58] and extending into mtMutS with downstream primer MUT3458R [59]. This last primer was later replaced by MUTChy3458R (Table 3), a novel primer that is a perfect match to primnoids, isidids and chrysogorgiids tested to date. Mitochondrial gene order is not conserved across the sub-order Calcaxonia [58]. In the isidid sub-family Keratoisidinae, mtMutS is flanked by cox3 at its 5′ end. Thus, primers CO3BAM5647F and MUT3458R were paired to amplify mtMutS in this group of taxa. Internal primers were used when DNA was degraded (primers 1–16, Table 3).

For a set of taxa, additional gene sampling was performed. These taxa were chosen based on their position on the mtMutS phylogeny to achieve two goals: (1) to assess if additional sequencing would retrieve more variation from clades/taxa that show little or no variation at mtMutS (e.g., Metallogorgia melanotrichos), and (2) to further resolve phylogenetic relationships among the Chrysogorgiidae (e.g., relationships between Radicipes and Chrysogorgia). For one set (n = 64), cox2-IGR-cox1 and 18S were amplified (primers 27–28 and 29–34). Among these (n = 42), the nearly complete mtMutS was amplified (2889–2997 bp; primers 1–26).

PCR was performed in 25 μL total volume using 1x TaKaRa Ex Taq buffer (Mg²⁺-free, proprietary composition), 1.5 mM of MgCl₂, 0.4 mM of dNTP mix, 0.5 U of Ex Taq polymerase (TaKaRa Bio USA Inc., now Clontech), 0.24 μM of each primer (Operon Biotechnologies, Inc., now Eurofin MWG Operon), and 40 ng of DNA template (quantified using BioRad VersaFluor fluorometer and/or Thermo Scientific Nanodrop ND-1000 spectrophotometer). 2.5 μg of acetylated BSA (Promega) was added for problematic samples. PCR amplification conditions were optimized for each primer pair (Table 3 provides cycling profiles for the most commonly-used pairs). PCR products were purified either by excising bands from low-melting point agarose gels followed by an agarase digestion (5 U; Sigma-Aldrich Co.; [54]) or by an Exo-SAP digestion (2 U of ExoS and 0.2 U of SAP/1 μL of DNA; Fermentas; [60]). The majority of purified PCR reactions were cycle-sequenced using the ABI BigDye Terminator v1.1 Cycle Sequencing Kit (1/4 reactions) and purified using either an EB/EDTA precipitation or Sephadex G-50 columns (Sigma-Aldrich). Purified products were electrophoresed on an ABI PRISM (R) 3100 or 3130xl Genetic Analyzer. A fraction of the PCR products were purified with AmPure XP beads, cycle-sequenced using the ABI BigDye Terminator v3.1 Cycle Sequencing Kit (1/32 reactions) and purified using an EB/NaODAcetate precipitation. These products were electrophoresed on an ABI PRISM (R) 3730xl Genetic Analyzer. All sequence traces were edited using Sequencer (TM) v4.7 (Gene Codes). DNA sequences of specimens representing each haplotype, for each biogeographic region, were submitted to GenBank (Table S1).

Data Analysis

mtMutS sequences were translated to amino acids (Mold-Protozoan mitochondrial code) and aligned using MAFFT 6 (L-ins-i method, [61,62]); TranslatorX was used to align nucleotides based on the amino-acid alignment [63]. There were no indels in the cox1 alignment. The 18S dataset contained only single-nucleotide indels, and could therefore be aligned by eye. Saturation plots and transition/transversion ratios were computed for each dataset using the ape 2.5–2 package [64] in R 2.12 [65]. The effect of complex indel motifs at mtMutS (particularly the 5′ region) on phylogenetic reconstruction was investigated by constructing trees with and without indels. Gblocks 0.91b [66,67] was used to eliminate indel regions that are poorly conserved, while keeping the regions that contain informative sites. All alignments are available from the authors upon request. The phylogenetic information content of all gene regions (5′ end of mtMutS, whole mtMutS, partial cox1 and 18S) and these regions in combination was evaluated by calculating the number of variable and parsimony-informative sites in MEGA 5 [68] and constructing maximum-likelihood (ML) trees using PhyML 3.0 [69]. Node support was evaluated with 1000 bootstrap replicates (500 replicates for mtMutS, cox1 and 18S concatenated). The most-likely model of evolution was inferred using jModelTest 0.1.1 [70], and PhyML was parametrized accordingly. MrBayes 3.1 [71,72] was used on the CIPRES Portal [73] to produce phylogenetic hypotheses based on Bayesian statistics (6 nucleotide substitution types, 4 × 4 substitution model with I+G among-site rate variation; 5 million generations, 2 runs, 4 chains, sampling every 1000 generations, burnin of 25% equaling 1250 samples). Convergence was assessed by checking that (1) standard deviations of split frequencies were <0.007, (2) potential scale reduction factors were close to one (they varied between 1 and 1.06), and (3) plots of log likelihood values did not show visible trends over time. Trees were rooted to the Pennatulacea (sea pens), which are the non-calcanxanorn octocorals that are the most closely related to the Calcaxonia, based on the results of [9].

Biogeography Database

Biogeographic data were compiled from the taxonomic literature (37 published papers, one in press, one in preparation), collection information from museums (the Australian Museum (Sydney), the Florida Museum of Natural History (University of Florida, Gainesville), the Harvard Museum of Comparative Zoology, the Museum and Art Gallery of the Northern Territory (Darwin, Australia), the Muséum national d’Histoire naturelle (Paris, France), the National Institute of
Water and Atmospheric Research (Wellington, New Zealand), the National Museum of Natural History (Smithsonian Institution), and the Yale Peabody Museum of Natural History (Yale University), and our collections (including specimens provided by colleagues). Duplicate records (i.e., fragments from individual colonies held in multiple museums, use of material in different manuscripts) were removed. Depth distributions were compiled using sampling station information. Thirty-five percent of the depth records (341 of 975) come from dredging or trawling, for which the minimum and maximum depth were reported. The average depth was computed for these records. However, in some cases the depth range was so large that it made the average meaningless. All records for which the depth range was more than half of the average depth were excluded from the dataset. A species-diversity profile across depth was computed for species belonging to the genera forming a monophyletic clade by counting the number of species found every 100 m between 0 and 4500 m. The biogeographic database is available from the authors upon request.

Supporting Information

Figure S1  Bayesian 50% majority rule consensus trees based on different markers (mtMutS, cox1 and 18S) and marker combinations. For mtMutS, the effect of indels on phylogeny inference was tested by removing them with Gblocks. Chrysogorgiidae taxa are either color coded (MCC) or have bolded branches (non-MCC). All trees are rooted to the Pennatulaceae. Table S3. PCR primers used in the present study to amplify targeted gene regions.

| Name       | Sequence (5’ –> 3’)         | Gene | Product size | Cycle | Reference |
|------------|-----------------------------|------|--------------|-------|-----------|
| CO38am5657F | gctgcattagtgattgtgcctcagt | cox3 | 1–15: 1014 bp | 94:20,55:30,72:50 | [58]     |
| ND4LA2475F | tagtttacttgccctcac          | nad4L|               |       |           |
| ND42625F   | tgacggaacaactgtcctc         | nad4L| 3–10: 476 bp  | 94:20,50:30,72:30 | [58]     |
| MSH12714F  | ctaatgaggagaatattc          | mtMutS|            |       |           |
| MSH2806F   | taactacgctgtgaagatgc        | mtMutS|            |       |           |
| msh2864r   | gaggcaactttgttaagggagtgtg  | mtMutS|            |       |           |
| MSH3101F   | ggttaaaagtttagactatattag    | mtMutS| 7–16: 448 bp | 94:20,55:30,72:30 |           |
| MSHLA3034R | cttcagatagtctgttagttaaggccc  | mtMutS|            |       |           |
| MSH3055R   | ggaagattaaacactgagycag     | mtMutS|            |       |           |
| MSH3101R   | gatatacaatgaaatctcctg       | mtMutS|            |       |           |
| MSH3186F   | gccatagggggcatatagata       | mtMutS|            |       |           |
| msh3208R   | atccgcyactttgctcctcgtc     | mtMutS|            |       |           |
| MSH3323F   | cttataaagggttggaagaa        | mtMutS|            |       |           |
| MSH3350F   | gccatagggggcatatagata       | mtMutS|            |       |           |
| MUF3458R   | tsgagcaaaaggcatcccc         | mtMutS| 2–15: 940 bp | 94:20,50:30,72:50 | [59]     |
| MUTFchy3458R | tsgaagaaagggcatccct       | mtMutS| 2–16: 940 bp | 94:20,50:30,72:50 |           |
| MSH3841F   | ctcgctttagaggagattgckac     | mtMutS|            |       |           |
| MSH4094F   | cagctgcacctcatctgtaatcgtc   | mtMutS|            |       |           |
| MSH4332R   | gaaaggctacacctctctctcg      | mtMutS| 14–19: 920 bp | 94:20,50:30,72:50 |           |
| MSH4757R   | gacctgccccgacacattcctcg     | mtMutS|            |       |           |
| MSH4759F   | tgaacgctgataattag           | mtMutS|            |       |           |
| MSH4915R   | cgacctcaaaagacactgctacc    | mtMutS| 18–22: 830 bp | 94:20,50:30,72:50 |           |
| MSH5056F   | gcacaaagtaggaaatgtagc       | mtMutS|            |       |           |
| MSH5075R   | gtagtagamgarcaaatagac       | mtMutS|            |       |           |
| MSH5376R   | agctcaccatattcccacac        | mtMutS|            |       |           |
| 16S5PR     | tcacgctttcatccagatg         | 16S  | 21–26: 900 bp |       |           |
| COI8068xF  | ccataacaggrctwgcagcatc      | cox2 |            |       |           |
| COIoctR    | acatagcatgacatccatc         | cox1 | 27–28: 1080 bp |       |           |
| 18S-Af     | aacctgctggactgctcgactgtctc | 18S  |            |       |           |
| 18S-Lr     | ccaactcagccttttatactgctctg | 18S  | 29–30: 620 bp | 94:20,60:30,72:40 | [77]     |
| 18S-Cf     | cgtgagctcggctcctctgcctg    | 18S  |            |       |           |
| 18S-Yr     | cagacacagctgctccacactcc    | 18S  | 31–32: 710 bp | 94:20,60:30,72:40 | [77]     |
| 18S-Of     | aagggccacccacaggttgagag    | 18S  |            |       |           |
| 18S-Br     | gatgctctccagagagctgccctc   | 18S  | 33–34: 620 bp | 94:20,60:30,72:40 | mod. [76]|

Predicted fragment sizes (approximate, in bp) and PCR cycle profiles (temperature in °C: time in seconds) are given for the most commonly used primer pairs. Primer combinations are listed in the product size column, prior to the predicted fragment size, using the primer numbers defined in the first column. (mod.: modified from). Between 30 and 45 cycles were used for PCR.

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laccia, except trees using the entire miMatS gene (rooted to the Ellisiellidae). (PDF)

**Table S1** Collection date, geographic coordinates, depth, and genetic markers sequenced for specimens used in this study. GenBank accession numbers representing each haplotype and biogeographic region are given in the final 3 columns. (XLS)

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**Author Contributions**

Conceived and designed the experiments: EGP SCF. Performed the experiments: EGP. Analyzed the data: EGP. Contributed reagents/materials/analysis tools: EGP SCF AC CC SS CSLM EW. Wrote the paper: EGP SCF.
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