Phylogenetic Diversity of the Cold Water Octocoral *Paragorgia arborea* (Linnaeus, 1758) off the East Coast of Canada

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Abstract

Commonly called "bubblegum coral', *Paragorgia* is a genus of cold-water gorgonian coral that has a broad global distribution and is eurybathic from depths of meters to kilometers. Such ecological breadth, however, may be confounded by the presence of cryptic species. In this study the genetic distances of various *Paragorgia* spp. across their distribution and depth range along the Canadian mid-Atlantic margin using 18S ribosomal DNA sequences were compared. Morphometric and 18S evidence show that although appearing closely related, only one species of cold-water *Paragorgia*, called *P. arborea*, predominates in this region. However, 18S evidence from corals collected at deep depths located at one site, called the Stone Fence, indicate that a significant amount of divergence exists and other species may also be present. Such new light provides a need for further genetic testing using alternative methods (e.g. microsatellites), which could reveal new lineages that may have considerable ecological and/or taxonomic importance.

Keywords: Cold-water coral, Deep-sea coral, Paragorgiidae, *Paragorgia*, 18S rDNA

1. Introduction

The gorgonian corals of the genus *Paragorgia* (Paragorgiidae; Octocorallia) are among the largest sessile benthic organisms commonly observed on continental shelves, seamounts, lithoherms, and canyons (Freiwald et al., 2004; Sanchez, 2005). Most species are fan-shaped and concave, with thick branches, reaching in excess of 2 m in height (Figure 1). Paragorgiids are unusual in that they lack a corneous or calcareous axial skeleton typical of most branching gorgonians (Sanchez, 2005). The massive structure is instead supported by calcitic sclerites, sometimes referred to as spicules (Bayer, 1993). They exhibit a wide color variation including red, salmon pink, white, black, brown, pumpkin orange and yellow (Diechmann, 1936). Descriptions of longevity vary from years (Koslow et al*.,* 2001) to thousands (Rogers, 2004), which reflect varied growth conditions of each corals' specific habitat. In the northern Pacific regions *Paragorgia* sp. are reported to grow ~2 cm yr^{-1} which is ten times faster than the same species growing in the Atlantic (Andrews et al*.,* 2002; Tsao, 2005); a two meter tall coral colony, then, is ~112 years of age. Based upon measurements of radioactive isotopes in the coral's skeleton, Mullineaux and Mills (2004) suggest that a 2 m tall *Paragorgia* specimen is at least 500 years of age. Because of their age and the complexity of their three-dimensional morphologies, *Paragorgia* and other deep-sea coral habitats may be analogous to condominiums hosting a plethora of bacteria (Penn et al*.,* 2006) and micro- and macro-organisms (Buhl-Mortensen and Mortensen, 2004, 2005; Mortensen and Buhl-Mortensen, 2005; Nedashkovskaya et al*.,* 2005). For example, Buhl-Mortensen and Mortensen (2005) identified 47 species (1,264 organisms) in thirteen *Paragorgia* colonies. *Paragorgia* sp. have also been identified as important fishery habitat for a variety of commercial fish, including *Gadus macrocephalus* (Pacific cod), *Anoplopoma fimbria* (sablefish), *Pleurogrammus monopterygius* (Atka mackerel), and *Sebastes marinus* (redfish) (Husebø et al., 2002; Auster et al., 2005; Heifetz et al., 2005). Due to the destructive practice of bottom trawling, many agencies worldwide are

implementing marine protected areas (MPAs) in an attempt to delay any destruction they may experience and to allow scientists and opportunity to better describe the taxonomic status of *Paragorgia* and the commercial importance of this species (e.g. see Hall-Spencer et al., 2009; Gilkinson and Edinger, 2009).

Generally, *Paragorgia* spp. is believed to be eurybathic, with a global depth range between 18 and 5800 m (Tendal, 1992; Cairns and Bayer, 2009). They seem to prefer channels between submarine canyons, fishing banks, and deep ocean basins where strong unidirectional currents are present. We speculate that the resilience of such populations depends upon the supply of new recruits. Having been documented in the Aleutian Islands (Heifetz et al., 2005) and Seamounts in the Gulf of Alaska (T. Shirley, pers. comm.), Pacific Ocean (Nedashkovskaya et al., 2005; Baco, 2007), Atlantic and Indian Oceans (Bayer, 1964; Strychar et al., 2005), and in the Gulf of Mexico and Straits of Florida (NOAA, 2009), we hypothesize that these species have very high larval dispersal ability or exist as a result of suitable spacing of reef patches to allow for larval mixing (Vrijenhoek, 1997; Samadi et al., 2006; Morrison et al., 2008). Such populations are not likely self-seeded, as that would lead to long-term consequences and reduced chances of re-colonization (Morrison et al., 2008).

Using a variety of phenotypic traits (e.g. sclerites), *Paragorgia* spp. has been placed taxonomically within the Phylum Cnidaria, Class Anthozoa, Subclass Octocorallia, Order Gorgonacea, Suborder Scleraxonia, Family Paragorgiidae (Sanchez and Cairns, 2004). There may be as many as 19 characterized species of *Paragorgia*, however, controversy exists regarding their descriptive accuracy (Table 1). In the most recent revision of the genus, Sanchez (2005) suggests that only 14 *Paragorgia* species exist, and that *Paragorgia nodosa* and *P. pacifica* should be subsumed within *P. arborea* on the basis of the location of the siphonozooids, the autozooids having eight grooved notches associated with the aperture, and a common sclerite morphology. Previously, Verrill (1922) cast doubt as to whether *P. pacifica* is a valid species as the only diagnostic character was based on gross morphology. He felt that it too may be a variety of *P. arborea*. In accordance with Grasshoff (1979), Sanchez (2005) suggests that no phenotypic differences exist between *P. boschmai* and *P. johnsoni*, concluding that the taxonomic name "*P. boschmai*" is a junior synonymy of *P. johnsoni*. In addition, Bayer (1964) who examined sclerite morphologies of two *Paragorgia* species, indicates that *P. dendroides* is a junior synonymy of *P. regalis*. *Paragorgia antilla*, however, has not been reviewed since Bayer (1964), but is catalogued (numbers 56994, 50913) by the Smithsonian Institute.

Molecular studies have addressed the phylogenetic relationships of the cold water ahermatypic corals using 18S rDNA (Berntson et al., 1999, 2001), 16S rRNA and large subunit rRNA (Sanchez et al., 2003), mitochondrial loci (Wirshing et al., 2005), rDNA (Waller et al., 2007), and inter-transcribed spacer region (ITS), 28S rDNA, and COI (Herrera et al., 2010). Using nuclear markers, Berntson et al. (1999, 2001) suggest that there are three major subclasses: (1) Octocorallia, (2) Hexacorallia, and (3) Ceriantipatharia. However, Bayer (1992) and Sanchez et al. (2001, 2003, 2005) provide evidence that the Families Paragorgiidae and Coralliidae may not be monophyletic. Herrera et al. (2010) examined *Paragorgia*, the Coralliidae, and a group of undescribed specimens resembling *Sibogagorgia* and they suggest that their data support the monophyly of *Paragorgia*, Coralliidae, and *Sibogagorgia*. None of these studies, however, have specifically attempted to discern the interspecific morphological relationships within *Paragorgia* using molecular markers.

In previous studies, Strychar et al. (2005) have reported on the small-subunit 18S rDNA sequence variation from six species of Anthozoa, including *Paragorgia* sp. from the Canadian Atlantic, Pacific and the Bahamas. Initial results from that study showed high levels of sequence divergence between geographically distant *Paragorgia* taxa. Specimens of *P. arborea* from the Canadian Atlantic were very divergent from a Canadian Pacific specimen. In this paper, different levels of DNA sequence variation seen among 29 additional samples of *Paragorgia* taxa collected from three different localities and depths ranging from 186 to 630 m along Canada's eastern coast were studied. Data collected from this study and previously published sequences were used to independently test the current taxonomic status of the *Paragorgia* taxa.

2. Materials and methods

2.1 Specimens

Cold-water coral (CWC) were sequenced using a portion of the 18S rDNA from 29 specimens of *P. arborea* collected from three different locations (Figure 2) which included the Northeast Channel (NEC; ~42° N, 65° W), the Gully ("G"; ~43° N, 58° W) and the Stone Fence (SF;~44° N, 56°W); these specimens were collected at depths from 186 to 630 m (Table 2). All samples used were preserved in ethanol, however, samples provided by the Smithsonian Institute had been stored as dry specimens for periods ranging from 2 to 60 years and subsequently, were suspended in 90% ethanol. Fresh specimens used for this project were either collected from remote operated vehicles (ROVs) or fishing practices that include bottom dragging and/or long-line fishing. All

specimens collected were frozen at -20°C until subsampled into 90% ethanol. Sclerite morphology was examined in all 29 of the putative *P. arborea* specimens and showed the typical morphology of that species.

2.2 DNA extraction and polymerase chain reaction (PCR) amplification protocol

Phosphate buffered saline solution (PBS; Fisher Scientific, USA) was used to wash the sample and eliminate ethanol and surface contaminants. DNA was obtained by dissecting ~100 mg of tissue and extracting the DNA using a DNeasy tissue kit (Qiagen, Canada) according to the manufacturer's protocol.

A modified nested-PCR approach following Berntson et al. (1999, 2001) was used to amplify CWC 18S rDNA. Two overlapping PCR products (primers A1 and B1; Table 3) were used to amplify the 18S region of interest. Amplification consisted of 1 nmole of A1 or B1 primers and a corresponding internal oligonucleotide primer (primers A2-5, B2-5; Table 3), 200 nmoles of each dNTP, 1 unit of *Taq* DNA polymerase (MBI Fermentas, Canada), and ~50 to 100 ng of DNA template, in a final volume of 50 µl. Specimens, including archival samples (i.e. from the Smithsonian Institute), that yielded no visible PCR product were amplified *via* a second PCR using 0.5 to 5 µl from the initial PCR using universal eukaryotic coral primers A1 and B1. These samples were then purified and used as template for further reactions using our constructed internal primers (A2-A5, B2-B5; Table 3); ~1 µl of negative control from PCR methods that did not result in a visible product initially were used as template for a second negative PCR control. Thirty-nine cycles of PCR amplification using a MJ Dyad (MJ Research, USA) consisted of denaturation at 94°C for 60 s, annealing at 57°C for 30 s, and extension at 72°C for 60 s, followed by one cycle with a 10 min extension at 72°C. All PCR products were purified using a QIAquick PCR Purification (Qiagen) kit according to the manufacturer's protocol. To determine the molecular size and quality of the PCR products, all samples were run on 1% agarose gels and stained with ethidium bromide or SYBR safe (Invitrogen) and visualized on a Gene Genius (Syngene, UK).

2.3 Sequencing of amplified 18S rDNAs

Eight to ten 18S rDNA-specific primers were used in the sequencing reactions (Table 3). Approximately 40-80 ng of the PCR amplicons (quantity determined using PicoGreen©; Invitrogen-Molecular Probes, Canada) were sequenced using the BigDye Terminator Cycling Sequencing Ready Reaction Kit v 3.1 (Applied Biosystems, USA) following the manufacturer's recommendations. Sequencing reaction products were size fractionated on a BaseStation (MJ Research, USA) using 6% acrylamide (FragPack, MJ Research, USA) and 20 cm plates or 5% acrylamide (BasePack, MJ Research, USA) and 30 cm plates. The electropherograms were visualized using Cartographer software (MJ Research, USA).

2.4 Phylogenetic analysis

In addition to the 29 samples we collected for sequencing, three other *Paragorgia* samples from distant locations (e.g. the Bahamas - *Paragorgia johnsoni),* provided by the Smithsonian Institute, were also sequenced. Twelve additional sequences including out-groups were acquired from GenBank (Table 2).

Assembly and evolutionary analysis of the sequenced amplicons was done using GeneStudio Pro©. All sequences used in this project were compared against the sequences in the National Centre for Biotechnology Information (NCBI) GenBank site using the BLAST function in GeneStudio Pro© and matching homologous 18S rDNA sequences were retained for multiple alignment. A data set of 44 sequences with a length of 1,634 nucleotides (including indels) was assembled using aligned nucleotides with CLUSTAL W (Thompson et al., 1994) and Clustal X2 (Larkin et al., 2007). Using Mega 4.0.2 (Tamura et al., 2007), pair-wise sequence differences (p-distance) among sequences were computed, and phylogenetic trees using maximum likelihood (ML), minimum evolution (ME), and Bayesian likelihood analysis (BL) were constructed. Different tree-forming methods were used to reduce the likelihood of poor tree representation given the fact that each method is designed on the basis of different evolutionary theories and different algorithms (Steel and Penny, 2000). Maximum likelihood analysis was carried out using PAUP (Phylogenetic Analysis Using Parsimony, version 4.0b10; Swofford, 2002). In ML methods, a search for the topology that maximizes the chance of observing a given probabilistic model of nucleotide substitutions was used. Data sets comprised of more than seven sequences are computationally demanding and time-consuming and as a result, a quartet-puzzling algorithm (PHYLIP; Felsenstein, 1990) using the bootstrap function set to one million and a 50% majority-rule consensus was used. A best-fit ME model using p-distance sequence divergence was performed using MEGA (Molecular Evolutionary Genetics Analysis V2.1). Minimum evolution analysis, unlike ML methods, assumes the shortest evolutionary route. One million bootstrap replications were used to test relative nodal support in the topology. Mr Bayes Version 3.0b4 (Huelsenbeck and Ronquist, 2001) was used for bayesian analysis utilizing the gamma function, "4-by-4" model, sampling one million generations, with Markov chains sampled every 100 iterations. The analysis was conducted three times using identical settings to ensure convergence on the same l nL values

(Huelsenbeck and Bollback, 2001). The number of random iterations generated prior to stationarity (20,000; called burn-in generations) were discarded and the subsequent generations used to establish the posterior probabilities. Bayesian trees using the software program BEAST 1.5.0 (Drummond and Rambaut, 2007) were also constructed for comparative analysis. In the program BEAST, we applied a relaxed clock uncorrelated lognorm, and the best-fit model of nucleotide substitution supported by the Akaike Information Criterion (AIC; Akaike, 1974) in jModeltest 0.1.1 (Posada, 2008). The MCMC chain length was set to 100 million generations with sampling every 1,000 links, and the first 10% of the dataset was discarded as burn-in. TreeView version 1.6.0 (Page, 1996) was used to display all phylogenetic trees. The out-group *Beroe cucumis* (Table 2) was used as previous studies have shown it to reliably root coral phylogenies (Berntson et al., 2001).

3. Results

3.1 Northeast Channel

All *P. arborea* specimens sequenced from this location were closely related as they appear to group together (Figures 3-5). In particular, samples N-1a,b (246 m, 249 m respectively) were very comparable (100% nodal support; Figure 5) *vs.* N-1c (250 m), which appears to be more divergent and related to samples N-3b,c at depths 509 m and 542 m, respectively (Figure 4 nodal support = 95%). Two specimens from 262-380 m at this location also appear relatively comparable (N-2a,b; Figure 5 – nodal support 99%) to samples obtained from deeper depths (e.g. N-3a, 100% node support, Figure 5). In addition, at 262-456 m a mixture of samples from the Northeast Channel with specimens from shallower depths (~186 m) from the Stone Fence and from distant locations (e.g. Bahamas and Bermuda; Figures 4,5) were observed.

3.2 The Gully

P. arborea from the Gully at shallow depths (G-1 a-c; Figure 5 nodal support = 85%) appear related (convergent). As depth increased in the Gully, greater diversity of *P. arborea* mixing with specimens from other locations was observed. For example, two specimens (G-2a and G-2b; Figure 5) were related (nodal support = 74%) at depths 472 m. One sample (G-2c; Figure 5), however, was more comparable to species from the Northeast Channel and the Stone Fence (nodal support = 95%; Figure 5). As depth increased (≥556 m; Figure 5) each specimen sampled from the Atlantic appeared divergent: G-3a was more related to *P. arborea* from the Stone Fence (nodal support = 99%; Figure 5), G-3 b was comparable to samples from the Pacific (99% nodal support; Figure 5), whilst G-3c appears closely related to species belonging to the Family Acanthogorgiidae (> 74% nodal support; Figures 3-5).

3.3 Stone Fence

Specimens of *P. arborea* sampled from the Stone Fence at shallow depths (186 m; S-1 a-c) from Atlantic Canada show the greatest diversity of species "mixing" with samples from various locations (e.g. Bermuda, Canadian-Pacific; Figure 5) and depths. At deeper depths (262-332 m), a high degree of divergence of S-2 a-c (Figures 3-5) was observed. One sample (S-2b; node support > 60%; Figures 3, 5), however, was closely related to samples from the Gully, sample S-2a (node support > 70%; Figures 3, 5) was more comparable to samples from the Northeast Channel, and S-2c (Figure 5) appeared closely associated with *Anthomastus* sp. (92% nodal support; Figure 5) and *P. arborea* from the Gully (99% nodal support; Figure 5). At deeper depths (>588-630 m) all three samples (S-3 a-c) were found to be closely associated with samples taken from shallower depths (< 472 m) from the Gully (Figure 4).

3.4 Generalizations

Specimens of the geographically distant *Paragorgia* taxa show an interesting picture from the North Atlantic and Pacific compared to the Bermuda (denoted as Bahamas-1; Table 2) and Bahamas (labeled as Bahamas-2; Table 2) samples (see Figures 3-5). *P. arborea* from the North Pacific (British Columbia, Canada) are related to *P. johnsoni* from Bermuda (> 89% nodal support; Figures 4, 5). *P. arborea* from the North Atlantic (Nova Scotia, Canada) however, appear divided. Specimens found in deeper waters, for example in the Gully (472-556 m; Figure 4) and the Stone Fence (262-630 m; Figure 4), appear more related to *P. johnsoni* from Bermuda (node support = Figure 3 - 81%; Figure 4. - 60%; Figure 5 - 93%). In shallower waters (< 250 m), *P. arborea* from Atlantic Canada collected from the Northeast Channel and the Stone Fence (Figure 3 – 99%; Figure 4 – 86%; Figure 5 – 100%) were more closely related to the Bahamas specimen. The similarity of one sample of *P. arborea* (G-3c; Figures 3-5 and G-3b; Figure 4) relative to *Chrysogorgia chryseis* and *Acanthorgorgia* sp., both from Hawaii (100% node support; Figure 5), and *Siphonogorgia* sp. from Palua (node support = 99%; Figure 3 and 74%; Figure 4) was unexpected. Similarily, the placement of *Anthomastus* sp. relative to these taxa was also unanticipated (Figures 3-5). *Anthomastus* sp. appears closely related to *P. arborea* from the Stone Fence at

depths ranging from 262-332 m and the Gully at ≥ 556 m (nodal support = 75%; Figure 3; 51%; Figure 4 and 92%; Figure).

Generally, samples from Atlantic Canada appear more related to each other (Figures 4, 5) compared to other anthozoans collected from various sites found globally (see Table 2). Base-pair differences observed on the 18S molecule were scattered and did not occur in a particular area or within loop structures. Maximum sequence divergence within *Paragorgia* sp. measured 0.428% overall taxa. Among the *Paragorgia* samples, there were minimal nucleotide differences (i.e. two to three base pair differences) with a consensus of 1.07 (± 0.03 standard error) transition/transversion ratio. The greatest sequence divergence was between families considering they possessed the largest genetic distances and the lowest percent identities.

4. Discussion

Phylogenetic analyses of nuclear 18S rRNA show that specimens of *P. arborea*, using the ME (minimum evolution) and ML (maximum likelihood) methods, both produce two groups. One includes *Paragorgia* samples from Northeast Channel, Stone Fence, and Gully at all depths. The other includes *Paragorgia* samples from only the Stone Fence and Northeast Channel. In this group the Northeast Channel samples are from all depths but only the shallow group is included; in the ML tree, Bahamas-2 is added to this group. This would seem as if there are two "species" as other very distant taxa separate the samples. The BL (bayesian likelihood) method, however, gives a tree which is more consistent with current taxonomic concepts. The two groups are still maintained but they join together and include other *Paragorgia* taxa before linking with other genera (except for the *Anthomastus* sample). The division of *Paragorgia* samples into two different topologies are related to the treeing algorithms. Minimum evolution trees are estimated using the least-squares criterion in which a tree with the smallest sum of branch lengths with negative branch lengths, are disallowed. However, ML trees generally use all sequenced positions to determine branch lengths that have the highest probability of evolving a particular phylogeny (Felsenstein, 1981). In this study, more than seven sequences were used, hence, ML analyses would have been computationally demanding and time consuming and as a result, we used the quartet-puzzling algorithm. It is plausible that the 50% majority rule imposed relatively short evolutionary routes, which are consistent with ME analyses, and consequently produced comparable homologies.

The BL method is a much faster inference of phylogeny in which branch support is expressed as posterior probabilities, and when combined with bootstrap re-sampling procedures, Douady et al. (2003) suggests "very strong correlations" exists with traditional ML methods using bootstrapped maximum likelihood percentages. In this study, the BL method used was designed to be computationally demanding and analogous to the ML method, but without the time constraint. We believe this method produced the most robust phylogenetic model of evolutionary lineages that would be comparable to traditional computationally intensive ML analyses indicating two groups of *Paragorgia* exist, but shortly, in terms of evolution, two new species will likely result.

Upon examination of six CWC samples including three Paragorgia specimens from the North Pacific, Atlantic (Canada), and from Bermuda, Strychar et al. (2005) suggest that those samples from geographically different regions appear closely related. Attempts to explain why deep cold-water *Paragorgia* isolates from geographically different regions and depths appear closely related are hampered by a lack of knowledge of transmission of larvae from one region to another. It is possible that the isolation of distinct deep CWC genes here, and the classification of such corals into clades and subclades described by Berntson et al. (2001, 2002) is related to hydrography and circulation patterns along the Nova Scotian shelf (see Han et al., 1997; Townsend et al., 2004) and global ocean circulation patterns (Benzie, 1999). Along the Scotian Shelf (Figure 6), it becomes conceivable that recruitment of larvae may be transported between geographically different regions across the continental shelf (Davis et al., 1998) and possibly, circulation patterns of the Gulf Stream connect southern deep CWC species. The primary criticism of this hypothesis is that recruitment of *Paragorgia* larvae have not been described, even though gametes have been observed (pers. observ.). Further, contemporary currents shaping distribution patterns of species today are probably not operative as they were 100 years ago when samples used in this study, would have recruited.

There are ~2000 known octocoral species, of which knowledge regarding their reproductive strategies (i.e. production of gametes and sperm) is only known in a few dozen. Asexual reproduction in these species occur *via* simple fission (Benayahu and Loya, 1985), partial mortality and separation of a colony (Farrant, 1985), survival and reattachment of fragmented colonies (Lasker, 1984), stolon and runner development from a parent colony (Dineson, 1985), fragmented colonial autotomy (Dahan and Benayahu, 1997) and parthenogenesis *via* planula development (Brazeau and Lasker, 1989). Two authors, Mullineaux and Mills (2004), suggested that reproduction of CWC is likely through budding *via* breakage and settling near a parent colony, indicating that

many colonies of local distance are genetic clones. In addition, even though sexual reproduction of CWC has not been reported, oocytes have been observed in CWC bamboo corals (Family Isididae; pers. observ.). The presence of oocytes indicates the CWC may be broadcast spawners and/or brooders. Reports by Coma et al. (1995) suggest that brooding may be more common with CWC species. In other coral species, Coll et al. (1995) suggests that broadcast spawning is most common with Alcyonaceans and brooding occurs most commonly in gorgonian corals. Furthermore, vitellogenesis of mature oocytes in several unidentified species of bamboo corals exist, suggesting that larvae transportation *via* deep-water currents is possible.

Reproductive environmental cues are also well documented in shallow tropical corals and include, but are not limited to synchronized monthly gamete releases (Coma et al., 1995), lunar cycles (Kruger et al., 1998), water temperature (Dahan and Benayahu, 1997), tidal cycle (Alino and Coll, 1989), food delivery (Brito et al., 1997) and a variety of genetic factors. In the Family Paragorgiidae (order Alcyonacea) gonads have been observed in both autozooids and siphonozooids (Bayer, 1973) and although the time of release (spawning) remains unknown, we speculate that it coincides with nutrient upwelling and/or downwelling of increased production of particulate matter. Within temperate water, Lawson (1991) suggests that gorgonian-type alcyonaceans (*e.g. Acanella arbuscula*) have a seasonal reproductive cycle, evidenced by seasonal gametogenetic development. Hartnoll (1975) also suggests that seasonal gametogenesis in some octocorals is strongly correlated with periods of food availability. These authors observed gamete development in the temperate-water alcyonacean *Alcyonium digitatum* during early spring and summer plankton blooms. Similarly, Zeevi Ben-Yosef and Benayahu (1999) reported that reproduction of the Red Sea gorgonian *Acabaria biserialis* only occurs after a major seasonal phytoplankton bloom.

The placement of *Anthomastus* sp. relative to *Paragorgia* sp. was unexpected (Figures 3-5), however, Berntson et al. (2001) suggest that these taxa share many morphological features and are dimorphic. Furthermore, given that one sample of *P. arborea* from geographically distant regions and depths along the Scotian shelf (Canada) appeared related to *Chrysogorgia chryseis* and *Acanthorgorgia* sp., both from Hawaii (100% node support; Figure 5), and *Siphonogorgia* sp. from Palua (node support = 99%; Figure 3 and 74%; Figure 4), we question the level of genetic similarity (ME = 58%, ML = <50%, BL = 64%; Figures 3-5). We propose several possible reasons that may explain some of the disparities between the topology of these taxa. In this study we used octocoral specific designed primers for *Paragorgia* sp. which may not consistently amplify with the same efficiency across different taxa; this explanation would be true if failure to amplify consistently occurred, however, we observed PCR product and were able to sequence that product. Such biases inherent to natural mixes of rRNA genes are in the early stages of being investigated (see von Wintzingerode et al., 1997). Gene templates with high G+C content, for example, are not as efficiently amplified as are those with low G+C content (Reysenbach et al., 1992). Moon-van der Staay et al., (2000) also suggest that non-complementarity of either PCR primer may decrease PCR yield through inhibition of binding targets during high annealing temperatures. Further Hansen et al. (1998) suggest that some amplified sequences may be inhibited by DNA flanking the template region. The presence of excess templates during re-annealing may also reduce amplification of templates initially present in high abundance (Suzuki and Giovannoni, 1996). In contrast, Moon-van der Staay et al., (2000) suggest that templates with G/C content, when amplified with degenerated primers, amplify better than those with A/T content. As a result primer specificity for some copies of the 18S rDNA may be responsible for the unusual results of such phylogenetic analyses. Another explanation may be that the gene may not be divergent enough to use for intraspecific work. Even internal transcribed spacer regions may be too conserved for this type of work (Barth et al., 2006). Future studies should perhaps include the mitochondrial cytochrome *c* oxidase (COI) gene since sequence discrimination amongst all animal phyla is reflected by the high rates of sequence change in this gene (COI) and intraspecific constraints on mitochondrial DNA divergence are due, in part, to interactions with the nuclear genome (Hebert et al., 2003). Lastly, PCR error rates may also account for some of the differences. *Taq* polymerase has the highest error rates of all known thermostable DNA polymerases (Ling et al., 1991). Further drawbacks associated with *Taq* polymerase are that the errors are biased towards A•T → G•C changes (Keohavong and Thilly, 1989) as was observed in this study; similar mutation bias has been reported by Kobayashi et al. (1998). In this study, the calculated error rate (5.7×10^{-5} / site / duplication) was computed using an equation given by Hayes (1965; 2 × observed error number / total length of DNA examined / effective number of duplications) and is consistent with reports by Flaman et al. (1994) and Cline et al. (1996). Kobayashi et al. (1999) suggest that the fidelity of PCR can be increased substantially by adding *Pfu* to *Taq* during the PCR reaction.

4.1 Northeast Channel

In this study, some *Paragorgia* specimens from the Northeast Channel appear closely related, probably due to

good mixing between surface and deep ocean waters. This is supported by Drinkwater et al. (1997) who reported that the strong tidal currents (as high as 3 to 5 m) and formation of strong eddies, which last from a few days to over one year and extend as deep as 1000 m, result in a nearly homogenous top to bottom water column regardless of season.

Paragorgia samples that appear related to the Stone Fence and from distant locations are likely the result of larval transport due to strong bottom ocean currents. Generally, circulation patterns along the Nova Scotian shelf appear to only flow along a southwest direction (i.e. flowing from the Stone Fence toward the Northeast Channel; Figure 5). A better perception of larval transport, and hence genetic diversity among geographically isolated populations, however, is to consider deep-ocean circulation patterns. Han et al. (1997) shows Gulf Stream ocean circulation patterns moving toward the North and Northwest. Such evidence implies that *Paragorgia* sp. mixing may occur from the Northeast Channel toward the Stone Fence, which lends support to observations in this study.

4.2 The Gully

The appearance of genetically comparable samples of *Paragorgia* at this location to other locations along the Scotian shelf is likely related to larval dispersal during summer months. Particle trajectories (Lacey, 2007a) indicate that >65% of the water at the Gully travels Southwest toward the Northeast Channel. It is not surprising that some similarities exist between *Paragorgia* sp. at this location since ~16% of the water forms eddies whilst 16% of the water travels west toward the shelf. In deeper water, similar trajectories are observed, however, ~16% of the water travels northwest toward the Stone Fence as opposed to the Southwest as is observed in shallow water. We speculate that larval dispersal is unlikely to occur during the winter months since particle trajectories near the surface and in deeper water indicate that ~83% of the water eddies near the mouth of the Gully and/or travels toward the shelf with the remainder of the water mass eventually exiting and traveling Southwest toward the Northeast Channel; little or no water appears to travel from the Gully toward the Stone Fence during the winter season (Lacey, 2007b).

4.3 Stone Fence

Paragorgia sp. found at the Stone Fence are well clustered with other specimens at this location, from other locations along the Scotian Shelf, and amongst specimens found globally (i.e. Bermuda and the Bahamas). This result was not unexpected since the Stone Fence is a habitat found within a much larger inlet and river system called the Laurentian Channel (Figure 2). Further, seasonal water circulation patterns vary significantly to include tremendous mixing during the winter (Loder et al., 1993) *via* gyres and eddies, enormous amounts of water flushing/exchange (as much as 700,000 m^3/s; see Townsend et al., 2004) of the Channel during the spring to a predominately south-westerly flow of water out of the Channel. In addition, water that travels along the Scotian Shelf connects to the Gulf Stream (Figure 2) helping to promote further mixing between samples found in Canada with samples found amongst other, seemingly remote geographical locations (Davis et al., 1998).

5. Conclusion

Summarizing our data, this study demonstrates for the first time that *Paragorgia* sp. from eastern and western Canada, although appearing related in morphology, are genetically divergent. However, Sanchez (2005) suggests that two very comparable morphometric characteristics be used to subsume separate species (e.g. *P. nodosa.* and *P. pacific* into *P. arborea*) into one. This presumption is flawed since it is likely that these corals are displaying convergent evolution – that is, distantly related species evolving similar appearances due to comparable ecological niches. The evidence, then, suggests that molecular studies be used to address phylogenetic relationships between CWCs; even Verrill (1922) suggested that gross morphology is an inaccurate methodology for cryptic taxa. Further, other genera appear more closely related to *Paragorgia* then previously observed. The relatedness observed in this study is believed to be accurate since gene flow between organisms has been previously established in other coral studies (Ayre and Hughes, 2000; 2004) and over long distances (e.g. 4000 km, see Hellberg, 2004; and 500-1200 km, Fuchs et al., 2006). Hence, it is plausible that ocean currents are responsible for mixing and transmission of larvae thereby mediating gene flow between geographically separated species found along the Scotian Shelf. This new light potentially provides a novel assessment of the relative taxonomic distribution and abundance of the various groups of deep CWCs, associated fauna, and cryptic taxa and to their morphologies. It may also reveal additional lineages that may have considerable ecological and/or taxonomic importance.

Acknowledgments

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Table 1. Summary of morphological characters and the depth and locations of 19 *Paragorgia* species. Characters are defined as branch width, coral (coenenchyme) color, type and size of sclerites, and presence or absence of autozoids. Condition / Position are the descriptive trait(s) associated with each character. Differences in character types (e.g. size) are numbered, and are subsequently used to delineate between particular *Paragorgia* species. Note: "-" represents an unknown morphological characteristic; reference based on Smithsonian Institute's catalogue of species

Characters	Condition / Position	*Paragorgia*																		
		arborea	*antilla*	*johnsoni*	*splendens*	*coralloides*	*regalis*	*alisonae*	*kaupeka*	*maunga*	*aotearoa*	*yutlinux*	*stephencairnsi*	*wahine*	*whero*	*tapachtli*	*nodosa*	*pacifica*	*boschmai*	*dendroides*
Terminal branch width	(1) < 4 mm, (2) > 5 mm	2	-	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1
Coenenchyme color	(1) colorless, (2) pink, (3) red, (4) colorless with dark purple autozooids	3	-	3	2	2	2	3	2	3	3	4	4	2	2	2	3	3	3	2
Sclerite surface form	(1)Symmetrical, (2) Asymmetrical	1	-	1	1	2	2	1	2	2	1	1	1	1	1	2	1	1	1	2
Sclerite surface rays	(1) Globular and fused, (2) smooth (unfused), (3) grooved, (4) lobulated, (5) > 5 lobes	3	-	2	5	1	3	5	3	3	2	4	4	5	5	1	3	3	2	3
Avg. Sclerite surface length (mm)	(1) 0-0.025, (2) 0.026-0.05, (3) 0.051-0.075, (4) 0.076-0.1	2	-	3	3	2	3	4	2	3	4	2	3	3	4	2	2	2	3	3
Avg. Sclerite surface width (mm)	(1) 0-0.025, (2) 0.026-0.05, (3) 0.051-0.075	2	-	2	2	2	2	3	2	2	2	2	2	2	3	2	2	2	2	2
Dominant surface sclerite ornamentation	(1) 6-radiates, (2) 7-radiates, (3) 8-radiates	1	-	3	3	3	2	3	2	3	3	1	2	3	3	3	1	1	3	2
Medulla spindles	(1) Smooth, (2) Ornate	2	-	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Autozooid tentacle sclerites	(0) Absent, (1) Ornate	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Oceans	(1) North Pacific, (2) South Pacific, (3) North Atlantic, (4) South Atlantic, (5) Indian, (6) Southern, (7) Artic	1-6	3	3	1, 5	5	1	2	2	2	2	1	1	2	2	1	1-6	1-6	3	1
Depths (m)	(1) 18-500, (2) 501-1000, (3) 1001-1500, (4) 1501-2000, (5) >2001	1-4	2	2-5	2	2	1-3	2-3	2	2-3	2	2	1	2	2	4	1-4	2	2	2

Table 2. Accession numbers of 18S rDNA gene sequences of deep cold-water taxa used in this study begin with the prefix "HM437". All sequences consist of >1650 nucleotides. Accession numbers are related to National Centre for Biotechnology Information (NCBI) coding. *Paragorgia* sp.* represents a "control" gene from Berntson *et al.,* (1999, 2001); *Beroe cucumis* (GenBank Accession # D15068) represents an out-group used for tree formation. Deep cold-water samples are from Atlantic Canada unless otherwise mentioned. Symbols B = Bermuda; CA = Canadian Atlantic; CP = Canadian Pacific; G = the Gully; N = Northeast Channel; S = Stone Fence (see Figure 1); "not. cat." = not catalogued. Numbers "1" = 186 to 250 m, "2" = 262 to 472 m, "3" = 509-630 m; letters "a, b, c" correspond to number of replicates at each depth

Accession number	Scientific name	Strain	Origin	Depth range (m)	Actual Depth (m)	Nucleotides (bp)	Citation
	Phylum Ctenophora						
D15068	*Beroe cucumis*	not. cat.	not. cat.	not. cat.	not. cat.	1676	Kobayashi *et al., *
	Phylum Cnidaira						
	Class Hydrozoa						
	Family Hormathiidae						
AF052890	Hormathiidae gen. sp.	AD2294-2	Fieberling Guyot	not. cat.	640	1800	Berntson *et al.,* 1999
	Order Alcyonacea						
	Family Alcyoniidae						
AY838564	*Anthomastus* sp. (CA)	SeqM-2	G-3	> 500	not. cat.	1792	Strychar *et al.,* 2005
	Family Nidaliidae						
AF052927	*Siphonogorgia* sp.	58584	Augulpelu Island, Palau	not. cat.	110 ft	1823	Berntson *et al.,* 2001
	Suborder Scleraxonia						
AF052922	*Anthothela nuttingi*	CR106-1	Cross Seamount, Hawaii	not. cat.	1,010	1822	Berntson *et al.,* 2001
	Family Paragorgiidae						
AF052917	*Paragorgia* sp.*	AD2301-1	Fieberling Guyot	not. cat.	490	1799	Berntson *et al.,* 2001
AY838559	*P. arborea* (CP)	Seq7-2	Can-Pacific	18 – 250	265	1800	Strychar *et al.,* 2005
AY838562	*P. johnsoni* (B)	Seq9-2	Bahamas-1	not. cat.	not. cat.	1734	Strychar *et al.,* 2005
AY838563	*P. arborea* (CA)	SeqJ-2	Can-Atlantic	18 – 250	18 – 250	1802	Strychar *et al.,* 2005
	P. arborea	Par-12	Bahamas-2	not. cat.	not. cat.	1798	HM437199
	P. arborea	G-1	S-1a	186 – 250	186	1738	HM437174
	P. arborea	F-1	S-1b	186 – 250	186	1743	HM437175
	P. arborea	Par-1	S-1c	186 – 250	186	1798	HM437200
	P. arborea	Seq J-2	S-2a	262 – 472	262	1688	HM437201
	P. arborea	I-1	S-2b	262 – 472	321	1696	HM437176
	P. arborea	K-2	S-2c	262 – 472	332	1736	HM437177
	P. arborea	E-1	S-3a	509 – 630	630	1688	HM437178
	P. arborea	E-2	S-3b	509 – 630	588	1688	HM437179
	P. arborea	F-2	S-3c	509 – 630	588	1688	HM437180
	P. arborea	J-1	G-1a	186 – 250	236	1692	HM437181
	P. arborea	H-2	G-1b	186 – 250	236	1688	HM437182
	P. arborea	J-2	G-1c	186 – 250	236	1694	HM437183
	P. arborea	I-2	G-2a	262 – 472	472	1740	HM437184
	P. arborea	I-3	G-2b	262 – 472	472	1689	HM437185
	P. arborea	D-1	G-2c	262 – 472	472	1690	HM437173
	P. arborea	K-1	G-3a	509 – 630	556	1700	HM437186
	P. arborea	G-2	G-3b	509 – 630	556	1686	HM437187
	P. arborea	H-1	G-3c	509 – 630	556	1711	HM437188
	P. arborea	Par-9	N-1a	186 – 250	246	1798	HM437189
	P. arborea	Par-11	N-1b	186 – 250	249	1798	HM437190
	P. arborea	Par-2	N-1c	186 – 250	250	1798	HM437191
	P. arborea	Par-5	N-2a	262 – 472	262	1798	HM437192

	P. arborea	Par-6	N-2b	262 – 472	380	1798	HM437193
	P. arborea	Par-10	N-2c	262 – 472	411	1798	HM437194
	P. arborea	Par-7	N-2d	262 – 472	456	1802	HM437195
	P. arborea	Par-8	N-3a	509 – 630	573	1798	HM437196
	P. arborea	Par-3	N-3b	509 – 630	509	1798	HM437197
	P. arborea	Par-4	N-3c	509 – 630	542	1798	HM437198
	Family Acanthogorgiidae						
AF052925	*Calcigorgia spiculifera*	not. cat.	Aleutian Islands, Alaska	not. cat.	431	1852	Berntson *et al.,* 2001
AF052907	*Acanthogorgia* sp.	BI104-3	Bishop Seamount, Hawaii	not. cat.	1,295	1852	Berntson *et al.,* 1999
	Family Plexauriidae						
AF052920	*Paramuricea* sp.	CR105-5	Cross Seamount, Hawaii	not. cat.	1145	1850	Berntson *et al.,* 2001
	Family Gorgoniidae						
AF052928	*Leptogorgia chilensis*	not. cat.	Santa Catalina Island, California	not. cat.	15	1823	Berntson *et al.,* 2001
AJ133545	*Acalycigorgia inermis*			not. cat.	not. cat.	1822	Won *et al.,* 2001
	Family Chrysogorgiidae						
AF052913	*Chrysogorgia chryseis*	CR106-2	Cross Seamount, Hawaii	not. cat.	1,010	1811	Berntson *et al.,* 2001

Table 3. Primers used to amplify and sequence 18S rDNA from deep-water fauna. Primers are listed as: "A1-A5" representing forward primers and "B1-B5" representing reverse primers. Primers A1 and B1 were located according to the DNA sequence of the 18S rRNA gene from *Paragorgia* sp. (AF052917; Berntson *et al.,* 2001); the remaining primers were from Strychar et al. (2005). Primer locations (PL) are given with respect to the sequence given in AF052917

Deep-water Fauna 18S rDNA PCR and Sequencing Primers					
Forward Compliment (A1 – A5)		PL		Reverse Compliment (B1 – B5)	PL
A1	AACCTGGTTGATCCTGCCAGT	2-22	B1	TGATCCTTCTGCAGGTTCACCTAC	1776-1799
A2	TGAAACTGCGAATGGCTCAT	81-100	B2	TTTGACCAACTTCTCGGCGG	1715-1734
A3	TAATTCCAGCTCCAATAGCG	582-601	B3	CTGGACCTGGTAAGGTTTCC	1202-1221
A4	ACGATGCCGACTAGGGATG	1031-1050	B4	CCTGCTTTGAACACTCTAATTTCT	759-782
A5	CGTCGTGATGGGAATAGATC	1531-1550	B5	GGGACTTTTCGCATGTATTAGC	163-184
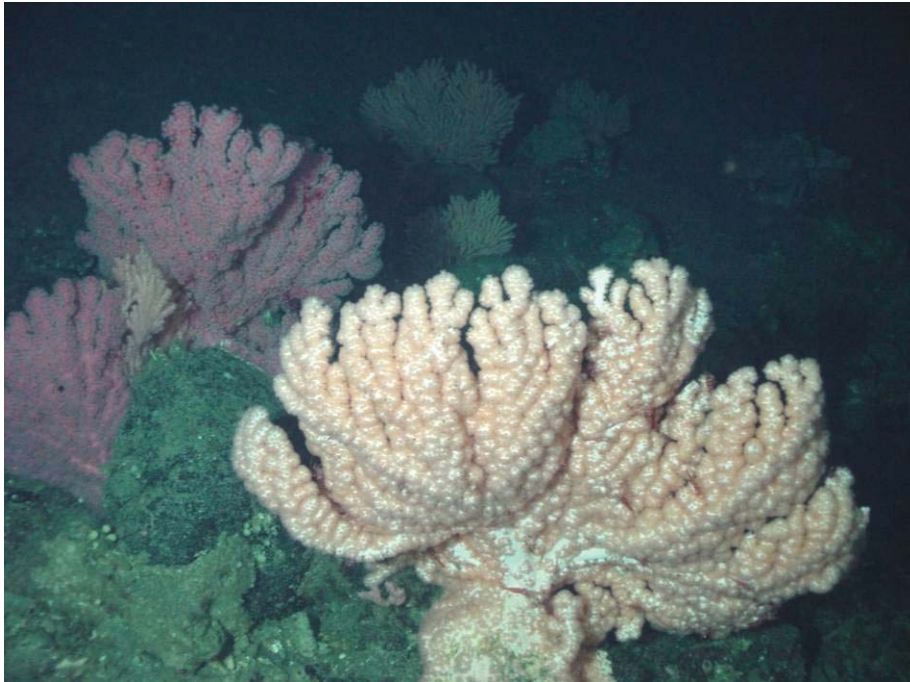
Figure 1. *Paragorgia arborea* photographed during the 2006 Discovery Cruise on CCGS Hudson from the ROPOS submersible at approximately 950 m depth in the Northeast Channel
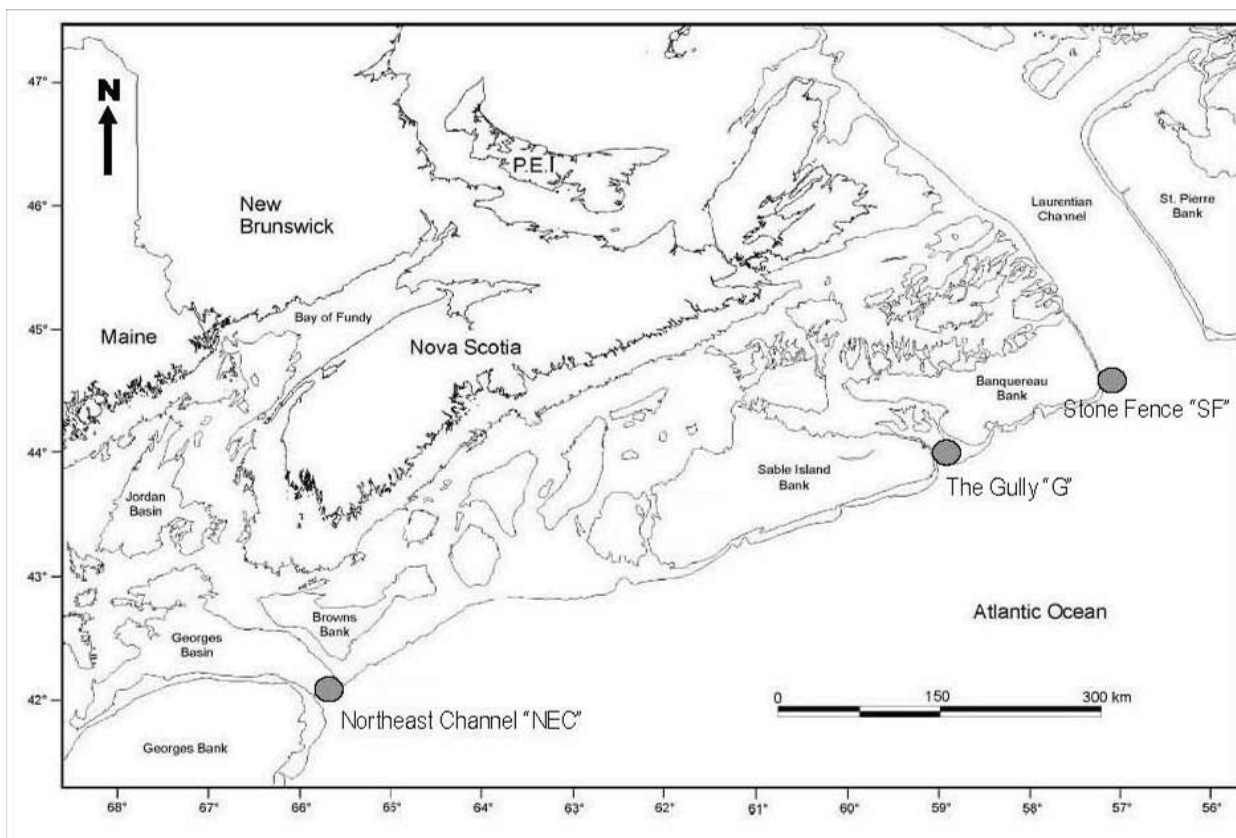
Figure 2. Map of Nova Scotia (Canada) showing sample locations of deep cold-water corals (CWCs)

Figure 3. Phylogenetic tree from minimum-evolution (ME) analysis. Bootstrap analysis (n = 1,000,000 replicates) provided an estimate of support for internal nodes ranging from 50 to 100% (on top of corresponding branch; values <50% are not shown). The number in brackets indicates substitutions per nucleotide position (values <0.001 are not shown). *Paragorgia* sp.* represents "control" gene from Berntson *et al.,* (1999, 2001); out-group used for tree construction was *Beroe cucumis* (D15068; Berntson *et al.,* 2001). Symbols: Bahamas-1 = sample collected from Bermuda; Bahamas-2 = specific site of location unknown, but region is Bahamas; CA = Canadian Atlantic; CP = Canadian Pacific; G = the Gully; N = Northeast Channel; S = Stone Fence. Numbers "1" = 186-250 m, "2" = 262-472 m, "3" = 509-630 m; letters "a, b, c" correspond to number of replicates at each depth
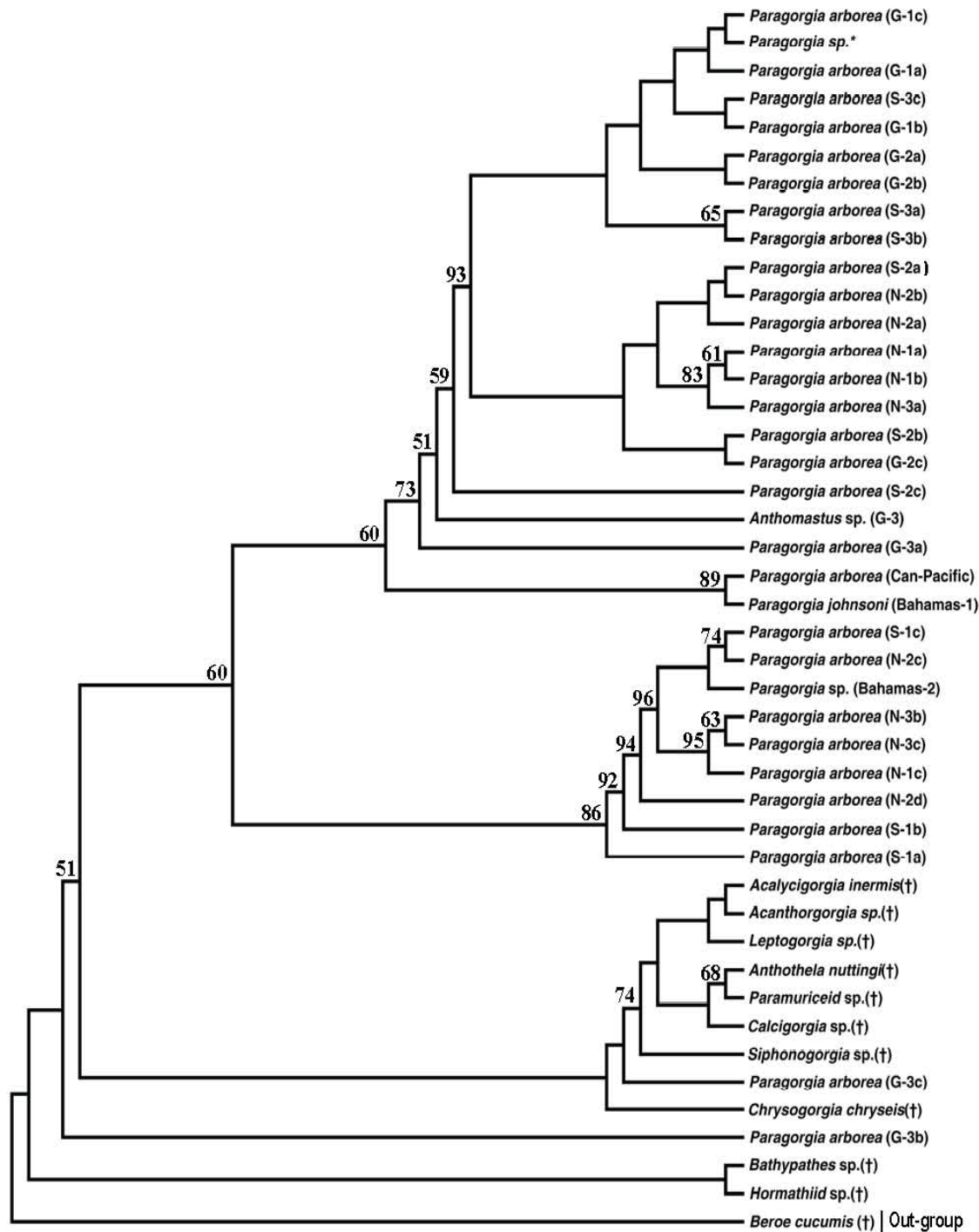
Figure 4. Phylogenetic tree from maximum likelihood (ML) quartet-puzzling analysis using PAUP (v. 4.010b; Swoford 2002). Bootstrap analysis (n = 1,000,000 puzzling steps) provided an estimate of support for internal nodes ranging from 50 to 100 %. Note that branch lengths are arbitrary and only the pattern is important. *Paragorgia* sp.* represents "control" gene from Berntson *et al.,* (1999, 2001); out-group used for tree construction was *Beroe cucumis* (D15068; Berntson *et al.,* 2001). Symbols: Bahamas-1 = sample collected from Bermuda; Bahamas-2 = specific site of location unknown, but region is Bahamas; CA = Canadian Atlantic; CP = Canadian Pacific; G = the Gully; N = Northeast Channel; S = Stone Fence. Numbers "1" = 186-250 m, "2" = 262-472 m, "3" = 509-630 m; letters "a, b, c" correspond to number of replicates at each depth
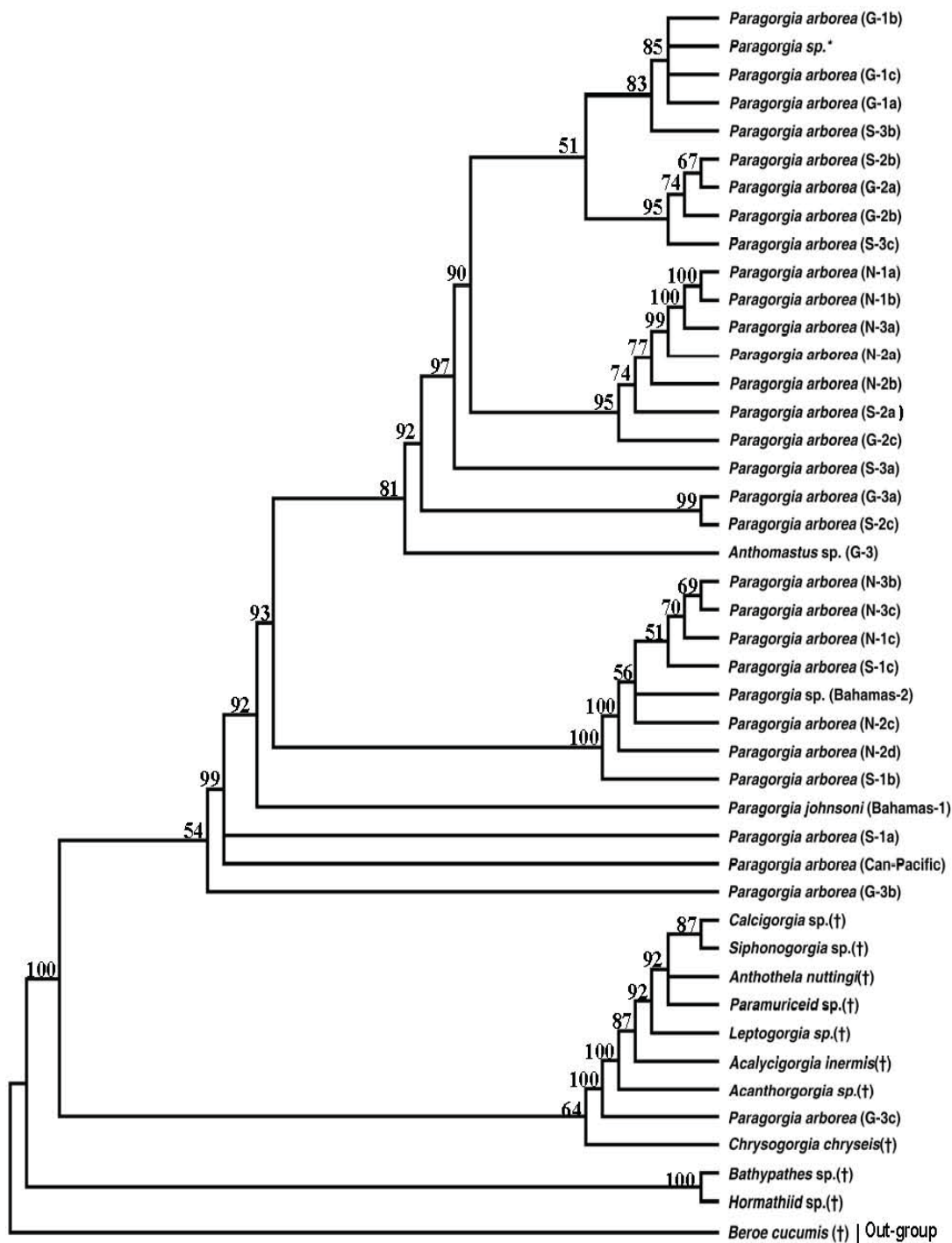
Figure 5. Phylogenetic tree from bayesian-likelihood (BL) analysis using the gamma function, 4-by-4 model, sampling 1,000,000 generations, with Markov chains sampled every 100 iterations. Above node support is the 50 %-majority-rule consensus from the sampled trees. Values <50 % are not displayed. Note that branch lengths are arbitrary and only the pattern is important. *Paragorgia* sp.* represents "control" gene from Berntson *et al.,* (1999, 2001); outgroup used for tree construction was *Beroe cucumis* (D15068; Berntson *et al.,* 2001). Symbols: Bahamas-1 = sample collected from Bermuda; Bahamas-2 = specific site of location unknown, but region is Bahamas; CA = Canadian Atlantic; CP = Canadian Pacific; G = the Gully; N = Northeast Channel; S = Stone Fence. Numbers "1" = 186-250 m, "2" = 262-472 m, "3" = 509-630 m; letters "a, b, c" correspond to number of replicates at each depth
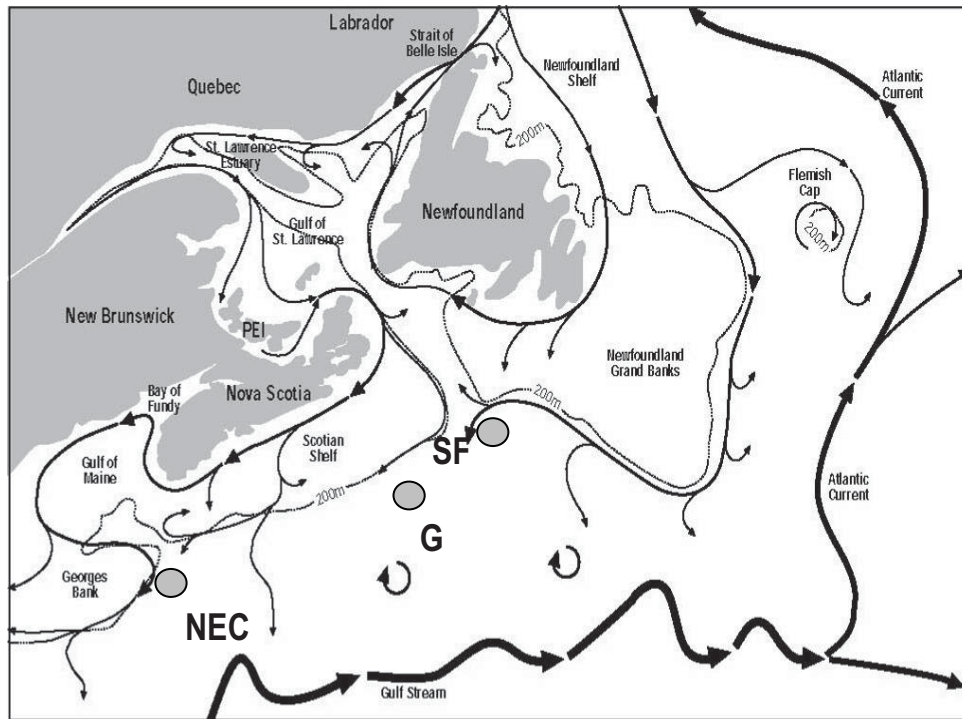
Figure 6. Scotian Shelf ocean circulation patterns along the eastern coast of Nova Scotia, Canada (adapted from Davis *et al.,* 1998). Symbols G = the Gully; NEC = Northeast Channel; SF = Stone Fence