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Gorgoniapolynoe caeciliae (Annelida: Phyllodocida) revisited: previously unknown characters and undescribed species from the Equatorial North Atlantic

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Gorgoniapolynoe caeciliae (Annelida: Phyllodocida) revisited: previously unknown characters and undescribed species from the Equatorial North Atlantic.

Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

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Jamie M. Maxwell

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Resumo

As poliquetas são o grupo mais diverso e abundante do Phylum Annelida. Depois dos nematodes são os metazoários mais difundidos no ambiente marinho bentónico. Devido à sua diversidade e distribuição alargada, formam associações estreitas com outros invertebrados marinhos. A grande maioria das poliquetas simbiontes são monoxênicas, isto é, apenas têm uma espécie hospedeira. Espécies que são heteroxénicas e que têm mais de uma espécie hospedeira, tendem a estar associados a espécies hospedeiras intimamente relacionadas; isso sugere um alto grau de especificidade quando se trata de poliquetas simbiontes. A maioria das poliquetas simbiontes forma relações comensais, existindo cerca e 490 espécies de poliquetas descritas envolvidas em 1229 relações comensais. Os membros da família Polynoidae representam 48,86% de todas as relações comensais. Trinta por cento dessas relações comensais ocorrem com hospedeiros do Phylum Cnidaria e envolvem 279 espécies de poliquetas.

Poliquetas comensais de profundidade associados a hospedeiros de coral são um grupo pouco estudado. Actualmente, existem 84 espécies de corais de profundidade envolvidos em 115 relações comensais com um total de 53 espécies de poliquetas, que representam cinco famílias. É provável que esse número esteja sub-estimado devido a vários factores: a ideia correntemente aceite de que as espécies de poliquetas apresentam uma distribuição global; a tendência para espécies de poliquetas estreitamente relacionadas com um plano corporal conservado; a natureza delicada das poliquetas, o que significa que as amostras geralmente são danificadas ou incompletas; técnicas de amostragem não seletivas e destrutivas; número limitado de taxonomistas especializados em poliquetas.

A aplicação de técnicas moleculares com análise morfológica detalhada revelou que muitas espécies com distribuição global constituem várias espécies distintas. Frequentemente, quando a análise molecular revela uma divergência, uma análise morfológica secundária detalhada revela diferenças subtis que antes eram ignoradas e podem ser usadas como caracteres diagnosticantes para delimitar espécies. Se não houver diferenciação morfológica mas divergência genética, essas novas espécies serão chamadas de espécies crípticas. Há duas razões comuns para que a divergência genética ocorra sem alterações morfológicas. Ou a

espécie utiliza sinais não visuais de acasalamento (químico), e portanto, não há pressão de seleção sobre caracteres morfológicos ou as espécies existem em ambientes que impõem uma seleção estabilizadora na morfologia. Estas explicações não são mutuamente exclusivas. E ambas as razões podem ser aplicadas às poliquetas e explicar o porquê dos limites das espécies poderem não se correlacionar com as algumas das transformações morfológicas.

Neste estudo, a variação morfológica e genética da poliqueta comensal de profundidade *Gorgoniapolynoe caeciliae* foi estudada. Esta poliqueta é encontrada em associação com os corais da ordem Alcyonacea. A taxonomia desta espécies e as suas estratégias de vida indicam que pode-se tratar de um exemplo de especiação críptica. As amostras foram amostrados em quatro picos submarinos do lado Oeste do Atlântico Equatorial Norte, abrangendo uma distância de quase 3000 quilómetros. Um fragmento do gene mitocondrial citocromo oxidase *c* sub-unidade I (*COI*) foi amplificado para 127 amostras. Esses espécimes representam indivíduos de cada local e provenientes de vários hospedeiros (corais da ordem Alcyonacea). Foram igualmente utilizadas sequências de COI provenientes do Genbank de indivíduos do género Gorgoniapolynoe amostrados no sul do Oceano Índico. Realizámos análises de inferência Bayesiana e máxima verosimilhança, bem como testes de delimitação de espécies (AGBD e bPTP). Também foi realizada análise morfológica com caracteres que pudessem ter sido negligenciados anteriormente. Cinco espécimes por localidade foram selecionados para amplificação adicional do gene mitocondrial ribossomal 16S rRNA e dos genes nucleares ribossomais 18S rRNA e 28S rRNA. Estes foram alinhados com sequências de outras espécies de polinóides originárias do GenBank, para inferir a posição filogenética da espécie *Gorgoniapolynoe caeciliae* na família Polynoidae.

A análise morfológica dividiu as amostras em dois grupos distintos. Os caracteres morfológicos que marcaram os limites desses dois grupos estão de acordo com os caracteres previamente atribuídos por Pettibone (1991) como variação morfológica intraespecífica. Esses caracteres diferenciaram o seguinte: 1) a forma e a distinção do lobo pós-setal neuropodial; 2) a área quitinosa do primeiro élitro modificado; 3) a presença de áreas bulbosas na base dos cirros ventrais. O uso de um microscópio eletrónico de varrimento revelou um novo tipo de notochaeta num dos grupo. Estruturas semelhantes a flagelos, ainda não descobertas, também igualmente observadas emergindo do élitro. Essas estruturas flagelares foram encontradas nos

dois grupos e podem ser um caráter definidor do género. A função desses flagelos é ainda desconhecida uma vez que não foram relatados anteriormente em nenhum outro polinóide. É possível que o flagelo seja um órgão sensorial, somatossensorial ou quimiosensorial. A análise histológica revelou a presença de células em forma de copo dentro do élitro, que eram semelhantes às células vistas no élitro de outros polinóides que podem produzir bioluminescência.

Palavras-chave: poliquetas comensais de profundidade; espécies crípticas; élitro; delimitação de espécies; morfologia; flagelos.

Abstract

Gorgoniapolynoe caeciliae (Fauvel 1913) is a deep-sea commensal polychaete that is lives in association with several genera of corals from the order Alcyonacea. The wide geographic range of *G. caeciliae,* coupled with it having multiple host coral species, and the evolution of its taxonomic description mean that it could potentially be more than one species. This study investigated the morphological and genetic differentiation in *G. caeciliae,* sampled from four seamounts in the Equatorial North Atlantic. It is the first large scale study to be carried out on the species. Analysis of cytochrome *c* oxydase subunit I, in tandem with detailed morphological analysis was carried out. Species delimitation models (AGBD and bPTP) inferred that *G. caeciliae*, as previously described, is in actual fact two distinct species, with a possible third cryptic species which can only be delimited using molecular markers. 16S, 18S and 28S markers were also used infer the species phylogenetic placement within family Polynoidae. Markers from individuals, identified as G. caeciliae, from the South Indian Ocean, were included in the analysis. These may also be a new species, as they are genetically diverged from the Atlantic species. Additionally, scanning electron microscopy revealed the presence of previously undescribed characters; a new type of notochaeta, unique to the redescribed *G. caeciliae*; and flagellum-like structures that are part of the micropapillae on the elytra. These flagellum-like structures, which were found on all specimens examined with a scanning electron microscopy, are assumed to have a sensory function and may be a new defining character of the genus *Gorgoniapolynoe.*

Keywords: deep-sea commensal polychaete, cytochrome *c* oxydase subunit I, cryptic species, Equatorial North Atlantic, elytra, species delimitation, morphology

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List of Abbreviations

BA = Bayesian analysis

bp = base pairs

BS = bootstrap support

COI = cytochrome *c* oxydase subunit I

ML = maximum likelihood

PP = posterior probability

SOI = South Indian Ocean

16S = mitochondrial 16S rRNA

18S = nuclear 18S rDNA

28S = nuclear 28S rDNA

Chapter 1 Introduction

1.1 Commensal polychaetes

Polychaetes are the most diverse and abundant group within the Phylum Annelida. After nematodes they are the most pervasive metazoans in the benthic marine environment (Fauchald and Foundation 1977). Due to their diversity and ubiquitous distribution, they commonly form close associations with other marine invertebrates. In fact, there are currently 618 reported polychaete species involved in 1626 symbiotic relationships across the whole of the marine realm (Martin and Britayev 2018), a number that has doubled since the last review of the topic (Martin and Britayev 1998). There are three well recognised forms of symbiosis: commensalism, mutualism and parasitism. There are three well recognised forms of symbiosis: commensalism (association in which one organism gets benefit from another without harming or providing a benefit to it), mutualism (both organisms get a benefit from the association) and parasitism (one organism is harmed from the association).

The vast majority of symbiotic polychaetes are monoxenous (i.e. associated with one host species) and those that are polyxenous (associated with two or more host species) tend to be associated with closely-related host species, which suggests there is a high degree of specificity when it comes to symbiotic polychaetes (Martin and Britayev 1998). At a family level there are some polychaetes which are strictly associated with a given host taxon e.g. the Serpulids with hermatypic corals, which suggests a monophyletic origin of symbiosis in these families (Martin and Britayev 2018). However, it is more common that a given polychaete family will have associations with hosts across classes within a phylum, indicating that the symbiotic mode of life has evolved several times within these polychaete families (Martin and Britayev 1998).

The majority of symbiotic polychaetes form commensal relationships, with a reported 490 polychaete species involved in 1229 commensal relationships (Martin and Britayev 1998, 2018). 30% of these commensal relationships are with hosts from the phylum Cnidaria, representing 279 polychaete species. Species from the family Polynoidae represent ca. 50% of all known commensal polychaetes.

Deep-sea commensal polychaetes associated with coral hosts are a group which is in need of investigation. At present there are 84 deep-sea coral species recorded as being in 115 commensal relationships with a total of 53 polychaete species, representing five families (Martin and Britayev 1998, 2018). This number is likely to be an underestimation, since in

depth knowledge on these associations is limited and is dispersed across many different subject fields, often as anecdotal evidence. Traditional sampling of the deep-sea is normally done using non-selective gear, resulting in dislodgement of associates from their hosts, with associations going unreported or the species being classed as free living. Added to which the number of already described species may also not be an accurate reflection of the true number due to historical beliefs in polychaete taxonomy. Advances in molecular techniques in recent decades have allowed for quick and easy identification of species and has uncovered the presence of cryptic species within many polychaete groups e.g. (Grassle and Grassle 1976; Carr *et al.* 2011; Nygren and Pleijel 2011; Nygren 2014; Borda *et al.* 2015; Brasier *et al.* 2016; Álvarez-Campos *et al.* 2017; Nygren *et al.* 2018; Simon *et al.* 2019)

1.2. A short history of polychaete taxonomy

1.2.1. Early descriptions and beliefs

Historically in polychaete taxonomy there was a belief that many species had high morphological variation, resulting in species being described as having very wide geographic or cosmopolitan ranges (Hutchings and Kupriyanova 2018, and references within). The root of this belief stems from the early morphological descriptions generally being very short and lacking in detail (e.g. Fauvel's 1913 description of *Polynoe caeciliae*, which is just over 200 words long). Many of these early descriptions were based on specimens collected in European waters, as that was where many early taxonomists were based. When specimens from other areas of the world's ocean were examined, they were commonly identified using keys for existing European species. Furthermore, characters seen in the non-European specimens were frequently added to the original species descriptions, resulting in descriptions which were composites of geographically distant specimens (Hutchings and Kupriyanova 2018).

Additionally, the quality of collected specimens was (and continues to be) an issue. Polychaetes, being soft-bodied and segmented, are particularly prone to becoming fragmented and to losing appendages. These traits are compounded by collection and sorting techniques, such as sieving, which will increase the likelihood of damage. The removal of unique features can result in identification being based on more robust features which can be more conserved across genera (Maxwell *pers obs*).

The limited number of taxonomists working in the field was another factor in upholding the belief in cosmopolitan polychaete distributions. Pierre Fauvel (1866-1958), John Day (1909-

1989), and Olga Hartman (1900-1974), three of the most influential early polychaete taxonomists, were all staunch believers in cosmopolitan distributions and have hundreds of species descriptions to their names. For example, many of the illustrations in "*Annelida Polychaeta. The Fauna of India, including Pakistan, Ceylon, Burma and Malaya*." (Fauvel 1953) were originally from Fauvel's earlier work on European polychaetes.

1.2.2. A new wave in polychaete taxonomy

In the 1980s there was a gradual shift away from believing in cosmopolitan and widespread polychaete distributions, due to a rise in the number of polychaete taxonomists, a greater global effort, and more detailed morphological investigations (Hutchings and Kupriyanova 2018). Subtle morphological differences between specimens of widely distributed species, which had previously been overlooked, combined into a generic description, or had been attributed to intraspecific variation, were discovered to be geographically unique and were therefore assigned as new species. An example of this can be seen in the commensal scaleworm *Harmothoe lunulata* (Delle Chiaje, 1830) which was believed to be widespread and associated with a wide variety of hosts (e.g. asteroids, ophiuroids, holothuroids, cnidarians, polychaetes, sipunculids and balanoglossids). A detailed taxonomic study revealed that *H. lunulata* was in fact 15 different species in three different genera (Pettibone 1993). Furthermore, each new species was restricted geographically and was associated with one or a few closely related host species. Further examples are found in Hutchings and Kupriyanova's (2018) review on cosmopolitan polychaetes. The introduction of molecular analysis went one step further in dismantling the assumption of cosmopolitan/widely distributed polychaetes as it made it possible to delimit cryptic species.

1.2.3 Discovery of cryptic species

There are several definitions of cryptic species. In taxonomy cryptic species refers to two or more species, that are or previously have been considered as a single species due to being at least superficially morphologically indistinguishable (Bickford *et al.* 2007). Before the advent of molecular analysis uncovering these cryptic species was possible with detailed examination of their life histories, reproductive biology, habitat preference or tolerances to abiotic parameters (Nygren 2014). One of the first studies to uncover cryptic polychaete species using molecular techniques was that of Grassle and Grassle (1976). In their study electrophoretic analysis, in conjunction with life history and reproductive analyses, was carried out on what was believed to be a single species, *Capitella capitata*. The results

revealed the presence of six distinct species, delimited using allelic differences, and life histories and/or reproductive modes (Grassle and Grassle 1976). The rise in the use of molecular analysis since then has augmented the number of cryptic polychaete species discovered (see Nygren 2014 and ref. within).

In his review, Nygren (2014) notes that no particular group of polychaetes is more likely to contain cryptic species than any other. Additionally, cryptic species do not need to be separated by large geographic distances. For example, the populations sampled for the aforementioned Grassle and Grassle (1976) study were mostly sampled from around Woods Hole in Massachusetts. An even more extreme example can be seen in Nygren and Pleijel (2011), where five cryptic species of the Phyllodocid, *Eumida sanguinea* (Örsted, 1843) were found on a single coralline alga. Across all animal groups which contain cryptic species, there are two common reasons as to how genetic divergence occurs without morphological change. Firstly, the species uses nonvisual mating (chemical) signals and therefore there is no selection pressure on physical characters. Secondly, that they exist in an environment which imposes a stabilizing selection on morphology (Bickford *et al.* 2007). These explanations are not mutually exclusive. And both reasons, can be readily applied to polychaetes and explain why species boundaries may not correlate with morphological changes for many species.

1.4. Hypotheses for cryptic speciation in polychaetes

1.4.1. Chemical signalling

Polychaetes can be free living (errant) or tubicolous/burrowing (sedentary). For sedentary polychaetes which either are either sessile or infaunal having visual mating signals makes little sense as they most likely rely on environmental cues and chemical signalling in their reproductive cycles. Intraspecific chemical signalling is also used by some polychaetes in larval recruitment (Lindsay 2009).

Errant nereidid polychaetes have been shown to use pheromones in their mating behaviours (Hardege 1999). Mating takes place during synchronised spawning events, which are firstly triggered by environmental cues. Both males and females swim to the surface, where the females attract the males using a pheromone. Once the male detects a female, he circles round her. The female detects a pheromone he is releasing causing her to emit a “sperm release” pheromone. As well as sperm, the resulting “sperm cloud” contains an egg release pheromone which in turn causes the female to release her eggs for fertilisation. Spawning controlled by pheromones is also reported in the sedentary *Arenicola marina* (Lindsay 2009).

While the understanding of how polychaetes use pheromones in their mating strategies is limited, from the example above it can be seen to be complex and there are more factors at play than a general description would infer. However, it is not unreasonable to hypothesise that if a change in the chemical composition of a pheromone inferred a positive selection on fecundity, that genetic divergence could take place without morphological change (Bickford *et al.* 2007).

1.4.2 Morphological stasis

Many of the habitats in which polychaetes live have extreme environmental conditions that enforce morphological stasis. There are the obvious habitats such as the deep sea, where there is extreme hydrostatic pressure, cool temperatures and complete darkness and hydrothermal vent environments were extreme high temperatures are coupled with high hydrostatic pressures. But there are also habitats like sandy intertidal shores or mudflats, where many polychaetes live, that are subject to regular emersion periods with associated changes in water saturation, temperature, and interstitial chemistry. In these environments there are a limited number of ways an organism can adapt to live there, causing a convergence of morphological characteristics (Bickford *et al.* 2007). But they may diverge genetically due to isolation by distance, genetic drift or due to changes in pheromones, as previously discussed.

The deep sea is a habitat where conditions may promote morphological stasis in polychaetes. In Antarctic waters this may be particularly prevalent as a recent study into polychaete diversity found that 50% of species samples contained previously unknown cryptic diversity (Brasier *et al.* 2016). That study analysed the mitochondrial genes *COI* and *16S* rRNA of 15 target species to investigate intraspecific genetic divergence between individuals. Any species that had genetic divergence between individuals underwent a secondary detailed morphological analysis to determine whether there were any congruent morphological characters. Brasier et al. (2017) concluded that there were ten new morphospecies species discovered i.e. new species that could be delimited using morphology but that were previously considered one species due to subtle morphological differences being previously overlooked. Additionally, ten cryptic species were uncovered purely from molecular analysis. Subsequent analysis of cryptic species distribution patterns revealed the majority of these species were widely distributed and 60% occurred sympatrically (Brasier *et al.* 2017). The cryptic species most likely diverged from each other when they became isolation during glaciation periods, with subsequent secondary contact. The widespread present-day

distributions can be explained by connectivity provided by the oceanic currents circling the Antarctic continent (Allcock and Strugnell 2012; Brasier *et al.* 2016, 2017).

1.5. Deep-sea commensal polychaetes, *Gorgoniapolynoe*

Due to their diversity and ubiquitous distribution, polychaetes commonly form close associations with other marine invertebrates (Martin and Britayev 1998, 2018). Members of the polynoid genus *Gorgoniapolynoe* are all deep-sea commensal polychaetes that live in association with Alcyonacea corals (Pettibone 1991). All members of *Gorgoniapolynoe* are also colonial, meaning many individuals can be found living on a single coral host (Pettibone 1991). There are six identified species in *Gorgoniapolynoe*, plus several only identified to genus level. In total, these species are in 23 relationships with six coral genera from Alcyonacea (Martin and Britayev 1998, 2018). The polychaetes live inside tunnels on the coral branches. These tunnels are not excavated into the coral, rather the polychaete appears to have a way to induce the corals to modify their sclerites to form these tunnels (Pettibone 1991; Britayev *et al.* 2014;). Within *Gorgoniapolynoe*, *Gorgoniapolynoe caeciliae* has the largest reported number of different coral hosts, having been found in association with five different genera (Martin and Britayev 1998, 2018; Serpetti *et al.* 2017) . The coral species they have been recorded in association with are: *Acanthogorgia armata* (Verrill, 1878)*, A. aspera* (Pourtalès, 1867)*, Candidella imbricata* (Johnson, 1862)*, Hemicorallium bayeri* (Simpson & Watling, 2011)*, Hemicorallium niobe* (Bayer, 1964), *Hemicorallium tricolor* (Johnson, 1899)*, Corallium johnsoni* (Gray, 1860)*, Pleurocorallium secundum* (Dana, 1846), *Narella* sp. (Gray, 1870) and *Isididae* sp. (Lamouroux, 1812). The locations from of these records range from the eastern United States and the Caribbean, across the Atlantic to the western Europe and down into the South Indian Ocean.

1.6. Gorgoniapolynoe caeciliae

Gorgoniapolynoe caeciliae is a species whose taxonomic description, and subsequently its reported distribution, may be incorrect due to the historical taxonomic issues discussed in section 1.2.1. Added to which it also has the potential to harbour cryptic species, due to the habitat in which it lives and life history strategy, see section 1.4. Firstly, the evolution of *G. caeciliae's* taxonomic description will be examined, followed by looking at its potential to be composed of two or more cryptic species.

1.6.1. The early history of *Gorgoniapolynoe caeciliae*'s description based on morphology

Gorgoniapolynoe caeciliae was originally designated *Polynoe caeciliae* and was described by Fauvel in 1913. The description was based on two specimens collected from different areas; Northern Portugal and the Cape Verde Islands, living in association with *Corallium johnsoni.* The original description was just over 200 words long and is very basic. The illustrations accompanying the description (Figure 1) were of a parapodia, a dorsal view of the mid-body, and a single notochaeta and neurochaetae (Fauvel 1913:24, Fig. 7). In 1914, Fauvel provided a slightly more detailed description of the same two specimens, with additional descriptions of the neurochaetae, giving more details on the structure of the parapodium and comparison of the characters with other polynoid species. The accompanying plates also contained additional illustrations (Figure 1); the different neurochaetae, the anterior end and a detailed illustration of an elytrum were all added (Fauvel 1914:69, pl. 4: figs. 1-6, 18-19). There are two syntypes for the species however where both are held was unable to be established at the time of writing. They were originally logged in the Monaco Oceanographic Museum. Repeated attempts to contact the museum to enquire about the syntypes went unanswered. What is known is that two parapodia that are recorded as being from one syntype are held at Smithsonian National Museum of Natural History (USNM 80098, Pettibone 1991: Fig 12 E-F).

In 1953 a description was included in the *Catalog of Polychaetes types from the Oceanographic Museum of Monaco* (Belloc 1953), where the syntypes were originally held. It was a copy of Fauvel's original description on illustrations. Then 32 years later, Hartmann-Schroder (1985) provided another description using species from the Canary Islands and Maderia.

1.6.2. Synonymization

In 1991 the species was re-described by Pettibone and placed in the new genus *Gorgoniapolynoe*. Pettibone provided a detailed description of the species, including for the first time the presence of a pair of modified elytra that cover the prostomium. No other description acknowledged these elytra, probably since they become detached very easily. The modified elytra have a clear chitinous area and Pettibone assigns them as a defining characteristic of genus *Gorgoniapolynoe*. In addition to examining the parapodia syntypes for

the re-description, Pettibone also examined specimens collected from different locations in the Atlantic, however all the specimens, bar one, came from waters off the eastern United States and the Caribbean. The only specimen examined from the Eastern Atlantic was collected from Portugal. None of these additional specimens examined were found on *Corallium johnsoni* the host coral on which the syntypes were found. They were collected off other species of Alcyonacean corals: *Corallium niobe, Candidella imbricata* and *Acanthogorgia aspera.*

1.6.3. The introduction of two potential morphotypes

From the specimens examined for the re-description, Pettibone (1991) illustrated two different individuals (Figure 1.1), one from the Caribbean (USNM 21123, Pettibone 1991: Fig 14) and the individual from Portuguese waters (USNM 133356, Pettibone 1991: Fig. 12 A-C and Fig 13). From these illustrations it can clearly be seen that there were differences in morphology between the two individuals. The most obvious differences are in the size and shape of the chitinous area in the modified first elytra, and in the shape and orientation of the neuropodial postsetal lobe (Fig.1.1). Pettibone also illustrates the presence of bulbous areas at the base of the ventral cirri on the Caribbean specimen, that were not observed in the specimen from Portugal. Presumably, Pettibone chose to illustrate both specimens due to the differences and believed that the differences were down to intraspecific morphological variation. However, this cannot be said with any certainty because these morphological differences are not acknowledged in the accompanying written description. The written description is generic when it comes to describing these features i.e. while the chitinous area of the first elytra is mentioned and said to be laterally positioned and crescent shaped, there is no mention of how the area which it takes up is very different between the two illustrated specimens.

The last re-description of *G. caeciliae* was in 2014. Britayev *et al.* (2014) discovered the presence of clavate papillae on the dorsal cirri on specimens found in association with *Corallium niobe* and *Candidella imbricate* off Northern Portugal (Fig. 1). The specimens examined had the same morphology as USNM 21123, one of the two morphotypes illustrated by Pettibone (1991).

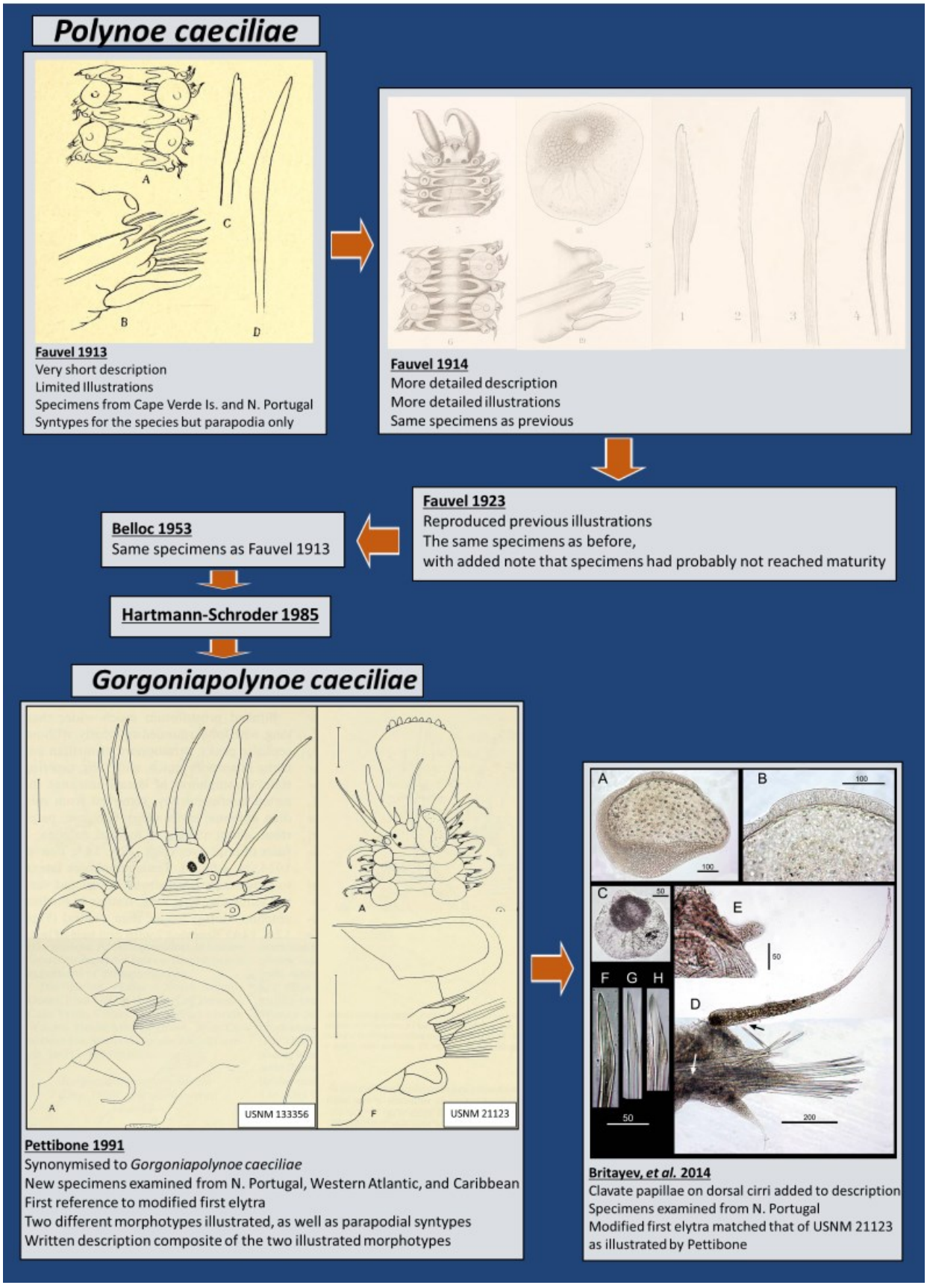

Figure 1.1. The evolution of the taxonomic description of *Gorgoniapolynoe caeciliae* from 1913 to the present day. Images taken from Fauvel (1913, 1914); Pettibone (1991); Britayev *et al*. (2014).

1.6.4. More than one species based on morphology?

The taxonomic description of *G. caeciliae* has potentially fallen into many of the problems previously highlighted for polychaete descriptions. Its initial description by Fauvel (1914) was not very detailed and no holotype was assigned. Remaining syntype consist of just a two parapodia, and the illustrations of the original whole-body specimen are lacking. When the species was re-described by Pettibone (1991) the vast majority of the specimens examined were sourced from areas far from the syntype specimen's location. Additionally, in the description two different morphologies were illustrated for the species but the written description was a generic composite (Pettibone 1991). Based on these potential issues, it is possible that the currently described *G. caeciliae* is actually composed of two distinct species.

1.6.5. Potential for cryptic species within *Gorgoniapolynoe caeciliae* based on its range

The wide geographic range and life history strategies reported for *G. caeciliae* make it a candidate to harbour cryptic species. *Gorgoniapolynoe caeciliae* has a distribution that stretches from the Gulf of Mexico and the Western Atlantic, across to Eastern Atlantic waters (Fauvel 1913; Pettibone 1991; Eckelbarger *et al.* 2005; Simpson and Watling 2011; Barnich *et al.* 2013; Britayev *et al.* 2014). It has also been reported in the South Indian Ocean (Stock 1986; Serpetti *et al.* 2017), which gives the species a range that stretches approximately 18,000 km.

Given the wide reported distribution range of *G. caeciliae*, there is a potential for genetic divergence due to isolation by distance. Unusually for deep-sea polychaetes there is some information on the reproductive biology of *G. caeciliae* (Eckelbarger *et al.* 2005). *Gorgoniapolynoe caeciliae* is a gonochoric species with no apparent sexual dimorphism. Histological analysis on specimens found in associated with *Candidella imbricata*, from New England seamounts revealed that oogenesis was intraovarian and sperm was simple ect-aquasperm. The presence of ect-aquasperm and no dimorphism suggests that fertilisation is not internal and that they are broadcast spawners. Furthermore, they appear to be Spring breeders, as sexual products were found in specimens sampled in May but not in ones sampled during July. They also have a relatively high fecundity, with over 3000 eggs recorded in a single female (Eckelbarger *et al.* 2005). If *G. caeciliae* is a broadcast spawner, then it's dispersal capabilities will be dictated by ocean currents and the length of their planktonic larval duration. It however would be unlikely for there be gene flow between

populations from the Gulf of Mexico and the South Indian Ocean. Theoretically the Mid-Atlantic Ridge could act as a stepping-stone between populations on the West and East of the Atlantic, as seen in some heterobranch gastropods (Eilertsen and Malaquias 2015).

Equally the Mid-Atlantic Ridge has the potential to restrict gene flow between the Atlantic basins due to its size and topography. This could produce a strong genetic population structure between basins. This disruption may occur if the vertical migration needed to overcome the ridge exceeds the physiological tolerance, hydrostatic pressure tolerances, of adults or larvae (Rex and Etter 2010). The presence of such a large topographic feature will also influence the hydrodynamics of the area. There is restricted gene flow between populations of the hydrothermal-vent associated polychaete *Ridgeia piscesae* (Sabellida) either side of the northeast Pacific ridge. Topographic steering of deep-water currents, whereby strong currents are forced to run parallel to the ridge, carry larvae along the ridge and prevent them crossing it (Young *et al.* 2008).

There has been only one study into the effect of the Mid-Atlantic Ridge on population structure of amphi-Atlantic commensal polychaetes (Shields *et al.* 2013). This study focused on another commensal polychaete, *Eunoe bathydomus*, which associates with the holothurian *Deima validum.* The polychaete lives on the exterior of the sea cucumber and it is hypothesised that the polychaete uses the host for transport and protection. There was no divergence in the *COI* marker analysed between specimens collected from the East and the West of the Mid-Atlantic Ridge (Shields *et al.* 2013). The populations sampled in this study were from along the axial trough of the ridge. The most northerly and southerly populations were separated by approximately 300 km, while the east and west populations separated by less than 70km. These populations may remain connected due to the topographic steering within the trough. Also, the lack of genetic divergence may be due to being associated with mobile hosts, which may take long migrations between food-rich areas, thus facilitating gene flow in the polychaetes (Shields *et al.* 2013).

1.6.6. Potential for cryptic species within Gorgoniapolynoe caeciliae based on its life history strategies

Ultimately fecundity, length of planktonic development and the hydrodynamics of the area will dictate the population structure of *G. caeciliae* across its range. Being a species living in an extreme environment, there is the potential that they may be in morphological stasis due to the previously stated reasons (see section 1.4). If this is the case, then speciation may have

occurred without morphological change. Additionally, it is possible that they utilise chemosensory capabilities at different stages of their lives, such as to synchronise gamete release or to facilitate larval settlement. As discussed previously these life history strategies may also facilitate speciation without morphological change.

Gorgoniapolynoe caeciliae has the potential to harbour cryptic species due to it being in a commensal relationship. The behavioural or physiological characters needed to adapt to a specific host are likely under strong selection pressure in some symbionts. Like non-visual mating cues, a change in these characters would be unlikely to produce any morphological change (Schonrogge *et al.* 2002). In the terrestrial environment, cryptic speciation as a result of host specific adaption pressures has been inferred in plant-parasitic nematodes (Palomares-Rius *et al.* 2014), parasitic wasps (Hambäck *et al.* 2013), avian lice (Malenke *et al.* 2009), feather mites (Doña *et al.* 2015) myrmecophilous hoverflies (Schonrogge *et al.* 2002) and Mordellid beetles (Blair *et al.* 2005).

Not only does *G. caeciliae* have the widest reported range of all *Gorgoniapolynoe*, but also the most recorded host-species. It has been found on seven coral genera, representing ten different species literature (Fauvel 1913; Hartmann-Schroder 1985; Stock 1986; Pettibone 1991; Eckelbarger *et al.* 2005; Simpson and Watling 2011; Britayev *et al.* 2014; Tu *et al.* 2015; Macpherson *et al.* 2016; Serpetti *et al.* 2017). Polyxenous symbiotic polychaetes make up 43% of all known symbiotic polychaetes, of which 76% have between two and five host species (Martin and Britayev 2018). With ten different host species reported, *G. caeciliae* is one of only 29 symbiotic polychaetes which have ten or more host species. *Gorgoniapolynoe caeciliae* seem to have the ability to induce their hosts to modify their sclerites to form the tunnels (Pettibone 1991; Britayev *et al.* 2014). It is possible that polychaetes initiating the modification by physical, chemical and/or physiological manipulations of the coral, as seen in insects which induce galls in terrestrial plants (Oliveira *et al.* 2016).

If *Gorgoniapolynoe caeciliae* manipulates the physiology or chemistry of their hosts to induce tunnel formation and presuming a variation in physiology and chemistry of different host species, then adaption to a new host would involve a change in the physiological or chemical manipulation techniques. This may provide a situation where genetic divergence may occur without morphological change (Bickford *et al.* 2007). This possibility may be more likely between hosts in different genera, as their physiology and chemistry are more likely to be distinctive compared to that of species in the same genera. Barnich *et al.* (2013) reported differences in tunnels formed on *Canidella imbricata* and *Acanthogorgia armata* by

G. caeciliae. The tunnels in *A. armata* were formed by a thin layer of sclerites between the inner axis and coral surface, while those in *C. imbricata* were formed by the enlargement of the characteristic sclerites at the base of the polyps. These differences may purely be down to the different morphological characteristics of the hosts rather than different methods of inducement by the polychaetes.

There has been one study into the whether polyxenous symbiosis can promote speciation in polychaetes. Meca *et al.* (2019) investigated if the *Oxydromus okupa* (Martin, Meca & Gil in Martin et al), a species which is found in association with different species of bivalves, had any significant morphological variation or genetic divergence that could be attributed to it being a polyxenous traits. The results suggest that the different host species produced phenotypic variation and genetic divergence in the polychaetes. This potentially be a mechanism by which speciation could be initiated (Meca *et al.* 2019)

1.7. Aims of this study

Given the potential that the descriptive taxonomic history based on morphology for *G. caeciliae* may not represent the true taxonomy and the additional possibility of the species harbouring cryptic species, the aims of this study were: 1) to utilise molecular techniques to analyse the genetic variation/divergence in *G. caeciliae* collected from both the eastern and western basins of the Atlantic; 2) to carry out detailed morphological analysis, in tandem with genetic analysis, to determine if morphological characters could define any new species boundaries found. This study, to the authors knowledge, is the first wide scale genetic investigation into a deep-sea commensal polychaete.

The current lack of understanding around the biological, chemical, and physical properties and functions of the deep ocean has been acknowledged by the UN Decade of Ocean Science for Sustainable Development (2021-2020). To address this they have set the goal of increasing the knowledge of the deep sea so that questions such as, what lives there, how is the system responding to climate change, and what are the effects of resource extraction, can be answered (Ryabinin *et al.* 2019). The results of this study will contribute towards this goal by increasing the knowledge of deep-sea polychaetes and contribute to a better understanding of the biodiversity of the deep sea.

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Chapter 2. Gorgoniapolynoe caeciliae (Annelida: Phyllodocida) revisited: previously unknown characters and undescribed species from the Equatorial North Atlantic

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Abstract

Gorgoniapolynoe caeciliae (Fauvel 1913) is a deep-sea commensal polychaete that is lives in association with several genera of corals from the order Alcyonacea. The wide geographic range of *G. caeciliae,* coupled with it having multiple host coral species, and the evolution of its taxonomic description mean that it could potentially be more than one species. This study investigated the morphological and genetic differentiation in *G. caeciliae,* sampled from four seamounts in the Equatorial North Atlantic. It is the first large scale study to be carried out on the species. Analysis of cytochrome *c* oxydase subunit I, in tandem with detailed morphological analysis was carried out. Species delimitation models (AGBD and bPTP) inferred that *G. caeciliae*, as previously described, is in actual fact two distinct species, with a possible third cryptic species which can only be delimited using molecular markers. 16S, 18S and 28S markers were also used infer the species phylogenetic placement within family Polynoidae. Markers from individuals, identified as G. caeciliae, from the South Indian Ocean, were included in the analysis. These may also be a new species, as they are

genetically diverged from the Atlantic species. Additionally, scanning electron microscopy revealed the presence of previously undescribed characters; a new type of notochaeta, unique to the redescribed *G. caeciliae*; and flagellum-like structures that are part of the micropapillae on the elytra. These flagellum-like structures, which were found on all specimens examined with a scanning electron microscopy, are assumed to have a sensory function and may be a new defining character of the genus *Gorgoniapolynoe.*

2.1. Introduction

The polynoid, *Gorgoniapolynoe caeciliae* (Fauvel 1913) is a commensal polychaete that is found in association with deep-sea corals from the order Alcyonacea (Fauvel, 1913; Pettibone 1991). It is one of the reported 490 polychaete species involved in 1229 commensal relationships with other marine invertebrates (Martin and Britayev 1998, 2018). All species of *Gorgoniapolynoe* are colonial, meaning many individuals can be found living on a single alcyonacean host (Pettibone 1991). The polychaetes live inside tunnels on the coral branches. These tunnels are not excavated into the coral, rather the polychaete appears to have a way to induce the corals to modify their sclerites to form these tunnels (Pettibone 1991; Barnich *et al.* 2013).

Of all the species of *Gorgoniapolynoe*, *Gorgoniapolynoe caeciliae* has the accumulated the most records in the literature (Fauvel 1913; Hartmann-Schroder 1985; Stock 1986; Pettibone 1991; Eckelbarger *et al.* 2005; Simpson and Watling 2011; Britayev *et al.* 2014; Tu *et al.* 2015; Macpherson *et al.* 2016; Serpetti *et al.* 2017). From these studies, G. caeciliae has been recorded in association with ten host species across 7 genera of Alcyonacea: *Acanthogorgia armata* (Verrill, 1878)*, A. aspera* (Pourtalès, 1867)*, Candidella imbricata* (Johnson, 1862)*, Hemicorallium bayeri* (Simpson & Watling, 2011)*, H. niobe* (Bayer, 1964), *H. tricolor* (Johnson, 1899)*, Corallium johnsoni* (Gray, 1860)*, Pleurocorallium secundum* (Dana, 1846), *Narella* sp. (Gray, 1870) and *Isididae* sp. (Lamouroux, 1812). The number of host species recorded for *G. caeciliae* makes it unusual, as it is one of only 29 species that have ten or more host species (Martin and Britayev 1998, 2018). Across all symbiotic polychaetes 57% are monoxenous i.e. having a single host species, 19% have two host species (polyxenous, having two or more relationships) and in total 33% have between two and five host species (Martin and Britayev 1998, 2018).

Gorgoniapolynoe caeciliae has a wide geographical range, which stretches from the eastern United States and the Caribbean, across the Atlantic to the western Europe and down into the

South Indian Ocean (Fauvel 1913; Hartmann-Schroder 1985; Stock 1986; Pettibone 1991; Eckelbarger *et al.* 2005; Simpson and Watling 2011; Britayev *et al.* 2014; Tu *et al.* 2015; Macpherson *et al.* 2016; Serpetti *et al.* 2017). This gives the species a range that stretches for approximately 18,000 km. Deep-sea taxa tend to have wider ranges than shallow-water or terrestrial taxa (McClain and Hardy 2010). The number or records for *G. caeciliae*, and the resultant geographic range, may be due to its existence being known of for over 100 years. There is a significant correlation between the time a deep-sea species is first described and the size of its range (Higgs and Attrill 2015). Higgs and Attrill (2015) suggest that this correlation may be explained due to wide-ranging species being more likely to be encountered earlier or that the longer a species has been described, the more records it will accumulate, thus giving it a larger range.

The taxonomic description of *G. caeciliae* (Fig. 1) has potentially fallen into some of the problems for polychaete descriptions, discussed in Hutchings and Kupriyanova (2018) i.e. poor early descriptions; 'European taxonomic bias'; composite generic re-descriptions based on two or more geographical distant specimens; assumption of wide variation of characters within species. For example, when the species was re-described by Pettibone (1991) the vast majority of the specimens examined were sourced from areas far from the syntype specimen's location. Additionally, two different morphologies were illustrated for the species (Fig. 1) but the written description was a generic composite and made no mention of the morphological differences observed (Pettibone 1991). Based on these potential issues, it is possible that the currently described *G. caeciliae* is actually composed of two distinct species based on morphology alone.

Gorgoniapolynoe caeciliae could potential harbour cryptic species also. Cryptic species have been uncovered within in many polychaete groups using molecular techniques (Grassle and Grassle 1976; Nygren and Pleijel 2011; Nygren 2014 and references within; Borda *et al.* 2015; Álvarez-Campos *et al.* 2017; Nygren *et al.* 2018; Simon *et al.* 2019). There are several definitions of cryptic species, in this context cryptic species refers to two or more species, that are or previously have been considered as a single species due to being at least superficially morphologically indistinguishable (Bickford *et al.* 2007). Across all animal groups which contain cryptic species, there are two common reasons as to how genetic divergence occurs without morphological change. Firstly, the species uses nonvisual mating (chemical) signals and therefore there is no selection pressure on physical characters.

Secondly, that they exist in an environment which imposes a stabilizing selection on morphology (Bickford *et al.* 2007). These explanations are not mutually exclusive.

Gorgoniapolynoe caeciliae live in an extreme environment i.e. the deep sea and being broadcast spawners (Eckelbarger *et al.* 2005) they may use chemical signalling to synchronise spawning events as seen in other polychaetes (Hardege 1999; Lindsay 2009). Given these factors *G. caeciliae* could potentially harbour cryptic species. Additionally, the behavioural or physiological characters needed to adapt to a specific host are likely under strong selection pressure in symbionts (Bickford *et al.* 2007). Like non-visual mating cues, a change in these characters would be unlikely to produce any morphological change (Schonrogge *et al.* 2002). In the terrestrial environment, cryptic speciation as a result of host specific adaption pressures has been inferred in plant-parasitic nematodes (Palomares-Rius *et al.* 2014), parasitic wasps (Hambäck *et al.* 2013), avian lice (Malenke *et al.* 2009), feather mites (Doña *et al.* 2015) myrmecophilous hoverflies (Schonrogge *et al.* 2002) and Mordellid beetles (Blair *et al.* 2005).

Given the potential that the descriptive taxonomic history based on morphology for *G. caeciliae* may not represent the true taxonomy and the additional possibility of the species harbouring cryptic species, the aims of this study were: (i) to utilise molecular techniques to analyse the genetic variation/divergence in *G. caeciliae* collected from both the eastern and western basins of the Atlantic; (ii) to carry out detailed morphological analysis, in tandem with genetic analysis, to determine if morphological characters could define any new species boundaries found. This study, to the authors knowledge, is the first wide scale morphological and genetic investigation into a deep-sea commensal polychaete. The results will increase the knowledge of deep-sea polychaetes and contribute to a better understanding of the biodiversity of the deep sea, contributing towards the goals set by the UN Decade of Ocean Science for Sustainable Development (2021-2020) to further the understanding of the deep sea (Ryabinin *et al.* 2019).

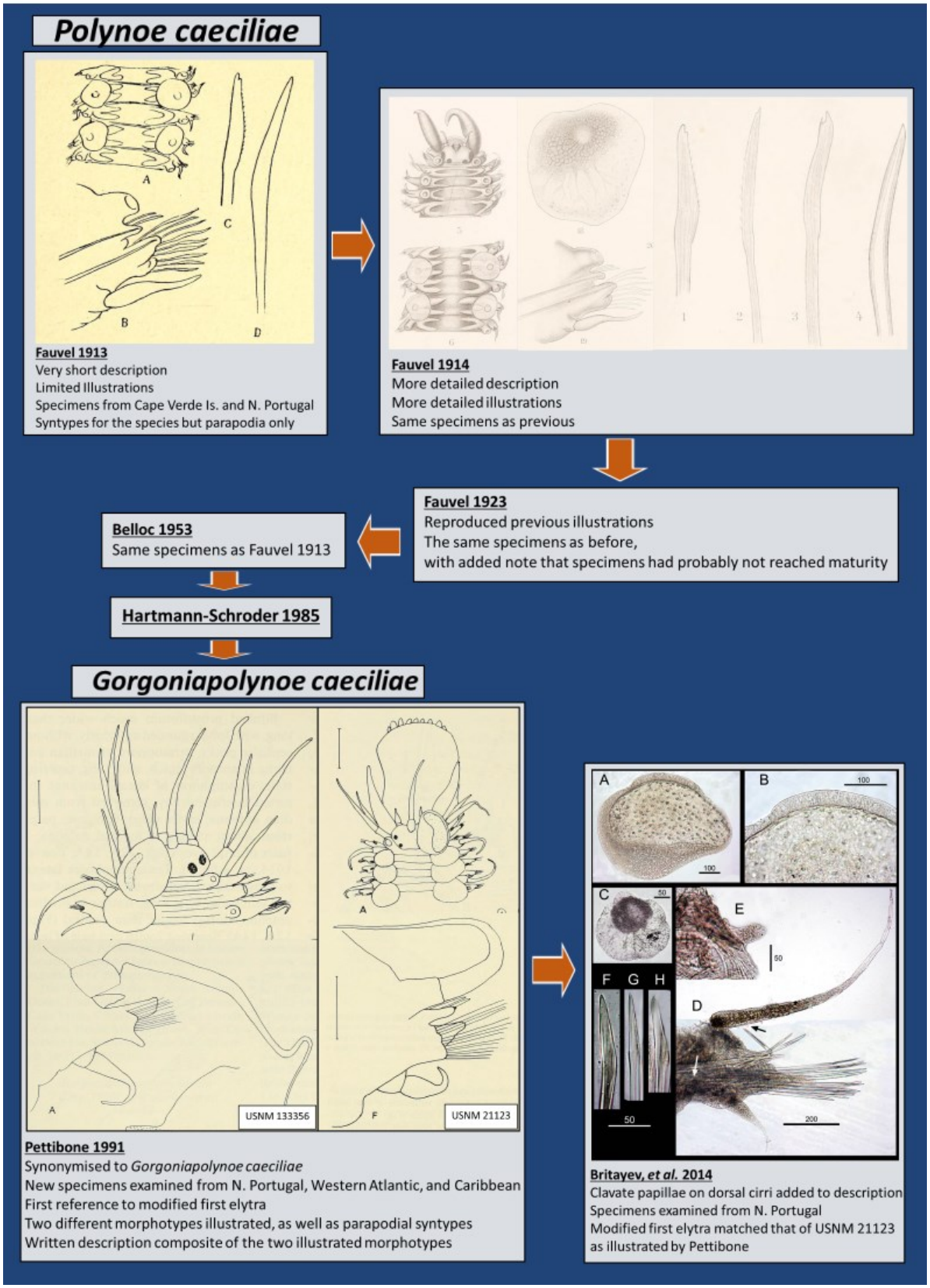

Figure 2.1. The evolution of the taxonomic description of *Gorgoniapolynoe caeciliae* from 1913 to the present day. Images taken from Fauvel (1913, 1914); Pettibone (1991); Britayev *et al*. (2014).

2.2. Methods

2.2.1 Specimen collection

The specimens were collected on board the National Oceanography Centre's vessel the *RRS James Cook* during the JC094 cruise in 2013. Sample collection was undertaken using the *ROV Isis.* The sampling locations were all between 05 °N and 15 °N in the Atlantic (Fig. 2.2). The sampling locations were either side of the Mid-Atlantic Ridge, with the seamounts Vayda and Vema being in the western basin and Carter and Knipovich in the eastern basin. The sampling depths ranged from 600 – 2340 m. All the specimens were found in association with deep-sea corals from the families Acanthogorgiidae, Primnoidae and Coralliidae (Order Alcyonacea). The specimens were found in tunnels within the coral tissues, with multiple individuals recovered from each coral. Once removed from the host the polychaetes were preserved in 70% ethanol and stored at -20 °C until further analysis could be carried out.

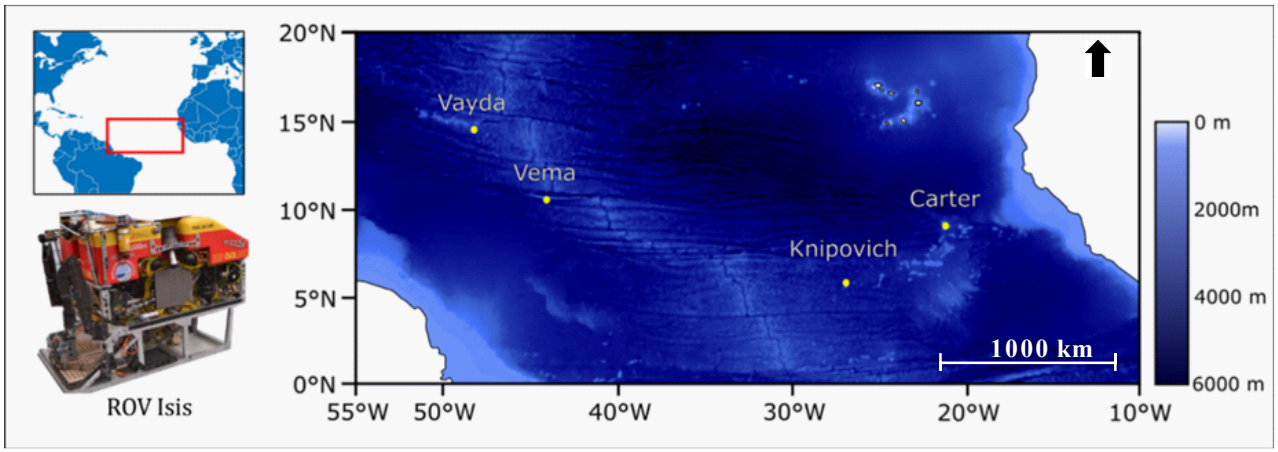

Figure 2.2. Map with the locations of the four seamounts where the sampling was undertaken. Sampling was carried out during the *RRS James Cook* JC094 cruise using the *ROV Isis.*

2.2.2. DNA extraction

Tissue (approximately half of the posterior part of each individual) was taken from 129 specimens, covering all sampling sites (Fig.2.2, Table 2.1). Extraction was done using DNeasy® Blood and Tissue kit (QIAGEN, Germany). The manufacturer's protocol was followed except for the final elution stage, where 75 µL of Buffer AE was used rather than the recommended 200 µL. The elution buffer was heated to 56 °C. After the initial wash, the buffer was reapplied to the spin column membrane and the wash was repeated. In doing so the amount of eluted DNA was maximised

Table 2.1. Summary of the specimens for which *COI* markers were amplified. Parent ID of host corals, coral type, sampling depth, polychaete specimen numbers (prefix) and number of individuals. * indicates that one individual with this prefix was also sequenced for 16S, 18S and 28S.

Vayda				
Parent	Coral	depth m	Specimen numbers	No. of individuals
96	Corallium	1416	1819, 1851, 1879	16
98	Corallium	772	2029, 2030, 2031	3
1454	Corallium	710	2054-2061, 2063, 2064, 2086, 2087, 2101	16
1466	Corallium	1622	2209, 2210, 2212-2218	9
1470	Corallium	1622	2169, 2170, 2171, 2173, 2191, 2194*	15
Vema				
Parent	Coral	depth m	specimen numbers	No. of individuals
91	Corallium	2190	1743*, 1771	9
515	Acanthogorgia	593	1714*, 1715	8
Knipovich				
Parent	Coral	depth m	specimen numbers	No. of individuals
83	Corallium	1445	1217*, 1238, 1260	16
Carter				
Parent	Coral	depth m	specimen numbers	No. of individuals
267	Primnoidae	1345	268	2
502	Candidella	1783	551, 552	2
24	Corallium	1364	602*	8
56	Corallium	1442	0811, 0812, 0814, 0820	4
61	Corallium	2343	680, 716	17
63	Primnoidae	1364	745	2
64	Corallium	1367	671	2

2.2.3. Amplification and sequencing

A fragment of the mitochondrial Cytochrome c oxidase subunit I (*COI*) gene was amplified for 129 specimens. These specimens represented individuals from each location and from multiple hosts (Table 2.1). Additionally, a fragment of the ribosomal mitochondrial gene 16S rDNA (*16S*), and the nuclear ribosomal genes 18S rDNA (*16S*) and 28S rDNA (*28S*) were amplified for five specimens (Table 2.1). These five specimens were selected based on genetic divergence inferred from analysis of the *COI* data and from features identified during the morphological analysis. The primer pairs used are presented in Table 2 and the PCR profiles used are summarised in Table 2.3.3. All markers were amplified using 10.5 µL of VWR Red Taq DNA Polymerase 1.1x Master Mix (VWR International bvba/sprl, Belgium), 0.5 µL of the forward and reverse primers, and 1 µL of DNA template. GelRed® (Biotium,

USA) was used to stain the PCR products, which were visualised using 1.5% agarose gel electrophoresis that was run for 30 min at 100 V. The PCR products were then purified and sequenced at the NHMUK's sequencing facilities.

Table 2.2. Primer pairs used for PCR and sequencing.

Primer	Sequence 5'-3'	Reference
COI		
LCO 1490	GGTCAACAAATCATAAAGATATTGG	Folmer *et al.*, 1994
HCO 2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al.*, 1994
16S		
arL	CGCCTGTTTATCAAAAACAT	Palumbi, 1996
brH	CCGGTCTGAACTCAGATCACGT	Palumbi, 1996
18S		
1F	TACCTGGTTGATCCTGCCAGTAG	Giribet *et al.*, 1996
5R	CTTGGCAAATGCTTTCGC	Giribet *et al.*, 1996
4F	CCAGCAGCCGCGCTAATTC	Giribet *et al.*, 1996
7R	GCAAATAACAGGTCTGTGATGCCC	Giribet *et al.*, 1996
a2.0	ATGGTTGCAAAGCTGAAAC	Whiting *et al.*, 1997
9R	GATCCTTCCGCAGGTTCACCTAC	Giribet *et al.*, 1996
28S		
a	GACCCGTCTTGAAACACGGA	Whiting *et al.*, 1997
rD5b	CCACAGCGCCAGTTCTGCTTAC	Whiting, 2002
C1	CCTGGTTAGTTTCTTTTCCTCCGCT	Vân Le *et al.*, 1993
C2	TGAACTCTCTCTTCAAAGTTCTTTTC	Vân Le *et al.*, 1993
F63.2	ACCCGCTGAAYTTAAGCATAT	Struck *et al.*, 2006
PO28R4	GTTCACCATCTTTCGGGTCCCAAC	Struck *et al.*, 2006

2.2.4. Phylogenetic analysis

The program Geneious v.10.1.3 (http://www.geneious.com, Kearse *et al.* 2012) was used to clean, assemble and trim the DNA sequence contigs. Consensus sequences were run through BLAST (Altschul *et al.* 1990) to check for contamination. Additional sequences were obtained from Genbank (Benson *et al.* 2005) (Tables 2.4 and 2.5). Sequence alignment was done in Geneious using the inbuilt MAFFT v.7.309 program (Katoh and Standley 2013) set at the default settings.

Table 2.3. The PCR programs used to amplify the selected gene fragments

	***COI*, LCO1490/HCO2198**		
Stage	Temp. (°C)	Time	Cycles
Initial denaturation	95	5 min	
Denaturation	95	1 min	
Annealing	58	1 min	} x38
Extension	72	1 min	
Final Extension	72	10 min	
	***16S*, arL/brH**		
Stage	Temp. (°C)	Time	Cycles
Initial denaturation	94	5 min	
Denaturation	94	1 min	
Annealing	58	45 sec	} x38
Extension	68	45 sec	
Final Extension	68	10 min	
	***18S* 1F/5R, 4F/7R, a2.0/9R**		
Stage	Temp. (°C)	Time	Cycles
Initial denaturation	94	5 min	
Denaturation	94	1 min	
Annealing	52	1 min	} x38
Extension	72	1 min	
Final Extension	72	10 min	
	***28S* a/Rd5b C1/C2**		
Stage	Temp. (°C)	Time	Cycles
Initial denaturation	94	5 min	
Denaturation	94	1 min	
Annealing	55	1 min	} x30
Extension	72	1 min	
Final Extension	72	10 min	
	***28S* F63.2/PO28R4**		
Stage	Temp. (°C)	Time	Cycles
Initial denaturation	95	5 min	
Denaturation	95	30 min	
Annealing	55	30 min	} x30
Extension	72	1.5 min	
Final Extension	72	10 min	

The *COI* sequences and sequences from individuals from the South Indian Ocean identified as *Gorgoniapolynoe caeciliae* and *Gorgoniapolynoe corralophila* (Tables 2.5 and 2.6) once aligned and trimmed were composed of 669 base pair (bp). A haplotype data file was created using DnaSP v6 (Rozas *et al.* 2017)(Supplementary material, Table S1).The best partition schemes and associated substitution models under AICc criterion were evaluated with PartitionFinder (Lanfear *et al.* 2016), using the greedy algorithm (Lanfear *et al.* 2012) (Table

2.7). Analysis was carried out using the model-based approaches of maximum likelihood (ML) analysis, implemented using RAxML v8.2.12 (Stamatakis 2014), and Bayesian inference (BI) analysis run in MrBayes v3.2.6 (Ronquist *et al.* 2012).

RAxML was run using the HKY+I+G model. The multiple tree search consisted of 100 alternative runs, and the multiparametric bootstrap analysis had 1000 iterations.

In MrBayes the Monte Carlo Markov Chains (MCMC) were run for five million generations, with tree samples every thousand generations using the evolutionary model HKY (lset nst=2) +I+G (rates=invgamma). Across the partitions, the parameters of substitution rates, nucleotide frequencies, invariant-sites proportion, and gamma shape were all unlinked. Burn-in was set at 25% percent. Tracer v1.7.1 (Rambaut *et al.* 2018) was used to check for convergence of the runs. Analysis was stopped once the standard deviation of split frequencies was <0.01, the potential scale reduction factor PSRF was around 1.00, the effective sample sizes were >200 and the trace plot had the appearance of a "hairy caterpillar".

For the mutli-marker analyses, all alignments were manually trimmed in Geneious resulting in 16S = 438 bp, 18S =1648 bp, 28S = 900 bp and *COI* = 567 bp. Additionally, the aligned 16S, 18S, and 28S sequences were run through Gblocks v.0.91b (Castresana 2000) to eliminate divergent regions and poorly aligned positions. The "minimum length of a block" was set at 5, "allowed gap positions" was set at "with half", "maximum number of contiguous non-conserved positions" was set at 10 and finally the "minimum number of sequences for a flanked position" was set at $\frac{2}{n} + 1$, where n = total number of sequences. Once each gene was aligned, trimmed and run through Gblocks, they were concatenated resulting in 3,553 bp.

RAxMl only allows for a single model of rate heterogeneity in partitioned analysis, so GTR+I+G was chosen as it was suggested for four out of the six partitions and had a low AICc score for the remaining two partitions. The other parameters were set as previous described for *COI*.

In MrBayes the Monte Carlo Markov Chains (MCMC) were run for ten million generations, with tree samples every thousand generations using the evolutionary models in 2.7. The parameters were set as before for *COI* and the analysis was halted once the same conditions had been met.

2.2.5. Species delimitation

Automatic Barcode Gap Discovery (ABGD, Puillandre *et al.* 2012.) and a Bayesian implementation of the Poisson tree processes model (bPTP Zhang *et al.* 2013) were used on the *COI* haplotype data to delimitate species.

ABGD was run online at https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html. The program relies on the inclusion of the priors, P_{max} and P_{min}, where P is divergence of intraspecific diversity. P_{min} was set at 0.001 and P_{max} at 0.37. These values were based on the range of intraspecific distances calculated for Polychaeta as a whole (Kvist 2016). 20 steps were run with a relative gap width (X) of 1.5. The number of bins for distance distribution was set to 30. The data input was an uncorrected p-distance matrix computed in Mega-X (Kumar *et al.* 2018) using the haplotype data file.

The bPTP model was run online at https://species.h-its.org/. The input file was the resulting tree from the *COI* RAxML analysis converted into Netwick format in Figtree v1.4.3 (Rambaut and Drummond 2012). The number of MCMC generations was set at 500000, with a thinning of 100 and a burn-in of 0.25. The outgroup was removed to improve results.

Analysis of molecular variance (AMOVA) (Excoffier and Lischer 2010) was run using Arlequin v3.5.2.2 under the hierarchy of location (seamount) > population (host). *F*-statistics were utilized to estimate the proportion of variability found between locations (F_{ct}), among populations within locations (F_{sc}), and within populations (F_{st}).

2.2.6. Morphological Analysis

The macroscopic morphological characters of all samples and voucher specimens were examined using a Leica MZ6 steromicroscope (Leica Microsystems, Germany). Parapodia and elytra were removed from selected specimens for further examination using an Olympus BX43 compound microscope (Olympus Corporation, Japan). Specimens were photographed using an Olympus UC50 camera and the cellSens Standard interface (Olympus Corporation, Japan) for both microscopes.

Four specimens were selected for analysis using a scanning electron microscope (SEM). Specimens were dehydrated in an ascending ethanol series from 70-100%, critical-point-dried in a Balzers CPD-030 dryer (Bal-Tec AG, Liechtenstein), mounted on stubs and coated with

gold (20nm). Imaging was preformed using Zeiss Ultra Plus Field Emission SEM (Zeiss Group, German) in the NHMUK imaging facilities.

2.2.7. Histological preparations

Two specimens were selected for histological analysis. One specimen was selected from each of the two morphotypes/clades observed as a result of the morphological and phylogenetic analyses, with the criteria for selection being the presence of the pair of modified first elytra, followed by the second pair. Both specimens were preserved in 70% ethanol and they were dehydrated in an ascending ethanol series up to 100%. Anterior portion of the specimen (prostomium and following two or three segments), was embedded in paraffin, sliced into 5 μm sections, stained with haematoxylin-eosin, and mounted with DPX (see supplementary material for method). Prepared slides were investigated using Olympus BX43 compound microscope (Olympus Corporation, Japan) and imaged using the Olympus UC50 camera and the cellSens Standard interface (Olympus Corporation, Japan).

Table 2.4. List of all the taxa (bar *Gorgoniapolynoe* species) used in phylogenetic analysis. The majority were sourced off GenBank, with the exception of *Harmothoe cf. bathydomus, Neopolynoe acanellae, Neopolynoe africana* and *Robertianella synophthalma* which were provided by Dr Sergio Taboada from the NHM.

Family	**Species**	**Voucher**	**Accession numbers**			
			18S	**28S**	**16S**	**COI**
Polynoidae	*Acholoe astericola*	SMNH118959	AY839567	JN852850	JN852888	AY839576
	Alentia gelatinosa	SMNH73632	AY839566	–	–	AY839577
	Antarctinoe ferox	–	KF713423	–	KF713463	KF713373
	Antipathypolyeunoa sp.	–	KU738169	KU738184	KU738149	KU738202
	Austropolaria magnicirrata	NHMUK:2012.95	JX863895	–	JX863896	–
	Bathykurila guaymasensis	–	DQ074765	–	–	DQ074766
	Branchinotogluma sandersi	SMNH118960	JN852821	JN852851	JN852889	JN852923
	Branchipolynoe symmytilida	–	–	–	AF315055	AY646021
	Brychionoe sp.	–	KU738182	KU738200	KU738167	–
	Bylgides elegans	SMNH118962	JN852822	JN852852	JN852890	JN852924
	Bylgides sarsi	SMNH118961	JN852823	JN852853	JN852891	JN852925
	Capitulatinoe cf. cupisetis		KF919301	KF919302	KF919303	
	Eunoe nodosa	SMNH118963	JN852833	JN852864	JN852892	JN852926
	Eunoe sp.	–	KU738183	KU738201	KU738168	KU738214
	Gastrolepidia clavigera	SMNH118964	JN852825	JN852855	JN852893	JN852927
	Gattyana cf. cirrhosa	–	KY823462	KY823462	KY823479	–
	Gattyana ciliata	USNM1077218	AY894297	DQ790035	–	AY894312
	Gattyana cirrhosa	SMNH118965	JN852826	JN852856	JN852894	JN852928
	Gesiella jameensis	–	Ky454403	Ky823476	Ky454412	Ky454429
	Halosydna brevisetosa	SMNH118966	JN852827	JN852857	JN852895	AY894313
	Halosydnella australis	–	KY823449	KY823463	KY823480	KY823495
	Harmothoe cf. bathydomus	TBC	TBC	TBC	TBC	TBC
	Harmothoe cf. imbricata	–	KY823450	KY823464	KY823481	KY823496
	Harmothoe glabra	SMNH118967	JN852828	JN852858	JN852896	JN852929

Table 2.4 continued

Family	Species	Voucher	Accession numbers			
			18S	**28S**	**16S**	**COI**
Polynoidae	*Harmothoe imbricata*	–	AY340434	AY340400	AY340463	AY839580
	Harmothoe impar	SMNH118968	JN852829	JN852859	JN852897	JN852930
	Harmothoe oculinarum	SMNH118969	AY894299	JN852860	JN852898	AY894314
	Harmothoe rarispina	–	KY657611	KY657624	KY657641	KY657659
	Harmothoe sp.	–	KU738178	KU738196	KU738163	–
	Hermenia verruculosa	SMNH118970	JN852830	JN852861	JN852899	JN852931
	Hyperhalosydna striata	SMNH118971	JN852831	JN852862	JN852900	JN852932
	Lepidasthenia elegans	SMNH118973	JN852832	JN852863	JN852901	JN852933
	Lepidonotus clava	SMNH118974	JN852833	JN852864	JN852902	JN852934
	lepidonotus squamatus	SNMH118975	AY894300	JN852865	JN852903	AY894316
	Lepidonotus sublevis	USNM107222	AY894301	DQ790039	–	AY894317
	Malmgreniella mcintoshi	SMNH118976	JN852834	JN852866	JN852904	JN852935
	Melaenis loveni	SMNH118977	JN852835	JN852867	JN852905	JN852936
	Neopolynoe acanellae	TBC	TBC	TBC	TBC	TBC
	Neopolynoe africana	TBC	TBC	TBC	TBC	TBC
	Neopolynoe paradoxa	SMNH118978	JN852836	JN852868	JN852906	JN852937
	Paradyte crinoidicola	SMNH118979	JN852837	JN852869	JN852907	JN852938
	Paralepidonotus ampulliferus	SMNH118980	JN852838	AF185164	JN852908	JN852939
	Pelagomacellicephala cf. illifei	ZMUC-POL-2396	–	–	KY454424	KY454440
	Pelagomacellicephala cf. illifei	ZMUC-POL-2394	KY454408	KY823474	KY454420	KY454435
	Pelagomacellicephala cf. illifei	ZMUC-POL-2397	KY454411	KY823475	KY454428	KY454443
	Pelagomacellicephala cf. illifei	ZMUC-POL-2392	KY454405	–	KY454416	KY454431
	Polyeunoa laevis	–	KU738177	KU738194	KU738161	KU738213
	Polynoe scolopendrina	SMNH118981	JN852839	JN852870	JN852909	JN852940
	Robertianella synophthalma	–	TBC	TBC	TBC	TBC
	Thormora jukesii	SMNH118983	JN852840	JN852871	JN852910	JN852941

Table 2.4 continued
Outgroups

Family	Species	Voucher	Accession numbers			
			18S	**28S**	**16S**	**COI**
Acoetidae	*Panthalis oerstedi*	SMNH118954	AY839572	JN852845	JN852881	AY839584
Aphroditidae	*Aphrodita aculeata*	SMNH118956	AY176281	JN852846	–	AY839578
Chrysopetalidae	*Bhawania heteroseta*	SMNH97305	EU555035	EU555025	EU555044	EU555053
Eulepethidae	*Grubeulepis mexicana*	SMNH118957	JN852817	JN852848	JN852884	–
	Mexieulepis weberi	SMNH118958	JN852818	–	JN852885	JN852920
Iphionidae	*Iphione sp.*	SMNH118972	JN852819	–	JN852886	JN852921
	Thermiphione sp.	SMNH118982	JN852820	JN852849	JN852887	JN852922
Sigalionidae	*Neoleanira tetragona*	SMNH118984	AY839570	JN852872	JN852911	AY839582
	Pholoe pallida	SMNH118986	AY894302	JN852874	JN852913	AY894318
	Sthenelais boa	USNM1077227	DQ779672	DQ779711	DQ779635	—

Table 2.5. The voucher and accession numbers of the Gorgoniapolynoe specimens sourced from GenBank, that were used in both the phylogenetic analysis and the species delimitation models

Species	Voucher	Accession no.	18S	28S	16S	COI	
Gorgoniapolynoe caeciliae SIO1	NSJC66_4277_1		KU738188	KU738153	KU738205	KU738172	
Gorgoniapolynoe caeciliae SIO2	NSJC66_804_2		KU738186	KU738151	KU738204	KU738171	
Gorgoniapolynoe caeciliae SOI3	NSJC66_104_1		KU738185	KU738150	KU738203	KU738170	
Gorgoniapolynoe corralophila SIO1	NSJC66_4279_S001		KU738192	KU738157	KU738209	KU738175	
Gorgoniapolynoe corralophila SIO2	NSJC66_3559_1		KU738191	KU738156	KU738208	KU738174	
Gorgoniapolynoe corralophila SIO3	NSJC66_133_S001		KU738189	KU738154	KU738206	KU738173	

Table 2.6. All the *Gorgoniapolynoe* specimens used in the phylogenetic analysis, the sampling depth, host coral and location. *marks all specimens collected as part of this study. All the rest are sourced from GenBank (see Table 2.4)

Species	Depth (m)	Coral	Area and Seamount	Lat	Long	ID
Gorgoniapolynoe sp. nov. 602*	1364	*Corallium*	East Central Atlantic, Carter	9.207633333	-21.30061667	Ggp_0602x7
Gorgoniapolynoe nov.sp. 1217*	1445	*Corallium*	East Central Atlantic, Knipovich	5.608726667	-26.95827833	Ggp_1217x6
Gorgoniapolynoe caeciliae A 1714*	593	*Acanthogorgia*	West Central Atlantic, Vema	10.71144	-44.42032167	Ggp_1714x7
Gorgoniapolynoe caeciliae A 2194*	1622	*Corallium*	West Central Atlantic, Vayda	14.86490333	-48.25581333	Ggp_2194x5
Gorgoniapolynoe caeciliae B 1743*	2190	*Corallium*	West Central Atlantic, Vema	10.76794833	-44.60063	Ggp_1743x3
Gorgoniapolynoe caeciliae SIO1	784	*Acanthogorgia*	South Indian Ocean, Atlantis Bank	32.70972222	32.70972222	NSJC66_4277_1
Gorgoniapolynoe caeciliae SIO2	1021	*Candidella imbricata*	South Indian Ocean, Melville	38.50888889	38.50888889	NSJC66_804_2
Gorgoniapolynoe caeciliae SOI3	1360	*Narella*	South Indian Ocean, Coral	41.35444444	42.92583333	NSJC66_104_1
Gorgoniapolynoe corralophila SIO1	894	Stylasteridae	South Indian Ocean, Atlantis Bank	50.45305556	50.45305556	NSJC66_4279_S001
Gorgoniapolynoe corralophila SIO2	1340	Stylasteridae	South Indian Ocean, Middle of What	37.95055556	50.45305556	NSJC66_3559_1
Gorgoniapolynoe corralophila SIO3	1357	Stylasteridae	South Indian Ocean, Coral	32.70972222	42.92555556	NSJC66_133_S001

Table 2.7. Alignment information and models of evolution for both phylogenetic analysis and species delimitation. As determined by PartionFinder under AICc criterion using the greedy algorithm

Phylogenetic Analysis			**COI data used in species delimitation**		
Gene and codon position	Partition delineation	Best model of evolution	Gene and codon position	Partition delineation	Best model of evolution
16S	1 - 438	GTR+I+G	COI – 1st	1 – 669/3	HKY+I+G
18S	439 - 2086	GTR+I+G	COI – 2nd	2 – 669/3	HKY+I+G
28S	2087 - 2986	GTR+I+G	COI – 3rd	3 – 669/3	HKY+I+G
COI – 1st	2987 – 3553/3	HKY+G			
COI – 2nd	2988 – 3553/3	SYM+I+G			
COI – 3rd	2989 – 3553/3	GTR+I+G			

2.3. Results and Discussion

2.3.1 Two species inferred by morphological analyses, with a possible third cryptic species based on molecular analysis

Morphological analysis divided the specimens into two distinct groups. The morphological characters which marked the boundaries of these two groups were congruent with the characters that differentiate the two specimens, USNM 133356 and USNM 21123, illustrated by Pettibone (1991). These differentiating characters where (i) the shape of the neuropodial postsetal lobe; (ii) the size and shape of the chitinous area of the modified first elytra; and (iii) the presence of bulbous areas at the base of the ventral cirri (Fig. 2.3). Individuals that had the morphological characters that matched USNM 21123 also had more notochaetae on anterior segments than individuals that had the morphology of USNM 133356. Not only were there more notochaetae on these individuals but two different types were observed using SEM. Individuals that had the morphology that matched 133356 only had one type of notochaetae. Clavate papillae on the dorsal cirri were also seen on individuals whose morphology matched USNM 21123. These papillae were described previous for *Gorgoniapolynoe caeciliae* by Britayev *et al.* (2014).

The molecular analysis was congruent with the morphological analysis, in that the two groups delimitated by morphology, formed two corresponding clades when the *COI* sequences were analysed (Fig. 2.3). The species delimitation models returned the clade with the morphology which matched USNM 133356 as a single species. Within the clade whose morphology matched that of USNM 21123, the species delimitation models inferred the presence of two species (the validity of this result will be discussed in section 2.3.2).

Based on the results, the species previously described as *Gorgoniapolynoe caeciliae* in fact represents two distinct species, with the possibility of a third cryptic species. One species can be distinguished using morphological characters alone i.e. the group whose morphology matched that of USNM 13356. This species will herein be referred to as *Gorgoniapolynoe* sp. nov. Whether or not the individuals whose morphology matches that of USNM 21123 represent one or two species, they can be distinguished from *Gorgoniapolynoe* sp. nov. based on the previously mention morphological characteristics. The individuals that are genetically distinct but morphologically identical had parapodia that closest resembled that of the *G. caeciliae* parapodial syntype. Therefore, they will be referred to as *Gorgoniapolynoe caeciliae* A and *Gorgoniapolynoe caeciliae* B (Fig. 2.3).

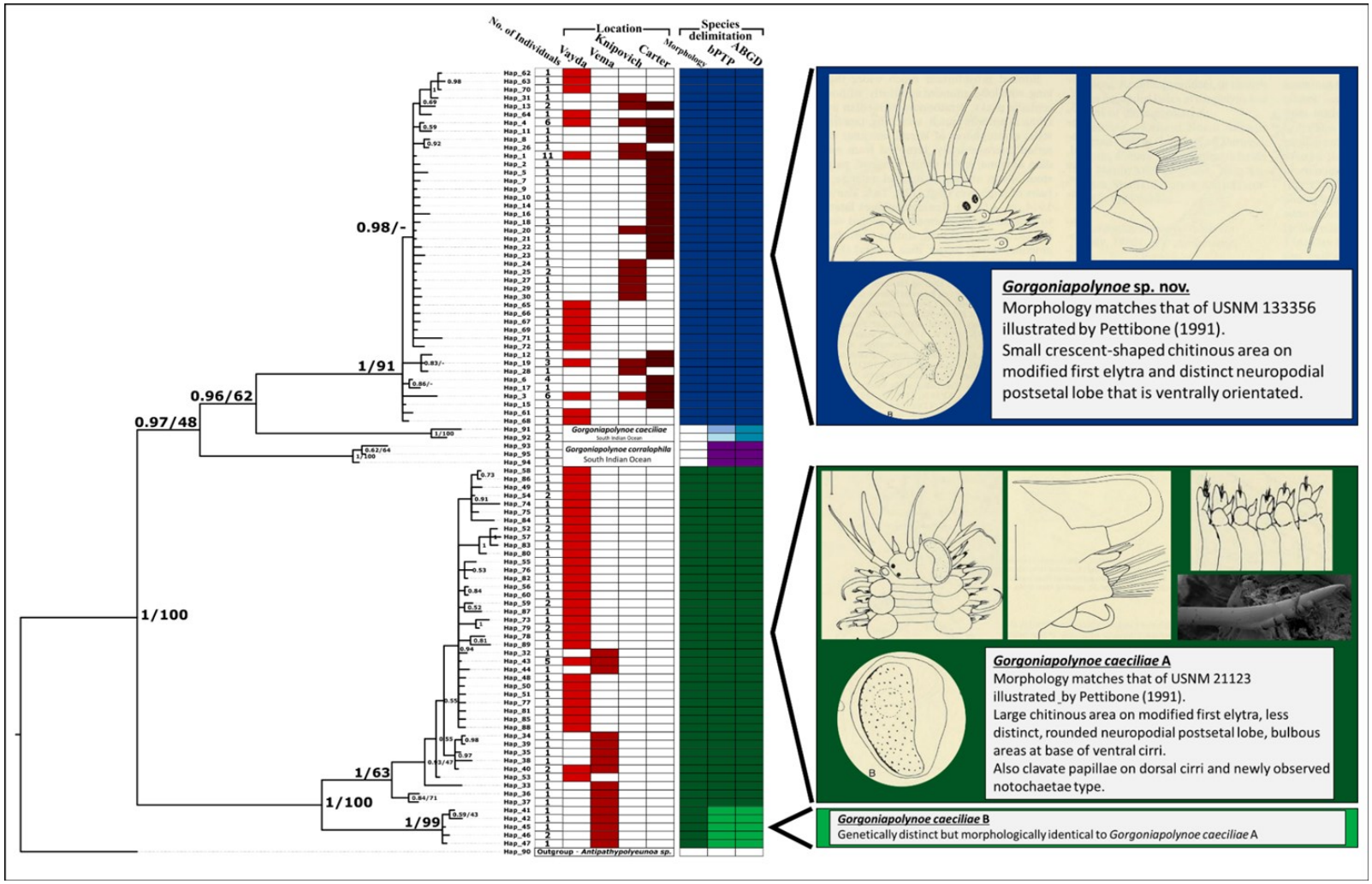

Figure 2.3. The cladogram recovered from the analysis of the *COI* haplotypes from individuals from the genus *Gorgoniapolynoe*. The tree topology is based on Bayesian Inference analysis. Node labels are the posterior probability from BI analysis, followed by the bootstrap support from Maximum likelihood analysis. To the right of the tree the number of individuals per haplotype and the locations from which the haplotypes are from. Included in the analysis were individuals from the Equatorial Atlantic collected for this study, individuals identified by Serpetti *et al*. (2017) as *G. caeciliae* and *G. corralophila* from the South Indian Ocean, and *Antipathypolyeunoa* sp. as the outgroup. Also included are the species delimitation as inferred from morphological analysis, and the species delimitation models ABGD (Puillandre *et al*. 2012) and bPTP (Zhang et al. 2013). The morphological characters which define the species boundaries are also presented. Illustrations were reproduced from Pettibone (1991). For haplotype details see supplementary material, Table S1.

2.3.2. *COI* analysis

In total there were 94 haplotypes recovered from the 127 individuals sequenced as part of this study and the six individuals sourced from GenBank (Table 2.5). The BI and ML analyses inferred the individuals from the Equatorial Atlantic sequenced for this current study are split into two distinct clades (Fig. 2.3). One clade contained 43 haplotypes representing 72 individuals, posterior probability (PP)=1, Bootstrap Support (BS)=91. This group is herein referred to as *Gorgoniapolynoe* sp. nov. The second clade contained 46 haplotypes representing 57 individuals (PP=1, BS=100). Within this clade a subclade was returned (PP=1, BS=100), representing six individuals, that were genetically distinct but without any corresponding morphological differences (see species delimitation below). The largest group within this clade (41 haplotypes, 66 individuals) is referred to as *Gorgoniapolynoe caeciliae* A, while the smaller subgroup (five haplotypes, six individuals) is be referred to as *Gorgoniapolynoe caeciliae* B

The topology inferred that *Gorgoniapolynoe caeciliae* A and *Gorgoniapolynoe caeciliae* B are closely related and are sister groups (Fig. 2.3). Gorgoniapolynoe *caeciliae* B was only found on one host *Corallium* sp. (parent ID 91) from Vema (supplementary material, Table 2.2) and was found in sympatry with *G. caeciliae* A. In turn, *Gorgoniapolynoe* sp. nov. are not closely related to *G. caeciliae* A and B, but appeared closely related to individuals identified as *G. caeciliae* by Serpetti *et al.* (2017) from the South Indian Ocean (PP=96, BS=62). Specimens identified as *Gorgoniapolynoe corralophila,* also from the South Indian Ocean (Serpetti *et al.* 2017), were returned as a sister group to *Gorgoniapolynoe* sp. nov. and *G. caeciliae* from the South Indian Ocean (PP=97, BS=48).

The three most common haplotypes found in *Gorgoniapolynoe* sp. nov. contained individuals from across three of the seamounts, Carter, Knipovich and Vayda (Fig. 2.3). The distance between Vayda and Cater is just under 3000 km. *Gorgoniapolynoe caeciliae* A had only two haplotypes that are shared by individuals from two seamounts from which they were found (Vema and Vayda), a distance of approximately 600 km. Of the six *Gorgoniapolynoe caeciliae* B, only two individuals shared a haplotype. A haplotype network based on the *COI* data for the Equatorial North Atlantic specimens can be seen in the supplementary material (Fig S1).

2.3.2. Species delimitation

The ABGD and bPTP models delimitated the haplotypes of individuals whose morphology matched that of USNM 133356 (Pettibone 1991), as a single species i.e. *Gorgoniapolynoe* sp. nov. (Fig. 2.3). The bPTP model had a PP of 0.72 for this being a single species (Supplementary material, Fig. S2). The inference by ABGD was based on a prior intraspecific difference range of P = 0.0065 – 0.0225. The ABGD model returned the two haplotypes from *G. caeciliae* from the South Indian Ocean as a single species. The bPTP model separated the two haplotypes into two different species (PP=0.51). Both models inferred *G. corralophila* to be a single species (bPTP, PP=0.68).

Individuals whose morphology matched that of USNM 21223 (Pettibone 1991) were delimitated into two separate species by both models i.e. *Gorgoniapolynoe caeciliae* A and *Gorgoniapolynoe caeciliae* B (Fig. 2.3). The bPTP PP for *G. caeciliae* B being a single species was 0.86. The PP for *G. caeciliae* A being a single species was 0.42 (see Supplementary material Fig S2). This is however is only based on one marker (*COI*) and the variance seen may represent intraspecific variation. Polychaetes have a large range in their interspecific and intraspecific variation, 0-52% and 0-37% respectively (Kvist 2016). In a study of Canadian polychaetes, Carr *et al.* (2011) a congeneric interspecific variation of 16.5% and an intraspecific variation of 0.38%. Carr *et al.* (2011) used ten times the intraspecific variation (3.8%) For ABGD, above the prior intraspecific difference of 0.0225 (2.25%), the model no longer recognised *G. caeciliae* B as a separate species from *G. caeciliae* A (Supplementary material, Fig. S3) but the other inferred delimitations remained. Above 5% intraspecific divergence the ABGD Model only returned one species for all samples. This suggests that the genus *Gorgoniapolynoe* may have low interspecific variation. To get a more robust result at least another marker would be needed to confirm whether or not *G. caeciliae* does in fact represent two morphologically but genetically distinct species. Brasier *et al.* (2016) used both *COI* and 16S sequences in their study on cryptic polychaetes. They showed that the inferred interspecific variation was many times higher than intraspecific variation in the *COI* compared to 16S. Based on this Brasier *et al.* (2016) suggest that there is no general rule for cryptic species delimitation in polychaetes and that the use of 16S in conjunction with *COI* can discriminate help in the differentiating between high intraspecific variation and potential cryptic species.

2.3.3. Phylogenetic analyses of 16S, 18S, 28S and *COI* concatenated sequences

The phylogenetic analyses for the family Polynoidae (Fig. 2.4), recovered the majority of members in the subfamily Polynoinae grouped together with strong support (PP=1, BS=99). However, the positions of *Paradyte crinoidicola* (Potts, 1910) and *Paralepidonotus ampulliferus* (Grube, 1878) mean that that Polynoinae was returned as polyphyletic group. *Paradyte crinoidicola* returned as a sister species to the two members of Arctoninae, *Capitulatinoe cupisetis* (Hanley & Burke, 1989) and *Gastrolepidia clavigera* (Schmarda, 1861)) that were included in the analysis (PP=1, BS=100). *Paralepidonotus ampulliferus* was recovered as the most basal taxa within Polynoidae (PP=0.90, BS=66). The only subfamily to return as monophyletic was Macellicepalinae (PP=1, BS=100). Members of the genus *Gorgoniapolynoe* were all recovered within a monophyletic clade composed of members of the family Polynoinae (PP=1, BS=100) (Figure 2.4). An *Antipathipolyeunoa* sp. (Pettibone 1991), *Robertianella synophthalma* (McIntosh, 1885) and *Brychionoe* sp. (Hanley & Burke, 1991) were returned closest to *Gorgoniapolynoe* but without good support (BB=0.6, BS=38). All are described as deep-sea commensal polychaetes, *Antipathipolyeunoa* sp. and *Brychionoe* sp. live on deep-sea antipatharian corals, and *R. synophthalma* on hexactinellid sponges (Martin and Britayev 1998). As a group (*Gorgoniapolynoe*, *Antipathipolyeunoa* sp., *R. synophthalma* and *Brychionoe* sp.) there is strong support for their position (BB=1, BS=86).

Specimens from each of the clades identified in Fig. 2.3, along with specimens sourced from Genbank (Tables 2.5 and 2.6), were used for the placement of the different Gorgoniapolynoe species in its phylogenetic context. Two individuals identified as *Gorgoniapolynoe* sp. nov. in this study (*Gorgoniapolynoe caeciliae* 602 and 1217) grouped together (Figure 2.4). The three *G. caeciliae* collected in the South Indian Ocean (*Gorgoniapolynoe caeciliae* SIO1, SOI2 and SOI3) were returned next to the two aforementioned specimens (PP =1, BS=86). The *G. corralophila* (*Gorgoniapolynoe corralophila* SIO1, SIO2 AND SIO3 was recovered outside the two previously mentioned groups (PP=1, BS=82). The rest of the Equatorial Atlantic specimens composed of two *Gorgoniapolynoe caeciliae* A (*Gorgoniapolynoe caeciliae* 714 and 2194) and one *Gorgoniapolynoe caeciliae* B (*Gorgoniapolynoe caeciliae* 1743) formed a sister group to the rest of the specimens (PP=1, BS=100). The topology of the multi-gene cladogram is congruent with the topology of the *COI* haplotype cladogram (Fig 2.3).

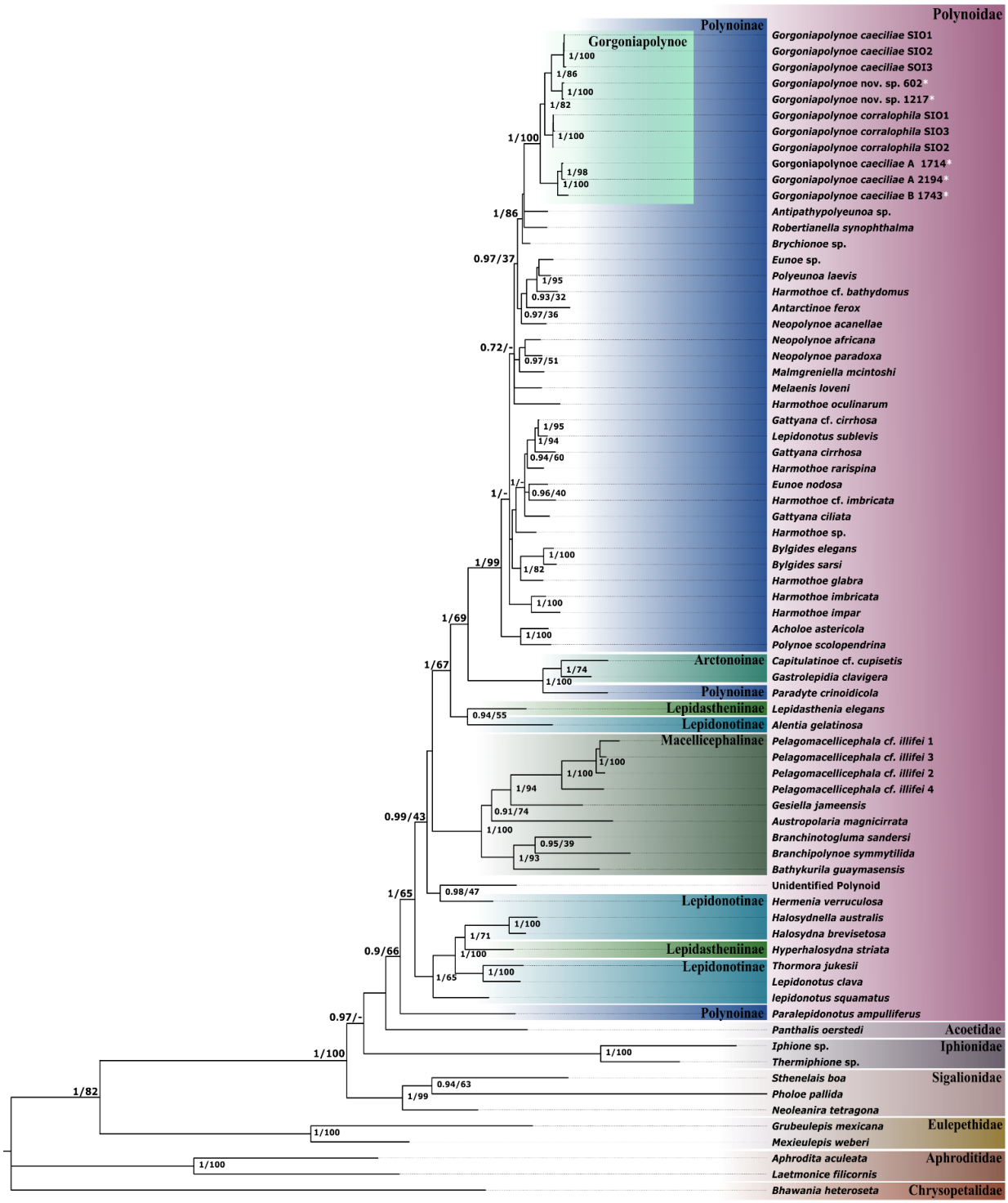

Figure 2.4. The phylogenetic tree of Polynoidae, recovered from analysis of the concatenated sequences of 16S, 18S, 28S and COI. The tree topology is based on Bayesian Inference analysis. Node labels are the posterior probability from BI analysis, followed by the bootstrap support from Maximum likelihood analysis. Only nodes with significant PP support (>0.95) are presented. The individuals from the genus *Gorgoniapolynoe* are highlighted and include individuals from this study (marked with *) and individuals sourced from GenBank from the South Indian Ocean, see Tables 2.5 and 2.6. For voucher IDs and accession numbers for all other specimens refer to Table 2.4.

2.3.4. Analysis of molecular variance (AMOVA)

For AMOVA the haplotype dataset was divided into the two clades i.e. one clade containing *Gorgoniapolynoe* sp. nov. and the other containing *Gorgoniapolynoe caeciliae* A and B. *Gorgoniapolynoe* sp. nov had no significant variation at any level ($p<0.05$) (Table 2.8). When the genetically distinct but morphological identical *Gorgoniapolynoe* sp. nov. A and *Gorgoniapolynoe* sp. nov. B was analysed as a single group, there was significant variation among hosts (populations) at a location and on individual hosts (Table 2.8). When the analysis was run again without *Gorgoniapolynoe* sp. nov. B, there was only significant variation found was within populations.

Table 2.8. The results of analysis of molecular variance (AMOVA) on the *COI* data for *Gorgoniapolynoe* from the Equatorial North Atlantic. Clades were determined by Bayesian inference and maximum likelihood analysis. * marks significant variation ($p<0.05$).

Source of variation	**d.f.**	**Sum of squares**	**% of variance**	***F*-statistics**	***P***
***Gorgoniapolynoe* sp. nov.**					
Between seamounts	2	1.763	-9.18	F_{CT} = -0.09182	0.9696
Between hosts within a location	7	17.886	11.05	F_{SC} = 0.10123	0.0649
On individual hosts	60	103.214	98.13	F_{ST} = 0.01871	0.1376
***G. caeciliae* A and B**					
Between seamounts	1	50.474	30.27	F_{CT} = 0.30270	0.0984
Between hosts within a location	3	41.902	18.83	F_{SC} = 0.27008	0.0085*
On individual hosts	52	138.465	50.9	F_{ST} = 0.49103	0*
***G. caeciliae* A only**					
Between seamounts	1	12.515	23.92	F_{CT} = 0.23920	0.1019
Between hosts within a location	3	6.229	0.64	F_{SC} = 0.00835	0.385
On individual hosts	46	88.021	75.44	F_{ST} = 0.24556	0.0009*

2.3.5. Taxonomic descriptions

Family Polynoidae Kinberg, 1856

Genus *Gorgoniapolynoe* Pettibone 1991

Genus diagnosis

Body dorso-ventrally flattened, becoming more pronounced towards the posterior, with approximately 60 segments. Paired elytra on segments 2, 4, 5, 7, alternative segments to 23, from which every third segment up to 32; anterior most segments the cover the dorsum, after which the mid-dorsum is exposed. First 1-3 pairs of elytra are modified, with a translucent, chitinous area. Prostomium bilobed, wider than long, with rounded to subtriangular lobes; with or without cephalic peaks; Long distal style median antenna inserted in anterior notch; a pair of short lateral antenna, removed from median antenna and inserted ventrally. Two pairs of eyes. First segment not distinct dorsally; Pair of dorsal and ventral tentacular cirri. First pair of elytrophores on buccal segment; long ventral buccal cirri that are lateral to the mouth. Eversible pharynx, with two pairs of jaws and nine pairs of border papillae.

Biramous parapodia, subconical notopodia are smaller and shorter than neuropodia; Neuropodia with presetal acicular lobe that is diagonally truncated; postsetal lobe much shorter. Notochaeta range from zero to seven, that are smooth, stout and acicular. Seven to fifteen neurochaetae, as stout as notochaeta but longer; usually bidentate, with spinose rows. Long dorsal cirri on non-elytrigerous segments. Ventral cirri, that are as long/longer than neuropodia.

Found in tubes formed by modified sclerites of a deep-sea alcyonacean coral.

Syntype of *Gorgoniapolynoe caeciliae* (Fauvel 1913)

Fauvel 1913:24, Fig. 7, b; 1914:69, pl. 4: Fig. 19; 1923:82, Fig. 31, c; Pettibone 1991: Fig. 12, E-F.

Material examined

Two parapodia from syntype of *Polynoe caeciliae*, Eastern North Atlantic Ocean: Gulf of Gascony, 45°05’N, 9°54’W, 1241 m, Prince de Monaco station 2743, 27/07/1908, on *Corallium johnsoni*, (parapodia only, USNM 80098).

Western North Atlantic Ocean: Caribbean Sea, West Indies, off St. Vincent, Lesser Antilles, 13°34’N, 61°03’W, 512 m, *Albatross* station 2753, 04/12/1886, on *Candidella imbricate*, two

specimens, removed by F. M. Bayer (USNM 21123); Off Portugal, 40°33'N, 9°26'W, 1170 m, *Thalassa* station Y405, 01/09/1972, on *Corallium niobe*, identified by M. Grasshoff, from G. Hartmann-Schröder, one specimen (USNM 133356)

Description

Two biramous parapodia, one is from an elytrigerous segment and the other is from a cirrigerous segment. The notopodium a subconical lobe while the neuropodium longer and wider, with presetal and postsetal lobes. The acicular presetal lobe is diagonally truncated and projects dorsally. The shorter postsetal lobe, is rounded and projects slightly ventrally. The orientation of the pre- and postsetal lobes cause the neuropodium to appear bilobed when viewed anteriorly. The postsetal lobe is more distinct in the elytrigerous parapodium compared to the cirrigerous one. 1-2 notochaetae, that are acicular, stout and slightly curved, anterior and slightly ventral to notopodium, longer than the notopodium. 13-15 notochaetae, which are as stout as the notochaeta but much longer, distal third widens becoming quill-like, with spinulose rows. Tips of neurochaetae are bidentate, with a slight change in form from dorsal to ventral, the mid-neurochaetae have the most pronounced bidentation, with a large curved primary tooth and a smaller pointed secondary tooth. The dorsal chaetae have a less pronounced secondary tooth, which is more rounded. The secondary tooth on the ventral chaetae is reduced to a rounded bump and the primary tooth is rounded rather than pointed. Both parapodium have tapering ventral cirri still attached. Slightly longer than the neuropodium.

Remarks

The two syntypes for the species *G. caeciliae* (originally *Polynoe caeciliae*) where originally collected by Fauvel from two locations, the Gulf of Gascony and Cape Verde Islands (Fauvel 1913). Fauvel only illustrated the one from the Gulf of Gascony (1913:24 Fig. 2.7 B; 1914:69, pl. 4: Fig. 19; 1923:82, Fig. 31 I), and described the other (from Cape Verde Islands) as being in bad condition but reporting that it did not differ from the illustrated specimen.

When Pettibone (1991) was redescribing the species she examined and illustrated two parapodia (Fauvel (1913, 1914) only illustrated one) and stated they were from the syntype from was from the Gulf of Gascony (Pettibone 1991: Fig 12 E-F). The parapodia were originally held by the Monaco Oceanographic Museum but are now deposited in the collections of Smithsonian National Museum of Natural History (USNM 80098). At the time

of writing it is unclear if the original full body syntypes still exist as the Monaco Oceanographic Museum was non-responsive to any enquiry. It would appear however that Pettibone only got to examine the parapodia and did not examine a whole-body specimen for the redescription. The two syntype parapodia that Pettibone examined and illustrated were the same two that were examined for this study.

The shape and orientation of the postsetal lobe and the bidentate neurochaetae tips are most like those seen on the specimen USNM 21123. Hence the reason for individuals whose morphology being congruent with USNM 21123 retaining the name *Gorgoniapolynoe caeciliae*

***Gorgoniapolynoe caeciliae* sp. A and B**

Fauvel 1913:24, Fig. 7 A-D; 1914:69, pl. 4: Figs. 1-6, 18-19; 1923:82, Fig. 31a-h. Belloc, 1953:4. Hartmann-Schröder, 1985:31, Fig. 1-11 (not Indian Ocean). Pettibone 1991: Fig. 14 (reproduced here as Fig. 2.5). Britayev et al, 2014:34, Figs. 5c-h, 8, 9, 10.

Figs. 2.5-2.10

Material examined

Western North Atlantic Ocean: Caribbean Sea, West Indies, off St. Vincent, Lesser Antilles, 13°34'N, 61°03'W, 512 m, *Albatross* station 2753, 04/12/1886, on *Candidella imbricate*, two specimens, removed by F. M. Bayer (USNM 21123); eight specimens, removed by M. Pettibone (USNM 80091). Straits of Florida, east of St. Lucie Inlet, 27°06'N, 79°32'W 659-677 m, *RV Gerda* cruise 6333 station G170, 29/06/1963, on holotype of *Corallium niobe*, three specimens, removed by F. M Bayer (USNM 133357). Off Georgia, 30°44'N, 79°26'W, 805 m, Albatross station 2415, 01/04/1885, on Acanthogorgia aspera, two specimens, removed by F. M. Bayer (USNM 80090).

Central West Atlantic, Vema Seamount, 10° 42'N, 44° 25'W, 593 m, JC094 station 41, on *Acanthogorgia sp.*, 13 specimens; 10°46'N, 44°36'W, 2190 m, JC094 station 42, on *Corallium sp.,* ten specimens, of which SEM stub was prepared from specimen 1771_4_Ve .

Central West Atlantic, Vayda Seamount: 14° 51'N, 48° 14'W, 1416 m JC094 station 45, on *Corallium sp*., 11 specimens on of which 1819_2_Va was used for histological analysis; 14° 51'N, 48° 15'W, 1622 m, JC094 station 49, on *Corallium sp*., 9 specimens, of which SEM stubs were prepared for specimens 2194__3_Va and 2191_5_Va.

Description

Description - USNM 21223, 2 specimens, one 11 mm long, 2.5 mm wide, with 38 segments; the other 14 mm long, 2.7 mm wide with 46 segments. Body almost cylindrical at anterior end, becomes progressively more dorso-ventrally flattened. Prostomium is bilobed, with rounded lobes without cephalic peaks, wider than long, two pairs of large eyes. Medial antenna present, long and tapering, attached to ceratophore in notch between prostomium lobes; subulate and short lateral antennae, inserted lateroventrally and removed from the medial antenna; two stout palps that are as long as the medial antenna (Figure 2.5). Tentaculophores lateral to the prostomium on achaetous first segment, Pair of dorsal and ventral tentacular cirri, as long/longer than medial antenna. Second segment has first pair of elytrophores, ventral buccal cirri and biramous parapodia.

15 pairs of elytra on segments 2, 4, 5, 7, then every second until 23, 26, 29 and 32. The first pair of elytra are large and cover the prostomium, modified with a large bean shaped, transparent, chitinous area, recessed and positioned on the lateral side of the elytron, covers an area over half the full area of the elytra, scattered rounded microtubercles and elongated, barrel-shaped micropapillae (Figure 2.5, A-B; Figure 2.6, A; Figure 2.7, A). The following elytra are smaller, oval with curved over borders, leave the mid-dorsum exposed, opaque, area around the elytrophore is darker and not opaque, very faint "veins" present that are only visible under microscope (Figure 2.5, C; Figure 2.6, B). Histological sections reveal that darker area corresponds to the presence of cup-like cells, possible photocytes (Figure 2.8, B). scattered cylindrical micropapillae also present. Emerging from the tips of all micropapillae, on all elytra, are 3-4 flagellum-like structures, many times longer than papillae, appear to be able to retract, at their distal end have flattened circular structures, which have scattered wart-like structures (Figure 2.7)

Dorsal cirri present on segments without elytra, long, around three times as long as the parapodia, sporadic clavate papillae present on proximal end (Britayev, *et al*. 2014: Fig. 2.8, D), cylindrical cirrophores from the posterior side of notopodium. Ventral cirri on all segments after second segment, base of cirri slightly inflated before tapering, reach past tip of neuropodium. From segment 10 bulbous, swollen area at base of ventral cirri causing them to project posteriorly (Figure 2.5, D). The posterior most segments without elytra have rows hair-like structures, dorsal, traverse segments between cirri (See Figure 2.14 for identical structures seen in *Gorgoniapolynoe* sp. nov.). Large, digitate nephridial papillae from

segment six, posterior-ventrally positioned on parapodium (Britayev, *et al*. 2014: Fig. 2.8, D-E).

Biramous parapodia, notopodium is a subconical acicular lobe; neuropodium longer and wider, acicular presetal lobe is diagonally truncated, projects dorsally, shorter flattened postsetal lobe that lays close to neurochaetae, rounded, projects slightly ventrally in anterior segments so that it can just be seen when viewed from anterior(Figure 2.5, E-F), in posterior segments becomes massively reduced. 3-7 notochaetae on anterior segments, reduced to 2-4 in posterior; two types present in anterior segments, a stout, acicular, slightly curved chaeta, with groove on outside of the curve and a stout, acicular slightly curved chaeta, with alternating spinous pockets from the apex of the curve to the tip (Figure 2.9). Both notochaetae are longer than notopodium and positioned anterior to the notopodium. 10-15 neurochaetae, as stout as notochaetae, last third or so becomes quill like, with spinulose rows; tips are bidentate to varying degrees, change in form from dorsal to ventral; dorsal chaeta have a large blunt tooth with a smaller rounded secondary tooth (Figure 2.10, B), mid chaeta have more pointed larger tooth which is slightly hooked, secondary smaller tooth slightly more distinct that those seen in dorsal chaetae (Figure 2.10, C), ventral chaeta have blunt rounded larger tooth with a highly reduced secondary tooth that is barely more than a bump (Figure 2.10, D).

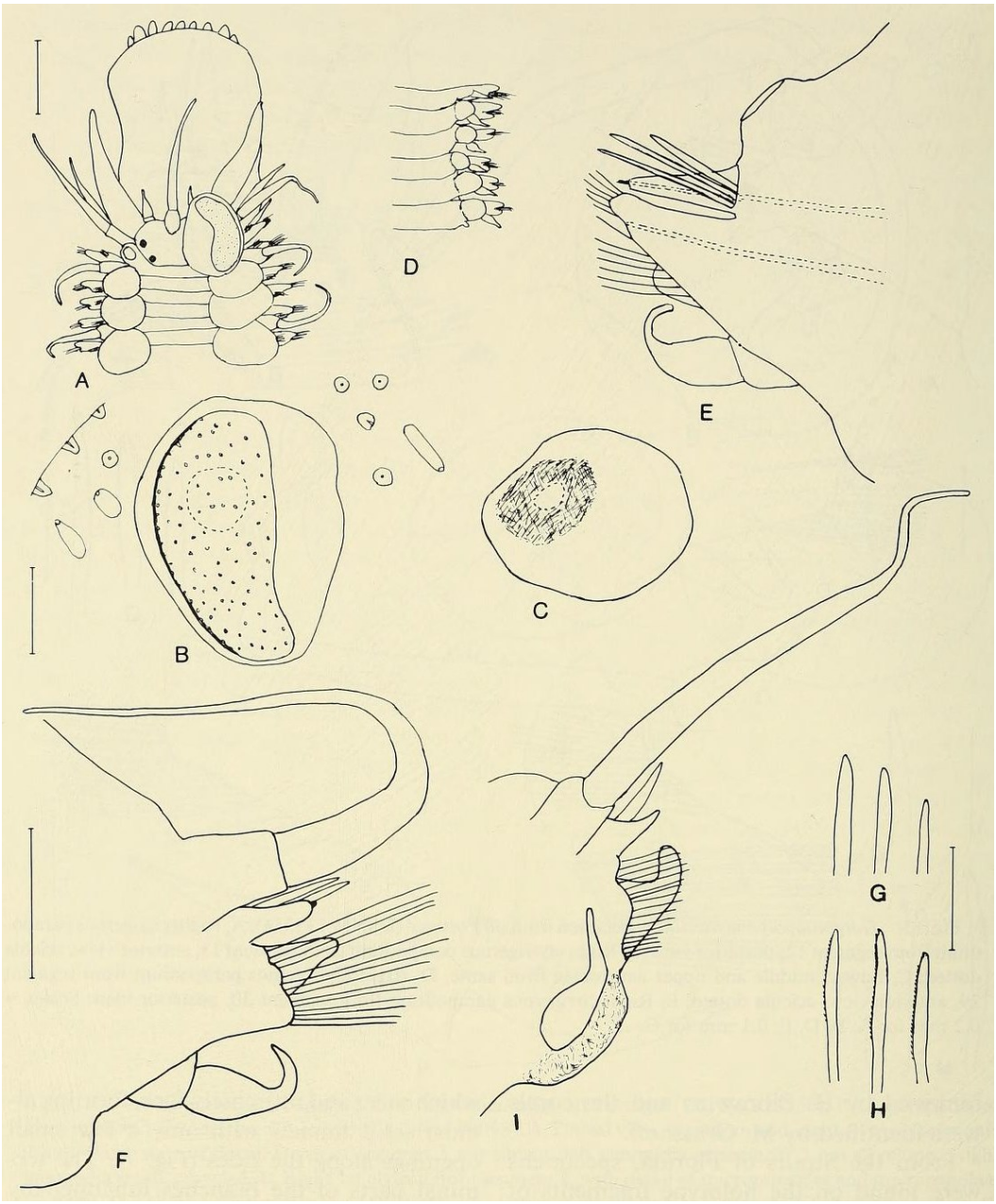

Figure 2.5. *Gorgoniapolynoe caeciliae* specimen from Lesser Antilles (USNM 21123), figure taken from Pettibone (1991): **A**. Dorsal view of anterior end, pharynx fully extended, left 1st elytron removed; **B**. Left 1st elytron from segment 2, with detail of microtubercles and micropapillae; **C**. Left middle elytron; **D**. Ventral view of left side of segments 9-14, showing bulbous areas near bases of ventral cirri; **E**. Right elytrigerous parapodium from anterior region, anterior view, acicula dotted; **F**. Right cirrigerous parapodium from anterior region, posterior view; **G**. Notochaetae from same; **H**. Upper, middle and lower neurochaetae from same; **I**. right cirrigerous parapodium from posterior region, posterior view. Scales = 0.5 mm for A, D; 0.2 mm for B, C, E, F, I; 0.1 mm for G, H.

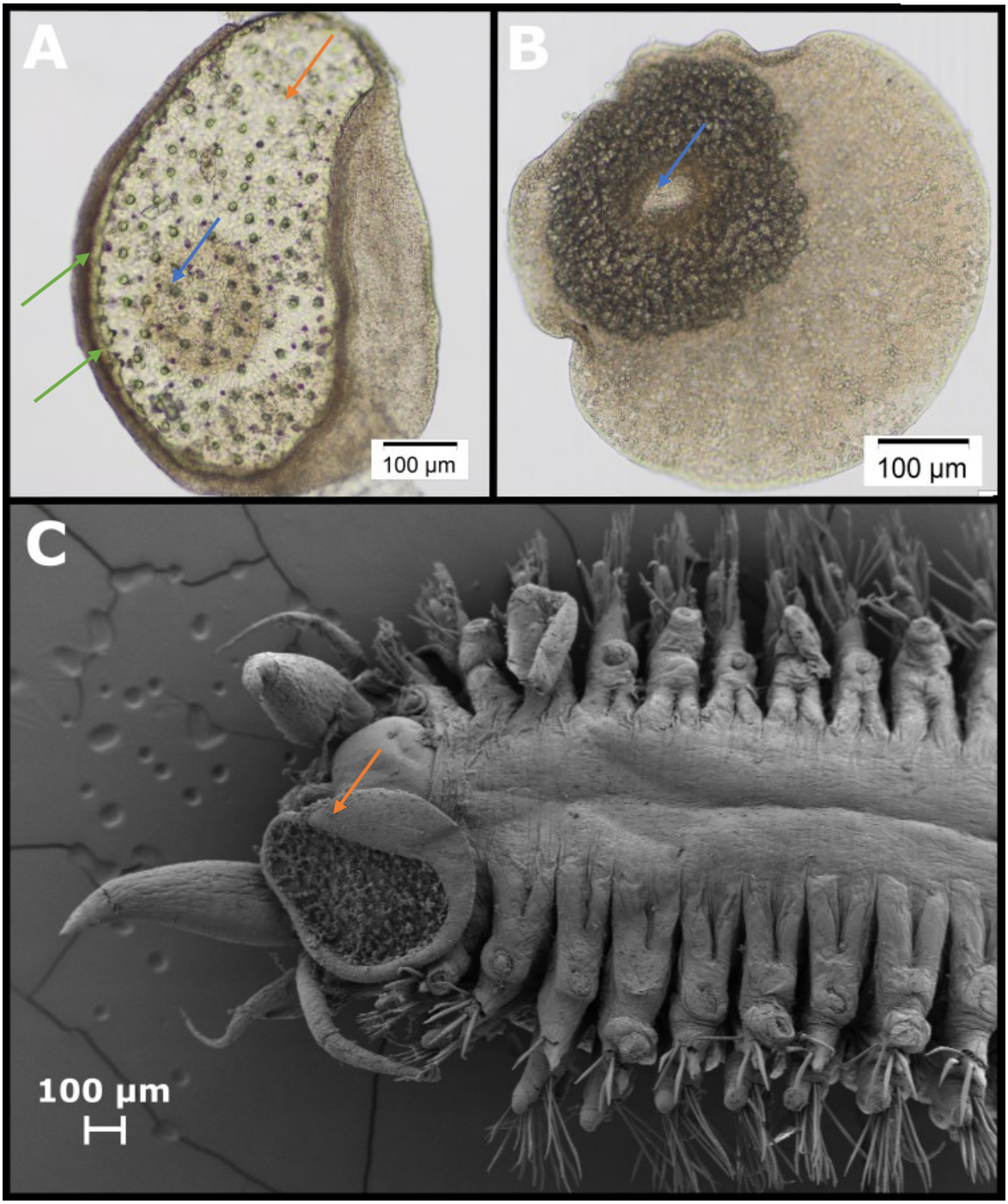

Figure 2.6. Elytra and anterior part of the body of *Gorgoniapolynoe caeciliae* **A.** The modified first elytron (left) from 1879_3_Va. **B.** The third elytron from same **C.** The anterior of Gorgoniapolynoe *caeciliae*, 2194__3_Va, with the left first modified elytron present. Arrows: Blue = elytrophore, Orange = chitinous area, Green = micropapillae.

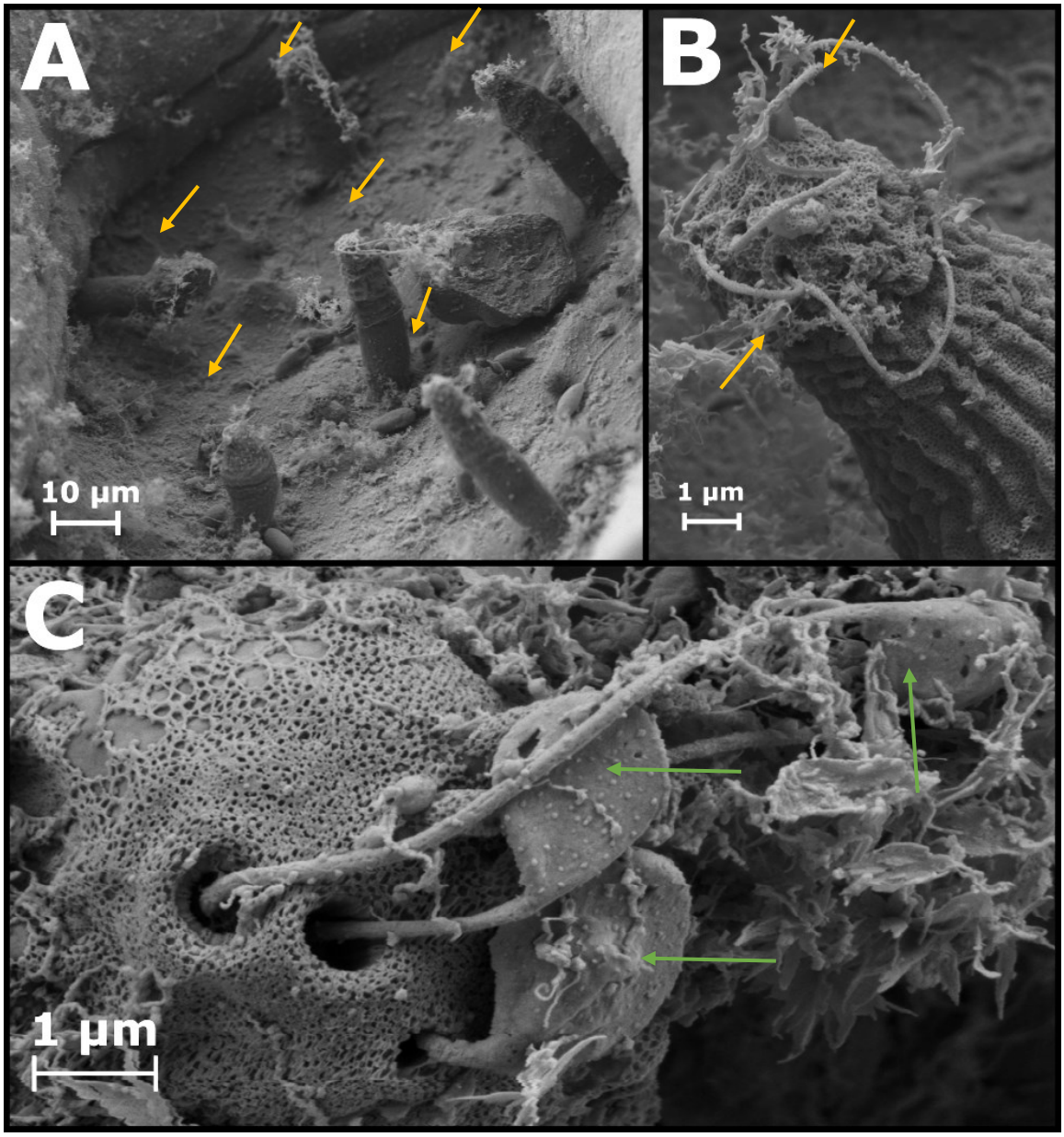

Figure 2.7. SEM micrographs of the flagella-like structures emerging from the tips of the papillae of the elytra of *Gorgoniapolynoe caeciliae* 2194__3_Va and *Gorgoniapolynoe* sp. nov. 602_8_Va. These features were observed on both species and may be a characteristic of genus *Gorgoniapolynoe* A. Papillae in the chitinous area of the first elytron of 602_8_Va. Yellow arrows mark the flagellum-like structures. **B**. The flagella-like structures emerging from a single papilla located in the chitinous area of the same elytron as A. **C.** The flattened ovoid structures at the distal end of the flagellum-like structures (green arrows) observed on *Gorgoniapolynoe caeciliae* 2194__3_Va

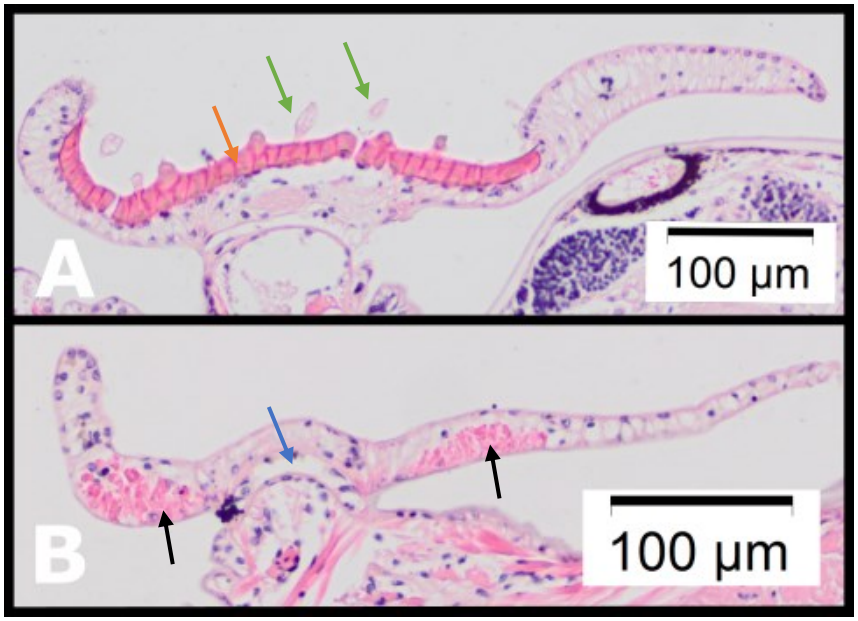

Figure 2.8. The histological sections of the elytra of Gorgoniapolynoe caeciliae, 1819_2_Va. **A.** The first modified elytron showing the chitinous area, with a papilla. **B.** Possible cup-shaped photocytes within the second elytra. **E**. shows the possible photocytes concentrated around the elytrophore, corresponding to the darker area in B. Arrows: Blue = elytrophore, Orange = chitinous area, Green = micropapillae, Black = possible photocytes.

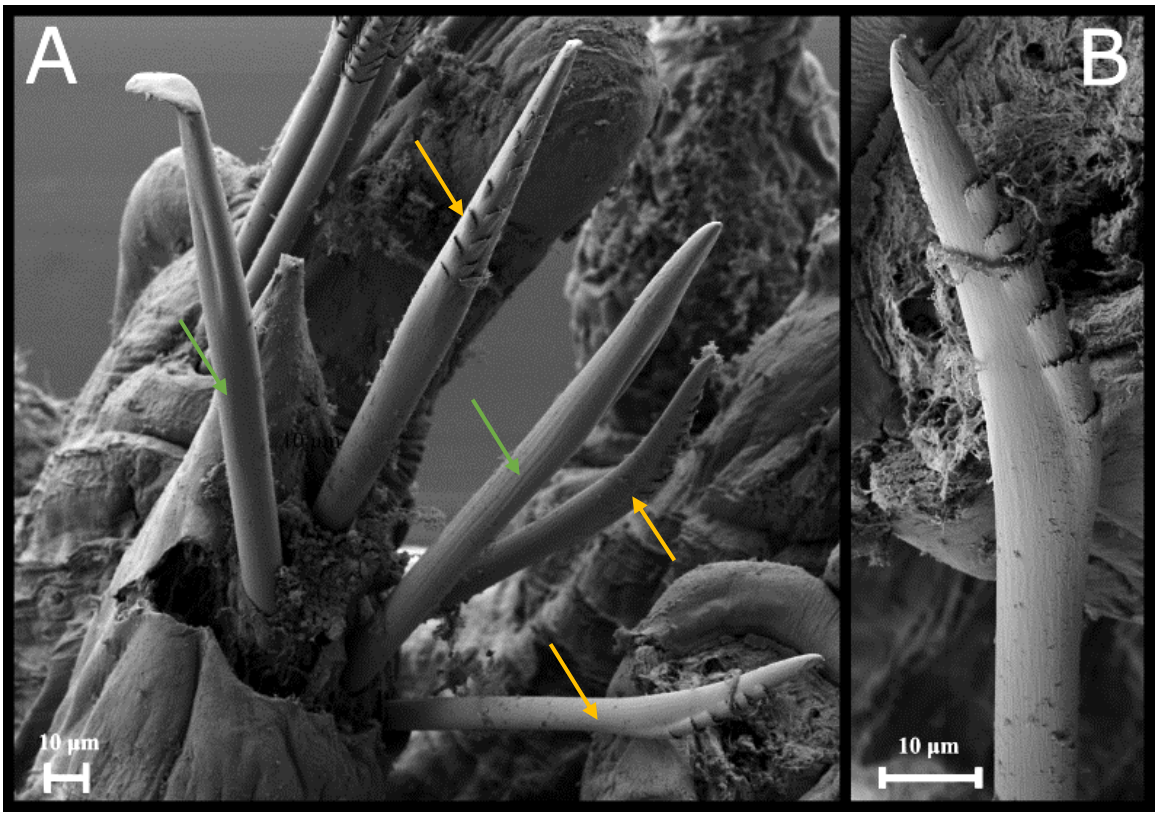

Figure 2.9. A SEM micrograph of the notochaeta on the third parapodia of a specimen identified as *Gorgoniapolynoe caeciliae* 1879_3_Va. **A**. Showing the two types of notochaeta, the newly observed chaetae with alternating spinous pockets (yellow arrows) and the smooth slightly curved chaetae (green arrows). **B**. The profile of the notochaeta with spinous pockets.

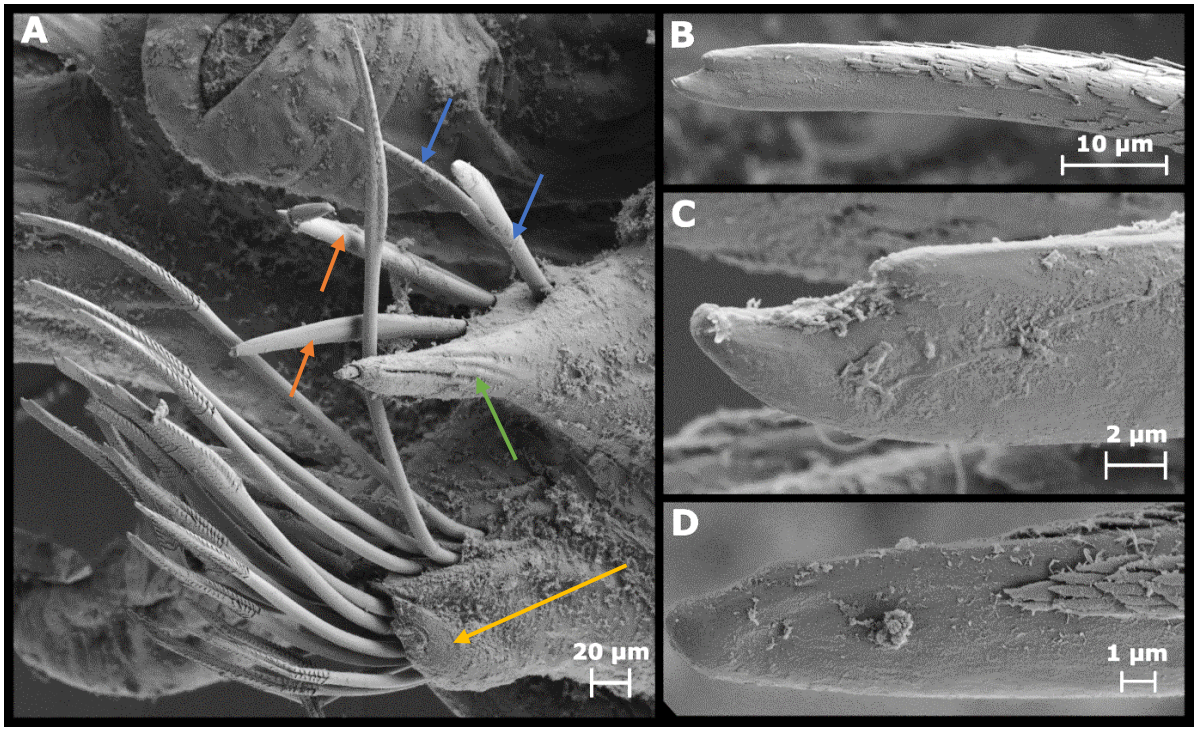

Figure 2.10. A SEM micrograph of the third parapodium of an individual identified as *Gorgoniapolynoe caeciliae*, 1771_4_Ve, with details of the tips of the neurochaetae. **A**. The posterior of the third parapodium. The notopodium (green arrow) and the flattened, rounded neuropodial postsetal lobe (yellow arrow) can be seen. Both types of notochaetae are also present, smooth (blue arrow) and spinous pocketed (orange arrow). **B**. The dorsal most neurochaetae. **C**. Mid-neurochaetae. **D**. ventral most neurochaetae. B, C and D are all from the same neuropodium

Remarks

The written description by Pettibone (1991) is an amalgamation of features from two different specimens (USNM 133356 and USNM 21123). The parapodial syntypes (USNM 80098) have the same morphology as the parapodia of USNM 21123 and herein defined as *G. caeciliae sens. strict.* The morphology of the Equatorial Atlantic specimens examined for the above description match that of USNM 21123. The presence of 3-7 notochaetae on anterior segments, observed for the Equatorial Atlantic specimens, are not mentioned or illustrated in Pettibone (1991). On re-examination of USNM 21123 (two specimens) there were 4-6 notochaetae observed to be present on segments three, five, six, eight of one specimen and segment three, five and ten on the other. The newly described notochaetae (Figure 2.9) in this study were only observable using SEM, so unable to confirm if they were present on the segments of the specimens of USNM 21223 that had 4-6 notochaetae. The newly observed notochaetae are similar in appearance to the notochaetae seen in *G. corralophila* (Day 1960). Additionally, the specimens USNM 80090, 80091 and 133357 (all of which were examined

by Pettibone for her description) agree with the morphological features observed on *G. caeciliae.*

The Equatorial Atlantic specimens also agree well with the re-description by Britayev *et al.* (2014: Figs. 5 C-H, 8). Britayev *et al.* (2014) added the clavate papillae on the dorsal cirri to Pettibone's 1991 description. The presence of the papillae, the form of the parapodia and modified first elytra seen in Britayev *et al.* (2014) suggest that the specimens observed in that Britayev *et al*. (2014) are *Gorgoniapolynoe caeciliae.*

Gorgoniapolynoe caeciliae is a separate species from *Gorgoniapolynoe* sp. nov. based on morphological and molecular evidence (Fig. 2.3). However, *Gorgoniapolynoe caeciliae* may contain cryptic species. The analysis of the *COI* sequences revealed that six of the 43 individuals identified as *G. caeciliae* were genetically distinct and were delimitated to be a separate species, *G. caeciliae* A and B, under ABCD and bPTP models (see section 2.3.2; (Fig. 2.3). Whether or not *G. caeciliae* A and *G. caeciliae* B are separate species needs more investigation. If they are indeed genetically distinct species then a DNA holotype will be need for each, as well as whole specimen paratypes. In the meantime, it is suggested that USNM 21223 is used as paratypes for identification, as they are whole body specimens unlike the syntypes.

Ecology

Unfortunately, the corals that *Gorgoniapolynoe caeciliae* were found on were not identified to species level. All the corals on which the specimens were found in association with were in the genus except for individuals from one location that were found on an *Acanthogorgia* sp. They were removed from galleries formed by modified sclerites of the host corals. Specimen USNM 21223 and USNM 80091 were removed from *Candidella imbricata*, USNM 80090 was on *Acanthogorgia aspera*, USNM 133357 came from *Corallium niobe* (Pettibone 1991). The specimens reported by Britayev *et al.* (2014) were found in association with *C. imbricata* and *C. niobe.* All of these USNM specimens were re-examined and confirmed to be *Gorgoniapolynoe caeciliae* based on their morphology. No genetics were available for these specimens however so it was not possible to assess if they may be cryptic species or not. The genetically distinct clade (possible cryptic species) *Gorgoniapolynoe caeciliae* B were found in association with A *Corallium sp*. Individuals that were genetically identified as *Gorgoniapolynoe caeciliae* A were also colonising the same host.

In this current study the species was only recorded to the west of the mid-Atlantic ridge at Vayda and Vema. The specimens examined by Pettibone (1991) and re-examined for this study were from the Caribbean Sea, St Lucia and off Georgia. Specimens, assumed to be *Gorgoniapolynoe caeciliae* based on the description and illustrations by Britayev *et al.* (2014), were recorded from the Galicia Bank, and the Aviles Canyon System to the north of the Iberian Peninsula. The total range for the species therefore stretches from the eastern continental shelf of the United States, across the Atlantic, and up the western continental shelf of southern Europe. Previous records for *Gorgoniapolynoe caeciliae* were sampled from depths ranging from 512-1585 m (Pettibone 1991, Britayev *et al.* 2014). Specimens from this current study were sampled from depths ranging from 593 – 2190 m, meaning they are the deepest record for the species.

***Gorgoniapolynoe* sp. nov.**

Pettibone 1991: Figs, 12, 13(reproduced here as Figure 2.11 Figure 2.12)

Figs 2.11-2.15

Off Portugal, 40°33'N, 9°26'W, 1170 m, *Thalassa* station Y405, 01/09/1972, on *Corallium niobe*, identified by M. Grasshoff, from G. Hartmann-Schröder, one specimen (USNM 133356)

Central East Atlantic, Carter Seamount: 9°12'N, 21°17'W 1783 m, JC094 station 07, on *Candidella sp*., one specimen; 9°12'N, 21°17'W 1442 m, JC094 station 07, on *Corallium sp.*, two specimens; 9°10'N, 21°16'W, 2343 m, JC094 station 11, on *Corallium sp.*, 16 specimens; 9°12'N, 21°18'W, 1364 m, JC094 station15 on Primnoidae, two specimens; 9°12'N, 21°18'W, 1364 m, JC094 station 15, on *Corallium sp.*, nine specimens including one specimen, 602_8_Va, for which a SEM stub was prepared; 9°12'N, 21°17'W, 1367 m, JC094 station 15, on *Corallium sp.*, five specimens

Central Atlantic, Knipovich Seamount, 5°36'N, 26°57'W, 1445 m, JC094 station 21, on *Corallium sp.*, 14 specimens.

Central West Atlantic, Vayda Seamount: 14°53'N, 48°9'W, 772 m, JC094 station 48, on *Corallium sp*., three specimens and 710 m, on *Corallium sp.*, 4 specimens

Description

Specimen USNM 133356 16 mm long, 3.2 mm wide, with 51 segments. Specimen 602_8_Va examined using SEM from Carter seamount, 13 mm long, 3 mm wide, with 38 segments.

Body almost cylindrical at anterior end, after approximately segment 13 it becomes progressively more dorso-ventrally flattened. Prostomium is bilobed, with rounded lobes without cephalic peaks, wider than long, two pairs of large eyes. Medial antenna present, long and tapering, attached to ceratophore in notch between prostomium lobes; subulate and short lateral antennae, inserted lateroventrally and removed from the medial antenna; two stout palps that are as long as the medial antenna (Figure 2.11, A) Tentaculophores lateral to the prostomium on achaetous first segment, Pair of dorsal and ventral tentacular cirri, as long/longer than medial antenna. Second segment has first pair of elytrophores, ventral buccal cirri and biramous parapodia.

15 pairs of elytra on segments 2, 4, 5, 7, then every second 23, 26, 29 and 32 (SEM specimen 602_8_Va only had elytrophores until segment 26, presumably not fully grown). The first pair of elytra are large and cover the prostomium, modified with a crescent-shaped, transparent, chitinous area, that is recessed and positioned on the lateral side of the elytron, scattered rounded microtubercles and elongated, cylindrical micropapillae (Figure 2.11, A-B; Figure 2.13, A). The following elytra are smaller, oval with curved over borders, leave the mid-dorsum exposed, opaque with vein-like pattern branching from the elytrophore (visible under stereoscope), area around the elytrophore is darker and not opaque (Figure 2.11, D-C;Figure 2.13, B). Histological sections reveal that darker area corresponds to the presence of cup-like cells, possible photocytes (Figure 2.13); scattered cylindrical micropapillae also present. Emerging from the tips of all micropapillae, on all elytra observed, are 3-4 long flagellum-like structures, appear to be able to retract, which at their distal end have flattened circular structures, which have scattered wart-like structures (Figure 2.7).

Dorsal cirri present on segments without elytra, long, extending past tips of parapodia, cylindrical cirrophores from the posterior side of notopodium. Ventral cirri on all segments after second segment, cylindrical cirrophore, base of cirri slightly inflated before tapering, reach past tip of neuropodium. Last 10 segments without elytra have rows of hair-like structures on dorsal side, traverse segments between cirri (Figure 2.14). Small, digitate nephridial papillae from segment six, posterior-ventrally positioned on parapodium.

Biramous parapodia, notopodium is a subconical acicular; neuropodium longer and wider, acicular presetal lobe is diagonally truncated that projects dorsally shorter postsetal lobe,

rounded, projects ventrally and slightly towards the posterior lobe projection of pre- and postsetal lobes cause neuropodium to appear bilobed when viewed anteriorly ((Figure 2.12, A-B, D; Figure 2.15, A), in posterior segments becomes reduced and is no longer projecting ventrally, takes same orientation as presetal lobe(Figure 2.12, E). 1-3 notochaetae, stout, acicular and slightly curved, small groove on dorsal side of the curve, anterior to notopodium, longer than notopodium (Figure 2.15, A). 10-15 neurochaetae (Figure 2.12, C), as stout as notochaetae, last third or so becomes quill like, with spinulose rows; tips are bidentate to varying degrees, change in form from dorsal to ventral; dorsal chaeta have slightly hooked, pointed larger tooth and a smaller, rounded secondary tooth (Figure 2.12, B), mid chaeta have more prominent secondary tooth that is more pointed than that of dorsal chaeta (Figure 2.12,C), ventral chaeta have a more rounded larger tooth with a highly reduced secondary tooth (Figure 2.12, D).

Remarks

Gorgoniapolynoe sp. nov. was previously illustrated by Pettibone (1991) as a morphotype of *Gorgoniapolynoe caeciliae.* The molecular analysis carried out in this current study revealed that individuals with the morphological characters described above are deeply genetically diverged from *G. caeciliae* (Fig. 2.3). It is suggested that specimen USNM 133356 becomes the holotype for Gorgoniapolynoe sp. nov.

Specimens identified as *G. caeciliae* by Serpetti *et al.* (2017) were recorded in the South Indian Ocean (SIO). The molecular data from this research is included in this current study (Table 2.5 Table 2.6; Figs. 2.3 and 2.4). The individuals from the SIO are possibly also *a new species of Gorgoniapolynoe.* The species delimitation models infer that these specimens represent either one separated species (AGBD) or two (bPTP) (Fig. 3). Further investigation is needed to confirm the phylogenetic positioning of individuals from the SIO. They may be another new species of *Gorgoniapolynoe*.

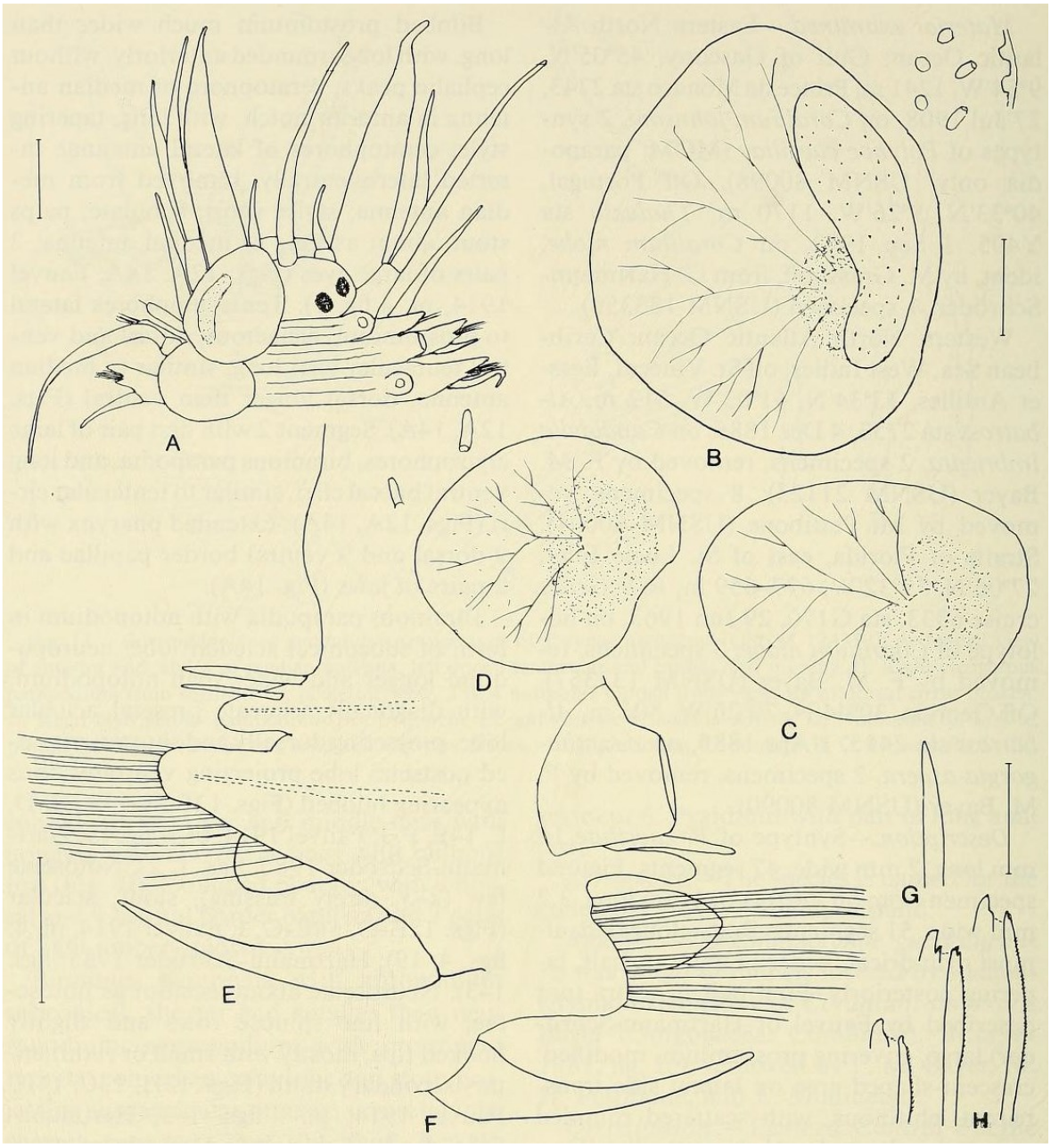

Figure 2.11. Gorgoniapolynoe sp. nov. A-D, specimen from off Portugal (USNM 133356); E-H, parapodia of syntype of Polynoe caeciliae (USNM 80098), figure reproduced from Pettibone (1991, Fig 12): **A**. Dorsal view of anterior end; **B.** Right 1st elytron from segment 2, with detail of microtubercles and micropapillae; **C**. Right 2nd elytron from segment 4; **D**. Right 7th elytron from segment 13, with detail of micropapillae; **E**. Right elytrigerous parapodium from anterior region, anterior view, acicula dotted; **F.** Right cirrigerous parapodium from anterior region, posterior view, style of dorsal cirrus broken off; **G**. Notochaetae from same; **H**. Lower, middle and upper neurochaetae from same, with detail of tips. Scales = 0.5 mm for A; 0.2 mm for B-E; F;0.1 mm for G, H.

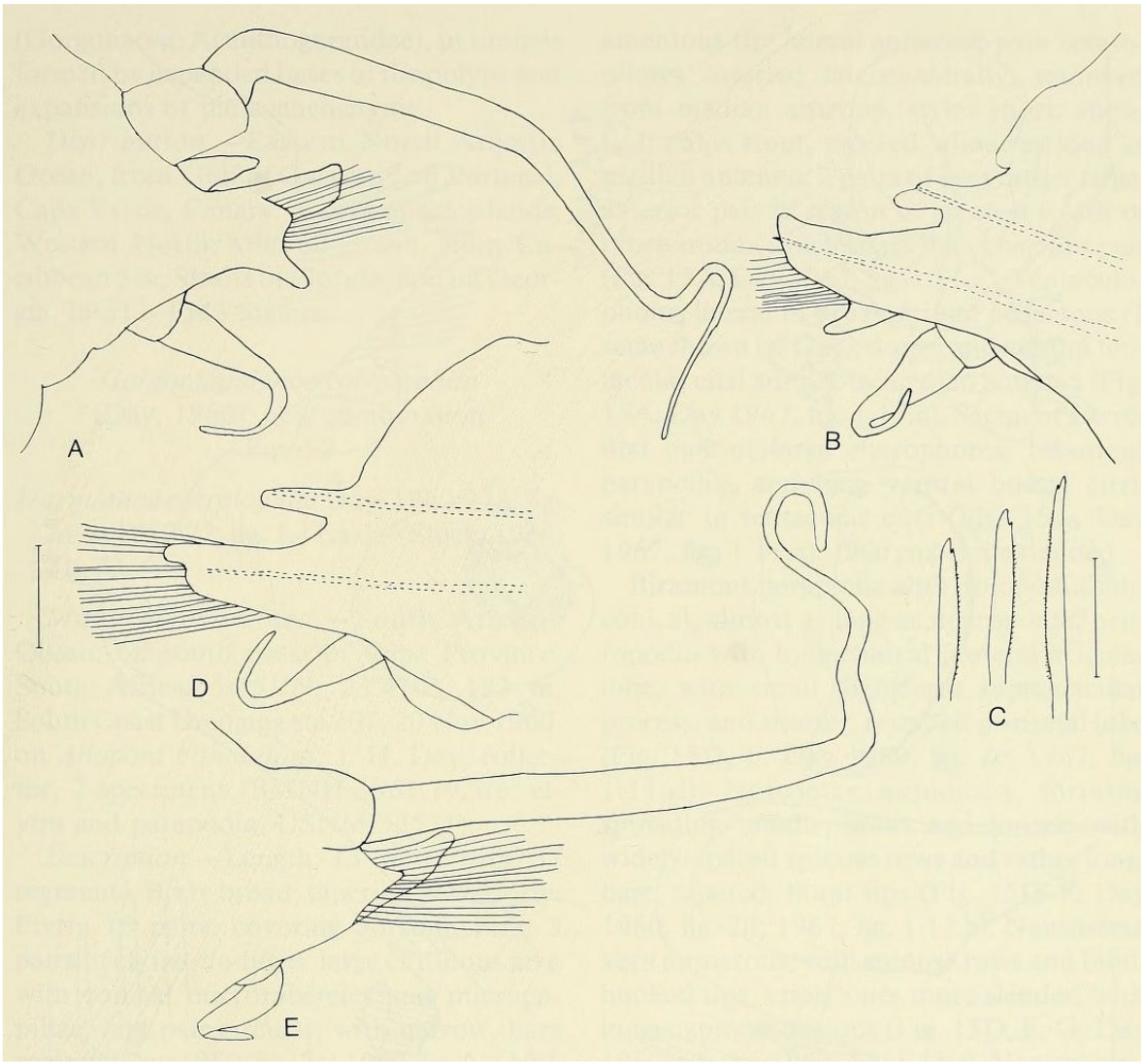

Figure 2.12. Gorgoniapolynoe sp. nov., specimen from off Portugal (USNM 1 33356): **A**. Right cirrigerous parapodium from segment 1 2, posterior view; **B**. Right elytrigerous parapodium from segment 13, anterior view, acicula dotted; **C**. Lower, middle and upper neurochaetae from same; **D**. Right elytrigerous parapodium from segment 29, anterior view, acicula dotted; **E**. Right cirrigerous parapodium from segment 30, posterior view. Scales = 0.2 mm for A, B, D, E; 0.1 mm for C.

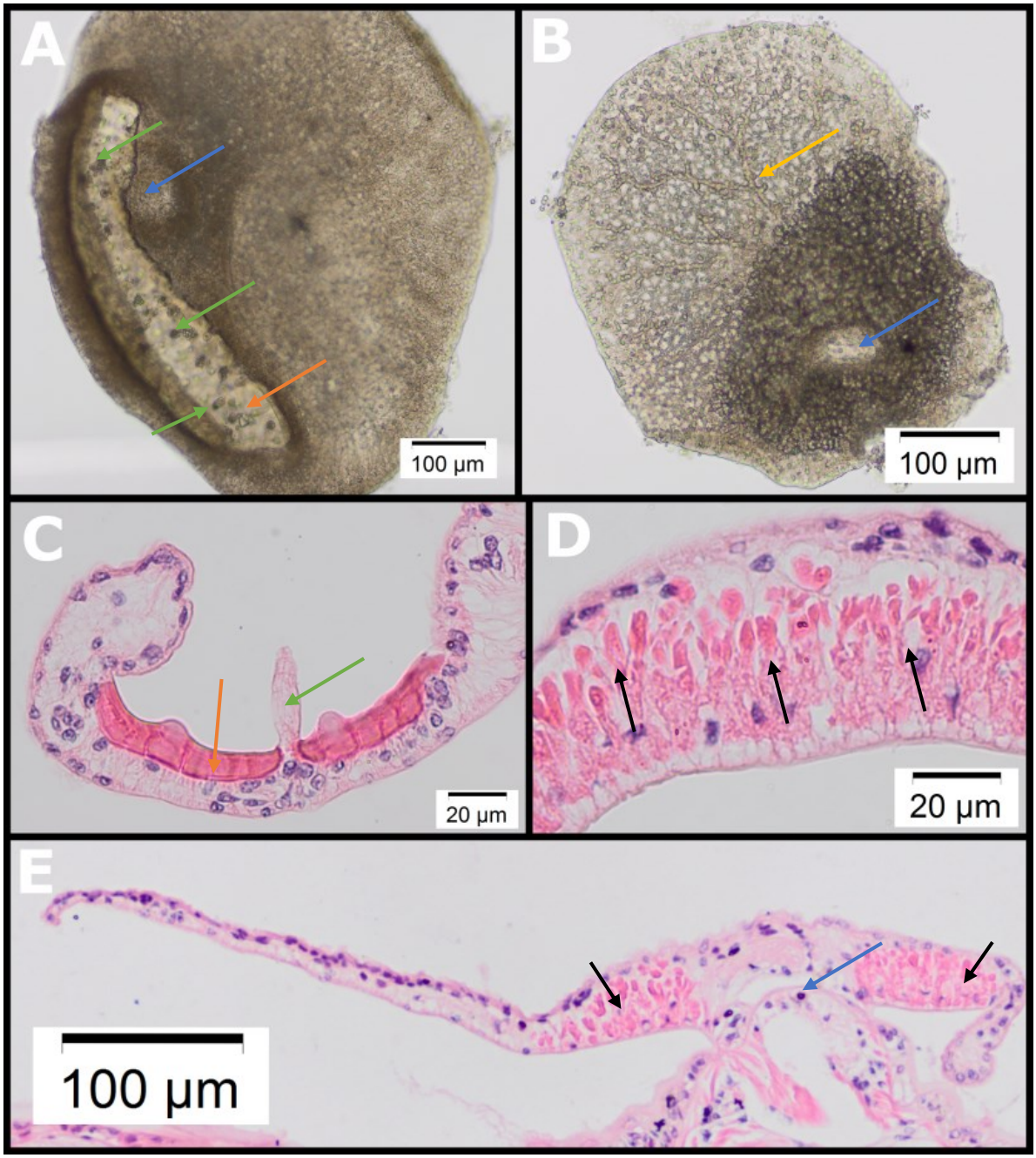

Figure 2.13. Elytra of *Gorgoniapolynoe* sp. nov., 602_1_Ca and histological sections of elytra from 680_4_Ca. **A**. Modified first elytron with small crescent-shaped chitinous area from 602_1_Ca **B.** The third elytron from the same individual as A. **C.** The histological section of the modified first elytra of 680_4_Ca , showing the chitinous area, with a papilla. Note the papillae is an extension of and connected to the cells below the chitin. **D.** Possible cup-shaped photocytes within the second elytra of 680_4_Ca **E**. shows the possible photocytes concentrated around the elytrophore, corresponding to the darker area in B. Arrows: Blue = elytrophore, Orange = chitinous area, Green = micropapillae, Yellow = "veins" in posterior elytra, Black = possible photocytes

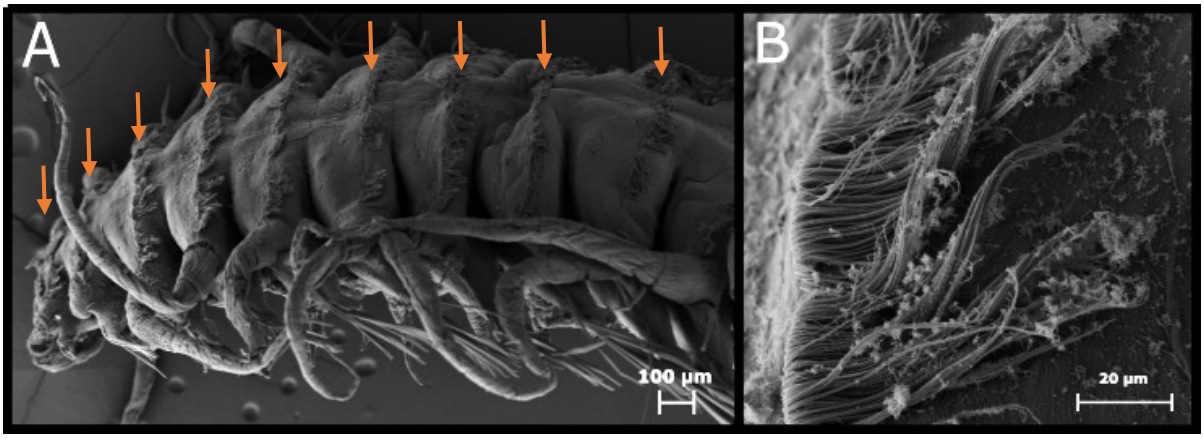

Figure 2.14. Hair-like structures of *Gorgoniapolynoe* sp. nov., 602_8_Ca, which are present on all segments after the segment containing the last elytra (orange arrows). **B.** The hair-like structures at a higher magnification.

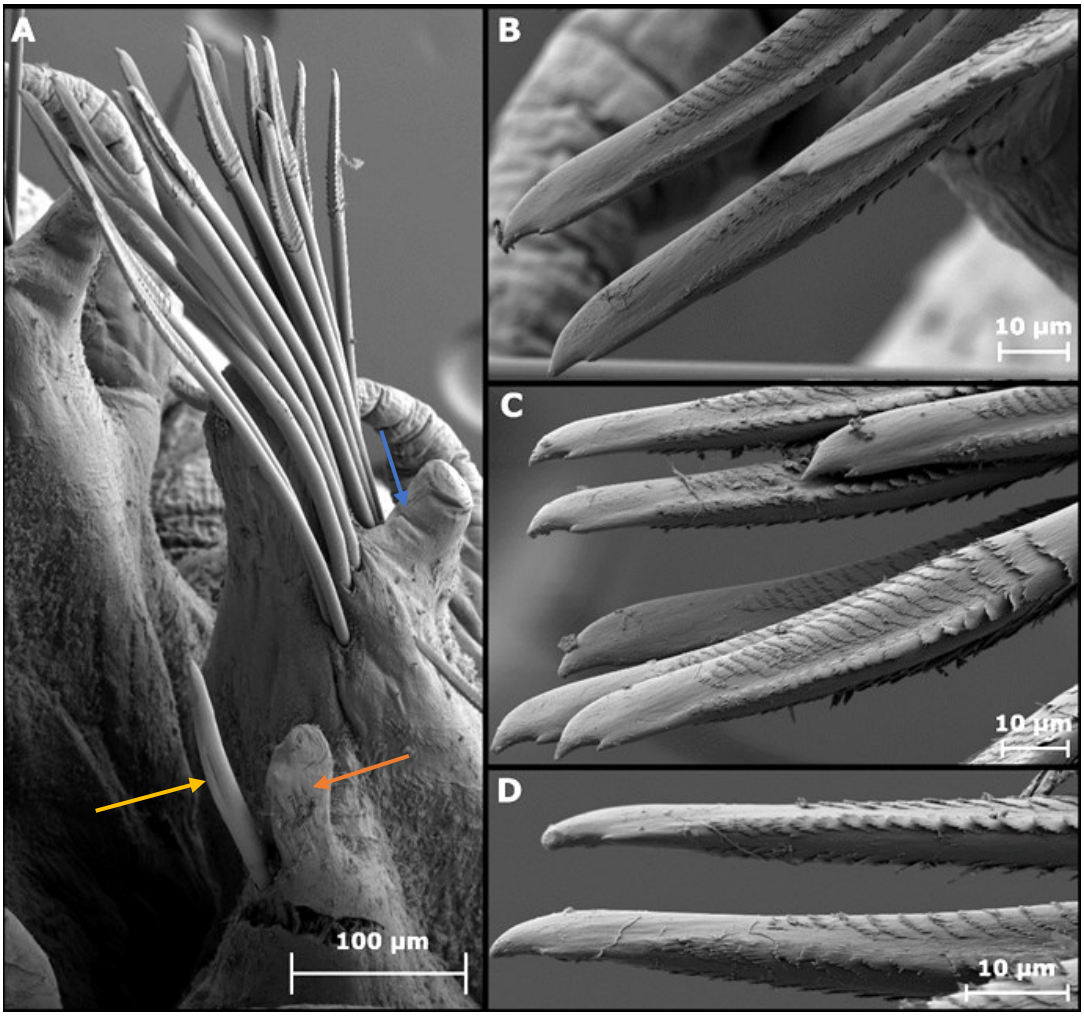

Figure 2.15. A SEM micrograph of the tenth parapodium of an individual identified as *Gorgoniapolynoe* sp. nov., 602_8_Ca with details of the tips of the neurochaetae. **A**. the tenth right parapodium. orange arrow = notopodium, yellow arrow = notochaeta, blue arrow = distinct postsetal neuropodial lobe. **B**. The dorsal most neurochaetae. **C**. Mid-neurochaetae. **D**. ventral most neurochaetae. B, C and D are all from the same neuropodium.

Ecology

Specimen USNM 133356, collected off Portugal in association with *Corallium niobe*. Unfortunately, the host corals of *Gorgoniapolynoe* sp. nov. from the Equatorial Atlantic region were not identified to species level. Specimens analysed in this study were found in association with species from the genera *Corallium*, *Candidella*, and unidentified Primnoidae. All specimens were removed from galleries formed by modified sclerites of the host corals. Some individuals which shared *COI* haplotypes were found on different host genera (Hap_1, Hap_25, supplementary material, Table 2). Additionally, specimens identified as *G. caeciliae* were found associated with on *Acanthogorgia armata* (Verrill 1878) and *Candidella imbricata* (Johnson, 1862) from the eastern Gulf of Mexico (Barnich *et al.* 2013). Based on the description and pictures provided by Barnich *et al.* (2013), The specimens in this study are likely to have been *G.* sp. nov.. In their paper Barnich *et al.* (2013) detail how the galleries formed by the modified sclerites differ between host coral species. This may just be a function of the morphological differences between the species sclerites.

The specimens in this study were sampled 593 – 2343 m. The previous reported locations for G. caeciliae (Pettibone 1991, Barnich *et al.* 2013) and the locations from this study, suggest that the species has a range which stretches from the Gulf of Mexico, across the Atlantic and up to the Western European continental shelf. In this study they were recorded from three of the four seamounts sampled (Carter, Knipovich and Vayda). The molecular analysis (Table 2.8) showed that for *COI* there was no significant structure between the three sampling sites, with the three most common haplotypes being found at all locations.

2.3.6. Newly discovered structures

The flagellum-like structures with the flattened ovoid distal tips, that emerge from the micropapillae (Figure 2.7), have not been observed before for any member of *Gorgoniapolynoe.* The only other record of similar structures is from *Pholoe minuta* (Fabricius, 1780), where short cilia-like structures with basal collars were found on the tips of their elytral papillae (Heffernan 1990). The cilia-like structures of *P. minuta* were hypothesized to have a sensory function. The function of the flagellum-like structures of *Gorgoniapolynoe* can similarly only be hypothesised. The fact that these structures were observed on all species (*G. caeciliae* A and B, and *G.* sp. nov.) may mean that they are a

characteristic of all *Gorgoniapolynoe*. Further investigation of the elytra of other species of Gorgoniapolynoe is needed.

The flagellum may be somatosensory organs to allow the polychaetes to feel and navigate their way around. Whether the elytra themselves have any sensory capabilities is unknown. But if they didn't it could be hypothesised that the flagellum structures could allow the polychaete to sense what was above in the sensory "blind spot" created by the elytra. The micropapillae are densest in the modified area of the first elytra, above the prostomium, which could lend credence to the idea that they sensory organs for navigation and orientation. The flattened ovoid tips of the flagellum could contain the sensory cells. The flagellum would appear to be retractable as they were observed to be different lengths.

It could be that the flagellum are chemosensory organs. Polychaetes have been shown to have a variety of chemosensory organs (Lindsay 2009). They may have a function in the reproductive cycle. Eckelbarger *et al.* (2005) provided evidence that Gorgoniapolynoe are broadcast spawners and would therefore need a mechanism that synchronised the release of sperm and eggs. It is possible that the flagellum play a role in synchronising spawning, thus increasing the likelihood of fertilization.

Another possible chemosensory function the flagellum may have is in host recognition. Commensal polychaetes have been suggested to be able to recognise their hosts at the recruitment stage by identifying compounds released by the host into the water column (Martin and Britayev 1998). While little is known on the exact mechanisms involved in host recognition, it is assumed to involves cilia which have been identified on larval forms in several polychaete genera (Lindsay 2009). However, the specimens examined for this study were all post-larval stage and established on their host, so would have no need for chemosensory organs associated with larval recruitment. If not for recruitment, the flagellum could have a role in the inducement and preservation of the tunnels in which they live. In a similar vein, they could have a role in interacting with the host. They could release compounds which prevent the coral from triggering their cnidocytes when the polychaete is hunting in the coral branches. Hunting behaviour in the branches has been observed by Barnich *et al.* (2013).

The flagellum may possibly be involved in the emittance of bioluminescence. The histological analysis of the elytra revealed the possible presence of cup-shaped photocytes (Figure 2.8 and Figure 2.13). Similar cells have been linked to bioluminescence in other

polynoids (Nicol 1953; Bassot and Nicolas 1995). If these cells were connected to the flagellum, they could emit bioluminescence, possibly as a defence mechanism or to attract prey. In other polynoids however, bioluminescence is emitted directly from the photocytes, causing the elytra to luminesce (Nicol 1953; Bassot and Nicolas 1995; Livermore *et al.* 2018). It is likely that *Gorgoniapolynoe* has the ability to produce bioluminescence in their elytra based on the presence of the cup-shaped cells. In order to confirm this either live specimens would need to be observed or to extract the protein from within the cells and test it for bioluminescent properties (Bassot and Nicolas 1995).

If the flagellum-like structures do have a chemosensory function, it could support the hypothesis that the *Gorgoniapolynoe* may be in morphological stasis. Added to which they are symbionts found in an extreme environment. These two factors combined this may account for only subtle morphological differences between species, despite large genetic divergence (Bickford *et al.* 2007). In fact, all described *Gorgoniapolynoe* to date have morphological features which vary very little between species (Pettibone 1991)

2.4. Conclusion

The redescription of *Gorgoniapolynoe caeciliae* by Pettibone (1991) was a composite based on two specimens with subtle morphological differences. The original descriptions by Fauvel (1913, 1914) which were short on detail and the lack of a whole-body holotype would have complicated Pettibone's redescription and synonymization of the species. Pettibone was the first to describe the modified first elytra of *G. caeciliae.* Presumably when presented with two variants of the elytra on different specimens, whose other morphological characters matched the vague description by Fauvel (1913, 1914), Pettibone accredited the differences as being down to intraspecific morphological variance. This current investigation has shown that the two morphological different specimens illustrated by Pettibone (1991) are distinct species, *Gorgoniapolynoe caeciliae strict. sens* and *Gorgoniapolynoe* sp. nov. Furthermore, analyses of the *COI* gene inferred the possibility of *G. caeciliae* being two morphologically identical but genetically distinct cryptic species. Subsequent investigation, using additional gene sequences, is needed to confirm the presence of cryptic species *in G. caeciliae*.

It is possible that due to the fact that *Gorgoniapolynoe* live in an extreme environment and have a symbiotic life history strategy that they are in morphological stasis. The subtle

morphological differences between the species and the discovery of the flagellum-like structures, which may be chemosensory in function, could support this hypothesis.

That all species were found at similar depths and locations, and may utilize the same host coral genera, suggests that speciation either occurred during times of isolation in the past before secondary contact, or in sympatry. As there is not a reliable fossil record or molecular clock for polychaetes at present (Nygren 2014), dating divergence to hypothesis causes of speciation is not currently possible. No coral hosted both *G. caeciliae* and *G.* sp. nov. A further study into the taxonomy of the host corals is needed to see if each polychaete species has specific host corals.

The specimens from Genbank, identified previously as *G. caeciliae,* from the South Indian Ocean (Serpetti *et al.* 2017) were found to be most closely related to *G.* sp. nov.. Additionally, species delimitation models inferred them to be a totally separate species. A full morphological and molecular investigation is needed into *Gorgoniapolynoe* in the South Indian Ocean to resolve their taxonomy and phylogeny. The central position of Gorgoniapolynoe *corralophila* within the cladograms i.e. between *G. caeciliae* and *G.* sp. nov, is interesting. The inclusion of *G. corralophila* in the genus *Gorgoniapolynoe* has previously been questioned due to its first three elytra being modified, and having very different notochaeta to the rest of the species in the genus (Britayev *et al.* 2014). The molecular evidence and the discovery of very similar notochaeta on *G. caeciliae* in this current study, suggest that it is correct to include *G. corralophila* within *Gorgoniapolynoe.*

This study highlights that the combination of morphological and molecular analyses in taxonomy is essential if the true diversity of a genus is to be uncovered and described. The results of this investigation contribute to the ongoing research effort into the understanding of the biodiversity of deep-sea polychaetes. It also contributes towards the larger goal, set by the UN Decade of Ocean Science for Sustainable Development (2021-2030) to increase the knowledge of the deep sea.

2.5. References

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Supplementary material

Table S1. *COI* haplotypes. Haplotype number, specimen number, parent coral number, coral host, sampling depth, area and seamount name. Hap_90 to Hap_95 composed of sequences obtained from GenBank.

Haplotype	Specimen no.	Parent	Coral Host	Depth (m)	Area	Seamount	Lat	Long
Hap_1	0268_1_Ca	267	Primnoidae	1345	Central Eastern Atlantic Ocean	Carter	9.207633	-21.3006
	0551_1_Ca	502	Candidella	1783	Central Eastern Atlantic Ocean	Carter	9.205877	-21.2978
	0671_1_Ca	64	Corallium	1367	Central Eastern Atlantic Ocean	Carter	9.207015	-21.3
	0680_5_Ca	61	Corallium	2343	Central Eastern Atlantic Ocean	Carter	9.181045	-21.2747
	0716_1_Ca	61	Corallium	2343	Central Eastern Atlantic Ocean	Carter	9.181045	-21.2747
	0716_7_Ca	61	Corallium	2343	Central Eastern Atlantic Ocean	Carter	9.181045	-21.2747
	0745_2_Ca	63	Primnoidae	1364	Central Eastern Atlantic Ocean	Carter	9.207633	-21.3006
	1238_3_Kn	83	Corallium	1445	Central Eastern Atlantic Ocean	Carter	5.608727	-26.9583
	1260_1_Kn	83	Corallium	1445	Central Eastern Atlantic Ocean	Carter	5.608727	-26.9583
	2056_0_Va	1454	Corallium	710	Central Eastern Atlantic Ocean	Vayda	14.88625	-48.1565
	2057_0_Va	1454	Corallium	710	Central Eastern Atlantic Ocean	Vayda	14.88625	-48.1565
Hap_2	0268_2_Ca	267	Primnoidae	1364	Central Eastern Atlantic Ocean	Carter	9.207633	-21.3006
Hap_3	0552_1_Ca	502	on Candidella	1783	Central Eastern Atlantic Ocean	Carter	9.205877	-21.2978
	0680_7_Ca	61	Corallium	2343	Central Eastern Atlantic Ocean	Carter	9.181045	-21.2747
	0716_9_Ca	61	Corallium	2343	Central Eastern Atlantic Ocean	Carter	9.181045	-21.2747
	1217_6_Kn	83	Corallium	1445	Central Eastern Atlantic Ocean	Carter	5.608727	-26.9583
	2031_1_Va	98	Corallium	772	Central Eastern Atlantic Ocean	Vayda	14.88873	-48.155
	2059_0_Va	1454	Corallium	710	Central Eastern Atlantic Ocean	Vayda	14.88625	-48.1565
Hap_4	0602_1_Ca	24	Candidella	1364	Central Eastern Atlantic Ocean	Carter	9.207633	-21.3006
	0680_4_Ca	61	Corallium	2343	Central Eastern Atlantic Ocean	Carter	9.181045	-21.2747
	0820_0_Ca	56	Corallium	1442	Central Eastern Atlantic Ocean	Carter	9.20549	-21.2972
	1217_2_Kn	83	Corallium	1445	Central Eastern Atlantic Ocean	Carter	5.608727	-26.9583
	2062_0_Va	1454	Corallium	710	Central Eastern Atlantic Ocean	Vayda	14.88625	-48.1565
	2102_1_Va	1454	Corallium	710	Central Eastern Atlantic Ocean	Vayda	14.88625	-48.1565
Hap_5	0602_3_Ca	24	Candidella	1364	Central Eastern Atlantic Ocean	Carter	9.207633	-21.3006

Table S1 continued

Haplotype	Specimen no.	Parent	Coral Host	Depth (m)	Area	Seamount	Lat	Long
Hap_6	0602_4_Ca	24	Candidella	1364	Central Eastern Atlantic Ocean	Carter	9.207633	-21.3006
	0680_1_Ca	61	Corallium	2343	Central Eastern Atlantic Ocean	Carter	9.181045	-21.2747
	0716_2_Ca	61	Corallium	2343	Central Eastern Atlantic Ocean	Carter	9.181045	-21.2747
	0716_3_Ca	61	Corallium	2343	Central Eastern Atlantic Ocean	Carter	9.181045	-21.2747
	0602_5_Ca	24	Candidella	1364	Central Eastern Atlantic Ocean	Carter	9.207633	-21.3006
Hap_7	0602_5_Ca	24	Candidella	1364	Central Eastern Atlantic Ocean	Carter	9.207633	-21.3006
Hap_8	0602_6_Ca	24	Candidella	1364	Central Eastern Atlantic Ocean	Carter	9.207633	-21.3006
Hap_9	0602_7_Ca	24	Candidella	1364	Central Eastern Atlantic Ocean	Carter	9.207633	-21.3006
Hap_10	0602_8_Ca	24	Candidella	1364	Central Eastern Atlantic Ocean	Carter	9.207633	-21.3006
Hap_11	0671_2_Ca	64	Corallium	1367	Central Eastern Atlantic Ocean	Carter	9.207015	-21.3
Hap_12	0680_2_Ca	61	Corallium	2343	Central Eastern Atlantic Ocean	Carter	9.181045	-21.2747
Hap_13	0680_3_Ca	61	Corallium	2343	Central Eastern Atlantic Ocean	Carter	9.181045	-21.2747
	1260_3_Kn	83	Corallium	1445	Central Eastern Atlantic Ocean	Knipovich	5.608727	-26.9583
Hap_14	0680_6_Ca	61	Corallium	2343	Central Eastern Atlantic Ocean	Carter	9.181045	-21.2747
Hap_15	0680_8_Ca	61	Corallium	2343	Central Eastern Atlantic Ocean	Carter	9.181045	-21.2747
Hap_16	0716_4_Ca	61	Corallium	2343	Central Eastern Atlantic Ocean	Carter	9.181045	-21.2747
Hap_17	0716_5_Ca	61	Corallium	2343	Central Eastern Atlantic Ocean	Carter	9.181045	-21.2747
Hap_18	0716_6_Ca	61	Corallium	2343	Central Eastern Atlantic Ocean	Carter	9.181045	-21.2747
Hap_19	0716_8_Ca	61	Corallium	2343	Central Eastern Atlantic Ocean	Carter	9.181045	-21.2747
	1238_7_Kn	83	Corallium	1445	Central Eastern Atlantic Ocean	Knipovich	5.608727	-26.9583
	2030_1_Va	98	Corallium	772	Central Eastern Atlantic Ocean	Vayda	14.88873	-48.155
Hap_20	0745_1_Ca	63	Primnoidae	1364	Central Eastern Atlantic Ocean	Carter	9.207633	-21.3006
	1217_1_Kn	83	Corallium	1445	Central Eastern Atlantic Ocean	Knipovich	5.608727	-26.9583
Hap_21	0811_0_Ca	56	Corallium	1442	Central Eastern Atlantic Ocean	Carter	9.20549	-21.2972
Hap_22	0812_0_Ca	56	Corallium	1442	Central Eastern Atlantic Ocean	Carter	9.20549	-21.2972
Hap_23	0814_0_Ca	56	Corallium	1442	Central Eastern Atlantic Ocean	Carter	9.20549	-21.2972
Hap_24	1217_3_Kn	83	Corallium	1445	Central Eastern Atlantic Ocean	Knipovich	5.608727	-26.9583
Hap_25	1217_4_Kn	83	Corallium	1445	Central Eastern Atlantic Ocean	Knipovich	5.608727	-26.9583

Table S1
continued

Haplotype	Specimen no.	Parent	Coral Host	Depth (m)	Area	Seamount	Lat	Long
	1238_6_Kn	83	Corallium	1445	Central Western Atlantic Ocean	Vema	5.608727	-26.9583
Hap_26	1217_5_Kn	83	Corallium	1445	Central Western Atlantic Ocean	Vema	5.608727	-26.9583
Hap_27	1238_2_Kn	83	Corallium	1445	Central Western Atlantic Ocean	Vema	5.608727	-26.9583
Hap_28	1238_4_Kn	83	Corallium	1445	Central Western Atlantic Ocean	Vema	5.608727	-26.9583
Hap_29	1238_5_Kn	83	Corallium	1445	Central Western Atlantic Ocean	Vema	5.608727	-26.9583
Hap_30	1260_2_Kn	83	Corallium	1445	Central Western Atlantic Ocean	Vema	5.608727	-26.9583
Hap_31	1260_4_Kn	83	Corallium	1445	Central Western Atlantic Ocean	Vema	5.608727	-26.9583
Hap_32	1714_1_Ve	515	Acanthogorgia	593	Central Western Atlantic Ocean	Vema	10.71144	-44.4203
Hap_33	1714_2_Ve	515	Acanthogorgia	593	Central Western Atlantic Ocean	Vema	10.76795	-44.6006
Hap_34	1714_3_Ve	515	Acanthogorgia	593	Central Western Atlantic Ocean	Vema	10.76795	-44.6006
Hap_35	1714_6_Ve	515	Acanthogorgia	593	Central Western Atlantic Ocean	Vema	10.76795	-44.6006
Hap_36	1714_7_Ve	515	Acanthogorgia	593	Central Western Atlantic Ocean	Vema	10.76795	-44.6006
Hap_37	1715_1_Ve	515	Acanthogorgia	593	Central Western Atlantic Ocean	Vema	10.76795	-44.6006
Hap_38	1715_2_Ve	515	Acanthogorgia	593	Central Western Atlantic Ocean	Vema	10.76795	-44.6006
Hap_39	1715_3_Ve	515	Acanthogorgia	593	Central Western Atlantic Ocean	Vema	10.76795	-44.6006
Hap_40	1743_2_Ve	91	Corallium	2190	Central Western Atlantic Ocean	Vema	10.76795	-44.6006
	2213_0_Va	1466	Corallium	1622	Central Western Atlantic Ocean	Vema	14.8649	-48.2558
Hap_41	1743_3_Ve	91	Corallium	2190	Central Western Atlantic Ocean	Vema	10.76795	-44.6006
Hap_42	1743_4_Ve	91	Corallium	2190	Central Western Atlantic Ocean	Vema	10.76795	-44.6006
Hap_43	1743_5_Ve	91	Corallium	2190	Central Western Atlantic Ocean	Vema	10.76795	-44.6006
	1851_3_Va	96	Corallium	1416	Central Western Atlantic Ocean	Vayda	14.8615	-48.2405
	2191_2_Va	1470	Corallium	1416	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
	2214_0_Va	1466	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
	2216_1_Va	1466	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_44	1771_1_Ve	91	Corallium	2190	Central Western Atlantic Ocean	Vema	10.76795	-44.6006
Hap_45	1771_2_Ve	91	Corallium	2190	Central Western Atlantic Ocean	Vema	10.76795	-44.6006
Hap_46	1771_3_Ve	91	Corallium	2190	Central Western Atlantic Ocean	Vema	10.76795	-44.6006

Table S1 continued

Haplotype	Specimen no.	Parent	Coral Host	Depth (m)	Area	Seamount	Lat	Long
	1771_4_Ve	91	Corallium	2190	Central Western Atlantic Ocean	Vema	10.76795	-44.6006
Hap_47	1771_5_Ve	91	Corallium	2190	Central Western Atlantic Ocean	Vema	10.76795	-44.6006
Hap_48	1819_1_Va	96	Corallium	1416	Central Western Atlantic Ocean	Vayda	14.8615	-48.2405
Hap_49	1819_2_Va	96	Corallium	1416	Central Western Atlantic Ocean	Vayda	14.8615	-48.2405
Hap_50	1851_1_Va	96	Corallium	1416	Central Western Atlantic Ocean	Vayda	14.8615	-48.2405
Hap_51	1851_2_Va	96	Corallium	1416	Central Western Atlantic Ocean	Vayda	14.8615	-48.2405
Hap_52	1851_4_Va	96	Corallium	1416	Central Western Atlantic Ocean	Vayda	14.8615	-48.2405
	1851_6_Va	96	Corallium	1416	Central Western Atlantic Ocean	Vayda	14.8615	-48.2405
Hap_53	1851_5_Va	96	Corallium	1416	Central Western Atlantic Ocean	Vayda	14.8615	-48.2405
Hap_54	1879_1_Va	96	Corallium	1416	Central Western Atlantic Ocean	Vayda	14.8615	-48.2405
	2215_0_Va	1466	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_55	1879_2_Va	96	Corallium	1416	Central Western Atlantic Ocean	Vayda	14.8615	-48.2405
Hap_56	1879_3_Va	96	Corallium	1416	Central Western Atlantic Ocean	Vayda	14.8615	-48.2405
Hap_57	1879_4_Va	96	Corallium	1416	Central Western Atlantic Ocean	Vayda	14.8615	-48.2405
Hap_58	1879_5_Va	96	Corallium	1416	Central Western Atlantic Ocean	Vayda	14.8615	-48.2405
Hap_59	1879_6_Va	96	Corallium	1416	Central Western Atlantic Ocean	Vayda	14.8615	-48.2405
	1879_7_Va	96	Corallium	1416	Central Western Atlantic Ocean	Vayda	14.8615	-48.2405
Hap_60	1879_8_Va	96	Corallium	1416	Central Western Atlantic Ocean	Vayda	14.8615	-48.2405
Hap_61	2029_1_Va	98	Corallium	772	Central Western Atlantic Ocean	Vayda	14.88873	-48.155
Hap_62	2054_0_Va	1454	Corallium	710	Central Western Atlantic Ocean	Vayda	14.88625	-48.1565
Hap_63	2055_0_Va	1454	Corallium	710	Central Western Atlantic Ocean	Vayda	14.88625	-48.1565
Hap_64	2058_0_Va	1454	Corallium	710	Central Western Atlantic Ocean	Vayda	14.88625	-48.1565
Hap_65	2060_0_Va	1454	Corallium	710	Central Western Atlantic Ocean	Vayda	14.88625	-48.1565
Hap_66	2061_0_Va	1454	Corallium	710	Central Western Atlantic Ocean	Vayda	14.88625	-48.1565
Hap_67	2063_1_Va	1454	Corallium	710	Central Western Atlantic Ocean	Vayda	14.88625	-48.1565
Hap_68	2064_1_Va	1454	Corallium	710	Central Western Atlantic Ocean	Vayda	14.88625	-48.1565
Hap_69	2086_1_Va	1454	Corallium	710	Central Western Atlantic Ocean	Vayda	14.88625	-48.1565
Hap_70	2087_1_Va	1454	Corallium	710	Central Western Atlantic Ocean	Vayda	14.88625	-48.1565

Table S1 continued

Haplotype	Specimen no.	Parent	Coral Host	Depth (m)	Area	Seamount	Lat	Long
Hap_71	2087_2_Va	1454	Corallium	710	Central Western Atlantic Ocean	Vayda	14.88625	-48.1565
Hap_72	2101_1_Va	1454	Corallium	710	Central Western Atlantic Ocean	Vayda	14.88625	-48.1565
Hap_73	2169_1_Va	1470	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_74	2170_0_Va	1470	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_75	2171_0_Va	1470	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_76	2173_0_Va	1470	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_77	2191_1_Va	1470	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_78	2191_3_Va	1470	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
	2191_5_Va	1470	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_79	2194_1_Va	1470	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
	2194_5_Va	1470	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_80	2194_2_Va	1470	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_81	2194_3_Va	1470	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_82	2194_4_Va	1470	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_83	2194_6_Va	1470	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_84	2194_7_Va	1470	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_85	2209_0_Va	1466	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_86	2210_0_Va	1466	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_87	2212_0_Va	1466	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_88	2217_0_Va	1466	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_89	2218_0_Va	1466	Corallium	1622	Central Western Atlantic Ocean	Vayda	14.8649	-48.2558
Hap_90	**An_sp**		**Outgroup**					
Hap_91	KU738203		Narella	1360	South Indian Ocean	Coral	41.35444	42.92583
Hap_92	KU738204		Candidella imbricata	1021	South Indian Ocean	Melville	38.50889	38.50889
	KU738205		Acanthogorgia	784	South Indian Ocean	Atlantis Bank	32.70972	32.70972
Hap_93	KU738206		Stylasteridae	1357	South Indian Ocean	Coral	32.70972	42.92556
Hap_94	KU738208		Stylasteridae	1340	South Indian Ocean	Middle of What	37.95056	50.45306
Hap_95	KU738209		Stylasteridae	894	South Indian Ocean	Atlantis Bank	50.45306	50.45306

Histological sample preparation for light microscopy

Paraffin mounting

Melt paraffin at 60ºC

Dehydrate the specimen in an ethanol series

50% EtOH for 30 min

70% EtOH for 30 min

90% EtOH for 30 min x2

100% EtOH for 30 min x2

Transfer samples to glass phials of EtOH-Xylene 1:1 for 10 min

100% Xylene for 15 min x2

Paraffin for 30 min, in oven at 60ºC

Place each sample in their stand and leave to set (8 hours)

Section blocks into 5 μm preparations and leave the slides to dry overnight.

Haematoxylin-Eosin staining

Xylene for 20 min

100% EtOH for 10 min

96% EtOH for 10 min

70% EtOH for 10 min

Distilled water for 10 min

Haematoxylin for 4 min

Run under tap water for 1 min

100:1 solution of 96% EtOH and 35% HCℓ for 1 sec x3

Run under tap water for 1 min

1% Eosin for 2 min

96% EtOH for 10 min

100% EtOH for 10 min

Xylene for 10 min

Leave overnight

Mount slides with DPX

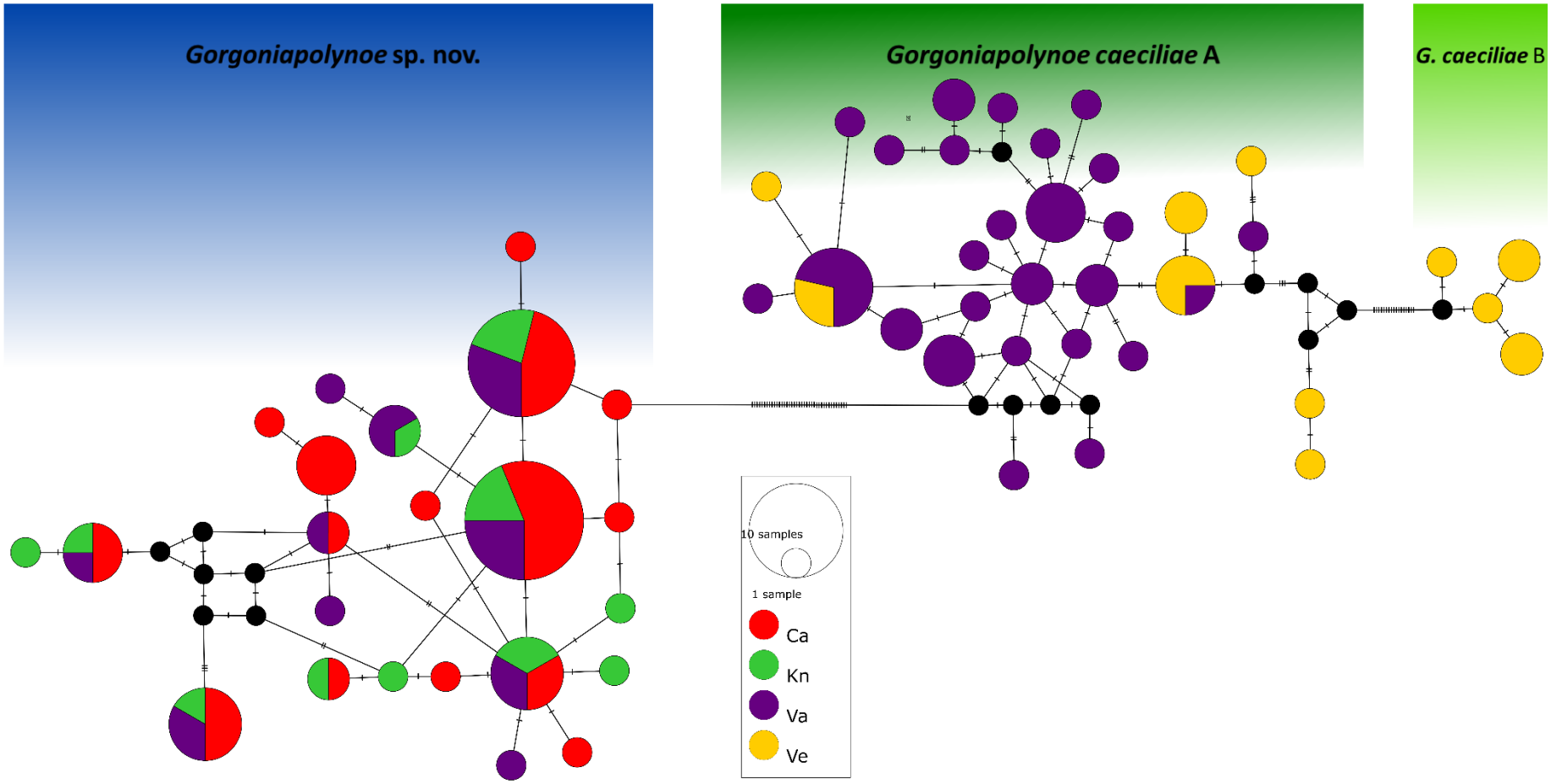


Figure S1. Haplotype network created using *COI* sequences of 129 individuals of *Gorgoniapolynoe* collected from four seamounts in the Equatorial North Atlantic; Ca = Carter seamount, Kn = Knipovich seamount, Va = Vayda seamount, and Ve = Vema seamount.

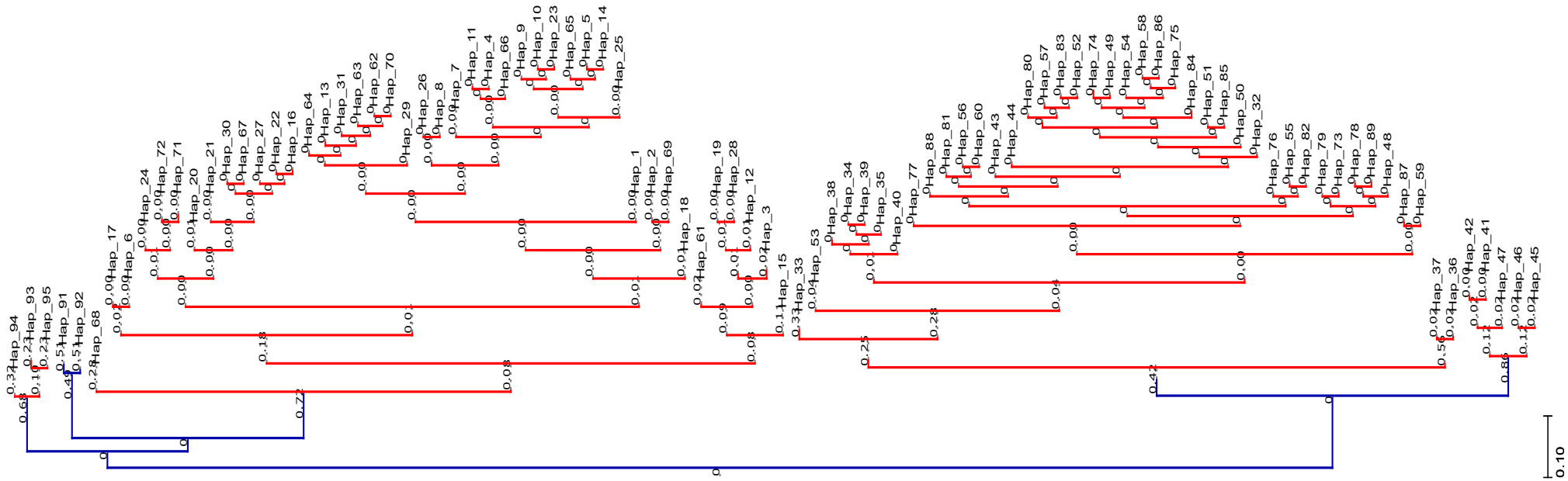
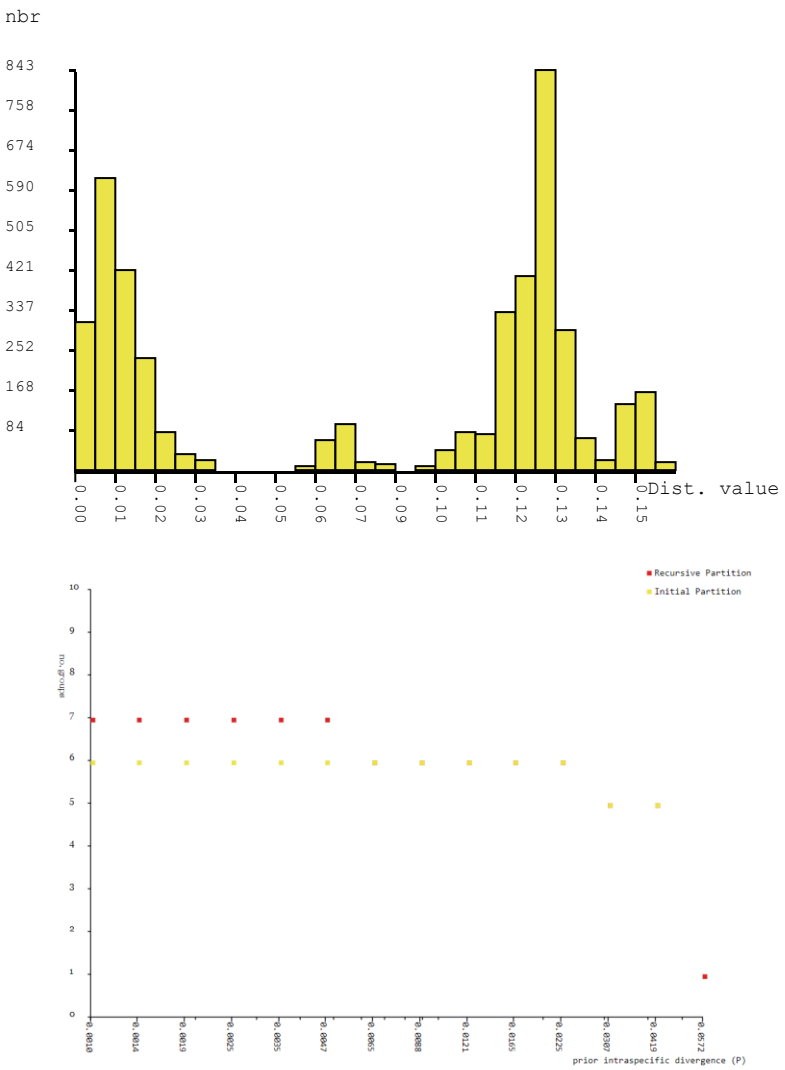


Figure S17. Bayesian support for bPTP model based on the output from maximum likelihood analysis of COI haplotype data implemented using RAxML v8.2.12 (Stamatakis 2014). For the bPTP model the number of MCMC generations was set at 500000, with a thinning of 100 and a burn-in of 0.25. The outgroup was removed to improve results.



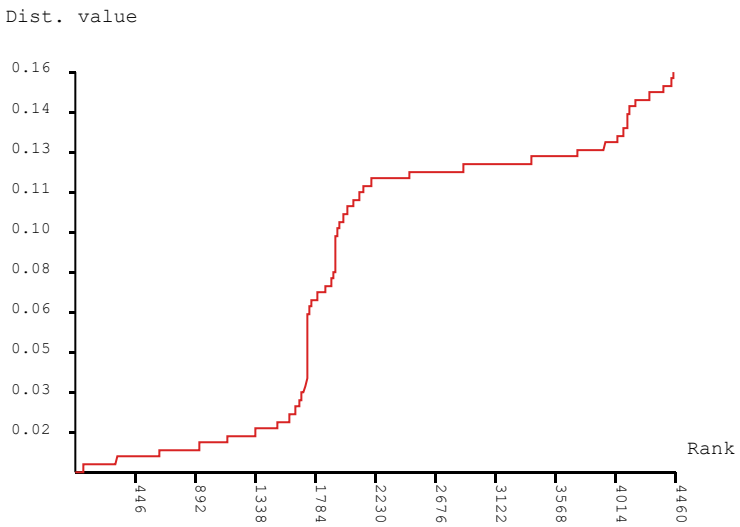


Figure S18. The outputs from Automatic Barcode Gap Discovery (ABGD, Puillandre et al. 2012.). run online at https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html. P_{min} was set at 0.001 and P_{max} at 0.37. The number of bins for distance distribution was set to 30. The data input was an uncorrected p-distance matrix computed in Mega-X (Kumar et al. 2018) using the COI haplotype data file.

Top left: Histogram showing pairwise differences between all samples

Top right: Ranked pairwise differences

Bottom left: The number of groups/species identified after partitioning and recursive partitioning. On the values on the x axis are prior intraspecific divergence values,