Towards an unravelling of the taxonomy of *Chrysaora* (Scyphozoa; Semaeostomeae; Pelagiidae) from around South Africa

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I declare that

"Towards an unravelling of the taxonomy of *Chrysaora* (Scyphozoa; Semaeostomeae; Pelagiidae) from around South Africa" is my own work, that it has not been submitted for any degree or examination at any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Signature: ______________

Date: 06/02/2017______

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Abstract

Historically, two species of *Chrysaora* are known from the Benguela Current Ecosystem: *C. fulgida* (Reynaud 1830) and *C. africana* (Vanhöffen 1902). However a third morphotype is now seen, which bears a resemblance to both. Thus a complete qualitative and quantitative analysis of the morphometric and meristic data of these three species was conducted, along with an in depth study into the cnidome as a potential tool of identification. These findings are supplemented by a genetic analysis using cytochrome *c* oxidase subunit I and internal transcribed spacer 1 gene markers.

Three species were unambiguously identified. The genetics and morphology showed considerable divergence, with some of the features used to separate them including: tentacle number and shape, colouration and shape of the oral arm, shape of the gastrovascular pouches and the number and shape of the marginal lappets. Although the mtDNA indicated clear separation of the three *Chrysaora*, the nucDNA displayed some ambiguity. The cnidome showed considerable divergence and succeeded in separating these species, while the rhopalia of the three species also showed distinct differences in the lengths of the rhopalal canals and basal stems. Much of the confusion surrounding jellyfish taxonomy has been the result of observations made on predominantly preserved specimens that are in subpar quality, a hinderence which this study endeavored to overcome.

Keywords: Agulhas, Benguela, COI, ITS1, Nematocysts, Scyphomedusae, Systematics

1. Introduction

Cnidarian history: Medusozoa vs. Anthozoa

Cnidaria is a diverse phylum comprised of approximately 11 000 relatively "simple" species that are characterized by their ability to synthesize a highly complex cellular organelle known as the cnida (Daly *et al.* 2007). The cnida is a diagnostic characteristic for the Cnidaria (Daly *et al.* 2007), and is often regarded as an encapsulated stinging cell possessing an eversible tubule (Weill 1934). In addition to the cnida, radial symmetry and the presence of a polyp (benthic attached phase) and planula (short-lived larval phase) stage within their development have also been considered diagnostic for this phylum. However, the latter mentioned features can prove problematic as many Cnidaria lack the planula larval stage within their development, whilst others do not always form a polyp. In addition, some Cnidaria display radial symmetry while others are "directionally asymmetrical" or bilateral (Dunn & Wagner 2006).

Due to this variability, much debate has surrounded a polypoid or medusoid (medusa phase) ancestry of the Cnidaria (Brooks 1886). Scientists such as Jagersten (1959), Patin (1960), Hadzi (1963) and Werner (1984), supported the notion that the ancestral Cnidaria possessed a benthic stage of life. This is concurrent with the hypotheses surrounding the origin of the coelom (Jagersten 1959; Hadzi 1963), which suggests that the radiation of the Eumetozoa required a primitive, bilateral, polypoid form, such as that of the Anthozoa. However, in contrast, many scientists also support a medusoid ancestry (Hyman 1940; Hand 1959). This disparity is likely due to the large degree of overlap which exists amongst the body plan and life histories between the Anthozoa and Medusozoa (Won *et al*. 2001). However, the Anthozoa and the Medusozoa do differ significantly, as all anthozoans are exclusively polypoid (Daly *et al.* 2007) being either solitary or colonial. Collins (2002) considered the ontogeny of the Anthozoa to be the most straight-forward owing to the simple life cycle of the settlement of a planula larva which develops into a sessile polyp. This exclusively polypoid form was often considered a synapomorphy (Brusca & Brusca 1990), but is now more generally considered a plesiomorphy since this trait is shared with some Medusozoa (Collins *et al.* 2006).

Although a clear consensus does not exist concerning cnidarian ancestry, and a multitude of contradictions exist within previous investigations into the relationships between cnidarian Classes (Petersen 1990; Bridge *et al.* 1992; Schuchert 1993; Collins 2002), with many early phylogenies suggesting anthozoan paraphyly (reviewed by Berntson *et al.* 1999; Collins 2002), a clear consensus has emerged in the last decade, which suggests the Cnidaria to be comprised of two monophyletic clades: the Anthozoa, which is made up of over 7 500 extant species and the Medusozoa which contains approximately 3 800 species (Bridge *et al.* 1992; Bridge *et al.* 1995; Collins 2002; Mianzan & Cornelius 1999; Daly *et al*. 2007). The distinction between the Anthozoa and Medusozoa is well supported by their genetics (Kim *et al.* 1999; Medina *et al.* 2001; Collins 2002), morphology and life histories (Bridge *et al.* 1995). Additionally, the presence of the apparently derived linear mtDNA structure of the Hydrozoa, Scyphozoa and Cubozoa, further support the hypothesis that these Classes do in fact form a monophyletic clade relative to the Anthozoa, with the Anthozoa appearing to be the more basal group (Bridge *et al.* 1992). It is important to note that although the taxonomy of Cnidaria largely reflects the phylogenetic structure, the ranks of many groups within taxonomic analyses are not compatible with that of the phylogenetic structure, for example: the Anthozoa comprises all of the members of the clade Anthozoa, while the Medusozoa contains the remaining Classes, indicating that Class and ranks have differing implications within their respective phylogenetic structure (Daly *et al.* 2007).

Medusozoa: historical vs. modern classifications

Medusozoa was originally considered to consist of the Hydrozoa, which contains approximately 3 500 valid species (Schuchert 2007) and, the Scyphozoa, which contains approximately 200 morpho-species (Mayer 1910; Kramp 1961). The Hydrozoa, which is the largest Class occurring within the Medusozoa, contain a number of diagnostic morphological apomorphies (Daly *et al.* 2007), some of which include: the presence of a velum; young medusae (ephyrae) are laterally budded from the polyp; the presence of two nerve rings (situated around the bell margin) and the lack of septa within the medusa and polyp phase (Boero & Bouillon 2004). They differ from the Scyphozoa in the way they bud (scyphozoans are budded via a process known as strobilation) as well as by the lack of septae in the polyp stage, as scyphozoan polyps always contain septae (reviewed by Marques & Collins 2004). Hydrozoans also possess unique sensory structures (rhopalia), which sense equilibrium and often light, suggesting that these organs have evolved independently of those observed within the Cubozoa, Staurozoa and Scyphozoa (reviewed by Marques & Collins 2004). This has resulted in a wide consensus regarding hydrozoan monophyly, which is now generally accepted (Bridge *et al.* 1992; Collins 2002; Marques & Collins 2004)

The Class Scyphozoa originally comprised the Orders: Cubomeduseae, Rhizostomeae, Coronatae, Semaeostomeae and Stauromedusae (Mayer 1910; Kramp 1961). However Haeckel (1880) was the first to recognize the Cubozoa as being distinct from the other Classes. Further investigation of the morphology by some authors (e.g. Thiel 1966; Russell 1970) determined that the Class Scyphozoa only be comprised of the Rhizostomeae, Coronatae and Semeaostomeae. This hypothesis has been reinforced by more recent molecular analyses conducted by Collins (2002), which also designated the Cubozoa and Staurozoa as distinct Classes. This is now generally accepted across the scientific community.

The Cubozoa

Amongst the Medusozoa, the Cubozoa, Werner 1975, is the most species-poor Class, containing only 36 formally described, valid species spread across two Families (Gershwin 2005, 2006a, 2006b). Cubomeduseae can be distinguished from all other Medusozoa by the presence of four perradial rhopalia (marginal sense organs situated at the end of canals originating directly from the stomach), which contain highly developed eyes containing lenses, retinas and ocelli (Pearse & Pearse 1978; Coates 2003). They also contain a velarium, a piece of subumbrellar tissue that reduces the width of the subumbrellar opening (Daly *et al.* 2007). The tentacles of the cubozoans are concentrated at four interradial corners, and this feature is also unique to the Class. One diagnostic feature for the Class, which is less readily observed, involves the metamorphosis of a polyp into a single juvenile medusa; a trait not observed in any other cnidarian (Daly *et al.* 2007). However, a study by Stangl *et al*. (2002) of *Carybdea marsupialis*, reported striking similarities between the aforementioned metamorphosis and that which is observed within the Stauromedusae.

Staurozoa vs. Medusozoa and Anthozoa

The next smallest Class within Medusozoa is Staurozoa, which comprises only 50 valid species (Mills & Hirano 2007), and is widely considered an attached medusa phase. It has an entirely benthic habit, similar to the Anthozoa, but is much more similar to the Hydrozoa, Scyphozoa and Cubozoa in its life history (Marques & Collins 2004) and genetic structure (Collins 2002). For example: similar to the Cubozoa, apical transformation of the polyp takes place without transverse fission, which results in a sessile polyp-like adult (Stangl *et al.* 2002). This is in contrast to the process of strobilation observed within most scyphozoans. Molecular analyses have consistently identified Staurozoa as a basally branching Class within Medusozoa (Collins 2002; Dawson 2004; van Iten *et al*. 2006).

The Scyphozoa: What are they? Problems with identification and traditional taxonomic frameworks

The Scyphozoa, which form the focus of this investigation, generally have a life cycle that alternates between a sessile polyp stage and a free swimming medusa stage (Russell 1970; Collins 2002; Morandini & Marques 2010). The benthic polyp stage, known as the scyphistoma, of many jellyfish is able to bud off numerous ephyrae (by a process known as strobilation), while also possessing the ability to bud off new polyps, via fission, vegetative budding or cyst formation, resulting in the mass production of free swimming medusae in favourable conditions (Arai 1997). In contrast to asexual budding by the polyp, the free swimming medusae reproduce sexually (Arai 1997). Often the mass production of eggs by female medusae results in the development of hundreds of planula larvae, following fertilization (Holst & Jarms 2007). The planula larvae usually undergo a short planktonic phase, following which they settle and metamorphose into new polyps (Holst & Jarms 2007). The diagnostic characters for this group (alluded to previously) thus include the process of polydisk strobilation in polyps and, the presence of marginal rhopalia and ephyrae (Marques & Collins 2004). It should however be noted that multiple species within the Rhizostomeae (Kolpophorae) display only monodisk strobilation (Russell 1970; Ax 1996).

Although our current understanding of the systematics of the Scyphozoa differs greatly to the original classification (with Cubozoa and Staurozoa now erected as separate Classes), further molecular analyses have also indicated the presence of only two monophyletic groups: Order Coronatae and Order Discomedusae. The Discomedusae contains Semeaostomeae which is paraphyletic with respect to Rhizostomeae (Collins 2002; Dawson 2004). The Sameaostomeae (Agassiz 1862) is now comprised of four Families, namely: Cyaneidae (Agassiz 1862), Pelagiidae (Gegenbaur 1856), Ulmaridae (Haeckel 1880) as well as the recently erected Family, Drymonematidae (Haeckel 1880), with the Family Ulmaridae appearing to be the sister taxon to Rhizostomeae, rather than to Cyaneidae or Pelagiidae (Collins 2002; Hamner & Dawson 2009) as previously suggested.

The Pelagiidae, Gegenbaur 1856, contains three genera: *Chrysaora* (Péron & Lesueur 1810), *Pelagia* (Péron & Lesueur 1810) and *Sanderia* (Goette 1886) (reviewed by Kramp 1961), and, contains approximately 20 formally described species across them. The diagnostic characters for this group are: a central stomach which gives rise to separate and unbranched gastric pouches; long, highly folded oral arms and the absence of a ring canal (Kramp 1961). The Pelagiidae have a worldwide distribution, and frequently swarm/bloom within the warmer waters of the Mediterranean, around Africa and the east coast of Brazil (Pitt & Lucas 2014), while frequently blooming within colder waters as well. As is the case with the annual *Aurelia* blooms occurring in Port Fidalgo off the coast of Prince William Sound, Alaska, suggesting the Pelagiidae to thrive in most productive shelf ecosystems (Pitt & Lucas 2014).

Traditional taxonomic and morphological phylogenetic frameworks have only been effective in dependably describing and reconstructing evolutionary relationships among a subset of taxa within the Scyphozoa (Bayha *et al.* 2010). As a result a wealth of contending phylogenetic hypotheses exist regarding their taxonomy (Bayha *et al.* 2010), and more accurate and detailed morphological and molecular descriptions are needed to resolve the systematics of various jellyfish taxa. Many descriptions that are still widely used today are fraught with errors and archaic, resulting in a variety of species being misidentified (Bayha *et al.* 2010). A host of species still lack descriptions for this reason and it is thus common for scientists to misidentify closely related species that share many similar morphological characteristics, often resulting in widespread synonymization between closely related species (Gershwin & Collins 2002).

The synonymization of jellyfish by taxonomists has occurred frequently, as was the case with the genus *Cassiopea*. Initially six species of *Cassiopea* were described and all subsequently synonymized into a single species, namely *C. andromeda* (Gohar & Eisawy 1960). Their synonymization was attributed to morphological crypsis. However, recent molecular analyses suggest *Cassiopea* to comprise six species: *C. frondosa* plus another five genetically distinct but morphologically cryptic species (Holland *et al.* 2004). Similarly, two species of *Aurelia*, *A. aurita* and *A. labiata* were originally recognized by taxonomists (reviewed by Russell 1970), however a total of 20 species of *Aurelia* have been described, based on morphology over the last century (Mayer 1910; Kramp 1961), while at least 13 distinct species have been confirmed by recent molecular analyses (Dawson & Jacobs 2001).

Molecular biology and jellyfish systematics

Molecular analyses of various genera are continuously revealing new species and often cryptic scyphozoan species (Dawson & Jacobs 2001; Dawson 2003; Dawson 2005a), resulting in a recent increase in the number of recognized species. Although molecular analyses provide valuable insight into species level relationships, it should be noted that jellyfish DNA is often highly variable (Dawson & Jacobs 2001) and robust molecular phylogenies are lacking. Despite recent progress, a number of morphological hypotheses still remain unresolved and unaddressed by molecular studies, leaving morphological descriptions as the only “go to” for taxonomists (Bayha *et al*. 2010). Dawson (2004) stated that scyphozoan relationships particularly, have never been the focus of any molecular analysis despite the inconsistently resolved phylogenies. As such complete objective analyses should still be undertaken on morphological data along with complete modern statistical analyses (Dawson 2003). Although morphological and molecular analyses may at times contradict each other, there is a need to integrate the two as this will facilitate a more robust and reliable phylogeny on which to base taxonomic decisions.

Using the cnidome to aid in scyphozoan systematics

Detailed descriptions of the medusae have been provided for several taxa in recent years, but very few have provided detailed descriptions of the cnidome (stinging cells on tentacles, oral arms and bell). The cnidome of jellyfish can provide additional taxonomic information at the species level (Peach & Pitt 2005). For example, Calder (1971, 1974) used the cnidae to distinguish between several species of Scyphozoa. In acontarian anemones they have also been used to distinguish between Families (Carlgren 1949). Three types of cnidae are found within the Cnidaria namely: nematocysts; ptychocysts and spirocysts, however, only one type of cnidae is found within scyphozoan jellyfish and they are known as nematocysts (Rifkin 1991). Each nematocyst sits within a host cell and consists of a collagenous capsule and an eversible coiled tube (Peach & Pitt 2005). These “host” cells are associated with mechanoreceptors and chemoreceptors that influence nematocyst discharge (Arai 1997). Comprehensive descriptions of the cnidome have not yet been provided for the majority of scyphozoan species (Peach & Pitt 2005), however, over 30 morphological types of nematocysts have been identified and various systems of nomenclature based on nematocyst morphology have been proposed for the Cnidaria (reviewed by Östman 2000).

The morphology of nematocysts vary between taxa. For example, enclosed tubules may vary, the shape of the outer nematocyst wall may vary, the tubule and its shaft (basal region of the tubule) may be differentiated in some but not in others, etc. (Östman 2000; Peach & Pitt

2005). Upon discharge, the thread of the nematocyst can be of variable length, structure and diameter and can bear several distinct types of spines (Mariscal 1974). The length, diameter and arrangement of spines on a thread are important in the identification of nematocyst type (Mariscal 1974). The common nematocyst types found in Schyphozoa are: holotrichous isorhizas, homotrichous and heterotrichous microbasic euryteles and atrichous isorhizas (Weill 1934). It is important to note that none of the nematocysts mentioned here are unique to Scyphozoa and these categories have all been observed in other Classes (Carlgren 1945), however the combination of types and their relative abundances tend to change with species and Class (Peach & Pitt 2005).

The differences between nematocysts can be used to distinguish between different species, where descriptions are robust (Östman 1982). Carlgren (1940) went so far as to state that cnidome size (i.e. length and width of the capsule and length of the thread) is of systematic value and that no species description is complete unless it includes information on the cnidome. Cnidome size becomes especially important for distinguishing species that possess the same type of cnidae (Östman 2000). Ideally the study of the cnidome is based on fresh, live material, however this is not always possible, creating major obstacles for many taxonomic studies (Mejía-Sánchez & Marques 2013). As such various methods have been developed for the study of discharged nematocysts from preserved material, some of which are applied in this study (Puce *et al*. 2006; Mejía-Sánchez & Marques 2013).

The isorhizas, which are found across all cnidarian Classes, have been suggested as the primitive cnidae for cnidarians (Marques & Collins 2004), and they are the most morphologically simple nematocyst. This is in agreement with the views of Bozhenova *et al*. (1988), but is in contrast to the views of Salvini-Plawen (1978), who suggested that the haploneme nematocysts are primitive for Cnidaria. Nematocysts may also have undergone convergent evolution which would explain why nematocyst type correlates well with the diet of many pelagic medusozoans (Purcell & Mills 1988). However it is more likely due to conservative evolution.

The genus *Chrysaora*

The genus *Chrysaora* Péron & Lesueur, 1810 is the most species-rich genus occurring within the Order Discomedusae Haeckel, 1880. However the systematics and taxonomy of many species within this genus remain confused, which is troubled by tangled synonymies and poor descriptions (Morandini & Marques 2010). Péron & Lesueur (1810) originally assigned 11 species to the genus *Chrysaora* and considered three of them to be doubtful. Other authors have listed variable numbers of species as belonging to this genus: Lesson (1843) listed 13, Agassiz (1862) listed nine, Haeckel (1880) listed 10, while Mayer (1910) listed a total of 15 species. The work of Mayer (1910) is often considered the first comprehensive review of the species richness within the *Chrysaora*. In more recent years, Gerswhin & Collins (2002) listed 10 species as belonging to the genus *Chrysaora* in their phylogenetic analysis of the Family Pelagiidae (Gegenbaur 1856). They noted two of these species to be undescribed. Morandini & Marques (2010) similarly, conducted a comprehensive review of the genus and subsequently identified a total of 13 species.

Around the coast of South Africa, two species of *Chrysaora*, *C. fulgida* and *C. africana* are known to occur (Neethling 2010), with their identities suffering much confusion in earlier years (Morandini & Marques 2010; Neethling 2010). *Chrysaora fulgida* was originally described as possessing 24 tentacles and 32 lappets with a rose-red coloured bell (Reynaud 1830), while *C. africana* was described as having 48 lappets and a pale bell with red markings (Vanhöffen 1902). In later years, Stiasny (1939) classified jellyfish off the South African coast with 32 lappets and 24 tentacles as well as jellyfish off the Namibian coast possessing 48 lappets, as both being *C. fulgida*, which effectively resulted in the initial confusion between these species. These classifications were generally accepted until Neethling (2010) succeded in unambiguously separating these two species. This highlights the need for more accurate and comprehensive morphological and molecular descriptions of species occurring within the genus. Neethling (2010) succeeded in further untangling the phylogeny of three species of *Chrysaora*, namely: *Chrysaora fulgida*, *Chrysaora africana* and *Chrysaora hysoscella,* by means of morphological, molecular and modern statistical techniques.

This study aims to determine the identity of an unknown species of *Chrysaora* occurring off the South African southern coast, possessing features of both *C. fulgida* and *C. africana,* by means of a complete statistical analysis of both the morphology and genetics of this species and, if new, to describe it. Additionally a full, comprehensive description of the cnidome will be provided with the aim of determining whether the cnidome can be used to designate between these species. Descriptions will also be provided for any characters of *C. fulgida* and *C. africana*, which Neethling (2010) failed to describe, or which were not adequately described.

2. Materials and Methods

2.1 Morphology

2.1.1 Data Collection

A total of 35 jellyfish specimens of the unknown *Chrysaora* (hereafter referred to as *Chrysaora* sp. X), were collected during 2012 – 2015, from either beach strandings or via boat collections at the locations listed in Table 1. Medusae were preserved in 5 % formalin in ambient seawater or 70 % ethanol and stored at room temperature until analyses. Sperm and eggs were released into water prior to preservation and a portion of that water was placed in a bucket to allow fertilization. Following fertilization, planulae were collected and settled following the protocols of Widmer (2005), after which polyps were reared in a laboratory environment until strobilation for the production of ephyrae (localities from which medusae were obtained for planula settlement highlighted in Table 1). Similarly, planulae were obtained from specimens of *Chrysaora fulgida* collected at Walvis Bay, Namibia during 2012. Polyps and ephyrae were then stored in 5 % formalin until analyses. A number of these specimens have been submitted to the South African Natural History Museum, Marine Invertebrate Department (MB).

Preservation often results in varying degrees of weight loss and shrinkage (Lucas 2008) and Thibault-Botha & Bowen (2004) suggest these effects are pronounced in gelatinous zooplankton due to their high water content. These preservation effects vary with the size of the organism (Thibault-Botha & Bowen 2004) and with the period of preservation (de Lafontaine & Legget 1989). That said, de Lafontaine & Legget (1989) suggest these effects stabilize after a minimum of 60 days in preservation. The present study did not correct for any preservation effects as all measurements were taken at least 90 days after preservation and it was assumed that the preservation effects had stabilized. In order to further control for inconsistencies between formalin preserved and ethanol preserved specimens, only specimens preserved in formalin (since this was the medium most frequently used) were analyzed in the present morphological study. High definition photographs were used to aid in the description of living specimens.

Vernier callipers were used to take all measurements (variables summarized in Table 2 & Figure 1 where possible) under a magnifying glass, as described in Neethling (2010). Comparative measures of *C. fulgida* and *C. africana* were obtained from Neethling (2010). Descriptive statistics for all measures from all three species (mean, median, standard deviation, variance, min and max) are provided in Appendix 1. Type material of *C. fuglida* and *C. africana* was not available for examination at the time of the study. Neethling (2010) did not provide a comprehensive description of the rhopalia, ephyrae or polyps of *C. fulgida* and *C. africana*. Differences are thus highlighted between the rhopalia, ephyrae and polyps (if available) of *Chrysaora* sp. X, *C. fulgida* and *C. africana* in this study. In order to determine whether sexes are separate within *Chrysaora* sp. X, multiple gonads and manubrium tissue from various specimens were mounted and studied, following the methods of Lucas and Reed (2009).

2.1.2 Data Analyses

Many morphological measures vary with animal size (Neethling 2010), and it is necessary to account for this in any comparisons between species using statistical techniques such as ANCOVA (Miller & Chapman 2001). Such an approach not only requires a wide size range of study material, but also a consistency in the size ranges between species. This was not possible, and so in an attempt to reduce any size-linked bias to data, measures were standardized by dividing all by bell diameter (S1).

Using SPSS 22 Ltd. 2016 (Statistical Package for Social Sciences), the data were tested for normality using the Shapiro-Wilk's test for homogeneity of variances. For variables that failed the test for normality, data were $\log_{10}$ transformed and again tested for normality and used in subsequent analyses if the data were normally distributed. Logarithmic transformations are commonplace when dealing with biological data as they place all variables onto a common scale of variation regardless of the original unit of measurement, making it ideal for use in both parametric and non-parametric analyses (Clarke & Green 1988). Logarithmic transformations compress the upper scales of measurement relative to smaller values, thereby reducing the influence of the larger values and in effect "centering" the data set (Waite 2000).

Correlations between standardized measures and bell diameter were assessed using Pearson's r (Zar 1999), in order to determine any effects of individual size on measured variables. Variables that failed the test for normality were analyzed using Spearman Rank correlations. Variables for which correlations were not significant are summarized in Table 3, as these measures are not related to the size of the bell and provide robust features that allow ready comparison between species.

In order to determine whether measured variables of *Chrysaora* sp. X were morphologically distinct from *C. fulgida* and *C. africana*, two-tailed *t*-tests were employed for those variables that were normally distributed. Similarly, Mann-Whitney U tests were performed comparing *Chrysaora* sp. X to both *C. fulgida* and *C. africana* in order to test for differences between those variables that failed the tests for normality (Zar 1999). All alpha levels were corrected for multiple comparisons using the Bonferoni adjustment (Quin & Keough 2002). All univariate statistics were considered significant at an alpha level equivalent to 0.05, unless otherwise corrected.

Although univariate analyses allow each variable to be explored individually, most variables change together with animal size and should be examined together in a multivariate space as well (Waite 2000). Biological and environmental data rarely conform to the assumptions made by parametric statistics, making non-parametric analyses the norm for many ecologists (Waite 2000). Non-parametric statistics impose considerably less stringent assumptions and make no assumptions about the underlying distribution of the dataset (Waite 2000). Such are appropriate for the present study.

The Canonical Analysis of Principle Co-ordinates (CAP) is a particularly useful constrained ordination procedure within biology and ecology, as it allows any distance of dissimilarity to be used. It is useful where *a priori* hypothesis concerns differences among groups. As such, using Primer 7.0.10 2016, the Canonical Analysis of Principle Co-ordinates (CAP) routine was executed, which seeks a set of axes that best distinguishes amongst *a priori* groups (Anderson *et al.* 2008): in this case species. This process generates numerous matrices and produces a set of canonical axes. The Canonical Discriminate Analysis, which is a subset of the Principle Co-ordinate Analysis (PCO) works by finding axes that best separate categories and is a means of visualizing similarity of individual cases within a dataset (Anderson *et al.* 2008). Generally the axes used are selected manually and are based on the number of variables in the original dataset. However, in order to determine the number of PCO axes in this study, the “leave one out” procedure proposed by Anderson *et al.* (2008) was employed. This procedure was executed as the number of measured variables approached the number of specimens. The PCO axes that were determined were all independent of each other and orthonormal. An orthonormalized data matrix, which is based on codes for groups identified by a factor associated with the Euclidean Distance Matrix, runs parallel to the this process. By relating the PCO axes to this data matrix, an additional matrix is created which yields canonical eigen values and eigen vectors that are then used to produce the CAP plot. The CAP axes were used to determine if the predefined groups were classified correctly. Furthermore, through a series of permutation tests, the CAP routine was used to test the null hypothesis of no dissimilarities amongst the positions of centroids among groups in a multivariate space (Anderson *et al.* 2008).

2.2 Cnidome

2.2.1 Data Collection

Tissues for analyses were prepared as described in Peach & Pitt (2005). In summary, samples of tissue were dissected from the tentacles and oral arms of formalin or alcohol preserved specimens for each of *Chrysaora* sp. X (four specimens), *C. fulgida* (six specimens) and *C. africana* (four specimens) as well as from ephyrae of *Chrysaora* sp. X (three specimens) and *C. fulgida* (four specimens). Nematocysts were isolated by placing the tissue in a 10 % solution of Sodium Thyocyanate (Mariottini *et al*. 2010), following which small pieces of tissue were removed and homogenized using a mortar and pestle, squashed beneath a cover slip and examined unstained at 1000 × magnification, using a compound microscope. Pieces of tissue removed from the above mentioned areas were also placed in a saline solution, and a current passed through it using a wire connected to a 12V battery in an attempt to discharge some of the nematocysts. This tissue was then squashed underneath a cover slip and examined using a compound microscope with differential contrast. In cases where the above mentioned methods did not allow nematocysts to discharge, one of the following methods were used: hydrating the tissue in distilled H_2O for three, five, eight or 10 days (mounting tissue on each subsequent day in order to determine whether cnidae had discharged) (Mejía-Sánchez & Marques 2013) or allowing the tissue on the slide to dry slightly before covering with a cover slip. In some cases discharge was simply not possible, perhaps owing to the extensive preservation period.

The maximum widths and lengths of the capsule as well as the lengths of the shaft were measured using a graticule for both the discharged and undischarged nematocysts. The relative abundance of each type of nematocyst seen was then determined by counting the number of nematocysts in each determined category in 10 randomly chosen fields of view. A total of 2 500 nematocysts were counted during this process. The lengths and widths of at least 10 discharged and at least 10 undischarged nematocysts were recorded for each type for both the oral arm and tentacle tissue resulting in a total of 700 nematocysts measured. Where possible, photographs were taken of discharged and undischarged nematocysts. Complete descriptions are provided for each nematocyst type observed within these tissues. Nematocysts were identified according to Mariscal (1974) and Östman (2000).

2.2.2 Data Analyses

Using the data for the various measures of size (widths, lengths and shaft lengths), Levene's test of homogeneity of variances was performed to determine if the measured variables were normally distributed between each specimen and tissue. If data were not normally distributed, the data were $\log_{10}$ transformed. In order to determine the differences between the width, length and shaft length of the different types of nematocysts between each specimen and tissue type for the discharged and undischarged nematocysts, a univariate ANOVA was performed. All measurements are presented as means (± SE). Post hoc tests were performed where measurements were statistically different. Two-tailed *t*-tests were performed between nematocyst types occurring on both the oral arm and tentacle within the same species as well to test for differences in nematocyst types occurring on different tissues between the three species. The Bonferoni procedure was applied to all statistics in order to control for Type I errors (Quinn & Keough 2002).

In order to determine whether the relative abundance of each nematocyst type differed between tissues and species, Chi-square tests were performed using SPSS 22 Ltd. 2016, for the null hypothesis of no dissimilarity between the relative abundance of nematocysts across the three species and two tissue types. To summarize the diversity of nematocysts across each tissue type and species, Simpson's Index (D) was calculated, which is a diversity index weighted by abundances of the most common types of nematocysts (Magurran 2004). In addition, Simpson's diversity index (1 – D) as well as Simpson's reciprocal index (1/D) are also calculated as described in Magurran (2004).

2.3 DNA Analyses

2.3.1 Tissue Collection

Material used in genetic analyses were obtained from the specimens collected for morphological analyses (outlined in section 2.1.1, above), as well as from material collected *ad hoc* in previous years. For all specimens used, oral arm tissue was cut off the specimen, before the balance was preserved in either formalin or alcohol. The oral arm tissue was placed in 90 % ethanol and stored at -18˚C until analysis.

2.3.2 DNA Extraction, Amplification and Sequencing

Total genomic DNA extractions, precipitations and quantifications were performed following a standard phenol-chloroform extraction (Wallace 1987; Sambrook & Russell 2001;

Neethling 2010). Cytochrome *c* oxidase subunit I (COI) and the internal transcribed spacer 1 (ITS1) markers were amplified using the primers listed in Table 4. A maximum of 720 base pairs were amplified from 12 samples for the COI marker using a ramp up cycle with reaction conditions: 94˚C for 8 min; then one cycle of 54.2˚C for 2 min, 72˚C for 2 min, 94˚C for 4 min; followed by one cycle of 55.2˚C for 2 min, 72˚C for 2 min, 94˚C for 45 sec; followed by 33 cycles of 56.2˚C for 45 sec, 72˚C for 1 min, followed by a final step of 72˚C for 5 min before storage at 4˚C. For the ITS1 marker, a maximum of 340 base pairs were amplified for 15 samples, using a ramp up cycle with reaction conditions: 94˚C for 8 min; then one cycle of 51.5˚C for 2 min, 72˚C for 2 min, 94˚C for 4 min; followed by one cycle of 52.5˚C for 2 min, 72˚C for 2 min, 94˚C for 45 sec; followed by 33 cycles of 53.5˚C for 45 sec, 72˚C for 1 min, followed by a final step of 72˚C for 5 min before storage at 4˚C. All PCR reaction were carried out using 25 μL reaction volumes on a Techne ® endurance TC-512 gradient thermal cycler (Barloworld Scientific). Two micro litres of the PCR product was visualized on a 7 % agarose gel, following which the remainder was then purified with a nucleoFast 96 PCR kit (Macherer-Nagel). Using BigDye chemistry, the cleaned products were then cycle sequenced and analysed with an ABI 3730 XL DNA Analyzer (Applied Biosystems Inc.) at the Central Analytical Facility (University of Stellenbosch).

2.3.4 Sequence Processing

Misreads within sequences were corrected and poorly determined terminal portions discarded using Bioedit 7.2 (Hall 2005). This was done by visually checking the sequences and chromatograms. Using the ClustalW alignment tool in Bioedit 7.2 (Hall 2005), the forward and reverse sequences were aligned and used to create consensus sequences. The consensus sequence was then verified using Blast in Genbank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Additional sequences were obtained from Genbank where possible in order to create more robust networks. Sequences obtained from Genbank are indicated by their succession numbers as it appears on Genbank.

2.3.5 Phylogenetic Analyses

Mean pairwise sequence differences using uncorrected "*P*", as well as maximum parsimony trees were then calculated in PAUP* 10.4b (Swofford 2001), for both COI and ITS1 sequences. The maximum parsimony tree generated for each gene implies the evolutionary phylogenetic relationships between species. The tree was calculated by performing a heuristic parsimony analyses, using a branch swapping algorithm, also known as the tree-bisection-reconnection method, with all characters assigned an equal weight and left unordered. A bootstrap procedure was then performed in order to test the stability of the nodes, using 1 000 resampling replicates and the tree-bisection-reconnection method. Only bootstrap values above 75 % were considered to be well supported and retained in the final tree, however only those with values of 90 - 95 % or higher were considered to be strong support (Felsentein 1985).

The Akaike information criterion was used in the programme JModeltest v. 2.1.2 (Darriba *et. al*. 2012) in order to determine the "best fit model of evolution" for both gene fragments (Akaike 1973; Nylander 2004). Bayesian analyses were then performed in Mr. Bayes v. 3.2 (Ronquist & Hulsenbeck 2003), using this model as a guide and included five paralleled Monte Carlo Markov chains. The resultant chains were sampled every 1 000th generation and a total of 10 million generations were used. In order to determine statistical stationarity, the "sump" command in Mr. Bayes was used to summarize the generated samples. Based on these results, 25 % were discarded as burn-in. To assess whether the data were adequately sampled, the potential scale reduction factor was determined (PSRF) (Ramhaut & Drummond

2007). Following this the “sumt” command was executed in Mr. Bayes in order to summarize trees. These trees were then visualized using the programme Fig tree v. 1.4.2 (http://tree.bio.edu.ac.uk/software/gtree) and those nodes with posterior probabilities $p < 0.95$ were considered not significantly supported.

Following individual analyses, a concatenated dataset was created and analyzed using Parsimony and Bayesian analyses as described above, in a partitioned fashion for both gene fragments (ITS1 and COI). Here the Bayesian analyses were run for 25 million generations and the tree again visualized using FigTree v. 1.4.2. In order to incorporate some population level processes, higher level gene clustering was assessed using splits tree v. 4.5 (Huson & Bryant 2006). Splits tree employs the Neighbour-net algorithm, which essentially uses a distance matrix as an input and works by agglomerating clusters (Bryant & Moulton 2004). This can however result in overlapping clusters which do not form a clear hierarchy (Bryant & Moulton 2004). These clusters are then represented by a phylogenetic network known as a splits network, which essentially represents character change distances. As such, uncorrected “*P”* distances were used to draw a neighbour network for each gene (Bryant & Moulton 2004), using equal angle splits to present the relationships (Dress & Huson 2004).

3. Results

3.1 Quantitative Descriptions

3.1.1 General Quantitative Description of *Chrysaora* sp. X

Jellyfish have an umbrella measuring, on average, 12 cm in diameter; umbrella thickened centrally with central portion approximately four times thicker than thinner marginal portion (Appendix 1); eight rhopalia divide umbrella into equal octants. Umbrella margin cleft into 32 lappets, four per octant: two rhopalial lappets and two velar lappets (Figure 2 a); velar lappets wider (L = 0.55 ± 0.25, W = 1.16 ± 3.72) than rhopalial lappets are long (L = 1.35 ± 4.58, W = 0.68 ± 0.31) (Appendix 1, Figure 2 a).

Twenty-four marginal tentacles situated around bell, three per octant: one primary tentacle and two secondary tentacles (Figure 2 a & c); primary tentacle (11.14 ± 6.4) generally as long as bell is wide in smaller specimens, becoming up to 30 % longer than bell diameter in larger specimens; secondary tentacles (9.83 ± 5.35) 1 cm – 2 cm shorter than primary tentacles (Appendix 1); at base, secondary tentacles (0.19 ± 0.10) generally half the width of the primary tentacle (0.22 ± 0.13).

On the subumbrellar, radial septa arise from periphery of a central stomach, dividing the gastrovascular cavity into 16 approximately equal pouches. From stomach arises a manubrium (2.22 ± 1.27), approximately 15 % – 20 % of bell diameter (10 ± 4.97) in length. From the manubrium extend four oral arms, typically 15 % – 25 % longer than diameter of bell in mature specimens (12.02 ± 4.09); maximum width of oral arm (4.78 ± 2.95) 1/3 of total arm length. Four highly folded gonads, slightly wider than long, are situated within the stomach; widths and length of gonads similar in smaller specimens (< 4 cm). One ostium situated between each adjacent gonad (Figure 2 b); ostia length (1.09 ± 0.84) typically half the length of gonads (2.27 ± 1.26).

3.1.2 Quantitative Description of the Cnidome of *Chrysaora* sp. X

Medusae

Chrysaora sp. X contained five distinct nematocyst types, summarized in Table 5. Upon discharge, holotrichous A- and O- isorhizas possessed tubules up to 20 times the capsule length (Table 5, Figure 3 a – d); capsules large measuring up to 18 µm; capsules often twice the length of other nematocysts. In the case of holotrichous A-isorhizas (Figure 3 f – g), tubule length significantly longer in tentacles (82.36 ± 2.54) than oral arms (53.2 ± 3.93) (DF = 19, F = 40.31, $p < 0.001$); holotrichous O-isorhizas showed no variation between tissue types (OA = 95.75 ± 11.85; T = 114.1 ± 3.54) (DF = 20, F = 2.213, $p = 0.653$: Table 6). Atrichous isorhizas occurred only on tentacles and had capsules 0.5 times the length of other isorhizas (10.3 ± 0.26), possessing tubules similar in length (125.5 ± 1.28) to those of holotrichous A- and O-isorhizas, tubule often longer than holotrichous A- and O-isorhizas (Tables 5 – 6, Figure 3 e).

Heterotrichous microbasic bi-rhopaloids were the most abundant type of nematocyst in the tentacles and oral arms; with two distinct types of spines, shaft less than two times capsule length with two clear dilations: one at the base of the shaft and another at the distal end (Table 5, Figure 4 a – d). Tubules (OA = 67.7 ± 7.32; T = 135.95 ± 24.24) more than 10 times capsule length (OA = 11.55 ± 0.32; T = 15.1 ± 0.94). Shaft length similar to holotrichous A- and O- isorhizas.

Heterotrichous microbasic euryteles only found in oral arms, with shaft less than two times capsule length (12.81 ± 0.37) and bearing a short tubule (24.1 ± 0.94: Table 5, Figure 5 a – c); possessing two distinct types of spines: one type on shaft and one on distal tubule.

Ephyrae

Two distinct nematocysts occured in the ephyrae: heterotrichous microbasic bi-rhopaloids and holotrichous O-isorhizas: rhopaloids (L = 11.2 ± 0.13; W = 10.3 ± 0.21) larger than isorhizas (L = 7.8 ± 0.2; W = 7.4 ± 0.16), the latter being more abundant than the former, with thousands often occurring within one lappet.

3.2 Comparative Statistics

3.2.1 Morphological Comparisons Between the Three *Chrysaora*

Chrysaora sp. X strongly resembles *C. africana* superficially, while appearing highly dissimilar to *C. fulgida*. *Chrysaora* sp. X and *C. fulgida*, do however share a number of morphological features, some of which include: shape of gonads and point of attachment; number and shape of lappets and the star shaped colouration pattern on the exumbrellar surface (Appendix 2). However, of the 25 standardized morphometric features compared between *Chrysaora* sp. X and *C. fulgida* in the quantitative univariate analysis, 13 features were significantly different (Tables 7). Some of these features included those relating to bell height (S2 & S3); diameter of the mouth (S20); inter-ostial width (S27) and length of the oral arms (S29). *Chrysaora* sp. X also had a number of features in common with *C. africana* (Tables 8), including the size and shape of gonads; typical star shaped colouration pattern on the exumbrellar surface and the colouration and shape of the oral arms. Of the 25 features compared between *Chrysaora* sp. X and *C. africana*, in the quantitative univariate analysis, 15 were significantly different (Tables 8). Some of the features that differed included those relating to the height of the bell (S2 & S3); rhopalial and velar lappet widths and lengths (S6, S7, S8 & S9); Mouth diameter (S20); inter-ostia width (S27) and width of the ostia (S25). Lastly, *Chrysaora* sp. X was also compared to *C. hysoscella* and of the 21 features they had in common, eight were found to differ significantly (Tables 9). These results indicate a closer morphological similarity between *Chrysaora* sp. X and *C. fulgida*, despite its strong superficial resemblance to *C. africana*.

The CAP plot was performed on standardized variables shown by all species that were considered to be diagnostic by Neethling (2010), and included: manubrium length (S22), rhopalial lappet length (S6), velar lappet length (S8), mouth diameter (S20), ostia width (S25), number of velar lappets (S5), manubrium depth (S24) and maximum width of the oral arm (S31). For the CAP analysis, a subset of eight PCO axes was used, based on the “leave-one-out” diagnostic, which accounted for 96.61 % of the total variation in the species data and resulted in 3.79 % miss-classification error (Table 10 & Figure 6). The first and second squared canonical correlations (δ_1^2) were high: 0.976 and 0.917 respectively, and the results of the permutation tests with *C. fulgida* were significant ($p < 0.0001$; trace statistic: 2.013; $p < 0.0001$). The results of the CAP analysis show a clear dissimilarity between *Chrysaora* sp. X, *C. africana* and *C. fulgida*, though they indicates closer morphological similarity between *Chrysaora* sp. X and *C. fulgida* (Figure 6). The analyses failed to separate *C. fulgida* and *C. hysoscella*.

In addition to the quantitative analysis, a number of qualitative features also distinguish *Chrysaora* sp. X from *C. fulgida* and *C. africana*. The most obvious features relate to the overall colouration of the medusa. *Chrysaora* sp. X has a strong purple/maroon colouration with white oval spots scattered throughout the exumbrellar (Figure 7 a – d). *C. fulgida* is a maroon or reddish brown colour when mature (Figure 8 b). The colouration of *Chrysaora* sp. X is more similar to *C. africana* which also has a purple colouration (Figure 9 a – b), with a cream to white base (Figures 7 a – c & 9 b). *Chrysaora fulgida* exhibits a large degree of variation in its colouration as the animal grows with smaller specimens always lacking the characteristic star shaped pattern on the exumbrellar of the bell and often exhibiting a pale pink colour rather than a deep maroon (Figure 8 a). This variation has not been observed in *C. africana* nor *Chrysaora* sp. X, both of which are strongly pigmented when small.

The number, shape and colouration of the lappets also differ greatly between these species. *Chrysaora* sp. X and *C. fulgida* both have 32 marginal lappets that are rounded or semi-circular in shape, while *C. africana* has 48, triangular lappets. Additionally, the lappets of *C. fulgida* contains an intricate network of canals around the outer edges, while *Chrysaora* sp. X possesses a similar but less defined network of canals within its lappets. *C. africana* lacks these canals entirely. Tentacles originate in clefts between adjacent lappets and therefore the number of tentacles is thus associated with the number of lappets. Subsequently, owing to differences in lappet number, *C. fulgida* and *Chrysaora* sp. X have up to 24 tentacles, while *C. africana* has 40 tentacles. The colour and shape of these tentacles varied between these three species as well. *C. fulgida* and *C. africana* both possess tentacles that are heavily pigmented with the tentacles of *C. fulgida* being a deep maroon and that of *C. africana* being a deep purple colour (Figures 8 b & 9 a). The tentacles of *C. fulgida* are cylindrical throughout however, the tentacles of *C. africana* are laterally flattened. The tentacles of *Chrysaora* sp. X are laterally flattened for approximately one third of their length proximally and becoming more cylindrical distally, and are white to cream in colour for the most part (Figure 7 a – c). The base width of the tentacles of *Chrysaora* sp. X is wider than the tentacles of *C. fulgida*. As a result, the secondary tentacles of *C. fulgida* readily break off and are often missing upon collection thus leaving the specimens appearing to have only eight tentacles. This is not observed within *Chrysaora* sp. X, which has persistent primary and secondary tentacles.

The oral arms of the three species also show a high degree of differentiation (Figures 7 – 9). *C. fulgida* has the most distinct oral arms, being orange (Figure 8), highly folded/spiraled resulting in their oral arms appearing to be comparatively shorter than that of *C. africana* and *Chrysaora* sp. X (Figures 7 & 9). *Chrysaora africana* possesses long, trailing, unspiralled oral arms that are cream in colour (Figure 9 a). In the case of *Chrysaora* sp. X, the oral arms are spiralled basally but become less spiralled distally; they are generally a cream colour (Figure 7 b) but may have some red pigmentation at the centre of the arm (Figure 7 a & c).

In addition to the variation observed within the lappets, tentacles and oral arms between these three species, the gastrovascular pouches and gonads also differ. In both *Chrysaora* sp. X and *C. fulgida*, the tentacular gastrovascular pouches dilate and contract distally, terminating at the periphery of the rhopalial lappets resulting in a pear shape at the distal end, while the tentacular pouches of *C. africana* dilate distally and terminate at the cleft between velar and rhopalial lappets resulting in a triangular shape at the distal end (shown in Figure 10 a − c). Lastly, the point of attachment of the gonads differed between the three *Chrysaora*. In *C. fulgida*, the gonads are found in thin membranous sacs, attached to the subumbrellar surface in the central stomach, while in *C. africana* the gonads are attached to the periphery of the ostia. The gonads of *Chrysaora* sp. X are found within the central stomach, attached to the subumbrellar surface, similar to *C. fulgida*, but they lack the membranous sac observed in *C. fulgida*. The qualitative analysis thus revealed *Chrysaora* sp. X to be more similar to *C. fulgida* than *C. africana* but is however highly dissimilar to both.

3.2.2 Comparison of the Cnidome

Medusae

A total of five distinct nematocyst types occurred within *Chrysaora* sp. X and *C. africana* while four distinct types occurred within *C. fulgida* (summarized in Table 5). For those nematocysts types found in both the oral arms and tentacles, within the same species, little variability was observed in the length and width of the capsule (Table 6). However, shaft lengths showed significant differences across all three species between the oral arms and tentacles for most of the nematocysts (Table 6).

Furthermore, nematocysts showed much variation across the three *Chrysaora* for the measured variables. The univariate ANOVA showed heterotrichous microbasic euryteles to differ significantly in their shaft lengths (DF = 28, F = 62.91, $p < 0.001$) between *Chrysaora* sp. X and *C. africana*, while the atrichous isorhiza differed in the width of the capsule (DF = 19, F = 19.19, $p < 0.001$: Tables 11 − 12) and length of the shaft (DF = 19, F = 136.61, $p < 0.001$; Table 11 − 12) between these species. The holotrichous A-isorhizas differed in the lengths (DF = 60, F = 5.41, $p < 0.001$) and widths (DF = 60, F = 5.78, $p < 0.001$) of the capsule, but did not differ significantly in the shaft lengths (DF = 60, F = 0.36, $p = 0.69$) across all three species (Tables 11 − 12). The holotrichous O-isorhiza differed in the capsule lengths (DF = 60, F = 7.24, $p < 0.001$) and widths (DF = 60, F = 4.56, $p = 0.014$) across all three species while the shaft lengths did not differ significantly (DF = 60, F = 1.439, $p = 0.245$) (Tables 11 − 12). No variability was observed across the remaining nematocysts.

In all three species of *Chrysaora*, the oral arms had a comparatively higher diversity of nematocysts (contained rhopaloids, isorhizas and euryteles) than the tentacles (contained only rhopaloids and isorhizas) (Table 13). The Chi-square analysis revealed the relative abundances across the species to differ significantly (DF = 2, Chi-square = 31.11, $p < 0.001$). The Chi-square analysis also revealed the relative abundances to differ between the oral arms and tentacles (DF = 1, Chi-square = 7.20, $p = 0.027$). The Simpson's Diversity Index (D) indicated a relatively low diversity of nematocysts across the three species (< 3), however did reveal some differences (Table 13). The highest levels of diversity occurred within the oral arms of both *C. fulgida* and *Chrysaora* sp. X, while the diversity of nematocysts in *C. africana* was slightly lower. *Chrysaora fulgida* also had the highest diversity of nematocysts within the tentacles.

Ephyrae

Two distinct types of nematocysts were found within the ephyrae of *C. fulgida* and *Chrysaora* sp. X namely: heterotrichous microbasoc bi-rhopalods and holotrichous O-isorhiza. For both these nematocyst types, the results of the *t*-tests revealed the capsule length and width to differ significantly between the two species, while the lengths of the shafts did not differ significantly (Table 12). The *t*-test also indicated shaft lengths and capsule lengths to differ significantly between the ephyrae and medusa for both nematocyst types, however capsule widths often indicated no significant differences (Table 12).

3.3 Genetic Analyses

3.3.1 Gene Characteristics

For the internal transcribed spacer 1 (ITS1), a 340 bp region was amplified from eight *Chrysaora* sp. X, three *C. africana*, three *C. fulgida* and one *C. hysoscella* specimen (Appendices 3 – 6) and subsequently truncated to 310 bp. For cytochrome *c* oxidase subunit I (COI) a 720 bp region was amplified from seven *Chrysaora* sp. X, two *C. africana*, two *C. fulgida* and one *C. hysoscella* specimens and was subsequently truncated to 655 bp

(Appendices 7 – 10). The parsimony analysis revealed 154 (23.5 %) characters to be parsimony informative, while 501 (76.5 %) were considered invariable for the COI gene region, while for ITS1 15 (4.4 %) characters were determined to be parsimony informative and 295 (95.6 %) were considered uninformative.

3.3.2 Pairwise divergence and phylogenetic reconstruction

The parsimony analysis of the COI gene region revealed the presence of three monophyletic lineages (Figure 11), which supports the complete distinction of *Chrysaora africana* and *Chrysaora fulgida*. It also showed *Chrysaora* sp. X to be genetically distinct from both *C. fulgida* and *C. africana*. The South African haplotype of *C. fulgida* formed a clade with *C. hysoscella*, while the Namibian haplotype showed little differentiation from *Chrysaora* sp. X. The mtDNA sequence data (Table 14) showed an average of 32.17 % pairwise sequence difference between *Chrysaora* sp. X and *C. africana*, an average of 16.16 % pairwise sequence difference between *Chrysaora* sp. X and *C. fulgida*, while an average of 24.4 % pairwise sequence difference was observed between *Chrysaora* sp. X and *C. hysoscella*. The mean pairwise sequence difference between *Chrysaora* sp. X and *C. fulgida* are comparatively lower than all other species (Table 14), but are however still substantial at 16.16 %. Since Dawson and Jacobs (2001) suggest differences of 10 – 20 % between COI sequences is the standard for species level divergence, the pairwise sequence differences observed here were sufficient to designate *Chrysaora* sp. X as a distinct lineage.

The analysis of the ITS1 gene region clearly distinguished between *C. africana* and *C. fulgida* but failed to distinguish between *C. fulgida* and *Chrysaora* sp. X (Table 14, Figure 12). Here *C. hysoscella* formed a clade with *C. africana*, however many results were ambiguous due to low pairwise divergences of below 5 % (Table 14). Although the pairwise sequence differences observed between *Chrysaora* sp. X and *C. fulgida* (2.24 %) as well as between *Chrysaora* sp. X and *C. hysoscella* (6.43 %) lie below the standard 5 – 15 % differences, suggested by Dawson and Jacobs (2001) to designate between species, considerable divergence is still evident and is more clearly reflected by the COI sequences. The separation of the three species is supported by intra-lineage divergences that are markedly lower than intra-lineage divergences.

The results of the parsimony analysis are perhaps best illustrated by the neighbour-net analysis which showed complete differentiation between *Chrysaora* sp. X, *C. fulgida* and *C. africana*, however showed the Namibian haplotype of *C. fulgida* to not be distinct from the South African *Chrysaora* sp. X for the COI gene region (Figure 13). Similarly to what was observed in the parsimony analysis for the ITS1 gene region, the neighbour-net analysis showed clear separation of *C. africana*, while the results of *Chrysaora* sp. X, *C. fulgida* and *C. hysoscella* appear highly ambiguous (Figure 14).

The maximum parsimony and Bayesian analyses of the concatenated dataset provided some further resolution (Figure 15). Although some ambiguity was still observed, here *Chrysaora africana* appeared a completely distinct lineage once again, while *C. fulgida* again formed a clade with *Chrysaora* sp. X, but did however show a larger degree of separation. A similar situation could be observed within the neighbor-net analysis (Figure 16), which showed a similar result to that of the maximum parsimony, however the genes separated more unambiguously in this analysis.

3.4 Summary

The nuclear and mitochondrial DNA both display considerable molecular differentiation between all four species investigated. This, coupled with the strong dissimilarity displayed by both the quantitative and qualitative analyses of the morphology for these species, show clear and distinct separations between the four *Chrysaora* investigated. The cnidome provides further support for the separation of *Chrysaora* sp. X, *C. fulgida* and *C. africana*, showing some variation between the three species. It is therefore concluded that *Chrysaora* sp. X is morphologically and genetically distinct from *C. fulgida* and *C. africana*, and is a new species. Due to these results, a complete description is provided for this third species of *Chrysaora* in the following section. Descriptions of components of *C. fulgida* and *C. africana* (as previously indicated) are included where necessary for comparative purposes and to supplement existing descriptions.

4. Formal Descriptions

4.1 *Chrysaora* sp. X

SYSTEMATICS

SUB-ORDER Semaeostomeae Agassiz, 1862

FAMILY Pelagiidae Gegenbaur, 1856

GENUS *Chrysaora* Péron & Lesueur, 1810

SPECIES *Chrysaora agulhensis* sp. nov.

[FIGURES: 2 a – c, 7 a – d, 10 a, 17 a – e, 18, 19 & 20]

Type specimens. HOLOTYPE**:** (MB-A088455) (14.8 cm in diameter, 22 June 2014, preserved in 5 % formaldehyde in ambient seawater, Fish Hoek beach, South Africa, opposite train station, V. Ras col.). Paratypes: (MB-A088456) (~12 cm in diameter, 22 April 2014, preserved in 5 % formaldehyde in ambient seawater, Fish Hoek beach, South Africa, opposite train station, V. Ras col.); (MB-A088457) (~13 cm in diameter, 13 November 2012, preserved in 5 % formaldehyde in ambient seawater, Whale Rock off Robben Island, South Africa, D. Cox col.).

Examined material: Holotype**:** (MB-A088455). Paratypes: (MB-A088456); (MB-A088457). Seven specimens collected by boat at Whale Rock off Robben Isand in November 2012 (-33.8076073, 18.3712309); 11 beach stranded specimens collected at Muizenberg in April 2014 (-34.1087476, 18.4730991) (MB-A088458); Four specimens collected by net at Gouritzmond River Mouth in October 2014 (-34.347336, 21.888564); Ten beach stranded specimens collected at Zewenwacht Beach in March 2011 (-34.1038628, 18.7882734); Five ephyrae (MB-A088460) and five polyps (MB-A088459) of *Chrysaora agulhensis* obtained from specimens collected at Whale rock off Robben Island; Two *Chrysaora africana* from

False Bay, collected in June 2010; Three *Chrysaora fulgida* from False Bay, collected in 2012; Three ephyrae (MB-A088461) and polyps of *Chrysaora fulgida* from the coast of Walvis Bay, Namibia collected in 2012.

Type locality. Fish Hoek beach, False Bay, Cape Town, South Africa.

Distribution. Range stretches from Table Bay along the west coast of South Africa toward Port Elizabeth along the east coast of South Africa (Agulhas Bank): endemic.

Diagnosis. *Chrysaora* of medium size; 32 rounded marginal lappets, four per octant; no more than 24 persistent tentacles; tentacles laterally flattened with pronounced bases, and ribbon-like; oral arms longer than bell, folded spirally at base; characteristic star shape pattern on exumbrellar surface always visible, created by the radial pattern of deep maroon/purple triangles; white spots scattered throughout the surface of the exumbrellar; mouth becomes substantially larger as organism grows.

Holotype description. Umbrella hemispherical in shape, diameter 14.8 cm. Exumbrellar with small raised nematocyst warts, slightly granular, translucent brown in colour (preserved) (Figure 17 a), with 16 elongated triangles extending outward from central apex on bell; apices of triangles pointed toward central apex (Figure 17 a); colouration of triangles alternate between darker brown pigmentation and little to no pigmentation, forming characteristic star –shaped pattern; central apex visible as unpigmented and translucent circle (Figure 17 a); white spots scattered throughout exumbrellar due to raised nematocyst warts. Umbrella centrally thickened; central mesoglea 3.5 times thicker than margin. Umbrella margin cleft into 32 rounded lappets, four lappets per octant: two rhopalial lappets situated next to rhopalium and two velar lappets situated between rhopalial lappets. Rhopalial lappets not as wide as velar lappets, thus velar lappets appearing elongated while rhopalial lappets appear pointed; lappets equally pigmented on upper and lower surface, appearing dark brown. **Rhopalia**: eight rhophalia situated in deep clefts between adjacent rhopalial lappets projects from margin of umbrella into rhopalar canal. Rhopalium protected by sensory niche and an extension from subumbrellar margin forms a protective layer or “hood” above rhopalium (Figure 17 e). Base of rhopalium attached to a ridge, running to proximal wall of sensory niche. Thickened endoderm covers surface of sensory niche on subumbrellar, thickest along proximal wall (Figure 17 d). Thickened endoderm extends outwards for short distance (equal in length to rhopalar canal) (Appendix 11) alongside lappets. Deep, cone shaped sensory pit situated above rhopalium. Rhopalium itself, consists of a statocyst, (Figure 17 d) and short, hollow, basal stem (approximately equal in length to statocyst) (Appendix 11). Basal stem clasped by subumbrellar bulb and receives rhopalar canal which is approximately twice as long as the statocyst (Figure 17 d – e; Appendix 11). No ocelli observed. One primary tentacle found in each octant, located in clefts between adjacent velar lappets, along with two well-developed secondary tentacles situated in clefts found between adjacent velar and rhopalial lappets (arrangement 2:1:2), for a total of 24 tentacles (Figure 17 c). Tentacles laterally flattened and “ribbon-like”; tentacles less pigmented on ventral surface and cream in colour, light brown on dorsal surface; tentacles cream and unpigmented at base, becoming more pigmented distally and light brown toward tentacle tip. Subumbrellar translucent white and smooth; gastrovascular pouches covering central stomach granular (Figure 17 c); radial septa arise from periphery of central stomach, dividing gastrovascular cavity into 16 pouches; septa span entire length of coronary muscle and fuse at periphery of rhopalial lappets; tentacular pouches dilate and contract distally; rhopalial pouches contract and dilate distally (shown in Figures 2 a & 10 a). Manubrium and gastrovascular pouches cream in colour;

manubrium arising from central stomach forms thin, tubular, slightly elongated structure with thickened mesoglea; oral opening (mouth) cruciform and situated in centre of manubrium; manubrium wall divided into four oral arms distally. Oral arms cream and translucent (Figure 17 b), appearing to have lancelet shape, with distal portion of oral arm much thinner than proximal and central portion; V-shaped in cross section; oral arms spiralled proximally, becoming less spiralled distally; oral arms 15 % longer than bell diameter. Basal portion of manubrium fused and thickened to form four gonadal pouches with four oval orifices or ostia situated between them (Figure 17 c); gonads attached to periphery of ostia and highly folded/convoluted into semi-circular shape (Figure 17 c); one ostia situated between two adjacent gonads. No sperm sacs, quadralinga or gastric cirri were observed in this specimen.

Description of other specimens and additional data

<u>**Medusae:**</u> All material examined were preserved; no live specimens examined apart from photographs. The umbrella diameter of investigated material ranged from 2.95 cm to 20.75 cm, all hemispherical in shape (slightly flattened in smaller specimens). Central apex always unpigmented and translucent in smaller specimens (Figure 18 a – b), but become more pigmented in larger specimens (Figure 7 d).White spots scattered throughout the exumbrellar, but become more clearly visible in larger specimens where maroon/purple pigmentation is more prominent (Figure 7 a – d). The mesoglea becomes proportionately thicker as the specimen grows. Marginal lappets containing a series of canals around the outer edges that are not well defined. Subumbrellar becomes slightly maroon/purple in larger specimens (Figure 2 b – c). Manubrium and gastrovascular pouches cream in colour, however the largest specimens display uniform pigmentation on the pouches, with pigmentation appearing as light brown oval spots (Figure 2 b). The oral arms become slightly more maroon/purple in the larger specimens (Figure 7 a & c). In larger specimens, the central, slightly more rigid portion

of the oral arms are cream or white, while the edges and frills are maroon/purple, with some specimens displaying some maroon/purple colouration in the central portions as well (Figures 2 c & 7 a). Oral arms are also darker at the base in the larger specimens and lose colour distally (Figure 7 a). Oral arms typically 15 % to 25 % longer than bell diameter and become proportionately longer as the jellyfish grows. No sperm sacs, quadralinga or gastric cirri were observed in any of these specimens. Sexes are separate. All colouration observed became a dark brown in specimens preserved for more than two years.

Polyp: Typically conical in shape, up to 3.5 mm in height (Figure 19 a – b). Oral disk roughly half the length of the polyp (Appendix 11). Possesses up to16 tentacles, up to five times the length of the polyp (Appendix 11). Posseses four gastric septa and a cruciform mouth. White in colour unless strobilating in which case the upper half becomes brown (Figure 19 a – b).

Ephyrae: At one to 14 days post liberation: ephyrae possessing eight elongated lappet stems containing rhopalial canals, each bearing two rhopalial lappets thus containing 16 round, spatula like lappets (Figure 20 a – c). Lappet length approximately equal to length of stem (Appendix 11). Rhopalial canals forked. Rhopalia situated in clefts between pairs of rhopalial lappets at end of each lappet stem (Figure 20 a). Statocysts dark and clearly visible, with little to no protection (Figure 20 a – c). Four gastric filament sockets present with one to two gastric filaments and no tentacle buds or tentacles observed. Manubrium prominent, relatively wide and fairly long, approximately 55 % of the central disk diameter (Figure 20 a – c, Appendix 11). Nematocyst clusters are situated at the base of the lappets and along rhopalial and velar canals. Ephyrae usually transparent with some light brown pigmentation due to nematocysts and some thickend areas along canals. At 30 days post liberation (Figure

20 d – e): ephyrae possesses many gastric filaments that are long and thick, velar canals well developed, gastric and tentacular pouches well formed.

Cnidome:

Haplonemes

Holotrichous A- and O- isorhiza – Oval/circular shaped capsule with no prominent shaft visible within the undischarged capsule. Tubule is inverted and isodiametric and begins to form a coil close to the aperture. The coils then form loops from wall to wall perpendicularly within the capsule (Figure 3 g). Tubule is tightly coiled and usually more than 10 times the length of the capsule (Table 5). The tubule supports spines of uniform length throughout (Figure 3 c – d). Here, "A" indicates a more oval, leaf-like shape (Figure 3 f – g), while "O" indicates a more circular/rounded shape (Figure 3 a – d).

Atrichous isorhiza – Oval shaped capsule without a visible, prominent shaft within the undischarged capsule. The tubule is inverted and isodiametric and begins to form a coil close to the aperture. The coils then form loops from wall to wall perpendicularly within the capsule. Tubule is tightly coiled and is usually more than eight times the length of the capsule: without spines (Figure 3 e).

Heteronemes

These nematocysts are distinguished from haplonemes by the presence of a prominent, clearly visible shaft within the unfired capsule (Figures 4 a & 5 a).

Heterotrichous microbasic bi-rhopaloid – In the undischarged capsule, the shaft generally forms a straight line or axial rod in the centre of the capsule and is microbasic with a relatively long tubule (Table 5). Two distinct dilations are also visible on the shaft, one at the base close to the aperture and one at the dorsal end. These shafts typically have large, loosely set spines, while the tubule bears smaller, more closely set spines (Figure 4 b – e). Tubules are often tightly coiled in the undischarged capsule.

Heterotrichous microbasic euryteles – Euryteles have broad and prominent shafts both within the undischarged capsule and upon discharge (Figure 5 a – c). Within the capsule the shaft forms a straight line or axial rod across the centre of the capsule (length-wise) and is less than three times the length of the capsule (Figure 5 a – b). The short distal tubule makes the first loop toward the aperture of the capsule directly after the end of the shaft. Heterotrichous euryteles (Figure 5 c) supports large spines throughout the shaft and smaller spines are present on the tubule.

Biological data: Species displays external fertilization.

Etymology. "*agulhensis*" referring to the endemic distribution of this *Chrysaora* across the Agulhas Bank along the Southern Coast of South Africa.

4.2 Descriptions of Additional Taxonomically Important Features for *C. fulgida* and *C. africana* (excluded in previous descriptions of Neethling (2010))

4.2.1 Description of the Marginal Sense Organ (Rhopalia)

C. fulgida: Rhopalium as in *C. agulhensis* sp. nov., but with a thickened endoderm that extends outwards for a relatively long distance (0.3 times the length of the rhopalar canal)

(Appendix 11) along the sides of the lappets (Figure 21 a – b). The rhopalium itself, consists of a statocyst and a short, hollow, basal stem that is 1.5 times the length of the statocyst (Appendix 13), which is clasped by a subumbrellar bulb and receives the rhopalar canal which is approximately twice as long as the statocyst (Figure 21 a – b). No ocelli observed.

C. africana: Rhopalium as in *C. agulhensis* sp. nov. and *C. fulgida*, but possessing a highly thickened protective layer or "hood" above the rhopalium. In addition, directly above the sensory niche, an additional smaller protective layer covers the rhopalal canal but ends at the base of the rhopalar canal (Figure 21 c – d). A thickened endoderm extends outwards for a relatively short distance (0.3 times the length of the rhopalar canal) along the sides of the lappets (Appendix 11). The rhopalium itself consists of a statocyst, a short, hollow, basal stem that is three times the length of the statocyst, which is clasped by a subumbrella bulb and receives the rhopalar canal, which is 3 times the length of the statocyst (Figure 21 c, Appendix 11). No ocelli observed.

4.2.2 *Chrysaora fulgida* Polyp and Ephyrae Description

Polyp: Typically conical in shape, up to 4 mm in height (Appendix 11 & Figure 22 a – b). Oral disk roughly half the length of the polyp. Usually possesses 16 tentacles, up to four times the length of the polyp height (Appendix 11). Posseses four gastric septae and a cruciform mouth. Pale peach in colour unless strobilating in which case the upper half becomes brown (Figure 22 a – b).

Ephyrae: At one to 14 days post liberation: ephyrae possessing eight elongated lappet stems containing rhopalial canals, each bearing two rhopalial lappets for a total of 16 round, spatula like lappets (Appendix 11 & Figure 23 a – b). Lappet length approximately equal to the length of the stem (Appendix 11). Lappet stems and lappets relatively slender and long. Rhopalial canals are forked. Rhopalia situated in clefts in between pairs of rhopalial lappets at the end of each lappet stem (Figure 23 a – b). Statocysts dark and clearly visible, with no protection or hood. Statocysts slender and relatively small (Figure 23 a – b). Four gastric filament sockets containing onr gastric filament each, present along with a single tentacle bud, situated in between each adjacent stem. Manubrium prominent, relatively narrow but long, approximately 30 % of the central disk diameter (Appendix 11). However manubrium length equals central disk diameter. Nematocyst clusters are situated at the tips of the lappets and are scattered throughout the central disk. Ephyrae usually transparent, with little to no pigmentation. At 30 days post liberation (Figure 23 c): gastric filaments not well developed (one per gastric filament socket), rhopalial lappets still fairly long, velar canals still not well developed, with a very long manubrium and narrow mouth.

5. Remarks

The identities of *Chrysaora* within the Benguela have been fraught with errors, confusion and synonymy (e.g. Pagès *et al*. 1992; Mianzan & Cornelius 1999). Originally, a 32 lappet medusa containing 24 tentacles with a star shaped colouration on the exumbrellar surface was described by Reynaud (1830) as *Medusa (Rhyzostoma) fulgidum*. Later, Haeckel (1880) also identified a 32 lappet medusa occurring within the Benguela as *C. fulgida*. Vanhöffen (1902) then described a medusa from the Benguela region possessing 48 lappets and 40 tentacles as *Dactylometra africana*. Confusion ensued when Mayer (1910) synonymized *C. fulgida* with *C. hysoscella,* and the similarity between these two species has been noted subsequently by, amongst others, Stiasny (1934), who identified a 32 lappet medusa occurring within the Benguela as *C. fulgida*. Stiasny (1939) then described a medusa from the Benguela region possessing 48 lappets and 40 tentacles as *C. fulgida* and commented that *C. fulgida* could simply be the "*Chrysaora*" (younger) stage of *D. africana* (adult/older stage). It became common place to assume *C. fulgida* and *D. africana* as two various life phases of the same species, however Kramp (1955) considered this highly doubtful. Kramp (1961) then noted that *C. fulgida* could possess up to 48 lappets and 40 tentacles. Since then, numerous studies have uncritically referred to the 32 lappet *Chrysaora* found in the Benguela as *C. hysoscella* (Pagès *et al*. 1992; Brierley *et al.* 2001; Beucher *et al*. 2001; Mills 2001; Sparks *et al*. 2001; Brierley *et al.* 2004, 2005; Lynam *et al*. 2006; Flynn & Gibbons 2007; Purcell *et al*. 2007).

Morandini & Marques (2010), in their monographic treatment of the genus, have stressed the fact that *C. fulgida* and *C. hysoscella* are two separate species. Neethling (2010) similarly attempted to differentiate between these *Chrysaora* and succeeded in unambiguously separating *C. africana*, *C. fulgida* and *C. hysoscella,* however, similar to this study, found little variation between the ITS1 data of *C. fulgida* and *C. hysoscella*. Neethling (2010) did not manage to sequence the COI gene region for *C. hysoscella*, which this study however managed to do, validating the results of Neethling (2010).

The present study confirms the existence of three distinct species of *Chrysaora* around the South African coast. *Chrysaora fulgida* and *C. africana* are both found within the Benguela current occurring off the west coast of Southern Africa and are known to have overlapping distributions (Neethling 2010). The range of *Chrysaora africana* extends up the west coast of Africa to the Gulf of Guinea and is rare within South African waters, while the range of *C. fulgida* stretches into the Northern Benguela but is common off the west and south coasts of South Africa (Mpohlo 2014). *Chrysaora agulhensis* sp. nov. is essentially an Agulhas Bank endemic, with reported sightings stretching from Table Bay eastwards to Algoa Bay (Mpohlo 2014). Interestingly, Vanhöffen (1902) described specimens of *Chrysaora* found at Algoa Bay, as having purple stripes and white netted spots, similar to *C. agulhensis* sp. nov. Whilst Vanhöffen (1902) noted that this material was distinct from *C. africana*, the specimens were too damaged to allow a detailed description, and he described it as *Chrysaora* sp., noting that it was likely to be *C. fulgida*. Although we cannot ascertain whether the species Vanhöffen (1902) observed is the same species investigated within this study, it does indicate the potential presence of this species off the South African coast for over a century.

Many species of jellyfish are frequently, overlooked, synonymized with others or misidentified as a result of the large degree of morphological stasis exhibited by jellyfish and particularly scyphozoans (Brickford *et al*. 2007). Scyphozoans are also known to display distinct morphological variation when populations are separated or geographically isolated (Bolton & Graham 2004; Dawson 2005b), often in response to variable environmental conditions (Dawson & Martin 2001). Since much of our understanding of jellyfish taxonomy has been based solely on morphological comparisons, which Dawson (2004) has suggested may underestimate true phylogenetic diversity, it becomes apparent why so much confusion exists. As with Vanhöffen's (1902) description of the purple striped medusa, morphological descriptions are often incomplete and lacking standardization, which further exacerbates difficulties in untangling jellyfish phylogenies where morphological descriptions form the basis of comparative studies. An extreme case of this is seen in the newly designated jellyfish Family, Drymonematidae (Bayha & Dawson 2010). Three genera (*Cyanea*, *Desmonema* and *Drymonema*) were originally described as belonging to the Family Cyanidae by Haeckel (1880) (reviewed by Bayha & Dawson 2010). For all variables considered diagnostic for this Family, *Drymonema* seemed the most extreme (Haeckel 1880): *Cyanea* and *Desmonema* both have 16 gastrovascular pouches, while *Drymonema* has over 100. Furthermore, the rhopalia of *Drymonema* are found "in deep subumbrellar niches", a third of the bell radius from the margin, while the rest have the rhopalia at the bell margin (Haeckel 1880; Vanhöffen 1902; Bayha & Dawson 2010). Despite these seemingly large disparities, Haeckel (1880) in his original publications did not consider these differences big enough to warrant its designation as a new Family. Bayha & Dawson (2010) however, have now distinguished it as a new Family with three separate species occurring within a single genus.

Due to the issues surrounding the use of morphological data as the basis of phylogenetic studies, molecular analyses are being used more frequently in scyphozoan systematics (Dawson 2004; Dawson 2005a; Dawson 2005b; Neethling 2010). Molecular studies allow the use of more objective characters in contrast to the use of impractical or inappropriate morphological features often used when looking at morphology only (Dawson 2004). There have, however, been cases where the sole use of molecular data have failed to differentiate between species that do show distinct morphological, physiological and behavioural differences (Dawson 2005a), as was shown by the study of Bayha *et al*. (2010). Bayha *et al*. (2010) attempted to resolve the phylogenetic relationships of various jellyfish Families by means of complete taxon sampling. Their results (Bayha *et al*. 2010) strongly affirmed many of the morphological phylogenies currently in use, while refuting several others. They also refuted a number of molecular phylogenies that were based on incomplete taxon sampling.

Another interesting example, and a study very similar to this study, is that of the *Mastigias* (Dawson 2003). Populations of *Mastigias*, which occupy various habitats within Palau in Micronesia, were found to display significant morphological variation, but did not show significant molecular variation to warrant distinction at the species level (Dawson 2003). Dawson (2003) thus suggested an integrative approach that combines additional morphological, molecular, ecological and geographical information on the investigated medusa. Similar to the *Mastigias*, the molecular variation for the ITS1 data between *Chrysaora agulhensis* sp. nov. and *C. fulgida* was relatively low and below the standard to designate a new species. The low levels of differences observed within the nuclear DNA could be the result of the retention of ancestral polymorphisms and as such future studies should make use of other genetic markers (e.g Ribosomal DNA) instead. Additional data (morphological, COI and cnidome), as proposed by Dawson (2003), however, reinforced the designation of *Chrysaora agulhensis* sp. nov. and *C. fulgida* as two separate species. The above mentioned studies highlight the importance of complete morphological descriptions of the medusa and the jellyfish at all stages within its life history. Although this is extremely difficult, the present study provides detailed information on the cnidome, ephyrae and polyp, lacking in previous descriptions.

As previously suggested, the cnidome can be of great value in resolving certain taxonomic problems (Calder 1972). That said, much debate surrounds the exact number of nematocyst categories occurring within Scyphozoa (Mariscal 1974; Fautin 1988; Östman & Hyman 1997; Östman 2000). This study identified only five distinct nematocyst types falling into three broader categories, which support the findings of Fautin (1988), who suggested Scyphozoans to possess atrichous isorhizas, holotrichous haplonemes and heterotrichous microbasic euryteles. By contrast, Östman (2000) suggests this variety to be markedly greater when Scanning Electron Microscopy (SEM) is employed. This is supported by Morandini and Marques (2010) who noted the difficulty in reliably distinguishing between certain types of nematocysts when light microscopy was solely used. Morandini and Marques (2010) determined *C. fulgida* to contain the same diversity of nematocysts as this study suggests, however we managed to identify and analyze heterotrichous microbasic euryteles whereas they chose to exclude them owing to difficulties with identification. As such, the variety of nematocysts in these *Chrysaora* may indeed be greater and can be further refined when SEM's are applied to these species.

Östman (2000) suggests that cnidome size becomes especially important for distinguishing between species that possess the same type of cnidome, as is reflected in the results of this study which indicated multiple differences between the size and relative abundance of nematocysts between these species. The usefulness of the cnidome within taxonomy cannot be overlooked as it has in the past, distinguished between two varieties of *C. quinquecirrha* (Desor 1848), on the bases of slight (2 µm) differences within the dimensions of the nematocysts occurring on their tentacles (Papenfuss 1936).

Different nematocysts have different functions. Euryteles (Wiebering *et al*. 2010) and large rhopaloids (Peach & Pitt 2005), which were distributed generously across the tissue of these *Chrysaora*, are generally used to capture prey by penetration. Isorhizas may serve various functions to either entangle prey (Wiebering *et al*. 2010) or for defense (Mariscal 1974). Atrichous isorhizas are thought to assist animals to adhere to the substrate during locomotion (Mariscal 1974). All three species of *Chrysaora* contained a large number of rhopaloids and isorhizas, indicating perhaps a tendency to preying on crustaceans such as copepods (Peach & Pitt 2005). Many differences exist between the nematocysts of the various species of *Chrysaora* and although no clear standard exists to compare and properly quantify these differences, the cnidome could provide important information on the identity and feeding preferences of these species. It can thus be thought of as a vital component of a species description and future studies should endeavour to provide detailed information on the ontogeny of the cnidome. Mariscal (1974), Peach & Pitt (2005) and Wiebering *et al*. (2010) all mention the possibility that the compliment of nematocyst occurring within various species could potentially change as the species grows or between variable environments as the food preference or availability changes. Since no strict record was kept affirming that all tissue analyzed was taken from specimens of similar sizes and at similar life history stages, it is difficult to know whether the differentiation observed is “real” or not.

The polyp/scyphistoma and ephyrae may also serve a useful role in the identification of medusa. Although size at liberation and proportional lengths of the marginal lappets may be highly variable between ephyrae within the same species (Russell 1970), a number of useful diagnostic characters are found within the ephyrae of different species even at that point (Russell 1970). In the case of *Chrysaora*, the end of the radial canal of the ephyrae have rounded processes at the corners of the ends of the radial canals and these ephyrae can also be distinguished from those of other taxa by the presence of nematocyct clusters occurring at the base of the marginal lappets. The numbers of gastric filaments may also serve as a useful diagnostic feature between various genera (reviewed by Russell 1970; Straehler-Pohl & Jarms 2010). *Chrysaora agulhensis* sp. nov. possessed numerous gastric filaments while *C. fulgida* possessed only a few. In addition rhopalar and velar canals were well formed at 30 days post liberation in *C. agulhensis* sp. nov., while in *C. fulgida* they were not. These features could thus serve as diagnostic features between the ephyrae of *C. agulhensis* sp. nov. and *C. fulgida*. The diagnostic characters within the schyphistoma are much more complex. Although differences were observed between the diameter of the oral disk, tentacle length and overall height of the polyps of *Chrysaora fulgida* and *C. agulhensis* sp. nov., it is difficult to assess whether these differences were “real”, as numerous studies investigating diagnostic features of the scyphistoma of *Chyrsaora* (Claus 1877, 1883, 1890; Hein 1900) suggest these features to be reflective of their surrounding physical environment (reviewed by Russell 1970). Straehler-Pohl & Jarms (2010), with reference mainly to the ephyrae also stated many characters to be unreliable in distinguishing species within the same genus. These findings thus highlight the need for increased descriptions of the various life history stages within *Chrysaora* and other jellyfish, to establish improved points of comparison between the various life history phases and not only focus on the medusa. Ephyrae in particular, serve as an early warning system for potential blooms (Straehler-Pohl & Jarms 2010), however accurate identification cannot be made unless robust descriptions for all ephyrae exist.

Species within the genus *Chrysaora* seem to have a biology and life history that enable them to rapidly capitalize on changes in the environment and to create mass aggregations. The implications of a third *Chrysaora* within the Benguela are widespread. *Chrysaora* tend to bloom in productive coastal waters such as upwelling systems (Arai 2001): the Bering Sea

(Brodeur *et al*. 2002), the coast of California (Graham & Largier 1997) and off the coast of Chile (Hamner & Dawson 2008). *Chrysaora fulgida* has sustained high biomass in the northern Benguela ecosystem (Lynam *et al.* 2006), perhaps in part due to the broad shelf of the Namibian coast (Roux *et al*. 2013). The upwelling environment off Namibia is not as dynamic as that off the west coast of South Africa, and the double cell circulation pattern here (Barange *et al*. 1992) allows for water retention that can result in the mass build-up of jellyfish (Hutchings *et al*. 2009; Roux *et al*. 2013).

In contrast to this, upwelling in the southern Benguela is dynamic, and high levels of offshore advection prevent the mass build-up of jellyfish populations there; though they are still prevalent in these waters seasonally (Hutchings *et al*. 2009; Roux *et al*. 2013). Along the southern coast of South Africa, however, the broad width of the Agulhas Bank is similar to that observed off Namibia, which could potentially allow the build-up of jellyfish along this coast (Hutchings *et al*. 2009). The South African electricity generating public entity, Eskom, is proposing to build a new nuclear power plant somewhere along this coastal region. The locality of which, suggested to be St. Francis Bay, is situated in an area where *Chrysaor*a *agulhensis* sp. nov. is known to occur. Potential mass build-up of jellyfish along these shores could negatively impact this power plant if conditions along this shore were to cause the blooming of this *Chrysaora*, which has been known to bloom here in the past (pers. obs.). Many strategies however, exist to manage the effects of jellyfish blooms: beach closures, bloom predictions, use of protective nets, etc. (Gibbons & Richardson 2013). If Eskom were to develop a power plant along this coast however, there would be a need for immediate mitigation and prevention strategies and more effective measures would have to be developed.

No behavioural or ecological work was conducted during this study and as a result little is known about the blooming tendencies or population dynamics of this *Chrysaora* species. Future studies should as such focus on extensive sampling of jellyfish along the entire western shelf of South Africa, extending up to Namibia as well as along the eastern shelf in order to establish population data and provide more detailed morphological and molecular data based on larger sample sizes. Behavioural and ecological studies should also be undertaken in an attempt to better understand this species and its possible origin.

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Table 1: Localities with their corresponding GPS co-ordinates for *Chrysaora* sp. X specimens, collected throughout 2012 – 2015 around the southern coast of South Africa, investigated in this study. Localities of meduasae from which planulae were obtained indicated with an asterix.

Location	GPS co-ordinates
Whale rock off Robbin Island*	-33.8349802, 18.311071
Zevenwacht beach	-34.1038628, 18.7882734
Muizenberg beach	-34.1087476, 18.4730991
Gouritzmond river mouth	-34.347336, 21.888564
Fish Hoek beach	-34.137357, 18.434072

Table 2: Morphological features observed and measured for *Chrysaora* sp. X collected around the southern coast of South Africa during 2012 − 2015. Material was preserved in 5 % formalin in ambient seawater upon collection.

Morphological feature (MF)	Morphological feature description (measured in cm)
S1	Maximum umbrella diameter
S2	Maximum umbrella height
S3	Minimum umbrella height
S4	Number of rhopalia
S5	Number of lappets in octant (Rhopalial+velar)
S6	Rhopalial lappet length
S7	Rhopalial lappet width
S8	Velar lappet length
S9	Velar lappet width
S10	No. primary tentacles
S11	No. secondary tentacles
S12	Width at base of primary tentacle
S13	Width at base of secondary tentacle
S14	Depth of primary tentacle
S15	Depth of secondary tentacle
S16	Length of intact primary tentacles
S17	Length of intact secondary tentacle
S18	Number of gastrovascular pouches
S19	Description of gastrovascular pouches
S20	Mouth diameter
S21	Manubrium length(total)
S22	Manubrium length(section)
S23	Manubrium depth(total)
S24	Manubrium depth(section)
S25	Width of ostia
S26	Length of ostia
S27	Inter-ostia width
S28	Number of oral arms
S29	Length of intact oral arm
S30	Width of oral arm originating from umbrella
S31	Maximum width of oral arm
S32	Maximum frill width
S33	Minimum frill width
S34	Length of oral arm pillars
S35	Width of oral arm pillars
S36	Gonad sac width
S37	Gonad sac length
S38	Sperm sacs
S39	Number of rhopalia
S40	Number of ostia

Table 3: Summary of the variables for which Pearson's r correlations were not significant. Bell diameter (raw data) was correlated against standardized measures for *Chrysaora* sp. X collected around the southern coast of South Africa during 2012 − 2015.

	Pearson's r		Spearman Rank	
Variable	**Manubrium length (section)**	**Manubrium depth (section)**	**Oral arm pillar width**	**maximum width of oral arm**
Bell diameter	r = -0.0876 N = 27 p = 0.767	r = 0.3567 N = 27 p = 0.346	r = 0.4187 N = 23 p = 0.212	r = 0.103 N = 15 p = 0.383

Table 4: Forward and reverse primers used to amplify the gene markers cytochrome *c* oxidase subunit I (COI) and internal transcribed spacer one (ITS1) with their associated authors.

Gene region	Primer name	Sequence	Author/s
Cytochrome *c* oxidase subunit I (COI)	LCOjf	5’-ggtcaacaaatcataaagatattggaac-3’	Dawson 2005c
	HCOcato	5’-ctccagcaggatcaaagaag-3’	Dawson 2005c
Internal transcribed spacer one (ITS1)	jfITS1-5f	5’-ggtttcgtaggtgaacctgcggaaggatc-3’	Dawson & Jacobs 2001
	jfITS1-3r	5’-cgcacgagccgagtgatccaccttagaag-3’	Dawson & Jacobs 2001

Table 5: Widths, lengths and shaft lengths (raw data), of various nematocysts occurring on the tentacles and oral arms of *Chrysaora* sp. X, *Chrysaora fulgida* and *Chrysaora africana*. All measurements shown as mean ± standard error.

		Measures		
Species	**Tissue**	**Shaft/tubule Length**	**Length**	**Width**
C. africana	**Oral arm (OA)**			
	Atrichous isorhiza	149.6 ± 1.63	10.1 ± 0.23	7.4 ± 0.16
	Heterotrichous microbasic bi-rhopaloid	51.05 ± 90.4	11.45 ± 0.40	8.1 ± 0.52
	Holotrichous A-isorhiza	80.1 ± 0.9	11.2 ± 0.39	8.4 ± 0.16
	Holotrichous O-isorhiza	116.1 ± 1.61	9.3 ± 0.30	8.9 ± 0.35
	Tentacle (T)			
	Heterotrichous microbasic bi-rhopaloid	159.2 ± 27.36	13.55 ± 0.76	8.4 ±0.52
	Holotrichous O-isorhiza	116.9 ± 6.82	11.2 ± 0.36	10.7 ± 0.30
C. fulgida	**Ephyrae**			
	Heterotrichous microbasic bi-rhopaloid	54.6 ± 1.46	9.9 ± 0.18	8.4 ± 0.27
	Holotrichous O-isorhiza		10.2 ± 0.25	8.8 ± 0.25
	Oral arm (OA)			
	Heterotrichous microbasic eurytele	12.11 ± 0.63	11.89 ± 0.35	8.78 ± 0.49
	Heterotrichous microbasic bi-rhopaloid	56.55 ± 6.74	10.95 ± 0.22	7.75 ± 0.22
	Holotrichous A-isorhiza	13.36 ± 0.43	12.91 ± 0.31	9.45 ± 0.28
	Holotrichous O-isorhiza	68.2 ± 10.48	12.1 ± 0.53	11.6 ± 0.50
	Tentacle (T)			
	Heterotrichous microbasic bi-rhopaloid	112.71 ± 17.5	14.57 ± 0.72	10.38 ± 0.55
	Holotrichous A-isorhiza	79.4 ± 1.50	11.4 ± 0.40	7.4 ± 0.27
	Holotrichous O-isorhiza	128 ± 2.47	11.6 ± 0.54	11 ± 0.85

		Measures		
Species	**Tissue**	**Shaft/tubule Length**	**Length**	**Width**
***Chrysaora* sp. X**	**Ephyrae**			
	Heterotrichous microbasic bi-rhopaloid		11.2 ± 0.13	10.3 ± 0.21
	Holotrichous O-isorhiza		7.8 ± 0.2	7.4 ± 0.16
	Oral arm (OA)			
	Heterotrichous microbasic eurytele	24.1 ± 0.94	12.81 ± 0.37	9.38 ± 0.56
	Heterotrichous microbasic bi-rhopaloid	67.7 ± 7.32	11.55 ± 0.32	7.7 ± 0.30
	Holotrichous A-isorhiza	53.2 ± 3.93	11.6 ± 0.48	7.8 ± 0.29
	Holotrichous O-isorhiza	95.75 ± 11.85	11.58 ± 1.10	10.42 ± 0.87
	Tentacle (T)			
	Atrichous isorhiza	125.5 ± 1.28	10.3 ± 0.26	6.4 ± 0.16
	Heterotrichous microbasic bi-rhopaloid	135.95 ± 24.24	15.1 ± 0.94	9.7 ± 0.73
	Holotrichous A-isorhiza	82.36 ± 2.54	12.64 ± 0.28	7.91 ± 0.25
	Holotrichous O-isorhiza	114.1 ± 3.54	18.1 ± 1.30	14.7 ± 0.93

Table 6: Results of the analysis of variance (ANOVA) illustrating differences between the log lengths, log widths and log shaft lengths for those nematocysts occurring on both the oral arms and tentacles within the same species. Nematocysts were measured for: *Chrysaora* sp. X collected around the southern coast of South Africa during 2012 – 2015 and, *Chrysaora fulgida* and *Chrysaora africana* collected on the "Goby and Hake Cruise", conducted on the R.V. *G. O. Sars*, from the 31st of March to 11th of April 2008 off the Namibian coast (Utne Palm *et al.*, unpublished data). Results considered significant at $p \leq 0.0063$ indicated in bold (after Bonferoni adjustment).

		log Length				**log Width**				**Log Shaft Length**			
Nematocyst	**Species (oral arm vs. tentacle)**	**DF**	**Sum of squares**	**F**	***p***	**DF**	**Sum of squares**	**F**	***p***	**DF**	**Sum of squares**	**F**	***p***
Holotrichous O-isorhia	*Chrysaora* sp. X	20	0.215	12.85	< **0.001**	20	0.13	11.45	< **0.001**	20	0.409	2.213	0.653
	C. fulgida	18	0.002	0.368	0.551	18	0.003	0.586	0.454	18	17880.2	30.85	< **0.001**
	C. africana	18	0.033	17.81	< **0.001**	18	0.033	15.66	< **0.001**	18	0	0.016	< **0.001**
Heterotrichous microbasic bi-rhopaloid	*Chrysaora* sp. X	38	0.105	11.6	< **0.001**	38	0.066	4.64	0.038	38	0.254	1.937	0.172
	C. fulgida	39	0.135	22.05	< **0.001**	39	0.14	19.04	< **0.001**	39	0.508	4.661	-0.04
	C. africana	38	0.045	6.305	0.016	38	0.002	0.201	0.656	38	3.125	13.62	< **0.001**
Holotrichous A-isorhia	*C. fulgida*	26	0.003	0.456	0.505	26	0.001	0.087	0.77	26	5.729	255	< **0.001**
	Chrysaora sp. X	19	0.008	3.88	0.064	19	0	0.097	0.759	19	4455.09	40.31	< **0.001**

Table 7: Results of the two-tailed *t*-tests and Mann-Whitney U tests illustrating differences in the standardized morphometric data (MF) common to both *Chrysaora* sp. X and *Chrysaora fulgida* (Results considered significant at $p \leq 0,003$ after Bonferoni correction, indicated in bold).

MF	***t***	**DF**	**Sig. (2-tailed)**	**Mann-Whitney U**	***Z***	**Sig. (2-tailed)**
S1	21.958	46	**0.000**	-	-	-
S2	-3.504	45	**0.001**	-	-	-
S3	-4.652	44	**0.000**	-	-	-
S6	-4.042	45	**0.000**	-	-	-
S7	-8.776	45	**0.000**	-	-	-
S8	-8.373	45	**0.000**	-	-	-
S9	-3.638	44	**0.001**	-	-	-
S12	-2.714	40	0.010	-	-	-
S13	-3.034	40	0.004	-	-	-
S20	6.597	39	**0.000**	-	-	-
S23	-18.199	39	**0.000**	-	-	-
S25	9.574	41	**0.000**	-	-	-
S26	2.384	41	0.022	-	-	-
S27	-10.654	40	**0.000**	-	-	-
S29	6.611	12	**0.000**	-	-	-
S30	1.923	33	0.063	-	-	-
S21	-	-	-	78.000	-3.261	**0.001**
S31	-	-	-	38.000	-2.135	0.033
S35	-	-	-	38.000	-2.135	0.033
S5	-	-	-	256.000	0.000	1.000
S41	-	-	-	256.000	0.000	1.000
S40	-	-	-	240.000	-0.834	0.404
S4	-	-	-	256.000	0.000	1.000
S11	-	-	-	240.000	-0.834	0.404
S28	-	-	-	256.000	0.000	1.000

Table 8: Results of the two-tailed *t*-test and Mann-Whitney U tests illustrating differences in standardized morphometric data (MF) common to both *Chrysaora* sp. X and *Chrysaora africana* (Results considered significant at $p \leq 0,0031$ after Bonferoni correction, indicated in bold).

MF	*t*	DF	Sig. (2-tailed)	Mann-Whitney U	*Z*	Sig. (2-tailed)
S1	21.958	46	**0.000**	-	-	-
S2	-3.504	45	**0.001**	-	-	-
S3	-4.652	44	**0.000**	-	-	-
S6	-4.042	45	**0.000**	-	-	-
S7	-8.776	45	**0.000**	-	-	-
S8	-8.373	45	**0.000**	-	-	-
S9	-3.638	44	**0.001**	-	-	-
S12	-2.714	40	0.01	-	-	-
S13	-3.034	40	0.004	-	-	-
S20	6.597	39	**0.000**	-	-	-
S23	-18.199	39	**0.000**	-	-	-
S25	9.574	41	**0.000**	-	-	-
S26	2.384	41	0.022	-	-	-
S27	-10.654	40	**0.000**	-	-	-
S29	6.611	12	**0.000**	-	-	-
S30	1.923	33	0.063	-	-	-
S21	-	-	-	78	-3.261	**0.001**
S31	-	-	-	38	-2.135	0.033
S35	-	-	-	38	-2.135	0.033
S41	-	-	-	256	0	1
S5	-	-	-	0	-6.749	**0.000**
S40	-	-	-	240	-0.834	0.404
S4	-	-	-	256	0	1
S11	-	-	-	240	-0.834	0.404
S28	-	-	-	256	0	1

Table 9: Results of the two-tailed *t*-test and Mann-Whitney U test illustrating differences in standardized morphometric data (MF) common to both *Chrysaora* sp. X and *Chrysaora* (Results considered significant at $p \leq 0{,}0042$ after Bonferoni correction, indicated in bold).

MF	***t***	**DF**	**Sig. (2-tailed)**	**Mann-Whitney U**	***Z***	**Sig. (2-tailed)**
S1	17.058	46	**0.000**	-	-	-
S2	-18.133	43	**0.000**	-	-	-
S3	-8.448	43	**0.000**	-	-	-
S7	-2.166	45	0.036	-	-	-
S9	-1.25	44	0.218	-	-	-
S12	3.788	44	**0.000**	-	-	-
S20	5.408	37	**0.000**	-	-	-
S23	-20.019	37	**0.000**	-	-	-
S25	1.43	39	0.161	-	-	-
S26	-1.134	39	0.264	-	-	-
S27	-4.365	38	**0.000**	-	-	-
S30	0.566	30	0.576	-	-	-
S21	-	-	-	56	-3.484	**0.000**
S31	-	-	-	29	-1.557	0.119
S35	-	-	-	29	-1.557	0.119
S5	-	-	-	256	0	1.000
S41	-	-	-	256	0	1.000
S40	-	-	-	256	0	1.000
S4	-	-	-	256	0	1.000
S11	-	-	-	256	0	1.000
S28	-	-	-	256	0	1.000

Table 10: Canonical Analysis of Principal Co-ordinates (CAP) results for the standardized morphometric data showing number of individuals of *Chrysaora* sp. X, *Chrysaora fulgida*, *Chrysaora africana* and *Chrysaora hysoscella*, assigned to each species. (Permutation test statistic reported (species significantly different at $p < 0.005$)

Original group	***C. fulgida***	***C. hysoscella***	***C. africana***	***Chrysaora* sp. X**	**Total**	**% correct**	**Total correct**	**Mis-classification error**	***p***
C. fulgida	17	1	0	0	18	94.44	96.61 %	3.39 %	**0.0001**
C. hysoscella	0	14	0	0	14	100			
C. africana	0	0	16	0	16	100			
***Chrysaora* sp. X**	1	0	0	10	11	90.91			

Table 11: Results of the one-way ANOVA performed between the species *Chrysaora* sp. X, *Chrysaora fulgida* and *Chrysaora africana* to test for overall differences in the log width, log length and log shaft/tubule length for those nematocysts they had in common. The ANOVA also tested for differences between those nematocysts occurring on both the oral arm and tentacles. (Results considered significant at $p < 0.0051$ after Bonferoni correction, indicated in bold).

		Log Length			**Log Width**			**Log Shaft/tubule Length**		
Nematocysts	**Comparisons**	**DF**	***F***	***p***	**DF**	***F***	***p***	**DF**	***F***	***p***
Atrichous anisorhiza	*Chrysaora* sp. X & *C. africana*	19	0.299	0.591	19	19.193	**< 0.001**	19	136.61	**< 0.001**
	Between tissue	1	0.299	0.591	1	19.193	**< 0.001**		136.61	**< 0.001**
Heterotrichous microbasic eurytele	*Chrysaora* sp. X & *C. africana*	29	2.216	0.148	29	0.212	0.649	29	62.912	**< 0.001**
	Between tissue	-	-	-	-	-	-	-	-	-
Heterotrichous microbasic bi-rhopaloid	All species	140	0.67	0.5134	140	1.83	0.169	140	1.382	0.255
	Between tissue	140	36.903	**< 0.001**	140	13.618	**< 0.001**	140	29.021	**< 0.001**
Holotrichous O-isorhiza	All species	60	7.24	**< 0.001**	60	4.557	0.014	60	1.439	0.245
	Between tissue	60	9.04	**< 0.001**	60	8.859	**< 0.001**	60	7.608	**< 0.001**
Holotrichous A-isorhiza	All species	60	5.413	**< 0.001**	60	5.778	**< 0.001**	60	0.368	0.694
	Between tissue	60	6.473	**0.014**	60	0.073	**0.788**	60	29.99	**< 0.001**

Table 12: Results of the independent *t*-tests performed between the species *Chrysaora* sp. X, *Chrysaora fulgida* and *Chrysaora africana*. Independent t-tests were also performed comparing the nematocysts between the ephyrae of *Chrysaora* sp. X and *C. fulgida* as well comparing ephyrae to adult medusa to test for individual differences in the log width, log length and log shaft/tubule length for those nematocysts they had in common. Only nematocysts which showed differences in the one-way ANOVA analysis, and were present in more than two species have been included here (results considered significant at $p < 0.0046$ after Bonferoni correction, indicated in bold).

			Log Length			**Log Width**			**Log Shaft/tubule Length**		
Nematocysts	**Species**		**DF**	***t***	***p***	**DF**	***t***	***p***	**DF**	***t***	***p***
Holotrichous O-isorhiza	*Chrysaora* sp. X	*C. fulgida*	40	-1.694	0.098	40	-0.787	0.436	40	-0.004	0.997
		C. africana	40	-3.343	**< 0.001**	40	-2.707	**< 0.001**	40	1.572	0.1238
	C. fulgida	*C. africana*	38	-3.102	**< 0.001**	38	-2.851	**< 0.001**	38	1.852	0.072
Holotrichous A-isorhiza	*Chrysaora* sp. X	*C. fulgida*	47	2.028	0.048	47	3.119	**< 0.001**	47	-0.368	0.715
		C. africana	29	-1.872	0.071	29	1.877	0.071	29	1.267	0.215
	C. fulgida	*C. africana*	36	-2.748	**< 0.001**	36	-1.335	0.1901	36	1.409	0.165
Heterotrichous microbasic bi-rhopaloid	*Chrysaora* sp. X ephyrae	*C. fulgida* ephyrae	18	5.717	**< 0.001**	18	5.254	**< 0.001**	**-**	-	**-**
		Chrysaora sp. X medusa	48	-1.808	0.07693	48	2.265	0.02806	**-**	-	**-**
	C. fulgida medusa	*C. fulgida* ephyrae	49	3.27	**< 0.001**	49	0.712	0.4797	49	1.458	0.151
Holotrichous O-isorhiza	*Chrysaora* sp. X ephyrae	*C. fulgida* ephyrae	18	7.511	**< 0.001**	18	4.835	**< 0.001**	**-**	-	**-**
		Chrysaora sp. X medusa	29	-4.682	**< 0.001**	29	-4.883	**< 0.001**	29	-13.511	**< 0.001**
	C. fulgida	*C. fulgida* ephyrae	28	-2.702	0.012	28	-4.252	**< 0.001**	28	-15.919	**< 0.001**

Table 13: Relative abundance of various nematocysts occurring within the tentacles and oral arms of *Chrysaora* sp. X, *Chrysaora fulgida* and *Chrysaora africana*. Table also indicates Simpson's diversity index (D) as well as Simpson's reciprocal index (1/D).

Species/Tissue	**Holotrichous O-isorhiza**	**Heterotrichous microbasic bi-rhopaloid**	**Holotrichous A-isorhiza**	**Heterotrichous microbasic eurytele**	**Atrichous isorhiza**	**D^**	**1/D^**
***Chrysaora* sp. X oral arm**	62	174	3	12	0	0.26	3.88
***Chrysaora* sp. X tentacles**	112	343	2	0	5	0.30	3.37
***C. africana* oral arm**	101	262	0	0	4	0.34	2.94
***C. africana* tentacles**	94	269	0	0	0	0.28	3.54
***C. fulgida* oral arm**	61	132	17	0	1	0.33	3.07
***C. fulgida* tentacles**	232	170	92	0	0	0.29	3.42

Table 14: Uncorrected mean pairwise distance matrix for cytochrome *c* oxidase subunit I (COI) and the internal transcribed spacer one (ITS1) markers, amplified from specimens of *Chrysaora* sp. X, *Chrysaora fulgida*, *Chrysaora africana* and *Chrysaora hysoscella* .

		COI		
	C. hysoscella	***C. africana***	***C. fulgida***	***Chrysaora* sp. X**
C. hysoscella	-			
C. africana	0.25677	-		
C. fulgida	0.31568	0.347035	-	
***Chrysaora* sp. X**	0.24396	0.321725	0.1616	-
		ITS		
C. hysoscella	-			
C. africana	0.39406	-		
C. fulgida	0.060455	0.385205	-	
***Chrysaora* sp. X**	0.0643375	0.38177	0.02242	-

Figure Headings

Figure 1: Schematic diagram of the subumbrellar view and exumbrellar view (top right) of a typical *Chrysaora* specimen with various variables indicates (see Table 2). Adapted from Morandini and Marques (2010).

Figure 2: Photographs of the subumbrellar of large (> 20 cm) *Chrysaora* sp. X specimens collected at whale rock in 2013, illustrating a) the shape of the tentacular and rhopalial pouches, showing radial septum fusing at periphery of rhopalial lappets. It also illustrates the arrangement of the primary and secondary tentacle (2:1:2) and illustrates how they are situated in deep clefts between adjacent lappets, b) shows highly folded gonads, position of ostia, pigmentation on manubrium covering central stomach and the purple hue of the manubrium and central stomach pouches and, c) showing pigmentation of oral arms at the centre, laterally flattened tentacles and spiraled oral arm shape.

Figure 3: Microscopic images (1000 ×) of the isorhiza nematocysts occurring on the oral arms/tentacles of *Chrysaora* sp. X, *Chrysaora fulgida* and *Chrysaora africana*: a – b) undischarged heterotrichous macrobasic O-isorhiza; c – d) discharged holotrichous O-isorhiza; e) discharged atrichous anisorhiza; f) discharged heterotrhichous macrobasic A-isorhiza; g) undischarged heterotrichous macrobasic A-isorhiza.

Figure 4: Microscopic images (1000 ×) of bi-rhopaloid nematocysts occurring on the oral arms/tentacles *Chrysaora* sp. X, *Chrysaora fulgida* and *Chrysaora africana*: a) undischarged heterotrichous microbasic bi-rhopaloid; b – e) discharged heterotrhichous microbasic bi-rhopaloid.

Figure 5: Microscopic images (1000 ×) of eurytele nematocysts occurring on the oral arms/tentacles of *Chrysaora* sp. X, *Chrysaora fulgida* and *Chrysaora africana*: a) undischarged eurytele; b – c) discharged microbasic euryteles.

Figure 6: Canonical analysis of principle co-ordinates (CAP) plot, showing eigenvectors and ordination of standardized morphometric data for *Chrysaora* sp. X, *Chrysaora fulgida*, *Chrysaora africana* and *Chrysaora hysoscella*.

Figure 7: Photographs of live specimens of *Chrysaora* sp. X found at False Bay South Africa in 2014, displaying the colour patterns of: a) - c) larger medusa, showing colouration of the lappets, ribbon-like tentacles and proximal spiraling of the oral arm and
d) colouration after preservation (at least one year).

Figure 8: Photographs of live *Chrysaora fulgida* in the northern Benguela ecosystem, illustrating colour patter variation between a) small and b) larger specimens. Highly folded oral arms are clearly represented in b.

Figure 9: Photographs of *Chrysaora africana* a) live b) juvenile showing tentacle pigmentation or colouration and c) preserved specimens of *Chrysaora africana*, in the northern Benguela ecosystem illustrating highly transparent bell/mesoglea with purple ccolouration and dark purple lappets and tentacles (© Simon Elwen, Namibian Dolphin Project).

Figure 10: Gastrovascular pouch shapes of a) *Chrysaora* sp. X, b) *C. africana* and c) *C. fulgida*, showing dilation and constriction at distal ends.

Figure 11: Molecular phylogenetic analysis by maximum parsimony method for cytochrome *c* oxidase subunit I (COI) sequences amplified from *Chrysaora* sp. X, *Chrysaora fulgida*, *Chrysaora africana* and *Chrysaora hysoscella*, for a maximum of 655 nucleotides. Strict consensus bootstrapped tree is shown here. Posterior probabilities are shown next to nodes, while bootstrap percentages are shown above branches.

Figure 12: Molecular phylogenetic analysis by maximum parsimony method for internal transcribed spacer 1 (ITS1) sequences amplified from *Chrysaora* sp. X, *Chrysaora fulgida*, *Chrysaora africana* and *Chrysaora hysoscella*, for a maximum of 310 neucleotides. Strict consensus bootstrapped tree is shown here. Posterior probabilities are shown next to nodes, while bootstrap percentages are shown above branches.

Figure 13: Splits tree illustrating gene clusters between *Chrysaora* sp. X, *Chrysaora fulgida*, *Chrysaora africana* and *Chrysaora hysoscella*, for cytochrome *c* oxidase subunit I (COI) gene fragment in which a maximum of 655 nucleotides were amplified.

Figure 14: Splits tree illustrating gene clusters between *Chrysaora* sp. X *Chrysaora fulgida*, *Chrysaora africana* and *Chrysaora hysoscella*, for the internal transcribed spacer 1 (ITS1) gene fragment in which a maximum of 310 nucleotides were amplified.

Figure 15: Molecular phylogenetic analysis by maximum parsimony method for cytochrome *c* oxidase subunit I (COI) and intertranscribed spacer 1 (ITS1) concatenated dataset for *Chrysaora* sp. X *Chrysaora fulgida*, *Chrysaora africana* and *Chrysaora hysoscella*, for a maximum of 965 neucleotides. Strict consensus bootstrapped tree is shown here. Posterior probabilities are shown next to nodes, while bootstrap percentages are shown above branches.

Figure 16: Splits tree illustrating gene clusters between *Chrysaora* sp. X *Chrysaora fulgida*, *Chrysaora africana* and *Chrysaora hysoscella*, for a concatenated dataset of the genes: internal transcribed spacer 1 (ITS1) gene fragment in which a maximum of 310 bp were amplified and cytochrome c oxidase subunit I (COI) in which 655 bp were amplified.

Figure 17: Photographs of the Holotype specimen (MB-A088455) of *Chrysaora* sp. X submitted to the South African Natural History Museum, collected at False Bay (Fish Hoek), South Africa in June 2014, showing a) exumbrellar surface showing the star- shaped colouration and raised nematocyst warts, b) subumbrellar surface showing colour and length of the oral arms and manubrium and, c) size and shape of the ostia. Enlarged images of the rhopalium are also included here which illustrates: c) the ventral view and d) the dorsal view.

Figure 18: Photographs of preserved *Chrysaora* sp. X collected at Whale Rock during November 2012: a) subumbrellar view of medium sized specimen (~12 cm) (Paratype: MB-A088456) displaying the lack of pigmentation and b) exumbrellar view of small sized specimen (< 8 cm), showing bean shaped gonads.

Figure 19: Enlarged images of the polyps of *Chrysaora* sp. X, settled from adult meduase collected at Robbin Island, South Africa in 2013: a) image illustrating two fully grown polyps; one strobilating (left) and one not strobilating (right), and b) showing a typical cluster of polyps.

Figure 20: Enlarged images of the ephyrae of *Chrysaora* sp. X, taken unfed, at a) two days post liberation, b) two weeks post liberation (indicating size of mouth), c) two weeks post liberation, d) 30 days post liberation indicating number of gastric filaments and well

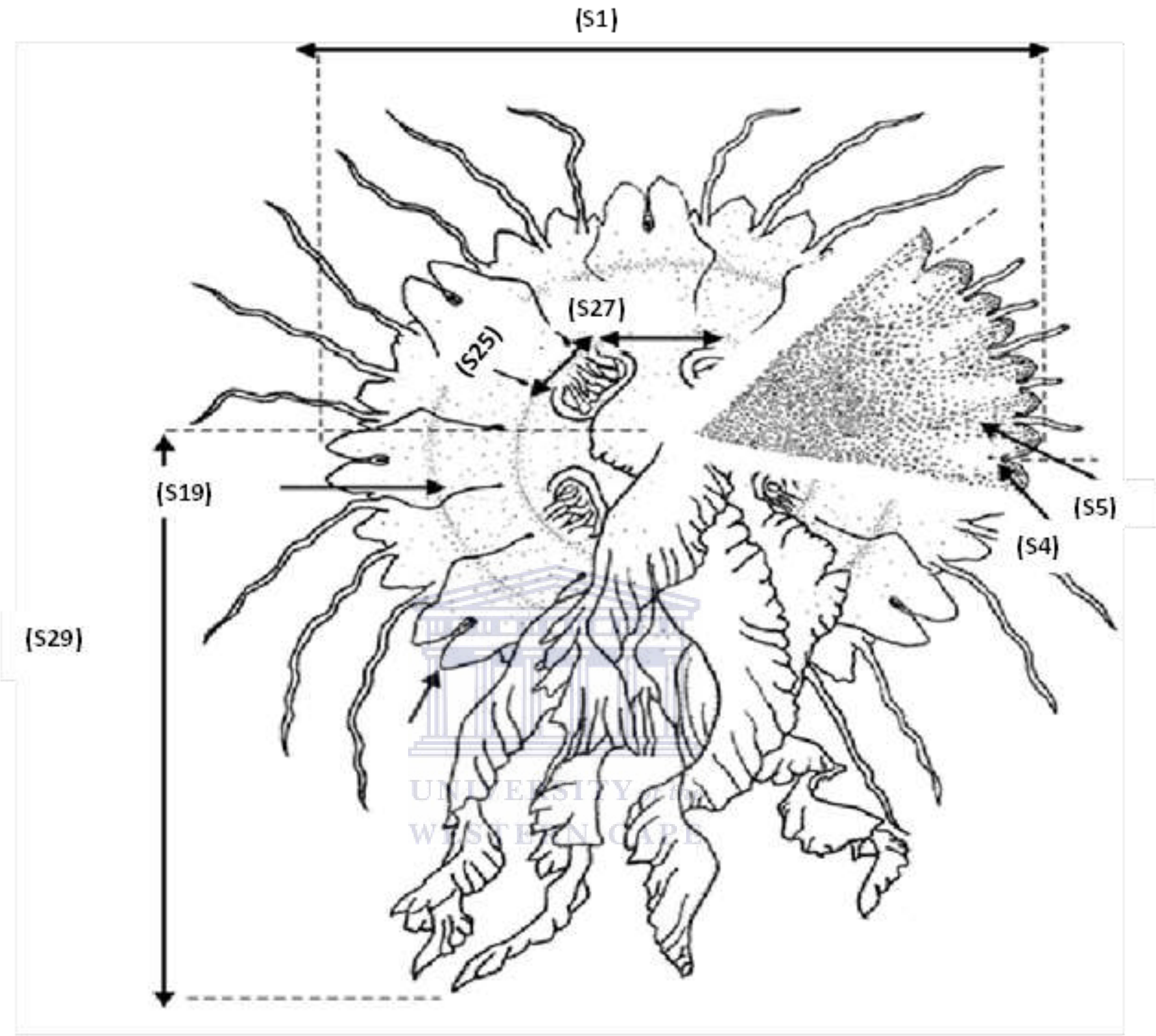
developed gastric cavity and e) 30 days post liberation indicating length and shape of lappet stems. Lappets stems, lappets, nematocyst clusters and gastric filaments clearly visible.

Figure 21: Enlarged images of the rhopalia of *C. fulgida*, , illustrating: a) the dorsal view and b) the ventral view, as well as the rhopalia of *C. africana*, illustrating : c) the ventral view and d) the dorsal view. Statocyst, rhopalal canal and hood clearly visible.

Figure 22: Enlarged images of the polyp of *C. fulgida* at time of strobilation, but with “non-strobilating polyps also clearly visible: a) and b) strobilating polyps appearing darker than others.

Figure 23: Enlarged images of the ephyrae of *C. fulgida*, taken unfed, at a) two days post liberation, b) two weeks post liberation and c) 30 days post liberation. Lappets stems, lappets, nematocyst clusters and gastric filaments clearly visible.

Figure 1:



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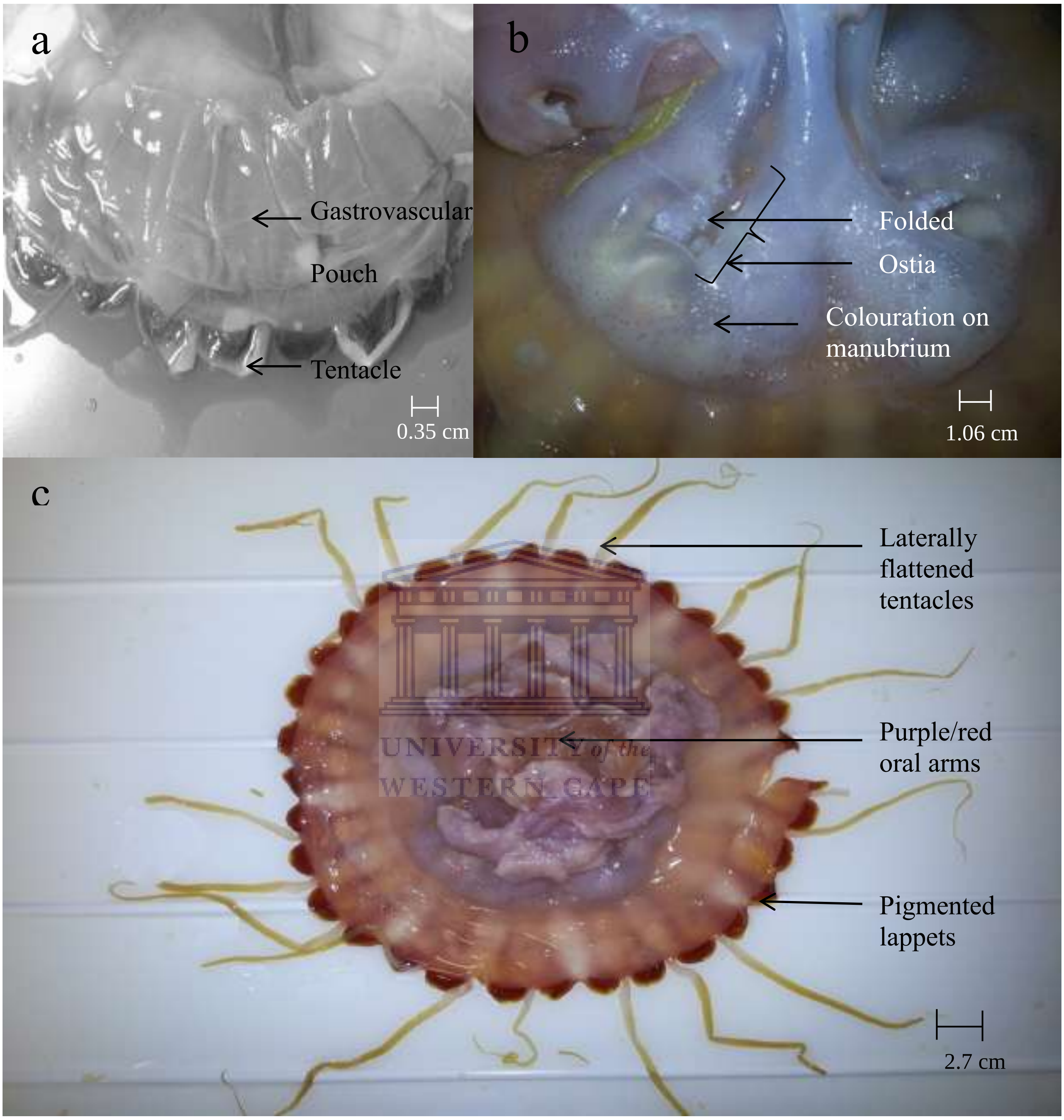
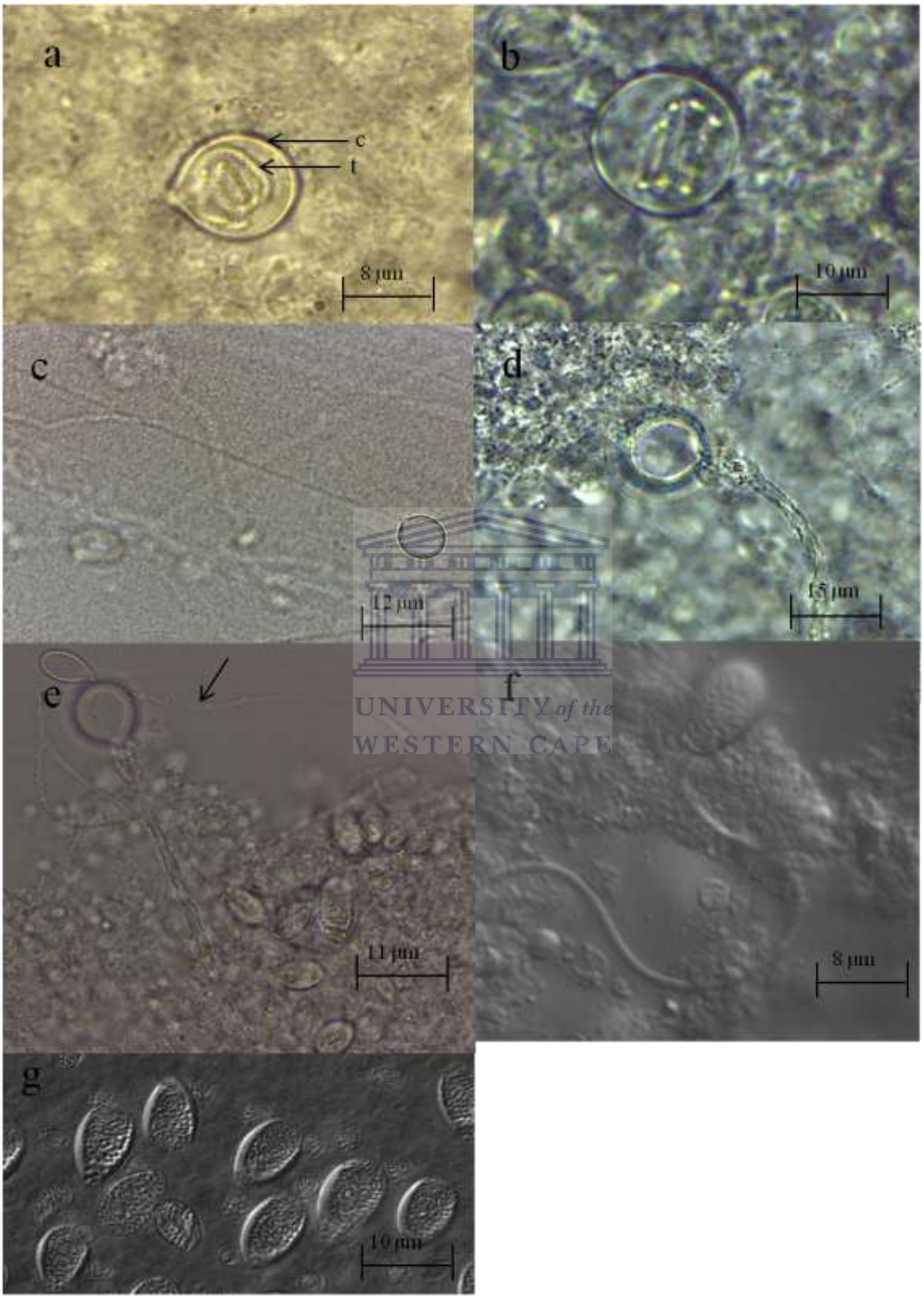

a
Gastrovascular
Pouch
Tentacle
0.35 cm
b
Folded
Ostia
Colouration on manubrium
1.06 cm
c
Laterally flattened tentacles
Purple/red oral arms
Pigmented lappets
2.7 cm

Figure 3:



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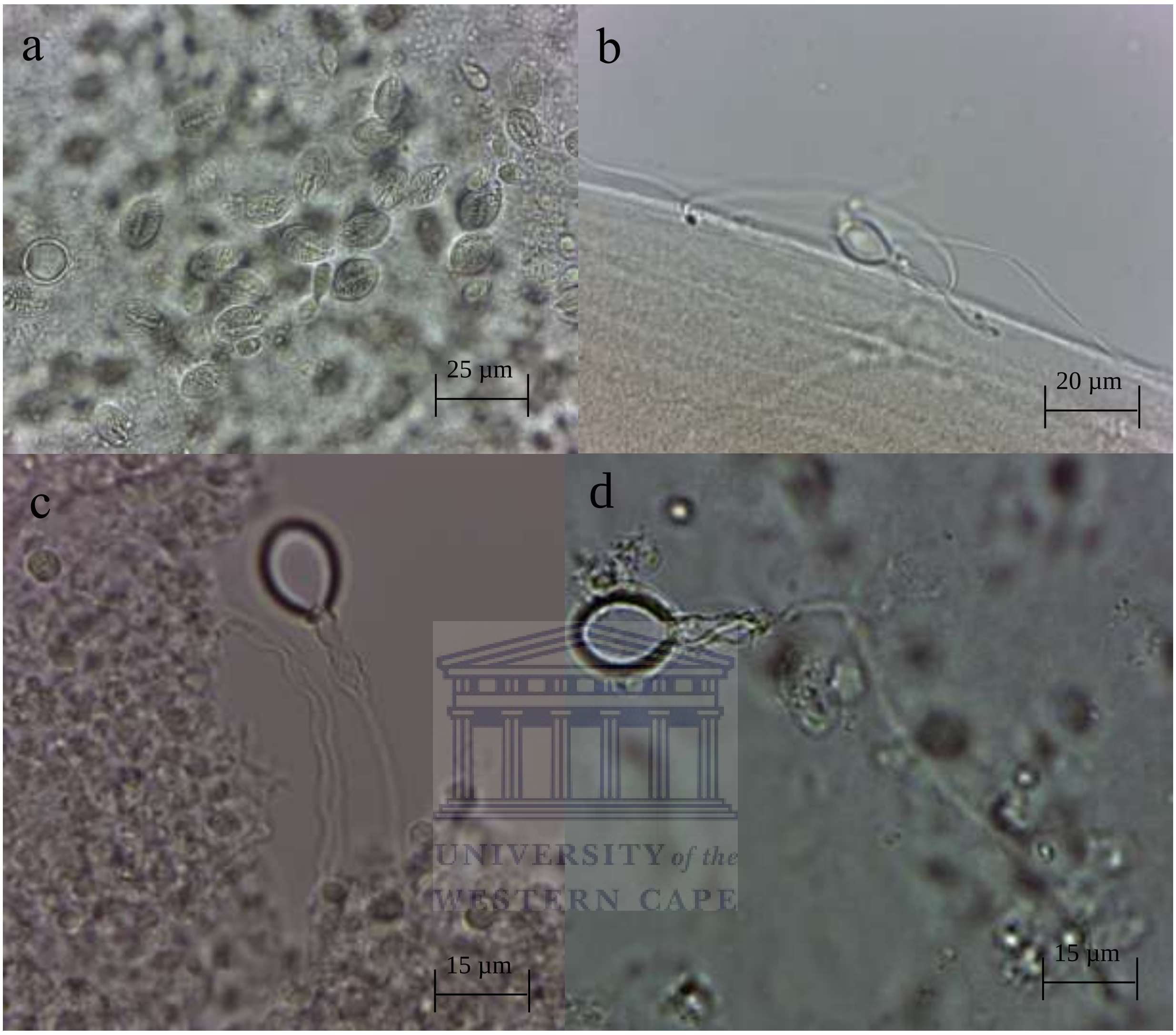

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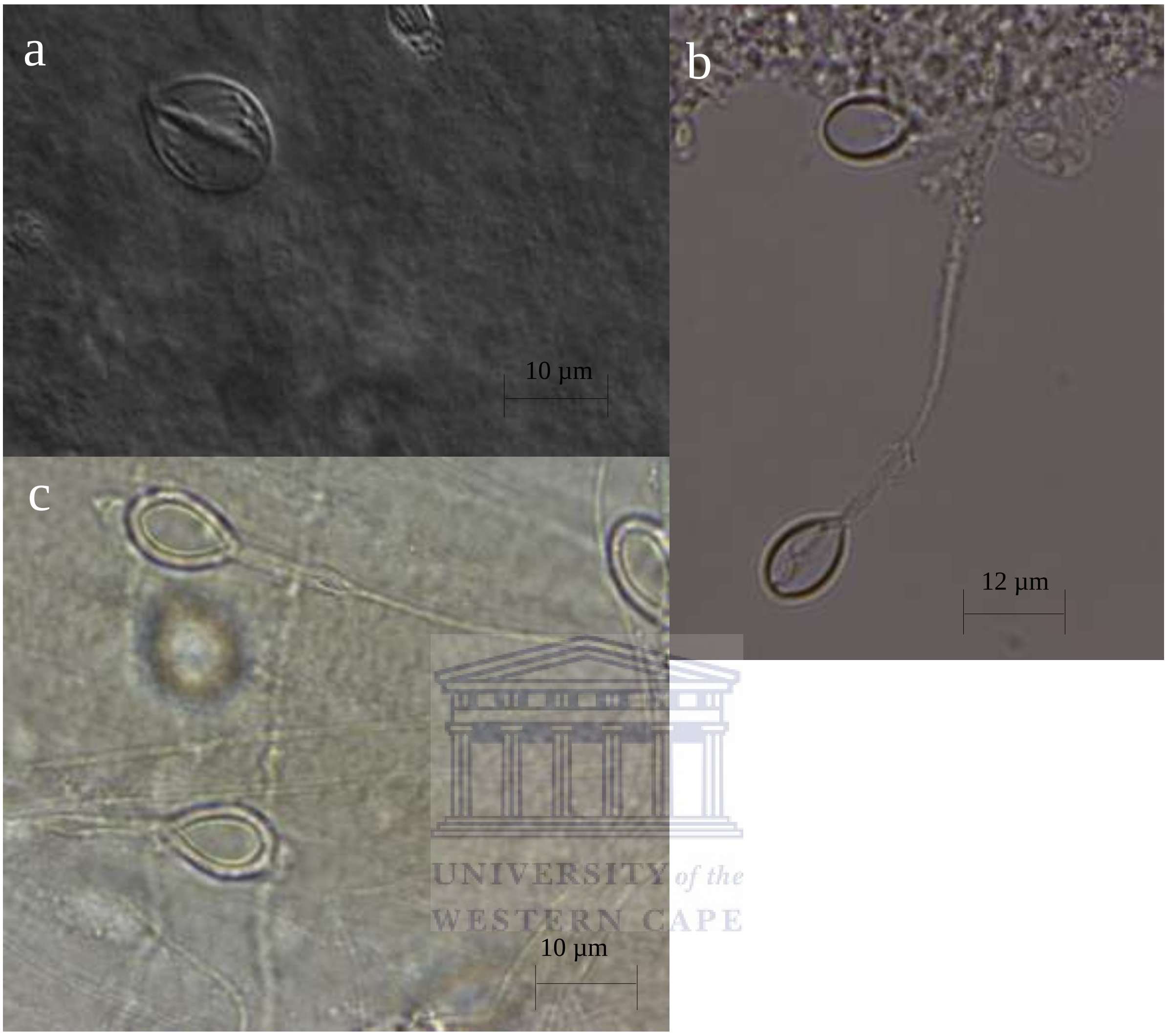

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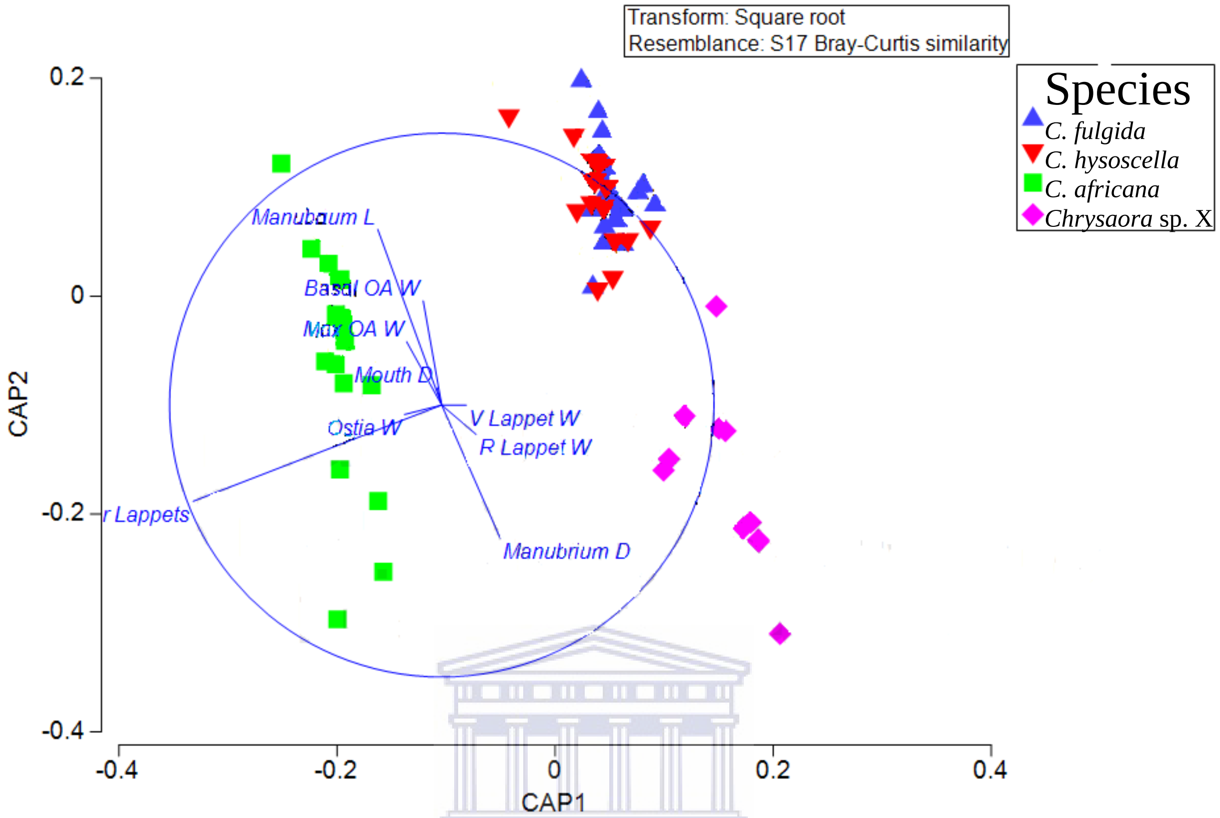
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Resemblance: S17 Bray-Curtis similarity
Species
▲ C. fulgida
▼ C. hysoscella
■ C. africana
◆ Chrysaora sp. X
Manubrium L
Basal OA W
Max OA W
Mouth D
Ostia W
V Lappet W
R Lappet W
Manubrium D
r Lappets
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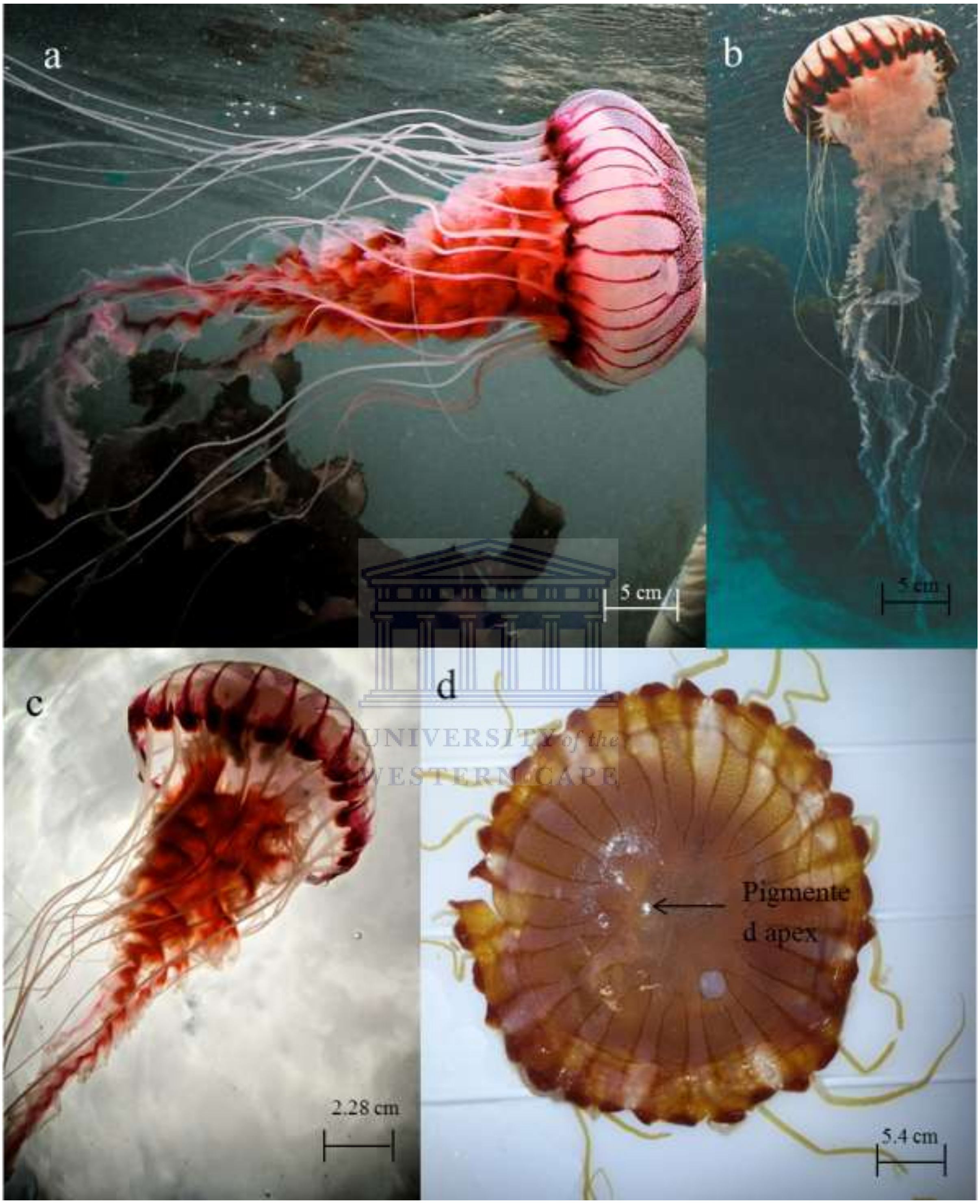
-0.2
-0.4
CAP2
-0.4
-0.2
0
0.2
0.4
CAP1

Figure 7:



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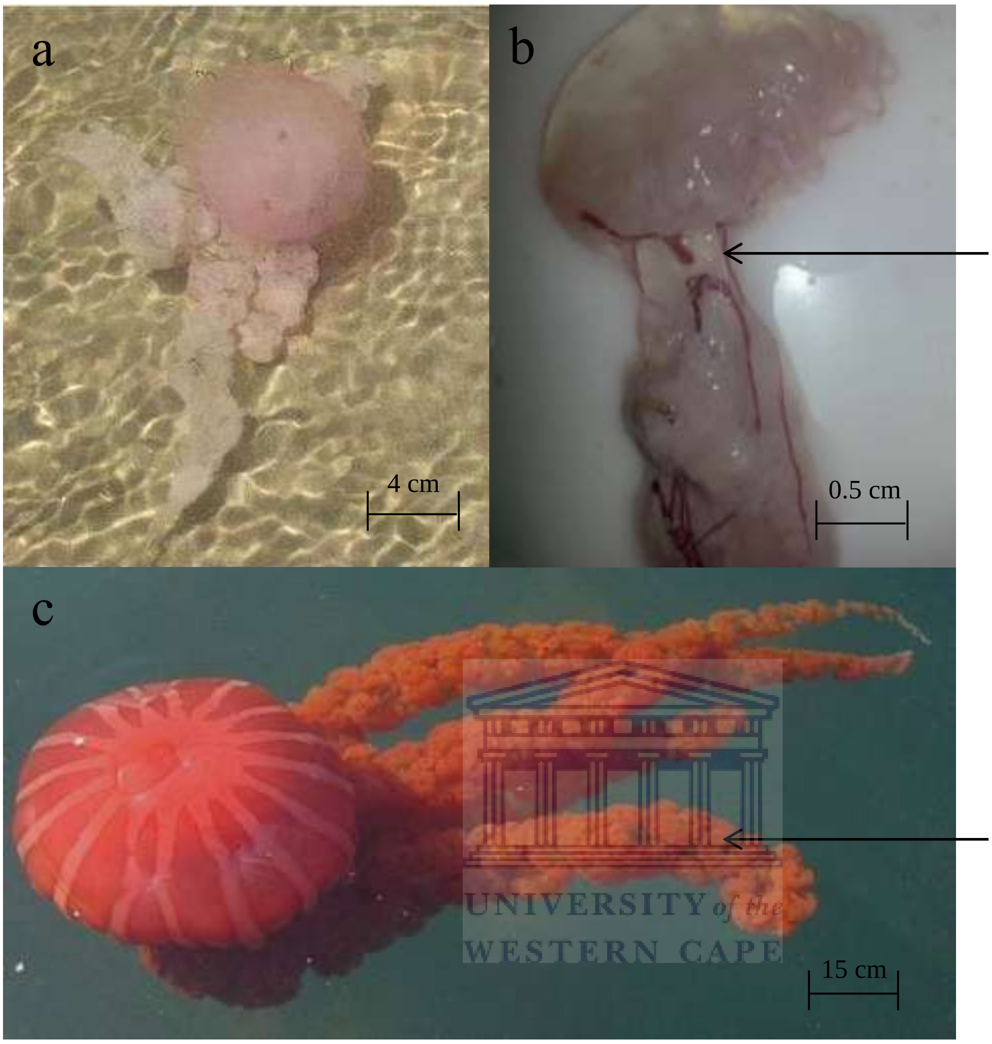
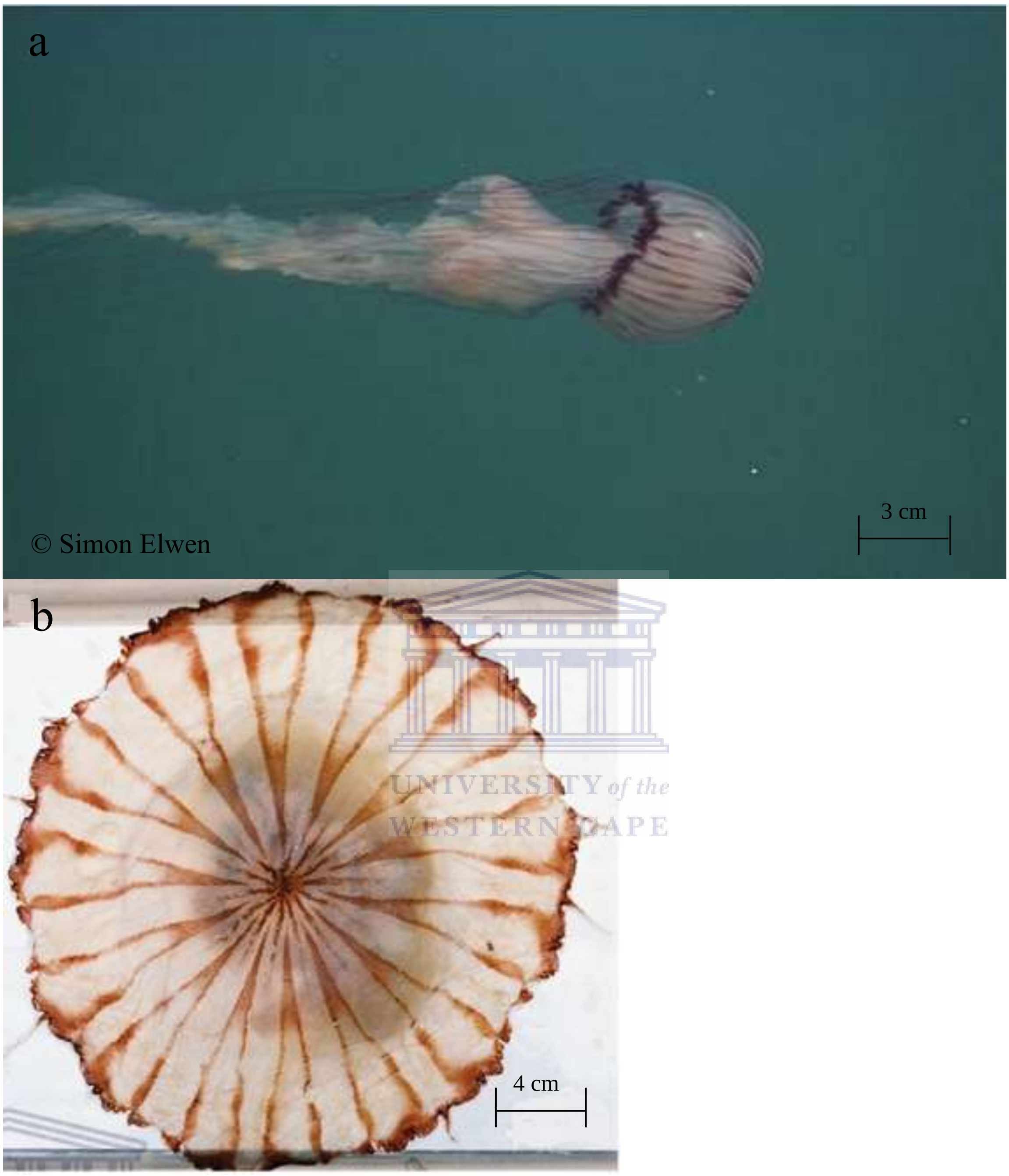


Maroon tentacles

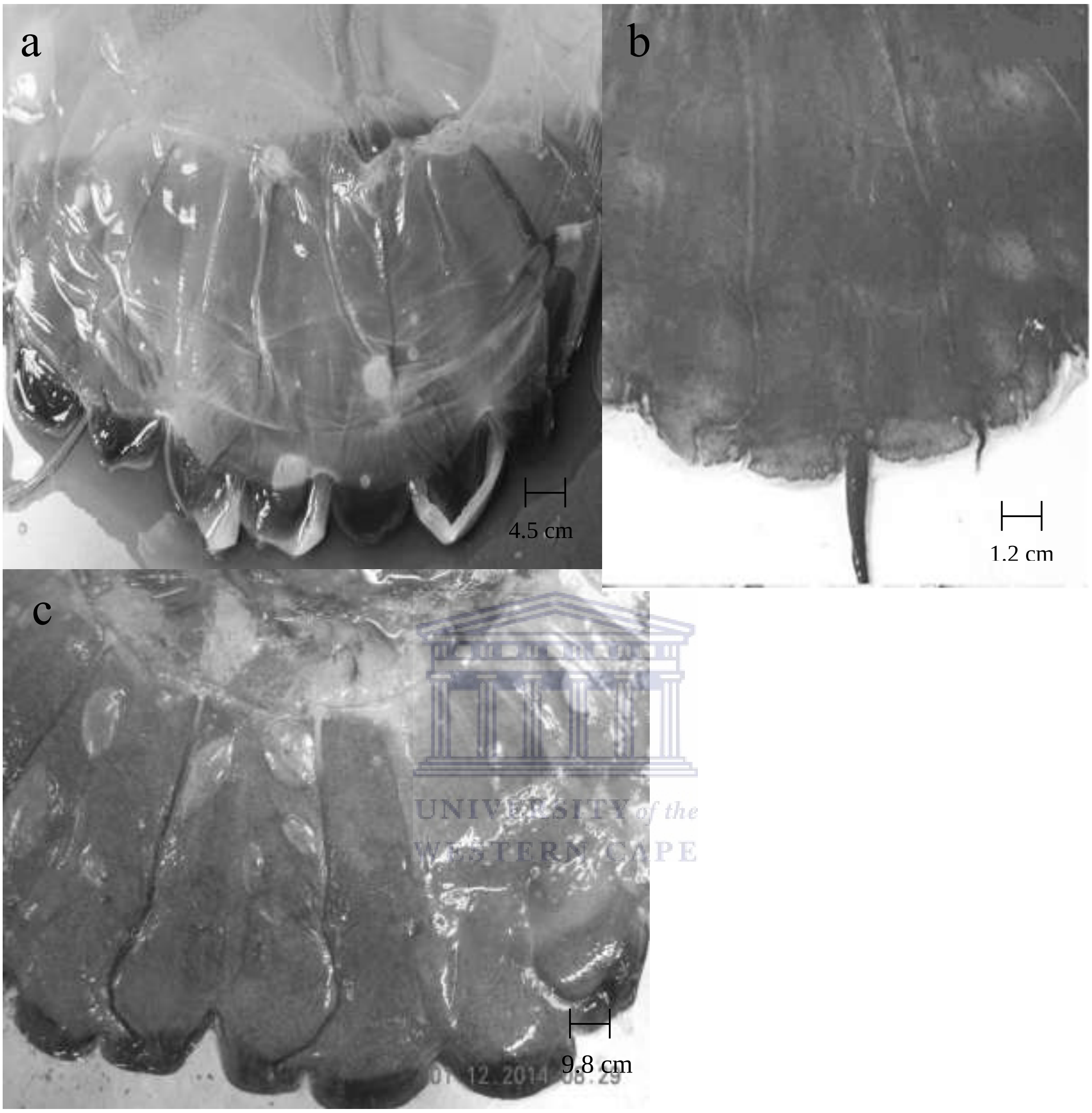
Highly folded oral arms

Figure 9:



© Simon Elwen

Figure 10:



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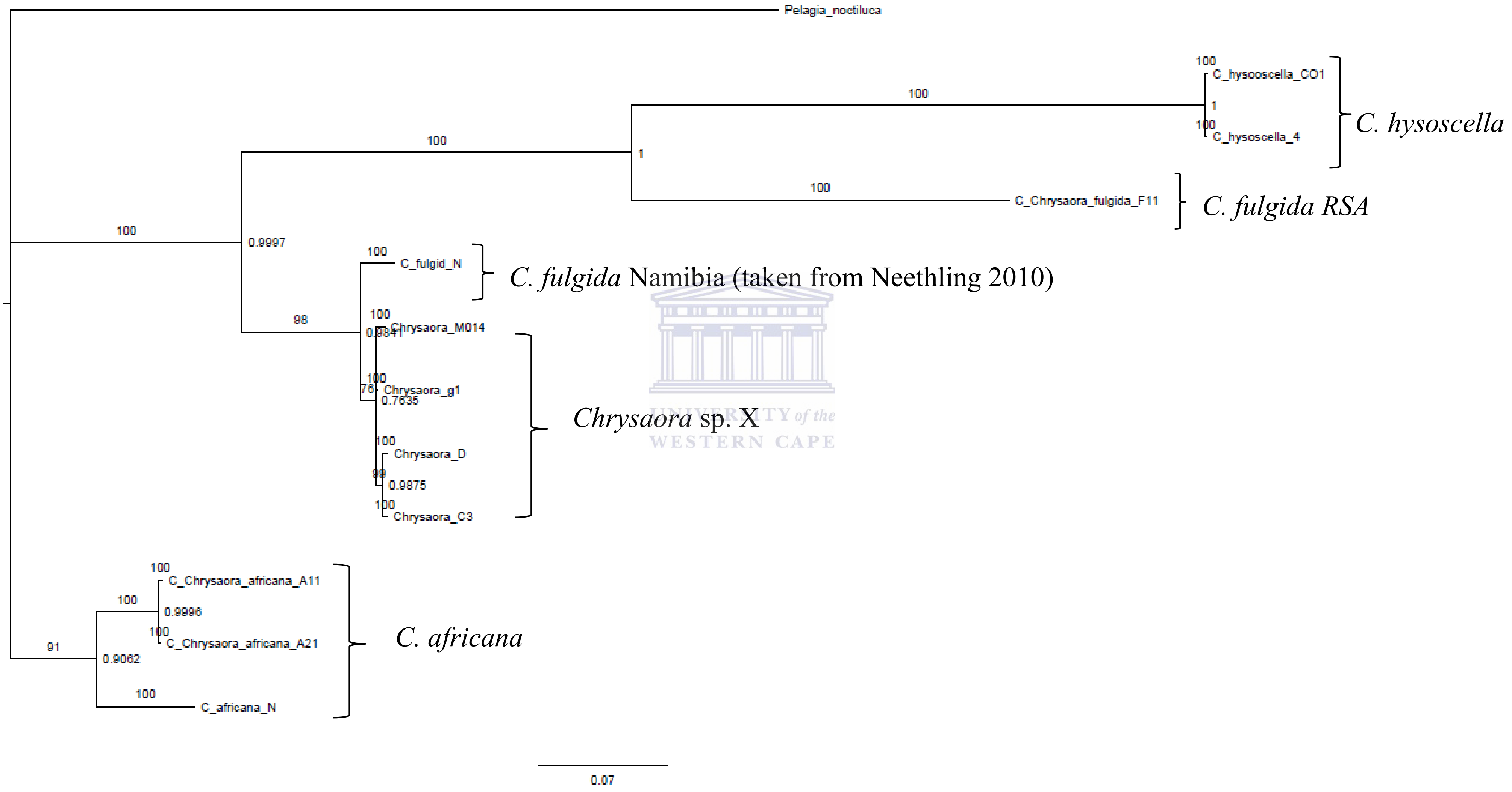
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1
100
C_hysoscella_4
C. hysoscella
100
100
1
100
C_Chrysaora_fulgida_F11
C. fulgida RSA
100
0.9997
100
C_fulgid_N
C. fulgida Namibia (taken from Neethling 2010)
98
100
0.9841
Chrysaora_M014
100
76
Chrysaora_g1
0.7635
100
Chrysaora_D
99
0.9875
100
Chrysaora_C3
Chrysaora sp. X
100
C_Chrysaora_africana_A11
100
0.9996
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C_africana_N
C. africana
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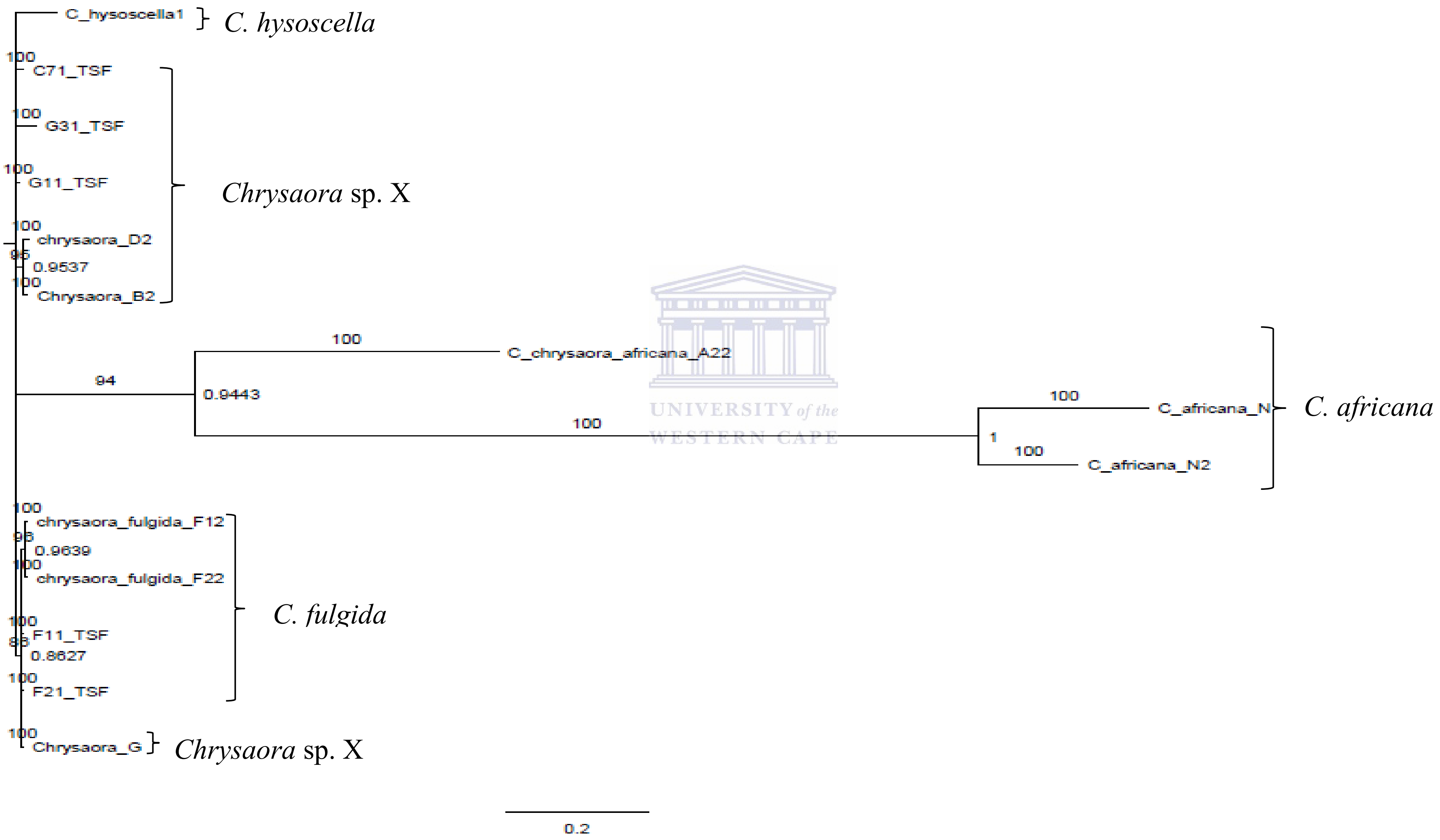
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C. hysoscella
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G31_TSF
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G11_TSF
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chrysaora_D2
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0.9537
100
Chrysaora_B2
100
C_chrysaora_africana_A22
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0.9443
100
100
C_africana_N
C. africana
1
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C_africana_N2
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chrysaora_fulgida_F12
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0.9639
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chrysaora_fulgida_F22
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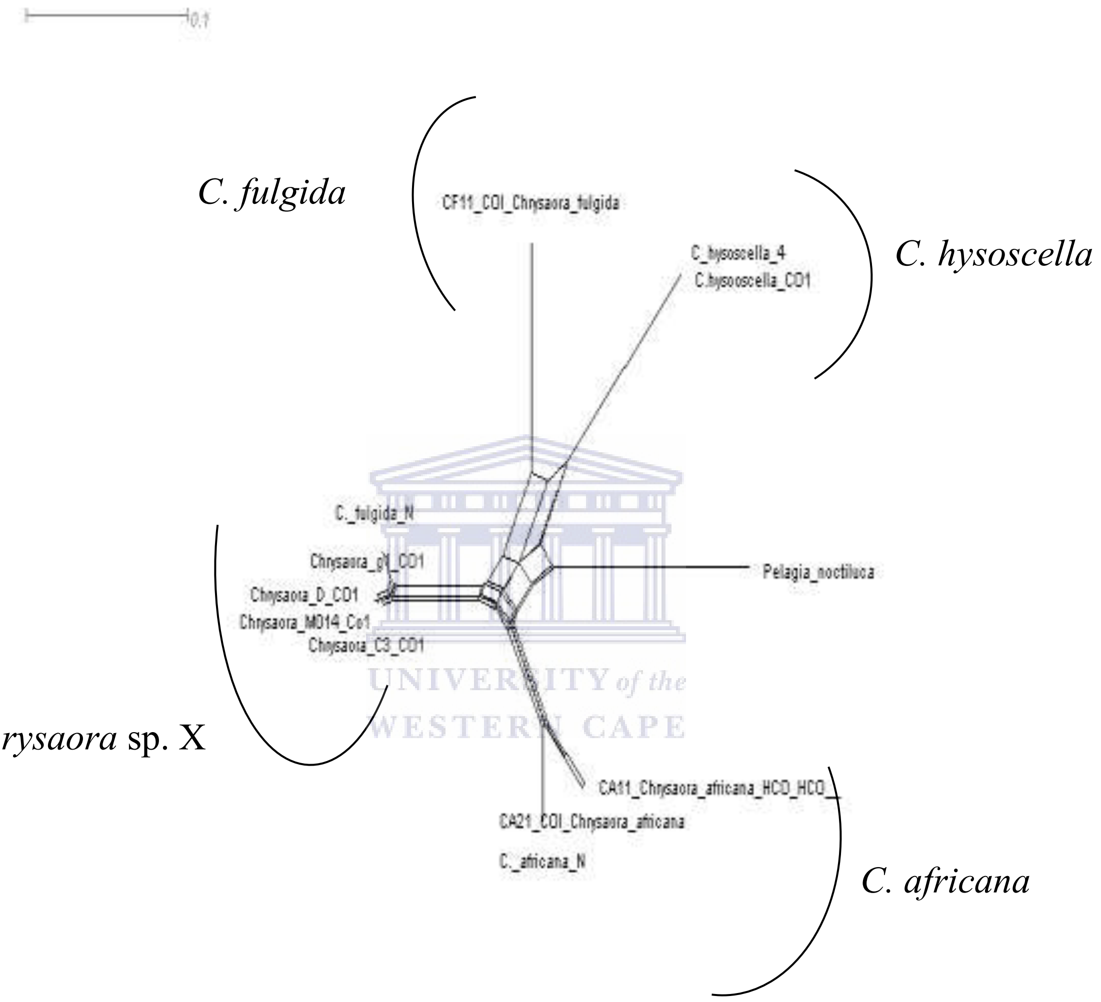
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C._fulgida_N
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Chrysaora_D_CO1
Chrysaora_M014_Co1
Chrysaora_C3_CO1
Pelagia_noctiluca
Chrysaora sp. X
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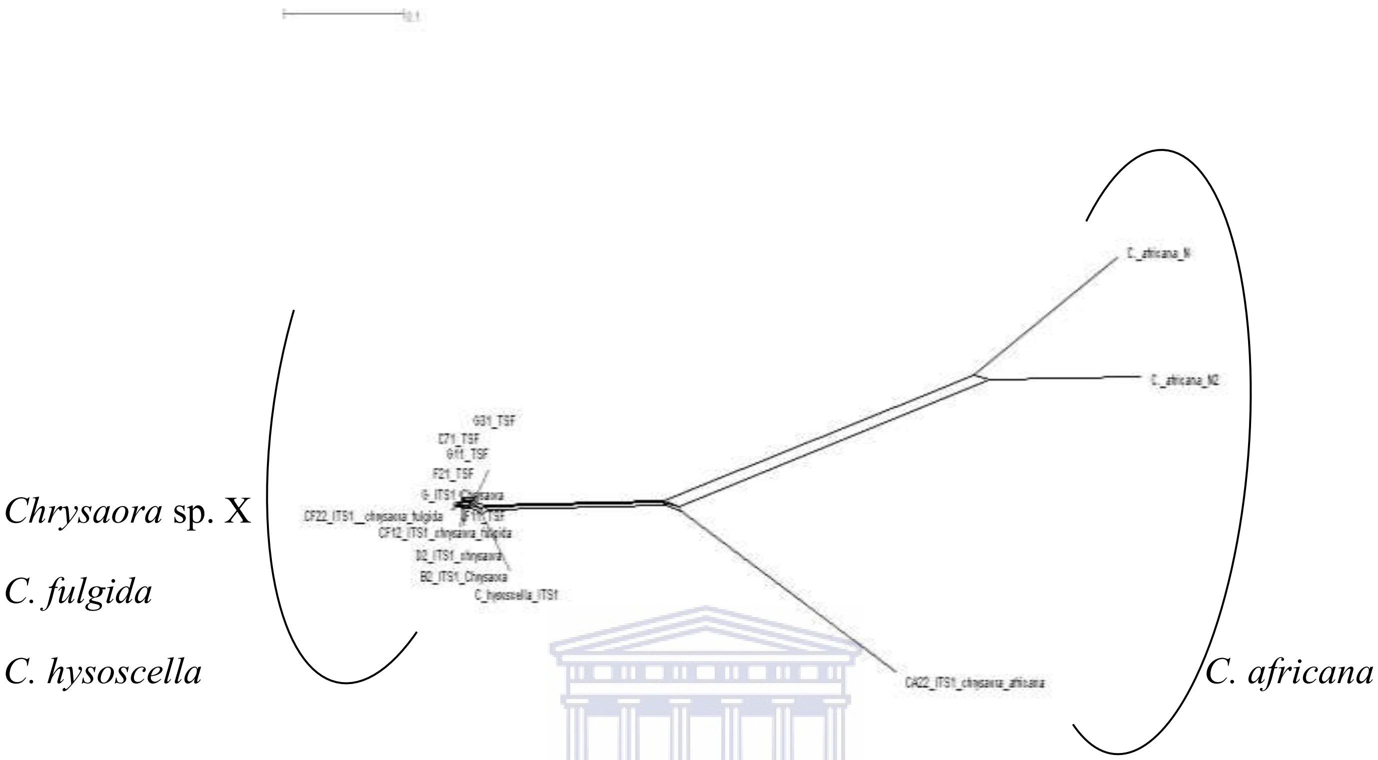
C. africana

Figure 14: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F21_TSF
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CF22_ITS1__chrysaora_fulgida
F11_TSF
CF12_ITS1_chrysaora_fulgida
D2_ITS1_chrysaora
B2_ITS1_Chrysaora
C_hysoscella_ITS1
C._africana_N
C._africana_N2
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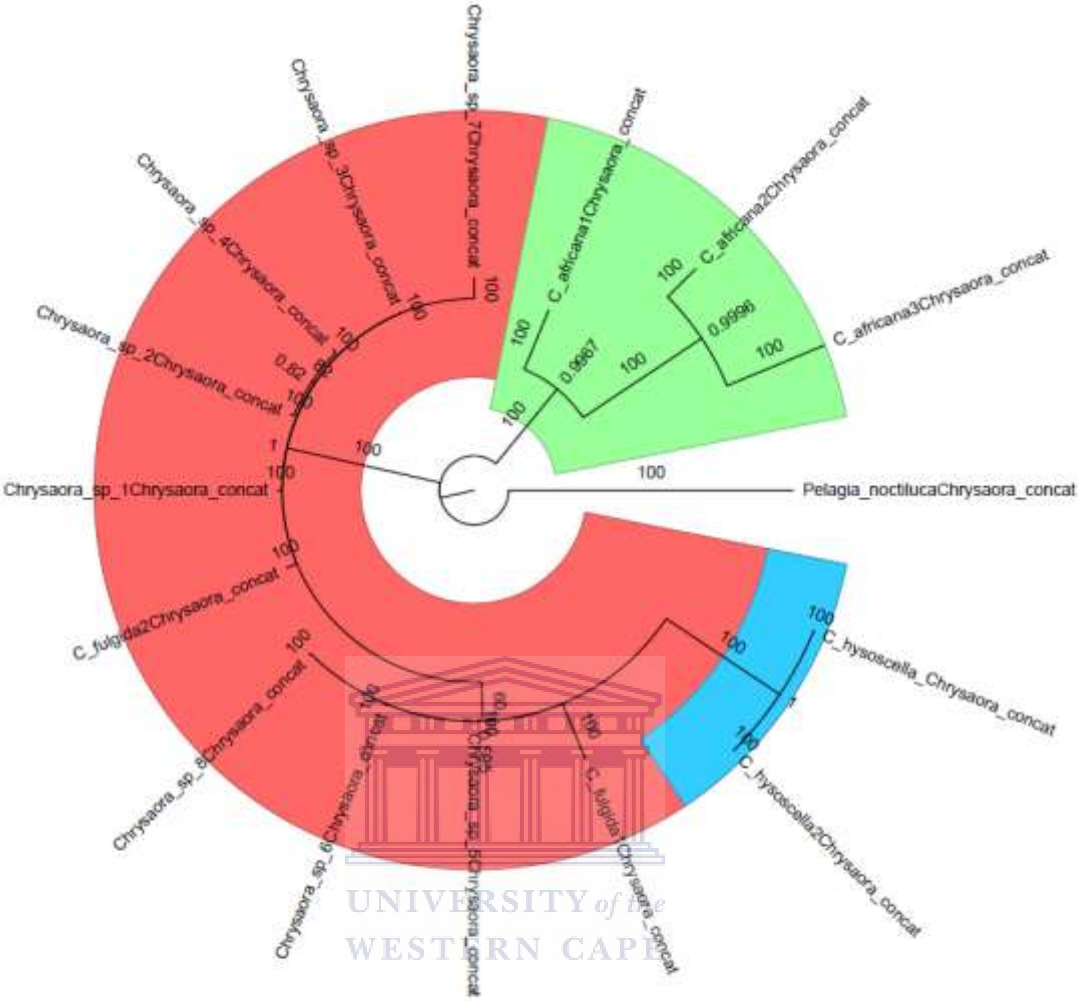
Chrysaora sp. X
C. fulgida
C. hysoscella
C. africana

Figure 15: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Chrysaora_sp_1Chrysaora_concat
C_fulgida2Chrysaora_concat
Chrysaora_sp_8Chrysaora_concat
Chrysaora_sp_6Chrysaora_concat
Chrysaora_sp_5Chrysaora_concat
C_fulgidaChrysaora_concat
C_hysoscella2Chrysaora_concat
C_hysoscella_Chrysaora_concat
C_africana3Chrysaora_concat
C_africana2Chrysaora_concat
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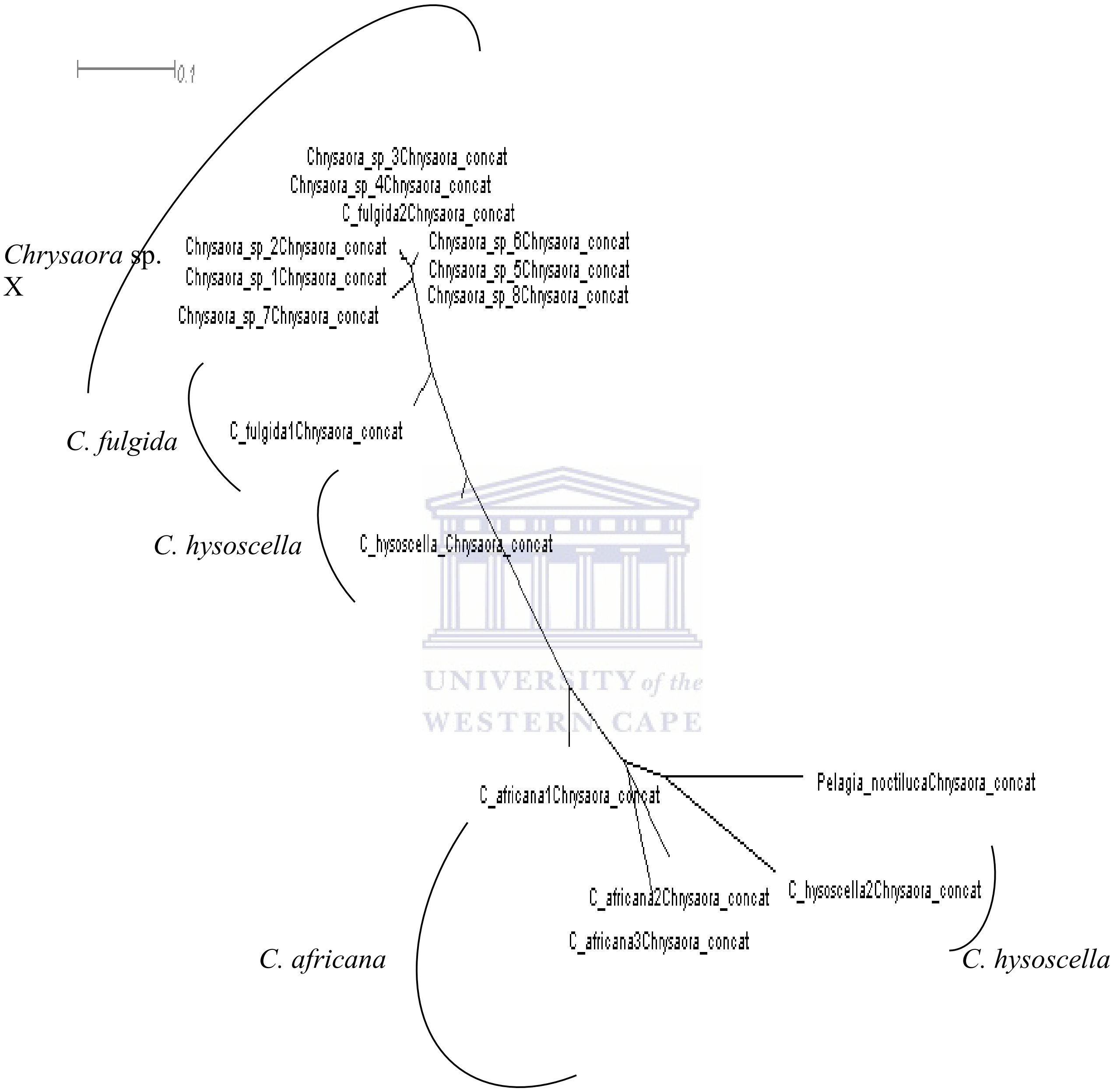
1

Figure 16:


0.1
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C. fulgida
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C. hysoscella
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C. hysoscella

Figure 17:

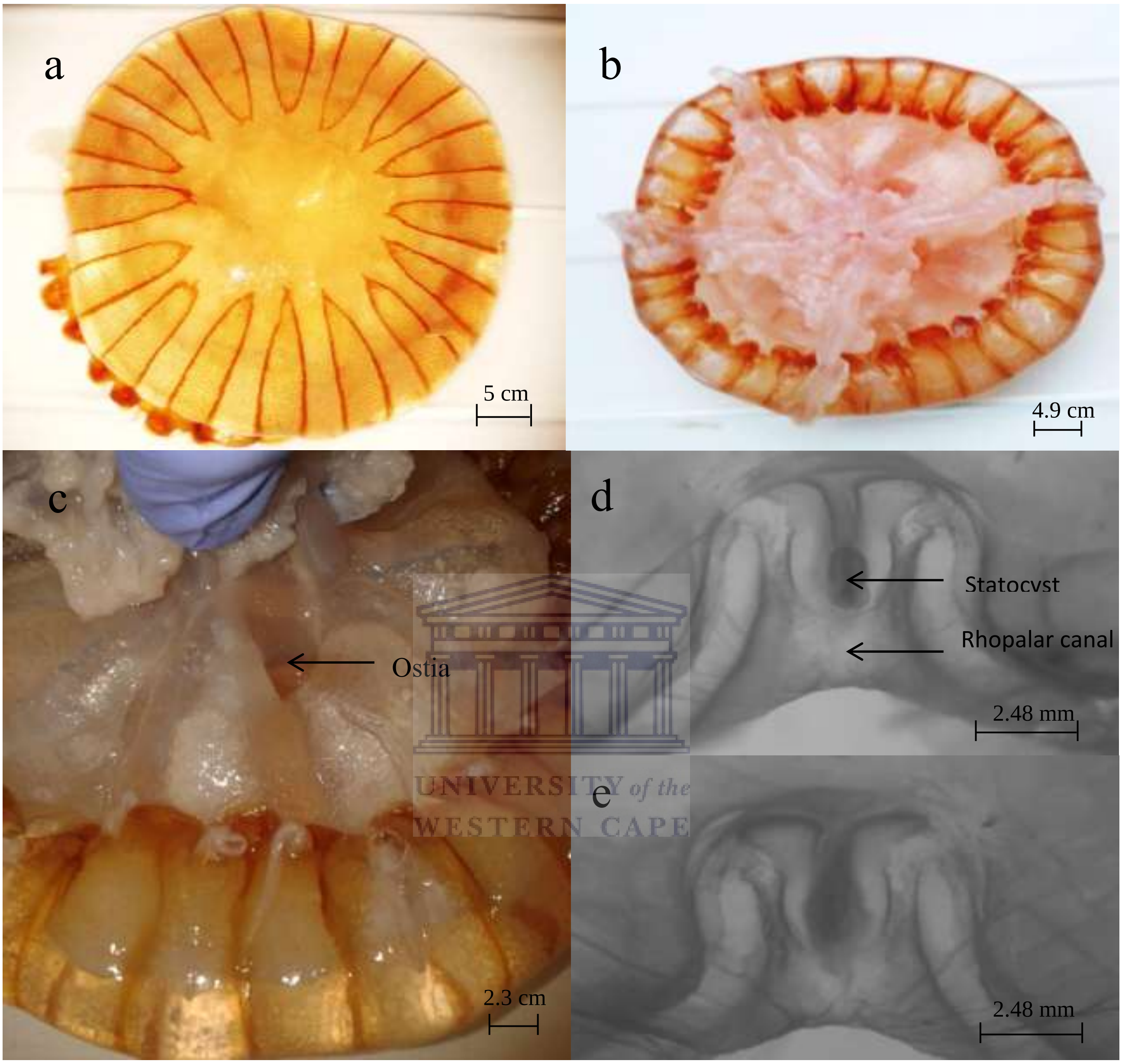

a
5 cm
b
4.9 cm
c
Ostia
2.3 cm
d
Statocyst
Rhopalar canal
2.48 mm
e
2.48 mm

Figure 18:

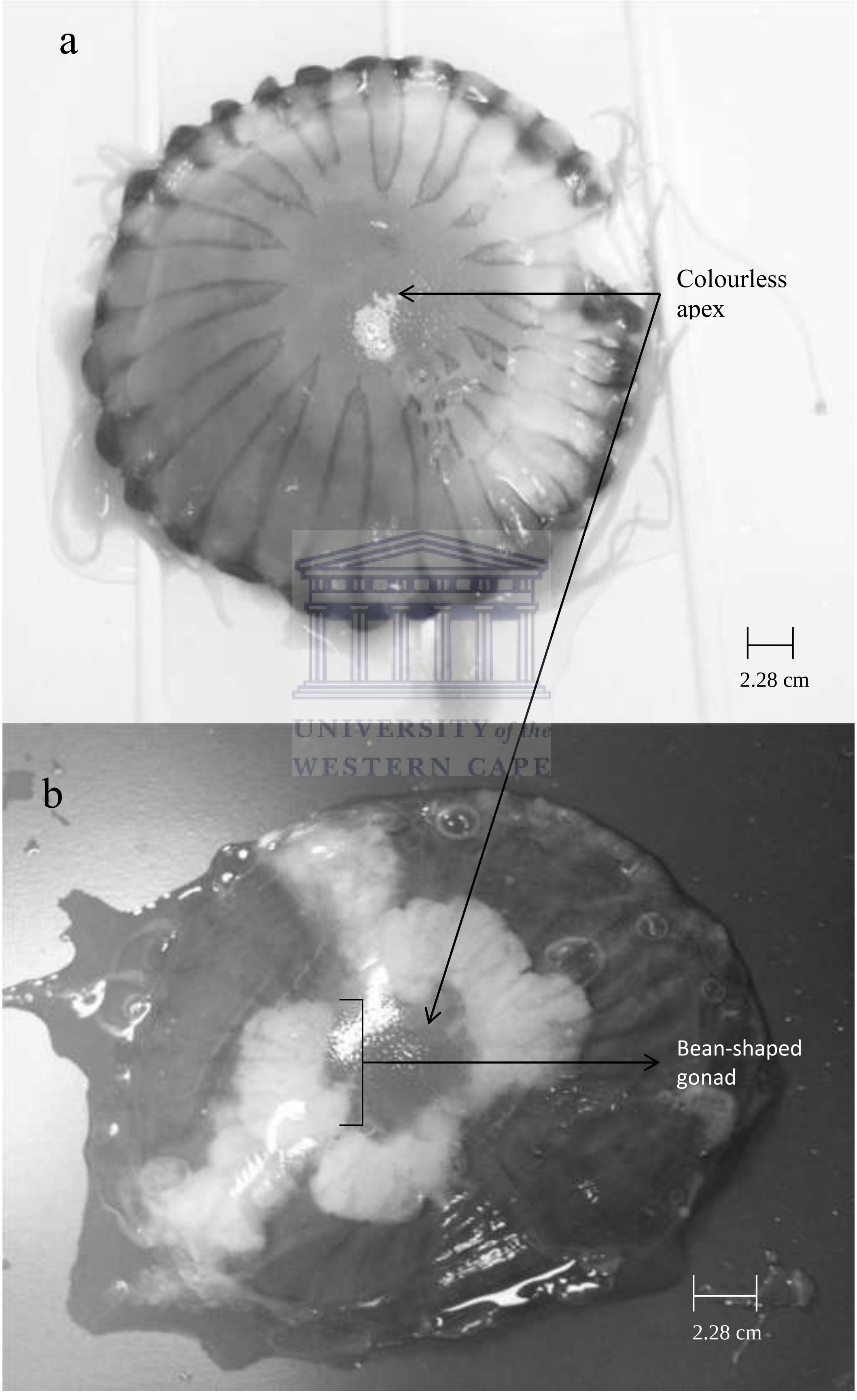
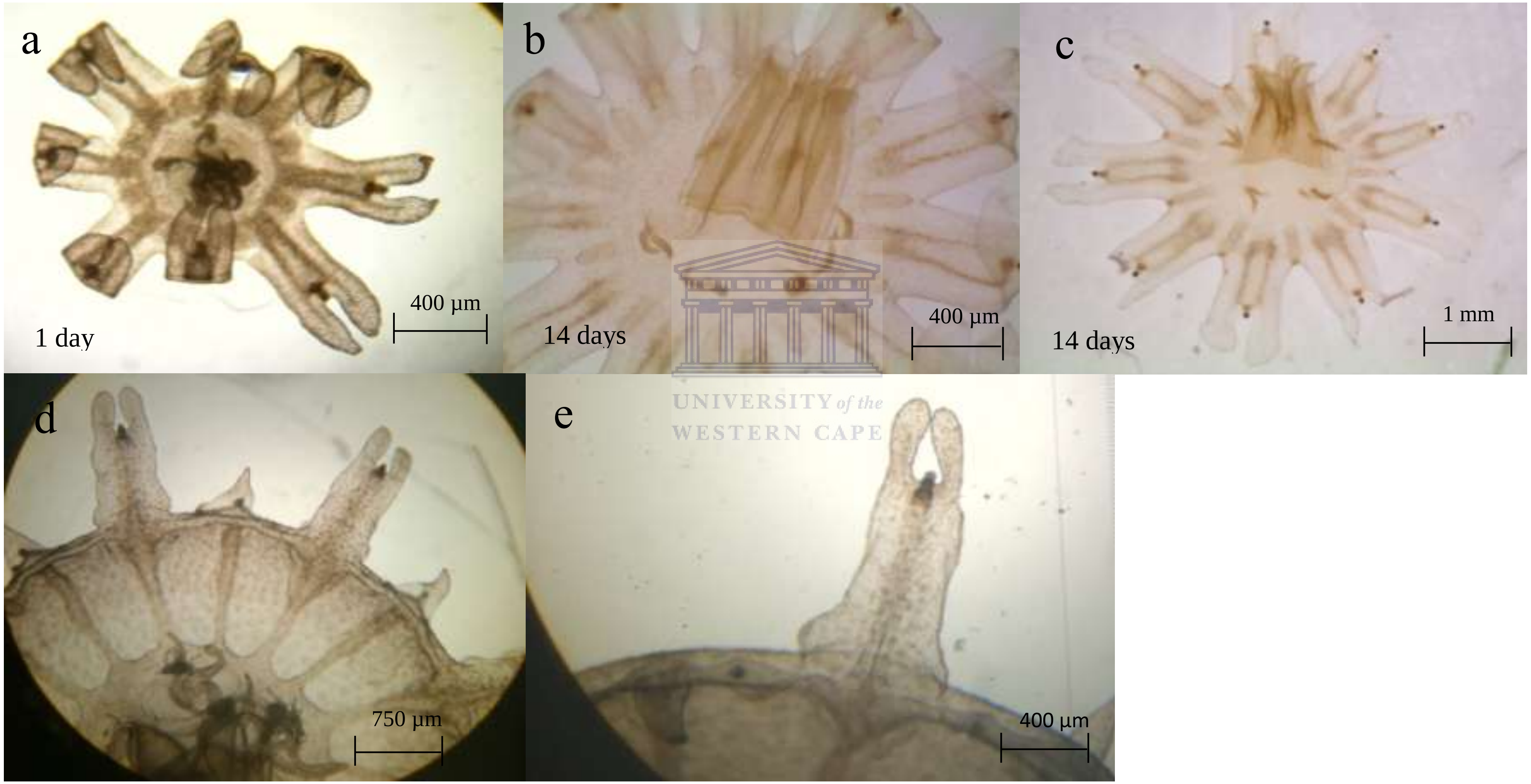


Figure 19:

Figure 20:



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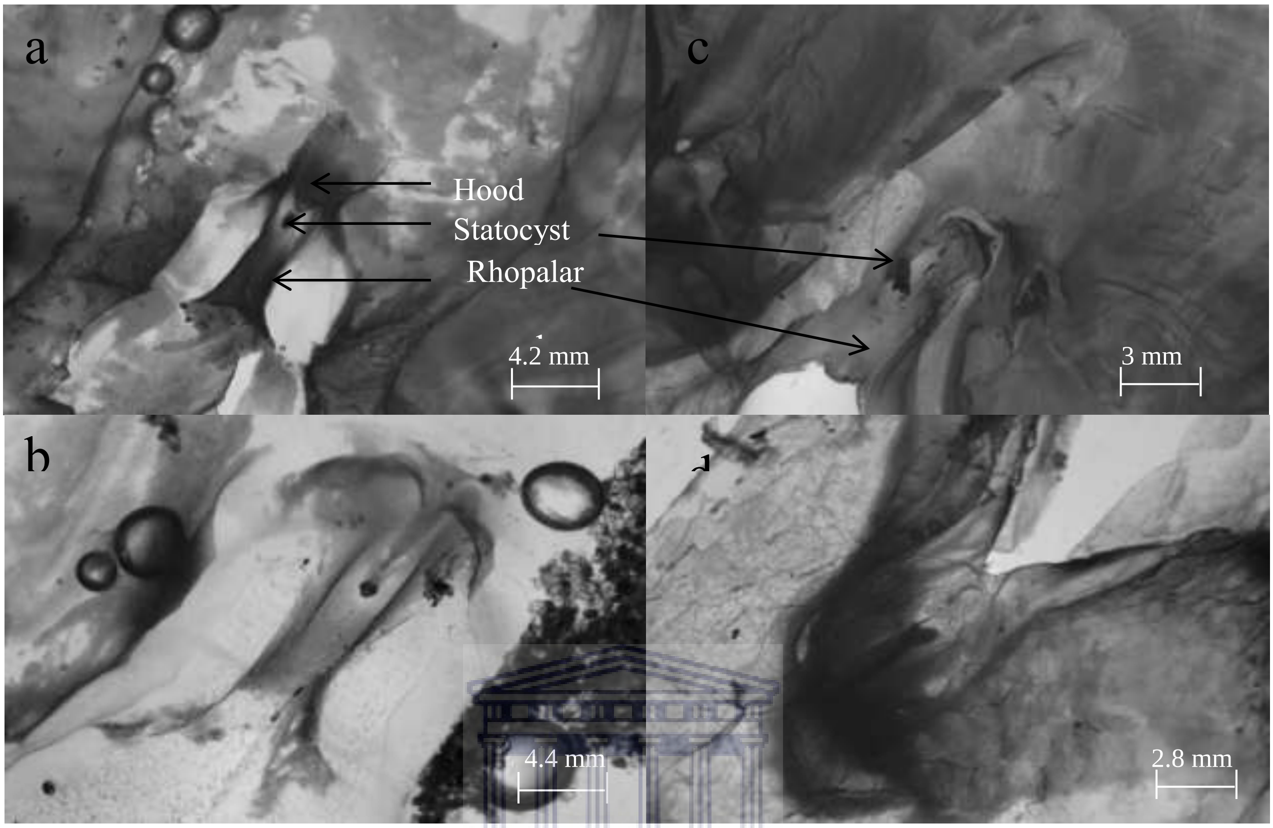

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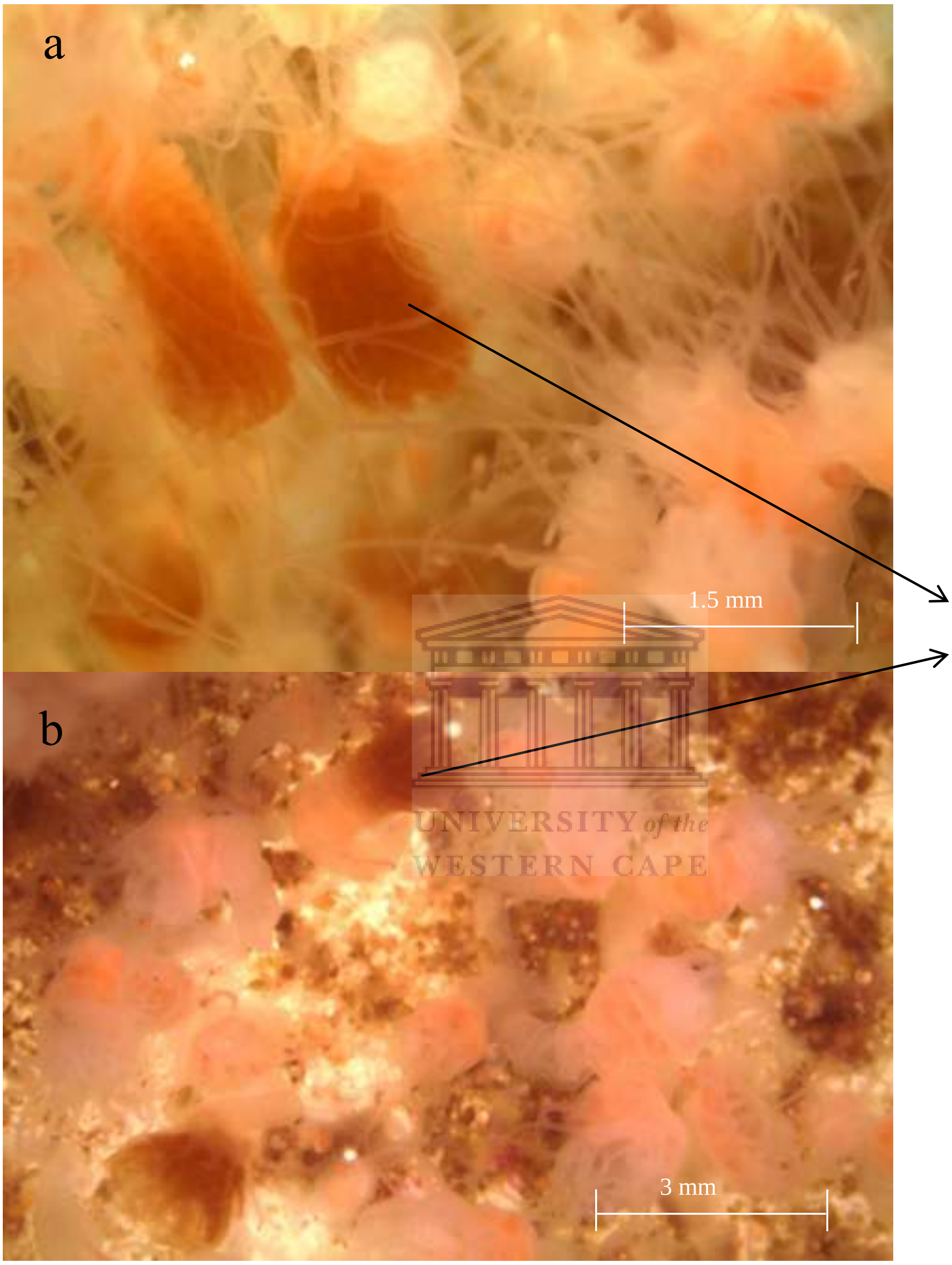

Strobilating polyps

Figure 23:

Appendix 1: Raw morphometric and meristic data, summarized for *Chrysaora fulgida*, *Chrysaora africana* and *Chrysaora* sp. X collected throughout 2008 – 2015.

Species	MF	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
C. fulgida	**Mean**	14.2	2.1	0.2	0.8	0.4	-	1.0	-	1.2	0.8	0.1
	N	4.0	4.0	4.0	4.0	4.0	-	1.8	-	1.8	3.9	4.0
	SD	7.0	9.5	0.6	0.0	0.0	-	0.8	-	1.0	0.1	0.2
	Var	490.3	894.7	3.1	0.0	0.0	-	5.9	-	9.4	0.1	0.5
	Median	12.0	0.6	0.1	0.8	0.4	-	0.8	-	0.9	0.8	0.0
	Min	5.9	0.3	0.0	0.8	0.4	-	0.4	-	0.4	0.3	0.0
	Max	40.7	60.4	3.6	0.9	0.4	-	3.1	-	3.9	0.8	1.1
	Range	34.8	60.1	3.6	0.1	0.0	-	2.7	-	3.4	0.5	1.1
C. africana	**Mean**	19.0	1.0	0.2	0.8	0.6	0.5	0.6	0.5	0.6	0.8	1.2
	N	1.6	1.6	1.5	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
	SD	5.6	0.4	0.1	0.0	0.0	0.1	0.2	0.1	0.2	0.0	0.4
	Var	317.2	1.6	0.0	0.0	0.0	0.1	0.3	0.1	0.4	0.0	1.9
	Median	18.0	1.1	0.2	0.8	0.6	0.5	0.6	0.5	0.6	0.8	1.4
	Min	10.5	0.2	0.1	0.7	0.6	0.3	0.3	0.3	0.3	0.7	0.1
	Max	31.2	1.6	0.3	0.9	0.6	0.7	0.9	0.6	0.9	0.9	1.6
	Range	20.7	1.4	0.2	0.2	0.0	0.4	0.6	0.4	0.7	0.2	1.5
***Chrysaora* sp. X**	**Mean**	10.1	1.0	0.4	0.8	0.4	1.3	0.7	0.5	21.2	0.8	1.6
	N	3.3	3.2	3.2	3.3	3.3	3.2	3.2	3.2	3.1	3.3	3.3
	SD	5.0	0.7	0.4	0.0	0.0	4.6	0.3	0.3	113.7	0.0	0.0
	Var	246.6	5.4	1.8	0.0	0.0	209.5	1.0	0.6	129320.7	0.0	0.0
	Median	8.3	0.8	0.2	0.8	0.4	0.5	0.7	0.5	0.7	0.8	1.6
	Min	3.2	0.3	0.1	0.8	0.4	0.2	0.2	0.2	0.4	0.8	1.6
	Max	20.8	3.0	1.7	0.8	0.4	26.4	1.5	1.2	633.9	0.8	1.6
	Range	17.5	2.7	1.7	0.0	0.0	26.2	1.3	1.0	633.5	0.0	0.0

Species	MF	S12	S13	S16	S17	S18	S20	S21	S23	S25	S26	S27
C. fulgida	**Mean**	0.3	-	-	-	1.6	2.4	5.1	0.1	0.8	1.3	1.3
	N	0.5	-	-	-	4.0	4.0	3.9	3.2	4.0	4.0	3.9
	SD	0.1	-	-	-	0.0	0.8	2.3	0.0	0.2	0.2	0.7
	Var	0.0	-	-	-	0.0	6.6	54.8	0.0	0.3	0.6	5.0
	Median	0.2	-	-	-	1.6	2.3	4.4	0.0	0.8	1.3	1.1
	Min	0.2	-	-	-	1.6	1.2	2.0	0.0	0.6	0.8	0.5
	Max	0.3	-	-	-	1.6	5.4	13.8	0.2	1.2	1.9	4.2
	Range	0.1	-	-	-	0.0	4.2	11.7	0.2	0.6	1.2	3.7
C. africana	**Mean**	0.3	0.2	-	-	1.6	5.2	6.3	0.2	2.8	2.4	1.2
	N	1.2	1.2	-	-	1.6	1.6	1.6	1.6	1.6	1.6	1.6
	SD	0.1	0.1	-	-	0.0	1.5	2.0	0.1	0.8	0.9	0.3
	Var	0.1	0.1	-	-	0.0	23.7	41.1	0.0	7.1	7.8	1.1
	Median	0.3	0.2	-	-	1.6	5.2	6.1	0.2	2.6	2.4	1.1
	Min	0.2	0.2	-	-	1.6	3.1	3.5	0.1	1.5	1.0	0.7
	Max	0.4	0.4	-	-	1.6	8.2	10.8	0.3	4.6	4.4	2.0
	Range	0.2	0.2	-	-	0.0	5.2	7.2	0.2	3.1	3.4	1.3
***Chrysaora* sp. X**	**Mean**	0.2	0.2	11.1	9.8	1.6	1.3	2.2	1.5	0.6	1.1	1.6
	N	3.1	3.1	8.0	8.0	3.3	2.6	2.6	2.6	2.8	2.8	2.7
	SD	0.1	0.1	6.4	5.4	0.0	0.8	1.3	0.9	0.3	0.8	0.8
	Var	0.2	0.1	40.9	28.6	0.0	6.7	16.2	7.9	1.1	7.0	7.0
	Median	0.2	0.1	12.3	11.8	1.6	1.1	1.5	1.4	0.5	0.7	1.3
	Min	0.0	0.0	4.5	3.7	1.6	0.3	0.6	0.2	0.2	0.3	0.7
	Max	0.5	0.4	22.6	16.6	1.6	3.2	4.8	3.3	1.3	3.2	3.7
	Range	0.4	0.3	18.1	12.9	0.0	2.9	4.2	3.1	1.1	2.9	3.0

Species	MF	S28	S29	S30	S31	S30	S34	S35	S40
C. fulgida	**Mean**	0.8	23.1	2.9	5.7	2.9	-	-	0.4
	N	4.0	2.9	4.0	4.0	4.0	-	-	4.0
	SD	0.0	9.7	1.2	3.1	1.2	-	-	0.0
	Var	0.0	948.7	14.8	95.6	14.8	-	-	0.0
	Median	0.8	21.7	2.7	5.1	2.7	-	-	0.4
	min	0.8	6.8	0.8	2.1	0.8	-	-	0.4
	max	0.9	60.8	7.8	18.0	7.8	-	-	0.4
	Range	0.1	54.0	7.0	16.0	7.0	-	-	0.0
C. africana	**Mean**	0.8	67.5	4.3	10.5	4.3	0.4	1.4	0.4
	N	1.6	0.5	1.5	1.4	1.5	1.3	1.4	1.6
	SD	0.1	30.0	2.3	4.3	2.3	0.2	0.4	0.0
	Var	0.0	9007.4	53.1	183.3	53.1	0.2	1.4	0.0
	Median	0.8	70.3	4.2	10.7	4.2	0.4	1.3	0.4
	min	0.6	36.5	1.2	3.9	1.2	0.2	1.0	0.4
	max	0.9	113.0	8.2	18.9	8.2	0.7	2.1	0.4
	Range	0.3	76.5	7.1	15.0	7.1	0.5	1.1	0.0
***Chrysaora* sp. X**	**Mean**	0.8	12.0	1.6	4.8	1.6	9.4	0.7	0.4
	N	3.3	0.9	2.1	1.1	2.1	2.5	2.5	3.3
	SD	0.0	4.1	1.4	2.9	1.4	37.0	0.4	0.0
	Var	0.0	166.9	19.8	86.9	19.8	13708.5	1.8	0.0
	Median	0.8	10.2	1.2	2.8	1.2	1.7	0.6	0.4
	min	0.8	5.5	0.5	1.4	0.5	0.7	0.3	0.4
	max	0.8	17.6	5.1	9.0	5.1	187.1	1.8	0.4
	Range	0.0	12.1	4.6	7.7	4.6	186.4	1.5	0.0

Appendix 2: Features considered diagnostic for *Chrysaora* sp. X, *C. fulgida* and *C. africana*, which allows ready identification in the field.

Features	***C. africana***	***C. fulgida***	***Chrysaora* sp. X**
Bell colour	transluscent-white with purple stripes	orange-brown	purple
Bell pattern	star-shaped	sometimes star-shaped	always star-shaped
Oral arms	transluscent white	orange to deep red	purple
Number of lappets	48	32	32
Number of rhopalia	8	8	8
Tentacle shape	cylindrical	cylindrical	laterally flattened
Tentacle colour	purple		cream
Number of tentacles	40	24	24
Gonads	attached to periphery of of four rounded subgenital ostia	situated in central stomach, attached to subumbrellar surface	situated in central stomach, attached to subumbrellar surface

Appendix 3: The consensus sequence of internal transcribed spacer 1 (ITS1) amplified from 11 *Chrysaora* sp. X specimens collected from the southern coast of South Africa throughout 2012 - 2015. Variable nucleotide bases are indicated, if present.

TTACGGAAAGTTGGCTACACGCTATCAACCCTTGACTGACCGTCAAAGGTATGCG

AACCACTGTGAACCCGTGCCAATCTGTGTGAGGTGACCAGAAGCAGGCCGGACT

GGCAGGCTGCAGTCGCTGTTGTCTGTGCTTAAATGGCAGGCGTCTTCCTGTCGGC

CTCACATGGAGTTTTTTTAGTGTTTTATTTTTTCAAACTTGAAATTCATGAATTCA

TAATTATTGACACATTAGTGGCAATAGTTGGAAAAGAAAAAAGTCAGAG

Appendix 4: The consensus sequence of internal transcribed spacer 1 (ITS1) amplified from one *Chrysaora hysoscella* specimens collected from either from Dingle Bay (52º 6' 54'' N -10º 20' 27'' W) or Cork Harbour (51º49'33.6"N, 8º16'8.4"W), Ireland. Variable nucleotide bases are indicated, if present.

TCTGGTTTCGTAGGTGAACCTGCGGAAGGATCATTACCGAAAGTTGGCTACACGC

TATCAGCTACTTGACTTAGCCGTCAAAGCTATGCGAACCACTGTGAACCCGTATC

GATCTGTGTGAGGTGACCAGAAGCAGGCCGGACTGGCAGGCTGCAGTCGCTGTT

GTCTGTGCTTAAATGGCAGGCGTCTTCCTGTCGGCCTCACATGGAGTTGTTTTTTA

TTCTTGTATTTTTTCAAACTTGAAATTCATGAATTCATAATTATTGACAACATTCA

TTGTCGTCGATAGTTGGAAATGAAAAAAGTCAGAGACAACTTCTAAGGTGGATC

ACTCGGCTCGTGCGA

Appendix 5: The consensus sequence of internal transcribed spacer 1 (ITS1) from five *Chrysaora fulgida* specimens collected on the "Goby and Hake Cruise", conducted on the R. V. *G. O. Sars*, from the 31st of March to 11th of April 2008 off the Namibian coast (Utne Palm *et al*., unpublished data). Variable nucleotide bases are indicated, if present.

TCGCACGAGCCGAGTGATCCACCTTAGAAGTTGTCTCTGACTTTTTTCAT

TTCCAACTATTCACACTAATGTGTCAATAATTATGAATTCATGAATTTCA

AGTTTGAAAAAATATAACACTAAAAAAACTCCATGTGAGGCCGACAGG

AAGACGCCTGCCATTTAAGCACAGACAACAGCGACTGCAGTCTGCCAGT

CCGGCCTGCTTCTGGTCACCTCACACAGATTGGCACGGGTTCACAGTGG

TTCGCATACCTTTGACGGTCAGTCAAGGGTTGATAGCGTGTAGCCAACT

TTCGGTAATGATCCTTCCGCAGGTTCACCTACGAAACCA

Appendix 6: The consensus sequence of internal transcribed spacer 1 (ITS1) amplified from two *Chrysaora africana* specimens collected on the “Goby and Hake Cruise”, conducted on the R. V. *G. O. Sars*, from the 31st of March to 11th of April 2008 off the Namibian coast (Utne Palm *et al.*, unpublished data). Variables nucleotide bases are indicated, if present.

T(G)C(G)CC(T)C(G)C(T)G(A)ACCGAG(T)GAT(C)CCCCTTAGAAG(T)TGT(C)TTGGTT

T(C)TTGGTATTATGAATGAATGATACAATGTCTCACTCAATC(T)CAACTCATGAA

TTTGCAAAAAAGTTTGTAAAAACAAAACACAAAAAAACTCCATGTGAGGCCG

GCAGGAAAACGCCTGCCATTTGAGCCCAGACGCCTGTCTGTCTCCCCGAG

ACATGCACAGACTCTGACCACCTCACACAGATCGGTACGAGTTCACAGTG

TATTATTGCCGTGTCCTGCACGCCACAATAATCTCTACGTCTCGAAAGAAC

G(A)TA(T)G(T)A(T)C(C)T(C)T(C)T(C)CGG(T)A(A)TG(A)(T)CCTTCC(G)CAGGT(T)CC

CCT(A)CAAA(A)CAA

Appendix 7: The consensus sequence of cytochrome *c* oxidase subunit I (COI) amplified from two *Chrysaora* sp. X specimens collected from the southern coast of South Africa from 2012 – 2015. Variables nucleotide bases are indicated, if present.

TTTRTAYA TAATTTTTGG CCMGCTTTTT CTGCTATGAT TGGTACAGCH

TTTAGTATGA TTATAAGACT AGAACTATC- ----TGAGCC CAGGCTCAAT

GTTAGGGGAY GACCAAATTTTATAACGTAG TAGTAACTGC CCACGCTTTA

ATAATGATAT TCTTTTTTGT AATGCCTGTATTAATAGGGG GATTTGGAAA

CTGATTTGTT CCTTTATATA TAGGTAGTCC TGATATGGCT TTTCCAAGAT

TAAATAATAT AAGTTTTTGA CTTTTACCTC CAGCTCTTTT ATTATTATTA

GGGTCTTCTC TTATTGAACA AGGAGCAGGG AACTGGTTGA ACTATATATC

CACCCCTATCTGCTATTCAA GCTCATTCCG GAGGATCTGT TGATATGGCA

ATTTTTAGTC TACATTTAGCAGGAGCTTCC TCTATAATGG GTGCTATTAA

CTTTATTACC ACAATTCTTA ACATGAGAGCCCCTGGGATG ACAATGGATA

GAATACCTCT ATTTGTTTGA TCTGTACTTA TTACAACAATACTTCTACTT

CTATCACTTC CAGTATTAGC TGGAGCCATT ACCATGTTAT

TAACAGATCAGAAATTTTAA TACTTCTTTT TTTGACCCTG CTGGAGGGGG

AGATCCTATT TTATTCCAACATT

Appendix 8: The consensus sequence of cytochrome *c* oxidase subunit I (COI) amplified from two *Chrysaora fulgida* specimens collected on the “Goby and Hake Cruise”, conducted on the R. V. *G. O. Sars*, from the 31st of March to 11th of April 2008 off the Namibian coast (Utne Palm *et al.*, unpublished data). Variables nucleotide bases are indicated, if present.

CATAAAGATATTGGAACTTTATACATAATTTTTGGCGCTTTTTCTGCTATGATTGG

TACAGCCTTTAGTATGATTATAAGACTAGAGTTATCTGGCCCAGGCTCAATGTTA

GGGGATGACCAAATCTATAACGTAGTAGTAACTGCCCACGCTTTAATAATGATAT

TCTTTTTTGTAATGCCTGTATTAATAGGGGGATTTGGAAACTGATTTGTTCCTTTA

TACATAGGTAGTCCTGATATGGCTTTTCCAAGATTAAATAACATAAGTTTTTGAC

T(A)TTTACCTCCAGCTCTTTTACTATT(G)CTAGGGTCTTCTCTAATTGAACAAGGA

GCAGGTACTGGTTGAACTGTATATCCACCCCTATCTGCTATTCAAGCTCATTCCG

GAGGATCTGTTGATATGGCAATTTTTAGTCTACATTTAGCAGGAGCTTCCTCTATA

ATGGGTGCTATTAACTTTATTACCACAATTCTAAACATGAGAGCCCCTGGGATGA

CAATGGATAGAATACCTCTATTTGTTTGATCTGTACTTATTACAGCAATACTTCTA

CTT(C)CTATCACTTCCAGTATTAGCTGGGGCCATTAC(T)ATGTTATTAACAGACAG

AAATTTTAATACTTCTTTCTTTGATCCTGCTGGAGGGGGAGATCCTATTTTATTCC

AACATTTATTTTGATTTTTTGGTCACCC

Appendix 9: The consensus sequence of cytochrome *c* oxidase subunit I (COI) amplified from two *Chrysaora africana* specimens collected on the "Goby and Hake Cruise", conducted on the R. V. *G. O. Sars*, from the 31st of March to 11th of April 2008 off the Namibian coast (Utne Palm *et al.*, unpublished data). Variables nucleotide bases are indicated, if present.

TTAAACTTCAGGGTGACCAAAAAAATCAAAATAAGTGTTGAAATAAAGAT

GGGGTCTCCTCCCCCTGCGGGGTCGAAGAAGGAAGTATTAAAATTTCTAT

CTGTTAATAGCATTGTAATAGCTCCAGCTAAAACGGGAAGTGAAAGTAAT

AAAAGAATTGCCGTAATAAAAACTGACCATACGAAAAGAGGTATTCTATC

CATTGTCATTCCAGGAGCTCTCATATTAATAATAGTAGTAATAAAATTTAT

TGCTCCCATTATGGATGAAGCTCCAGCTAAATGGAGACTGAAGATTGCCA

T(A)ATCTACTGA(G)CCCCCTGAATGTGCTTGGACAGCTGCAAGTGGGGGTA

A(G)ATAGTTCAACCTGTTCCTGCTCCTTGCTC(T)ATAAGAGAAGATCCTAATA

AAAGAAGAAGAGCGGGAGGAAGAAGTCAAAAGCTTATATTATTTAATCT

AGGAAAAGCCATGTCAGGACTTCCTATATATAAAGGAACAAATCAGTTTC

CAAATCCCCCTATTAAAACAGGCATAACAAAAAAGAAAATCATTATTAAG

GCATGAGCAGTTACAACTACGTTGTAAATTTGGTCATCTCCTAGCATAGAC

CCCGGTCCAGATAGTTCTAATCTAATAATCATACTAAATGCTGTTCCTATC

AT(A)TG(C)C(G)A(T)G(A)AAAATGCTCCAAATA(T)(T)ATA(T)ATA(A)A(C)AGTTCC

AATATCTTTATGATTTGTTGACCAGTTTAA

Appendix 10: The consensus sequence of cytochrome *c* oxidase subunit I (COI) amplified from one *Chrysaora hysoscella* specimen collected from either from Dingle Bay (52º 6' 54" N -10º 20' 27" W), Ireland. Variable nucleotide bases are indicated, if present.

TCTCTATTTAGTATTCGGAGCGTTTTCAGGAATGGTAGGTACAGCTTTAAGTATGT

TAATAAGATTAGAACTAGCAGGGCCAGGAGCCATGTTTGGTGATGATCATTTATA

CAATGTTATAGTTACAGCCCATGCCCTTATCATGATTTTTTTCTTAGTTATGCCTG

TTTTGATTGGAGGTTTCGGTAATTGATTTATTCCTTTATATATTGGAGCACCTGAT

ATGGCATTCCCAAGACTTAATAATTTAAGTTTTTGATTATTACCTCCCGCGTTGTT

CTTATTATTGGGTTCCTCTTTAATCGAACAAGGAGCAGGTACAGGATGAACCATG

TATCCTCCGTTATCTGGGCCTCAAACCCATTCTGGAGGTTCTGTTGATTTAGCTAT

ATTTAGTTTACATTGTGCGGGTGCCTCCTCCATTATGGGGGCTATTAATTTTATTA

CTACTATCTTAAATATGAGAGCCCCAGGAATGACAATGGACAAAATCCCTCTGTT

TGTTTGGTCCGTTTTAATTACAGCTATCTTATTACTATTATCTTTACCTGTATTAGC

AGGGGCGATTACGATGTTATTGACAGACAGAAATTTTAATACTACATTCTTTGAA

CCCCCAAGGGGGAG

Appendix 11: Means Measures of various features of the rhopalia, ephyrae and polyps of *Chrysaora* sp. X collected around the southern coast of South Africa throughout 2012 – 2015 and *C. fulgida* and *C. africana,* collected on the "Goby and Hake Cruise", conducted on the R.V. *G. O. Sars*, from the 31st of March to 11th of April 2008 off the Namibian coast (Utne Palm *et al.*, unpublished data).

	Rhopalia		
	Chrysaora sp. X	*C. fulgida*	*C. africana*
Length of rhopalar canal (mm)	5.1	5.7	6
Length of hood (mm)	5.5	1.5	2.3
Length of statocyst (mm)	2.2	2.75	1.78
Length of basal stem (mm)	2.45	3.2	4.76
	Ephyrae		
No. of lappet stems (mm)	8	8	
No. of lappets (mm)	16	16	
Lappet length (mm)	0.4	0.3	
Stem length (mm)	0.85	0.6	
Manubrium length (mm)	0.48	0.7	
Mouth diameter (mm)	0.63	0.38	
Length of rhopalar canal (mm)	0.38	0.33	
Oral disk diameter (mm)	0.75	0.93	
Diameter of ephyrae (mm)	2.35	2.1	
	Polyp		
Overall height (mm)	3.5	4.3	
Diameter of oral disk (mm)	1.82	2.5	
No. of tentacles (mm)	16	16	
Tentacle length (mm)	16.23	15.83	

Section B: The identification of a South African *Rhizostoma* (Scyphozoa: Rhizostomeae).

Abstract

The genus *Rhizostoma* is comprised of three valid species: *R. pulmo* (Macri 1778), *R. octopus* (Linnaeus 1788) and, *R. luteum* (Quoy & Gaimard 1827). Extensive blooming characterizes the genus *Rhizostoma* however *R. luteum* is the exception. Two species of *Rhizostoma*, *R. luteum* and *R. pulmo*, have been identified from around South Africa using nuclear (ITS1) and mitochondrial (COI) gene markers as well as more traditional qualitative and quantitative methods. The parsimony and Bayesian analyses however failed to confidently separate these species. Thus a complete revision of this genus is required. The morphological identification of *R. pulmo* was dependent on museum specimens at the South African Natural History Museum which suggested either temporal or spatial separation of these two South African morphotypes.

Keywords: Barrel jellyfish, COI, Ecosystem impact, Morphological analysis, Taxonomy

1. Introduction

The sub-order Rhizostomeae, Cuvier 1800, is characterized by: a) the presence of oral arms bearing suctoral mouths; b) oral arms that occur in four groups of two and are fused proximally, and c) the absence of tentacles around the bell margin (Kramp 1961a; Russell 1970). The genus *Rhizostoma* is further differentiated by the presence of a terminal, club shaped appendage at the end of each oral arm and the presence of scapulets (Kramp 1961a). The genus is comprised of three valid species: *R. octopus* (Linnaeus 1788); *R. pulmo* (Macri 1778) and *R. luteum* (Quoy & Gaimard 1827). *Rhizostoma octopus* was originally described from north-west Europe and is the only *Rhizostoma* occurring in the Southern North Seas and off the western and southern coasts of the British Isles (Russell 1970), while *R. pulmo* is known to occur within the Mediterranean and surrounding seas (Russell 1970; Ramšak *et al.* 2012). *Rhizostoma luteum* is known from the coast of Portugal (Stiasny 1931), the straits of Gibralter and the west coast of Africa (Quoy & Gaimard 1827).

The above mentioned *Rhizostoma* have few morphological characters that distinguish them making accurate identification difficult, a feature that appears common in jellyfish taxonomy. *Rhizostoma pulmo* and *R. octopus* however, can be separated by the number of velar lappets occurring within each octant, with *R. pulmo* having eight lappets per octant and *R. octopus* having 10 or more lappets per octant when mature (Macri 1778; Linnaeus 1788; reviewed by Russell 1970). *Rhizostoma luteum* can be separated from both *R. octopus* and *R. pulmo* by their size, rarely growing beyond 60 cm in diameter while both *R. octopus* and *R. pulmo* grow much bigger, frequently having diameters of up to 90 cm. The terminal appendages of *R. luteum* appear as long, thin stalks as opposed to the typical club shape observed within the other two species (Kramp 1961a). The taxonomic status of *R. luteum* is, however, uncertain with many authors rejecting the validity of this species (reviewed by Russell 1970). As a result no records of *R. luteum* have been published within the past 50 – 60 years, apart from a very recent account by Prieto *et al.* (2013).

Studies that have investigated patterns of jellyfish abundance within the Irish Seas (Graham *et al*. 2003; Houghton *et al*. 2007) have revealed species specific patterns of distribution (Doyle *et al*. 2007). *Rhizostoma octopus* (commonly known as the barrel jellyfish) is typically found in shallow embayments, in large aggregations (Doyle *et al*. 2007). These bays experience considerable variation in water depth, containment, tidal flow, temperature and salinity and trophic conditions (Lucas 2001). All bay areas within the Mediterranean Sea, where *R. octopus* is known to bloom receive appreciable fresh water and nutrient input from rivers (Lilley *et al*. 2009). Pérez-Ruzafa *et al*. (2005) suggested that often nutrient loads have a larger effect on blooms than trophic or hydrographic variables.

Rhizostoma blooms are widespread across Europe and *R. pulmo* is known to bloom extensively within European seas (Lilley *et al*. 2009). The extent of the blooms formed by these rhizostomes, coupled with their large size, suggests them to exert great top-down pressure on their ecosystems. Holst *et al*. (2007) determined that *R. octopus* polyps strobilate when temperatures are lowered from 15˚C to 10˚C or increased from either 5˚C to 10˚C or from 10˚C to 15˚C, while Pérez-Ruzafa (1997), suggest *R. pulmo* polyps to strobilate at warmer temperatures than that of *R. octopus*. The extensive *Rhizostoma* blooms experienced across many of the European seas throughout 2003 reflect this, as this was an exceptionally warm year across Europe. Many *R. pulmo* strandings were recorded from European coasts, from Tuscany, Italy and from Western France, during this year (reviewed by Lilley *et al*. 2009), suggesting that warmer waters potentially lead to increased abundance of *R. pulmo*.

Little ecological information exists regarding *R. luteum* due to its historical taxonomic confusion and rare status. As a result a need exists for further analysis investigating the underlying conditions resulting in blooms for these species and may also indicate where blooms of *R. octopus* and *R. pulmo* may be expected in the future. However, before any of these investigations can be conducted, it first becomes important to determine where *R. octopus* and *R. pulmo* individuals occur. Correct identification of these species is thus vital in managing their ecological impacts.

Traditional scyphozoan descriptions make use of only a few descriptive and qualitative characters (Bolton & Graham 2004; Dawson 2005a), which has resulted in a highly confused jellyfish taxonomy (Bolton & Graham 2004). Due to the incomplete morphological descriptions that dominate scyphozoan taxonomy, many species have been misidentified (Gershwin & Collins 2002; Bayha *et al*. 2010), synonymized with others (Stiasny 1939; Gohar & Eisawy 1960) or have been completely overlooked (Gohar & Eisawy 1960; Dawson & Jacobs 2001; Dawson 2003). Holst & Jarms (2006) suggested that detailed morphological and molecular analysis be performed on *Rhizostoma*, as is being done on multiple other genera, in order to resolve the relationships among *Rhizostoma* from different geographic areas.

A *Rhizostoma* bearing a strong resemblance and appearing, at least superficially, to be *R. octopus*, is widespread along the South African coast (a coastal system possessing numerous large embayment areas and river mouths bringing nutrient rich waters). This species occurs along the west coast and extends around the southern coast, into the warmer waters of East London along the east coast (Mpohlo 2014). They appear to be evident in the water column throughout the year but are more abundant in spring and autumn months, typical of *R.*

octopus populations in other parts of the world (Holst & Jarms 2006; Houghton *et. al.* 2007). Although the *Rhizostoma* occurring around the South African coast is seen throughout the year, no study has provided a detailed description of its morphology. In addition, virtually no modern descriptions of *Rhizostoma* have been published. This study thus aims to provide a detailed description of this species based on modern morphological and molecular frameworks in order to aid in the resolution of *Rhizostoma* relationships and to limit misidentification as phenotypic plasticity is common within this genus (reviewed by Russell 1970).

2. Materials and Methods

2.1 Specimen Collection

A total of nine specimens of *Rhizostoma* (hereafter referred to as *Rhizostoma* sp. 1) were collected during March 2014 at the locations specified in Table 1. Upon collection specimens were stored in 5 % formalin in ambient seawater and kept at room temperature (25˚C) until analysis. The current study did not correct nor account for any preservation effects as all measurements were taken at least 90 days after preservation and it was thus assumed that the preservation effects had stabilized (da Lafontaine & Legget 1989; Thibault-Botha & Bowen 2004; Lucas 2009). Vernier callipers were used to take all measurements (variables summarized in Table 2 & Figure 1 a − b where possible) under a magnifying glass. A single specimen of *Rhizostoma* (hereafter referred to as *Rhizostoma* sp. 2) was analyzed at the South African Natural History Museum (MB-A088462), which was also collected along the southern coast (-34.588300, 21.166700) of South Africa during 1994. Unfortunately the specimen had to be examined non-destructively and as such no genetic material could be obtained, however, five specimens of this *Rhizostoma* were collected at Hermanus, along the South-western coast of South Africa during 2015, from which some genetic material was obtained. No morphological information could be obtained from these specimens. No comparative material for, *R. octopus* and *R. luteum* was available at the time of the investigation and to further exacerbate comparative issues, little to no robust statistical investigations have ever been performed on any of these *Rhizostoma* in previous years. As such comparisons had to be made using previous morphological descriptions (where available) and where possible, some measurements (outlined in Appendix 1), when provided as part of these descriptions. The descriptions of Macri (1778) and Mayer (1910) for *R. pulmo* and Russell (1970) for *R. octopus*, formed the basis of the literature used for comparative purposes, however they were supplemented where necessary with more recent descriptions. Comparative statistics were performed using only those measures which could be established across all three species, however descriptive statistics are still provided for all measures analysed for *Rhizostoma* sp. 1 (Appendix 2).

2.2 Morphological Data Analyses

Many morphological measures vary with animal size (Neethling 2010), and it is customary to account for this in any comparisons between species using ANCOVA (Miller & Chapman 2001). Such an approach not only requires a wide size range of study material, but also a consistency in the size ranges between species. This was not possible, and so in an attempt to reduce any size-linked bias to data, measures were standardized by dividing all measures by bell diameter (S1), following which all ratios were $\log_{10}$ transformed, which is a common transformation used within biological datasets (Clarke & Green 1988). Using SPSS 22 Ltd. 2015 (Statistical Package for Social Sciences), the transformed, standardized data, were tested for normality using the Shapiro-Wilk's test for homogeneity of variances. For variables that failed the test for normality, standardized measures only (not transformed) were tested for normality and used in subsequent analysis if normally distributed. Correlations between standardized measures and bell diameter were assessed using Pearson's r (Zar 1999), in order to determine any effects of individual size on measured variables. Variables for which correlations are not significant are summarized in Table 3.

In order to determine whether *Rhizostoma* sp. 1 and *Rhizostoma* sp. 2 were morphologically distinct from *R. octopus*, *R. pulmo* and *R. luteum*, two-tailed *t*-tests were performed for those variables that were normally distributed and the alpha levels were corrected for multiple comparisons using the Bonferroni adjustment in order to control for Type I errors (Quin & Keough 2002). Similarly, Mann-Whitney U tests were performed comparing *Rhizostoma* sp.

1 and *Rhizostoma* sp. 2 to *R.octopus*, *R. pulmo* and *R.luteum* in order to test for differences between those variables that failed the tests for normality (Zar 1999). All univariate statistics were considered significant at an alpha level equivalent 0.05 after correction.

A constrained ordination was also performed (Anderson *et al*. 2008) which makes use of an *a priori* hypothesis to produce a plot. The Canonical Analysis of Principle Co-ordinates (CAP) is a particularly useful constrained ordination procedure within biology and ecology as it allows any distance of dissimilarity to be used (Anderson *et al.* 2008). It is useful where the *priori* hypothesis concerns differences among groups. As such, using Primer 7.0.10 Ltd., the CAP routine was executed, which seeks a set of axes that best distinguishes amongst *a priori* group (Anderson *et al.* 2008): in this case species. This process generates numerous matrices and produces a set of canonical axes. The Canonical Discriminate Analysis, which is a subset of the Principle Co-ordinate Analysis (PCO) works by finding axes that best separate categories and is a means of visualising similarity of individual cases within a dataset (Anderson *et al.* 2008). Generally axes used are selected manually and is based on the quantity of variables in the original dataset, however, Anderson *et al.*'s (2008) "leave one out" procedure was used to determine PCO axes. This method was executed as the number of measured variables approached the number of specimens. The PCO axes which were determined were all independent of each other and orthonormal. An orthonormalised data matrix, which is based on codes for groups identified by a factor associated with the Euclidean Distance Matrix, runs parallel to this process. By relating the PCO axes to this data matrix, an additional matrix is created which yields canonical eigen values and eigenvectors which are then used to produce the CAP plot. The CAP axes were used to determine if the predefined groups were classified correctly. Furthermore, through a series of permutation tests, the CAP routine was used to test the null hypothesis of no dissimilarities amongst the positions of centroids among groups in a multivariate space (Anderson *et al.* 2008).

2.3 DNA Analyses

Oral arm tissue samples were used in the genetic analysis which was obtained from the specimens collected for morphological analysis before they were preserved in formalin. In addition, four juvenile *Rhizostoma* (*Rhizostoma* sp. 2) were collected (mentioned above) from which additional genetic material was obtained. Tissues were then stored in absolute ethanol at -18˚C until analysis.

DNA extractions, precipitations and quantifications were performed following a standard phenol-chloroform extraction (Wallace 1987, Sambrook & Russell 2001, Neethling 2010). Cytochrome *c* oxidase subunit I (COI) and the internal transcribed spacer one (ITS1) region were amplified using the primers outlined in Table 4. A maximum of 720 base pairs were amplified from four samples for the COI gene region using a ramp up cycle with reaction conditions: 94˚C for 8 min; then one cycle of 54.2˚C for 2 min, 72˚C for 2 min, 94˚C for 4 min; followed by one cycle of 55.2˚C for 2 min, 72˚C for 2 min, 94˚C for 45 sec; followed by 33 cycles of 56.2˚C for 45 sec, 72˚C for 1 min, followed by a final step of 72˚C for 5 min before storage at 4˚C. A maximum of 340 base pairs were amplified for four samples for the ITS1 gene region using a ramp up cycle with reaction conditions: 94˚C for 8 min; then one cycle of 51.5˚C for 2 min, 72˚C for 2 min, 94˚C for 4 min; followed by one cycle of 52.5˚C for 2 min, 72˚C for 2 min, 94˚C for 45 sec; followed by 33 cycles of 53.5˚C for 45 sec, 72˚C for 1 min, followed by a final step of 72˚C for 5min before storage at 4˚C. All PCR reaction were carried out using 25 μL reaction volumes on a Techne ® endurance TC-512 gradient thermal cycler (Barloworld Scientific). Two micro litres of the PCR product was visualized on a 7 % agarose gel, following which the remainder was then purified with a nucleoFast 96 PCR kit (Macherer-Nagel). Using BigDye chemistry, the cleaned products were then cycle sequenced and analysed with an ABI 3730 XL DNA Analyzer (Applied Biosystems Inc.) at the Central Analytical Facility (University of Stellenbosch).

Misreads within sequences were corrected and poorly determined terminal portions discarded using BioEdit 7.2 (Hall 2005). This was done by visually checking the sequences and chromatograms. Using the ClustalW alignment tool in BioEdit 7.2 (Hall 2005), the forward and reverse sequences were aligned and used to create consensus sequences. The consensus sequence was then verified using Blast in Genbank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Additional sequences for *R. pulmo*, *R. octopus* and *R. luteum* were obtained from Genbank where possible for comparative purposes (accession numbers represented in Table 5)

Mean pairwise sequence differences using uncorrected "*P*", as well as maximum parsimony trees were then calculated in PAUP* 10.4b (Swofford 2001), for both CO1 and ITS1 sequences. The tree was calculated by performing a heuristic parsimony analyses, using a branch swapping algorithm, also known as the tree-bisection-reconnection method, with all characters assigned an equal weight and left unordered. A bootstrap procedure was then performed in order to test the stability of the nodes, using 1000 resampling replicates and the tree-bisection-reconnection method. Only bootstrap values above 75 % were considered to be well supported and retained in the final tree (Felsentein 1985).

The Akaike information criterion was used in the programme JModeltest v. 2.1.2 (Darriba *et. al.* 2012) in order to determine the "best fit model of evolution" for both gene fragments (Akaike 1973; Nylander 2004). Bayesian analyses were then performed in Mr.Bayes v. 3.2

(Ronquist & Hulsenbeck 2003), using this model as a guide and included five paralleled Monte Carlo Markov chains. The resultant chains were sampled every 1 000th generation and a total of 12 million generations were used. In order to determine statistical stationarity, the "sump" command in Mr. Bayes was used to summarise the generated samples. Based on these results, 25 % were discarded as burn-in. To assess whether the data were adequately sampled, the potential scale reduction factor was determined (PSRF) (Ramhaut & Drummond 2007). Following this the "sumt" command was executed in Mr. Bayes in order to summarize the trees. These trees were then visualized using the programme Fig tree v. 1.4.2 (http://tree.bio.edu.ac.uk/software/gtree) and those nodes with posterior probabilities $p < 0.95$ were considered not significantly supported.

Due to the general lack of ITS1 data available for *Rhizostoma* on Genbank, the COI and ITS1 datasets were concatenated and analysed using Parsimony and Bayesian analyses as described above, in a partitioned fashion for both gene fragments (ITS1 and COI). For the concatenated dataset 212 characters were determined to be parsimony informative and 356 were considered uninformative. Here the Bayesian analyses were run for 22 million generations and the tree again visualized using FigTree v. 1.4.2. In order to incorporate some population level processes, higher level gene clustering was assessed using splits tree v. 4.5 (Huson & Bryant 2006). Splits tree employs the Neighbour-net algorithm, which essentially uses a distance matrix as an input and works by agglomerating clusters (Bryant & Moulton 2004). This can however result in overlapping clusters which do not form a clear hierarchy (Bryant & Moulton 2004). These clusters are then represented by a phylogenetic network known as a splits network, which essentially represents character change distances. As such, uncorrected *P* distances were used to draw a neighbour network for each gene (Bryant & Moulton 2004), using equal angle splits to present the relationships (Dress & Huson 2004).

3. Descriptions

3.1 *Rhizostoma* sp. 1

SYSTEMATICS

ORDER Discomedusae Haeckel, 1880

SUBORDER: Dactyliophorae Stiasny, 1920

FAMILY: Rhizostomatidae Cuvier, 1800

SPECIES *Rhizostoma luteum* (Quoy & Gaimard, 1827)

(FIGURES: 2 a – c & 3 a – d)

Orithyia lutea: Quoy & Gaimard 1827; Eschscholtz 1829.

Pilema stylonectes: Haeckel 1880.

Rhizostoma luteum: Vanhöffen 1888; Stiasny 1921 (mentioned as a valid species); Stiasny 1931; Kramp 1955 (mentioned from the West coast of Africa).

R. pulmo var. lutea: Mayer 1910.

Holotype specimen: Not available.

EXAMINED MATERIAL: Two specimens from Still Bay (34.3642° S, 21.4336° E) collected on 17 March 2014; one specimen from Jeferry's Bay (34.0507° S, 24.9102° E) collected 19 March 2014; one specimen from Breede River (34.4° S, 20.8333° E) collected 21 march 2014; three specimens from Wilderness (33.997316° S, 22.568579° E) collected 25 March 2014 and two specimens from Bluewater Bay (33.85822° S, 25.63476° E) collected 30 March 2014. All localities occur along the southern coast of South Africa.

Type locality. Portugal shoreline.

Distribution. Found along the Atlantic coasts of Spain, along the Portugal shoreline, through the Strait of Gibraltar into the western Mediterranean and down the northwest coast of Africa.

Diagnosis. Scapulate Rhizostomeae with small epaulettes on base of manubrium; single terminal club on each oral arm which forms a long thin stalk; subgenital papillae in the form of thickened valves on the edges of subgenital pits; eight marginal lappets per octant.

Original specimen description (after Quoy & Gaimard 1827). Umbrella hemispherical; pale grey and thickened centrally; eight rhopalia divide bell into octants. Bell margin cleft into 80 lappets; ten per octant; all with short, oval shape. No marginal tentacles. Stomach square shaped, occupying approximately one third of bell. From the stomach extend 16 canals that connect to bell edge where a broad ring canal is situated approximately one third of radius from bell margin. Short manubrium bears 16 scapulets with numerous mouthlets and eight oral arms that are dark grey in parts furthest from manubrium. Arms with numerous mouthlets and long terminal appendage extending from oral arms. Terminal appendage longer than oral arm, and occurs in the shape of a long, thin basal stalk.

Description of other specimens and additional data. Bell diameter ranged from 40.7 cm to 72 cm bell. Umbrella dome shaped and thickened centrally; mesoglea thick and solid throughout bell; exumbrella rough with raised oval nematocyst warts, giving it a matte appearance in preservation (Figure 2 a); umbrella usually translucent white with light blue or purple hue (Figure 2 a). Eight rhopalia divide bell into eight octants; rhopalia protected by thickening of exumbrella which extends from umbrella margin; within which sits deep exumbrella sensory pit, forming shape of elongated cone; umbrella margin distal to sensory pit forms hood of rhopalium; sensory pit covered in epithelial cells which appear to form furrows or micro canals in mesoglea surrounding pit. Rhopalium consists of basal stalk and statocyst, with no ocelli. Marginal tentacles absent. Umbrella margin cleft into 64 lappets; eight per octant (Figure 2 a – b): two rhopalial and six velar; rhopalial lappets larger than velar lappets, all with short oval shape. Lappets translucent white with network of vessels/canals. Well-developed coronal muscle situated on subumbrella margin, form multiple folds (Figure 2 c), which extend to base of lappets. The four sided, square shaped stomach is situated in centre of subumbrella, with concave sides; 16 radial canals extend from periphery of stomach to umbrella margin (eight adradial, four perradial and four interradial); 16 radial canals join primary ring canal situated around bell margin (one third of radius inward from margin) but may be discontinuous in larger specimens. Manubrium extends from stomach, forms solid oral, tube-like structure; manubrium thick at base and short, possessing 16 epaulettes, grouped in eight pairs of two (Figure 3 a). Epaulettes three winged; with a leaf like structure, which divides into two similar structures; highly folded mouthlets cover edges of epaulette wings. Eight oral arms extend from manubrium directly below epaulettes, arranged in four pairs; with three wings each (one adaxial and two abaxial) containing mouthlets along wing edges (Figure 3 b). Three winged portion of oral arm followed by basal portion bearing no mouthlets, known as terminal club; terminal club longer than length of oral arms, translucent for two thirds, thereafter becoming pigmented (Figure 3 c). Epaulettes and oral arms all translucent cream. Terminal clubs grows proportionately in length as animal size increases, looking more like a filament. The subumbrella thickened interradially forming subgenital pits which extends to base of stomach; a bean shaped protuberance (Figure 3 d) extends from subumbrellar wall in each subgenital pit. Possesses four interradial gonads, situated above stomach; highly convoluted forming circle around subumbrella; gonads light brown.

Variation. Numerous specimens displayed variation in the colour of the bell, being more translucent white or grey.

Distribution around South Africa: Known to span across entire coast of South Africa.

Biological data: Species has a historically rare status, however blooms frequently throughout the year along the southern coast of South Africa, particularly during spring and autumn months.

3.2 *Rhizostoma* sp. 2

SPECIES *Rhizostoma pulmo* (Macri, 1778)

(FIGURE: 4 a - e)

Medusa (*Rhyzostoma*) *pulmo*: Macri 1778 (description); Forbes 1848 (description); Agassiz 1862.

Pilema pulmo: Haeckel 1880.

Rhyzostoma octopus: Stiasny 1927 (identified juvenile medusae as *R. octopus*); Stiasny 1928 (brief description).

Rhizostoma pulmo var. *octopus*: Mayer 1910 (description; later distinguished *R. octopus* to be a separate species); Kramp 1961 (description); Russell 1970 (description).

Rhizostoma pulmo: Conway 2012 (mention).

Holotype specimen: Not available

EXAMINED MATERIAL: One specimen of *Rhizostoma pulmo* examined at the South African Natural History Museum (MB-A088462), collected along the southern coast of South Africa (-34.588300, 21.166700) on 29 September 1994.

Type locality. Mediterranean Sea.

Distribution. Found in northeast Atlantic, Adriatic, Mediterranean Sea and Black Sea (Mayer 1910). Also known from the southern Atlantic off the western South African coast and into False Bay (Branch *et al.* 2010).

Diagnosis: Scapulate Rhizostomeae with small epaulettes on base of manubrium; single terminal club on each oral arm possessing characteristic club-like shape; subgenital papillae in the form of thickened valves on the edges of subgenital pits; typically ten marginal lappets per octant.

Original specimen description (after Macri 1778). Umbrella hemispherical, pale grey, with jelly thickened centrally. Bell divided into octants by eight marginal rhopalia. Eight lappets per octant; two rhopalial and six velar; rhopalial lappets triangular and velar lappets rounded. No marginal tentacles. Stomach square shaped, occupying third of bell. From the stomach extend 16 canals that connecting bell edge. A broad ring canal, situated approximately one third of radius from bell margin, gives rise to coarse, irregular anastomosing network of canals. This network does not connect with radial canals. Fine meshwork of canals on periphery of intermediate ring canal, becoming increasingly fine toward perimeter of bell. Short manubrium bears 16 scapulets with numerous mouthlets. Eight oral arms extend from manubrium also with numerous mouthlets and a translucent terminal club. Four convoluted gonads form a circle around bell.

Description of other specimens and additional data. Umbrella 15 cm in diameter; dome shaped and thickened centrally; mesoglea thick and solid throughout bell; exumbrella finely granular with raised nematocyst warts, giving umbrella matte appearance in preservation (Figure 9); umbrella translucent white (Figure 4 a). Eight rhopalia divide bell into eight octants; rhopalia protected by thickening of exumbrella which extends from umbrella margin, over the rhopalium; within which sits a deep exumbrella sensory pit, forming shape of elongated cone; umbrella margin distal to sensory pit forms hood of rhopalium; sensory pit covered in epithelial cells which appear to form furrows or micro canals in mesoglea surrounding pit. Rhopalium consists of basal stalk and statocyst, with no ocelli. Marginal tentacles absent. Umbrella margin cleft into 80 lappets, ten per octant: two triangular rhopalial lappets and eight rounded velar lappets (Figure 4 b); lappets pigmented on upper and lower surface. Well-developed coronal muscle situated on subumbrella margin, forming multiple folds around margin (Figure 4 c), extending to base of velar lappets. Four sided, square shaped stomach is situated in centre of subumbrella, with concave sides; 16 radial canals extend from periphery of stomach to umbrella margin (eight adradial, four perradial and four interradial); 16 radial canals join primary ring canal situated around bell margin (one third of radius inward from margin). Fine network of anastomosing canals occur between ring canal and umbrella margin, connecting radial and main ring canals (Figure 4 d). Manubrium extends from stomach, forms solid oral, tube-like structure; manubrium thick at base and short, possessing 16 epaulettes, grouped in eight pairs of two. Epaulettes three winged; with oval leaf like structure, which divides into two similar structures; highly folded mouthlets cover edges of epaulette wings. Eight oral arms extend from manubrium directly below epaulettes, arranged in four pairs; consisting of three wings (one adaxial and two abaxial), with folded mouthlets occurring along wing edges. Three winged portion of oral arm followed by a basal portion bearing no mouthlets, known as terminal club; terminal club possessing characteristic club-like shape (Figure 4 e), entire length of oral arm and terminal club roughly equals bell diameter. Epaulettes and oral arms all brown in colour. Subumbrella thickened interradially forming subgenital pits which extends to base of stomach. Mesoglea of stomach forms roof of pit and thickened into knob-like shape. Possesses four interradial gonads, situated above stomach are highly convoluted forming circle around subumbrella; gonads light pink in colour.

Biological data: None available.

4. Remarks

It is well known that the genus *Rhizostoma* has suffered much confusion and that misidentification is widespread due to the close resemblance of all three species occurring within this genus. Unfortunately, a robust statistical analysis was not possible due to the relative lack of measures available for *R. pulmo* and *R. octopus*, coupled with the complete lack of any available measures for *R. luteum*. However, using the available data (although limited), some statistical tests could be performed to illustrate some basic differences in the morphology of the aforementioned species. The results of the Mann-Whitney U test indicated that no statistical differences existed between any of the measures compared between *Rhizostoma* sp. 1, *Rhizostoma* sp. 2, *R. pulmo* and *R. octopus* (Table 6). This could be reflective of the difficulty which exists when making accurate measurements on these medusae. Thiel (1965) outlined some of these difficulties. Thiel (1965) pointed out that deciding on the actual boundaries between various parts of the manubrium is extremely difficult and subjective. Thiel (1965) also commented on the high degree of abnormality occurring within the various proportions of the manubrium within this genus, creating further difficulties with regards to accurate measurements.

A total of five variables were common across the *Rhizostoma* (variables for which at least one measurement could be found), resulting in an unreliable ordination plot for CAP analyses (Figure 5). The CAP analyses (Table 7 & Figure 5), showed clear dissimilarity on the CAP 1 axes and a high squared canonical correlation ($\delta^2 = 0.9592$, $p < 0.05$; Table 6). The mis-classification error was fairly low at 9. 09 %. These results only indicate some dissimilarity between *R. pulmo*, *R. octopus* and *Rhizostoma* sp. 1, however more complete sampling across the genus, and subsequent robust statistical analyses would need to be performed to confirm this dissimilarity.

The results of the genetics however, provided more clarity on the identities of the South African *Rhizostoma*. The cytochrome *c* oxidase subunit I (COI) DNA analysis indicated a low mean pairwise sequence difference of only 2.1 % between *Rhizostoma* sp. 1 and *R. luteum* , a 12 % mean pairwise sequence difference between *Rhizostoma* sp. 1 and *R. octopus*, and a 9.31 % mean pairwise sequence difference between *Rhizostoma* sp. 1 and *R. pulmo* (Table 8 & Figures 6 – 7). Furthermore a 5 % mean pairwise sequence difference occurred between *R. octopus* and *R. pulmo* while a 0.5 % mean pairwise difference occurred between *R. pulmo* and *Rhizostoma* sp. 2. Dawson & Jacobs (2001) suggest that differences of 10 % – 20 % between COI sequences set the standard for species level divergence, thus indicating *Rhizostoma* sp. 1 to be *R. luteum* and *Rhizostoma* sp. 2 to be *R. pulmo*. It also questions whether *R. pulmo* and *R. octopus* should be designated as two distinct species or whether they are in fact one species.

Although the COI indicates *Rhizostoma* sp. 1 to be *R. luteum*, the results of the ITS1 analysis contradicts this. DNA sequence data from the ITS1, showed an average of 3 % mean pairwise sequence differences between *Rhizostoma* sp. 1 and *R. pulmo* while *Rhizostoma* sp. 2 showed an average of 0.2 % differences (Table 9, Figures 8 – 9). These results fall below the 5 % – 15 % threshold as indicated by Dawson & Jacobs (2001), indicating *Rhizostoma* sp. 1 and *Rhizostoma* sp. 2 are both *R. pulmo*. The lack of ITS1 sequences available for both *R. luteum* and *R. octopus* on Genbank prevents the resolving of a stable ITS1 phylogeny for these species. It is however common for jellyfish to retain ancestral polymorphisms in their nuclear DNA which could provide an explanation for these ambiguous results. Other genetic markers, such as Ribosomal DNA, should thus be used for a more accurate resolution of the taxonomy of these species.

Due to the ambiguity which resulted from the individual gene regions, a concatenated dataset was also analysed. The results of the parsimony and Bayesian analyses once again showed *Rhizostoma* sp. 1 to form a clade with *R. luteum*, while *R. pulmo* formed a clade with *R. octopus* and *Rhizostoma* sp. 2 (Figure 10). This provided some further confidence in the identifications of *Rhizostom*a sp.1 and *Rhizostoma* sp. 2, however resulted in further uncertainty in the sequences present on Genbank for *R. octopus*.

In order to provide some further validity to these identifications, a qualitative comparison was conducted using the available descriptions for *R. luteum* and *R. pulmo*. Based on this analysis, *Rhizostoma* sp. 1 possesses features most similar to *R. luteum* (Appendix 3). Some of these features included the presence of eight, transparent marginal lappets, a relatively short manubrium, and a long, thin basal appendage at the end of each oral arm. Based on the qualitative analyses, and coupled with the low genetic variability observed between this species and the *R. luteum* sequences obtained from Genbank, we can confidently identify this South African species as the rare *R. luteum*. *Rhizostoma* sp. 2 however differed to *Rhizostoma* sp. 1 in many of its features for example: the presence of anastomosing canals found across the subumbrellar, the presence of two additional velar lappets (thus ten lappets) that are pigmented and the presence of a short terminal club, suggesting this specimen to be more similar to *R. pulmo*. The low mean pairwise sequence differences between the COI and ITS1 gene regions of these two species provide further support for this identification.

This study thus alludes to the presence of two South African *Rhizostoma*: *R. luteum* and *R. pulmo*, however this is somewhat inconclusive. The only means by which to validate this would be to conduct a full revision of this genus, in which real specimens could be analyzed for each species both in their morphometrics and their DNA. A large debate has surrounded the number of valid species occurring within the genus *Rhizostoma*, with the status of *R. luteum* being highly questioned until recently. *R. luteum* was not considered to be a valid species by many taxonomists (reviewed by Russell 1970) and often considered a variation of *R. pulmo* (Mayer 1910) as was *R. octopus* (reviewed by Russell 1970). *R. luteum* was however suggested to be a distinct and valid species as early as 1921 by Stiasny, while being confirmed as a valid species in 2013 by Prieto *et al*. Similarly Russell (1970) suggested *R. octopus* be treated as being separate to *R. pulmo*, however the current study questions this distinction.

Rhizostoma luteum is a rarely seen scyphozoan jellyfish. It was first described in 1827, and sightings were occasionally recorded up until 1955 but in the 60 years since then none were seen until two stranding events in 2012 and 2013, which washed up approximately 75 individuals, mostly on beaches along the southern coast of the Iberian Penninsula (Prieto *et al.* 2013). Both stranding events occurred following a water temperature decline of approximately 2˚C, however the reason for the sudden occurrences of these jellyfish is not known (Prieto *et al.* 2013). If these mass strandings are related to the temperature decline experienced during those years, these jellyfish could have the potential to bloom extensively within South African waters, as the waters within the southern Benguela, occuring along the south western coast of South Africa have been experiencing a decline in temperature. *R. pulmo* is also commonly known for its mass occurrences within the Mediterranean and surrounding seas (Fuentes *et al.* 2011; Lilley *et al.* 2011), and have the potential to produce these same blooms within South African waters due to the dynamic nature of this environment. This could have a devastating impact on the ecosystems surrounding South Africa.

The proliferation of jellyfish has been known to hamper the recruitment of pelagic fishes (Möller 1984) and in recent years, many scientists believe the rapid decline in global fishes to be attributed to increased jellyfish populations (Purcell *et al.* 1999). The South African *Rhizostoma*, could have far reaching impacts on our coastal ecosystems. This study has highlighted once more, the troubles surrounding jellyfish taxonomy, and while providing valuable information on these South African *Rhizostoma*, has not confidently determined their identities. Based on the results of the present study, the identity of not only these species but all three *Rhizostoma*, will require a complete revision both statistically and in terms of their DNA. Only then will we be in a position to determine what these *Rhizostoma* truly are.

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Table 1: Localities with their corresponding GPS co-ordinates for *Rhizostoma* sp. 1 specimens, collected around the southern coast of South Africa during March 2015, investigated in this study.

Location	GPS co-ordinates
Still Bay	34.3642° S, 21.4336° E
Jeffery's Bay	34.0507° S, 24.9102° E
Breede River	34.4° S, 20.8333° E
Wilderness	33.997316° S, 22.568579° E
Bluewater Bay	33.85822° S, 25.63476° E

Table 2: Morphological features observed and measured for *Rhizostoma* sp. 1 collected around the southern coast of South Africa during March 2015. Material was preserved in 5 % formalin in ambient seawater upon collection.

Morphological feature (MF)	Morphological feature description (measured in cm)
S1	Maximum umbrella diameter
S2	Maximum umbrella height
S3	Minimum umbrella height
S4	Number of octants
S5	Number of lappets in octant (Rhopalial+velar)
S6	Velar lappet width
S7	Velar lappet length
S8	Rhopalial lappet width
S9	Rhopalial lappet length
S10	No. coronal muscle folds
S11	No. of rhopalia
S12	No. of radial canals
S13	Number of epaulettes
S14	Epaulette width
S15	Epaulette length
S16	Oral arm length
S17	Maximum width of oral arm
S18	Terminal club length
S19	Terminal club width
S20	Mouth diameter
S21	Presence of sperm sacs
S22	Mouth open/closed
S23	Subgenital papillae length
S24	Subgenital papillae width
S25	Manubrium length
S26	Manubrium depth
S27	Ostia length
S28	Ostia width
S29	Width between ostia

Table 3: Summary of the variables for which Pearson's r correlations were not significant. Bell diameter (raw data) was correlated against standardized measures for *Rhizostoma* sp. 1 collected around the southern coast of South Africa during 2015.

Variables	**Bell diameter**	***p***	***R²***	**N**
Rhopalial lappet length	-0.997	0.051	0.994	9
Rhopalial lappet width	-0.991	0.085	0.982	9
Epaulette width	-0.864	0.335	0.747	9
Epaulette length	-0.635	0.562	0.403	9
Oral arm length	-0.984	0.114	0.968	7
Oral arm width	0.994	0.069	0.988	7
Subgenital pit length	0.493	0.672	0.243	3
Manubrium total length	-0.425	0.721	0.181	7

Table 4: Forward and reverse primers used to amplify the gene regions cytochrome *c* oxidase subunit I (COI) and internal transcribed spacer 1 (ITS1) with their associated authors. These genetic markers were amplified from *Rhizostoma* sp. 1 specimens collected around the southern coast of South Africa during 2015 as well as *Rhizostoma* sp. 2 specimens collected at Hermanus along the south-western coast of South Africa during 2015.

Gene region	Primer name	Sequence	Author/s
Cytochrome *c* oxidase subunit I (COI)	LCOjf	5’-ggtcaacaaatcataaagatattggaac-3’	Dawson 2005c
	HCOcato	5’-ctccagcaggatcaaagaag-3’	Dawson 2005c
Internal transcribed spacer one (ITS1)	jfITS1-5f	5’-ggtttcgtaggtgaacctgcggaaggatc-3’	Dawson & Jacobs 2001
	jfITS1-3r	5’-cgcacgagccgagtgatccaccttagaag-3’	Dawson & Jacobs 2001

Table 5: Genbank accession numbers for various species of *Rhizostoma* used in the genetic analyses of this study.

Species	Genbank accession No.
COI	
Rhizostoma octopus	HQ425479.1
Rhizostoma octopus	HQ425478.1
Rhopilema esculentum	HF930515.1
Rhizostoma luteum	HF545309.1
Rhizostoma luteum	HF937340.1
Rhizostoma pulmo	GQ999568.1
Rhizostoma pulmo	GQ999569.1
Rhizostoma pulmo	GQ999571.1
ITS1	
Rhopilema esculentum	AB377589.1
Rhizostoma pulmo	HQ902083.1
Rhizostoma pulmo	HQ902085.1
Rhizostoma pulmo	HQ902081.1
Rhizostoma pulmo	HQ902076.1
Rhizostoma pulmo	HQ902075.1

Table 6: Results of the Mann-Whitney U test illustrating differences in the morphometric data common to both *Rhizostoma* sp. 1 and compared to those measures obtainable for *R. pulmo*, *R. octopus* and *Rhizostoma* sp. 2 analyzed at the South African Natural History Museum. (Results considered significant at $p \leq 0,0056$).

	Rhizostoma* sp. 1 vs. *R. pulmo			***Rhizostoma* sp. 1 vs. *R. octopus***			***Rhizostoma* sp. 1 vs. *Rhizostoma* sp. 2**		
MF	***U***	***Z***	**Sig.**	***U***	***Z***	**Sig.**	***U***	***Z***	**Sig.**
S1	3.00	-.522	.602	3.00	-.522	.602	3.00	-.522	.602
S2	-	-	-	-	-		3.00	-.512	.613
S3	-	-	-	-	-	-	3.00	-.502	.611
S5	3.00	-.387	.699	3.00	-.387	.699	3.00	-.387	.699
S7	3.00	-.522	.602	3.00	-.522	.602	3.00	-.522	.602
S14	-	-	-	-	-	-	2.00	-.765	.442
S15	-	-	-	-	-	-	3.00	-.562	.545
S16	2.00	-.775	.439	3.00	-.387	.699	3.00	-.387	.699
S17	0.00	-1.549	.121	0.00	-1.549	.121	0.00	-1.549	.121
S18	0.00	-1.342	.180	0.00	-1.342	.180	0.00	-1.342	.180
S19							0.00	-1.256	.201
S25	0.00	-1.500	.134	0.00	-1.500	.134	0.00	-1.500	.134
S26	-	-	-	-	-	-	0.00	-1.513	.119
S27	-	-	-	-	-	-	0.00	-1.333	.176
S28	-	-	-	-	-	-	0.00	-1.243	.202

Table 7: Canonical Analysis of Principal Co-ordinates (CAP) results for the standardized morphometric data showing number of individuals of *Rhizostoma* sp. 1, *R. pulmo* and *R. octopus* for those measures obtainable from previous descriptions and *Rhizostoma* sp. 2, assigned to each species. (Permutation test statistic reported; species significantly different at $p < 0,005$)

Original group	*Rhizostoma* sp. 1	*R. pulmo*	*R. octopus*	*Rhizostoma* sp. 2	Total	% correct	miss-classification	*p*
***Rhizostoma* sp. 1**	9	0	0	0	9	100	25 %	**0.004**
R. pulmo	0	1	0	0	1	100		
R. octopus	0	1	0	0	1	0		
***Rhizostoma* sp. 2**	0	0	0	1	1	100		

Table 8: Uncorrected mean pairwise distance matrix for cytochrome *c* oxidase subunit I (COI) marker amplified from specimens of *Rhizostoma* sp. 1 and *Rhizostoma* sp. 2. Comparative sequences for *R. luteum*, *R. pulmo* and *R. octopus* were obtained from genbank.

	R. luteum	***R. pulmo***	***Rhizostoma* sp. 1**	***R. octopus***	***Rhizostoma* sp. 2**
R. luteum	-				
R. pulmo	14.78	-			
***Rhizostoma* sp. 1**	3.67	15.03	-		
R. octopus	14.11	5.38	14.82	-	
***Rhizostoma* sp. 2**	0.155	0.016	0.157	0.053	-

Table 9: Uncorrected mean pairwise distance matrix for internal transcribed spacer 1 (ITS1) fragments amplified from specimens of *Rhizostoma* sp. 1 and *Rhizostoma* sp. 2. Comparative sequences for *R. pulmo* were obtained from genbank.

	R. pulmo	***Rhizostoma* sp. 1**	***Rhizostoma* sp. 2**
R. pulmo	-		
***Rhizostoma* sp. 1**	3.2	-	
***Rhizostoma* sp. 2**	0.005	0.034	-

Figure Headings

Figure 1: Schematic representation of a typical *Rhizostoma*, indicating (where possible) various morphological features analyzed during this study: a) subumbrellar view and b) cross section (side view).

Figure 2: Photographs illustrating various features of *Rhizostoma* sp. 1: a) exumbrellar surface, showing the external colouration and nematocyst warts on the bell, b) lappet shape and number and c) the orientation of the coronal muscle around the bell margin.

Figure 3: Photographs of *Rhizostoma* sp. 1 illustrating the a) epaulette orientation as it extends from the manubrium, b) the oral arms, c) the long trailing terminal clubs and d) the bean-shaped protuberance situated on the exumbrellar surface.

Figure 4: Photographs of of *Rhizostoma* sp. 2 examined at the South African Natural History Museum during 2016, indicating a) the exumbrellar surface, displaying bell colouration and raised nematocyst warts, b) lappet shape and number, c) orientation of the coronal muscle around the bell margin, d) anastomosing canals situated on the subumbrellar surface and e) distal portion of the oral arm with the terminal club-like appendage.

Figure 5: Canonical analysis of principle co-ordinates (CAP) plot, showing ordination of standardized morphometric data for *Rhizostoma* sp. 1, collected around the southern coast of South Africa during March 2015, *Rhizostoma* sp. 2 examined at the South African Natural History Museum during 2016 as well as *R. pulmo* and *R. octopus* where measures were obtainable.

Figure 6: Molecular phylogenetic analysis by maximum parsimony method for cytochrome *c* oxidase subunit I (COI) sequences of *Rhizostoma* sp. 1, collected around the southern coast of South Africa during March 2015, *Rhizostoma* sp. 2 collected at Hermanus Lagoon during 2015, as well as *R. luteum, R. pulmo* and *R. octopus* where sequences were obtainable from Genbank, for a maximum of 720 neucleotides. Strict consensus bootstrapped tree is shown here. Posterior probabilities are shown next to nodes, while bootstrap percentages are shown on branches.

Figure 7: Splits tree illustrating gene clusters between *Rhizostoma* sp. 1, , *Rhizostoma* sp. 2, as well as *R. luteum, R. pulmo* and *R. octopus* where sequences were obtainable from Genbank, for a maximum of 720 nucleotides, for cytochrome *c* oxidase subunit 1 (COI).

Figure 8: Molecular phylogenetic analysis by maximum parsimony method for internal transcribed spacer 1 (ITS1) sequences amplified from *Rhizostoma* sp. 1, *Rhizostoma* sp. 2 and *R. pulmo* where sequences were obtained from Genbank, for a maximum of 320 nucleotides. Strict consensus bootstrapped tree is shown here. Posterior probabilities are shown next to nodes, while bootstrap percentages are shown on branches.

Figure 9: Splits tree illustrating gene clusters between *Rhizostoma* sp. 1, , *Rhizostoma* sp. 2 and *R. pulmo* where sequences were obtained from Genbank, for the internal transcribed spacer 1 (ITS1) gene fragment in which a maximum of 320 nucleotides were amplified.

Figure 10: Molecular phylogenetic analysis by maximum parsimony method for cytochrome *c* oxidase subunit 1 (COI) and internal transcribed spacer 1 (ITS1) concatenated dataset for *Rhizostoma* sp. 1, *Rhizostoma* sp. 2, as well as *R. luteum, R. pulmo* and *R. octopus* where

sequences were obtainable from Genbank, for a maximum of 1040 nucleotides. Strict consensus bootstrapped tree is shown here. Posterior probabilities are shown next to nodes, while bootstrap percentages are indicated above branches.

Figure 1:

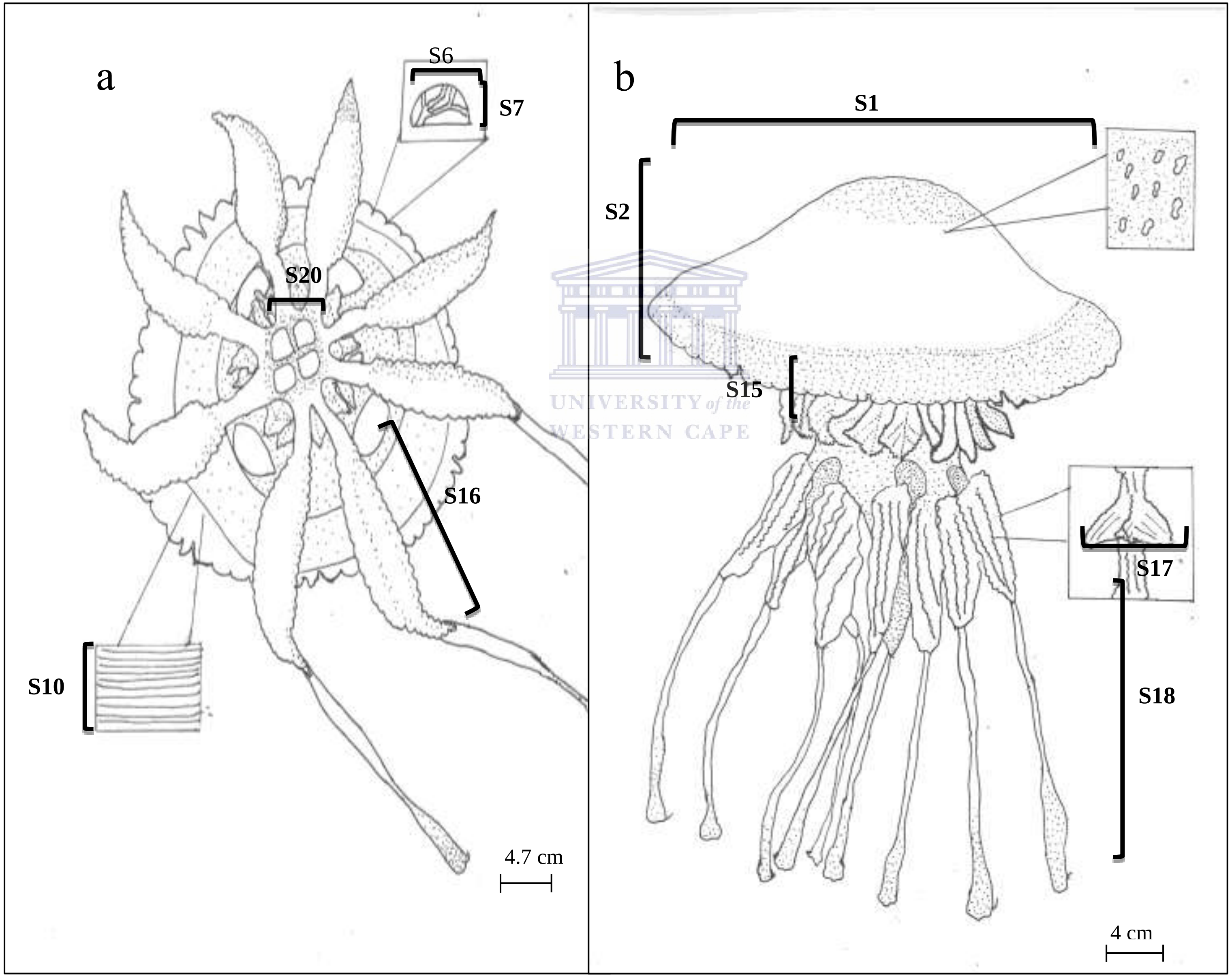


Figure 2:

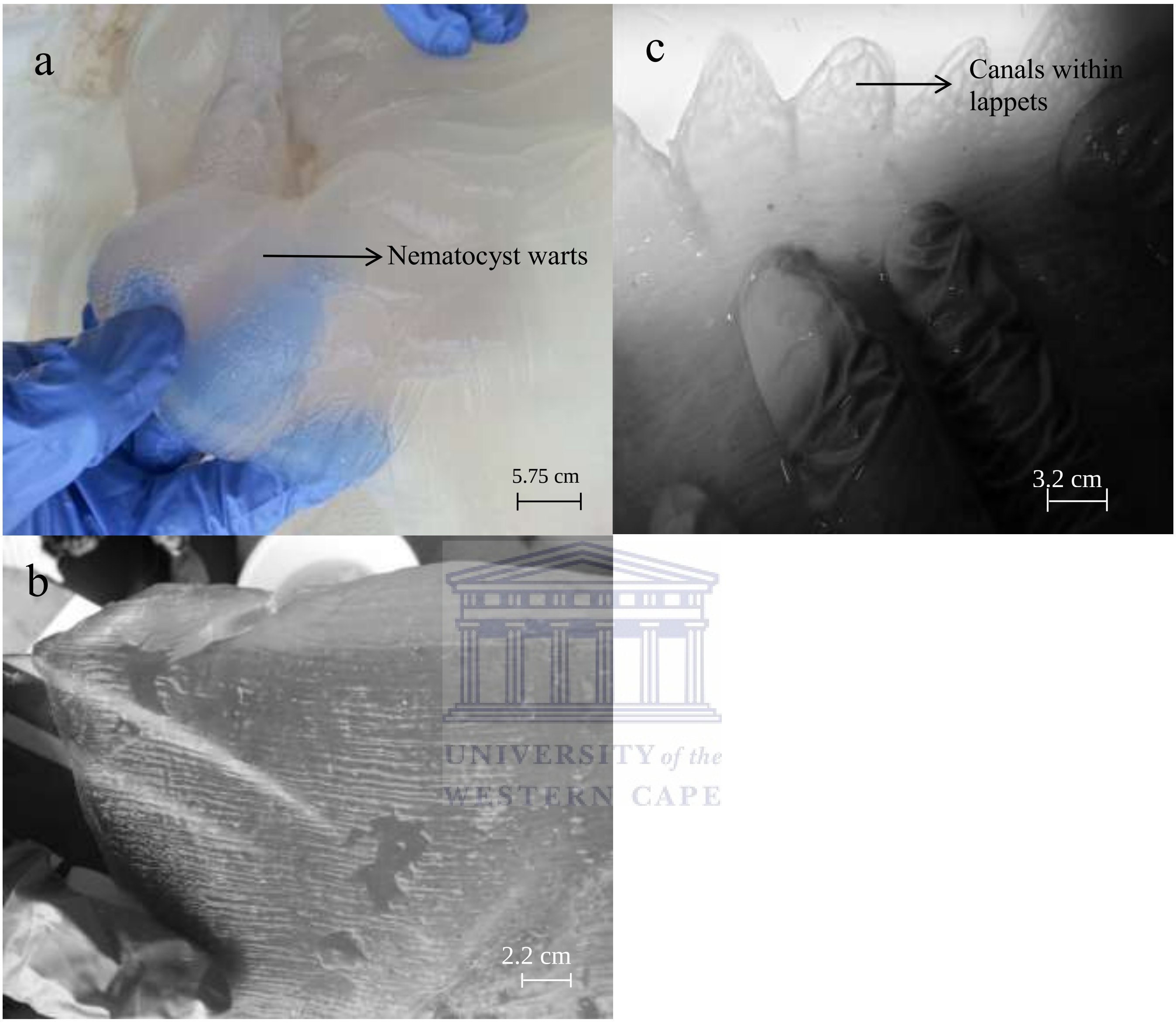

a
Nematocyst warts
5.75 cm
c
Canals within lappets
3.2 cm
b
2.2 cm

Figure 3:

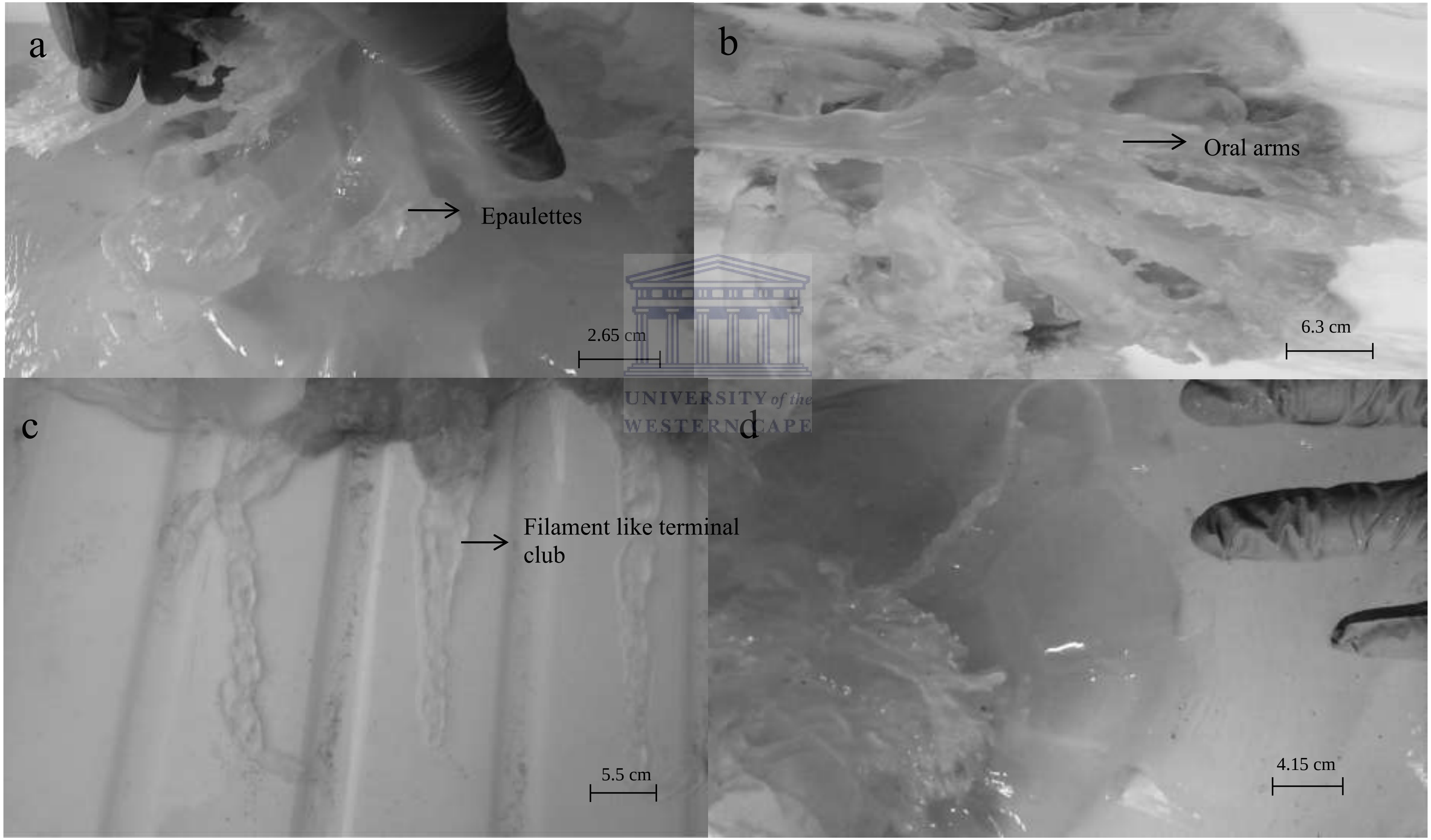

a
Epaulettes
2.65 cm
b
Oral arms
6.3 cm
c
Filament like terminal club
5.5 cm
d
4.15 cm

Figure 4:

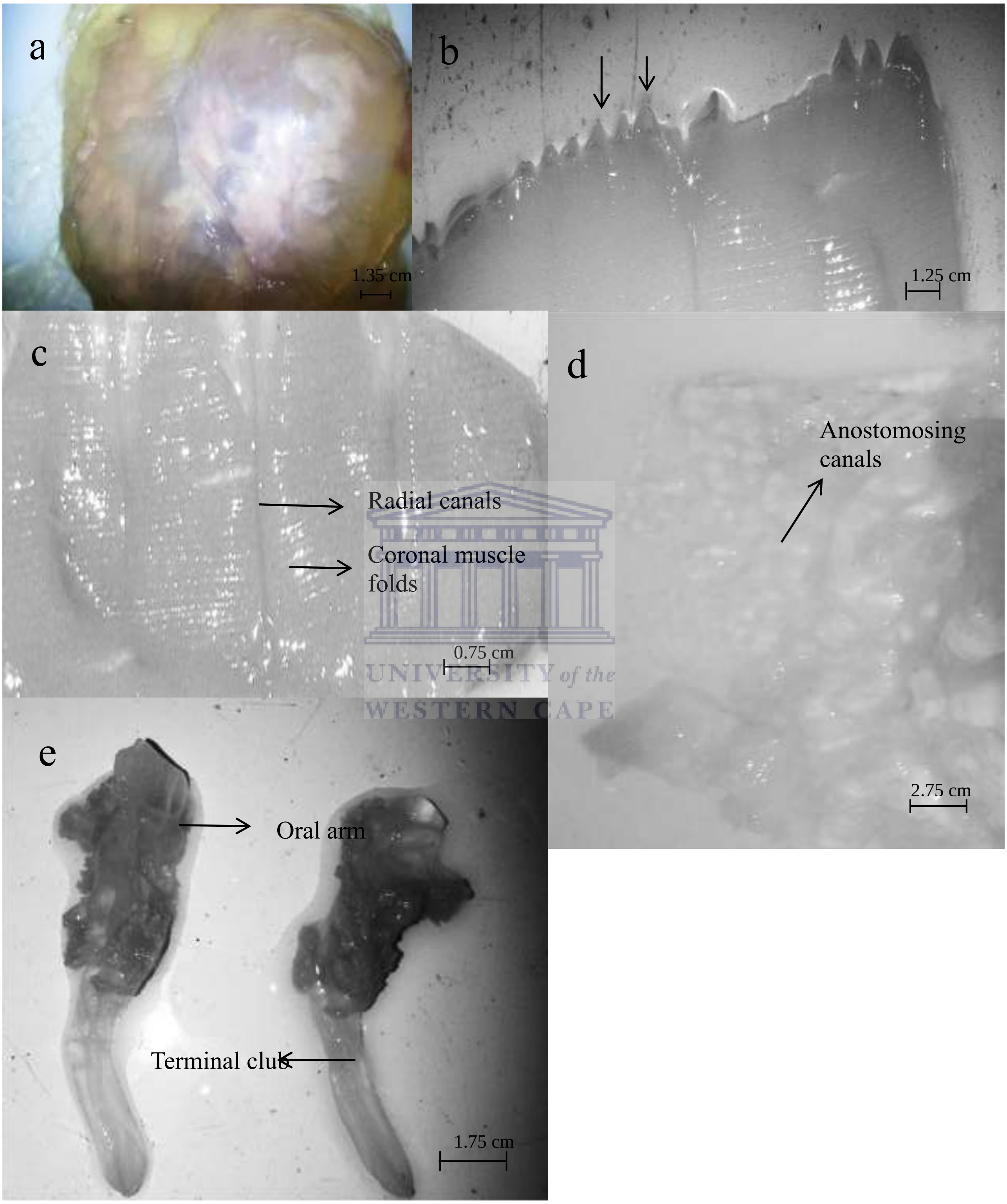

a
1.35 cm
b
1.25 cm
c
Radial canals
Coronal muscle folds
0.75 cm
d
Anostomosing canals
2.75 cm
e
Oral arm
Terminal club
1.75 cm

Figure 5:

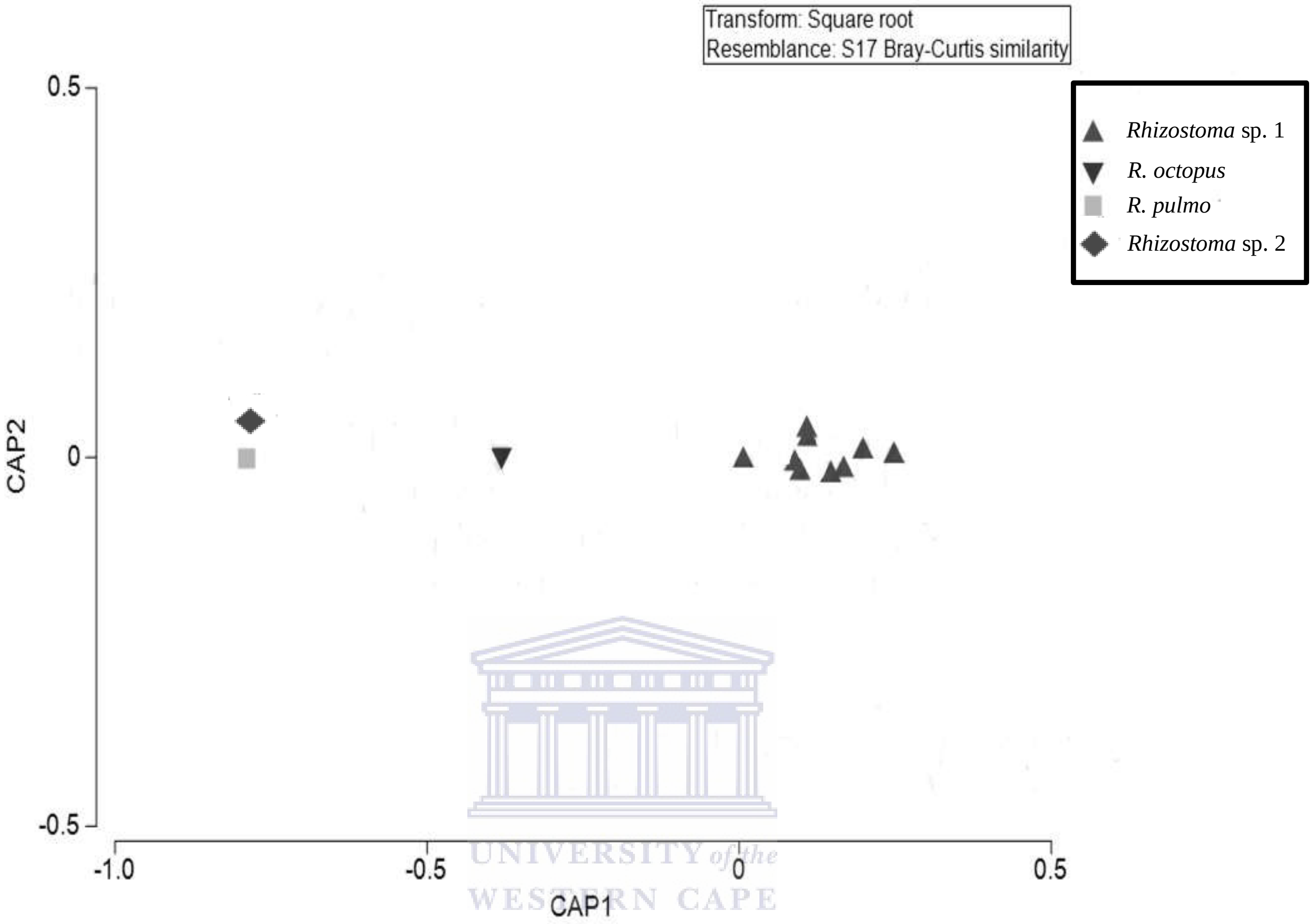

Transform: Square root
Resemblance: S17 Bray-Curtis similarity
▲ Rhizostoma sp. 1
▼ R. octopus
■ R. pulmo
◆ Rhizostoma sp. 2
CAP2
0.5
0
-0.5
CAP1
-1.0
-0.5
0
0.5

Figure 6:

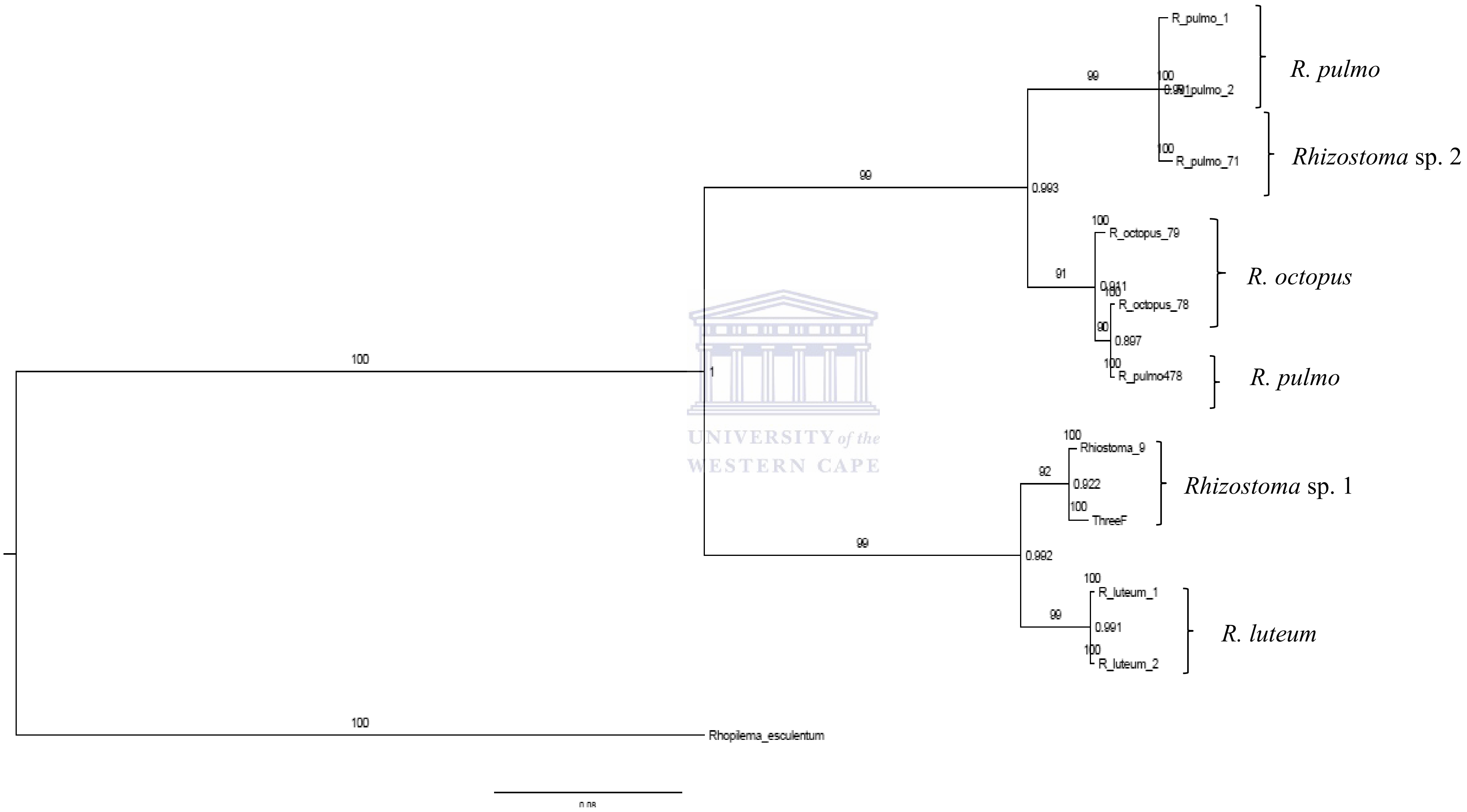
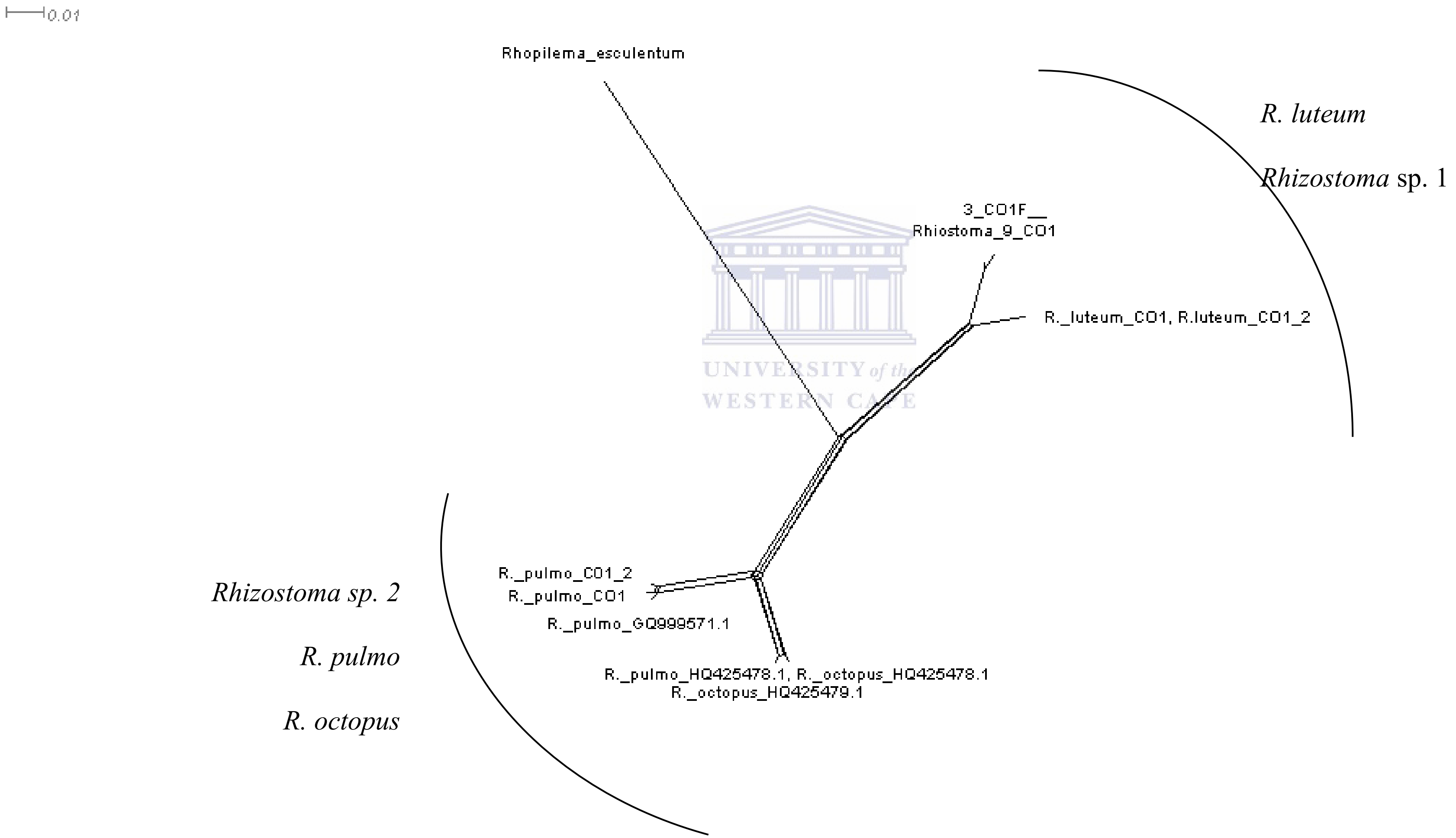

R_pulmo_1
R_pulmo_2
R_pulmo_71
R. pulmo
Rhizostoma sp. 2
99
100
0.991
100
99
0.993
R_octopus_79
R_octopus_78
R_pulmo478
100
91
0.911
100
90
0.897
100
R. octopus
R. pulmo
100
1
Rhiostoma_9
ThreeF
100
92
0.922
100
Rhizostoma sp. 1
99
0.992
R_luteum_1
R_luteum_2
100
99
0.991
100
R. luteum
100
Rhopilema_esculentum
0.08

Figure 7: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R. luteum
Rhizostoma sp. 1
3_CO1F__
Rhiostoma_9_CO1
R._luteum_CO1, R.luteum_CO1_2
Rhizostoma sp. 2
R. pulmo
R. octopus
R._pulmo_CO1_2
R._pulmo_CO1
R._pulmo_GQ999571.1
R._pulmo_HQ425478.1, R._octopus_HQ425478.1
R._octopus_HQ425479.1

Figure 8:

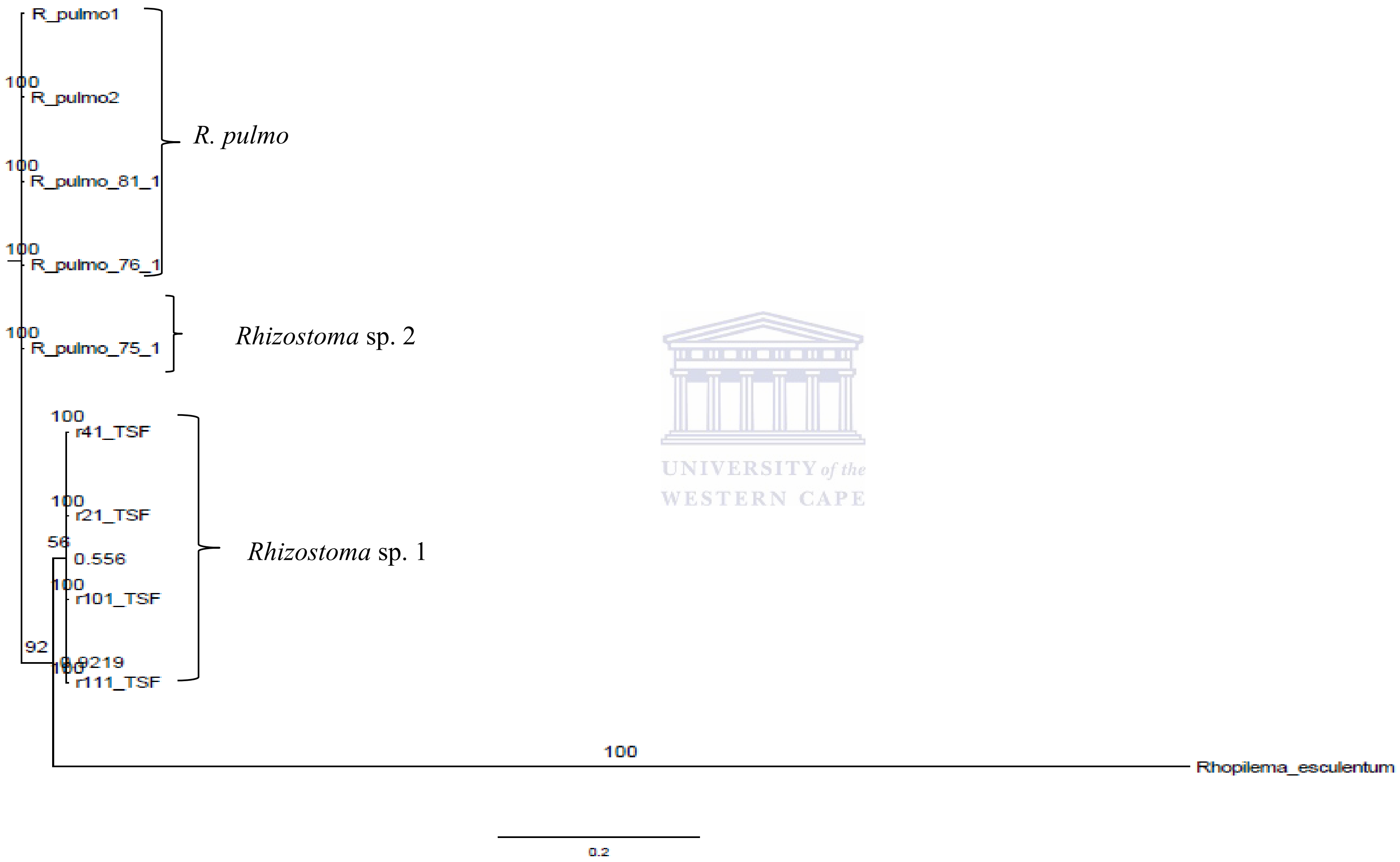

R_pulmo1
100
R_pulmo2
100
R_pulmo_81_1
100
R_pulmo_76_1
100
R_pulmo_75_1
100
r41_TSF
100
r21_TSF
56
0.556
100
r101_TSF
92
0.9219
100
r111_TSF
100
Rhopilema_esculentum
0.2
R. pulmo
Rhizostoma sp. 2
Rhizostoma sp. 1

Figure 9:

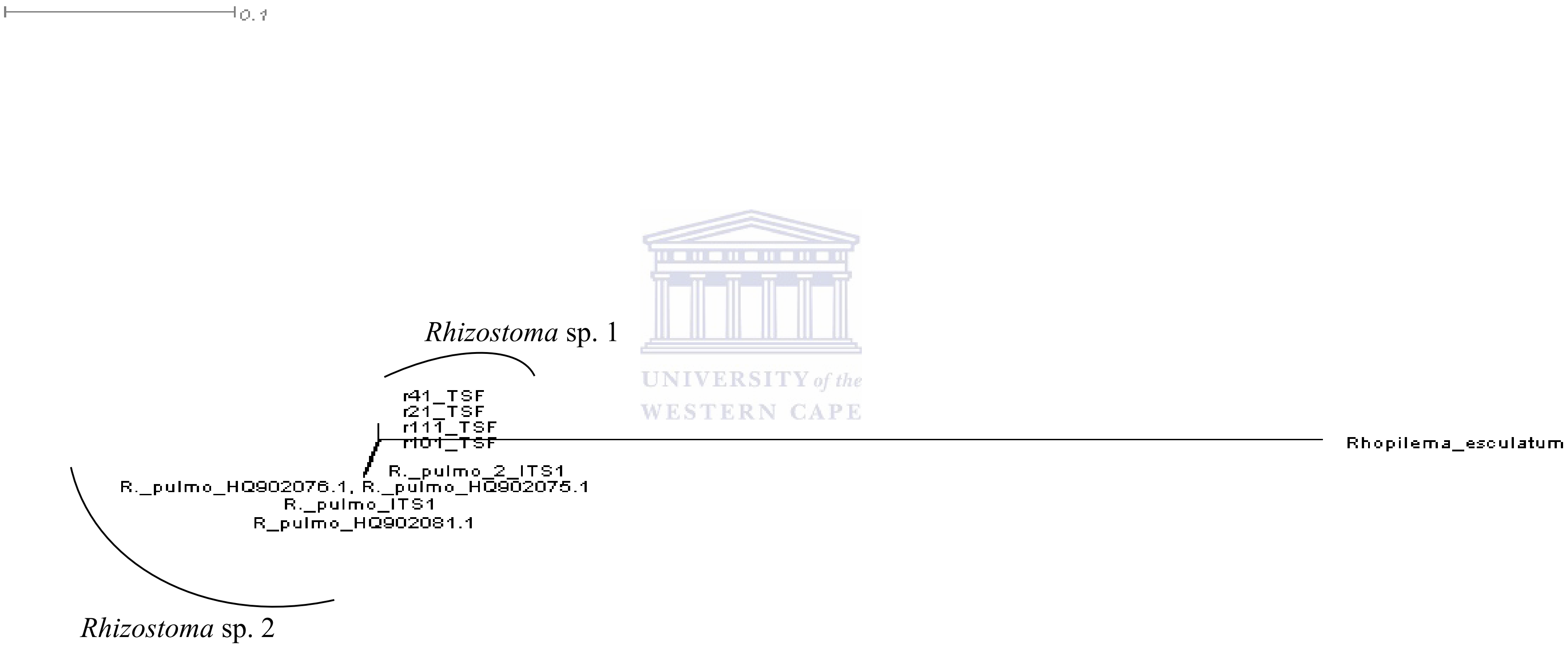
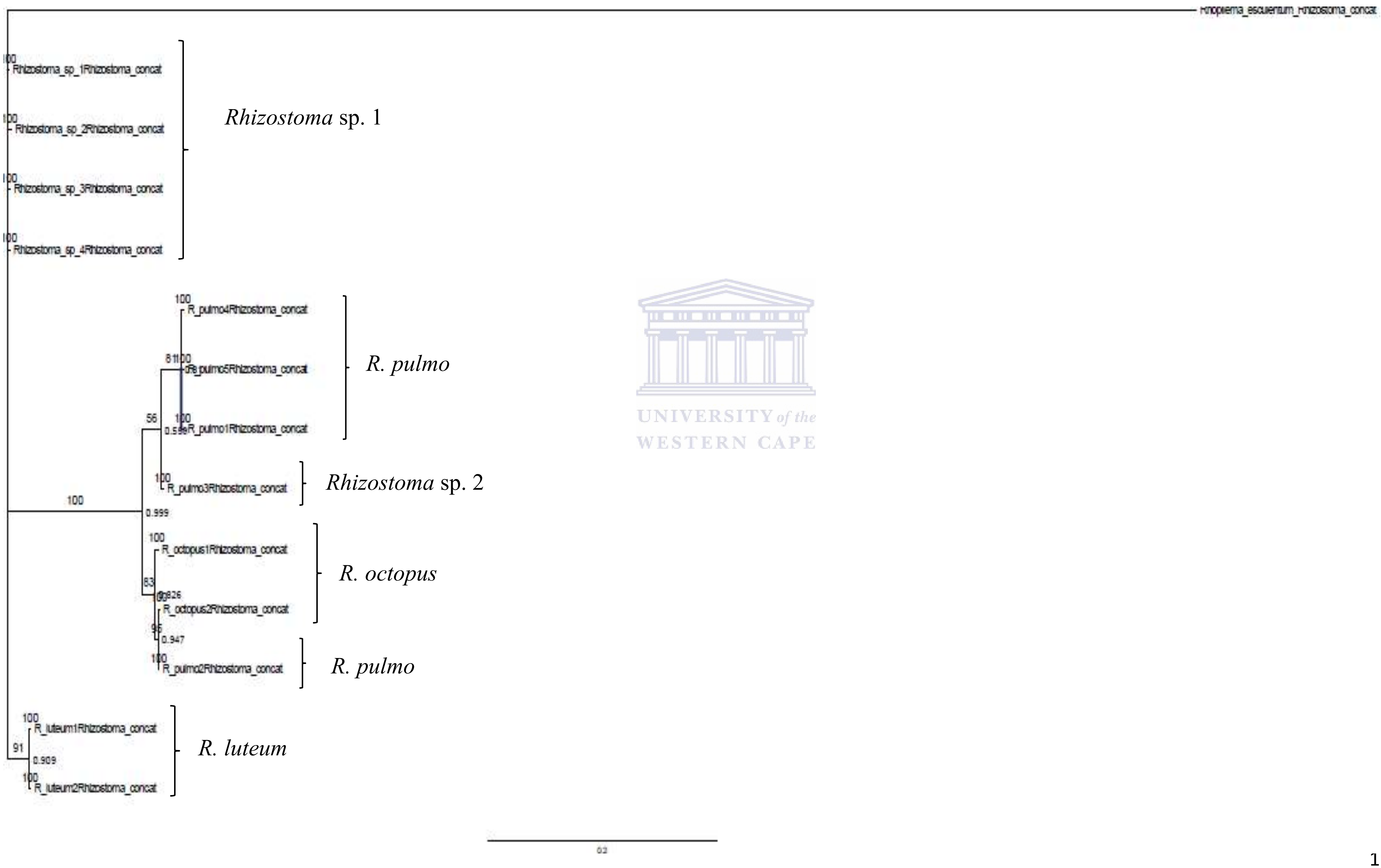

0.1
Rhizostoma sp. 1
r41_TSF
r21_TSF
r111_TSF
r101_TSF
Rhopilema_esculatum
R._pulmo_2_ITS1
R._pulmo_HQ902076.1, R._pulmo_HQ902075.1
R._pulmo_ITS1
R_pulmo_HQ902081.1
Rhizostoma sp. 2

Figure 10:


Rhopilema_esculentum_Rhizostoma_concat
100
Rhizostoma_sp_1Rhizostoma_concat
100
Rhizostoma_sp_2Rhizostoma_concat
100
Rhizostoma_sp_3Rhizostoma_concat
100
Rhizostoma_sp_4Rhizostoma_concat
Rhizostoma sp. 1
100
R_pulmo4Rhizostoma_concat
81100
R_pulmo5Rhizostoma_concat
R. pulmo
56
100
0.599
R_pulmo1Rhizostoma_concat
100
R_pulmo3Rhizostoma_concat
Rhizostoma sp. 2
100
0.999
100
R_octopus1Rhizostoma_concat
R. octopus
83
0.826
R_octopus2Rhizostoma_concat
95
0.947
100
R_pulmo2Rhizostoma_concat
R. pulmo
100
R_luteum1Rhizostoma_concat
91
0.909
R. luteum
100
R_luteum2Rhizostoma_concat
0.2

Appendix 1: Measures obtained for various variables for *Rhizostoma octopus* and *R. pulmo* used in the statistical analyses of this study and their associated sources.

	R. pulmo	***R. octopus***
	Obtained from: Macri 1778 and Mayer 1910	Obtained from Russell 1970
	Measures	
S1	60	57.6
S5	10	12
S7	1.234	1.212
S16	15.5	15.356
S17	13.756	13.547
S18	5.8	5.231
S25	15.8	15.432

Appendix 2: Standardized morphological data summarized for *Rhizostoma* sp. 1 collected around the southern coast of South Africa during 2015.

MF	Mean	N	Std. Deviation	Std. Error	Minimum	Maximum	Range	Variance
S1	1.711	9	0.119	0.040	1.513	1.857	0.344	0.014
S2	-0.727	9	0.074	0.025	-0.794	-0.627	0.167	0.006
S3	-1.948	9	0.128	0.043	-2.072	-1.761	0.311	0.016
S4	-0.838	7	0.107	0.040	-0.954	-0.725	0.229	0.011
S5	-0.736	8	0.100	0.035	-0.857	-0.628	0.229	0.010
S6	-1.634	9	0.071	0.024	-1.734	-1.543	0.191	0.005
S7	-1.713	9	0.063	0.021	-1.774	-1.589	0.185	0.004
S8	-1.575	9	0.057	0.019	-1.656	-1.515	0.141	0.003
S9	-1.503	9	0.054	0.018	-1.585	-1.418	0.167	0.003
S10	-0.808	9	0.119	0.040	-0.954	-0.610	0.344	0.014
S11	-0.808	9	0.119	0.040	-0.954	-0.610	0.344	0.014
S12	-0.507	9	0.119	0.040	-0.653	-0.309	0.344	0.014
S13	-0.507	9	0.119	0.040	-0.653	-0.309	0.344	0.014
S14	-1.148	9	0.168	0.056	-1.449	-0.945	0.504	0.028
S15	-0.934	9	0.028	0.009	-0.978	-0.891	0.087	0.001
S16	-0.559	8	0.031	0.011	-0.608	-0.527	0.081	0.001
S17	-0.736	8	0.085	0.030	-0.844	-0.653	0.190	0.007
S18	-0.386	3	0.217	0.125	-0.637	-0.255	0.381	0.047
S19	-1.615	3	0.233	0.134	-1.883	-1.459	0.423	0.054
S20	-0.907	8	0.053	0.019	-0.979	-0.857	0.121	0.003
S23	-1.041	7	0.025	0.009	-1.078	-1.007	0.071	0.001
S24	-1.026	7	0.049	0.019	-1.065	-0.947	0.118	0.002
S25	-0.476	6	0.014	0.006	-0.495	-0.459	0.036	0.000
S26	-0.356	6	0.046	0.019	-0.416	-0.298	0.118	0.002
S27	-0.866	6	0.129	0.053	-1.003	-0.710	0.294	0.017
S29	12.228	6	4.782	1.952	7.828	18.575	10.747	22.863

Appendix 3: Summary of features used to distinguish between: *Rhizostoma* sp. 1 collected around the southern coast of South Africa during 2015 as well as *R. octopus*, *R. pulmo* and *R. luteum* where features were obtained from previous descriptions and , *Rhizostoma* sp. 2 examined at the South African Natural History Museum during 2016.

Features	***Rhizostoma* sp. 1**	***Rhizostoma* sp. 2**	***R. pulmo***	***R. luteum***	***R. octopus***
No. of lappets per octant	10	10	10	10	12
Lappet shape	oval	pointed	semi-circular	short,oval	short, pointed
Terminal club length	usuallylonger than oral arm, with long slender basal stalk	equal in length to oral arm	shorter or equal in length to oral arm	Usually longer than oral arm, with long slender basal stalk	usually equal to oral arm in length with thin basal stalk, shorter than luteum
Bell colouration	Bell pale blue or tranparent	only observed in preservation	Bell pale yellow with blue lappet	warts on exumbrella appear brown; terminal club often deep brown	Bell pale yellow with blue lappet
Anostomosing canals	absent	present	present	absent	somewhat present
Length of upper oral arm	Longer than lower arm	Longer than lower arm	Longer than lower arm	Longer than lower arm	shorter than lower arm

Appendix 4: The consensus sequence of internal transcribed spacer 1 (ITS1) amplified from nine *Rhizostoma* sp. 1 specimens collected from the southern coast of South Africa during 2015. Variable nucleotide bases are indicated, if present.

ATATATTGAGGATGACCGTTCGATCCCGAACAGCCGTCTGTCGATTGCTGCTTGT

CACACTGATTGAAAAACCAGTTTTGACAAACACCACTGTGAACCTGTACCCATCC

GTGTGAGGTGGACAGAGTGCACGGATACGTACGTGTACGTCTGTGCTTAAATGA

AAGGCGATTCCCTTTCGGCCTCACATTGGAGTTTTCTCACATCGTATTCGGTCGGT

TGTTTTCGAATTCGTTCGGAAACAGTCAGCCACACTTTGTTGCACACATAAATAT

TTACTGAACATTTTGGACGTGCTTGCTTTGCTTGCACGGCGATTGGAAAATGAAA

TACAACTTCTAACGGTGGATCTCTTGGCTCG

Appendix 5: The consensus sequence of cytochrome *c* oxidase subunit I (COI) amplified from three *Rhizostoma* sp. 1 specimens collected from the southern coast of South Africa during 2015. Variables nucleotide bases are indicated, if present.

CACTATATTTGATATTCGGAGCTTTTTCTGCTATGATCGGAACTGCTTTTAGTATG

ATCATAAGATTAGAATTGTCTGGCCCAGGGTCAATGTTAGGTGACGATCAATTGT

ATAATGTTGTAGTAACAGCCCATGCATTGATTATGATATTTTTCTTCGTTATGCCA

GTACTAATAGGAGGCTTTGGAAACTGATTAGTTCCTTTGTACATAGGAGCGCCAG

ATATGGCTTTCCCAAGATTAAATAATATTAGCTTTTGATTATTACCACCTGCACTA

CTATTATTGTTGGGGTCCTCCCTTGTAGAACAGGGCGCAGGAACAGGTTGAACAA

TTTATCCCCCTCTTAGCTCAATTCAAGCCCATTCAGGAGGTTCTGTAGATATGGCT

ATCTTCAGTCTACATTTAGCGGGGGCTTCTTCTATAATGGGAGCTATTAATTTTAT

TACTACTATCTTAAATATGAGAGCCCCTGGTATGACCATGGATAAAATTCCCTTA

TTTGTATGGTCTGTCTTAGTTACAGCAATATTATTGTTGTTATCTCTACCTGTTTTA

GCTGGAGCTATTACAATGTTACTTACAGATAGAAATTTTAATACTTCTTTTTTTGA

TCCTGCAGGAGGACGGAGACCCAATACTATTTCAACACTTATTT

Appendix 6: The consensus sequence of internal transcribed spacer 1 (ITS1) amplified from two *Rhizostoma* sp. 2 specimens collected from the southern coast of South Africa during 2015. Variable nucleotide bases are indicated, if present.

GGAAGGATCATTACTGATATATTGAGGATGACCGTTCGATCACGAACAGCCGTCT

GTCGATTGCTTGTCACACTGATTGAAAAACCAGTTTTGACAAACACCACTGTGAA

CCTGTACCCATCCGTGTGAGGTGGACAGAGTGCACGAAGAAGCACTACATTGTA

CTCTCTTGGATTGGTACTGTCGTGTACGTGTACGTCTGTGCTTAAATGAAAGGCG

ATTCCCTTTCGGCCTCACATTGGAGTTTTCTTATATCGTATTCGGTCGGTTGTTTTC

GAATTCGTTCGAAAACAGTCAGCCACACTTTGCACACATAAATATTTACTGAATA

TTTTGGACGTGCTTGCTTTGCTGGCACGGCGATGGAAAAATGAAATACAACTTCT

AACGATGGATACTCTTGGCTCGT

Appendix 7: The consensus sequence of cytochrome *c* oxidase subunit I (COI) amplified from three *Rhizostoma* sp. 2 specimens collected from the southern coast of South Africa during 2015. Variables nucleotide bases are indicated, if present.

AACACTATATTTAATATTTGGAGCCTTTTCCGCTATGATAGGAACTGCTTTCAGTA

TGATTATAAGATTAGAATTATCTGGTCCAGGATCCATGTTGGGTGATGATCAACT

ATATAATGTTGTAGTGACAGCCCACGCATTGATTATGATATTTTTCTTTGTTATGC

CAGTGTTGATAGGAGGTTTTGGAAACTGGTTAGTTCCTTTATATATAGGGGCACC

AGACATGGCCTTCCCAAGGTTAAATAATATTAGTTTTTGATTACTTCCTCCTGCAC

TACTACTATTGCTAGGTTCTTCTCTTGTAGAACAGGGTGCAGGAACAGGTTGGAC

AATTTATCCTCCTCTAAGTTCGATCCAGGCCCATTCAGGAGGTTCTGTGGATATG

GCCATCTTCAGTTTACACTTAGCAGGAGCTTCCTCTATAATGGGAGCTATTAATTT

CATTACTACTATCTTGAATATGAGGGCTCCCGGTATGACTATGGATAAAATTCCT

TTGTTTGTATGGTCTGTTCTAGTCACAGCAATATTATTGTTATTGTCCTTACCTGTT

TTAGCTGGGGCTATTACAATGCTACTTACAGATAGAAATTTTAATACTTCTTTCTT

CGACCCAGCGGGAGGAGGAGATCCTATTTTATTTCAGCATTTGTTT