Re-descriptions of some southern African Scyphozoa: out with the old and in with the new

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To my family: Brian, Esther, Michelle and Joan, for their constant encouragement and support, for convincing me every day that anything is possible. To God, for providing me with the spiritual guidance to complete this thesis.

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Abstract

Two species of *Chrysaora* are described from the northern Benguela ecosystem: *C. fulgida* and *C. africana*. These species can be diagnosed by a combination of morphological features including lappet and tentacle number, shape of lappets, colouration patterns (alive), shape of the proximal portion of radial septa, gastrovascular pouch shape, point of attachment of gonads and the presence or absence of small raised nematocyst warts on the exumbrellar surface. Objective, quantitative statistical analyses coupled with molecular sequence data support the qualitative morphological dissimilarity observed, as these analyses unambiguously diagnose *C. fulgida* and *C. africana* as two distinct species. There is a strong superficial resemblance between the *C. fulgida* material described here and the preserved specimens of *C. hysoscella* examined at the Natural History Museum, London. Thorough investigation does however allow the separation of these two species. Morphological features found to be dissimilar were the proximal portion of the manubrium, gastrovascular pouch shape and the presence or absence of sperm sacs. Objective, quantitative statistical analyses support these findings. Nuclear sequence variation suggests considerable divergence between the two species but additional molecular work is needed.

Keywords: *Chrysaora*, northern Benguela ecosystem, taxonomy, systematics, morphological analyses, molecular analyses.

Introduction

The Benguela Current is one of the four major eastern boundary current systems. Prevailing coastal southerly and south-easterly winds along the west coast of southern Africa fuel the upwelling of cool, nutrient rich waters (Shannon, 1985). The Benguela ecosystem is traditionally divided, at Lüderitz, into northern and southern subregions where upwelling tends to be more seasonal (Shannon, 1985). The coastal region surrounding Lüderitz, where the continental shelf is narrowest and prevailing winds strongest, is characterized by perennial upwelling and it is considered southern Africa's most intense upwelling cell (Shannon, 1985). Upwelling of cool nutrient rich water prompts concentrated phytoplankton growth in the upper photic zone (Shannon, 1985) which in turn supports plentiful fish stocks and numerous seabirds, seals and sharks as top predators (Boyer *et al.*, 2000). Characteristic of an ecosystem with high levels of primary production is increased bacterial decomposition that can strip surrounding water of oxygen (Chapman and Shannon, 1985). This often leads to hypoxic and at times anoxic conditions that may be associated with sulphide eruptions (Bakun and Weeks, 2006) and mass mortalities of marine life (Boyer *et al.*, 2000): hypoxic waters have been linked to depleted abundances of commercially valuable fish species (Woodhead *et al.*, 1997). Environmental anomalies and anthropogenic activities are thought to have adversely affected the productive northern Benguela ecosystem resulting in a highly modified and deficient ecosystem (Boyer *et al.*, 2000).

The northern is traditionally considered the more productive of the two Benguela ecosystems (Carr, 2001). Intense upwelling is associated with high concentrations of diatoms, whereas quiescent or post-upwelling periods favour dinoflagellates; as upwelling intensities vary so do nutrient concentrations determining the dominant plankton group (Sakko, 1998). The

zooplankton community, dominated by copepods and euphausiids, all occur at low levels of species diversity but high abundance/biomass (Gibbons and Hutchings, 1996; Sakko, 1998). Prominent for its once abundant fish stocks, Namibian waters supported numerous commercially valuable species that in turn provided important resources to the Namibian economy. Species are generally divided into three groups dependant on the zone occupied in the marine environment (Sakko, 1998; Boyer *et al.*, 2000). In the epipelagic zone clupeiforms such as round herring *Etrumeus whiteheadi*, sardines *Sardinops sagax*, anchovy *Engraulis encrasicolis* well as juvenile horse mackerel *Trachurus trachurus capensis* (Perciformes) are found. Perciforms such as chub mackerel *Scomber japonicus*, horse mackerel and geelbek *Atractoscion aequidens* are found in the mesopelagic zone and the demersal zone is dominated by Cape hake *Merluccius capensis* and *M. paradoxus* (Gadiformes) and the bearded goby *Sufflogobius bibarbatus* (Perciformes). Unfortunately unsustainable fishing practices carried out in the late 20th century have resulted in radical, and for some species irreversible, declines in fish catches (Heymans *et al.*, 2004).

The overexploitation of fish during the past century was not confined to the northern Benguela ecosystem but was a worldwide issue and has resulted in a global decline in the mean trophic level of exploited resources (Pauly *et al.*, 1998). Industrial fishing in the northern Benguela dates back to the early 20th century but only subsequent to World War II, after purse seiners were introduced, has it taken place on a larger scale (Boyer *et al.*, 2000). Sardines, once the most abundant of the small pelagic fish off Namibia served as an important prey component in the diets of many mammals, seabirds and commercially valuable fish species (Cury and Shannon, 2004). Sardine catches peaked at ~5 million tonnes during the 1950s and doubled in the 1960s after good recruitment in the late 1950s and early 1960s (reviewed by Cury and Shannon, 2004). Unfortunately following the onset of heavy

industrial fishing in the early 1970s sardines suffered subsequent stock crashes (Heymans *et al.*, 2004). Heymans *et al.* (2004, pp. 182) estimated sardine biomass as "virtually zero during the 1980s". Sardine catches increased marginally during the 1990s (Heymans *et al.*, 2004), but due to sustained heavy fishing pressure, causing poor recruitment, the full recovery of sardine stocks had been prevented (Boyer *et al.*, 2000). Similar patterns were observed for other commercially valuable fish such as anchovies, chub mackerel and hake (Heymans *et al.*, 2004). Annual fish catches decreased to a mere ~3.5 tonnes wet weight km^{-2} during the 1990s, approximately half that caught during the 1970s and 1980s (Heymans *et al.*, 2004).

Overfishing has had complex knock on effects. Cury *et al.* (2000) and Cury and Shannon (2004) review a number of hypotheses regarding the mechanisms that control the trophic dynamics of upwelling systems and the origin of the regime shift experienced in the northern Benguela ecosystem. Small pelagic fish that constitute intermediate trophic levels form "wasp-waist" populations and are known to have significant roles in upwelling systems as these populations exert both bottom up control on top predators and top down control on zooplankton prey as well as influencing other small pelagic fish within the occupied trophic level (Cury *et al.*, 2000; Cury and Shannon, 2004). These "wasp-waist" populations comprise schooling fish and are dominated by either a species of sardine (or sardinella) or anchovy (Cury and Shannon, 2004). When a dominant species is removed, the subordinate species tends to be favoured, as has been exhibited in other upwelling systems such as that off Peru (see Bakun and Weeks, 2008). This latter system is known for the "colossal" quantity of commercially valuable fish produced in comparison to other upwelling systems and Bakun and Weeks (2008) identified a number of unique geographical and physical characteristics that permit this ecosystem to continually recover from alternations between a range of *Sardinops* species and the typically dominant anchovy *Engraulis ringens*, despite decreasing

fish biomass. The northern Benguela ecosystem, in contrast to other upwelling systems has displayed no clear shift between dominant fish species after the stock crash of sardines, as the predicted subordinate species (anchovy) was also overexploited (Cury and Shannon, 2004; Boyer *et al.*, 2000; Cury *et al.*, 2000). Instead, a wide range of opportunistic species such as jellyfish, the bearded goby and other mesopelagic fish have replaced the dominant group (Boyer and Hampton, 2001).

A number of theories have been put forward to explain the observed regime shift off Namibia. Bakun and Weeks (2006) suggested that overfishing altered school dynamics and thereby changed the reproductive behavioural patterns of species involved. Schooling fish possess inherent instincts to form schools which can be either pure schools when their respective populations are in great abundances or mixed schools when the abundances of the respective populations are diminished (Cury *et al.*, 2000). Schools can therefore be made up of a dominant population as well as subordinate populations in smaller numbers. The school's behavioural patterns are controlled by the dominant population, to the detriment of the subordinate population (Bakun and Cury, 1999). Bakun and Weeks (2006) present a translation of the "school trap" concept to the specific dilemma faced in the northern Benguela ecosystem. Bakun and Weeks (2006, pp. 324) state "much of the sardine stock biomass and the bulk of its reproductive output are believed to have been located in the near-coastal area north of Walvis Bay" as this region provided the most favourable conditions for reproductive success of sardines. But as fishing efforts were concentrated in and around the vicinity of Walvis Bay, fish with the instinct to migrate to this region were removed. The secondary, less productive zone located at the Angola – Benguela front consequently became favoured, as fish with the affinity to migrate come to dominate the schools. Bakun and Weeks (2006) further suggest that in addition to the altered reproductive migratory behaviour, this

adverse feedback loop also determines a schools' affinity to the Angola-Benguela front as the primary feeding habitat, instead of the intense upwelling cell located near Lüderitz. As a result, phytoplankton that does not sink to bottom waters is transported downstream to areas of high zooplankton numbers that are then able to exploit this unutilized resource. An increase in zooplankton prey results in an increase in those zooplanktivores not exploited, such as jellyfish and gobies. The primary reproductive area previously occupied by overfished schools now offers these opportunistic species a fertile vacant niche. Due to lowered grazing pressure on phytoplankton, exponential production results in much sedimentation and bacterial decomposition. This decomposition turns surrounding waters anoxic often leading to associated hydrogen sulphide eruptions (Weeks *et al.*, 2004). Anoxic bottom waters and hydrogen sulphide eruptions have obvious negative effects on those fish populations that cannot tolerate these conditions. Many of the currently observed opportunistic species, such as jellyfish and gobies, appear to be able to tolerate these altered environmental conditions (Arai, 1997; Richardson *et al.*, 2009; Staby and Krakstad, unpublished data; Utne Palm *et al.*, unpublished data).

The bearded goby *Sufflogobius bibabartus* is endemic to the Benguela ecosystem and is found in highest abundances on the central Namibian shelf (Staby and Krakstad, unpublished data). This habitat is characterized by a diatomaceous mud belt, anoxic waters and frequent sulphide events; conditions the bearded goby is well adapted to (Staby and Krakstad, unpublished data; Utne Palm *et al.*, unpublished data). Although historical quantitative data are lacking on the abundance of gobies over the past few decades, it is known to be one of a suite of partly-planktivorous fishes that have replaced the once dominant sardine (Boyer and Hampton, 2001). A study investigating the diet of seabird populations on islands off the Namibian coast has shown a marked change in prey species over time (Crawford *et al.*,

1985). Gobies have replaced sardines and made up a considerable part of these predators' diets (Crawford *et al.*, 1985). Crawford *et al.* (1985) also note that the bearded goby plays an important role in the ecosystem, as essentially all of its production is available for consumption by predators due to the lack of goby's commercial value.

A species of *Chrysaora* has been shown to be highly abundant in the northern Benguela ecosystem (Brierly *et al.*, 2001), and its biomass, in combination with that of *Aequorea forskalea* actually exceeds that of commercially valuable fish (Lynam *et al.*, 2006). Heymans *et al.* (2004) observed the negative impact an increase in jellyfish biomass has on energy flow through ecosystems. Jellyfish are often considered to be "trophic dead ends" because of their low nutritional value and consequently lack of predators (Sommer *et al.*, 2002) therefore the majority of the energy that flows to jellyfish appears to return straight back to detritus (Heymans *et al.*, 2004; Bakun and Weeks, 2006). However other literature suggests that this is an oversimplification as research reveals jellyfish to be an established prey item in marine ecosystems (Catry *et al.*, 2004; Arai, 2005; Houghton *et al.*, 2006). Whether energy flow to the benthos is skewed within the northern Benguela ecosystem the residing *Chrysaora* medusae have been shown to take advantage of this occurrence, as Flynn and Gibbons (2007) have noted its ability to consume benthic organisms when available. Jellyfish otherwise prey on a variety of zooplankton including fish eggs and larvae (Arai, 1997; Purcell, 1992; Purcell *et al.*, 1994; Sommer *et al.*, 2002; Lynam *et al.*, 2005; Flynn and Gibbons, 2007) and are classified as having a Type I functional feeding response as no satiation occurs at natural food densities (Arai, 1997). These attributes have negative knock on effects for declining fish populations, as competitive and predatory pressures exerted by jellyfish are suggested to prevent the recovery of depleted fish stocks (Richardson *et al.*, 2009).

When present in large aggregations, termed jellyfish blooms (Graham *et al.*, 2001); competitive and predatory pressures are potentially high. Hamner and Dawson (2009) hypothesized that jellyfish possessing traits favourable to, and therefore inclined to, bloom are found mainly within the cnidarian class Scyphozoa. Many jellyfish species are able to bud off numerous ephyrae from the benthic polyp stage (termed scyphistoma in scyphozoans) and a single polyp can bud off new polyps that can result in the mass production of large, conspicuous medusae (Purcell *et al.*, 2007). Hamner and Dawson (2009) note jellyfish that possess traits favourable to bloom belong to diverse clades, which imply blooming is an advantageous adaptation favoured by natural selection.

Evidence is accumulating that indicates jellyfish blooms are increasing in relative frequencies and intensities around the world in response to altered marine ecosystems (Purcell *et al.*, 2007). Blooms have a number of negative effects on humans. Fishing industries can suffer major financial losses as blooms damage expensive gear and ruin catches (Purcell *et al.*, 2007; Richardson *et al.*, 2009). Aquaculture establishments may also suffer financial loss such as that witnessed by the bloom of *Pelagia noctiluca* off the coast of Ireland that lead to mass mortalities of approximately 250 000 salmon in aquaculture farms (Doyle *et al.*, 2008). Power plants located along the coast use seawater for cooling and large numbers of jellyfish can block cooling intake systems forcing expensive shutdowns (Masilamoni *et al.*, 2000). Some jellyfish species are well known for their severe stings, harming and in rare cases causing the death of bathers, resulting in beach closures that ward off potential tourists (Purcell *et al.*, 2007). Although blooms are synonymous with adverse consequences, jellyfish fisheries do however form a profitable industry in Southeast Asian countries (Heish *et al.*, 2001; Omori and Nakano, 2001). Demands for the few rhizostome species that constitute this industry appears to be on the rise (Heish *et al.*, 2001; Omori and Nakano, 2001), and aside

from the cultural food value Heish *et al.* (2001) note that some may be considered to be of medicinal value.

Not all scientists agree that jellyfish are increasing worldwide as there is a paucity of long term data regarding jellyfish abundance. Mills (2001) and Purcell (2005) have proposed that some varying abundances could be linked to natural climate change on a decadal time scale. In some instances environmental conditions may inhibit jellyfish blooms while in others a decrease in abundances has been observed (Mills, 2001). But an increase in abundances still remains the dominant trend globally (Shiganova, 1998; Graham, 2001; Brodeur *et al.*, 2002; Link and Ford, 2006; Lynam *et al.*, 2006). Numerous anthropogenic activities have been postulated as the origin for increasing jellyfish biomass as changing oceanic conditions seem to favour gelatinous plankton over fish. Climate change associated with global warming and increasing water temperatures appear to promote jellyfish proliferation (Purcell *et al.*, 2007; Richardson and Gibbons, 2008; Richardson *et al.*, 2009). Heavy fishing pressure removes potential predators (Pauly *et al.*, 2002) and competitors as the diets of some fish and jellyfish species overlap (Purcell and Arai, 2001). As development increases along the coast, natural environments are modified; aquaculture farms, artificial reefs, docks, marinas, breakwater and oil platforms are all examples of infrastructure that provide ideal substrata for benthic polyps (Purcell *et al.*, 2007; Richardson *et al.*, 2009). Coastal development is also linked with eutrophication that increases biomass at all trophic levels, providing additional prey for polyps and medusae leading to escalating rates of proliferation (Purcell *et al.*, 2007). Eutrophication is also associated with hypoxic events to the detriment of much marine life, but as jellyfish exhibit tolerance to these conditions their continued success is certain (Richardson *et al.*, 2009). Arai (2001) however could not directly link an increase in nutrients to an increase in jellyfish abundances as eutrophication was not the only plausible factor

potentially increasing jellyfish abundance. Purcell *et al.* (2007) and Richardson *et al.* (2009) also highlight the probable synergistic effects of multiple environmental conditions causing or promoting jellyfish blooms.

The introduction of non-indigenous jellyfish species has certainly caused some of the blooms around the world and may be accelerated as alien populations can have certain advantages over indigenous species in the invaded habitat, such as a lack of native predators (see Ivanov *et al.*, 2000). Mills (2001) reviews a well documented case of a ctenophore invader, *Mnemiopsis leidyi*, in the Black Sea that illustrates the detrimental effects a non-indigenous species can have on highly stressed ecosystem. During the 1960s the Black Sea was subject to eutrophication and an exploitation of fish that caused "favourable bottom-up resource supply and weakening top-down pressure" for *Engraulis encraiclolus* (anchovy) (Oguz *et al.*, 2008, pp. 1386). Oguz *et al.* (2008) suggested that these favourable conditions allowed a dramatic increase in anchovy biomass from ~300 000 tonnes in the 1960s to ~1 500 000 tonnes in the 1970s. *Mnemiopsis leidyi*, which is thought to have been introduced via ballast water discharge, was first documented in the Black Sea in the early 1980s and by the end of that decade had occupied the entire ecosystem and spread to adjacent marine habitats (Graham and Bayha, 2007). The Black Sea became progressively more degraded due to persistent eutrophication favouring opportunistic and gelatinous species, which was dominated by *Aurelia aurita* and *M. leidyi* (Oguz *et al.*, 2008). In 1990 a drastic increase in *M. leidyi* biomass was observed that coincided with the collapse of anchovy (*Engraulis encrasicolus*) stock (Oguz *et al.*, 2008). Oguz *et al.* (2008) review two major theories postulated to explain the drastic decline in anchovy stock. Firstly intense overfishing caused the anchovy - *M. leidyi* shift and the second alternative theory caused by intense food competition and predation on anchovy eggs and larvae by *M. leidyi*. Oguz *et al.* (2008)

proposed that unfavourable temperatures caused the lag in *M. leidyi* outbreak. Favourable spring temperatures returned in 1989 – 1990 therefore allowing *M. leidyi* numbers to increase to bloom levels, and this phenomenon in combination with eutrophication, overfishing and climate changes caused the regime shift observed in the Black Sea. Ivanov *et al.* (2000) regards the success of this ctenophore in the Black Sea, and its subsequent invasion of the Caspian Sea, as a result of abundant available prey, suitable environmental conditions and a lack of native predators. It was only after the accidental introduction in 1998 of yet another ctenophore, *Beroe ovata* that feeds exclusively on other ctenophores, that *M. leidyi* showed a significant decrease in population size in the Black Sea (Oguz *et al.,* 2008).

Population explosions of the scyphozoan *Phyllorhiza punctata* caused major financial loss to the local shrimping industry in the Gulf of Mexico in 2000 (Graham *et al.*, 2003). Scyphozoans are also known to invade a single habitat on multiple occasions, such as the introduction of the jellyfish *Cassiopea andromeda* to the Hawaiian Islands during World War II (Holland *et al.*, 2004). These authors have suggested that this scyphozoan invaded the Hawaiian Islands once from the Indo-Pacific region and then again from the Atlantic Ocean. The introduction of non-indigenous jellyfish has been linked with the exchange of ballast water and transportation of polyps on ship hulls (Graham and Bayha, 2007). Polyps, however, have to endure adverse conditions often experienced in extensive journeys in ballast waters, on ship hulls and in new environments after an invasion. Some scyphozoans are known to produce podocysts, dormant cysts that develop beneath the pedal discs of scyphistomae, when present in unfavourable physical conditions (Arai, 1997). Podocysts may remain viable for extended periods of time and allow populations to survive under conditions of reduced food availability, harsh temperature changes and even predation (Arai, 1997;

2009). When favourable conditions return podocysts excyst and form scyphistomae (Kawahara *et al.*, 2006), which are capable of further podocyst formation. Given that a single polyp may form numerous podocysts, jellyfish populations are able to increase readily (Arai, 2009) and re-establish following unfavourable physical conditions (Kawahara *et al.*, 2006). Podocysts may therefore play a significant role in numerous scyphozoans species ability to bloom successfully (Arai, 2009).

The incidence of invasive species encountered globally may be underestimated due to confusion surrounding their identity. Graham and Bayha (2007, pp. 239) note that "incomplete historical systematic treatment, generally poor taxonomic appreciation by non-specialists, and species crypsis" all contribute to this dilemma. Cryptic species are increasingly being encountered in marine invertebrates present in a diverse range of habitats (Knowlton, 1993). In the past, it was assumed that marine species were characterised by broad dispersal ranges, due to the lack of geographical and environmental barriers (Palumbi, 1992). Rates of speciation were considered to be low and taxa were dominated by cosmopolitan species (Dawson and Jacobs, 2001), which is unlikely as the successful incidence of long distance dispersal of most marine taxa is extremely uncommon (Knowlton, 1993). A major obstacle in marine invertebrate taxonomy, including that of scyphozoans (e.g. *Aurelia* in Dawson and Jacobs, 2001; Scroth *et al.*, 2002), is the paucity of useful, diagnostic morphological features without which differentiation between valid species becomes problematical (e.g. Mayer, 1910 *in* Dawson, 2004), and this can lead to misidentification amongst closely related species that share similar morphological features (Gershwin and Collins, 2002).

Molecular studies are now revealing new cryptic scyphozoan species (Dawson and Jacobs, 2001; Scroth *et al.*, 2002; Dawson, 2003; Dawson, 2005a) resulting in a recent increase in species recognised by taxonomists (Dawson, 2004). Uncertainty surrounding cnidarian taxonomy has been a long standing, complex and unresolved topic of discussion. The scyphozoan *Aurelia aurita* has received the most attention in this regard due to its circumglobal presence (Dawson and Jacobs, 2001). Approximately 20 *Aurelia* species have been described over the past century (Mayer, 1910; Kramp, 1961) of which only two, *A. aurita* and *A. limbata*, were recognised by taxonomists (Russell, 1970; Arai, 1997). Subsequent molecular analyses have revealed at least 13 *Aurelia* species including the resurrected *A. labiata* (Dawson and Jacobs, 2001; Gershwin, 2001; Scroth *et al.*, 2002).

Synonymization is not unique to the genus *Aurelia*. Holland *et al.* (2004) noted that originally six species of *Cassiopea* were described from the Pacific all of which were subsequently synonymised into a single species *C. andromeda* (Gohar and Eisaway, 1960). The systematics of *Cyanea* has also suffered much disarray, as pointed out by Dawson (2005a), whereby species were synonymised by numerous taxonomists (e.g. Mayer, 1910; Kramp, 1961). Molecular analyses on these taxa have now unambiguously shown the presence of cryptic species. Molecular analyses indicated the presence of six *Cassiopea* species (Holland *et al.*, 2004) and three potential *Cyanea* species (Dawson, 2005a). These findings serve to confirm the underestimation of species diversity within these taxa. In some studies, although molecular data provide valuable insight into species-level relationships, a well-supported phylogeny has not been produced due to highly variable DNA sequences (Dawson and Jacobs, 2001; Scroth *et al.*, 2002). The lack of robust phylogenies using molecular data highlights the need to incorporate other analytical tools such as objective, quantitative morphological data and appropriate, modern statistical analyses (Dawson, 2003). It should be

noted that in some cases, however, the results of morphological and molecular data contradict each other. For example morphological measurements amongst populations of *Mastigias* exceed that which normally delineates species boundaries, whilst by contrast insignificant differences have been revealed in the molecular findings (Dawson, 2005b). Dawson (2005b, pp. 200) concluded that there is "no gold standard for designating species in the Scyphozoa." It is important to note that all the recent studies discussed above stress the integration of thorough molecular and morphological analyses if a robust phylogenetic relationships to base taxonomic decisions are desired.

Jellyfish commonly found in the northern Benguela ecosystem comprise two species; the hydrozoan *Aequorea forskalea* which is considered to be the most abundant and a *Chrysaora* species (Lynam *et al.*, 2006), commonly but recently, identified as *C. hysoscella*. A second *Chrysaora* species, had also been reported in the Benguela ecosystem but is not relatively widespread (Gibbons, 2007). Medusae identified as *C. hysoscella* are presently known to be highly abundant in the northern Benguela ecosystem (Brierley *et al.*, 2001; Lynam *et al.*, 2006), however, long-term quantitative studies concerning these medusae are lacking. Extensive studies carried out in the 1950s and 1960s on biota in the Benguela ecosystem fail to document the presence of this species (Hart and Currie, 1960; Stander and De Decker, 1969). This has lead some scientists to believe that it was relatively uncommon or perhaps non-existent in the region pre-1970s (Fearon *et al.*, 1992; Gibbons, 2007). King and O'Toole (1973) and Cram and Visser (1973) were the first to record these medusae in the northern Benguela ecosystem. Only a decade later Venter (1988) and Fearon *et al.* (1992) conducted the first semi-quantitative analyses on this species. Some scientists, are however sceptical about this theory as large medusae are notorious for damaging nets and are often disposed of during research cruises (Mills, 2001). Nonetheless it seems highly unlikely that scientists

identifying and providing exhaustive descriptions for highly inconspicuous gelatinous plankton (e.g. Hart and Currie, 1960) would neglect to do the same for large medusae filling up nets (Gibbons, 2007). A lack of records of these large medusae from various whaling companies and complaints by locals also implies that these *Chrysaora* medusae were relatively uncommon in the northern Benguela ecosystem in the early 1900s (Gibbons, 2007). These theories provide a critical link to the collapse of the pelagic fishing industry in Namibian waters and the rise in jellyfish abundances experienced in this region since the 1970s.

There is not only a lack of literature on jellyfish abundances but modern descriptions are scarce. Although archaic descriptions are still widely used in scyphozoan taxonomy they are fraught with errors that have lead to much confusion among taxonomists. A typical example of this disarray concerns the taxonomy of the genus *Chrysaora* in the Benguela ecosystem. Essentially three *Chrysaora* species have been described from the Benguela ecosystem: *C. hysoscella, C. africana* and *C. fulgida* (Reynaud, 1830; Haeckel, 1880; Vanhöffen, 1902; Mayer, 1910; Stiasny, 1934; Stiasny, 1939; Kramp 1961; Pagès *et al.*, 1992; Mianzan and Cornelius, 1999). The first taxonomic account of *Chrysaora* within the Benguela ecosystem was of *C. fulgida*. Medusae possessing twenty-four tentacles and, presumably, thirty-two lappets (Reynaud, 1830). Subsequently Vanhöffen (1902) described *C. africana* that possessed forty-eight lappets (forty tentacles). Stiasny (1934) identified a thirty-two lappet (twenty-four tentacle) medusae as *C. fulgida* but a few years later identified medusae possessing forty-eight lappets also as *C. fulgida* (Stiasny, 1939), instead of following the description provided by Vanhöffen (1902). Recent descriptions have identified the common thirty-two lappet medusae in the Benguela as *C. hysoscella*, possibly due to the confusion surrounding *C. fulgida* as medusae that possess forty-eight lappets instead of the original

description that suggests it has thirty-two (Reynaud, 1830). It could also be due to the similar morphological features that *C. hysoscella* and *C. fulgida* share as noted previously by taxonomists (Mayer, 1910). The fact that Mianzan and Cornelius (1999) excluded a description of *C. africana* and/or *C. fulgida* in their review of zooplankton in the South Atlantic highlights the uncertainty surrounding the identity of these species present.

The medusae of *Chrysaora*, which are the focus of this investigation, belong to the phylum Cnidaria, class Scyphozoa. Scientists have endeavoured to classify cnidarians since the late-19th century (eg. Haeckel, 1880; Mayer, 1910; Kramp, 1961) and over the past two decades numerous molecular studies have been undertaken to better resolve phylogenetic relationships within this phylum (Bridge *et al.*, 1992; Bridge *et al.*, 1995; Odorico and Miller, 1997; Kim *et al.*, 1999; Medina *et al.*, 2001; Collins, 2002; Dawson, 2004; Collins *et al.*, 2006). Some theories such as the basal placement of the class Anthozoa within Cnidaria and the monophyly of the clade Medusozoa comprising the classes Scyphozoa, Cubozoa and Hydrozoa have been readily accepted (Bridge *et al.*, 1992; Kim *et al.*, 1999; Collins, 2002). Traditionally, Scyphozoa consisted of the orders Cubomedsae, Stauromedusae, Coronatae, Semaeostomeae and Rhizostomeae (Mayer, 1910; Kramp, 1961). Cubozoa (formerly known as Cubomedusae) was erected as an independent class form Scyphozoa due to different developmental histories. Recent morphological and molecular studies have suggested that Stauromedusae be removed from Scyphozoa and be erected as a fifth cnidarian class and that the order Semaeostomeae appears to be paraphyletic with respect to Rhizostomeae; the subclass Discomedusae has been proposed to include both orders (Collins, 2002; Dawson, 2004; Marques and Collins, 2004; Collins *et al.*, 2006). The close relationship between semaeostomes and rhizostomes has been noted in studies dating as far back as the early-20th century due to similarities in the radial canal system (Collins *et al.*, 2006). Although

scyphozoan systematics has come a long way since the Linnaean classification system, the modern classification has been described as a "cumbersome mix of ordinal and higher taxonomic groupings" (Daly *et al.*, 2007; pp.169). So what is the way forward in jellyfish systematics? Dawson (2005c) puts forward a "total evidence approach" an integration of all types of available data into descriptions and diagnoses which, is what this investigation endeavours to do.

This study addresses the taxonomic confusion surrounding the *Chrysaora* genus within the northern Benguela ecosystem. It aims to statistically analyse the morphology and genetics of the *Chrysaora* species frequently found off the Namibian coast and compare it to previous taxonomic descriptions of *Chrysaora* sampled in the Benguela ecosystem in order to resolve the taxonomic confusion surrounding its identity. Consequently it attempts to determine if there is any evidence of crypsis with populations of *C. hysoscella* originally described in the Northern Hemisphere. Objective, quantitative morphological features and molecular analyses are utilized to resolve the dilemma of whether an additional *Chrysaora* species exists within the Benguela ecosystem. Material will also be compared it to previous taxonomic descriptions of *Chrysaora* sampled in the Benguela ecosystem in order to resolve its identity.

Materials and Methods

Morphological data collection

Jellyfish specimens for morphological analysis were 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). Various sampling gears (pelagic and bottom trawls, including MOCNESS) were used to collect a total of 56 *Chrysaora* medusae (Utne Palm *et al.*, unpublished data). Material was preserved in 5% formalin in ambient seawater immediately on collection. Medusae were grouped according to superficial appearance (colouration pattern on exumbrella surface, tentacle and lappet number) of the 56 specimens, 40 were categorized as *Chrysaora* sp.1 and the remaining specimens as *Chrysaora* sp.2. After a minimum of 50 days in preservation, morphometric and meristic features were measured from *Chrysaora* sp.1 and *Chrysaora* sp.2 specimens (summarized in Table 1 and illustrated where possible in Figure 1). Preservation is known to cause weight loss and shrinkage in several marine organisms (e.g. Lucas, 2009), these effects may however be more potent in jellyfish due to their high water content and lack of skeletal support (Thibault-Botha and Bowen, 2004). These effects have been documented in various gelatinous animals and may vary with the size of the specimen (Thibault-Botha and Bowen, 2004) and period of preservation (de Lafontaine and Leggett, 1989). However after a period of 60 days preservation effects appear to stabilize (de Lafontaine and Leggett, 1989). This study did not correct for any effects of preservation on size but given that specimens were all measured after approximately 60 days in preservation, we assume that its effects will have stabilised. All measurements were taken, using vernier callipers, under a magnifying glass or a dissecting microscope at various magnifications. Descriptive statistics (including mean,

mode, median, 25 % and 75 % quartiles) of all morphological features are summarized in Appendix 1. Type material was not available for examination.

Comparative material from elsewhere was examined from the collections at the Natural History Museum, London (Table 2). The morphological measurements outlined above (Table 1 and Figure 1) were replicated where possible on preserved material although some measurements had to be excluded as material had to be studied non-destructively.

Morphological data analyses

In order to determine the effect of individual size of on measured variables, they were correlated against maximum bell diameter (S 1) using Pearsons R correlations (Zar, 1999). All data were tested for normality visually and the Levene test of Homogenetiy of Variances was used to test for homoscedacity (Zar, 1999). Relationships between size (S 1) and measurements for those variables that failed tests of normality were examined using Spearman Rank Correlations (Zar, 1999). Correlations were then repeated on standardized morphometric (which were divided by S 1 and log transformed) to examine the relationship between relative proportions of measured variables and size. All correlations were corrected using the Bonferroni procedure, therefore adjusting alpha levels, to control for Type I errors in multiple test analyses (Quinn and Keough, 2002).

Standardized morphometric data were used in all subsequent statistical analyses (including multivariate tests) in order to eliminate size dependency. Clarke and Green (1988) highlight that logarithmic transformations are commonly used in statistical analyses, including non-

parametric tests, as measured variables are put on a common scale of variance and the relative weight of each measured variable can be determined. In order to test for differences between standardized morphometric data of the Namibian and comparative material, two-tailed *t*-tests were employed (Zar, 1999). Alpha levels were corrected for multiple comparisons using the Bonferroni adjustment (Quinn and Keough, 2002). Those data that failed tests for normality were investigated using Mann-Whitney-U tests (Zar, 1999), and results were again corrected for Type I errors using the Bonferroni adjustment (Quinn and Keough, 2002). All univariate statistical analyses were considered significant at the 5 % level (unless otherwise adjusted) and were executed using STATISTICA Version 7.

Non-parametric tests were used to examine morphological dissimilarity in a multivarite space. As non-parametric tests make no statistical assumptions about the underlying quality and distribution of original data, these tests are common practice among ecologists (Clarke and Green, 1988) and are most appropriate for the present study. The non-metric multi-dimensional scaling (MDS) routine in PRIMER 6 was used to illustrate the multivariate relationship between standardized morphometric features measured (Clarke, 1993). The MDS routine is an iterative procedure based on rank orders, as an alternative to qualitative values, in a Euclidean distance matrix generated from the original log transformed standardized morphometric features (Clarke and Warwick, 2001). Non-metric MDS utilizes an algorithm that attempts to preserve the ranked differences in a 2-dimensional ordination space (Clarke and Warwick, 2001). To quantify the deviation from the original ranking in the Euclidean distance matrix to that reflected in the 2-dimensional ordination space, a "stress" value is generated (McCune and Grace, 2002). Clarke and Warwick (2001) suggest that MDS plots with stress values > 0.2 should be treated with caution. Prior to generating the Euclidean distance matrix between specimens based on their standardized morphometric features, gaps were filled either by mean substitution (if there was no significant relationship

of the considered feature with size) or from regression equations. Meristic features were not included. The same Euclidean distance matrix was used in all subsequent multivariate tests.

The One-way analysis of similarities (ANOSIM) routine in PRIMER 6 was used to test the null hypothesis of no morphological dissimilarity between species (Clarke and Warwick, 2001). ANOSIM, a non-parametric method, executes this through two key processes (Clarke and Warwick, 2001). Firstly the routine computes an *R* statistic that measures the average distance between every specimen within a group and contrasts it to the average distance between every specimen from different groups. Distances are also based on ranking orders within a Euclidean distance matrix. ANOSIM then utilizes a series of permutation tests, whereby variables from each group being tested are randomly distributed between groups, recalculating the *R* statistic for each permutation. If the original *R* statistic is more extreme than 95 % of the permutation tests the null hypothesis is rejected by a $p < 0.05$. ANOSIM in PRIMER 6 ran 999 permutation tests. In order to determine what standardized morphometric features contributed the most to dissimilarity between species the Similarity Percentages (SIMPER) routine in PRIMER 6 was utilized (Clarke, 1993). SIMPER determines the average dissimilarity between all pairs of inter-group specimens (Clarke and Warwick, 2001). These averages are then disaggregated into percentages that each standardized morphometric feature contributes to dissimilarity amongst groups (Clarke and Warwick, 2001).

Finally the Canonical Analysis of Principal Co-ordinates (CAP) routine in PRIMER 6 & PERMANOVA+ that utilized predefined groups, in contrast to many other multivariate tests, was also executed. The CAP routine seeks a set of axes that best discriminates amongst *a priori* groups in a multivariate space (Anderson *et al.*, 2008). Anderson *et al.* (2008) describes the processes executed within this routine. Numerous matrices are generated to

produce a set of canonical axes. Conventionally in a canonical discriminant analysis a subset of Principal Co-ordinate (PCO) axes are chosen manually, based on the number variables in the original data matrix. However, in the present study, as the number of standardized morphometric features approached the number of specimens, Anderson *et al.* (2008) suggest "leave-one-out" diagnostics to determine the subset of PCO axes. The PCO axes determined are all orthonormal and therefore independent of each other. Running parallel to this process is a matrix based on codes for groups identified by a factor associated with the Euclidean distance matrix, also orthonormalised. An additional matrix is then generated by relating the subset of PCO axes to orthonormalised data matrix, yielding canonical eigenvalues and their associated eigenvectors which can be used to produce a CAP plot. These CAP axes, which are linear combinations of a subset of orthonormal PCO axes, were used to determine if predefined groups were correctly classified. The CAP routine was also used to test the null hypothesis of no differences in the positions of centroids among groups in a multivariate space through a series of permutation tests (Anderson *et al.*, 2008). This routine makes no assumptions about the underlying distribution of variables rendering it suitable for non-parametric analyses (Anderson *et al.*, 2008). All multivariate tests were repeated for *Chrysaora* sp.1 and *Chrysaora* sp.2 and were considered significant at the 5 % level.

DNA analysis

Material for genetic analysis was obtained on the R.V. *G.O. Sars* cruise. A small piece of oral arm tissue was cut out before specimens were preserved in formalin, and this was placed in absolute ethanol (99 %) and stored at -20 °C prior to analysis in the laboratory. Unfortunately comparative genetic material for *C. hysoscella* could not be obtained from locations where archived specimens were collected. However genetic material, identified as *C. hysoscella*,

was obtained by Dr. Tom Doyle (Coastal and Marine Resources Centre, Cork Harbour) from Dingle Bay (52° 6' 54" N -10° 20' 27" W) and Cork Harbour (51° 49' 33.6" N -8° 16' 8.4" W), Ireland.

DNA was extracted from ethanol-preserved oral arm tissues using a phenol-chloroform based method. Samples were placed in separate eppendorf tubes. Extraction Buffer (SDS 0.5 %; 50 Mm Tris; 0.4 M EDTA; pH 8.0) in quantities of 0.5 ml were pipetted over each sample. Tissue samples were then macerated. Proteinase K (20 mg/ml) in quantities of 10 µl was then added. Samples were vortexed and incubated at 55 °C for a minimum of three hours until majority of protein was digested. Samples were then mixed with 500 µl phenol:chloroform:isoamyl alcohol (24:24:1), finger vortexed, then centrifuged at low speed (5000 x g) for 10 minutes. Supernatants were removed and placed in new eppendorf tubes, mixed with 500 µl chloroform:isoamyl alcohol (24:1) and finger vortexed. Solutions were then centrifuged at low speed (5000 x g) for 10 minutes. Supernatants were removed and placed in new eppendorf tubes. DNA was precipitated with 45 µl Na acetate and 650 µl of ice cold ethanol and left to incubate at -18 ° C overnight. Samples were then centrifuged at full speed (13000 x g) for 10 minutes and supernatants were discarded. Eppendorf tubes were inverted and left to air dry for a minimum of an hour. Each DNA sample was finally resuspended in 50 µl TE buffer.

Cytochrome *c* oxidase subunit I (COI) was amplified using primers LCOjf (5'-ggtcaacaaatcataaagatattggaac-3') and HCOcato (5'-ctccagcaggatcaaagaag-3') (Dawson, 2005c) or HCO2198 (5'-taaacttcagggtgaccaaaaaatca-3') (Folmer *et al.*, 1994). Internal transcribed spacer one (ITS1) was amplified using the primers jflTS1-5f (5'-ggtttcgtaggtgaacctgcggaaggatc-3') and jflTS1-3r (5'-cgcacgagccgagtgatccaccttagaag-3')

(Dawson and Jacobs, 2001). Sequences were amplified through polymerase chain reaction (PCR) and PCR conditions were different for each fragment, summarized in Table 3 (adapted from Daryanabard and Dawson, 2008). PCR products were purified and sequenced at the Central Analytical Facility, University of Stellenbosch. Electopherograms were checked visually, misreads corrected and poorly resolved terminal portions of sequences were discarded using Sequencher 4.9. Forward and reverse sequences were then aligned, using default settings, in Sequencher 4.9. Sequence identifications were verified by BLAST in GenBank. All sequence lengths were then edited in Sequencher 4.9. Mean pairwise sequence differences, using uncorrected "*P*", distances were calculated in PAUP* 10.4b.

SYSTEMATICS

Order SEMAEOSTOMEAE L. Agassiz, 1862

Family PELAGIIDAE Gegenbaur, 1856

Genus *Chrysaora* Péron and Lesueur, 1810

Chrysaora fulgida (Reynaud, 1830)

(Figures 1-8, 12, 14; Tables 1, 3-17; Appendices: 1-3, 5)

Medusa (Rhyzostoma) fulgidum: Reynaud, 1830

Chrysaora fulgida: Haeckel, 1880; Vanhöffen, 1902; Stiasny, 1934

Chrysaora hysoscella var. *fulgida*: Mayer, 1910

Chrysaora hysoscella: Pagès *et al.*, 1992; Mianzan and Cornelius, 1999

Description

Umbrella diameter of the material investigated (previously referred to as *Chrysaora* sp.1) ranges between 59 – 407 mm, roughly hemispherical in shape. Exumbrella smooth, lacking raised nematocyst warts. In life smaller specimens' mesoglea relatively thin; exumbrellar translucent pink, oral arms pink-white; deep maroon marginal tentacles (Figure 2). Larger specimens exumbrellar and oral arms are translucent orange-red to deep red in colour; inner portion of oral arms opaque; deep maroon marginal tentacles (Figure 2). Some medusae possess characteristic star-shaped colouration pattern on exumbrellar formed by central apex with typically sixteen radially distributed triangles (apices pointed towards apex of the exumbrellar); always darker than under surface pigment (Figure 3). In preservation smaller specimens' exumbrellar transparent-cream; frilled edges of oral arms brown, inner central

portion of oral arms and manubrium transparent cream (Figure 4). Larger specimens' exumbrellar orange-brown with or without darker triangles radially distributed, gonads cream, inner central portion of oral arms and manubrium transparent cream, outer delicate frills brown in colour (Figure 4). Tentacles, in preservation are orange-brown in colour (Figure 4). Umbrella thickened centrally, thinning towards the margin. Larger specimens' mesoglea greatly thickened. Eight rhopalia divide the umbrella margin into octants. Umbrella margin cleft into thirty-two broadly rounded lappets; four per octant consisting of two rhopalial lappets adjacent to the sensory organ and two velar lappets (Figure 1). The peripheries of lappets are free of gastrovascular canals (Figure 5). Margin of rhopalial lappets do not overlap ("open rhopalium" condition; Morandini and Marques, in submission). Rhopalia are situated in deep clefts between adjacent rhopalial lappets. Each sensory organ consists of a statocyst and sensory bulb, without an ocellus and covered by an exumbrella hood. Immediately above each rhoplalium is a deep exumbrellar sensory pit, cone-shaped in longitudinal cross section funnelling towards the subumbrella. On the subumbrellar surface the edges of flanking rhopalial lappets (next to rhopalia) form a sensory niche, the rhopalium is attached at its base to a ridge running to the proximal wall of this niche. Oral openings are cruciform in shape. Medusae possess a maximum of eight primary tentacles one per octant, cylindrical in shape, located at umbrella margin in clefts between velar lappets. Five specimens possessed fully developed secondary tentacles located between rhopalial and velar lappets and if present were not present in all octants. Lateral protrusions arise from subumbrella between rhopalial and velar lappets in tentacular gastric pouches are observed, where fully developed secondary tentacles are lacking (Figure 5). Quadralinga absent. Thin, elongate manubrium, arising from gastric cavity form a short oral tube that is distally divided into four long oral arms approximately twice the length of umbrella diameter. Oral arms are v-shaped in horizontal cross section; "cartilaginous" inner central portion with delicate frilled

edges, distal ends spiralled. Proximal portion of oral arm wider in diameter in comparison to middle and distal portion; oral arms therefore lancet-shaped. Radial septa, proximal portion pear shaped (Figure 5), arise from periphery of central stomach dividing gastrovascular cavity into sixteen gastric pouches. Septa span the entire length of coronary muscle and fuse at the edge of rhopalial lappets; kinked towards secondary tentacles. Tentacular pouches therefore dilate and contract distally; whereas rhopalial pouches contract and dilate distally (Figure 5). Highly folded gonads found in four interradial circular pouches; situated in the central stomach, attached to the subumbrellar surface; readily protrude out of four rounded subgenital ostia. No sperms sacs were observed.

Variation

Variation in colour pattern was observed as some medusae lack the darkly pigmented central apex on the exumbrellar surface or lack the entire star-shaped colouration pattern typically observed on *Chrysaora.* One specimen possessed nine rhopalia. Although this deviated from the standard eight rhopalia found in the remaining thirty-nine specimens Gershwin (1999) highlighted that scyphozoans tend to display variability in relative numbers of body parts, including number of rhopalia. Five specimens possessed fully developed secondary tentacles ranging in number from four to eleven per specimen. Lateral protrusions from the subumbrellar surface, which in most specimens did extend beyond marginal lappets, were found where fully developed secondary tentacles were lacking (Figure 5). Tentacle length could not be determined as tentacles broke off readily.

Correlation analyses between umbrella diameter (S 1) and meristic as well as morphometric features (Tables 4 and 5) were either constant (most meristic features) or significantly

correlated with specimen size (most morphometric features). Constant features can be considered to be potentially diagnostic and could be applied to future identification of this species. When morphometric features were expressed as a ratio of umbrella diameter and log transformed (hereafter referred to as standardized), size dependency tended to disappear (Tables 6 and 7). These constant features are informative as they too could serve as diagnostic characteristics. Those standardized morphometric features still found to be significantly correlated with specimen size include: diameter of oral opening (S 14), ostia width (S 19) and length (S 20) (Tables 6 and 7). These features should be treated with caution when comparing specimens of different sizes. All standardized morphometric features that were significantly correlated to umbrella diameter were negative.

Remarks

There is a very strong superficial resemblance between the *Chrysaora fulgida* material described here and the preserved specimens of *C. hysoscella* examined at the Natural History Museum (NHM), London. Common morphological features include: number of rhopalia; rhopalium description; rhopalia condition; number and shape of marginal lappets; number and arrangement of tentacles; absence of quadralinga; absence of conspicuous nematocyst warts on exumbrella; oral arm description; elongate manubrium; typical star-shaped exumbrella colouration pattern; point of attachment and shape of gonads (summarised in Table 8). However of the nineteen standardized morphometric features compared between the *C. fulgida* material described here and of *C. hysoscella*, twelve were found to be significantly different (Tables 9 and 10). Some of the these features included those relating to bell height (S 2 and S 3); lappet width (S 7 and S 9); gonadal measurements (S 36 and S 37); maximum oral arm width (S 24) and inter-ostia width (S 18).

Multi-Dimensional Scaling (MDS) of the standardized morphometric features (stress value: 0.09; Figure 6) illustrated a clear differentiation between the *C. fulgida* material described here and that of *C. hysoscella*, although some degree of overlap is apparent in the plot. Further statistical analysis however reinforces this dissimilarity as there are significant differences between the *C. fulgida* material described here and of *C. hysoscella* (Global *R*: 0.61; $p < 0.001$; ANOSIM). SIMPER analysis identified four standardized morphometric features as being mostly responsible for these differences between the two groups studied. The variables contributing to the dissimilarities between species are highlighted in Table 11, foremost of which are features relating to the oral opening: diameter of oral opening (S 14, 29.3 %), oral pillar width (S 13; 12.51 %) and to umbrella height: minimum umbrella height (S 3; 15.56 %), maximum umbrella height (S 2; 9.95 %). However diameter of oral opening should be treated with caution as this standardized morphometric feature was found to be significantly correlated with size (Table 6). For the canonical procedure a subset of eight PCO axes were used based on the "leave-one-out" diagnostics which accounted for 94.51 % of the total variation in the species data and resulted in 1.79 % mis-classification error (Table 12). The first squared canonical correlation (d_1^2) was high: 0.88 and the permutation test results were significant at $p < 0.001$ (Table 12).

In addition to standardized features noted above, three key qualitative features also differed between the *C. fulgida* material described here and that of *C. hysoscella*. Firstly the manubrium of NHM specimens adheres to the description noted by Russell (1970) as the proximal portion is thickened forming four basal oral arm pillars, resembling a four-leafed clover, each oral arm pillar diverges to form an ostia then fuses with surrounding pillars to form a short continuous oral tube from which four oral arms arise (Figure 7). The manubrium of the *C. fulgida* material described here was similar to that of NHM in that distal regions

form a short continuous tube from which four oral arms arise. It differed however in that the manubrium of the *C. fulgida* material described here was found to be thin across the entire surface; lacking a thickened base associated with four oral arm pillars as noted in NHM specimens (Figure 7). Morphometric features relating to the manubrium such as oral opening (width of oral pillars: S 13; diameter of oral opening: S 14) and manubrium length were significantly different between Namibian and NHM specimens (Tables 9 and 10). Again caution should be met with the standardized morphometric feature: diameter of oral opening.

Gastrovascular pouch shape also differed between the *C. fulgida* material described here and *C. hysoscella* as the distal region of radial septa (in rhopalial gastrovascular pouches) contract in *C. hysoscella* specimens (as noted by Russell, 1970; pp. 89 and 90), whereas in *C. fulgida* the radial septa contract then are "kinked" towards respective secondary tentacles (Figure 8). The absence of sperm sacs on *C. fulgida* was the final qualitative feature found to distinguish the two groups of *Chrysaora* examined. *Chrysaora hysoscella* are known to be protandrous hermaphrodites (Russell, 1970; Arai, 1997), as observed on the NHM specimens examined (Figure 9), but little literature exists on physical cues that stimulate male and female gonad development. An increase in sample size, greater geographical distribution of sampling, seasonality and associated physical factors are probable rationalizations to explain the absence of sperm sacs observed in Namibian specimens, although this may be real as most scyphozoans sexually lack this feature (Arai, 1997).

It was interesting to note that of the forty Namibian specimens examined only five possessed fully developed secondary tentacles; where fully developed tentacles were lacking lateral protrusions arising from the subumbrella between rhopalial and velar lappet were observed (Figure 5). Regrowth of tentacles is a potential theory to explain the presence of these lateral

protrusions on sexually mature medusae, as Pagès *et al.* (1992, see Figure 61) noted tentacles of *Chrysaora* specimens, sampled in the Benguela ecosystem, readily broke off. Lateral protrusions originate from the subumbrellar surface; similar to the tentacle development noted in *Chrysaora* ephyrae (Russell, 1970; Tronolone *et al.*, 2002; Morandini *et al.*, 2004). In ephyrae protrusions develop under lappets that result in subsequent splitting of lappets to form new tentacular ones (Russell, 1970; Tronolone *et al.*, 2002) this is however not the case in the material presently examined as all tentacular lappets are fully formed in mature medusae. Primary tentacle width (S 30) was found to be significantly different between the *C. fulgida* material examined here and *C. hysoscella* specimens. Statistical analyses therefore reveal considerable morphological dissimilarity, coupled with the distinctive qualitative morphological features observed; suggest *C. fulgida* and *C. hysoscella* are two distinct species.

For internal transcribed spacer one (ITS1) a maximum length of 336 nucleotides was amplified from five *C. fulgida* specimens (Appendix 3) and 346 nucleotides from three *C. hysoscella* specimens (Appendix 4). DNA sequence data from ITS1 showed an average of 4.06 % pairwise sequence differences between the *C. fulgida* material examined here and *C. hysoscella* (Table 13). Dawson and Jacobs (2001) suggest that differences of 5 – 15 % between ITS1 sequences set the standard for species level divergence. Although pairwise sequence differences between *Chrysaora* medusae from Namibia and the UK lie below the standard percentage that suggests inter-species differences, ITS1 sequence variation is substantial and suggests considerable divergence between the two species. This implies that *C. fulgida* is a local species to the eastern South Atlantic, and not an "invasive" population of *C. hysoscella* from European waters. Although *C. fulgida* and *C. hysoscella* show strong superficial resemblance thorough investigation, including inspection of qualitative

morphological features, will allow the separation of these two species. Morphological data therefore further implies that *C. fulgida* is not a cryptic species.

Similar to this study, Dawson (2003) found significant morphological variation between populations of *Mastigias* occupying various habitats in Palau, Micronesia; molecular variation was however insignificant between populations. Dawson (2003, pp. 198) therefore suggested taking an "evolutionary perspective that incorporates heterogeneity in process" entailing the integration of additional ecological, morphological, molecular and geographical information on respective medusae. Although the molecular variation observed between the specimens in this study compared to that observed between *Mastigias* populations was considerably more; additional data, as proposed by Dawson (2003), could reinforce the designation of two different *Chrysaora* species in the UK and Namibia. For cytochrome *c* oxidase subunit I (COI) a maximum length of 689 nucleotides was amplified from two *C. fulgida* specimens (Appendix 5) Unfortunately it was not possible to sequence COI from *C. hysoscella*, as primers used before on this genus (LCOjf and HCO2198 used on *Chrysaora* sp. in Dawson, 2005a) as well as other potential primers (HCOcato used by Dawson, 2005c) failed to amplify samples. It was out of the scope of this project to generate new primers but ongoing molecular analyses on COI will be conducted.

Taxonomic confusion has surrounded the identity of the large *Chrysaora* species that possess thirty-two lappets (and twenty-four tentacles) in the Benguela ecosystem. Originally these medusae were described by (Reynaud, 1830) as *Medusa (Rhyzostoma) fulgidum*. Although the latter's report lacked detail it was informative as it noted that, apart from other morphological features, specimens so described possessed twenty-four tentacles; (presumably) thirty-two lappets; the typical star-shaped exumbrellar colouration pattern and

medusae were red/brown in colour. Haeckel (1880) and Stiasny (1934) followed suit and identified medusae in the Benguela ecosystem possessing thirty-two tentacles (and other common morphological features) as *C. fulgida*. Stiasny (1934) highlighted the morphological similarity between *C. fulgida* and *C. hysoscella*. Confusion arose however when Stiasny (1939) identified five *Chrysaora* medusae that displayed morphological dissimilarities to *C. fulgida* as *C. fulgida*. Perplexing the matter further was that a description matching Stiasny's (1939) record already existed. Vanhöffen (1902) had described a new species, *Dactylometra africana*, which possessed six lappets (and five tentacles) per octant in varying size classes as Stiasny (1939) described in his record of *C. fulgida*. Stiasny (1939) noted that the only real difference between the medusae he examined and Vanhöffen's (1902) description was colour. Vanhöffen (1902) noted medusae to possess a red star-shaped exumbrellar colouration pattern; Stiasny (1939) had observed dark brown colouration patterns on medusae. Differences in colour could however have been due to preservation which causes variation and even deterioration (Figures 2 and 4). Stiasny's description was dated the 18th March 1939 and the material he examined was collected at sea on the 31st August 1938 (sent in by Dr. Engel). Preservation is therefore a plausible reason for differences in colour noted when compared to Vanhöffen's (1902) description who had described the colouration patterns on medusae on board the research vessel immediately after sampling had taken place. Stiasny (1939) also considered the medusae he examined to be the *Dactylometra* stage of *C. fulgida*, as he believed these scyphozoans underwent a series of developmental stages. He considered the initial stage *Pelagia* that develops into *Chrysaora*, then *Dactylometra* and the final phase of development the *Kuragea* stage. Stages progressed according to lappet (and tentacle) number. Stiasny (1939) therefore concluded that all these representatives belonged to a single cosmopolitan species with a large number of local varieties that sexually mature at the *Chrysaora* stage. As Reynaud's (1830) description

preceded Vanhöffen's (1902) the material Stiasny (1939) examined was assigned to the species *C. fulgida.*

Numerous scientists use Kramp (1961) to aid with identification of jellyfish around the world. The synopsis has an informative bibliography, is "comprehensible" and is written in English. It is highly likely that much of modern literature either describing or studying an ecological component of these medusae possessing thirty two lappets (and twenty-four tentacles) use Kramp (1961) as a point of reference for identification (e.g. Pagès *et al.*, 1992). Kramp (1961) however describes *C. fulgida* to possess six lappets per octant and not four as the original description (Reynaud, 1830) portrays; confusion surrounding Stiasny's (1939) description could be a possible explanation. Kramp (1961) was cautious as his review to describe *C. africana* and *C. fulgida* as two separate species, but noted the former "Probably= *C.fulgida"* (Kramp, 1961; pp. 323). The similarity between these "two" species was most likely due to the fact that Kramp (1961) excluded significant details given by the original, yet vague, description (Reynaud, 1830), highlighting Stiasny's (1939) description. It is now clear, however that these taxonomists were indeed describing two distinct species (see below). Numerous modern studies relating to this species have uncritically identified the *Chrysaora* species possessing thirty-two lappets (and twenty-four tentacles) in the Benguela as *C. hysoscella* (Pagès *et al.*, 1992; Mianzan and Cornelius, 1999; Brierley *et al.,* 2001; Buecher *et al.,* 2001; Mills, 2001; Sparks *et al.,* 2001; Brierley *et al.,* 2004; Brierley *et al.*, 2005; Lynam *et al.,* 2006; Flynn and Gibbons, 2007; Purcell *et al.,* 2007; Palomares and Pauly, 2009), and this mistake is corrected here.

Mayer (1910) synonymized *C.fulgida* as a variety of *C. hysoscella* although it should be realised that his review was based solely on the published descriptions given by Reynaud

(1830), Haeckel (1880) and Vanhöffen (1902). Although his review highlighted the morphological similarity, as did Stiasny (1934), the present study shows significant morphological differentiation between the *C. fulgida* material described here and of *C. hysoscella*. Molecular data revealed some divergence but according to previous literature (Dawson and Jacobs, 2001) these differences may not be enough to designate the two species. *Chrysaora achylos*, *C. fuscescens*, *C. melanaster*, *C. plocamia*, *C. colorata* and *C. kynthia* are all species that also possess thirty-two lappets (and twenty-four tentacles), but a suite of other morphological features, as well as geographical distribution (Morandini and Marques, in submission) allow the separation of *C. fulgida* (Table 8).

This species of *Chrysaora* material in the Benguela ecosystem is therefore designated as *C. fulgida*, although ongoing molecular (COI) as well as cnidome studies are being conducted to confirm these findings. Future work on this species should also include broader spatial and temporal sampling to resolve issues such as the absence of sperm sacs on *C. fulgida*, and as Morandini and Marques (in submission, pp. 30) recommended "to sample intermediate areas in relation to the present known distributions". Additional ecological, physiological and behavioural data are also needed to facilitate an integrated approach to scyphozoan systematics (Dawson, 2003).

SYSTEMATICS

Order SEMAEOSTOMEAE L. Agassiz, 1862

Family PELAGIIDAE Gegenbaur, 1856

Genus *Chrysaora* Péron and Lesueur, 1810

Chrysaora africana (Vanhöffen, 1902)

(Figures 1, 10-14; Tables 1, 3-8, 11, 13-17; Appendices: 1-2, 6-7)

Dactylometra africana: Vanhöffen, 1902; Mayer, 1910

Chrysaora africana: Kramp, 1961

Chrysaora fulgida: Stiasny, 1939; Kramp, 1961; Pagès *et al.*, 2002

Description

Umbrella diameter of the material investigated (previously referred to as *Chrysaora* sp.2) ranges between 105 – 312 mm, roughly hemispherical in shape. Exumbrella possess small raised nematocyst warts. In life specimens are translucent-white with characteristic star-shaped colouration pattern on exumbrellar formed by a central apex with typically sixteen radiating lines, alternating with sixteen radially distributed triangles (apices pointed towards the apex of the exumbrellar), all dark-purple in colour (Figures 10 and 11). Lappets and dorsal surface of tentacles are dark-purple in colour; manubrium and oral arms translucent-white (Figures 10 and 11). In preservation all colouration patterns on the exumbrellar surface, including lappets and the dorsal surface of tentacles, are dark brown in colour (Figure 11). The background pigment remains translucent; the subumbrellar surface, manubrium and oral arms are translucent-cream; ventral surface of tentacles translucent-brown; gonads cream in

colour. Umbrella thickened centrally, thinning towards the margin. Eight rhopalia divide umbrella margin into octants. Umbrella margin cleft into forty-eight lappets; two rhopalial lappets (flanking the sensory organ), two velar lappets and two "tentacular" lappets (adjacent to primary tentacle) per octant (Figure 12). Peripheries of marginal lappets are free of gastrovascular canals. Rhopalial and velar lappets are triangular and narrower than the more "tentacular" lappets. Forty tentacles, laterally compressed at the base, situated at umbrella margin; one primary tentacle, two secondary tentacles and two tertiary tentacles per octant (Figure 12). Rhopalia are situated in deep clefts between adjacent rhopalial lappets. Margin of rhopalial lappets do not overlap ("open rhopalium" condition; Morandini and Marques in submission). Each sensory organ consists of a statocyst and sensory bulb, without an ocellus and covered by an exumbrella hood. Immediately above each rhoplalium is a deep exumbrellar sensory pit, cone-shaped in longitudinal cross section that funnels towards the subumbrella. On the subumbrellar surface the edges of flanking rhopalial lappets (next to rhopalia) form a sensory niche, the rhopalium is attached at its base to a ridge running to the proximal wall of this niche. Thin, "cartilaginous", elongated manubrium, arising from gastric cavity and distally divided into four long oral arms. Oral arms approximately four times the length of the umbrella diameter; v-shaped in horizontal cross section, inner "cartilaginous" central portion, delicate frilled edges, distal ends spiralled. Oral openings are cruciform in shape (Figure 13). Quadralinga absent. Radial septa, triangular shaped at the base, arise from periphery of central stomach dividing gastrovascular cavity into sixteen gastric pouches spans entire length of coronary muscle and fuse at the cleft between adjacent tentacle and rhopalial lappet (Figure 12). Rhopalial pouches therefore contract distally (pear-shaped) whereas tentacular pouches dilate distally (Figure 12). Highly folded gonads attached to the periphery of four interradial rounded subgenital ostia. No sperm sacs were observed.

Variation

Variation in the number rhopalia was observed as two specimens possessed seven rhopalia and one specimen nine. The number of primary tentacles varied with the number of rhopalia. This variation in overall symmetry and relative variation in morphological features is not unique to the material presently investigated, as Gershwin (1999) found similar occurrences in the scyphozoans *Chrysaora colorata* and *C. fuscescens*.

Correlation analyses between umbrella diameter (S 1) and meristic as well as morphometric features (Tables 4 and 5) were either constant (most meristic features) or significantly correlated with specimen size. Constant features can be considered to be potentially diagnostic and could be applied to future identification of this species. When morphometric features were expressed as a ratio of umbrella diameter and log transformed (hereafter referred to as standardized), size dependency tended to disappear (Tables 6 and 7). These constant features are informative as they too could serve as diagnostic characteristics. Tertiary lappet length (S 8) was still found to be significantly correlated with specimen size (S 8). This feature was negatively correlated with umbrella diameter.

Remarks

As noted previously confusion has largely surrounded the number of *Chrysaora* species present within the Benguela ecosystem (Pagès *et al.*, 1992). Vanhöffen (1902) and Stiasny (1939) have both described a *Chrysaora* medusa possessing forty-eight lappets (and forty tentacles) sampled within the Benguela as *Dactylometra africana* and *C. fulgida* respectively. As highlighted previously the only difference between the descriptions of Vanhöffen (1902)

and Stiasny (1939) were related to differences in colouration on the exumbrellar surface. Stiasny (1939) considered *D. africana* as the *Dactylometra* stage of *C. fulgida* and concluded that the medusae he examined were indeed *C. fulgida*. It is clear that Stiasny (1939) erroneously identified these forty-eight lappet (and forty tentacle) medusae as *C. fulgida* instead of *D. africana*. Subsequently scientists have refrained from including *D. africana* in cladistic analyses (Gershwin and Collins, 2002), reviews of zooplankton within the Benguela ecosystem (Mianzan and Cornelius, 1999) and revisions of the *Chrysaora* genus (Morandini and Marques, in submission).

The previously mentioned medusae in the present study possessing thirty-two lappets (and twenty-four tentacles) were tentatively identified as *C. fulgida*. *Chrysaora fulgida* compared to the *C. africana* material described here show strong superficial dissimilarity. Apart from the difference in lappet and tentacle number, *C. africana* material differs in colouration pattern when in the wild and in preservation compared to the *C. fulgida* material described here (Figures 2, 4, 10 and 11). *Chrysaora fulgida* is also a "weighty" animal with a heavier mesoglea in comparison to *C. africana* material described here (not quantified in this study due to preservation, but in general handling of these specimens on ship differences in weight were obvious). Comparisons between the two species described here reveal that of the sixteen standardized morphometric features compared between the species of *C. fulgida* and *C. africana* nine were found to be significantly different (Tables 14 and 15). These features included those relating to lappet width (S 7 and S 9); ostia (S 18 and S 19); manubrium (S 14, S 15 and S 16); length of oral arm (S 22) and primary tentacle width (S 30).

Multi-Dimensional Scaling (MDS) of the standardized morphometric features (stress value: 0.1; Figure 14) illustrated a clear differentiation between the *C. fulgida* and *C. africana*

material described here. Further statistical analysis, examined through ANOSIM, reinforces this dissimilarity as there are significant differences between *C. fulgida* and *C. africana* (Global R: 0.75; $p < 0.01$). SIMPER identified five standardized morphometric features as being mostly responsible for these differences between the two groups studied. The variables contributing to the dissimilarities between groups are highlighted in Table 11, foremost of which are features relating to the lappet width: velar lappet width (S 7, 14.74 %) and rhopalial lappet width (S 9, 12.19 %); ostia width (S 19, 12.47 %); maximum umbrella height (S 2; 11.85 %) and manubrium depth (S 16; 10.91 %). For the canonical procedure a subset of three PCO axes were used based on the "leave-one-out" diagnostics which accounted for 100 % of the total variation in the species data and resulted in 0 % mis-classification error (Table 16). The first squared canonical correlation (d_1^2) was high: 0.93 and the permutation test results were significant at $p < 0.001$, Table 16).

In addition to the morphometric features, a number of key, qualitative morphological features differed between *C. fulgida* and *C. africana* material described here. Firstly *C. africana* possess small elevated nematocyst warts on the exumbrellar surface which *C. fulgida* lack. The umbrella margin of *C. africana* is cleft into forty-eight triangular shaped lappets (six per octant), in contrast to the umbrella margin of *C. fulgida* that is cleft into thirty-two semi-circular shaped lappets (four per octant) (Figure 12). Tentacles are situated in clefts between lappets; as a result tentacle numbers are associated with the number of lappets present. *Chrysaora africana* possess two additional tentacles per octant, in this study termed tertiary tentacles, that *C. fulgida* lack (Figure 12). Tentacle shape also varies between the two groups examined as *C. africana* tentacles are laterally compressed at the base whereas *C. fulgida* tentacles are cylindrical in shape across the entire length (Figure 12). In addition to variations in lappet and tentacle shape, gastrovascular pouch and radial septa shapes also differs.

Tentacular gastrovascular pouches dilate distally and terminate at the cleft between rhopalial and velar lappets in *C. africana* specimens whereas these pouches in *C. fulgida* pouches dilate and contract distally terminating at the periphery of rhopalial lappets (Figure 12). The proximal portion of radial septa of *C. africana* is triangular whereas in *C. fulgida* radial septa are pear-shaped at the base. Another diagnostic feature observed between groups was point of attachment of gonads. Gonads are attached to the periphery of ostia in *C. africana* but are found in thin membranous sacs attached to subumbrellar surface in the central stomach in *C. fulgida.* Statistical analyses therefore reveal considerable morphological dissimilarity, coupled with the different meristic and qualitative morphological features observed; suggest *C. fulgida* and *C. africana* are indeed two distinct species.

For ITS1 a maximum region of 342 nucleotides was amplified from two *C. africana* specimens (Appendix 6). Novel DNA sequence data from ITS1 showed an average of 28.53 % pairwise sequence difference between *C. africana* and *C. fulgida* material described here (Table 13). Dawson and Jacobs (2001) suggest that differences of 5 – 15 % between ITS1 sequences set the standard for species level divergence. Pairwise sequence differences were therefore adequate to designate as two separate species. For COI a maximum region of 720 nucleotides was amplified from *C. africana* specimens. Novel DNA sequence data from COI showed an average of 16.5 % pairwise sequence difference between *C. africana* and *C. fulgida* material described here (Table 17). Dawson and Jacobs (2001) suggest that differences of 10 – 20 % between COI sequences set the standard for species level divergence therefore pairwise sequence differences were adequate to designate *C. fulgida* and *C. africana* as two separate species. Mitochondrial and nuclear DNA data reveal considerable molecular differentiation; in combination with statistical analyses of quantitative

morphological features that demonstrate two distinct morphological groups; unambiguously designate *C. fulgida* and *C. africana* as two distinct and valid species.

The present study therefore confirms the existence of two *Chrysaora* species within the Benguela ecosystem; an unresolved issue since the early 19th century (Reynaud, 1830; Haeckel, 1880; Vanhöffen, 1902; Mayer, 1910; Stiasny, 1934; 1939; Kramp, 1961). Pagès *et al.* (1992) noted an assortment of synonyms and lack of preserved material was responsible for this dilemma. *Chrysaora africana* conflicted with previous descriptions of *C. fulgida* sampled in the Benguela ecosystem (Reynaud, 1830; Haeckel, 1880; Stiasny, 1934). Morphological features of the present *Chrysaora* species under investigation agree with Vanhöffen (1902 as *D. africana*) and Stiasny (1939 as *C. fulgida*). *Chrysaora lactea*, *C. quinquecirrha*, *C. southcotti* and *C. chinensis* are all examples of medusae that also possess forty-eight lappets (and forty tentacles). But a suite of morphological features, as well as geographical distribution (Morandini and Marques, in submission); allow the separation of *C. africana* material described here (Table 8). Records concerning the abundance of *C. africana* are lacking. Vanhöffen (1902), sampled medusae off Namibia, commented that it was only common in the vicinity of Walvis Bay; Stiasny (1939) also examined medusae (presumably *C. africana*) sampled in Walvis Bay but neglected to comment on numbers observed at sea. At present *C. africana* is uncommon in the northern Benguela ecosystem in contrast to the abundant *C. fulgida* (personal observation). Both Reynaud (1830) and Haeckel (1880) sampled *C. hysoscella* in the False Bay area (South Africa) and commented on its great abundance (in thousands) in the southern Benguela ecosystem. The first records however, presumably, of *C. fulgida* in the northern Benguela ecosystem was only in the 1970s (King and O'Toole, 1973; Cram and Visser, 1973) and semi-quantitative analyses following a decade later (Venter, 1988; Fearon *et al.*, 1992). These records suggest *C. fulgida*

has successfully spread from the southern to northern Benguela ecosystem and has sustained high biomasses within this region (Lynam *et al.*, 2006). *Chrysaora fulgida*'s high biomass observed within the northern Benguela ecosystem, in contrast to *C. africana* could be due to a number of factors; including feeding habits, prey selection, behavioural or physiological factors. This study has however highlighted that the genus *Chrysaora* is in dire need of a taxonomic revision.

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Table 1: Morphological features and measurements (S #) of *Chrysaora* sp.1 and *Chrysaora* sp.2 specimens. Specimens were 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). Material was preserved in 5 % formalin in ambient seawater. Figure references are given where applicable. Morphological features excluded on comparative material from the NHM museum specimens are indicated by †. Additional morphological features measured on *Chrysaora* sp.2 are indicated by ‡.

Morphological feature number (MF)	Figure reference number (FRN)	Morphological feature description (measured in g or mm)	MF	FRN	Morphological feature description (measured in g or mm)
S 1	Figure 1	Maximum umbrella diameter	S 22	Figure 1	Length of intact oral arm
S 2		Maximum umbrella height	S 23		Width of oral arm originating from umbrella
S 3		Minimum umbrella height	S 24		Maximum width of oral arm
S 4		Number of octants	S 25		Difference in length from (S 22) to (S 23)
S 5	Figure 1	Number of velar lappets in octant	S 26		Maximum frill width
S 6		Velar lappet length	S 27		Minimum frill width
S 7		Velar lappet width	S 28	Figure 1	Number of primary tentacles
S 8		Rhopalial lappet length	S 29		Number of primary tentacles base stalks
S 9		Rhopalial lappet width	S 30		Width of primary tentacle base
S 10		Tertiary lappet width‡	S 31	Figure 1	Number of secondary tentacles
S 11		Tertiary lappet length‡	S 32		Width of secondary tentacle base
S 12		Number of gastrovascular pouches	S 33		Number of tertiary tentacles‡
S 13		Width of oral opening pillar	S 34		Width of tertiary tentacle base‡
S 14		Diameter of oral opening	S 35	Figure 1	Number of rhopalia
S 15		Manubrium length	S 36		Gonad width
S 16		Manubrium depth	S 37		Gonad length
S 17		Number of ostia	S 38		Presence of sperm sacs
S 18	Figure 1	Inter-ostia width	S 39	Figure 1	Presence of exumbrella warts
S 19	Figure 1	Width of ostia	S 40	Figure 1	Gastric pouch shape
S 20		Length of ostia	S 41	Figure 1	Marginal lappet shape
S 21		Number of oral arms			

Table 2: Details of the comparative material examined at the Natural History Museum, London.

Species	Specimen Collection No.	Collection Locality	Collector
Chrysaora hysoscella	25.8.11.1	Haven Gove Creek, Kent	FJ Lambert
C. hysoscella	25.8.11.1	Haven Gove Creek, Kent	FJ Lambert
C. hysoscella	26.3.10.21	Leigh Creek	FJ Lambert
C. hysoscella	27.1.18.8	-	FJ Lambert
C. hysoscella	1934.8.20.1	On a beach, Sea View	ES de Butts-Tavernes
C. hysoscella	1982.11.30.276	Sula Plymouth Laboratory	FS Russell
C. hysoscella	1982.11.30.276	Sula Plymouth Laboratory	FS Russell
C. hysoscella	1982.11.30.276	Sula Plymouth Laboratory	FS Russell
C. hysoscella	1982.11.30.276	Sula Plymouth Laboratory	FS Russell
C. hysoscella	1982.11.30.276	Sula Plymouth Laboratory	FS Russell
C. hysoscella	1982.11.30.276	Sula Plymouth Laboratory	FS Russell
C. hysoscella	1982.11.30.276	Sula Plymouth Laboratory	FS Russell
C. hysoscella	1982.11.30.307	-	FS Russell
C. hysoscella	1983.8.5.1	Margate, Kent	SJ Moore
C. hysoscella	1983.7.28.2	Ramsgate, Kent	SJ Moore
C. hysoscella	98.5.7.2	Salcombe, Devon	Norman Collection
C. africana	1987.10.8.1	St. Paul River, Mohrovia, Liberia	C Betterton
C. quinquechirrha	09.8.23.1	Lagoon of Lagos	GE Bruce
C. quinquechirrha	31.8.11.3-4	?	By exchange with Rÿks Museum van Natuurtijke Historie (G Stiasny)
C. quinquechirrha	31.8.11.3-4	?	By exchange with Rÿks Museum van Natuurtijke Historie (G Stiasny)
C. quinquechirrha	32.2.6.16	?	By exchange with G Stiasny
C. fulgida	1937.7.19.344	Saldanha Bay, South Africa	-
C. lactea	1997.998-999	Valeria del Mar (Pinamar), Argentina	H Mianzan
C. lactea	1997.1001	Pto. Ingro. White, Argentina	H Mianzan

Table 3: PCR conditions used to amplify cytochrome *c* oxidase subunit I (COI) and internal transcribed spacer one (ITS1) from Namibian *Chrysaora* sp.1 and *Chrysaora* sp.2 and United Kingdom (UK) *C. hysoscella* specimens (adapted from Daryanabard and Dawson, 2008).

Number of Cycles	PCR steps	*Chrysaora* sp.1		*Chrysaora* sp.2		UK *C. hysoscella*
		CO1	ITS1	CO1	ITS1	ITS1
One	Initial denaturation	8 min at 94 °C	8 min at 94 °C	8 min at 94 °C	8 min at 94 °C	8 min at 94 °C
	Annealing	2 min at 54.2 °C	2 min at 51.5 °C	2 min at 49 °C	2 min at 51.5 °C	2 min at 51.5 °C
	Extension	2 min at 72 °C	2 min at 72 °C	2 min at 72 °C	2 min at 72 °C	2 min at 72 °C
	Denaturation	4 min at 94 °C	4 min at 94 °C	4 min at 94 °C	4 min at 94 °C	4 min at 94 °C
One	Annealing	2 min at 55.2°C	2 min at 52.5°C	2 min at 50°C	2 min at 52.5°C	2 min at 52.5°C
	Extension	2 min at 72 °C	2 min at 72 °C	2 min at 72 °C	2 min at 72 °C	2 min at 72 °C
	Denaturation	45 sec at 94 °C	45 sec at 94 °C	45 sec at 94 °C	45 sec at 94 °C	45 sec at 94 °C
Thirty-three	Annealing	45 sec at 56.2 °C	45 sec at 53.5 °C	45 sec at 51 °C	45 sec at 53.5 °C	45 sec at 53.5 °C
	Extension	1 min at 72 ° C	1 min at 72 ° C	1 min at 72 ° C	1 min at 72 ° C	1 min at 72 ° C
	Final extension	5 min at 72 °C	5 min at 72 °C	5 min at 72 °C	5 min at 72 °C	5 min at 72 °C
Final hold		4 °C	4 °C	4 °C	4 °C	4 °C

Table 4: Morphological features (raw data) of *Chrysaora fulgida* and *C. africana* (collected on the "Goby and Hake Cruise", 2008 conducted on the R.V. *G.O. Sars*) correlated with specimen size (S 1) using Pearsons product-moment correlation test. *Chrysaora fulgida* correlations significant at $p \leq 0.003$ and *C. africana* correlations at $p \leq 0.005$ after Bonferroni corrections; indicated by *).

MF	*C. fulgida*			*C. africana*		
	R	N	*p*	R	N	*p*
S 2	0.64	40	≤ 0.001*	0.34	16	0.19
S 3	0.20	40	0.22	0.53	15	0.04
S 6	-	-	-	0.65	16	0.006
S 8	-	-	-	0.46	16	0.08
S 10	-	-	-	0.86	16	≤ 0.001*
S 11	-	-	-	0.54	16	0.03
S 12	0.00	40	1	0.00	16	1
S 13	0.88	39	≤ 0.001*	-	-	-
S 15	0.99	39	≤ 0.001*	0.93	16	≤ 0.001*
S 16	0.88	32	≤ 0.001*	0.56	16	0.02
S 17	0.00	40	1	0.00	16	1
S 18	0.96	39	≤ 0.001*	0.51	16	0.04
S 21	0.00	40	1	0.00	16	1
S 24	0.97	40	≤ 0.001*	0.86	14	≤ 0.001*
S 25	0.93	40	≤ 0.001*	0.78	14	≤ 0.001*
S 26	0.85	38	≤ 0.001*	-	-	-
S 27	0.93	34	< 0.05*	-	-	-
S 32	0.84	5	0.07	0.66	12	0.02
S 33	-	-	-	-0.08	16	1
S 34	-	-	-	0.46	12	0.13
S 37	0.97	40	≤ 0.001*	-	-	-

Table 5: Morphological features (raw data) of *Chrysaora fulgida* and *C. africana* (collected on the "Goby and Hake Cruise" 2008, conducted on the R.V. *G.O. Sars*) correlated with size of specimens (S 1) using Spearmans rank correlation test (R values are reported, *C. fulgida* and *C. africana* correlations significant at $p \leq 0.003$ after Bonferroni corrections; indicated by *).

MF	*C. fulgida*	*C. africana*
S 4	0.23	-0.44
S 5	-0.08	-0.02
S 7	0.9*	0.64
S 9	0.9*	0.64
S 13	0.77*	-
S 14	0.79*	0.84*
S 19	0.15	0.83*
S 20	0.34	0.75*
S 22	0.78*	1
S 23	0.59*	0.8*
S 28	-0.15	-0.5
S 29	0.52	-
S 30	0.9*	0.64
S 31	-0.16	0.42
S 35	0.23	-0.65
S 36	0.73*	-

Table 6: Standardized morphometric data (logged ratios) of *Chrysaora fulgida* and *C. africana* (collected on the "Goby and Hake Cruise", 2008 conducted on the R.V. *G.O. Sars*) correlated with specimen size (S 1) using Pearsons product-moment correlation test. *Chrysaora fulgida* correlations significant at $p \leq 0.004$ and *C. africana* correlations at $p \leq 0.003$ after Bonferroni corrections; indicated by *). All merisitc features were excluded as previous correlations illustrated no significant relationship with varying specimen size.

MF	*C. fulgida*			*C. africana*		
	R	N	*p*	R	N	*p*
S 2	-0.42	40	0.14	-0.23	16	0.40
S 3	-0.15	40	0.36	-0.31	15	0.27
S 6	-	-	-	-0.67	16	0.004
S 7	0.58	18	0.01	0.04	16	0.90
S 8	-	-	-	-0.76	16	≤ 0.001*
S 10	-	-	-	-0.14	16	0.60
S 11	-	-	-	-0.65	16	0.006
S 14	-0.62	40	≤ 0.001*	-0.24	16	0.36
S 19	-0.87	40	≤ 0.001*	-0.28	16	0.29
S 20	-0.87	40	≤ 0.001*	0.01	16	0.98
S 22	-0.06	29	0.77	0.91	5	0.03
S 23	-0.29	40	0.07	0.49	15	0.07
S 25	0.30	40	0.06	0.30	14	0.30
S 26	-0.07	38	0.68	-	-	-
S 27	0.32	34	0.06	-	-	-
S 30	-0.80	5	0.10	-0.71	12	0.009
S 32	0.08	5	0.90	-0.26	12	0.42
S 34	-	-	-	-0.56	12	0.06
S 37	-0.16	40	0.31	-	-	-

Table 7: Standardized morphometric data (logged ratios) of *Chrysaora fulgida* and *C. africana* (collected on the "Goby and Hake Cruise" 2008, conducted on the R.V. *G.O. Sars*) correlated with size of specimens (S 1) using Spearmans rank correlation test (R values are reported, *C. fulgida* correlations significant at $p \leq 0.006$ and *C. africana* correlations at $p \leq 0.008$ after Bonferroni corrections; indicated by *). All merisitc features were excluded as previous correlations illustrated no significant relationship with varying specimen size.

MF	*C. fulgida*	*C. africana*
S 9	-0.13	-0.46
S 13	-0.34	-
S 15	-0.27	0.12
S 16	0.19	-0.41
S 18	0.19	-0.6
S 19	-0.83	-0.26
S 24	-0.16	0.26
S 36	-0.21	-

Table 8: A matrix of morphological features compared amongst described *Chrysaora* species and the material presently under investigation (*Chrysaora* sp.1 and *Chrysaora* sp.2). Table adapted from Gershwin and Collins (2002); additional morphological information sourced from Morandini and Marques (in submission). *Chrysaora fulgida* comparison based solely on holotype description given by Morandini and Marques (in submission) as well as Reynaud (1830), Haeckel (1880), Stiasny (1934) and examination of specimen 1937.7.19.344 at the Natural History Museum, London. *Chrysaora africana* comparison based on Vanhöffen (1902) and Stiasny (1939, including Abb. 2-7).

	Chrysaora species examined																
Morphological feature	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Rhopalia number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhopalial pits	1	1	1	1	1	1	1	1	1	1	?	0	?	1	1	1	1
Distal septa shape	{1,2}	2	1	{0,1}	2	{0,2}	{1,2}	1	2	2	1	?	?	3	4	3	4
Septa termination	0	0	0	0	0	0	0	0	0	0	0	?	?	0	2	0	2
Spiral oral arms	1	1	0	0	{0-1}	0	0	0	1	0	?	0	0	0	0	0	0
Manubrium length	1	0	0	0	0	0	0	0	1	?	?	?	?	?	0	0	0
Tentacle number	2	2	2	{2-4}	2	{2-5}	2	{2-4}	0	{2-4}	{2,3}	4	{2-3}	2	4	2	4
Lappet number	2	2	2	{2-4}	2	{2-5}	2	{2-4}	2	4	2	4	{1-4?}	2	4	2	4
Lappets in size classes	0	0	0	0	0	1	0	1	0	1	0	1	1	0	1	0	1
Warts/papillae	0	0	0	0	0	0	0	0	0	0	?	1	?	0	1	0	?
Maximum umbrella diameter observed check	2	1	1	1	2	0	2	1	2	0	0	0	0	2	1	2	1
Dominant colour	0	1	1	2	1	1	1	{1,2}	0	1	2	1	?	1	0	1	0
Star-shaped exumbrellar mark	1	1	1	1	1	1	1	1	1	1	0	0	?	1	1	1	1
Quadralinga	1	0	0	0	0	0	1	0	1	0	?	?	?	0	?	0	0
Gonad within pouch	1	1	1	?	1	1	1	1	1	?	?	?	?	?	?	1	0
Gonad shape	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0
Rhopalium condition	0	1	1	?	1	1	1	0	0	{0-1}	1	?	?	1	1	1	1

Taxa: (1) *Chrysaora achylos*; (2) *C. fuscescens*; (3) *C. hysoscella*: (4) *C. lactea*: (5) *C. melanaster*; (6) *C. pacifca*; (7) *C. plocamia*; (8) *C. quinquecirrha*; (9) *C. colorata*; (10) *C. chinensis*; (11) *C. kynthia*; (12) *C. southcotti*; (13) *C. wurlerra*; (14) *C. fulgida*; (15) *Chrysaora* sp.1; (16) *Chrysaora* sp.2 . Characters: (1) rhopalia number: 0 = 8, 1 = 16; (2) Rhopalia pits: 0 = shallow, 1 = deep, 2 = absent; (3) septa shape: 0 = straight, 1 = bent, 2 = s-shaped, 3 = kinked at extreme end, 4 = pear-shaped; (4) septa termination: 0 = near tentacle, 1 = near rhopalium, 2 = between; (5) spiral oral arms: 0 = no, 1 = yes; (6) manubrium length: 0 = elongated, 1 = short; (7) tentacle number: 0 = 8, 1 = 16, 2 = 24,3 = 32; 4 = 40; (8) lappet number: 0 = 24, 1 = 32, 2 = 48; (9) lappets in size classes: 0 = no, 1 = yes; (10) warts/papillae: 0 = inconspicuous, 1 = conspicuous; (11) maximum bell diameter: 0 = < 20 cm, 1 = 20 - 40 cm, 2 = 41 - 100 cm; (12) dominant colour: 0 = purple, 1 = brown/red, 2 = colourless; (13) exumbrellar marks: 0 = no star, 1 = star; (14) quadralinga: 0 = indistinct/absent, 1 = distinct; (15) gonads within pouch: 0 = no, 1 = yes; (16) gonad shape: 0 = semi-circular ring, 1 = inverted W, 2 = M-shaped; (17) rhopalial condition: 0 = rhopalial lappets overlapping (closed) rhopalium, 1 = open.

Table 9: Results of the two-tailed *t*-test illustrating differences of standardized morphometric data (MF) common to *Chrysaora fulgida* collected on the "Goby and Hake Cruise" 2008, conducted on the R.V. *G.O. Sars* and *C. hysoscella* examined at the Natural History Museum (NHM) (relationships significantly different at $p \leq 0.0045$ after Bonferroni corrections; indicated by *).

MF	*C. fulgida*			*C. hysoscella*					
	Valid N	Mean	Std.Dev.	Valid N	Mean	Std.Dev.	df	*p*	t-value
S 2	14	-1.31	0.09	14	-1.45	0.17	13	= 0.001*	1.29
S 3	40	-2.02	0.32	14	-2.36	0.24	52	= 0.001*	3.69
S 7	18	-1.08	0.04	16	-1.19	0.08	32	= 0.001*	5.66
S 14	40	-0.75	0.08	14	-0.60	0.09	52	= 0.001*	-5.63
S 20	40	-1.03	0.15	14	-1.08	0.11	52	0.25	1.16
S 22	29	0.22	0.09	-	-	-	27	-	-
S 23	40	-0.69	0.12	12	-0.80	0.09	50	0.0047	2.96
S 25	40	-0.94	0.11	9	-0.97	0.13	47	0.42	0.82
S 26	38	-1.35	0.12	1	-1.26	0.00	37	0.46	-0.75
S 27	34	-1.92	0.13	-	-	-	32	-	-
S 30	5	-1.64	0.03	16	-1.52	0.07	19	0.002*	-3.64
S 32	5	-1.97	0.09	16	-1.75	0.20	19	0.03	-2.35
S 37	40	-0.88	0.05	13	-0.73	0.07	51	= 0.001*	-7.84

Table 10: Mann-Whitney-U results illustrating differences of standardized morphometric data (MF) between *Chrysaora fulgida* collected on the "Goby and Hake Cruise", 2008 and *C. hysoscella* examined at the Natural History Museum (NHM) (relationships significantly different significant at $p \leq 0.006$ after Bonferroni corrections; indicated by *).

MF	*C. fulgida* Rank Sum	Valid N	*C. hysoscella* Rank Sum	Valid N	U	Z	Z adjusted	p-level	2*1sided exact p
S 9	422.00	18	173.00	16	37.00	3.69	3.69	= 0.001*	= 0.001
S 13	842.00	39	536.00	13	62.00	-4.05	-4.05	= 0.001*	= 0.001
S 15	902.00	39	529.00	14	122.00	-3.05	-3.05	0.002*	0.002
S 16	808.00	32	273.00	14	168.00	1.34	1.34	0.18	0.19
S 18	831.00	39	600.00	14	51.00	-4.48	-4.48	= 0.001*	= 0.001
S 19	1031.00	40	454.00	14	211.00	-1.36	-1.36	0.17	0.18
S 24	1116.00	40	109.00	9	64.00	3.00	3.00	0.003*	0.002
S 36	833.00	40	598.00	13	13.00	-5.11	-5.11	= 0.001*	= 0.001

Table 11: Standardized morphometric data (MF) that contributed to dissimilarity between *Chrysaora fulgida* and *C. hysoscella* specimens examined at the Natural History Museum (NHM); *C. fulgida* and *C. africana* collected on the "Goby and Hake Cruise", 2008 as determined by SIMPER analysis.

Groups	MF	contribution %	cumulative %
Chrysaora fulgida vs. *C. hysoscella*	S 2	9.95	67.32
	S 3	15.56	44.86
	S 9	1.83	91.33
	S 13	12.51	57.37
	S 14	29.3	29.3
	S 16	4.26	82.66
	S 19	2.89	85.55
	S 20	2.07	87.62
	S 23	1.88	89.5
	S 32	5.24	78.40
	S 36	5.83	73.16
Chrysaora fulgida vs. *C. africana*	S 2	11.85	51.24
	S 3	8.18	70.33
	S 7	14.74	14.74
	S 9	12.19	39.39
	S 13	3.14	89.88
	S 16	10.91	62.15
	S 18	2.83	92.71
	S 19	12.47	27.21
	S 20	3.44	83.56
	S 22	5.31	75.64
	S 23	3.18	86.74
	S 30	4.48	80.12

Table 12: Canonical Analysis of Principal Co-ordinates of standardized morphometric data showing number of individuals from *Chrysaora fulgida* collected on the "Goby and Hake Cruise", 2008 and *C. hysoscella* examined at the Natural History Museum (NHM) (left column) assigned to each species (top column). Permutation test statistic reported (species significantly different at $p < 0.005$).

Species	*C. fulgida*	*C. hysoscella*	Sample size	% correctly allocated to respective species	Mis-calculation error	*p* value
C. fulgida	40	0	40	100	1.79 %	0.001
C. hysoscella	1	15	16	93.75		

Table 13: Uncorrected pairwise distance matrix for internal transcribed spacer one (ITS1) fragments from specimens of *Chrysaora fulgida* (1 – 5) and *C. africana* (9, 10) collected on the "Goby and Hake Cruise", 2008, off the coast of Namibia and *C. hysoscella* specimens sampled off the coast of the United Kingdom (6 – 8).

	1	2	3	4	5	6	7	8	9	10
1	-									
2	0.000	-								
3	0.003	0.003	-							
4	0.003	0.003	0.000	-						
5	0.000	0.000	0.003	0.003	-					
6	0.042	0.042	0.039	0.039	0.042	-				
7	0.004	0.042	0.039	0.039	0.000	0.042	-			
8	0.042	0.042	0.039	0.039	0.000	0.042	0.000	-		
9	0.272	0.272	0.268	0.268	0.280	0.272	0.280	0.280	-	
10	0.301	0.301	0.298	0.298	0.310	0.301	0.310	0.310	0.028	-

Table 14: Results of the two-tailed *t*-test illustrating differences of standardized morphometric data (MF) common to *Chrysaora fulgida* and *C. africana* collected on the "Goby and Hake Cruise" 2008, conducted on the R.V. *G.O. Sars*, preserved in 5% formalin in ambient seawater (relationships significantly different significant at $p \leq 0.0045$ after Bonferroni corrections; indicated by *).

MF	*C. fulgida*			*C. africana*							
	Valid N	Mean	Std.Dev.	Valid N	Mean	Std.Dev.	df	F-ratio Variances	p Variances	p	t-value
S 2	16	-1.31	0.09	16	-1.30	0.20	15	3.36	0.01	0.41	-0.28
S 3	40	-2.02	0.32	15	-1.98	0.13	53	6.05	0.00	0.65	-0.45
S 6	-	-	-	16	-1.61	0.10	14	-	-	-	-
S 7	18	-1.08	0.04	16	-1.52	0.10	32	6.59	0.00	≤ 0.001*	17.63
S 8	-	-	-	16	-1.56	0.13	14	-	-	-	-
S 10	-	-	-	16	-1.33	0.07	14	-	-	-	-
S 11	-	-	-	16	-1.56	0.11	14	-	-	-	-
S 14	40	-0.75	0.08	16	-0.57	0.07	54	1.29	0.61	≤ 0.001*	-7.78
S 20	40	-1.03	0.15	16	-0.90	0.10	54	2.18	0.10	0.0046	-2.95
S 22	29	0.22	0.09	5	0.46	0.13	32	2.36	0.16	≤ 0.001*	-5.21
S 23	40	-0.69	0.12	15	-0.71	0.19	53	2.47	0.03	0.61	0.52
S 25	40	-0.94	0.11	14	-0.84	0.13	52	1.47	0.35	0.007	-2.83
S 26	38	-1.35	0.12	-	-	-	36	-	-	-	-
S 27	34	-1.92	0.13	-	-	-	32	-	-	-	-
S 30	5	-1.64	0.03	12	-1.82	0.11	15	14.48	0.02	0.0043*	3.35
S 32	5	-1.97	0.09	12	-1.96	0.11	15	1.59	0.70	0.88	-0.16
S 34	-	-	-	12	-1.91	0.13	10	-	-	-	-
S 37	40	-0.88	0.05	-	-	-	38	-	-	-	-

Table 15: Mann-Whitney-U results illustrating differences of standardized morphometric data (MF) common to *Chrysaora fulgida* and *C. africana* collected on the "Goby and Hake Cruise" 2008, conducted on the R.V. *G.O. Sars*, preserved in 5% formalin in ambient seawater (relationships significantly different significant at $p \leq 0.008$ after Bonferroni corrections; indicated by *).

	C. fulgida		*C. africana*						2*1sided
MF	Rank Sum	Valid N	Rank Sum	Valid N	U	Z	Z adjusted	p-level	exact p
S 9	459.00	18	136.00	16	0.00	4.97	4.97	≤ 0.001*	0.00
S 15	1275.00	39	265.00	16	129.00	3.39	3.39	≤ 0.001*	0.0004
S 16	542.00	32	634.00	16	14.00	-5.29	-5.29	≤ 0.001*	0.00
S 18	1306.00	39	234.00	16	98.00	3.97	3.97	≤ 0.001*	0.00002
S 19	820.00	40	776.00	16	0.00	-5.81	-5.81	≤ 0.001*	0.00
S 24	968.00	40	517.00	14	148.00	-2.61	-2.61	0.009	0.01

Table 16: Canonical Analysis of Principal Co-ordinates of standardized morphometric data showing number of individuals from *Chrysaora fulgida* and *C. africana* collected on the "Goby and Hake Cruise", 2008 (left column) assigned to each species (top column). Permutation test statistic reported (species significantly different at $p < 0.005$).

Species	*C. fulgida*	*C. africana*	Sample size	% correctly allocated to respective species	Mis-calculation error	*p* value
C. fulgida	40	0	40	100	0 %	0.001
C. africana	0	16	16	100		

Table 17: Uncorrected pairwise distance matrix cytochrome *c* oxidase subunit I (COI) fragments from specimens of *Chrysaora fulgida* (1 and 2) and *C. africana* (3 and 4) collected on the "Goby and Hake Cruise", 2008, off the coast of Namibia.

	1	2	3	4
1	-			
2	0.010	-		
3	0.16657	0.16514	-	
4	0.16495	0.16353	0.003	-

Figure Captions

Figure 1: A schematic diagram of the subumbrellar view and exumbrellar view (top right) of a *Chrysaora* specimen. Numerous morphological measurements are indicated (S #, see Table 1). Oral arms, gonads and tentacles are represented (adapted from Morandini and Marques, in submission).

Figure 2: Photographs of live *Chrysaora fulgida* in the northern Benguela ecosystem illustrating colour pattern variation between small and large specimens. Umbrella with trailing oral arms showing typical colour variation for small medusae (A) and larger medusae (B) (©Kolette Grobler, MFMR, Lüderitz, Namibia).

Figure 3: Photographs of *Chrysaora fulgida* found in the northern Benguela ecosystem illustrating colour pattern variation of larger medusae (in the wild) with sixteen radially distributed triangles (A) (©Kolette Grobler, MFMR, Lüderitz, Namibia) and a preserved medusa without (B) (©Simone Neethling).

Figure 4: Photographs of preserved *Chrysaora fulgida* collected on the "Goby and Hake Cruise", 2008 in the northern Benguela ecosystem illustrating colour pattern variation of between large (A) and small specimens (B) (©Simone Neethling).

Figure 5: Subumbrellar view of preserved *Chrysaora fulgida* (A, stained with rose-bengal) collected on the "Goby and Hake Cruise", 2008 in the northern Benguela ecosystem illustrating pear shaped proximal portion of radial septa and shapes of tentacular and rhopalial gastrovascular pouches. Enlarged photograph showing radial septum contracting then dilating distally (B), and fusing at the periphery of a rhopalial lappet (a); position of secondary tentacle where lateral protrusion originates from subumbrella between a rhopalial and velar lappet (b); periphery of marginal lappets free of gastrovascular canals (c); a fully developed primary tentacle situated in a deep cleft between velar lappets (d) (©Simone Neethling).

Figure 6: Non-metric multi-dimensional scaling (MDS) ordination of standardized morphometric data between *Chrysaora hysoscella* (black triangles) examined at the Natural History Museum (NHM) and *C. fulgida* (grey triangles) collected on the "Goby and Hake Cruise", 2008 in the northern Benguela ecosystem (stress value indicated).

Figure 7: Photographs of preserved *Chrysaora fulgida* collected on the "Goby and Hake Cruise", 2008 in the northern Benguela ecosystem (A-stained smaller specimen; B-larger specimen) showing thin, elongated manubrium forming four rounded subgenital ostia and a short continuous tube from which four oral arms arise. Pear shaped proximal portion of radial septa is also shown (A). The manubrium of *C. hysoscella* (C), examined at the Natural History Museum is thickened at the proximal portion forming four basal oral arm pillars, resembling a four-leafed clover (©Simone Neethling).

Figure 8: Photographs illustrating the difference in shape of rhopalial gastrovascular pouches when comparing *C. hysoscella* (specimen number: 25.8.11.1) from the Natural History Museum (NHM) and *Chrysaora fulgida* collected on the Goby and Hake Cruise, 2008 in the Benguela ecosystem. Rhopalial gastrovascular pouches of NHM *C. hysoscella* contract distally (A) whereas in Namibian *C. fulgida* (photograph shows a stained specimen) contract then dilate distally fusing at the periphery of rhopalial lappets (B) (©Simone Neethling).

Figure 9: *Chrysaora hysoscella* specimen (specimen number: 25.8.11.1) examined at the Natural History Museum showing male gonads (sperm sacs) indicated by arrows. A subumbrellar (A) and exumbrellar (B) view of sperm sacs on the lappet region; sperm sacs on oral arm (C) (©Simone Neethling). When examining *C. hysoscella* specimens, Russell (1970), identified sperm sacs on the gastrovascular cavity and on the oral arms. Morandini and Marques (in submission) also noted male gonads on the gastrovascular cavity on preserved *C. hysoscella* specimens.

Figure 10: A photograph of live *Chrysaora africana* collected on the "Goby and Hake Cruise", 2008 in the northern Benguela ecosystem illustrating bell with radially distributed purple triangles, dark purple lappets and trailing tentacles (©Simon Elwen, Namibian Dolphin project).

Figure 11: Photographs of *Chrysaora africana* collected on the "Goby and Hake Cruise", 2008 in the northern Benguela ecosystem showing differences in colour of live (A) and preserved (B) (©Simone Neethling) specimen.

Figure 12: Photographs illustrating difference in gastrovascular shape, lappet and tentacle number between *Chrysaora fulgida* and *C. africana* collected on the "Goby and Hake Cruise", 2008 in the northern Benguela ecosystem. *Chrysaora fulgida* possess four lappets and up to three tentacles, cylindrical in shape, per octant (A) whereas *C. africana* possess six lappets and up to five tentacles, laterally compressed at the base, per octant (B). The tentacular gastrovascular pouch of *C. fulgida* dilate and contract distally (C) whereas in *C. africana* dilate distally (B) (©Simone Neethling).

Figure 13: Photograph of *Chrysaora africana* collected on the "Goby and Hake Cruise", 2008 in the northern Benguela ecosystem showing cruciform shape of oral opening (©Simone Neethling).

Figure 14: Non-metric multi-dimensional scaling (MDS) ordination of standardized morphometric data showing morphological dissimilarity among *Chrysaora fulgida* (grey triangles) and *C. africana* (black triangles) collected on the "Goby and Hake Cruise", 2008 in the northern Benguela ecosystem (stress value indicated).

Figure 1

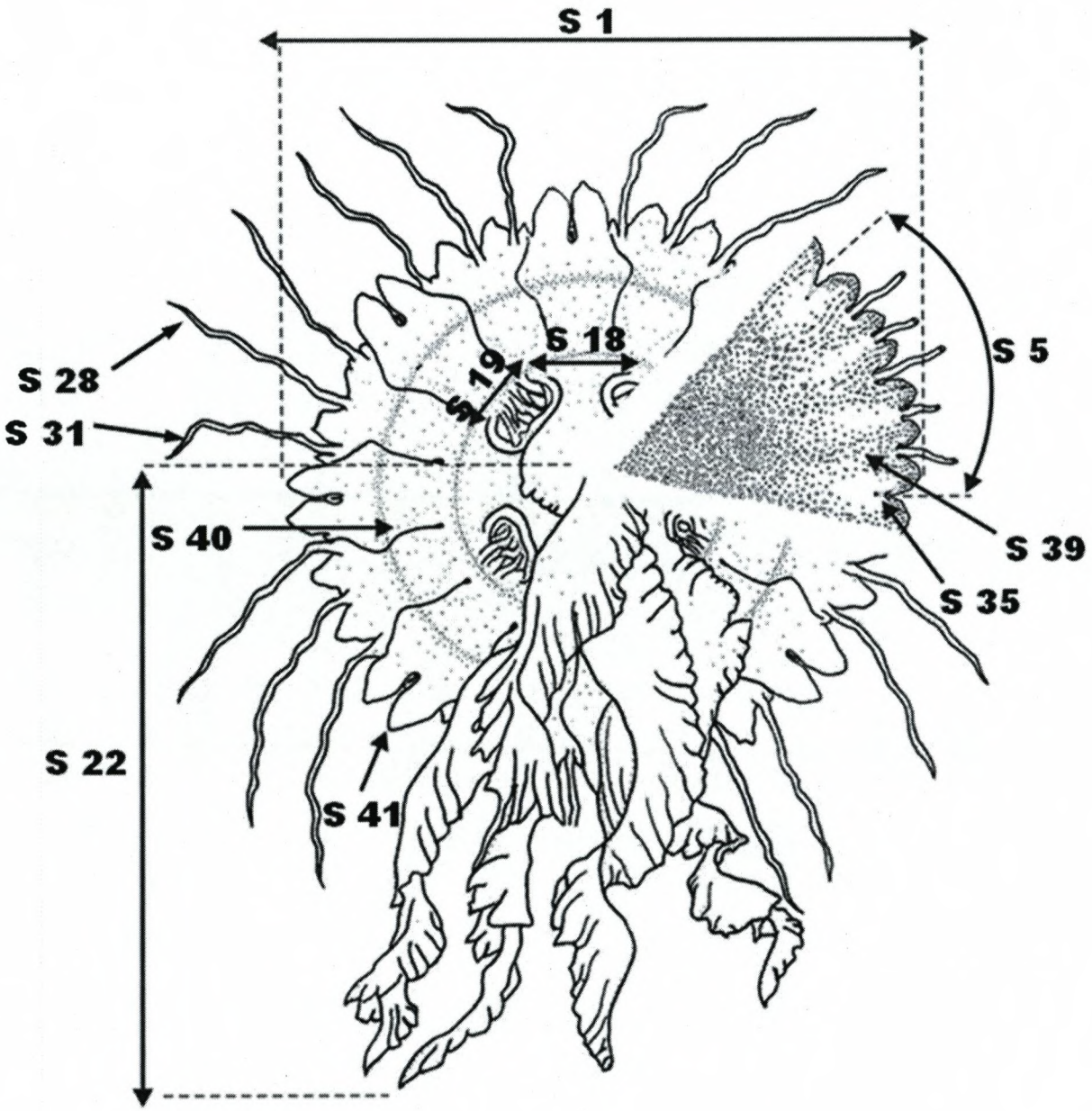


Figure 2

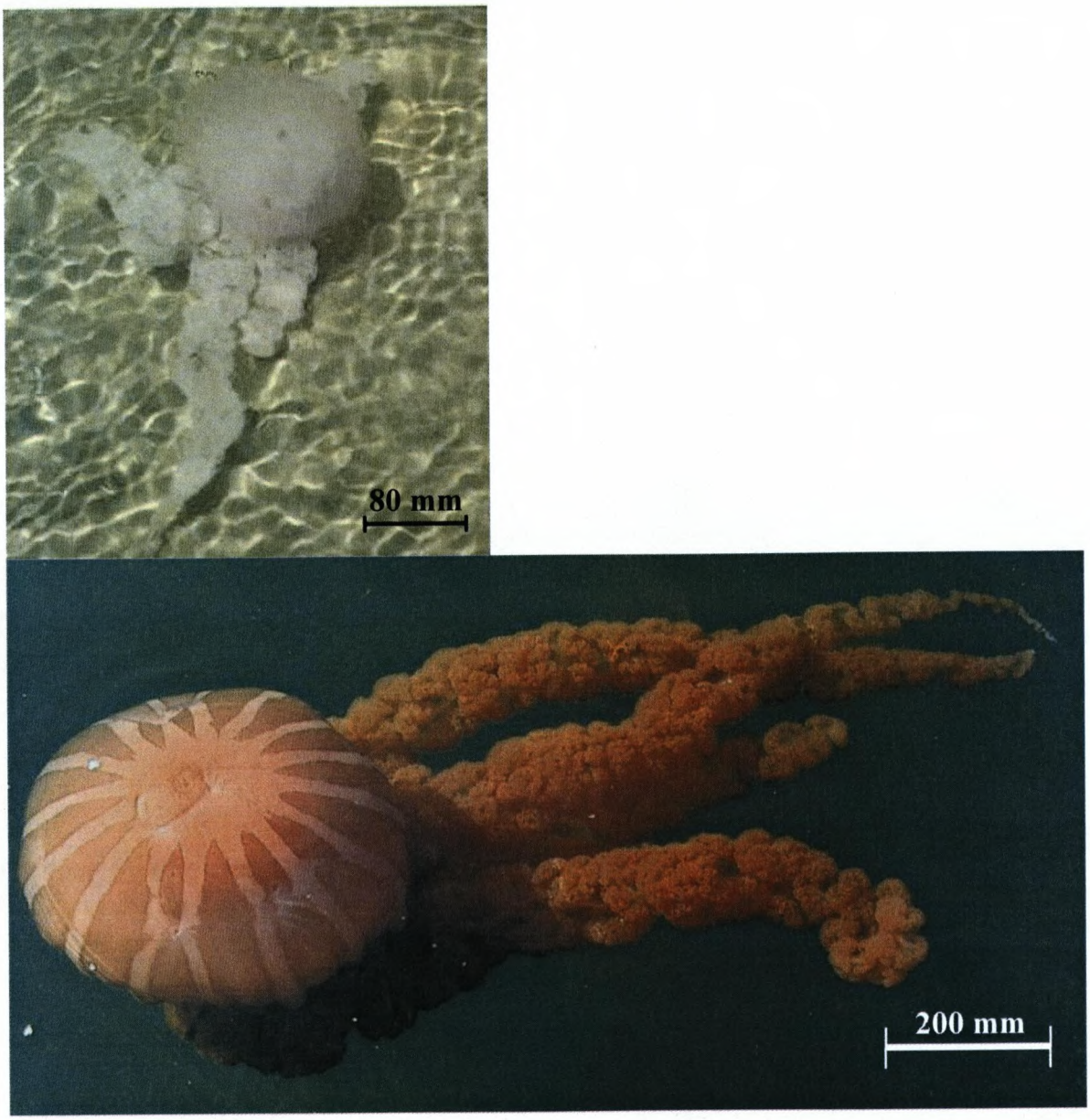


Figure 3

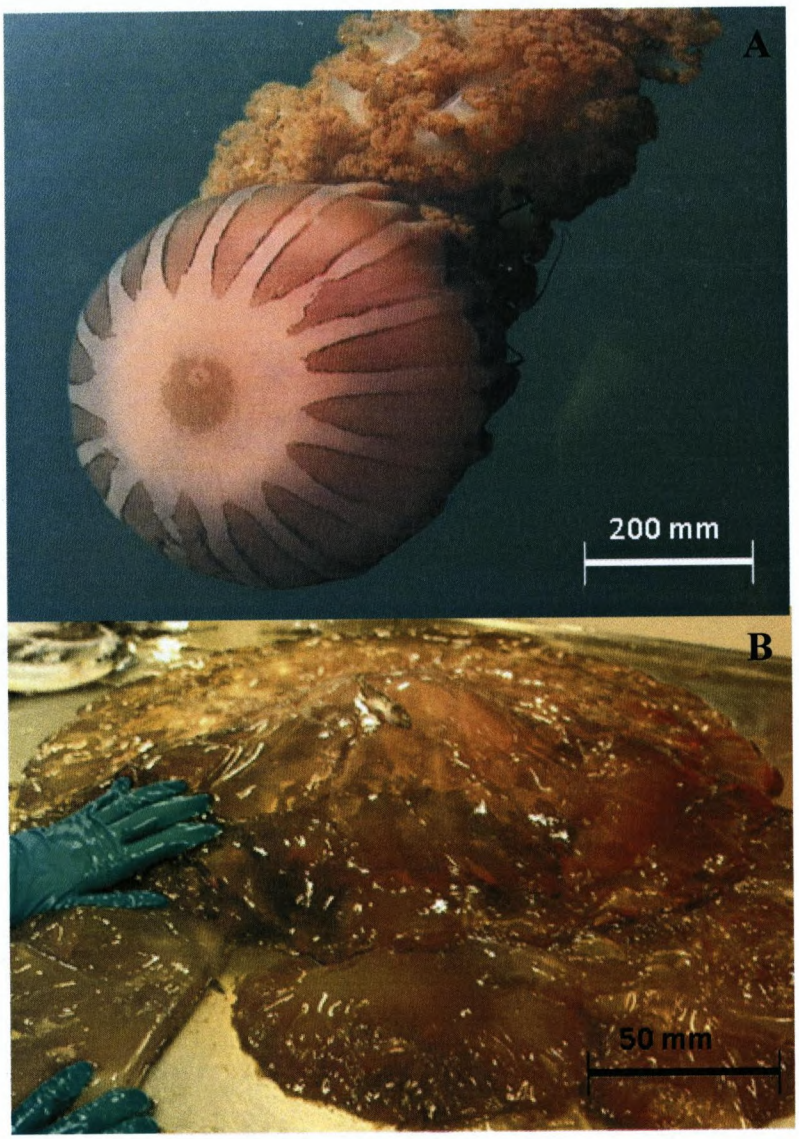


Figure 4

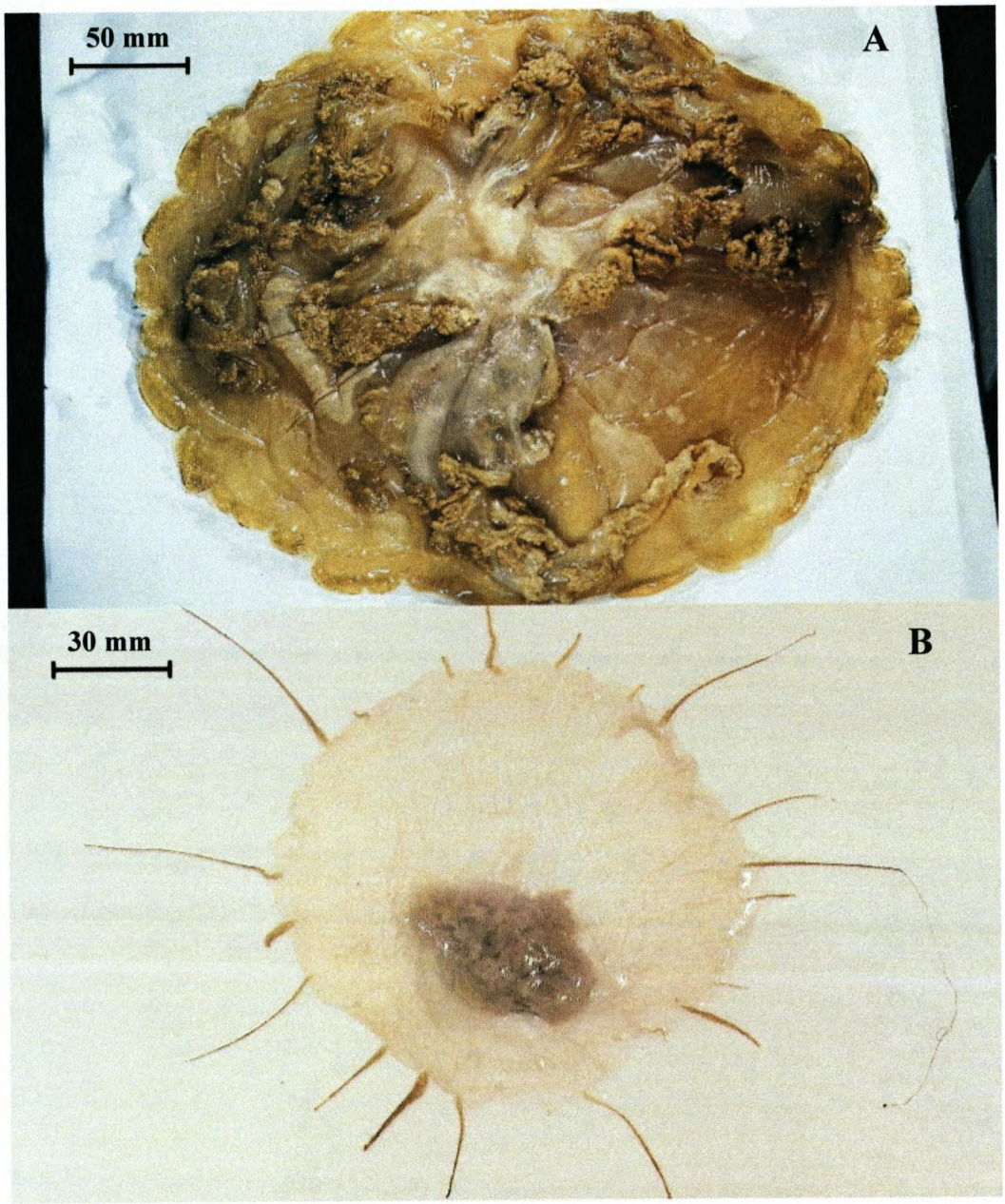


Figure 5

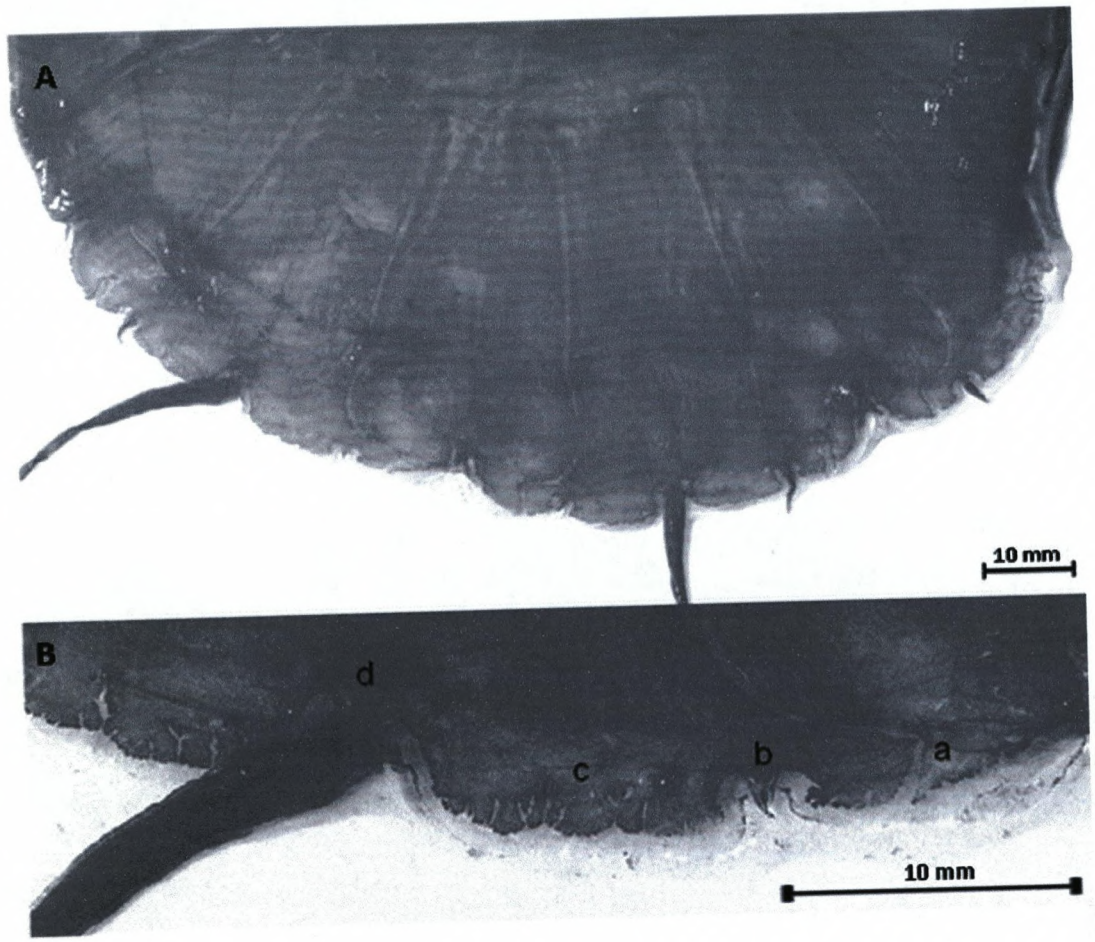


Figure 6

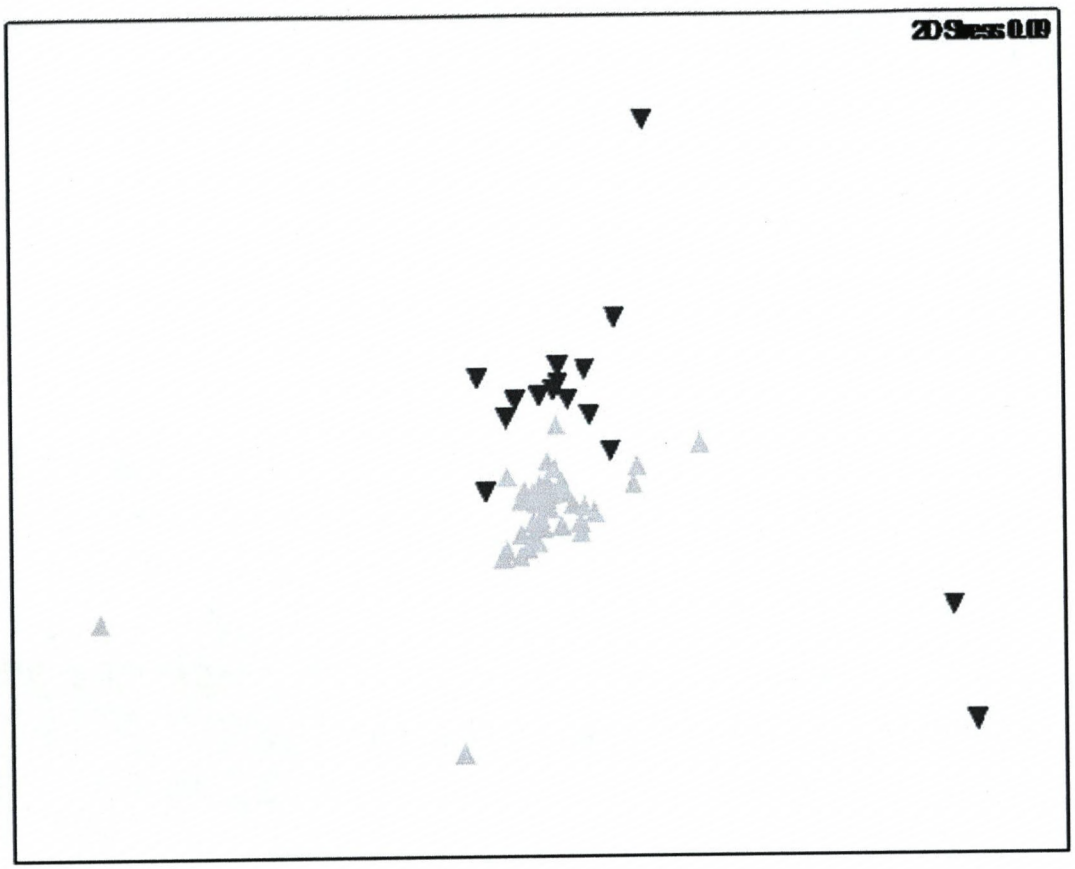
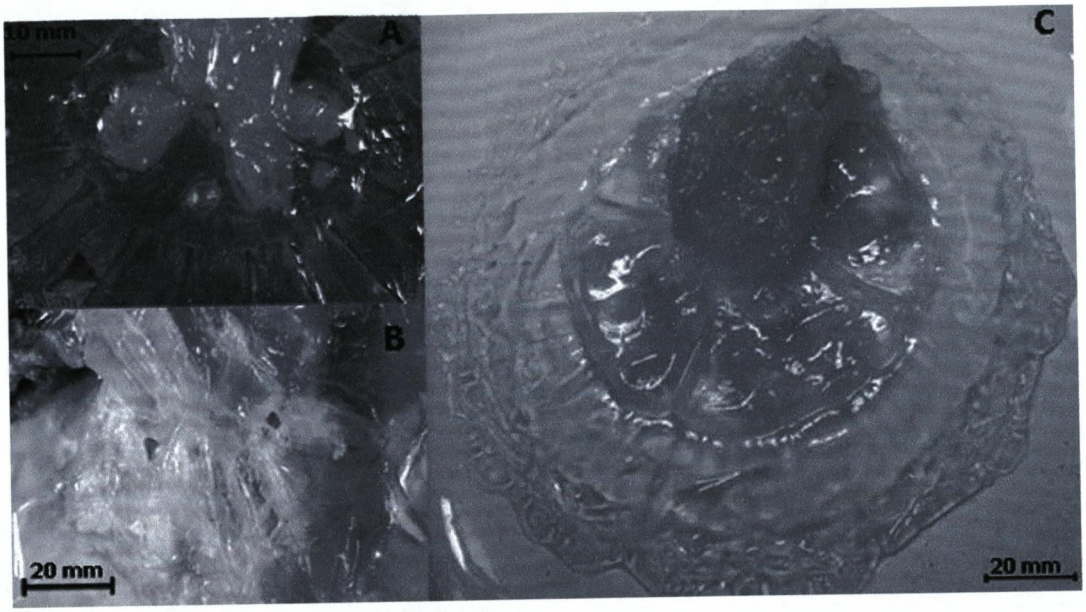


Figure 7



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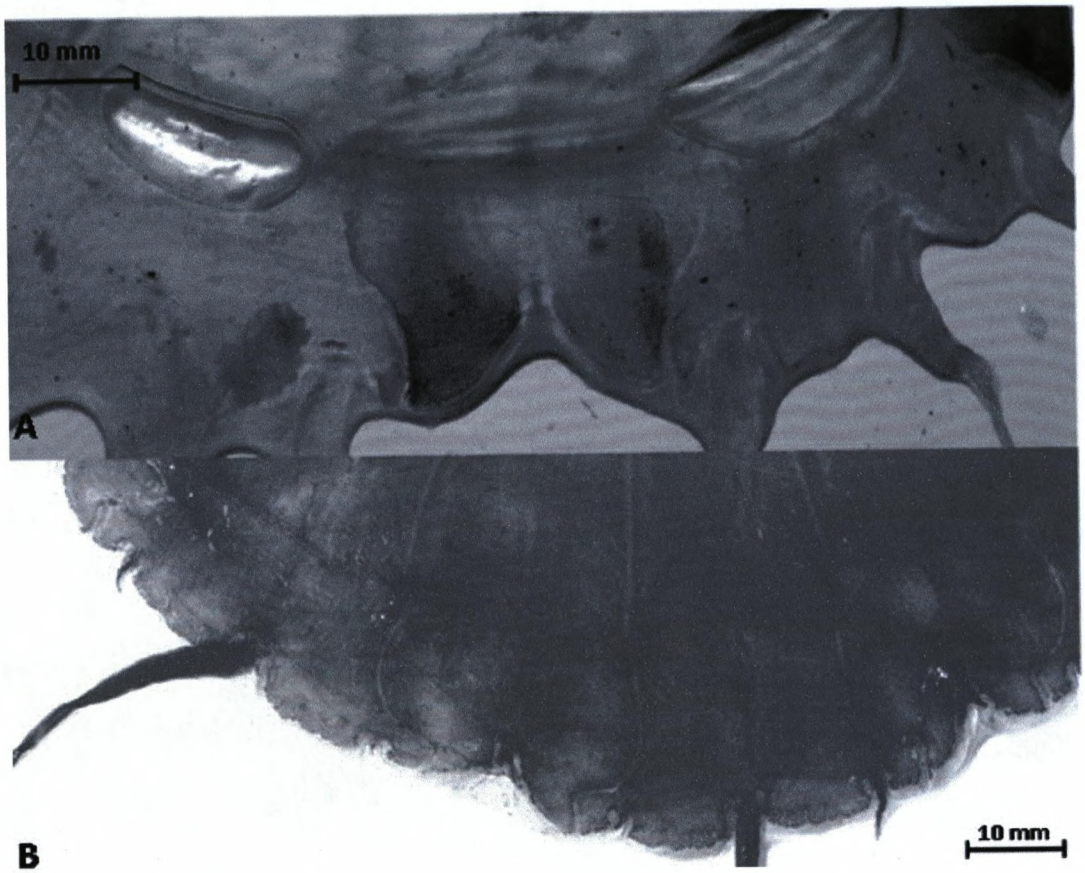


Figure 9

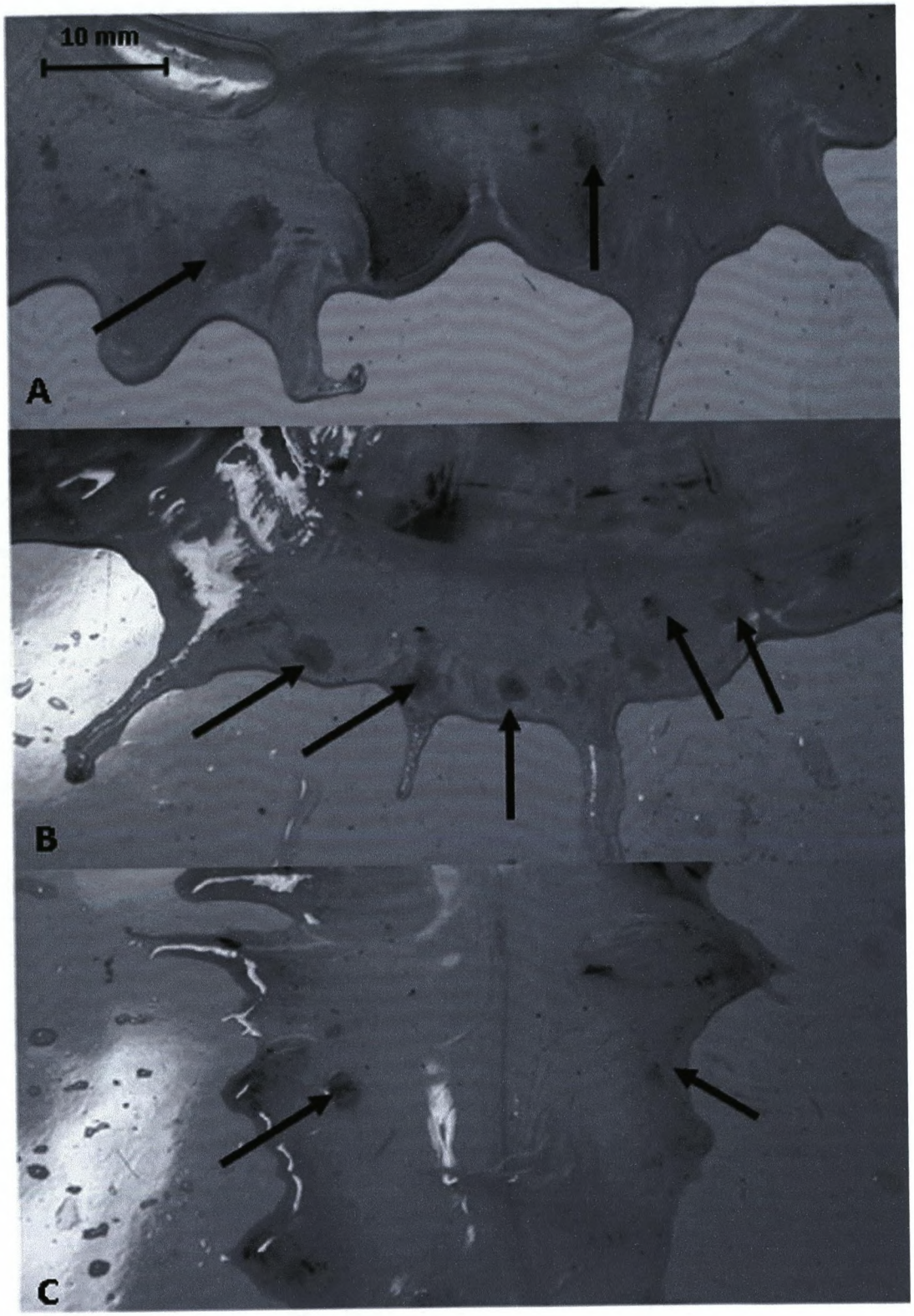


Figure 10

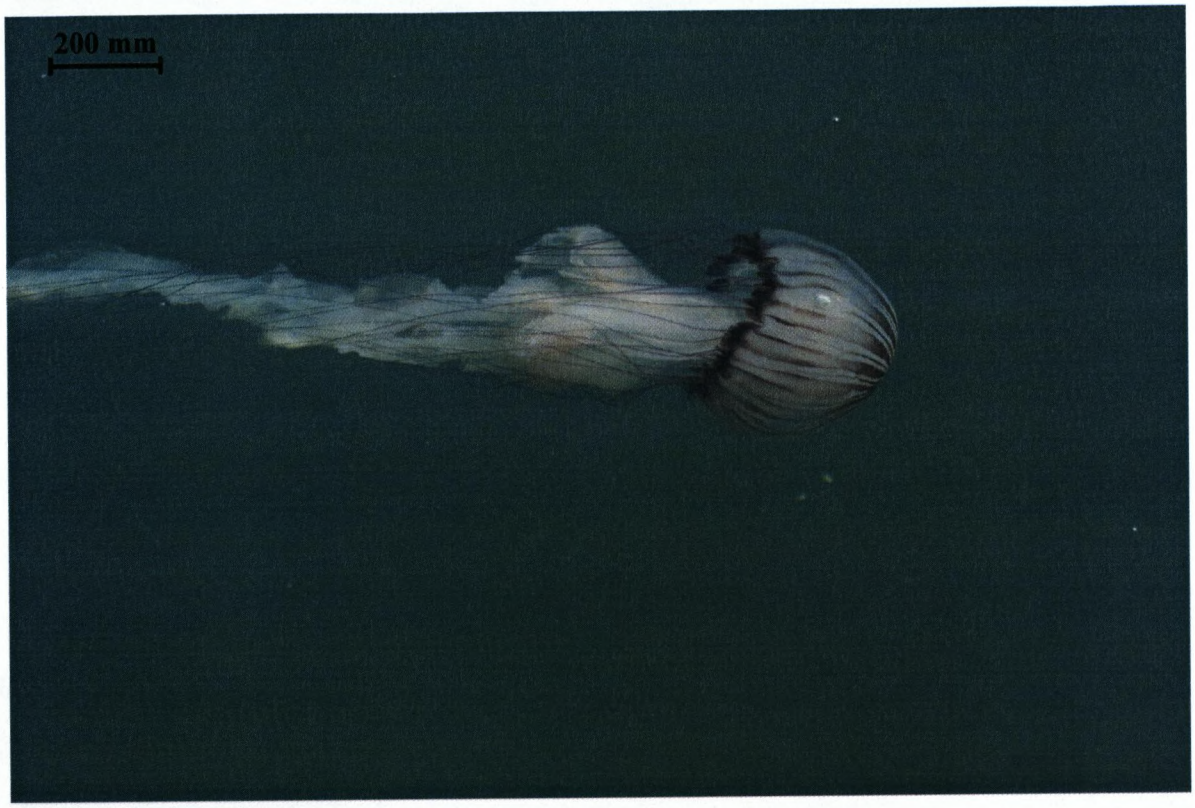


Figure 11

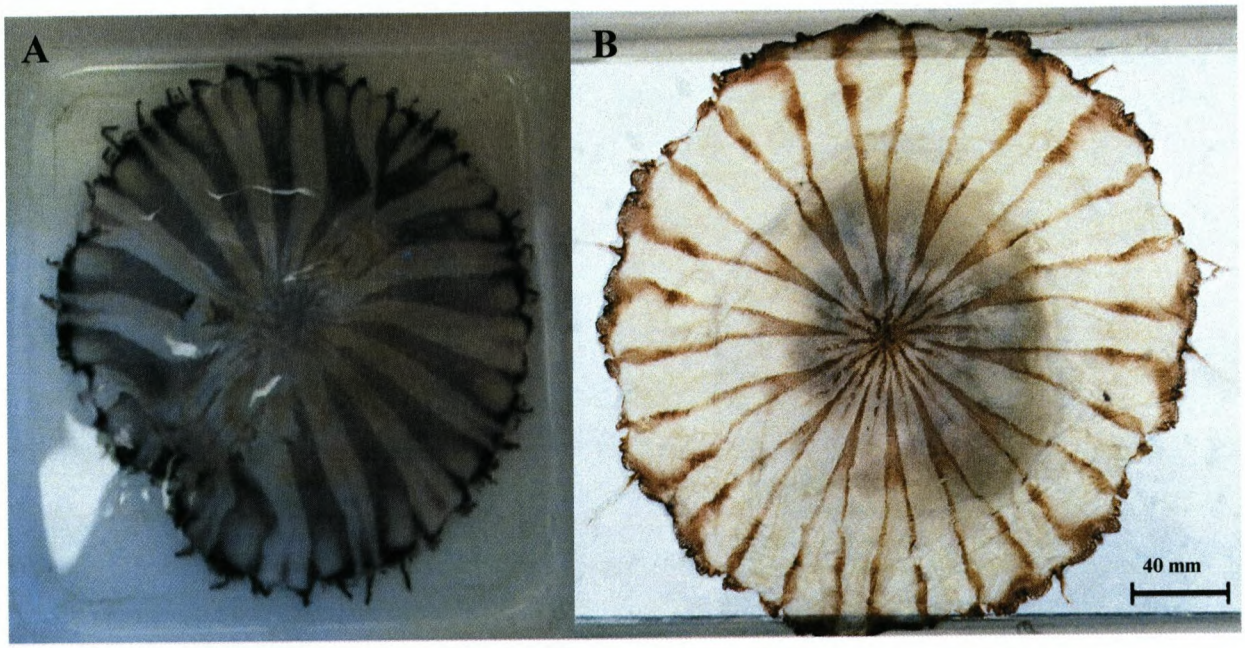


Figure 12

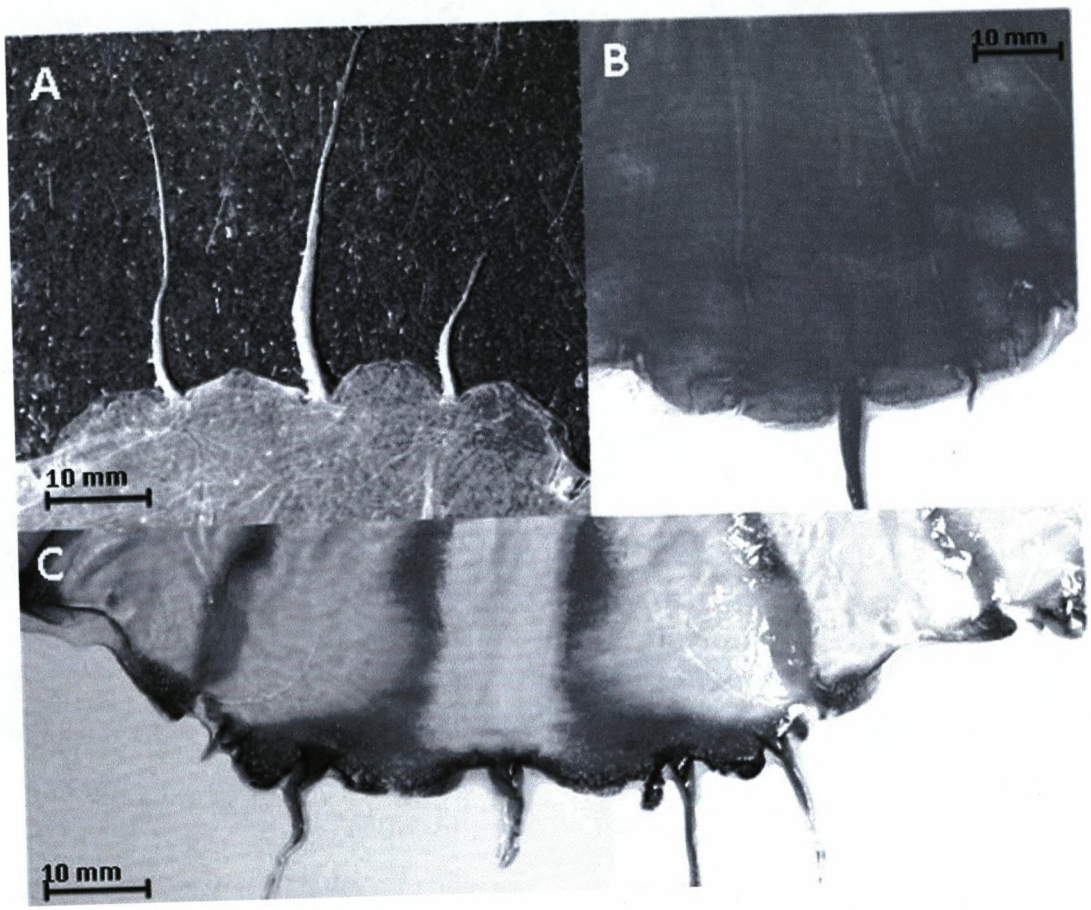


Figure 13

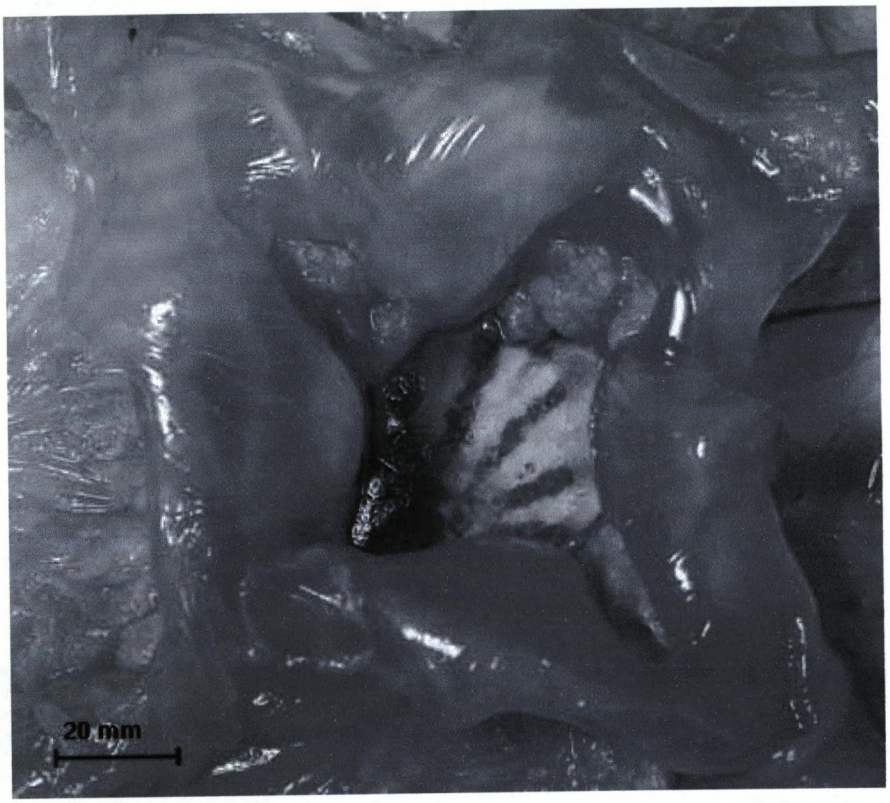
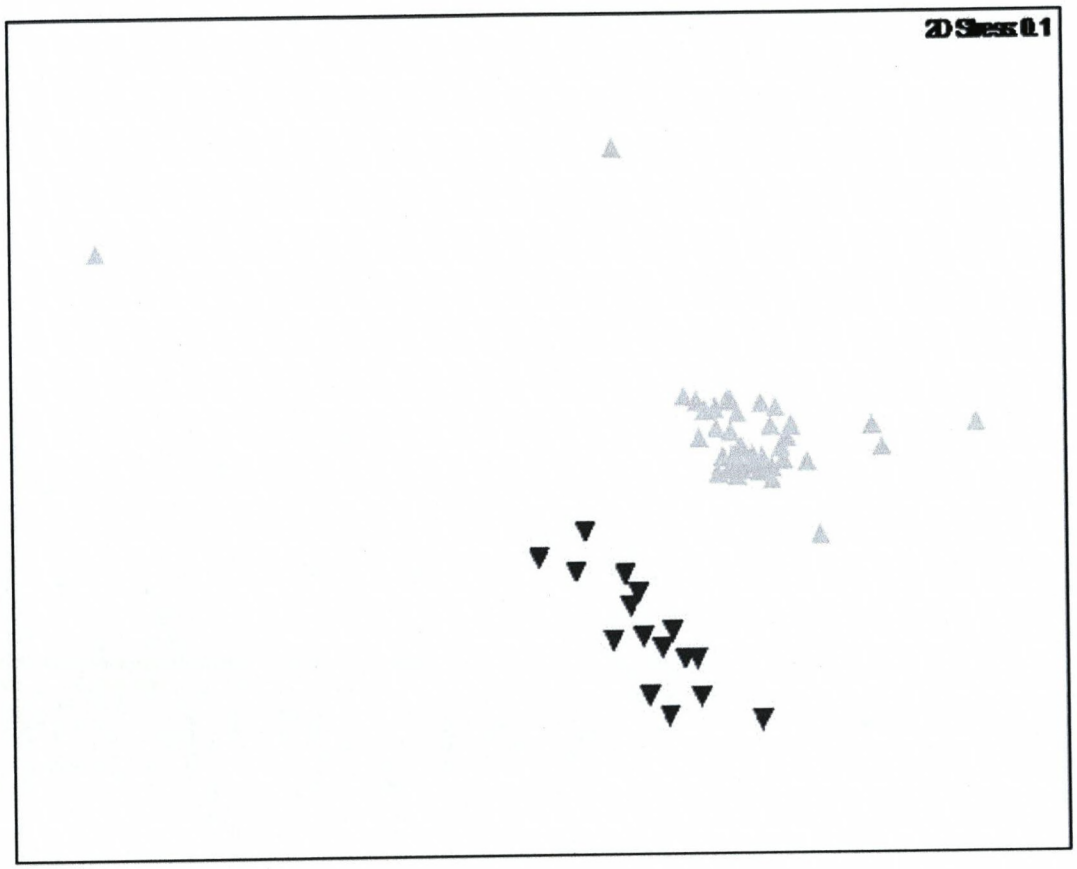


Figure 14



Appendix 1: Morphological data summarized for *Chrysaora fulgida* (Cf), *C. africana* (Ca) collected on the "Goby and Hake Cruise", 2008 as well as *C. hysoscella* (Ch NHM), *C. africana* (Ca NHM), *C. quinquechirrha* (Cq NHM), *C. fulgida* (Cf NHM) and *C. lactea* (Cl NHM) examined at the Natural History Museum (NHM), London. Measurements are taken in mm.

MF	Descriptive statistics	Cf	Ch NHM	Ca	Ca NHM	Cq NHM	Cf NHM	Cl NHM
S 1	Mean	141.65	131.26	190.00	48.00	92.85	324.00	90.10
S 1	N	40	16	16	1	4	1	2
S 1	Std.Dev.	70.02	43.24	56.32	0.00	42.70	0.00	10.47
S 1	Minimum	59.00	28.40	105.00	48.00	57.00	324.00	82.70
S 1	Maximum	407.00	178.00	312.00	48.00	147.00	324.00	97.50
S 1	25% Quartile	108.50	111.50	158.00	48.00	58.70	324.00	82.70
S 1	Median	120.00	145.00	180.00	48.00	83.70	324.00	90.10
S 1	75% Quartile	149.50	165.50	230.00	48.00	127.00	324.00	97.50
S 44	Mean	49.61	-	77.71	-	-	-	-
S 44	N	40	-	16	-	-	-	-
S 44	Std.Dev.	125.80	-	60.64	-	-	-	-
S 44	Minimum	1.05	-	7.69	-	-	-	-
S 44	Maximum	588.00	-	223.93	-	-	-	-
S 44	25% Quartile	7.79	-	38.92	-	-	-	-
S 44	Median	12.47	-	66.50	-	-	-	-
S 44	75% Quartile	26.70	-	96.36	-	-	-	-
S 25	Mean	17.45	16.51	30.59	-	10.53	30.50	-
S 25	N	40	9	14	-	4	1	-
S 25	Std.Dev.	12.13	5.55	13.22	-	4.94	0.00	-
S 25	Minimum	5.84	10.13	10.47	-	6.38	30.50	-
S 25	Maximum	61.70	27.00	60.94	-	17.00	30.50	-
S 25	25% Quartile	10.90	12.50	21.88	-	6.69	30.50	-
S 25	Median	13.75	14.67	30.05	-	9.38	30.50	-
S 25	75% Quartile	18.76	19.77	38.58	-	14.38	30.50	-
S 42	Mean	2.46	-	5.20	-	-	-	-
S 42	N	40	-	16	-	-	-	-
S 42	Std.Dev.	5.88	-	6.10	-	-	-	-
S 42	Minimum	0.10	-	0.06	-	-	-	-
S 42	Maximum	31.00	-	23.45	-	-	-	-
S 42	25% Quartile	0.45	-	0.87	-	-	-	-
S 42	Median	0.64	-	4.27	-	-	-	-
S 42	75% Quartile	1.45	-	6.75	-	-	-	-
S 37	Mean	18.83	26.07	-	-	-	-	-
S 37	N	40	13	-	-	-	-	-
S 37	Std.Dev.	9.42	8.53	-	-	-	-	-
S 37	Minimum	9.68	7.55	-	-	-	-	-
S 37	Maximum	54.10	37.17	-	-	-	-	-
S 37	25% Quartile	13.83	23.50	-	-	-	-	-
S 37	Median	16.02	25.45	-	-	-	-	-

MF	Descriptive statistics	Cf	Ch NHM	Ca	Ca NHM	Cq NHM	Cf NHM	Cl NHM
S 37	75% Quartile	20.04	31.60	-	-	-	-	-
S 36	Mean	17.86	35.38	-	-	-	-	-
S 36	N	40	13	-	-	-	-	-
S 36	Std.Dev.	10.21	15.52	-	-	-	-	-
S 36	Minimum	9.24	8.05	-	-	-	-	-
S 36	Maximum	57.75	62.00	-	-	-	-	-
S 36	25% Quartile	12.73	26.40	-	-	-	-	-
S 36	Median	14.68	32.73	-	-	-	-	-
S 36	75% Quartile	18.13	41.00	-	-	-	-	-
S 22	Mean	230.78	-	674.90	-	-	-	-
S 22	N	29	-	5	-	-	-	-
S 22	Std.Dev.	97.40	-	300.12	-	-	-	-
S 22	Minimum	68.00	-	365.00	-	-	-	-
S 22	Maximum	607.50	-	1130.33	-	-	-	-
S 22	25% Quartile	182.00	-	445.00	-	-	-	-
S 22	Median	216.50	-	702.50	-	-	-	-
S 22	75% Quartile	262.00	-	731.67	-	-	-	-
S 16	Mean	0.62	0.55	1.87	-	1.44	1.78	0.75
S 16	N	32	14	16	-	3	1	2
S 16	Std.Dev.	0.42	0.37	0.59	-	0.72	0.00	0.17
S 16	Minimum	0.16	0.10	0.99	-	1.00	1.78	0.63
S 16	Maximum	1.97	1.53	2.86	-	2.28	1.78	0.87
S 16	25% Quartile	0.41	0.35	1.43	-	1.00	1.78	0.63
S 16	Median	0.48	0.43	1.81	-	1.05	1.78	0.75
S 16	75% Quartile	0.63	0.60	2.43	-	2.28	1.78	0.87
S 2	Mean	20.93	5.21	10.14	2.40	3.85	5.00	5.75
S 2	N	5.98	14	16	1	4	1	2
S 2	Std.Dev.	40	2.11	3.99	0.00	1.06	0.00	0.35
S 2	Minimum	2.60	2.20	2.40	2.40	3.00	5.00	5.50
S 2	Maximum	3.00	9.00	16.40	2.40	5.20	5.00	6.00
S 2	25% Quartile	16.00	3.40	7.10	2.40	3.00	5.00	5.50
S 2	Median	4.15	5.30	10.50	2.40	3.60	5.00	5.75
S 2	75% Quartile	5.60	7.00	12.50	2.40	4.70	5.00	6.00
S 26	Mean	6.64	9.00	-	-	-	-	-
S 26	N	38	1	-	-	-	-	-
S 26	Std.Dev.	3.94	0.00	-	-	-	-	-
S 26	Minimum	2.74	9.00	-	-	-	-	-
S 26	Maximum	21.27	9.00	-	-	-	-	-
S 26	25% Quartile	4.34	9.00	-	-	-	-	-
S 26	Median	5.67	9.00	-	-	-	-	-
S 26	75% Quartile	6.94	9.00	-	-	-	-	-
S 3	Mean	2.20	0.69	2.02	0.20	0.78	0.60	1.00
S 3	N	40	14	15	1	4	1	2
S 3	Std.Dev.	5.54	0.47	0.69	0.00	0.26	0.00	0.57
S 3	Minimum	0.20	0.30	1.00	0.20	0.50	0.60	0.60
S 3	Maximum	36.00	2.00	3.20	0.20	1.00	0.60	1.40
S 3	25% Quartile	0.90	0.30	1.40	0.20	0.55	0.60	0.60

MF	Descriptive statistics	Cf	Ch NHM	Ca	Ca NHM	Cq NHM	Cf NHM	Cl NHM
S 3	Median	1.00	0.60	2.00	0.20	0.80	0.60	1.00
S 3	75% Quartile	1.68	0.80	2.60	0.20	1.00	0.60	1.40
S 27	Mean	1.85	-	-	-	-	-	-
S 27	N	34	-	-	-	-	-	-
S 27	Std.Dev.	1.22	-	-	-	-	-	-
S 27	Minimum	0.65	-	-	-	-	-	-
S 27	Maximum	5.61	-	-	-	-	-	-
S 27	25% Quartile	1.03	-	-	-	-	-	-
S 27	Median	1.47	-	-	-	-	-	-
S 27	75% Quartile	2.29	-	-	-	-	-	-
S 15	Mean	51.01	56.85	63.41	-	41.13	129.00	37.13
S 15	N	39	14	16	-	3	1	2
S 15	Std.Dev.	23.42	16.79	20.27	-	15.46	0.00	7.73
S 15	Minimum	20.46	26.13	35.25	-	26.13	129.00	31.67
S 15	Maximum	137.85	83.00	107.68	-	57.00	129.00	42.60
S 15	25% Quartile	37.89	44.75	51.18	-	26.13	129.00	31.67
S 15	Median	44.23	55.01	61.26	-	40.25	129.00	37.13
S 15	75% Quartile	57.29	73.25	79.43	-	57.00	129.00	42.60
S 41	Mean	23.18	-	28.18	-	-	-	-
S 41	N	40	-	14	-	-	-	-
S 41	Std.Dev.	60.52	-	18.81	-	-	-	-
S 41	Minimum	0.50	-	2.03	-	-	-	-
S 41	Maximum	313.00	-	61.73	-	-	-	-
S 41	25% Quartile	4.28	-	16.66	-	-	-	-
S 41	Median	7.66	-	26.54	-	-	-	-
S 41	75% Quartile	15.36	-	35.15	-	-	-	-
S 14	Mean	24.50	34.06	51.70	-	23.95	-	28.80
S 14	N	40	14	16	-	3	-	2
S 14	Std.Dev.	8.14	8.75	15.38	-	8.46	-	5.37
S 14	Minimum	12.15	22.50	30.50	-	16.25	-	25.00
S 14	Maximum	53.65	52.15	82.10	-	33.00	-	32.60
S 14	25% Quartile	20.10	27.25	35.83	-	16.25	-	25.00
S 14	Median	22.88	32.50	51.80	-	22.60	-	28.80
S 14	75% Quartile	27.80	39.60	60.60	-	33.00	-	32.60
S 24	Mean	57.40	45.76	104.56	-	51.44	145.50	-
S 24	N	40	9	14	-	4	1	-
S 24	Std.Dev.	30.92	12.24	42.81	-	34.58	0.00	-
S 24	Minimum	20.56	31.38	39.13	-	21.00	145.50	-
S 24	Maximum	180.18	64.55	189.30	-	90.00	145.50	-
S 24	25% Quartile	43.89	36.75	77.90	-	22.25	145.50	-
S 24	Median	51.05	43.02	106.57	-	47.38	145.50	-
S 24	75% Quartile	59.64	52.67	128.58	-	80.63	145.50	-
S 12	Mean	16.00	16.00	16.00	16.00	16.00	16.00	16.00
S 12	N	40	16	16	1	4	1	2
S 12	Std.Dev.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S 12	Minimum	16.00	16.00	16.00	16.00	16.00	16.00	16.00
S 12	Maximum	16.00	16.00	16.00	16.00	16.00	16.00	16.00

MF	Descriptive statistics	Cf	Ch NHM	Ca	Ca NHM	Cq NHM	Cf NHM	Cl NHM
S 12	25% Quartile	16.00	16.00	16.00	16.00	16.00	16.00	16.00
S 12	Median	16.00	16.00	16.00	16.00	16.00	16.00	16.00
S 12	75% Quartile	16.00	16.00	16.00	16.00	16.00	16.00	16.00
S 5	Mean	4.00	4.00	5.96	4.00	5.94	4.00	5.69
S 5	N	40	16	16	1	4	1	2
S 5	Std.Dev.	0.00	0.00	0.07	0.00	0.13	0.00	0.09
S 5	Minimum	4.00	4.00	5.75	4.00	5.75	4.00	5.63
S 5	Maximum	4.00	4.00	6.00	4.00	6.00	4.00	5.75
S 5	25% Quartile	4.00	4.00	5.94	4.00	5.88	4.00	5.63
S 5	Median	4.00	4.00	6.00	4.00	6.00	4.00	5.69
S 5	75% Quartile	4.00	4.00	6.00	4.00	6.00	4.00	5.75
S 4	Mean	8.03	8.00	7.88	8.00	8.00	8.00	8.00
S 4	N	40	16	16	1	4	1	2
S 4	Std.Dev.	0.16	0.00	0.62	0.00	0.00	0.00	0.00
S 4	Minimum	8.00	8.00	6.00	8.00	8.00	8.00	8.00
S 4	Maximum	9.00	8.00	9.00	8.00	8.00	8.00	8.00
S 4	25% Quartile	8.00	8.00	8.00	8.00	8.00	8.00	8.00
S 4	Median	8.00	8.00	8.00	8.00	8.00	8.00	8.00
S 4	75% Quartile	8.00	8.00	8.00	8.00	8.00	8.00	8.00
S 17	Mean	4.00	4.00	4.00	4.00	4.00	4.00	4.00
S 17	N	40	16	16	1	4	1	2
S 17	Std.Dev.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S 17	Minimum	4.00	4.00	4.00	4.00	4.00	4.00	4.00
S 17	Maximum	4.00	4.00	4.00	4.00	4.00	4.00	4.00
S 17	25% Quartile	4.00	4.00	4.00	4.00	4.00	4.00	4.00
S 17	Median	4.00	4.00	4.00	4.00	4.00	4.00	4.00
S 17	75% Quartile	4.00	4.00	4.00	4.00	4.00	4.00	4.00
S 28	Mean	7.69	8.00	8.00	8.00	8.00	8.00	8.00
S 28	N	39	16	16	1	4	1	2
S 28	Std.Dev.	0.86	0.00	0.37	0.00	0.00	0.00	0.00
S 28	Minimum	3.00	8.00	7.00	8.00	8.00	8.00	8.00
S 28	Maximum	8.00	8.00	9.00	8.00	8.00	8.00	8.00
S 28	25% Quartile	8.00	8.00	8.00	8.00	8.00	8.00	8.00
S 28	Median	8.00	8.00	8.00	8.00	8.00	8.00	8.00
S 28	75% Quartile	8.00	8.00	8.00	8.00	8.00	8.00	8.00
S 29	Mean	1.08	0.00	-	-	-	-	-
S 29	N	12	16	-	-	-	-	-
S 29	Std.Dev.	1.38	0.00	-	-	-	-	-
S 29	Minimum	0.00	0.00	-	-	-	-	-
S 29	Maximum	5.00	0.00	-	-	-	-	-
S 29	25% Quartile	0.00	0.00	-	-	-	-	-
S 29	Median	1.00	0.00	-	-	-	-	-
S 29	75% Quartile	1.00	0.00	-	-	-	-	-
S 35	Mean	8.03	8.00	7.94	8.00	8.00	8.00	8.00
S 35	N	40	16	16	1	4	1	2
S 35	Std.Dev.	0.16	0.00	0.44	0.00	0.00	0.00	0.00
S 35	Minimum	8.00	8.00	7.00	8.00	8.00	8.00	8.00

MF	Descriptive statistics	Cf	Ch NHM	Ca	Ca NHM	Cq NHM	Cf NHM	Cl NHM
S 35	Maximum	9.00	8.00	9.00	8.00	8.00	8.00	8.00
S 35	25% Quartile	8.00	8.00	8.00	8.00	8.00	8.00	8.00
S 35	Median	8.00	8.00	8.00	8.00	8.00	8.00	8.00
S 35	75% Quartile	8.00	8.00	8.00	8.00	8.00	8.00	8.00
S 31	Mean	0.75	16.00	11.69	0.00	7.50	0.00	15.00
S 31	N	40	16	16	1	4	1	2
S 31	Std.Dev.	2.25	0.00	4.32	0.00	8.70	0.00	1.41
S 31	Minimum	0.00	16.00	1.00	0.00	0.00	0.00	14.00
S 31	Maximum	11.00	16.00	16.00	0.00	16.00	0.00	16.00
S 31	25% Quartile	0.00	16.00	9.00	0.00	0.00	0.00	14.00
S 31	Median	0.00	16.00	13.50	0.00	7.00	0.00	15.00
S 31	75% Quartile	0.00	16.00	15.00	0.00	15.00	0.00	16.00
S 33	Mean	-	-	15.25	0.00	6.00	0.00	13.50
S 33	N	-	-	16	1	4	1	2
S 33	Std.Dev.	-	-	1.61	0.00	6.98	0.00	0.71
S 33	Minimum	-	-	12.00	0.00	0.00	0.00	13.00
S 33	Maximum	-	-	18.00	0.00	13.00	0.00	14.00
S 33	25% Quartile	-	-	14.00	0.00	0.00	0.00	13.00
S 33	Median	-	-	16.00	0.00	5.50	0.00	13.50
S 33	75% Quartile	-	-	18.00	0.00	11.00	0.00	14.00
S 21	Mean	4.00	4.00	4.00	4.00	4.00	4.00	4.00
S 21	N	40	16	16	1	4	1	2
S 21	Std.Dev.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S 21	Minimum	4.00	4.00	4.00	4.00	4.00	4.00	4.00
S 21	Maximum	4.00	4.00	4.00	4.00	4.00	4.00	4.00
S 21	25% Quartile	4.00	4.00	4.00	4.00	4.00	4.00	4.00
S 21	Median	4.00	4.00	4.00	4.00	4.00	4.00	4.00
S 21	75% Quartile	4.00	4.00	4.00	4.00	4.00	4.00	4.00
S 20	Mean	12.52	11.60	24.34	-	10.35	38.33	7.30
S 20	N	40	14	16	-	4	1	2
S 20	Std.Dev.	2.35	3.60	8.82	-	6.77	0.00	0.99
S 20	Minimum	7.72	5.00	10.16	-	4.00	38.33	6.60
S 20	Maximum	19.36	19.55	44.26	-	19.00	38.33	8.00
S 20	25% Quartile	10.65	10.73	17.71	-	5.04	38.33	3.05
S 20	Median	12.63	11.16	24.35	-	9.20	38.33	1.00
S 20	75% Quartile	13.76	13.28	30.28	-	15.67	38.33	0.00
S 19	Mean	8.07	9.95	27.54	-	11.20	42.33	3.38
S 19	N	40	14	16	-	4	1	4
S 19	Std.Dev.	1.66	3.30	8.44	-	7.50	0.00	2.33
S 19	Minimum	5.90	5.15	15.05	-	5.00	42.33	2.00
S 19	Maximum	12.39	14.73	45.56	-	20.83	42.33	0.32
S 19	25% Quartile	6.85	7.00	20.50	-	5.25	42.33	3.38
S 19	Median	7.71	10.50	26.20	-	9.48	42.33	3.89
S 19	75% Quartile	8.81	12.50	30.88	-	17.15	42.33	4.40
S 30	Mean	2.56	3.97	2.82	0.55	2.48	11.75	2.10
S 30	N	5	16	12	1	4	1	3
S 30	Std.Dev.	0.52	1.41	0.72	0.00	0.77	0.00	

MF	Descriptive statistics	Cf	Ch NHM	Ca	Ca NHM	Cq NHM	Cf NHM	Cl NHM
S 30	Minimum	1.92	0.85	1.94	0.55	1.75	11.75	0.00
S 30	Maximum	3.36	5.65	4.07	0.55	3.50	11.75	
S 30	25% Quartile	2.40	3.00	2.35	0.55	1.90	11.75	2.10
S 30	Median	2.48	3.95	2.66	0.55	2.33	11.75	2.33
S 30	75% Quartile	2.63	5.30	3.30	0.55	3.05	11.75	2.55
S 13	Mean	6.48	25.76	-	-	-	-	
S 13	N	39	13	-	-	-	-	
S 13	Std.Dev.	2.62	56.48	-	-	-	-	2.88
S 13	Minimum	2.77	6.15	-	-	-	-	2.00
S 13	Maximum	15.99	213.43	-	-	-	-	0.14
S 13	25% Quartile	4.75	7.30	-	-	-	-	
S 13	Median	6.27	10.20	-	-	-	-	
S 13	75% Quartile	7.29	13.68	-	-	-	-	
S 8	Mean	-	-	5.16	-	3.26	10.50	2.78
S 8	N	-	-	16	-	4	1	3
S 8	Std.Dev.	-	-	1.03	-	1.73	0.00	4.54
S 8	Minimum	-	-	3.39	-	0.98	10.50	2.00
S 8	Maximum	-	-	7.07	-	4.85	10.50	0.12
S 8	25% Quartile	-	-	4.87	-	1.95	10.50	2.78
S 8	Median	-	-	5.18	-	3.61	10.50	2.88
S 8	75% Quartile	-	-	5.60	-	4.58	10.50	2.98
S 9	Mean	10.31	8.11	5.68	-	3.91	21.38	4.45
S 9	N	18	16	16	-	4	1	5
S 9	Std.Dev.	7.71	3.57	1.74	-	2.17	0.00	1.68
S 9	Minimum	4.06	1.18	3.05	-	1.90	21.38	2.00
S 9	Maximum	31.42	12.35	9.05	-	6.95	21.38	0.18
S 9	25% Quartile	7.04	6.35	4.16	-	2.45	21.38	4.45
S 9	Median	7.90	8.71	5.61	-	3.39	21.38	4.54
S 8	75% Quartile	8.87	11.30	6.79	-	5.36	21.38	4.63
S 32	Mean	1.25	2.61	2.11	0.25	1.88	7.50	1.55
S 32	N	5	16	12	1	4	1	2
S 32	Std.Dev.	0.41	1.01	0.74	0.00	0.73	0.00	1.80
S 32	Minimum	0.80	0.10	0.99	0.25	0.93	7.50	2.00
S 32	Maximum	1.75	4.25	3.37	0.25	2.70	7.50	0.35
S 32	25% Quartile	0.98	2.12	1.59	0.25	1.39	7.50	1.55
S 32	Median	1.09	2.61	1.99	0.25	1.95	7.50	1.68
S 32	75% Quartile	1.62	3.19	2.67	0.25	2.38	7.50	1.80
S 10	Mean	-	-	5.15	-	3.63	-	1.55
S 10	N	-	-	16	-	4	-	2
S 10	Std.Dev.	-	-	1.20	-	2.26	-	3.13
S 10	Minimum	-	-	3.36	-	1.25	-	2.00
S 10	Maximum	-	-	7.47	-	6.68	-	0.32
S 10	25% Quartile	-	-	4.19	-	2.13	-	1.55
S 10	Median	-	-	5.20	-	3.30	-	1.80
S 10	75% Quartile	-	-	6.07	-	5.14	-	2.05
S 11	Mean	-	-	9.01	-	5.13	-	2.90
S 11	N	-	-	16	-	4	-	3

MF	Descriptive statistics	Cf	Ch NHM	Ca	Ca NHM	Cq NHM	Cf NHM	Cl NHM
S 11	Std.Dev.	-	-	2.94	-	3.19	-	
S 11	Minimum	-	-	4.89	-	2.35	-	0.00
S 11	Maximum	-	-	16.19	-	9.65	-	
S 11	25% Quartile	-	-	7.40	-	2.96	-	2.90
S 11	Median	-	-	8.74	-	4.25	-	3.13
S 11	75% Quartile	-	-	10.28	-	7.29	-	3.35
S 44	Mean	75.25	344.00	119.29	-	-	-	
S 44	N	40	10	14	-	-	-	
S 44	Std.Dev.	190.75	262.77	82.98	-	-	-	2.08
S 44	Minimum	1.66	100.00	9.77	-	-	-	2.00
S 44	Maximum	888.00	800.00	307.99	-	-	-	0.11
S 44	25% Quartile	13.55	160.00	70.96	-	-	-	
S 44	Median	22.07	190.00	93.17	-	-	-	
S 44	75% Quartile	41.02	600.00	123.47	-	-	-	
S 34	Mean	-	-	2.34	-	0.85	-	2.00
S 34	N	-	-	12	-	2	-	2
S 34	Std.Dev.	-	-	0.79	-	0.04	-	2.51
S 34	Minimum	-	-	1.62	-	0.83	-	2.00
S 34	Maximum	-	-	3.94	-	0.88	-	0.12
S 34	25% Quartile	-	-	1.72	-	0.83	-	2.00
S 34	Median	-	-	2.11	-	0.85	-	2.08
S 34	75% Quartile	-	-	2.64	-	0.88	-	2.15
S 6	Mean	-	-	4.61	-	1.68	7.68	2.43
S 6	N	-	-	16	-	4	1	3
S 6	Std.Dev.	-	-	0.95	-	1.12	0.00	2.46
S 6	Minimum	-	-	2.51	-	0.48	7.68	2.00
S 6	Maximum	-	-	6.22	-	2.95	7.68	1.29
S 6	25% Quartile	-	-	4.10	-	0.76	7.68	2.43
S 6	Median	-	-	4.70	-	1.65	7.68	2.51
S 6	75% Quartile	-	-	5.22	-	2.60	7.68	2.60
S 7	Mean	11.98	8.66	5.90	-	2.16	17.95	1.55
S 7	N	18	16	16	-	4	1	3
S 7	Std.Dev.	9.71	3.39	1.95	-	1.29	0.00	14.80
S 7	Minimum	4.13	2.05	2.53	-	0.83	17.95	2.00
S 7	Maximum	38.58	12.53	9.46	-	3.43	17.95	0.99
S 7	25% Quartile	8.39	6.39	4.84	-	1.06	17.95	1.55
S 7	Median	9.15	9.49	6.21	-	2.19	17.95	2.46
S 7	75% Quartile	9.87	11.41	7.01	-	3.25	17.95	3.38
S 18	Mean	12.63	16.08	11.77	-	6.65	14.80	14.10
S 18	N	39	14	16	-	3	1	16
S 18	Std.Dev.	7.10	4.82	3.35	-	0.88	0.00	
S 18	Minimum	5.02	7.88	7.15	-	5.85	14.80	0.00
S 18	Maximum	42.06	22.60	20.03	-	7.60	14.80	
S 18	25% Quartile	8.47	12.25	9.67	-	5.85	14.80	14.10
S 18	Median	10.78	15.46	11.01	-	6.50	14.80	14.80
S 18	75% Quartile	15.12	21.50	12.99	-	7.60	14.80	15.50
S 23	Mean	28.93	22.43	42.53	-	22.64	71.00	-

MF	Descriptive statistics	Cf	Ch NHM	Ca	Ca NHM	Cq NHM	Cf NHM	Cl NHM
S 23	N	40	12	15	-	4	1	-
S 23	Std.Dev.	12.18	7.40	23.05	-	16.34	0.00	-
S 23	Minimum	7.90	11.73	11.55	-	6.00	71.00	-
S 23	Maximum	78.27	33.87	82.49	-	45.00	71.00	-
S 23	25% Quartile	21.64	17.39	20.93	-	11.88	71.00	-
S 23	Median	26.84	22.41	41.91	-	19.78	71.00	-
S 23	75% Quartile	33.44	27.11	61.25	-	33.40	71.00	-

Appendix 2: Standardized morphometric data (logged ratios) summarized for *Chrysaora fulgida* (Cf), *C. africana* (Ca) collected on the "Goby and Hake Cruise", 2008 as well as *C. hysoscella* (Ch NHM), *C. africana* (Ca NHM), *C. quinquechirrha* (Cq NHM), *C. fulgida* (Cf NHM) and *C. lactea* (Cl NHM) examined at the Natural History Museum (NHM), London.

MF	Descriptive statistics	Cf	Ch NHM	Ca	Ca NHM	Cq NHM	Cf NHM	Cl NHM
S 25	Mean	0.12	0.11	0.15	-	0.11	0.09	-
S 25	N	40.00	9.00	14.00	-	4.00	1.00	-
S 25	St. Dev.	0.03	0.03	0.05	-	0.01	-	-
S 37	Mean	0.13	0.19	-	-	-	-	-
S 37	N	40.00	13.00	-	-	-	-	-
S 37	St. Dev.	0.02	0.03	-	-	-	-	-
S 36	Mean	0.13	0.25	-	-	-	-	-
S 36	N	40.00	13.00	-	-	-	-	-
S 36	St. Dev.	0.02	0.06	-	-	-	-	-
S 22	Mean	1.68	-	2.97	-	-	-	-
S 22	N	29.00	-	5.00	-	-	-	-
S 22	St. Dev.	0.34	-	0.93	-	-	-	-
S 16	Mean	-	-	0.01	-	0.01	0.01	0.01
S 16	N	-	-	16.00	-	3.00	1.00	2.00
S 16	St. Dev.	-	-	0.00	-	0.00	0.00	0.00
S 2	Mean	0.18	0.04	0.06	0.05	0.04	0.02	0.06
S 2	N	40.00	14.00	16.00	1.00	4.00	1.00	2.00
S 2	St. Dev.	0.86	0.01	0.02	0.00	0.01	0.00	0.01
S 26	Mean	0.05	0.06	-	-	-	-	-
S 26	N	38.00	1.00	-	-	-	-	-
S 26	St. Dev.	0.01	0.00	-	-	-	-	-
S 3	Mean	0.01	0.01	0.01	0.00	0.01	0.00	0.01
S 3	N	40.00	14.00	15.00	1.00	4.00	1.00	2.00
S 3	St. Dev.	0.02	0.00	0.00	0.00	0.00	0.00	0.01
S 27	Mean	0.01	-	-	-	-	-	-
S 27	N	34.00	-	-	-	-	-	-
S 27	St. Dev.	0.00	-	-	-	-	-	-
S 15	Mean	0.36	0.41	0.33	-	0.40	0.40	0.41
S 15	N	39.00	14.00	16.00	-	3.00	1.00	2.00
S 15	St. Dev.	0.03	0.05	0.05	-	0.03	0.00	0.04
S 14	Mean	0.18	0.25	0.28	-	0.23	-	0.32
S 14	N	40.00	14.00	16.00	-	3.00	-	2.00
S 14	St. Dev.	0.03	0.05	0.04	-	0.03	-	0.02
S 24	Mean	0.41	0.31	0.52	-	0.51	0.45	-
S 24	N	40	9	14	-	4	1	-

MF	Descriptive statistics	Cf	Ch NHM	Ca	Ca NHM	Cq NHM	Cf NHM	Cl NHM
S 24	St. Dev.	0.05	0.09	0.13	-	0.15	0.00	-
S 20	Mean	0.1	0.09	0.13	-	0.10	0.12	0.08
S 20	N	40.00	14.00	16.00	-	4.00	1.00	2.00
S 20	St. Dev.	0.03	0.02	0.03	-	0.03	0.00	0.02
S 30	Mean	0.02	0.03	0.02	0.01	0.03	0.04	0.03
S 30	N	5.00	16.00	12.00	1.00	4.00	1.00	2.00
S 30	St. Dev.	0.00	0.00	0.00	0.00	0.00	0.00	0.01
S 13	Mean	0.05	0.20	-	-	-	-	-
S 13	N	39.00	13.00	-	-	-	-	-
S 13	St. Dev.	0.01	0.46	-	-	-	-	-
S 8	Mean	-	-	0.03	-	0.04	0.03	0.03
S 8	N	-	-	16.00	-	4.00	1.00	2.00
S 8	St. Dev.	-	-	0.01	-	0.02	0.00	0.00
S 9	Mean	0.07	0.06	0.03	-	0.04	0.07	0.05
S 9	N	18.00	16.00	16.00	-	4.00	1.00	2.00
S 9	St. Dev.	0.00	0.01	0.01	-	0.02	0.00	0.01
S 32	Mean	0.01	0.02	0.01	0.01	0.02	0.02	0.02
S 32	N	5.00	16.00	12.00	1.00	4.00	1.00	2.00
S 32	St. Dev.	0.00	0.01	0.00	0.00	0.01	0.00	0.00
S 11	Mean	-	-	0.03	-	0.04	-	0.02
S 11	N	-	-	16.00	-	4.00	-	2.00
S 11	St. Dev.	-	-	0.01	-	0.02	-	0.00
S 10	Mean	-	-	0.05	-	0.05	-	0.03
S 10	N	-	-	16.00	-	4.00	-	2.00
S 10	St. Dev.	-	-	0.01	-	0.01	-	0.00
S 34	Mean	-	-	0.01	-	0.01	-	0.02
S 34	N	-	-	12.00	-	2.00	-	2.00
S 34	St. Dev.	-	-	0.00	-	0.00	-	0.00
S 6	Mean	-	-	0.03	-	0.02	0.02	0.03
S 6	N	-	-	16.00	-	4.00	1.00	2.00
S 6	St. Dev.	-	-	0.01	-	0.02	0.00	0.00
S 7	Mean	0.08	0.07	0.03	-	0.03	0.06	0.03
S 7	N	18.00	16.00	16.00	-	4.00	1.00	2.00
S 7	St. Dev.	0.01	0.01	0.01	-	0.02	0.00	0.02
S 18	Mean	0.09	0.12	0.07	-	0.07	0.05	0.16
S 18	N	39.00	14.00	16.00	-	3.00	1.00	2.00
S 18	St. Dev.	0.01	0.02	0.02	-	0.02	0.00	0.01
S 23	Mean	0.21	0.16	0.21	-	0.23	0.22	-
S 23	N	40.00	12.00	15.00	-	4.00	1.00	-
S 23	St. Dev.	0.05	0.03	0.08	-	0.09	0.00	-

Appendix 3: The consensus sequence of internal transcribed spacer one (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 4: The consensus sequence of internal transcribed spacer one (ITS1) amplified from three *Chrysaora hysoscella* specimens collected from either from Dingle Bay (52° 6' 54" N -10° 20' 27" W) or Cork Harbour (51° 49' 33.6" N -8° 16' 8.4" W), Ireland. Variable nucleotide bases are indicated, if present.

TCTGGTTTCGTAGGTGAACCTGCGGAAGGATCATTACCGAAAGTTGGCTACACGC
TATCAGCTACTTGACTTAGCCGTCAAAGCTATGCGAACCACTGTGAACCCGTATC
GATCTGTGTGAGGTGACCAGAAGCAGGCCGGACTGGCAGGCTGCAGTCGCTGTT
GTCTGTGCTTAAATGGCAGGCGTCTTCCTGTCGGCCTCACATGGAGTTGTTTTTTA
TTCTTGTATTTTTTCAAACTTGAAATTCATGAATTCATAATTATTGACAACATTCA
TTGTCGTCGATAGTTGGAAATGAAAAAAGTCAGAGACAACTTCTAAGGTGGATC
ACTCGGCTCGTGCGA

Appendix 5: 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

TTTTACCTCCAGCTCTTTTACTATT(G)CTAGGGTCTTCTCTAATTGAACAAGGAGC

(A)

AGGTACTGGTTGAACTGTATATCCACCCCTATCTGCTATTCAAGCTCATTCCGGA

GGATCTGTTGATATGGCAATTTTTAGTCTACATTTAGCAGGAGCTTCCTCTATAAT

GGGTGCTATTAACTTTATTACCACAATTCTAAACATGAGAGCCCCTGGGATGACA

ATGGATAGAATACCTCTATTTGTTTGATCTGTACTTATTACAGCAATACTTCTACT

TCTATCACTTCCAGTATTAGCTGGGGCCATTAC(T)ATGTTATTAACAGACAGAAA

(C)

TTTTAATACTTCTTTCTTTGATCCTGCTGGAGGGGGAGATCCTATTTTATTCCAAC

ATTTATTTTGATTTTTTGGTCACCC

Appendix 6: The consensus sequence of internal transcribed spacer one (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.

TCCCCCG(A)ACCGAG(T)GAT(C)CCCCTTAGAAG(T)TGT(C)TTGGTTTTTGG
(G) (G) (T) (G) (T)
TATTATGAATGAATGATACAATGTCTCACTCAATC(T)CAACTCATGAATTT
(C)
GCAAAAAAGTTTGTAAAAACAAAACACAAAAAAACTCCATGTGAGGCCG

GCAGGAAAACGCCTGCCATTTGAGCCCAGACGCCTGTCTGTCTCCCCGAG

ACATGCACAGACTCTGACCACCTCACACAGATCGGTACGAGTTCACAGTG

TATTATTGCCGTGTCCTGCACGCCACAATAATCTCTACGTCTCGAAAGAAC

GTAGACTTTCGG(T)A(A)TG(A)(T)CCTTCC(G)CAGGT(T)CCCCT(A)CAAA(A)
(A) (T) (T)(T) (C) (C) (C) (C)
CAA

Appendix 7: 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

TATCTACTGA(G)CCCCCTGAATGTGCTTGGACAGCTGCAAGTGGGGGGTA

(A)

AATAGTTCAACCTGTTCCTGCTCCTTGCTC(T)ATAAGAGAAGATCCTAATA

(G)

AAAGAAGAAGAGCGGGAGGAAGAAGTCAAAAGCTTATATTATTTAATCT

AGGAAAAGCCATGTCAGGACTTCCTATATATAAAGGAACAAATCAGTTTC

CAAATCCCCCTATTAAAACAGGCATAACAAAAAAGAAAATCATTATTAAG

GCATGAGCAGTTACAACTACGTTGTAAATTTGGTCATCTCCTAGCATAGAC

CCCGGTCCAGATAGTTCTAATCTAATAATCATACTAAATGCTGTTCCTATC

ATTGCAGAAAATGCTCCAAATA(T)(T)ATA(T)ATA(A)A(C)AGTTCCAATAT

(A)(C) (G) (T) (A)

CTTTATGATTTGTTGACCAGTTTAA

Jellyfish amongst the crocodiles: a new record of *Crambionella stuhlmanni* (Scyphozoa: Rhizostomeae) from St. Lucia Estuary, South Africa

Abstract

A new record of *Crambionella stuhlmanni*, a rhizostome from the Greater St. Lucia Wetland Park situated on the east coast of South Africa, is reported. The material is described from quantitative morphological data, mitochondrial (cytochrome c oxidase subunit I) and nuclear (internal transcribed spacer one) sequence data. The species can be diagnosed by a combination of morphological features including the presence of conical projections on velar lappets, the absence of orbicular appendages among mouthlets and low ratio (Mean 0.17 ± 0.04) between the lengths of the terminal club and oral arm. The close proximity of St. Lucia to the known geographic range of *C. stuhlmanni* reinforces this finding. Mitochondrial sequence data unambiguously delineate *C. stuhlmanni* as a separate species from *C. orsini*; subsequent phylogenetic analyses support its placement within the monophyletic genus: Crambionella. However future work is needed to resolve family-level relationships within the order Rhizostomeae.

Keywords: Rhizostomeae, taxonomy, systematics, morphological analyses, molecular analyses.

Introduction

Scyphozoans are considered to be true jellyfish (Mianzan and Cornelius, 1999). They are carnivores that feed on a wide diversity of prey from protists to chordates (Sommer *et al.*, 2002). They have the potential to compete with fish for food (Lynam *et al.*, 2005) and can consume large numbers of fish eggs and larvae which means that scyphozoans can have detrimental impacts on fish recruitment (Purcell and Arai, 2001). Scyphozoans and other jellyfish often display seasonal fluctuations in population size (Mills, 2001) and can reach high densities in enclosed embayments and at physical discontinuities (Graham *et al.*, 2001). Although these blooms may be a natural phenomenon (Purcell, 2005) there is growing evidence to suggest that they are occurring more frequently and for longer periods of time in recent years in response to altered marine ecosystems (Mills, 2001; Purcell, 2005; Purcell *et al.*, 2007). A number of anthropogenic factors have been blamed for causing these increases, and it is likely that these act synergistically (Purcell *et al.*, 2007; Richardson *et al.*, 2009). These include global warming (Mills, 2001; Purcell *et al.*, 2007), overfishing (Bakun and Weeks, 2006; Lynam *et al.*, 2006), eutrophication (Arai, 2001; Purcell, 2005; Purcell *et al.*, 2007) and proliferation in hard substrata (Richardson *et al.*, 2009). Alien species have often been involved (Mills, 2001; Graham and Bayha, 2007; Oguz *et al.*, 2008). Jellyfish blooms have a number of negative implications for regional economies, ranging from fishing (Purcell *et al.*, 2007) and aquaculture (Doyle *et al.*, 2008) through to coastal power production (Masilamoni *et al.*, 2000) to tourism (Purcell *et al.*, 2007). That said jellyfish are considered an important food resource in some SE Asian countries (Hsieh, 2001; Omori and Nakano, 2001).

Most scyphozoans have both benthic and pelagic life history phases and approximately 200 species have been recorded (Mianzan and Cornelius, 1999). Unfortunately, our understanding of diversity in this group is poor because scyphozoan systematic is subject to much disagreement and debate (Bolton and Graham, 2004). The chief cause of this controversy is that the original descriptions are archaic and use only a few subjective, qualitative characters in their diagnoses (Bolton and Graham, 2004; Dawson, 2005a). This has caused much confusion, and is further exaggerated by phenotypic plasticity (Dawson *et al.*, 2001; Dawson, 2005a) and the presence of cryptic species (Knowlton, 1993; Féral, 2002). Although traditional morphological descriptions are useful, taxonomists have had to revise these descriptions using modern statistical and molecular analyses (Dawson and Jacobs, 2001; Schroth *et al.*, 2001; Dawson, 2003; Dawson, 2004; Holland *et al.*, 2004; Dawson, 2005b; 2005c).

An unknown species of *Crambionella* Stiasny, 1921 can be found in St. Lucia Estuary which forms part of the Greater St. Lucia Wetland Park; a world heritage site situated on the NE coast of South Africa. Historically the St. Lucia Estuary has demonstrated to be a naturally variable system often subjected to various disturbances such as flooding, mouth closures (Fielding *et al.*, 2001) and recently extended periods of drought (Jerling *et al.*, 2010). These perturbations have obvious effects on local species but the St. Lucia Estuary has still supported a high diversity of flora and fauna (Fielding *et al.*, 2001) making it a unique system; well documented compared to other South African ecosystems (Pillay and Perissinotto, 2008) . However, in this system an erratically, abundant species of *Crambionella* (Perissinotto, pers. comm.) remained unidentified. Species of *Crambionella* are found in various parts of the Indian Ocean and are known for their seasonal blooms (Billet *et al.*, 2006; Daryanabard and Dawson, 2008). There are three described species within this

genus *C. orsini* (Vanhöffen, 1888), *C. stuhlmanni* (Chun, 1896) and *C. annandalei* Rao, 1931. This investigation aims to identify the *Crambionella* species using objective, quantitative morphological features and molecular analysis which can be applied to any future study of this genus.

Materials and Methods

Morphological data collection

During December 2005; Mr. Ashok Bali of Marine and Coastal Management, Cape Town, South Africa collected 48 specimens of *Crambionella* (Figure 1) from the St. Lucia Estuary (28°0′0″ S 32°30′0″E) (Figure 2). Specimens were collected by dip-net and were immediately preserved in 5% formalin in ambient seawater. After 22 months in preservation, thirty-six morphological features were measured from 44 specimens (summarized in Table 1 and illustrated where possible in Figures 3, 4, 5, 6). Preservation is known to cause weight loss and shrinkage in several marine organisms (e.g. Lucas, 2009), these effects may however be more potent in jellyfish due to their high water content and lack of skeletal support (Thibault-Botha and Bowen, 2004). These effects have been documented in various gelatinous animals and may vary with the size of the specimen (Thibault-Botha and Bowen, 2004) and period of preservation (de Lafontaine and Leggett, 1989). However after a period of 60 days preservation effects appear to stabilize (de Lafontaine and Leggett, 1989); therefore this study did not utilize any correction factors to account for the effects of preservation. After removal of the oral arms, the radial canal system was injected with coloured latex to highlight arrangement and number of canals. All measurements were taken under a magnifying glass or a dissecting microscope (under numerous magnifications), using vernier callipers. Type material was not available for examination.

Five preserved specimens of *C. orsini* (Specimen numbers: 1950.3.25.343; 1950.3.25.346; 1950.3.25.347; 1950.3.25.356; 1950.3.25.357) from the Natural History Museum (NHM),

London were examined for comparative purposes. Some measurements were excluded on *C. orsini* as specimens had to be studied non-destructively.

Morphological data analyses

In order to determine the effect of individual size on measured variables, Pearsons R correlations were computed following log transformation of data (Zar, 1999). Relationships between size (external bell diameter to tip of lappets: S 1) and measurements for those transformed variables that failed tests of normality were examined using Spearman Rank Correlations (Zar, 1999). Some morphological measures were expressed as ratios following Chun (1896), Kramp (1961), Mayer (1910), Menon (1930, 1936), Rao, (1931) and Stiasny (1937). These included: oral disc diameter (S 13) to external umbrella diameter (S 1); length of the distal oral arm portion (S 7) to length of the proximal oral arm portion (S 6); length of terminal club (S 11) to total oral arm length (S 6 and S 7); ostia width (S 15) to inter-ostia width (S 15) and umbrella height (S 3) to external umbrella diameter (S 1). In order to determine if the ratios changed in a size dependant way, these too were log transformed and correlations with bell diameter (S 1) were investigated using Pearson's R (Zar, 1999). Those data that failed tests for normality were tested for size dependency using Spearman Rank Correlations (Zar, 1999). All correlations were corrected using the Bonferroni procedure to control for Type I errors in multiple test analyses (Quinn and Keough, 2002). In order to test for morphological differences between individual ratios, or between meristic measures, of the *Crambionella* specimens from the St. Lucia Estuary and those of *C. orsini* two-tailed *t*-tests were employed (Zar, 1999). Those data that failed tests for normality were investigated using Mann-Whitney-U tests (Zar, 1999). Two sample results were corrected for Type I errors by

adjusting alpha levels using the Bonferroni procedure (Quinn and Keough, 2002). All univariate statistical analyses were executed using STATISTICA Version 7.

Raw morphometric data were standardized by dividing by S 1 and log transformed (hereafter referred to as standardized). Ratios were just log transformed. Standardized morphometric data were used in all subsequent statistical analyses in order to eliminate size dependency. Clarke and Green (1988) highlight that logarithmic transformations are commonly used in statistical analyses, even in non-parametric tests, as measured variables are put on a common scale of variance and determines the relative weight of each measured variable. Non-parametric tests were used to examine morphological dissimilarity in a multivarite space. As non-parametric tests make no statistical assumptions about the underlying quality and distribution of original data, these tests are common practice among ecologists (Clarke and Green, 1988) and are most appropriate for the present study. The non-metric multi-dimensional scaling (MDS) routine in PRIMER 6 was used to illustrate the multivariate relationship between standardized morphometric features measured (Clarke, 1993). It is an iterative procedure based on rank orders, as an alternative to qualitative values, in a Euclidean distance matrix generated from the original standardized morphometric features (Clarke and Warwick, 2001). Non-metric MDS utilizes an algorithm that attempts to preserve the ranked differences in a 2-dimensional ordination space (Clarke and Warwick, 2001). To quantify the deviation from the original ranking in the Euclidean distance matrix to that reflected in the 2-dimensional ordination space, a "stress" value is generated (McCune and Grace, 2002). Clarke and Warwick (2001) suggest that MDS plots with stress values > 0.2 should be treated with caution. Prior to generating the Euclidean distance matrix between specimens based on their standardized morphometric features, gaps were filled either by mean substitution (if there was no significant relationship of the considered feature with size) or from regression

equations. Meristic features were not included. The same Euclidean distance matrix was used in all subsequent multivariate tests.

The One-way analysis of similarities (ANOSIM) routine in PRIMER 6 was used to test the null hypothesis of no morphological dissimilarity between species (Clarke and Warwick, 2001). ANOSIM, a non-parametric method, executes this through two key processes (Clarke and Warwick, 2001). Firstly the routine computes an *R* statistic that measures the average distance between every specimen within a group and contrasts it to the average distance between every specimen from different groups. Distances are also based on ranking orders within a Euclidean distance matrix. ANOSIM then utilizes a series of permutation tests, whereby variables from each group being tested are randomly distributed between groups, recalculating the *R* statistic for each permutation. If the original *R* statistic is more extreme than 95 % of the permutation tests the null hypothesis is rejected by a $p < 0.05$. ANOSIM in PRIMER 6 ran 999 permutation tests. In order to determine what standardized morphometric features contributed the most to dissimilarity between species the Similarity Percentages (SIMPER) routine in PRIMER 6 was utilized (Clarke, 1993). SIMPER determines the average dissimilarity between all pairs of inter-group specimens (Clarke and Warwick, 2001). These averages are then disaggregated into percentages that each standardized morphometric feature contributes to dissimilarity amongst groups (Clarke and Warwick, 2001).

Finally the Canonical Analysis of Principal Co-ordinates (CAP) routine in PRIMER 6 & PERMANOVA+ that utilized predefined groups, in contrast to many other multivariate tests, was also executed. The CAP routine seeks a set of axes that best discriminates amongst *a priori* groups in multivariate space (Anderson *et al.*, 2008). Anderson *et al.* (2008) describes the processes executed within this routine. Numerous matrices are generated to produce a set

of canonical axes. Conventionally in a canonical discriminant analysis a subset of Principal Co-ordinate (PCO) axes are chosen manually, based on the number variables in the original data matrix. However, in the present study, as the number of standardized morphometric features approached the number of specimens, Anderson *et al.* (2008) suggest "leave-one-out" diagnostics to determine the subset of PCO axes. The PCO axes determined are all orthonormal and therefore independent of each other. Running parallel to this process is a matrix based on codes for groups identified by a factor associated with the Euclidean distance matrix, also orthonormalised. An additional matrix is then generated by relating the subset of PCO axes to orthonormalised data matrix, yielding canonical eigenvalues and their associated eigenvectors which can be used to produce a CAP plot. These CAP axes, which are linear combinations of a subset of orthonormal PCO axes, were used to determine if predefined groups were correctly classified. The CAP routine was also used to test the null hypothesis of no differences in the positions of centroids among groups in a multivariate space through a series of permutation tests (Anderson *et al.*, 2008). This routine makes no assumptions about the underlying distribution of variables rendering it suitable for non-parametric analyses (Anderson *et al.*, 2008). All multivariate tests were considered significant at the 5 % level.

DNA analysis

Three specimens of *Crambionella* were collected from the St. Lucia Estuary at Charters Creek on the Lake Shore during September 2008. Specimens were collected by Professor Renzo Perissinotto from the University of Kwazulu Natal, Durban, South Africa, preserved in absolute ethanol (99 %) and once received was stored at -20 °C prior to analysis in the laboratory.

DNA was extracted from ethanol-preserved oral arm tissues using a phenol-chloroform based method. Samples were placed in separate eppendorf tubes. Extraction Buffer (SDS 0.5 %; 50 Mm Tris; 0.4 M EDTA; pH 8.0) in quantities of 0.5 ml were pipetted over each sample. Tissue samples were then macerated. Proteinase K (20 mg/ml) in quantities of 10 µl was then added. Samples were vortexed and incubated at 55 °C for a minimum of three hours until majority of protein was digested. Samples were then mixed with 500 µl phenol:chloroform:isoamyl alcohol (24:24:1), finger vortexed, then centrifuged at low speed (5000 x g) for 10 minutes. Supernatants were removed and placed in new eppendorf tubes, mixed with 500 µl chloroform:isoamyl alcohol (24:1) and finger vortexed. Solutions were then centrifuged at low speed (5000 x g) for 10 minutes. Supernatants were removed and placed in new eppendorf tubes. DNA was precipitated with 45 µl Na acetate and 650 µl of ice cold ethanol and left to incubate at -18 ° C overnight. Samples were then centrifuged at full speed (13000 x g) for 10 minutes and supernatants were discarded. Eppendorf tubes were inverted and left to air dry for a minimum of an hour. Each DNA sample was finally resuspended in 50 µl TE buffer.

Cytochrome *c* oxidase subunit I (COI) was amplified using primers LCOjf (5'-ggtcaacaaatcataaagatattggaac-3') and HCOcato (5'-ctccagcaggatcaaagaag-3') (Dawson, 2005d) or HCO2198 (5'-taaacttcagggtgaccaaaaaatca-3') (Folmer *et al.*, 1994). Internal transcribed spacer one (ITS1) was amplified using the primers jfITS1-5f (5'-ggtttcgtaggtgaacctgcggaaggatc-3') and jfITS1-3r (5'-cgcacgagccgagtgatccaccttagaag-3') (Dawson and Jacobs, 2001). Sequences were amplified through polymerase chain reaction (PCR) and PCR conditions were different for each fragment analysed. PCR conditions (adapted from Daryanabard and Dawson, 2008) are summarised in Table 2. PCR products were purified and sequenced at the Central Analytical Facility, University of Stellenbosch.

Electopherograms were checked visually, misreads corrected and poorly resolved terminal portions of sequences were discarded using Sequencher 4.9. Forward and reverse sequences were then aligned, using default settings, in Sequencher 4.9.Sequence identifications were verified by BLAST in GenBank. Phylogenetic analyses were utilized to examine family level relationships using COI rhizostome sequences (received from Professor MN Dawson). *Aurelia aurita*, a representative of the order: Semaeostomeae collectively with Rhizostomeae has been suggested to form the subclass: Discomedusae (Collins, 2002; Dawson, 2004; Marques and Collins, 2004; Collins *et al.*, 2006), was most suitable to be used as an outgroup. Sequence data for *A. aurita* was downloaded from GenBank (EF010537). Prior to further analyses, all sequence lengths were edited in Sequencher 4.9. A parsimony analysis was performed under Direct Optimization in the program POY 4.1.1 (Varón *et al.*, 2009) which simultaneously optimizes nucleotide homology and tree costs, thereby reducing the set of assumptions throughout the analysis. Bootstrap analyses (1500 pseudoreplicates) were performed to assess support of branch nodes. Mean pairwise sequence differences, using uncorrected "*P*", distances were calculated in PAUP* 10.4b.

SYSTEMATICS

Order RHIZOSTOMEAE Cuvier, 1799

Suborder DAKTYLIOPHORAE Stiasny, 1921

Superfamily INSCAPULATAE Stiasny, 1921

Family CATOSTYLIDAE Gegenbaur, 1857

Genus *Crambionella* Stiasny, 1921

Crambionella stuhlmanni (Chun, 1896)

(Figures 1-11; Tables 1-9; Appendices 1-2)

Crambessa stuhlmanni: Chun, 1896; Stiasny 1922

Catostylus stuhlmanni: Mayer, 1910

Crambionella stuhlmanni: Stiasny, 1921; Stiasny, 1937; Ranson, 1945; Kramp, 1961; Kramp, 1970

Description

Umbrella diameter of the material examined ranges between 62 – 81 mm (Table 3). Specimens possess a finely granular, hemispherical, in some cases dome shaped umbrella, with eight three-winged oral arms. The umbrella margin lacks tentacles but is cleft into narrow velar lappets separated by deep furrows. Eight rhopalia (mode: 8, range: 6-10, Table 3) separate the umbrella margin into octants. Oral arms are divided into a naked proximal and a three-winged distal portion consisting of an adoral and two aboral rows (all possessing mouthlets and club-shaped appendages). Oral arms terminate in a naked pyramidal club, proportion of terminal club length to oral arm length low (mean: 0.17, St.Dev.: 0.04, Table

3). The adoral row of mouthlets originates higher than the two aboral rows at the base of the oral arm and extends further than the two dorsal rows onto the back of the terminal club. Proportion of distal portion of oral arm length approximately double the length of proximal portion length (mean: 2.78, St.Dev.: 0.86, Table 3). In life specimens' exumbrella transparent-white, oral arms transparent-white bearing mouthlets and appendages light-brown in colour; terminal clubs transparent-white (Figure 1). In preservation exumbrella, oral arms and terminal clubs are transparent-cream in colour; mouthlets and appendages on oral arms loose colour and become transparent-cream.

The canal system consists of a continuous ring canal, sixteen radial canals of which eight (mode: 8, range: 5-8, Table 3) are rhopalial and the other eight are inter-rhopalial canals (mode: 8, range: 5-8, Table 3). An intra-circular network of anastomosing canals originates from the ring canal and does not communicate with the gastric cavity except through the rhopalial and inter-rhopalial canals and is less dense than that of the extra-circular network. The intra-circular network of anastomosing canals connects to both adjacent rhopalial and inter-rhopalial canals (Figure 7). Rhopalial canals reach the umbrella margin and inter-rhopalial canals terminate at the ring canal. Occasionally it may appear that the inter-rhopalial canals extend beyond the ring canal but on closer inspection these extensions are much thinner, and at times more subdivided than those of the rhopalial canals (Figure 8).

Rhopalia are found in pits with radiating furrows (Figure 9). Two ocular lappets are found at the edge of each rhopalium in each octant, which are smaller and elevated in comparison to velar lappets (Figure 9). Both velar and ocular lappets are free of any anastomosing canals. Velar lappets possess conical projections (mode: 12, range: 1 - 19, Table 3) on the dorsal median line (Figure 9). Specimens possess annular muscles (mode: 84; range: 40 – 111,

Table 3) on the subumbrellar surface, interrupted by rhopalial canals. There are four crescent shaped ostia that lead from the gonadal and gastro-vascular cavity. Proportions of ostia width to inter-ostia width are approximately equivalent (mean: 0.61, St.Dev.: 0.16, Table 3). Gonads at the time of sampling were thin and elongated when immature, but became mature and plump when external bell diameter reached ~ 100 mm.

Variation

Many of the measures were size dependant (Tables 4 and 5), although some were not. The latter typically included the meristic measures and ratios as well as umbrella height, oral disc diameter and gonadal diameter along the adradial axis. These features are highlighted as they can be useful in species-level comparisons.

Remarks

A comparison of the key morphological and meristic features that can be used to distinguish the three recognised species of *Crambionella*, together with the appropriate data from this study is shown in Table 6. From this it can be seen that the number of velar lappets in each octant, of the present material under investigation, was similar to that of *C. stuhlmanni* (Chun, 1896). The presence of conical projections on the dorsal median line of each lappet was also consistent with observations for *C. stuhlmanni*, and this feature can be used to distinguish the material from *C. orsini* (Menon, 1930; 1936; Stiasny, 1937) but not from *C. annandalei* (Rao, 1931; Stiasny, 1937). However, the high ratio of terminal club length to the oral arm length as well as the ratio between distal winged portion to naked proximal portion of the oral arm separate *C. annandalei* (Menon, 1930; Rao, 1931) from the present

material. Both *C. annandalei* and *C. orsini* possess accessory orbicular mouth appendages (Rao, 1931; Menon, 1936; Stiasny, 1937; Kramp, 1961) which the material lacks, a feature consistent in *C. stuhlmanni* (Stiasny, 1922; Kramp, 1961).

Although meristic differences (Table 6) between the present material and *C. orsini* are pronounced enough to allow ready separation (see also Table 3), and generally agree with the literature (Vanhöffen, 1888; Chun, 1896; Mayer, 1910; Stiasny, 1922; 1923; Menon, 1930; Rao, 1931; Menon, 1936; Stiasny, 1937; Ranson, 1945; Nair, 1946; Kramp, 1956; 1961; 1970) there are differences in some of the ratio data (Tables 7 and 8). The results of the MDS analysis (Figure 10) show that the two species are well separated, and even though the stress value is relatively high CAP was able to successfully categorize all specimens into the correct group (the permutation test results were significant: $p < 0.001$). For the canonical procedure a subset of three PCO axes were used based on the "leave-one-out" diagnostics which accounted for 66.74 % of the total variation in the species data. The first squared canonical correlation (d_1^2) was high: 0.56. Similar results were also obtained from the ANOSIM contributed (Global R: 0.67; $p < 0.001$). The variables contributing to the dissimilarities between species are highlighted in Table 9, foremost of which are features: length of terminal club (24.10 %), the ratios terminal club length to total oral arm length (17.89 %) and length of the distal portion to length of the proximal portion of the oral arm (12.77 %). All of which refer to various characteristics of the oral arm.

For cytochrome *c* oxidase subunit I (COI) a maximum length of 660 nucleotides was amplified from three *Crambionella* specimens sampled in the St. Lucia Estuary (Appendix 1) and compared to two *C. orsini* specimens (sequences downloaded from GenBank, accession numbers: EU363341 and EU363342). DNA sequence data from COI showed an average of

11.84 % pairwise sequence difference between the present material examined and *C. orsini*. Dawson and Jacobs (2001) suggest that differences of 10 – 20 % between COI sequences set the standard for species level divergence. Phylogenetic analyses computed using COI demonstrate a monophyletic *Crambionella* clade (Figure 11). The consensus tree was supported by generally high bootstrap values, except at the branch that illustrated Catostylidae to be paraphyletic to the other rhizostome families represented. This is in contrast to previous molecular phylogenetic analyses executed on rhizostomes using COI (Daryanabard and Dawson, 2008) and future work is needed to verify the findings in the present study. For internal transcribed spacer one (ITS1) a maximum length of 335 nucleotides was amplified from two *Crambionella* specimens sampled in the St. Lucia Estuary (Appendix 2); no comparative data for *C. orsini* was available.

Although on balance the material most closely resembles *C. stuhlmanni*, which is in agreement with its geographical distribution (Table 6), there was one feature at odds with previous descriptions. In the present specimens the intra-circular anastomosing canal network sometimes connected to both the rhopalial and inter-rhopalial canals (Figure 7), whilst in the original descriptions the anastomosing canals were only connected to rhopalial canals (Stiasny, 1922). It is unlikely that these discrepancies reflect erroneous observations on the part of Stiasny; it is probable that previous descriptions overlooked this rare feature due to small sample sizes examined. Scyphozoans often display considerable intra-specific morphological variation between geographically isolated or separated populations (Brewer, 1991; Bolton and Graham, 2004; Dawson, 2005a). Morphological variation is often as a result of phenotypic plasticity, a response to variable environmental conditions (Bolton and Graham, 2004; Dawson *et al.*, 2001). Dawson (2005b) highlights the importance of thorough

geographic sampling, in combination with adequate sample sizes (as observed in this study), to get a more accurate representation of morphological variation.

Molecular analyses are increasingly being used in scyphozoan systematics (Dawson and Jacobs, 2001; Schroth *et al.*, 2002; Dawson, 2003; Dawson, 2004; Holland *et al.*, 2004; Dawson, 2005a; 2005b; 2005c; 2005d; 2005e) and the decision about whether to use molecular or morphological analyses when describing species is subject to much debate (Dawson, 2005f). Molecular data increase the number of objective characters used, which enhances the likelihood of distinguishing taxa and permit phylogenetic reconstruction, free of impractical or inappropriate morphological features (Dawson, 2004). However, in some studies molecular analyses have failed to differentiate groups that showed significant morphological, behavioural and physiological differences (Dawson, 2005a). An approach which combines all data available is therefore required in scyphozoan systematics (Knowlton, 1993; Dawson, 2003; Dawson, 2005f). Although this study did not utilize ecological or behavioural data, integrating molecular and morphological data is an important stepping stone for future work on this species.

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Table 1: Morphological features (S #) of *Crambionella* specimens used in data analyses. Specimens were collected from St. Lucia estuary, on the NE coast of South Africa, during December 2005 and preserved in 5% formalin in ambient seawater. Figure references are given where applicable.

Morphological feature number	Figure reference number	Morphological feature description (measured in mm)	Morphological feature number	Figure reference number	Morphological feature description (measured in mm)
S 1	-	External umbrella diameter to tip of lappets	S 19	-	Width of oral pillars
S 2	-	External umbrella diameter to base of lappets	S 20	-	Internal umbrella diameter to tip of lappets
S 3	Figure 3	Umbrella height	S 21	-	Internal umbrella diameter to base of lappets
S 4	Figure 3	Umbrella thickness	S 22	Figure 5	Ring canal diameter
S 5	-	Width of oral arm base	S 23	Figure 5	Gonadal diameter along perradial axis
S 6	Figure 3	Length of the proximal (naked) portion of the oral arm	S 24	Figure 5	Gonadal diameter along adradial axis
S 7	Figure 3	Length of the distal portion (winged and terminal club) of the oral arm	S 25	Figure 5	Number of velar lappets in octant
S 8	Figure 3	Depth of oral arm (including naked and ventral winged portion)	S 26	-	Number of conical projections on velar lappets
S 9	-	Depth of naked portion of oral arm	S 27	-	Number of rhopalia
S 10	Figure 3	Depth of winged portion of oral arm	S 28	Figure 5	Number of rhopalial canals
S 11	Figure 3	Length of terminal clubs of oral arms	S 29	-	Point of termination for rhopalial canals
S 12	-	Width of terminal clubs of oral arms	S 30	Figure 5	Number of inter-rhopalial canals
S 13	Figure 4	Oral disc diameter	S 31	-	Point of termination for inter-rhopalial canals
S 14	Figure 4	Inter-ostia width	S 32	Figure 6	Number of anastomoses connecting with the ring canal
S 15	Figure 4	Width of ostia	S 33	Figure 6	Number of anastomoses connecting with adjacent inter- and rhopalial canals
S 16	-	Length of ostia	S 34	Figure 6	Number of anastomoses connections within the network
S 17	Figure 4	Depth of oral pillars	S 35	Figure 6	Number of primary folds in each section of gonads
S 18	-	Length of oral pillars	S 36	Figure 5	Number of annular muscles

Table 2: PCR conditions used to amplify cytochrome *c* oxidase subunit I (COI) and internal transcribed spacer one (ITS1) from *Crambionella* specimens collected from St. Lucia estuary, on the NE coast of South Africa, during December 2005 and preserved in absolute ethanol (adapted from Daryanabard and Dawson, 2008).

Number of Cycles	PCR steps	CO1	ITS1
One	Initial denaturation	8 min at 94 °C	8 min at 94 °C
	Annealing	2 min at 49 °C	2 min at 51.5 °C
	Extension	2 min at 72 °C	2 min at 72 °C
One	Denaturation	4 min at 94 °C	4 min at 94 °C
	Annealing	2 min at 50°C	2 min at 52.5°C
	Extension	2 min at 72 °C	2 min at 72 °C
Thirty-three	Denaturation	45 sec at 94 °C	45 sec at 94 °C
	Annealing	45 sec at 51 °C	45 sec at 53.5 °C
	Extension	1 min at 72 ° C	1 min at 72 ° C
One	Final extension Final hold	5 min at 72 °C 4 °C	5 min at 72 °C 4 °C

Table 3: A summary of all measurements of *Crambionella* specimens from the St. Lucia Estuary and *C. osini* specimens examined at the Natural History Museum, London.

	Crambionella material under investigation							*C. orsini*						
MF	Max	Min	Mean	StDev	N	Median	Mode	Max	Min	Mean	StDev	N	Median	Mode
S 1	181	62	119.53	29.75	38	121	135	165	114	147.6	19.53	5	152	152
S 2	158	54	96.79	24.70	38	98	102	144	100	132.8	18.46	5	139	139
S 4	29.4	4.7	13.90	6.17	44	11.8	11	-	-	-	-	-	-	-
S 5	9.2	1	4.43	1.24	44	4.4	5	8	3.5	5.61	1.03	5	5.5	5
S 6	19	1	9.35	3.13	44	10	11	14.5	5	9.27	2.78	5	8	8
S 7	45.4	1.6	24.83	8.16	44	26.75	30	52	26	40.04	6.85	5	41	41
S 8	19	3	10.66	3.09	44	11	11	17	8	12.07	2.26	5	12	12
S 9	10	1.5	5.67	1.72	44	6	6	8.5	3.5	5.91	1.41	5	6	6
S 10	29	3	15.19	4.26	44	15.6	15	26	12	18.85	3.68	5	18	17
S 11	12.7	1	5.71	2.05	42	6	6	22	11	16.63	3.1	5	17	17
S 12	11	1.4	5.79	1.78	42	6	5	15.5	1	11.06	2.84	5	11.75	13
S 13	58.4	20.5	37.86	8.28	43	38.75	41	-	-	-	-	-	-	-
S 14	26.4	7	15.62	4.40	44	15.4	17	26.5	17.5	22.81	2.99	4	24	25
S 15	17	3.3	9.25	2.65	44	9	8	17.5	12	14.34	1.94	4	13.75	13
S 16	11	2	4.47	1.24	44	4.1	4	6	3.5	4.79	0.72	3	6	5
S 17	12	1	4.51	1.45	44	4.4	5	-	-	-	-	-	-	-
S 18	25	6.4	16.52	3.71	44	16.9	19	19	7	11.65	2.85	5	11	8
S 19	13	1	4.91	1.87	44	4.4	4	11	3	6.96	2.12	5	6.75	6
S 20	122	40	86.66	20.68	38	24.9	78	-	-	-	-	-	-	-
S 21	113.5	29.4	71.10	18.58	38	78.95	78	-	-	-	-	-	-	-
S 22	90.5	28	63.59	15.68	43	63	50	-	-	-	-	-	-	-
S 23	57	18	36.97	8.97	40	35.8	43	-	-	-	-	-	-	-
S 24	12.6	1	5.61	1.99	40	5.2	6	-	-	-	-	-	-	-
S 25	29	4	13.31	1.62	38	13	12	20	13	6.39	1.28	5	16	16
S 26	19	1	10.18	3.36	34	10	12							
S 27	10	6	7.91	0.75	34	8	8	8	8	8	0	5	8	8
S 28	8	5	7.76	0.71	33	8	8	-	-	-	-	-	-	-
S 29	8	5	7.67	0.85	33	8	8	-	-	-	-	-	-	-
S 30	8	5	7.72	0.77	32	8	8	-	-	-	-	-	-	-
S 31	8	8	8	0	32	8	8	-	-	-	-	-	-	-
S 32	11	4	7.14	1.29	41	7	8	-	-	-	-	-	-	-
S 33	4	0	0.22	0.37	41	0	0	-	-	-	-	-	-	-
S 34	52	0	17.22	7.48	41	16	16	-	-	-	-	-	-	-
S 35	33	6	18.15	4.17	39	18	19	-	-	-	-	-	-	-
S 36	111	40	81.34	14.91	38	81.5	84	-	-	-	-	-	-	-
S 3: S 1	0.37	0.28	0.32	0.03	7	0.32	-	-	-	-	-	-	-	-
S 7: S 6	5.32	1.05	2.78	0.86	44	2.71	-	8.00	2.71	4.65	1.27	5	4.63	3
S 13: S 1	0.41	0.24	0.32	0.04	37	0.32	-	-	-	-	-	-	-	-
S 15: S 14	0.99	0.3	0.61	0.16	44	0.60	-	0.72	0.50	0.63	0.069	4	0.65	0.54
S 11: Oral arm length	0.243	0.1	0.17	0.04	42	0.17	-	0.44	0.24	0.34	0.053	5	0.65	0.31
Oral arm length: S 1	0.35	0.18	0.28	0.04	38	0.29	-	-	-	-	-	-	-	-

Table 4: Log transformed morphological features of *Crambionella* specimens (collected from St. Lucia estuary, during December 2005, and preserved in 5% formalin in ambient seawater) correlated with size of specimens (indicated by external umbrella diameter, S 1) using Pearsons product-moment correlation test (correlations significant at $p \leq 0.001$ after Bonferroni corrections; indicated by *). Comparisons are described by linear regression and reported in the form of: $y = mx + c$ for significant correlations.

		Pearson				
Morphological feature	Valid N	R	*p*	m	c	r^2
External diameter to tip of lappets	45	0.95	< 0.001*	0.97	-0.03	0.9
Length of proximal portion of the oral arm	45	0.78	< 0.001*	1.04	-1.2	0.61
Length of distal portion of the oral arm	45	0.82	< 0.001*	1.3	-1.3	0.68
Length of terminal clubs of oral arms	43	0.78	< 0.001*	1.14	-1.6	0.6
Depth of oral arm	45	0.81	< 0.001*	1.03	-1.1	0.66
Depth of naked portion of oral arm	45	0.9	< 0.001*	1.11	-1.55	0.81
Depth of winged portion of oral arm	45	0.89	< 0.001*	1.09	-1.08	0.79
Width of terminal clubs of oral arms	42	0.85	< 0.001*	1.2	-1.71	0.73
Width of oral arm base	45	0.85	< 0.001*	0.95	-1.32	0.73
Inter-ostia width	45	0.86	< 0.001*	0.92	-0.72	0.74
Width of ostia	45	0.72	< 0.001*	0.79	-0.68	0.52
Length of ostia	45	0.7	< 0.001*	0.67	-0.74	0.49
Depth of oral pillars	45	0.85	< 0.001*	1.12	-1.7	0.72
Length of oral pillars	45	0.89	< 0.001*	0.92	-0.76	0.79
Width of oral pillars	45	0.8	< 0.001*	1.06	-1.52	0.63
Internal umbrella diameter to base of lappets	38	0.9	< 0.001*	1	-0.14	0.81
Internal umbrella diameter to tip of lappets	38	0.9	< 0.001*	0.89	0.1	0.81
Umbrella thickness	38	0.77	< 0.001*	1.31	-1.59	0.6
Ring canal diameter	38	0.9	< 0.001*	0.92	-0.1	0.81
Gonadal diameter along perradial axis	34	0.9	< 0.001*	1.05	-0.6	0.8
Gonadal diameter along adradial axis	34	0.43	> 0.05			
Number of velar lappets in octant	38	0.21	> 0.05			
Number of conical projections on velar lappets	34	0.63	< 0.001*	1.18	-1.48	0.4
Number of anastomoses connecting with ring canal	38	0.59	< 0.001*	0.44	-0.06	0.35
Number of anastomoses connecting with adjacent inter- and rhopalial canals	16	-0.17	> 0.05			
Number of anastomoses connections within network	38	0.71	< 0.001*	1.22	-1.31	0.5
Number of primary folds in each section of gonads	33	0.9	> 0.05			
Number of annular muscles	38	0.38	> 0.05			
Umbrella height	7	0.84	>0.05			
Oral disc diameter	37	0.88	< 0.001*	0.003	1.19	0.77
Oral disc diameter: external umbrella diameter (S 2)	37	-0.47	<0.001*	-0.001	-0.4	0.22
Length of the distal portion of the oral arm: length of proximal portion of the oral arm	45	0.19	> 0.05			
Club length: oral arm length	43	-0.15	> 0.05			
Ostia width: inter-ostia width	45	-0.12	> 0.05			
Umbrella height: external umbrella diameter to tip of lappets	7	0.29	> 0.05			

Table 5: Log transformed morphological features of *Crambionella* specimens (collected from St. Lucia estuary, during December 2005, and preserved in 5% formalin in ambient seawater) correlated with size of specimens (indicated by external umbrella diameter, S 1) using Spearmans correlation test (correlations significant at $p \leq 0.01$ after Bonferroni corrections; indicated by *).

Morphological feature	Valid N	Spearman	
		p	R
Number of rhopalia	37	> 0.05	0.2
Number of rhopalial canals	37	> 0.05	0.22
Number of inter-rhopalial canals	36	> 0.05	0.27

Table 6: A character matrix highlighting morphological features that differ among the three *Crambionella* spp. (Vanhöffen, 1888; Chun, 1896; Mayer, 1910; Stiasny, 1922; 1923; Menon, 1930; Rao, 1931; Menon, 1936; Stiasny, 1937; Ranson, 1945; Nair, 1946; Kramp, 1956; 1961; 1970) and the *Crambionella* material under investigation. Recorded geographical ranges are also given for all species.

Feature	*C. orsini*	*C. annandalei*	*C. stuhlmanni*	*Crambionella* material under investigation
Umbrella diameter	55 – 210mm	80-200 mm	80-200 mm	62-181 mm (Table 3)
Proportion of umbrella height to umbrella diameter	0.3	0.3	0.3-0.5	Mean: 0.32 ±0.03 (Table 3)
Number of velar lappets in each octant	16	14	12	Mode: 12; range: 4-29 (Table 3)
Conical projections on velar lappets	Absent	Present	Present	Present
Number of conical projections	-	14-16	15-18	Mode: 12; range: 1-19 (Table 3)
Proportion of oral disc to external umbrella diameter	0.5-0.6	≤ 0.5	0.5	Mean: 0.32 ±0.04 (Table 3)
Accessory orbicular mouth appendages on distal winged portion	Present	Present	Absent	Absent
Proportion of distal winged portion to naked proximal portion	Three to four times as long	More than six times as long	Two to three times as long	Mean: 2.78 ±0.86 (Table 3)
Proportion of terminal club length to oral arm length	0.125	0.5	0.33	Mean: 0.17 ±0.04 (Table 3)
Proportion of ostia to inter-ostia width	⅓ - ½ as wide as inter-ostial columns	½ as wide as inter-ostial columns	¼ - ⅓ as wide as inter-ostial columns	Mean: 0.61 ±0.16 (Table 3)
Inter-rhopalial canals termination	Ring canal	Ring canal	Ring canal	Ring canal
Number of intra-circular anastomosing canals connected to ring canal	Rare	Rare	Rare	Rare (Table 3)
Intra-circular anastomosing canal connections to inter-rhopalial or rhopalial canal	Inter-rhopalial canals	-	Rhopalial canals	Connections to both inter- and rhopalaial canals (Figure 7)
Geographical range	SW and SE coast of India, Krusadai Islands, Persian Gulf to Red Sea and Kenya to Seychelles Islands	Bay of Bengal and Andaman Islands	Along the coasts of Mozambique and Madagascar	St. Lucia estuary (Figure 2)

Table 7: Two-tailed *t*-test results showing differences between *C. orsini* from the Natural History Museum, London and the *Crambionella* material under investigation (relationships significant at $p \leq 0.0028$ after Bonferroni corrections; indicated by *).

Morphological feature	*C. orsini*			*Crambionella* material under investigation					
	Valid N	Mean	StDev	Valid N	Mean	StDev	df	*p*	t-value
External diameter to tip of lappets	5	0.90	0.04	38	0.81	0.07	41	0.01	2.58
Length of proximal portion of oral arm	5	0.06	0.01	38	0.08	0.02	41	0.03	-2.21
Length of terminal club	5	0.11	0.01	36	0.05	0.01	39	< 0.001*	11.36
Depth of oral arm	5	0.08	0.01	38	0.09	0.01	41	0.24	-1.20
Depth of proximal porion of oral arm	5	0.04	0.01	38	0.05	0.01	41	0.03	-2.24
Depth of distal portion of oral arm	5	0.13	0.02	38	0.13	0.02	41	0.82	0.23
Width of terminal club	5	0.07	0.01	36	0.05	0.01	39	< 0.001*	5.60
Width of oral arm base	5	0.04	0.01	38	0.04	0.01	41	0.46	0.74
Inter-ostia width	5	0.08	0.01	38	0.14	0.02	41	< 0.001*	-8.15
Width of oral pillars	5	0.05	0.01	38	0.04	0.01	41	0.25	1.18
Number of velar lappets	5	0.11	0.02	38	0.12	0.03	41	0.63	-0.48
Width of ostia	4	0.10	0.01	38	0.08	0.02	40	0.03	2.31
Length of ostia	3	0.03	0.01	38	0.04	0.01	39	0.11	-1.64
Number of rhopalia	5	0.06	0.01	34	0.07	0.02	37	0.17	-1.41
Total oral arm length	5	0.33	0.02	38	0.28	0.04	41	0.02	2.50
Terminal club length: total oral arm length	5	0.34	0.04	42	0.17	0.04	45	< 0.001*	9.73
Distal portion of oral arm length: proximal portion oral arm length	5	4.65	0.98	44	2.78	0.86	47	< 0.001*	4.56
Ostia width: inter-ostia width	4	0.63	0.07	44	0.61	0.16	46	0.82	0.23

Table 8: Mann-Whitney-U results showing differences between *C. orsini* from the Natural History Museum, London specimens and the *Crambionella* material under investigation (relationships significant at $p \leq 0.017$ after Bonferroni corrections; indicated by *).

	C. orsini		*Crambionella* material under investigation							
	Valid N	Rank Sum	Valid N	Rank Sum	U	Z	p-level	Z adjusted	p-level	2*1sided exact p
Length of distal portion of oral arm	5	203	38	743	2	3.52	0.00	3.52	< 0.001*	0.00
Oral disc diameter	5	96	37	807	81	-0.45	0.66	-0.45	0.66	0.68
Inter-ostia width	4	145	38	758	17	2.53	0.01	2.53	0.01*	0.01

Table 9: Standardized morphometric data (MF) that contributed the most to dissimilarity between *Crambionella* material collected from the St. Lucia Estuary and *C. orsini* specimens examined at the Natural History Museum, London as determined by SIMPER analysis.

MF	contribution %	cumulative %
Length of the terminal club	24.10	24.10
Terminal club length: total oral arm length	17.89	41.99
Length of the distal portion of the oral arm: length of proximal portion of the oral arm	12.77	54.76
Width of terminal club	6.78	61.54
Length of oral pillar	5.81	67.35
Length of distal portion of oral arm	4.13	71.48
Length of proximal portion of oral arm	4.11	75.60
Width of ostia	3.20	78.80
Oral disc diameter	2.81	81.61
Length of ostia	2.76	84.37
Width of oral pillar	2.53	86.91
Ostia width: inter-ostia width	2.49	89.40
Depth of naked portion of oral arm	2.01	91.41

Figure Captions

Figure 1: A photograph of a live specimen from St. Lucia estuary (©Ricky Taylor).

Figure 2: A map showing the geographical location of the Greater St. Lucia Wetland Park and a close up of St. Lucia estuary (modified from http://www.bibs.co.za/st_lucia.htm and http://commons.wikimedia.org/wiki/Category:Greater_St._Lucia_Wetland_Park).

Figure 3: A schematic diagram of a longitudinal section along the perradial axis of a specimen (adapted from Dawson 2005e). Only two of the eight oral arms are represented.

Figure 4: A schematic diagram of the oral disc, from a subumbrella view, showing two of the eight oral arms (adapted from Dawson 2005e). Only two of the eight oral arms are fully represented.

Figure 5: A photograph showing the subumbrella view of a *Crambionella* medusa collected in the St. Lucia Estuary illustrating various morphological measurements taken (©Simone Neethling).

Figure 6: A photograph showing the subumbrellar view of a *Crambionella* medusa collected in the St. Lucia Estuary illustrating the intra-circular and extra-circular anastomosing canal networks, after injecting coloured dye latex (©Simone Neethling).

Figure 7: A photograph showing the subumbrellar view of a *Crambionella* medusa collected in the St. Lucia Estuary illustrating anastomosing canal network connections to both the rhopalial and inter-rhopalial canals, after injecting coloured dye latex (©Simone Neethling).

Figure 8: Photographs showing the subumbrellar view of a *Crambionella* medusa collected in the St. Lucia Estuary illustrating inter-rhopalial canals that appear to extend beyond the ring canal. On closer inspection more than one canal originated from ring canal section and was thinner than canals that preceded the ring canal. Inter-rhopalial canals were therefore accepted to terminate at the ring canal (©Simone Neethling).

Figure 9: A schematic diagram of a rhopalium, terminating in two ocular lappets, with adjacent velar lappets. Velar lappets possess conical projections on the dorsal median line. Anastomosing canal networks can be seen.

Figure 10: Non-metric multi-dimensional scaling (MDS) of standardized morphometric data illustrating the morphological dissimilarity between *Crambionella* medusa collected in the St. Lucia Estuary (grey) and *C. orsini* (black) examined at the Natural History Museum, London. Stress value is indicated.

Figure 11: A consensus tree of Rhizostomeae (sequence data received from Professor MN Dawson), and outgroup, based on 474 nucleotides from cytochrome *c* oxidase subunit I (COI). Analyzed by Direct Optimization in POY. Bootstrap values are indicated.

Figure 1

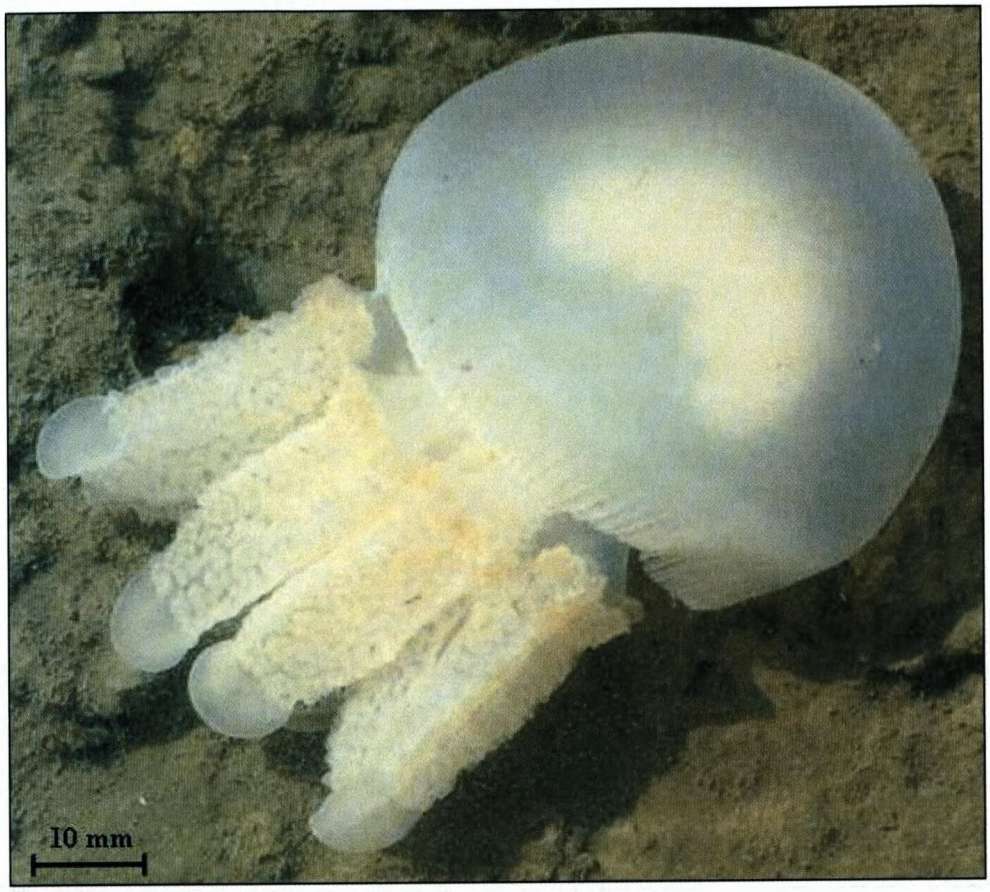
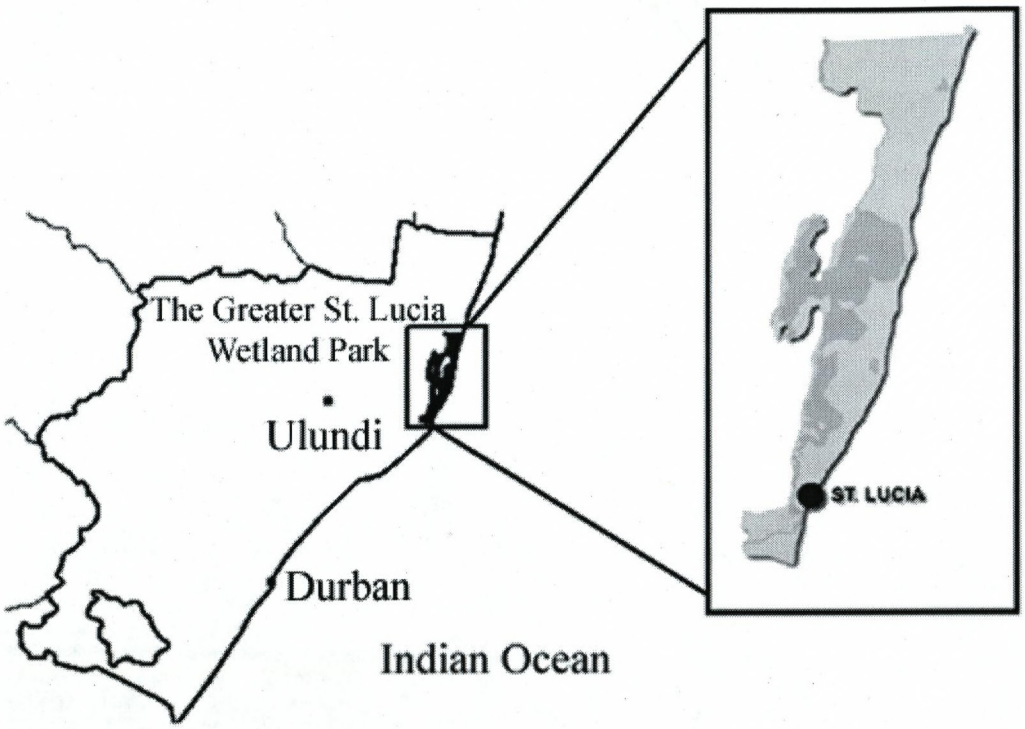


Figure 2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Wetland Park
Ulundi
Durban
Indian Ocean
ST. LUCIA

Figure 3

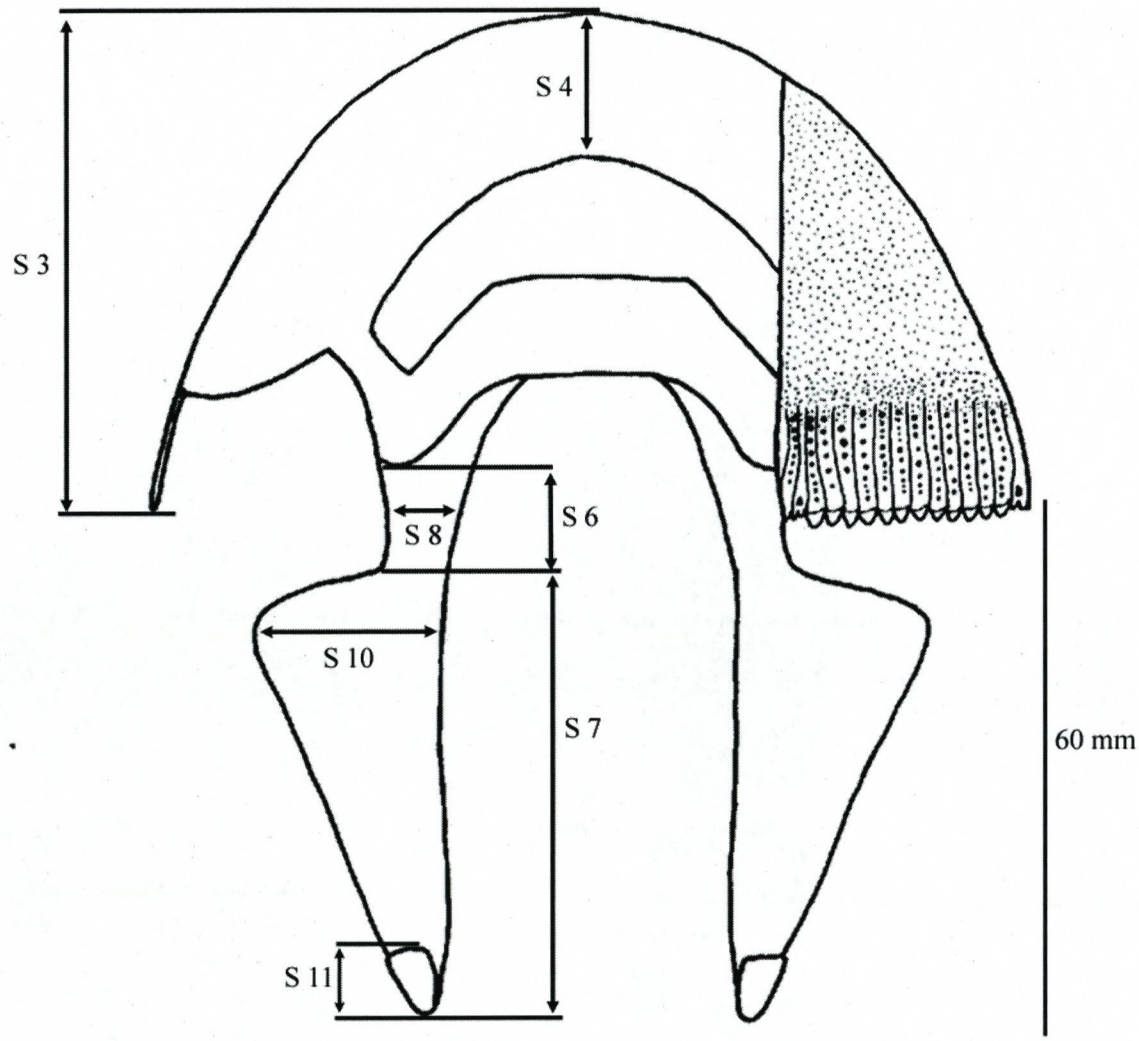
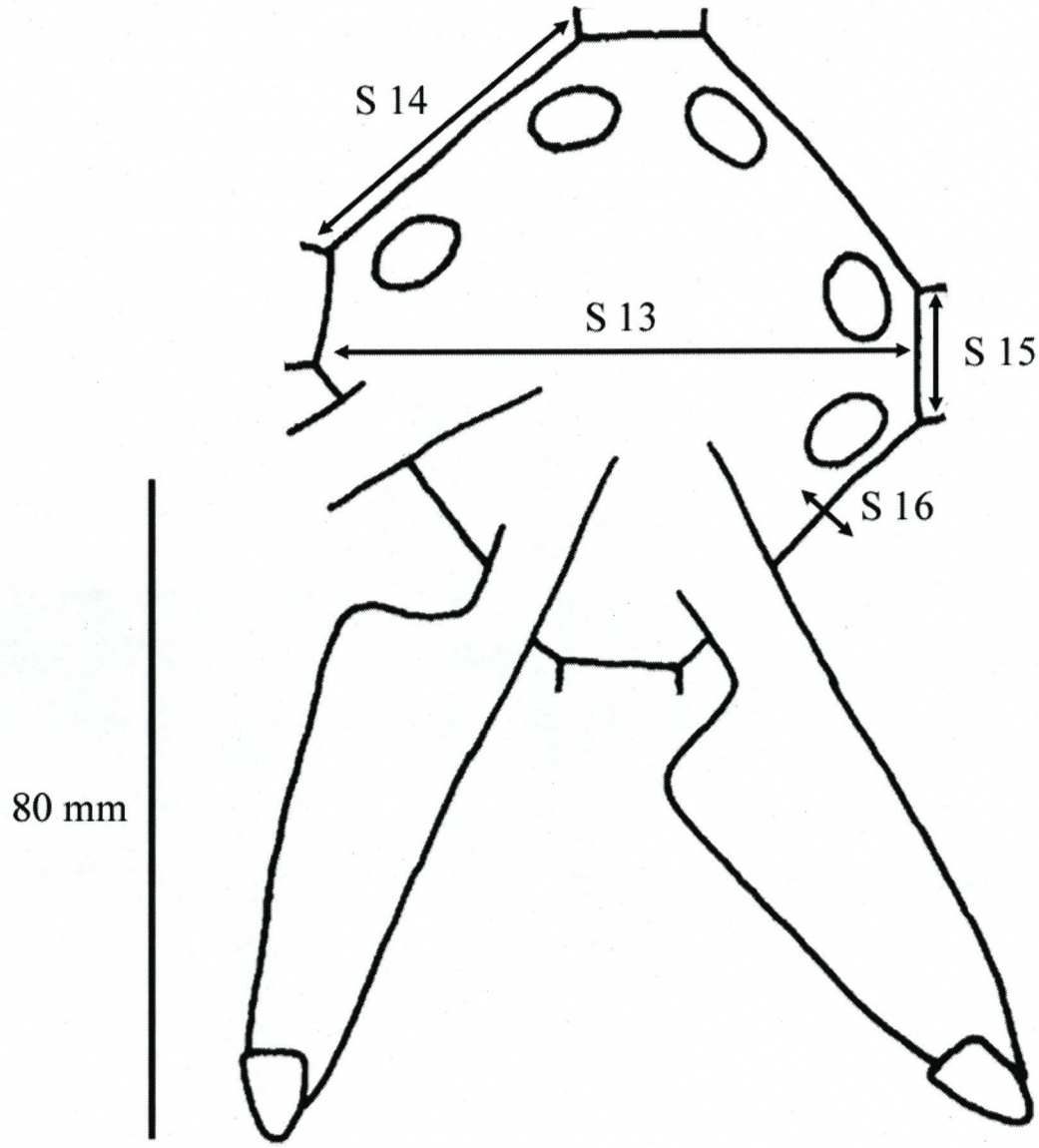


Figure 4



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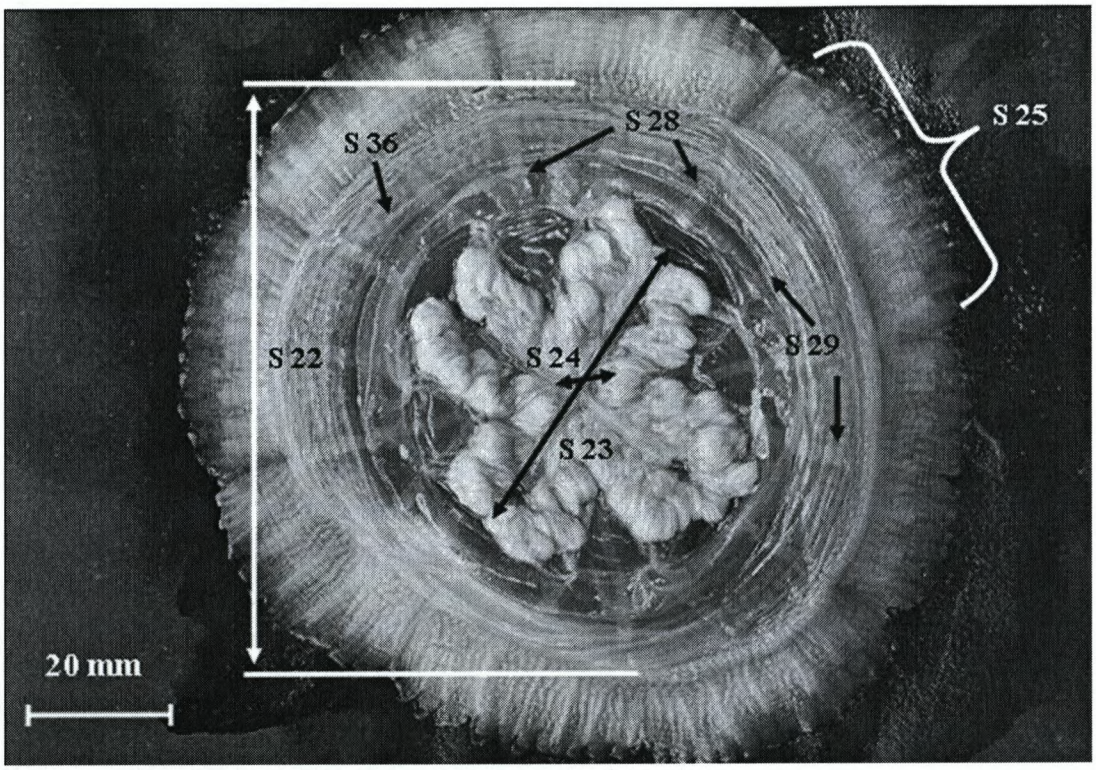

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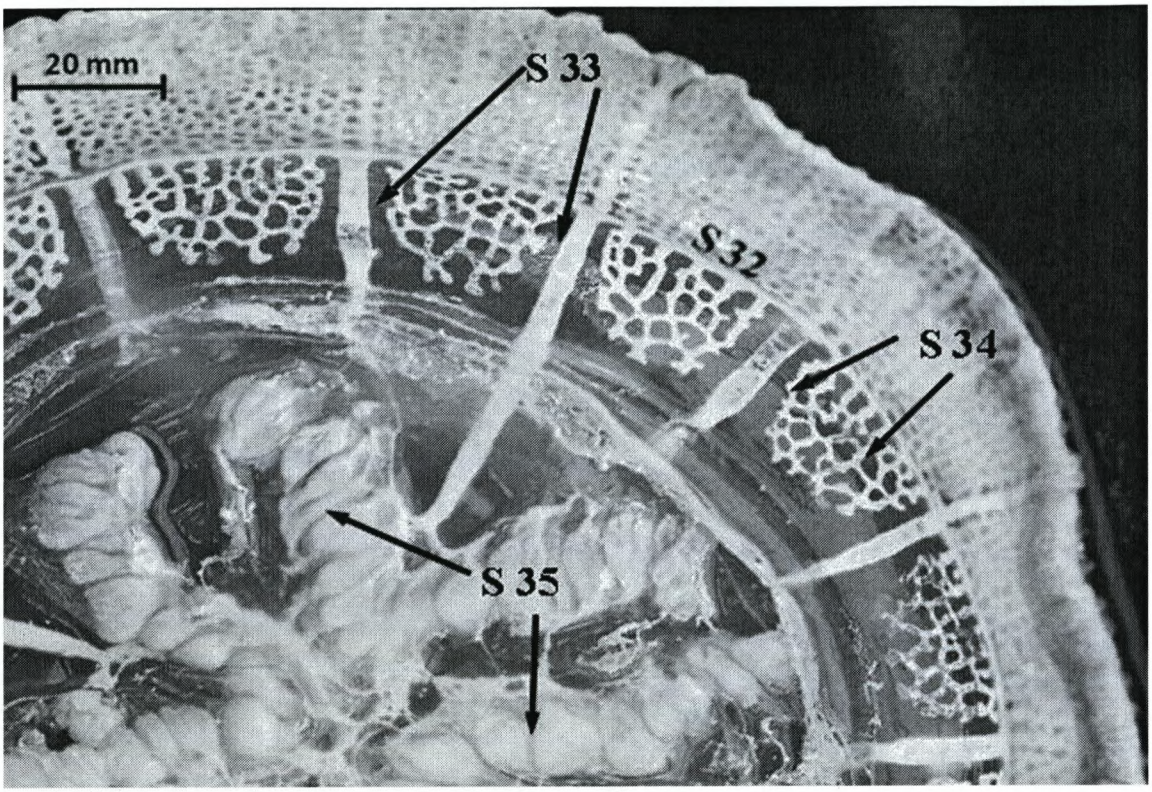


Figure 7

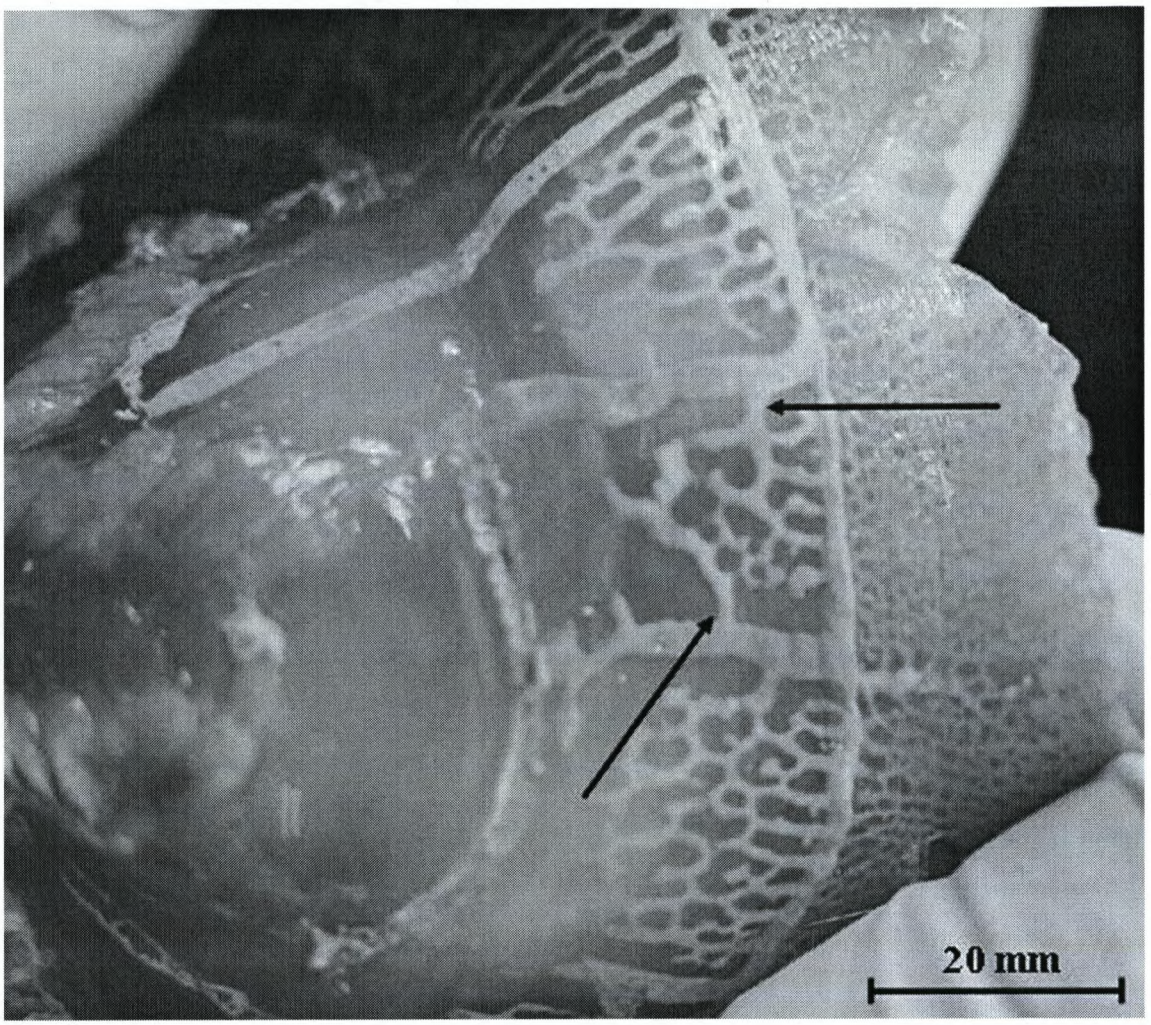


Figure 8

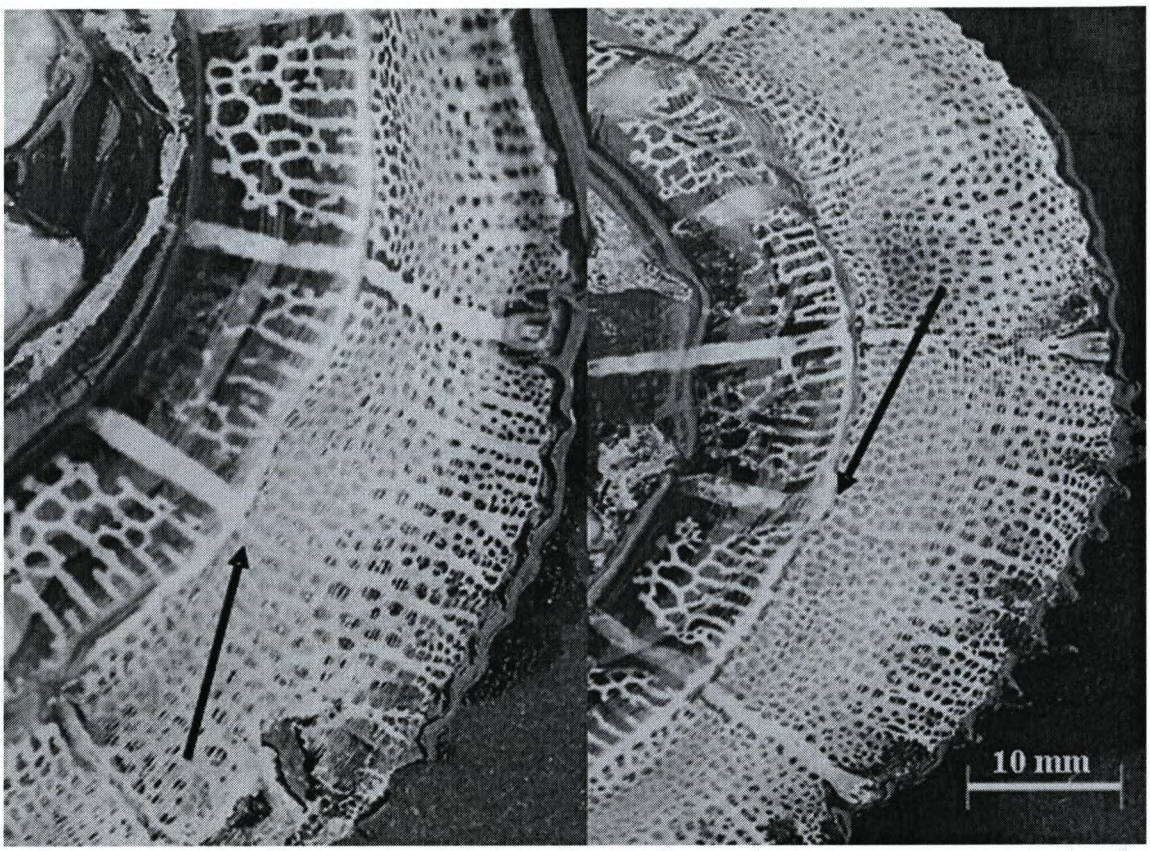


Figure 9

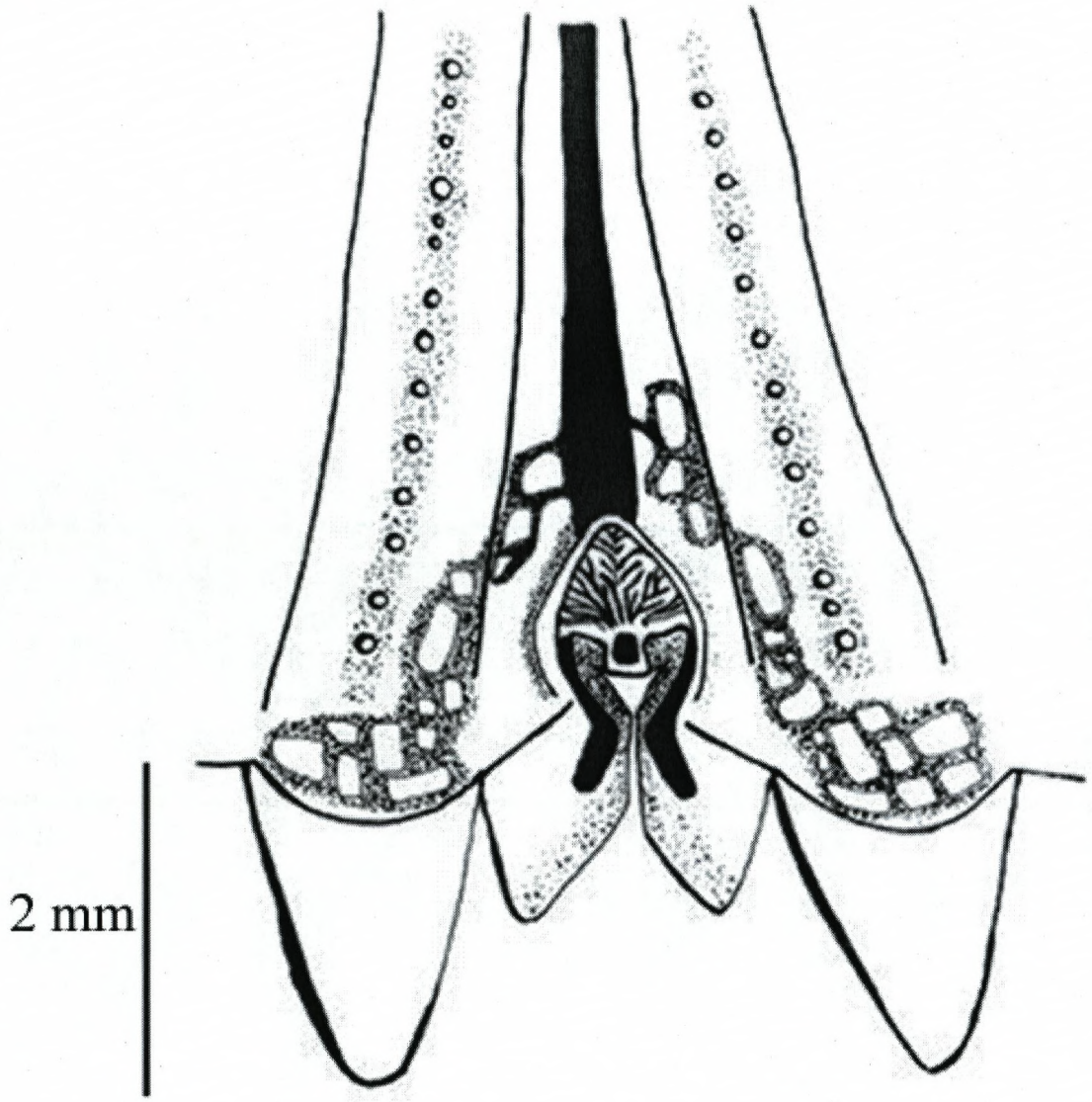


Figure 10

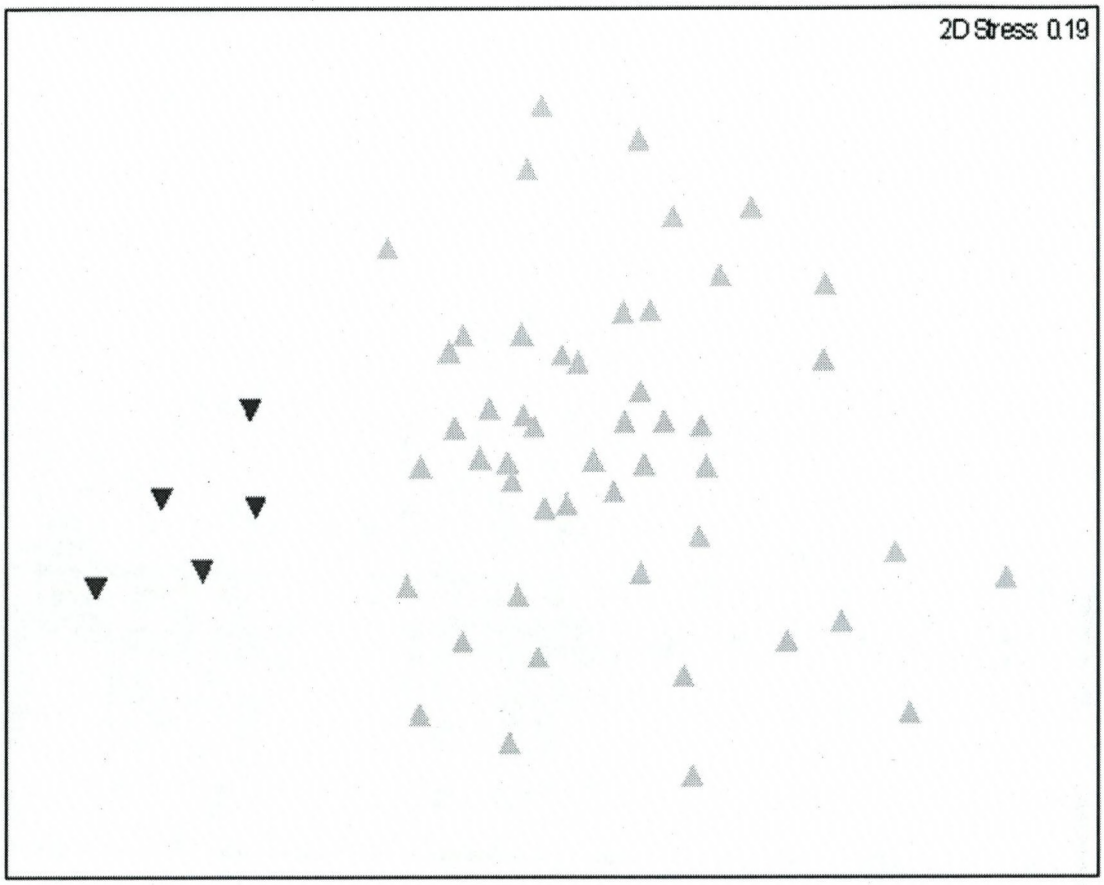
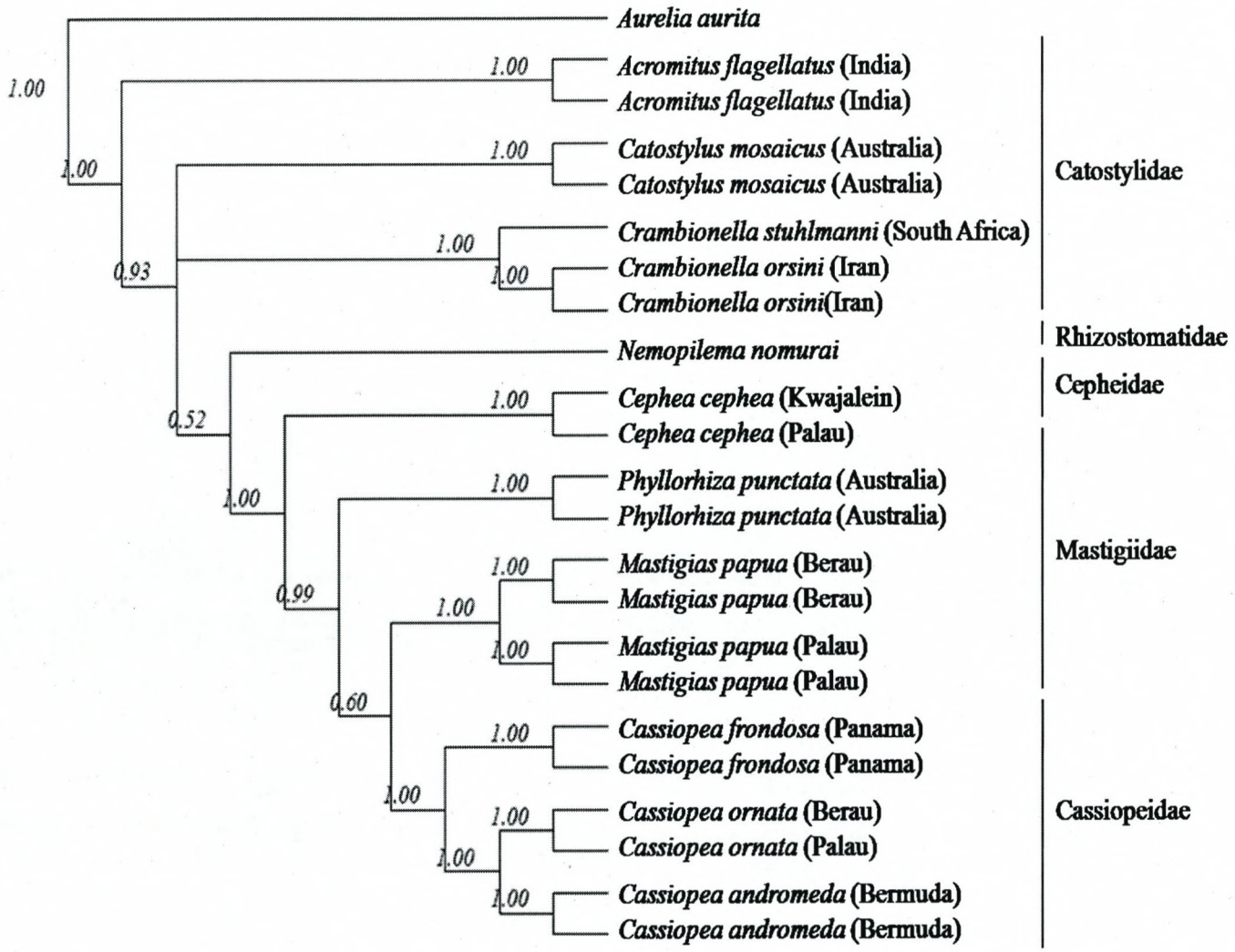


Figure 11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Acromitus flagellatus (India)
Catostylus mosaicus (Australia)
Catostylus mosaicus (Australia)
Crambionella stuhlmanni (South Africa)
Crambionella orsini (Iran)
Crambionella orsini(Iran)
Nemopilema nomurai
Cephea cephea (Kwajalein)
Cephea cephea (Palau)
Phyllorhiza punctata (Australia)
Phyllorhiza punctata (Australia)
Mastigias papua (Berau)
Mastigias papua (Berau)
Mastigias papua (Palau)
Mastigias papua (Palau)
Cassiopea frondosa (Panama)
Cassiopea frondosa (Panama)
Cassiopea ornata (Berau)
Cassiopea ornata (Palau)
Cassiopea andromeda (Bermuda)
Cassiopea andromeda (Bermuda)
Catostylidae
Rhizostomatidae
Cepheidae
Mastigiidae
Cassiopeidae

Appendix 1: The consensus sequence of cytochrome *c* oxidase subunit I (COI) amplified from three *Crambionella* specimens collected from the St. Lucia Estuary at Charters Creek on the Lake Shore during September 2008. Variable nucleotide bases and gaps, if present, are indicated.

consensus	**T**	**C**	**C**	**T**	**C**	**N**	**A**	**G**	**C**	**A**	**G**	**G**	**A**	**T**	**C**	**A**	**A**	**A**	**G**	**A**	**A**	**A**	**G**	**A**	**A**	**G**	**T**	**A**	**T**	**T**
cs 1	C	·	T	·	—	—	—	·	G	T	C	A	·	C	A	·	·	T	C	·	T	·	A	·	G	A	·	·	·	·
cs 2	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**G**	**A**	**A**	**A**	**T**	**T**	**T**	**T**	**C**	**T**	**G**	**T**	**C**	**T**	**G**	**T**	**T**	**A**	**A**	**T**	**A**	**G**	**—**	**—**	**—**	**—**	**—**	**—**	**—**	**—**
cs 1	·	·	·	·	C	·	C	·	T	·	A	·	T	·	·	A	·	·	·	·	T	·	G	T	G	C	T	T	T	T
cs 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	—	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**—**	**C**	**A**	**T**	**A**	**G**	**T**	**G**	**A**	**T**	**A**	**G**	**C**	**T**	**C**	**C**	**A**	**G**	**C**	**C**	**A**	**A**	**T**	**A**	**C**	**A**	**G**	**G**	**T**	**A**
cs 1	T	·	T	G	C	A	·	·	·	·	·	·	G	·	A	·	·	·	·	·	T	T	C	·	—	G	T	A	·	G
cs 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**G**	**N**	**G**	**A**	**T**	**A**	**G**	**A**	**A**	**G**	**T**	**A**	**A**	**A**	**A**	**G**	**T**	**A**	**T**	**C**	**G**	**C**	**T**	**G**	**T**	**T**	**A**	**C**	**T**	**A**
cs 1	A	T	T	·	·	·	A	G	·	C	·	C	G	·	·	T	·	·	·	·	—	—	·	·	G	·	C	·	·	G
cs 2	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**A**	**T**	**A**	**C**	**T**	**G**	**A**	**C**	**C**	**A**	**T**	**A**	**C**	**A**	**A**	**A**	**C**	**A**	**A**	**G**	**G**	**G**	**A**	**A**	**T**	**T**	**T**	**T**	**A**	**T**
cs 1	G	·	T	·	·	A	T	G	T	T	A	G	G	·	G	·	T	G	·	C	C	A	·	C	·	·	·	·	C	A
cs 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**C**	**C**	**A**	**T**	**A**	**G**	**T**	**C**	**A**	**T**	**C**	**C**	**C**	**T**	**G**	**G**	**G**	**G**	**C**	**T**	**C**	**T**	**C**	**A**	**T**	**A**	**T**	**T**	**T**	**A**
cs 1	A	T	G	·	T	·	·	T	G	·	·	A	·	C	G	–	–	–	·	·	·	A	·	G	C	T	·	·	A	·
cs 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**A**	**A**	**A**	**T**	**T**	**G**	**T**	**A**	**G**	**T**	**A**	**A**	**T**	**G**	**A**	**A**	**A**	**T**	**T**	**A**	**A**	**T**	**A**	**G**	**C**	**T**	**C**	**C**	**C**	**A**
cs 1	T	·	·	·	G	A	·	T	T	·	T	T	·	C	T	T	C	G	·	T	·	·	G	C	·	·	G	T	A	T
cs 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**T**	**T**	**A**	**T**	**A**	**G**	**A**	**G**	**G**	**A**	**G**	**G**	**C**	**C**	**C**	**C**	**T**	**G**	**C**	**T**	**A**	**A**	**A**	**T**	**G**	**T**	**A**	**A**	**A**	**C**
cs 1	·	·	·	·	·	·	G	C	·	·	A	T	T	·	–	–	–	–	–	–	–	–	–	–	·	G	·	·	·	·
cs 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**T**	**A**	**A**	**A**	**T**	**A**	–	**T**	**N**	**C**	**C**	**C**	**A**	**T**	**A**	**T**	**C**	**C**	**A**	**C**	**A**	**G**	**A**	**T**	**C**	**C**	**T**	**C**	**C**	**A**
cs 1	·	G	·	T	·	·	G	·	A	·	·	T	C	·	T	·	A	T	·	T	·	·	G	·	G	·	·	·	·	T
cs 2	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**G**	**A**	**G**	**T**	**G**	**A**	**G**	**C**	**T**	**T**	**G**	**G**	**A**	**T**	**T**	**G**	**A**	**A**	**C**	**T**	**A**	**A**	**G**	**T**	**G**	**G**	**T**	**G**	–	–
cs 1	·	·	T	A	T	G	·	·	A	·	T	T	C	C	·	A	G	·	·	·	·	·	·	A	·	A	A	·	A	T	T
cs 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**G**	**G**	**T**	**A**	**T**	**A**	**T**	**A**	**G**	**T**	**T**	**C**	**A**	**T**	**C**	**C**	**T**	**G**	**T**	**C**	**C**	**C**	**G**	**A**	**C**	**T**	**C**	**C**	**T**	**T**
cs 1	A	·	·	T	·	T	·	G	·	·	·	A	T	·	A	·	C	G	C	·	A	G	C	·	·	·	·	·	·	A
cs 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**G**	**T**	**T**	**C**	**T**	**A**	**C**	**T**	**A**	**A**	**G**	**G**	**A**	**T**	**G**	**A**	**G**	**C**	**C**	**A**	**A**	**G**	**T**	**A**	**G**	**C**	**A**	**A**	**T**	**A**
cs 1	T	·	G	·	·	·	·	·	T	G	·	C	T	C	A	T	C	·	T	T	·	·	·	·	·	A	·	C	A	·
cs 2	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·

consensus	**A**	**G**	**A**	**G**	**T**	**G**	**C**	**T**	**G**	**G**	**C**	**G**	**G**	**T**	**A**	**A**	**T**	**A**	**A**	**C**	**C**	**A**	**A**	**A**	**A**	**A**	**C**	**T**	**A**	**A**
cs 1	G	G	A	G	T	C	G	G	G	A	C	A	G	G	A	T	G	A	A	C	T	A	T	A	T	A	C	C	–	–
cs 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**T**	**A**	**T**	**T**	**A**	**T**	**T**	**T**	**A**	**G**	**T**	**C**	**T**	**A**	**G**	**G**	**A**	**A**	**A**	**T**	G	**C**	**C**	**A**	**T**	**A**	**T**	**C**	**A**	**G**
cs 1	C	·	C	C	·	C	·	·	·	·	·	T	C	·	A	T	C	C	·	A	G	·	T	C	A	C	·	·	T	G
cs 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**G**	**A**	**G**	**C**	**A**	**C**	**C**	**T**	**A**	**T**	**A**	**T**	**A**	**A**	**A**	**G**	**G**	**G**	**G**	**T**	**A**	**C**	**T**	**A**	**G**	**T**	**C**	**A**	**G**	**T**
cs 1	·	·	·	G	·	T	·	·	G	·	G	G	·	T	·	T	·	·	·	G	·	–	·	·	T	·	T	·	·	·
cs 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**T**	**T**	**C**	**C**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**G**	**A**	**A**	**T**	**C**	**C**	**G**	**C**	**C**	**T**	**A**	**T**	**T**	**A**	**A**	**T**
cs 1	·	·	A	·	A	T	T	T	A	G	C	A	G	G	·	G	C	C	T	·	C	T	·	·	·	·	A	·	T	G
cs 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**A**	**C**	**A**	**G**	**G**	**C**	**A**	**T**	**A**	**A**	**C**	**G**	**A**	**A**	**G**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**T**	**C**	**A**	**T**	**T**	**A**	**T**	**T**
cs 1	G	G	·	·	C	T	·	·	T	·	A	T	T	T	C	·	T	T	·	C	T	A	C	A	·	·	·	T	·	A
cs 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**A**	**A**	**A**	**G**	**C**	**G**	**T**	**G**	**A**	**G**	**C**	**–**	**–**	**–**	**G**	**G**	**T**	**G**	**A**	**C**	**A**	**A**	**C**	**A**	**A**	**C**	**A**	**T**	**T**	**G**
cs 1	·	·	T	A	T	·	A	·	·	·	·	C	C	C	A	·	G	·	·	T	G	·	·	T	·	T	G	G	A	T
cs 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**T**	**A**	**A**	**A**	**G**	**T**	**T**	**G**	**G**	**T**	**C**	**A**	**T**	**C**	**T**	**C**	**C**	**T**	**A**	**A**	**C**	**A**	**T**	**A**	**G**	**A**	**A**	**C**	**C**	**A**
cs 1	A	·	·	·	T	·	C	C	C	·	T	G	·	T	T	G	T	A	T	G	G	T	C	·	·	T	A	T	T	A
cs 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**G**	**G**	**A**	**C**	**C**	**A**	**G**	**–**	**–**	**A**	**T**	**A**	**A**	**T**	**T**	**C**	**G**	**A**	**G**	**T**	**C**	**T**	**T**	**A**	**T**	**A**	**A**	**T**	**C**	**A**
cs 1	·	T	·	A	·	·	·	C	G	·	·	·	C	·	·	T	T	·	C	·	T	C	·	·	·	C	C	C	T	·
cs 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**T**	**N**	**C**	**–**	**T**	**G**	**A**	**A**	**G**	**G**	**C**	**T**	**G**	**T**	**A**	**C**	**C**	**T**	**A**	**T**	**C**	**A**	**T**	**T**	**G**	**C**	**A**	**G**	**A**	**A**
cs 1	C	C	T	G	·	A	T	T	·	·	·	·	·	G	·	G	·	·	·	·	·	·	C	·	A	T	G	–	–	–
cs 2	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**A**	**A**	**A**	**G**	**C**	**A**	**C**	**C**	**A**	**A**	**A**	**T**	**A**	**T**	**C**	**A**	**A**	**A**	**T**	**A**	**A**	**A**	**G**	**A**	**G**	**T**	**T**	**C**	**C**	**A**
cs 1	–	–	–	–	–	–	–	T	T	·	T	·	·	A	·	·	G	·	C	·	G	·	A	·	–	·	·	T	·	·
cs 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
cs 3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

consensus	**A**	**T**	**A**	**T**	**C**	**T**	**T**	**T**	**A**	**T**	**G**	**A**	**T**	**T**	**T**	**G**	**T**	**T**	**G**	**–**	**A**	**C**	**C**	**A**	**G**	**A**	**G**	**G**	**A**	
cs 1	·	·	·	C	T	·	C	·	T	·	C	T	·	·	G	A	·	C	C	T	G	·	T	G	·	·	·	·	·	
cs 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
cs 3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	–	

Appendix 2: The consensus sequence of internal transcribed spacer one (ITS1) amplified from two *Crambionella* specimens collected from the St. Lucia Estuary at Charters Creek on the Lake Shore during September 2008. Variable nucleotide bases and gaps, if present, are indicated.

TCGCACGAGCCGAGTGATCCACCTTAGAAGTTGTCTCTGACTTTTTTCATTTCCAACT
ATTCACACTAATGTGTCAATAATTATGAATTCATGAATTTCAAGTTTGAAAAAATAT
AACACTAAAAAAACTCCATGTGAGGCCGACAGGAAGACGCCTGCCATTTAAGCACA
GACAACAGCGACTGCAGCCTGCCAGTCCGGCCTGCTTCTGGTCACCTCACACAGATT
GGCACGGGTTCACAGTGGTTCGCATACCTTTGACGGTCAGTCAAGGGTTGATAGCGT
GTAGCCAACTTTCGGTAATGATCCTTCCGCAGGTTCACCTACGAAACCAA