Molecular systematics of the genus *Hypnea* (Rhodophyta) in South Africa, with the description of a new genus, *Tenebris* (Cystocloniaceae, Rhodophyta)

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DECLARATION

I declare that this thesis is my own, unaided work and has not been submitted in this or any form to another university. Where use has been made of the research of others, it has been duly acknowledged in the text.

Work discussed in this thesis was carried out under the supervision of Professor JJ Bolton of the Department of Biological Sciences, University of Cape Town, Associate Professor RJ Anderson of Department of Agriculture, Forestry and Fisheries and the Department of Biological Sciences, University of Cape Town, and Dr Lydiane Mattio of the Department of Biological Sciences, University of Cape Town and CSIRO (Australia)

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Abstract

Hypnea, an economically important genus of red algae (containing κ-carrageenan) found globally on tropical and subtropical shores, is shrouded in taxonomic confusion due to morphological plasticity of species and general lack of clear morphological characters. Of 113 species described worldwide only half of that number is accepted taxonomically. Of the 11-recorded species in South Africa there are currently 8 recognised (*Hypnea arenaria, H. ecklonii, H. intricata, H. musciformis, H. rosea, H. spicifera, H. tenuis and H. viridis*). Some of these species are difficult to tell apart and appear to differ only in dimensions, which overlap in some species. In addition, some entities do not fit the descriptions currently in existence for these species and some have not been recorded since description. This study aimed to use morphological and molecular techniques to re-examine as many South African *Hypnea* entities as possible, in order to improve the taxonomic understanding of this group. Collections were done along the coast of South Africa, Mozambique, Madagascar and Europa Island. Samples were pressed as herbarium specimens, preserved in 5% formalin in sea water for morphological and anatomical analysis, and dried in silica gel for sequencing. DNA was extracted from dried samples and mitochondrial (*cox1*) and plastid (*rbcL*) DNA sequences were obtained. Sequences were assembled, aligned by eye, and analysed using maximum likelihood, Bayesian analyses, and genetic distance (GD) matrices for molecular analysis. Specimens were sectioned and photographed for morphological analysis. The results uncovered different organisation of the *Hypnea* genus than previously recorded. Specimens which fitted the description of *Hypnea rosea* were found to form a complex containing two molecularly distinct morphotypes (1,7% GD in *rbcL*; 6,8% GD *cox1*) with a link to *H. cervicornis. Hypnea viridis* is shown to be closely linked to the tropical *Hypnea pannosa* (1,7% GD in *rbcL*)*, Hypnea spicifera,* although very morphologically variable, was found to comprise one species. There is a clade which could represent *Hypnea cf. intricata* – a species with a very brief type description that matches with the morphology of these specimens. *Hypnea musciformis* does not exist here, because none of the specimens that fitted the South African description were even closely linked to *H. musciformis* from close to the type locality (Trieste, Italy). They came out in the *H. rosea* clades and the *H. cf. intricata* clade. *Hypnea ecklonii* could not be recognised among any of the specimens that were studied. There are two new records of *Hypnea cf. pannosa* and *Hypnea cf. cervicornis* for South Africa.

All of the specimens that fitted the descriptions of *H. tenuis* were molecularly unrelated to any of the *Hypnea* species for which DNA sequences were available (*rbcL* GD >11%). However, they fit in the Cystocloniaceae with their closest relatives being *Calliblepharis ciliata, Hypnea viridis* and *Hypnea pannosa* (*rbcL* GD >8%). These GD values are large enough that these two species form a separate genus. Therefore, I describe a new genus of Cystocloniaceae, *Tenebris* V. Johnson, J. Bolton, L. Mattio, R. Anderson gen. nov. This appears morphologically very similar to *Hypnea*, but differs molecularly. The morphological differences include size ratio of central filament to periaxial cells – where present, *Hypnea* central cells are significantly smaller than pericentral cells while *Tenebris* pericentral cells are similar in size to the central filament. *Tenebris spp.* are also much smaller than the *Hypnea spp. Hypnea tenuis* Kylin is re-assigned to *Tenebris tenuis* (Kylin) V Johnson, JJ Bolton, L Mattio, RJ Anderson *comb. nov.* The second species is based on only one specimen, and although it is somewhat similar to the type description of *Hypnea arenaria*, without more evidence, it is provisionally named *Tenebris* sp.

In total, there are 13 molecular clades of South African sequences in this study: 7 *Hypnea* clades identified to species level, 2 unidentified *Hypnea* which cluster with no other *Hypnea* species, 2 unidentified and distinct sequences which are not *Hypnea*, and 2 species assigned to a new genus *Tenebris.* In conclusion, the 7 South African *Hypnea* are: *Hypnea cf. cerviconis, H. cf. intricata, H. cf. pannosa, H. rosea sp. 1, H. rosea sp. 2, H. spicifera,* and *H. viridis.* The unidentified *Hypnea spp.* are in clades G and I. The unidentified sequences from a different genus are in clade H. There are 2 species *Tenebris tenuis* and *Tenebris sp.* assigned to the new genus.

Introduction

South African marine macro-algae

South Africa has a long coastline (ca 3000km), extending from tropical waters in the north of Kwazulu-Natal (KZN), through warm temperate waters on the south coast to cool temperate waters on the west coast. Rocky substrata are common along the coast, so that it is no surprise that the diversity of seaweeds is relatively high (Stegenga et al. 1997, Bolton & Anderson 1997). In South Africa, there are to date just over 800 species of algae recorded, a rich diversity according to Lüning (1990), with roughly a 1:1:4 split between green algae (Chlorophyta), brown algae (Phaeophyceae), and red algae (Rhodophyta), respectively (Bolton 1999, Bolton & Stegenga 2002, Anderson et al. 2016). The continuous residence of the seaweed flora in the warm temperate conditions of coastal waters of South Africa, coupled with the long time period of geological isolation has been proposed to have led to a high degree of species endemism along the coast (Hoek 1984, Bolton 1999, Bolton & Stegenga 2002). South African seaweed diversity has been relatively well documented in catalogues and floras, but much of the original taxonomic information is in old, hard-to-find literature (Bolton 1999). However, the advent of the online 'Biodiversity Heritage Library' has made it much easier to access.

Taxonomic history

Bolton (1999) compiled a history of South African seaweed systematics over the past 2 centuries. The work done by taxonomists since the 19th century provided the groundwork for the two catalogues listing seaweed species names and synonyms that were compiled by Seagrief (1984) and Silva et al. (1996) – the latter being an extension of the Indian Ocean seaweed catalogue started by Papenfuss (1947). A comprehensive flora of the seaweeds of the west coast of South Africa, including detailed descriptions of close to 400 species, was published by Stegenga et al. (1997). These coupled with the approximately 700 South African species documented in Silva et al. (1996) provided a workable census of South African algae until the establishment of Algaebase, a literature-based online catalogue of global seaweed diversity (Guiry and Guiry 2017). The past few decades have shown a huge increase in South African taxonomic work spurred by the need for extensive and up-to-date studies of South African seaweeds.

The Coastal Environment of South Africa

Bolton & Anderson (1990) showed that seaweed species composition has a strong correlation with the temperature of coastal waters. The east coast of South Africa is strongly influenced by the Indian Ocean and along this coast we find seaweeds with warm-water affinities, while the west coast is

largely influenced by the Benguela current and we find seaweeds adapted to colder water temperatures.

The seaweed distribution records further support the idea of three biogeographic Marine Provinces in South Africa, (Fig. 1) (Bolton & Anderson 1990; Stegenga et al. 1997; Anderson et al. 2009): the cool temperate West Coast (Benguela Marine Province), the warm temperate South Coast (Agulhas Marine Province), and a Tropical Western Indian Ocean flora in northern KwaZulu-Natal. Broad overlap zones lie between these marine provinces. Between the Benguela and Agulhas Marine Provinces lies a transition zone extending from Cape Point to Cape Agulhas, and most of the east coast represents an overlap between the warm temperate seaweeds of the Agulhas marine provinces and the tropical flora of extreme northern KwaZulu-Natal (Maputaland).

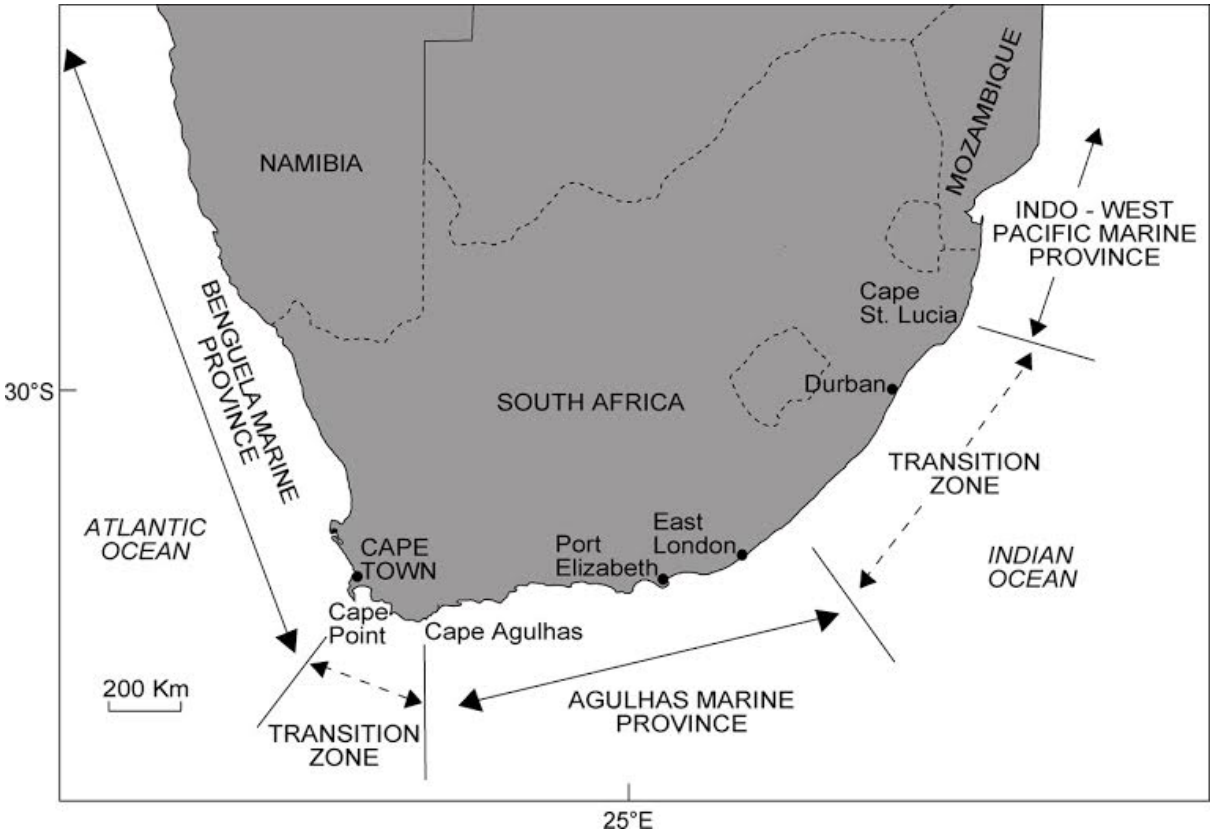

Figure 1: South African Coastal map displaying the three Marine Provinces and the transition zones (Anderson et al. 2009)

Red algae (Rhodophyta)

The red algae (Rhodophyta) are a significant group of organisms, comprising just under 8000 species worldwide according to the latest estimates (Guiry & Guiry 2017). Apart from a few freshwater taxa and some unicellular taxa most Rhodophyta are marine macroalgae. They form distinct zones on rocky seashores and are an important component of the subtidal algal flora, down to near the limit of light penetration (Robba et al. 2006). These primary producers are vital to maintaining fish,

mollusc and crustacean populations by providing food and shelter and are thus fundamental components that maintain the balance and structure of the near-shore food web (Amado Filho et al. 2006; Robba et al. 2006). In addition to their ecological importance they contain compounds used in industry and have long been harvested or cultivated for human food and phycocolloids such as agar and carrageenan (Robba et al. 2006; Diaz et al. 2011). The classification of the Rhodophyta has traditionally relied upon morphology, anatomy, and reproductive features, the latter based largely on the work of Kylin (1956) who revised previous classification schemes of the order Florideophyceae by Schmitz (1889) and Oltmanns (1898).

Morphological variability

Many red algal species are morphologically variable and character traits overlap between species, which complicates species identification (Vázquez-Delfín et al. 2016; Rangel Miguel et al. 2014). This is common within the Rhodophyta, so that the total number of species is unknown (Robba et al. 2006). Morphological plasticity results in under- or over-estimation of species numbers, leading to incorrect inferences of biodiversity, difficulty in identifying individuals, and the creation of classification schemes that are very confusing (Belton et al. 2014). This, however, has not dissuaded taxonomists from describing new species and commenting on biodiversity even though there is still much disagreement over which traits and features to use as species-defining tools, and over what is the nature of a species (Leliaert et al. 2014; Leliaert & De Clerck 2017).

Red algae have complex life-histories which often consist of morphologically distinct phases. It is only through identifying the different stages and the morphology of each stage that one can be sure of accurate species identification. Algae in these different stages were often described in the literature as separate, distinct species before it was discovered, through laboratory culture, that they are in fact different life stages of one species (Robba et al. 2006).

The genus *Hypnea*

Morphological variation

The genus *Hypnea* (Cystocloniaceae, Rhodophyta) was described by Lamouroux (1813) based on the lectotype *H. musciformis* (Wulfen) J.V. Lamouroux. This species was originally described under the name *Fucus musciformis* Wulfen from the type locality of Trieste, Italy (Stegenga et al. 1997). Species from the genus *Hypnea* grow in abundance in the lower intertidal and shallow subtidal in tropical, subtropical and warm temperate coastlines throughout the world (Yamagishi & Masuda 2000; Rangel Miguel et al. 2014; Ganesan et al. 2006; Guiry & Guiry 2017; Wolf et al. 2011) and comprise some of the most abundant seaweeds in the Indo-Pacific intertidal zone (Geraldino et al. 2010).

Characters used historically in distinguishing species of the genus *Hypnea* include the dimensions of axes and branches, size of thallus, growth form, cell size and structure of and differences between life history phases. Reproductive structures are often used in species delimitation, (Lamouroux 1813; Kylin 1938; Kützing 1849; Agardh 1852; Papenfuss 1947) however these are not always present in collected samples.

Environment is known to influence the morphological variation found within species of *Hypnea* (Yamagishi & Masuda 2000; Vázquez-Delfín et al. 2016; Wolf et al. 2011). Vázquez-Delfín et al. (2016) found that at the microhabitat level significant differences within *Hypnea* species occurred, such as colour of the thallus, abundance of branchlets, and the shape of the apex. Although they were unable to find a direct correlation between morphological variation and environmental factors in nature, when *H. musciformis* was grown in culture it lost its hooked apices, indicating that this is a plastic character and serves its purpose for attachment only in nature. The different species of *Hypnea* are often difficult to distinguish morphologically as they all have terete to compressed thalli, short tapering branches which can terminate in characteristic hooks in some species, and when fertile they produce globular cystocarps and zonate tetrasporangia (Geraldino et al. 2010).

Taxonomic history – global

Hypnea species, along with the parasitic species of the genus *Hypneocolax* Borgesen, used to be grouped in their own family, the Hypneaceae (Gigartinales, Rhodophyta), based on their single carposporangia differing from the Cystocloniaceae which have paired or chained carposporangia (Saunders et al. 2004; Guiry & Guiry 2017). However, vegetative and reproductive features of *Hypnea* and *Hypneocolax* are virtually identical to the family Cystocloniaceae, and Min-Thein & Womersley (1976) viewed the differences in carposporangia as too slight a difference to warrant familial separation and suggested that further study into *Hypnea* species was required. *Hypnea* and *Hypneocolax* have since been included in the Cystocloniaceae based on molecular evidence (Saunders et al. 2004).

Initially comprising 6 species (Lamouroux 1813), the genus *Hypnea* has grown significantly as species have been added. J. G. Agardh (1852) described a further 19 species, dividing these into 3 groups based on their growth forms: *Spinuligerae, Pulvinitae,* and *Virgatae*. Those grouped in section *Spinuligerae* have an intricate-caespitose thallus with short spine-like branches and branchlets arranged alternately along main axes. The *Pulvinitae* have fertile upper branchlets and anatomising branchlets extending from a creeping, intricate, cushion-like matted thallus. Members of the *Virgatae* have a thallus with a main axis with caespitose lateral branchlets that are dense and erect but not intricate (J. G. Agardh 1852; Geraldino et al. 2010). Since then, there have been at least 121

Hypnea species described worldwide, although only 60 of these names are currently accepted (Guiry & Guiry 2018).

Taxonomic history – South Africa

South African *Hypnea* comprises 11 described species (Stegenga et al. 1997, De Clerck et al. 2005, Guiry & Guiry, 2017). Some have not been collected since they were described, while the distinction between others appears to be based on size, and in some cases remains unclear, resulting in taxonomic confusion (pers. obs.). The identifying features of South African *Hypnea* were described by J. G. Agardh (1852), Kylin (1938) and Papenfuss (1947), who mostly relied upon cell size and structure, colour differences, and thallus measurements. Species recorded or described from South Africa are *Hypnea arenaria* Kylin*, H. ceramioides* Kützing*, H. ecklonii* Suhr*, H. fruticulosa* Kŭtzing*, H. intricata* Kylin*, H. musciformis, H. rosea* Papenfuss*, H. spicifera* (Suhr) Harvey in J. Agardh*, H. spinella* (C. Agardh) Kŭtzing*, H. tenuis* Kylin and *H. viridis* Papenfuss.

Of these 11 species listed for South Africa, only 8 feature in recent taxonomic literature: *H. arenaria, H. ecklonii, H. intricata, H. musciformis, H. rosea, H. spicifera, H. tenuis* and *H. viridis* (Stegenga et al. 1997; De Clerck et al. 2005). The status of the 3 remaining species is uncertain and warrants further investigation: *Hypnea ceramioides* has been listed as a synonym for *H. ecklonii* (Price et al. 1992; Harper & Garbary 1997; LLuch 2002; Guiry & Guiry 2017); *H. fruticulosa* (Type locality: Cape of Good Hope, South Africa) is only mentioned in the diagnosis (Kutzing 1849) and has been catalogued by Seagrief (1984) and Silva et al. (1996) with the disclaimer that the record needs investigation; and *H. spinella* (Type locality: West Indies) is only recorded for South Africa by Barton (1893, 1896) and is included in subsequent marine algal catalogues based solely on Barton's record (Delf et al. 1921; Seagrief 1984; Silva et al. 1996; De Clerck et al. 2005).

Several species of *Hypnea* appear to be widely distributed along the South African coast, extending across two or even three of the Marine Provinces (Table 1). *Hypnea arenaria, H. intricata, H. rosea, H. spicifera, H. tenuis* and *H. viridis* are all endemic to South Africa and have been recorded along the East Coast. *H. ecklonii, H. musciformis, H. rosea, H. spicifera* and *H. tenuis* are all reported from regions along the South African South and West Coast and are all South African endemics apart from *H. musciformis* (Stegenga et al. 1997; De Clerck et al. 2005). *Hypnea arenaria* and *H. intricata* do not appear in the recent publications on marine algae from South Africa. There are 5 *Hypnea species* listed by GRIIS (2018) as invasive, however, no South African species appear on this list (with the exception of *H. musciformis* which this study shows does not exist in South Africa).

Brief species descriptions

The most widespread and abundant of the currently recognised species is *Hypnea spicifera*. It has been recorded from Northern KwaZulu-Natal on the East Coast to within 50 km north of Port Nolloth on the West Coast (Type locality: Algoa Bay, Cape Province) (Guiry & Guiry 2018). *Hypnea spicifera* is characterised by its lack of apical hooks common in the other *Hypnea* species, and is highly morphologically variable depending on where it is found (Isaac & Hewitt 1953). When found in the upper section of the shore *H. spicifera* is described to be short, bright green and has upright fronds that lose their colour towards the apices. At the lowest level of the intertidal, or shallow subtidal zone, where there is extensive wave action, *H. spicifera* is described to have long, dark, fronds with minimal branchlets. *Hypnea spicifera* ranges in size from a few centimetres high in the upper tidal region to up to 60cm high in the lower regions (Isaac & Hewitt 1953; Stegenga et al. 1997).

Hypnea rosea is another widespread species, recorded from the South and East Coasts of South Africa and sporadically collected from False Bay, immediately east of Cape Point (Stegenga et al. 1997; De Clerck et al. 2005) (Type locality: Umtwalumi, Natal) (Guiry & Guiry 2018). This is also a morphologically variable species and it has often been confused with the more globally widespread *H. musciformis* in field identifications from South Africa as they both have the apical hooks typical of many *Hypnea* species (Stegenga et al. 1997).

The Southern African endemic species *Hypnea tenuis* has a relatively wide distribution, and is recorded from Swartklip (False Bay) to Southern Mozambique (De Clerck et al. 2005), yet remains relatively morphologically uniform across the different Marine Provinces (Stegenga et al. 1997; De Clerck et al. 2005; Anderson et al. 2016) (Type locality: Isipingo Beach, Natal) (Guiry & Guiry 2018). *Hypnea tenuis* is, as its name suggests, a much smaller *Hypnea,* growing intertwined in other algal species found in the intertidal zone (Stegenga et al. 1997; De Clerck et al. 2005).

Hypnea viridis is an iridescent warm water species with noted similarities to the tropical *Hypnea pannosa* (De Clerck et al. 2005; Geraldino et al. 2010). It is found on the East Coast from Isipingo (Kwazulu-Natal) to southern Mozambique (De Clerck et al. 2005) (Type locality: Umhlali Beach, Natal) (Guiry & Guiry 2018). Lacking the typical apical hooks, *H. viridis* is bright iridescent blue when submerged and greenish brown when out of water, and forms a low cushion-like habit of interspersed axes (De Clerck et al. 2005).

Hypnea arenaria is a small creeping species recorded on sand-covered rock in the upper intertidal from East London to Northern KwaZulu-Natal (type locality: Inyone Rock, Kwazulu-Natal). *Hypnea intricata* is another small species of *Hypnea,* recorded from Tsitsikamma to Durban (type locality:

Port Elizabeth, Eastern Cape) described briefly by Kylin (1938). *Hypnea ecklonii* is reported to be a slender, epiphytic *Hypnea* (Type locality: Algoa Bay, Cape Province) (Guiry & Guiry 2018). Stegenga et al. 1997 record a distribution only from Pearly Beach to Namibia. Since they were described, none of these three species have been recorded without some doubt of their identification.

Table 1: Identifying features of current species described from South Africa (Stegenga et al. 1997; De Clerck et al. 2005; Anderson et al. 2016)

Species	**Thallus height**	**Main axis diameter**	**Central filament**	**Medullary cell height**	**Colour**	**Hooks**
H. arenaria	3-5mm	180µm	Unknown	Unknown	Unknown	Absent
H. ecklonii	<20cm	400-500µm	50µm	80µm	Bright to dark red	Scarce/Absent
H. intricata	2-3cm	500µm	Unknown	Unknown	Unknown	Present
H. musciformis	<15cm	1mm	Absent	100µm	Reddish brown	Present
H. rosea	<17cm	1mm	Absent	250µm	Bright red to reddish brown	Present
H. spicifera	<60cm	<3mm	Absent	150µm	Bright green to dark purple	Absent
H. tenuis	2-3cm	250-400µm	70µm	70µm	Dark red to black	Present
H. viridis	2cm	1mm	Present	150µm	Bright blue – iridescent	Absent

Industrial uses of Hypnea

Hypnea is an important source of kappa-carageenan and was ranked as the second most important source of carrageenan in the tropics after *Eucheuma* (Mshigeni & Chapman 1994). This has resulted in a wide interest in its harvesting and cultivation potential on a global scale. Intermittent harvesting of *Hypnea musciformis* is done in Senegal, Vietnam, USA, Philippines, India, Brazil, Burma, Bangladesh and the Bahamas and this species is an essential source of carrageenan in Brazil (Ganesan et al. 2006).

In addition to carrageenan extraction, various *Hypnea* species have been assessed for other useful properties. *Hypnea valentiae* (Turner) Montagne for example, from India, contains much lower levels of carrageenan and has thus been used instead as a biosorbent for cadmium removal (Rathinam et al. 2010). Cosenza et al. (2014) refer to other studies which have assessed the polysaccharides of *H. musciformis,* focusing on their structure or rheological properties, or their antioxidant, anti-inflammatory, or cytotoxic properties. *H. musciformis* is attracting attention for use in aquaculture due to its fast growth rate and its ability to tolerate a wide range of environments (Cosenza et al. 2014). The harvesting potential for South African *Hypnea* is largely unknown, besides a feasibility study conducted on *H. spicifera* (Van Zyl 1993) which suggested poor recovery after harvesting and advised against it. Isaac & Hewitt (1953), however, noted that it would be unwise to disturb the rhizomatous creeping holdfasts of *H. spicifera* and therefore that only the upper thallus should be harvested. Further investigations into harvesting potential of *Hypnea* species are required.

Molecular systematics

Overcoming taxonomic challenges

One of the biggest challenges in algal taxonomy is the current uncertainty shrouding the circumscription and nomenclature of taxa. The findings from molecular data are often difficult to match with existing or previously described taxa, especially as species lists contain numerous synonyms, many names that are no longer in use, and there are many infra-specific taxa (Belton et al. 2014; Leliaert & De Clerck 2017). In *Hypnea*, in particular, because of the apparently variable morphology within species and its worldwide distribution, there have been recent global attempts to critically reassess the phylogeny of this genus (e.g. Geraldino et al. 2010; Nauer et al. 2014a; Nauer et al. 2014b; de Jesus et al. 2016).

Gene regions used in red algal systematics

Molecular data can be used not only to differentiate *Hypnea* from other genera but also to delimit species within the genus (Rangel Miguel et al. 2014). For *Hypnea,* it seems the most popular and effective choices for molecular studies are the plastid *rbcL* gene region and the mitochondrial *cox1* gene region. Saunders et al. (2004) used SSU rDNA sequences to create a familial phylogeny, but *rbcL* and *cox1* have been widely used in generic phylogenies of *Hypnea* from Brazilian and Asian coasts and in the Indian Ocean (e.g. Yamagishi & Masuda 2000; Geraldino et al. 2006; Geraldino et al. 2009; Geraldino et al. 2010; Wolf et al. 2011; Bast et al. 2014; Nauer et al. 2014a; Nauer et al. 2014b; Geraldino et al. 2015; de Jesus et al. 2016). The chloroplast gene region *rbcL* is commonly used in the study of red algae as many primers are available for efficient amplification and sequencing, its evolution rate is relatively slow, and it is large (about 1400 base pairs) therefore providing numerous characters for genetic comparisons (Freshwater & Rueness 1994).

Single marker barcoding vs multi-locus marker and morphology

Various trends have been noted in the last few decades with regards to how many genetic markers to use. The use of multiple markers increased from the late 1990s to the early 2000s, yet subsequent studies tended to use single markers coupled with morphological data, because in a lot of instances single markers were shown to be just as effective in delimiting species as using a multi-marker approach (Maggs et al. 2007). This led to the establishment of DNA barcoding, a technique where a single gene region is used across a range of organisms to effectively identify them by comparing results to a database of gene sequences (Maggs et al. 2007; Geraldino et al. 2006; Leliaert et al. 2014; Robba et al. 2006; Bast et al. 2014; Payo et al. 2013). Maggs et al. (2007) and Freshwater et al. (1994) suggested that the *rbcL* gene region be used as the DNA barcode region for the red algae as it

is by far the most available sequence found on GenBank and it has high resolution in defining species boundaries. Even though the gene region *cox1* has been shown to be effective in delimiting species of red algae, significantly fewer *cox1* sequences are available for the red algae, again showing that *rbcL* would be a more appropriate choice (Geraldino et al. 2006).

There has been some resistance to the establishment of DNA barcoding as many traditional taxonomists are worried that newly discovered species will no longer be formally described in as much detail as previously (Robba et al. 2006; De Clerck et al. 2013; Leliaert et al. 2014). Another concern is that molecular systematics will be mandatory for taxonomic studies, replacing traditional methods and destroying a longstanding approach to species descriptions (Páll-Gergely 2017). Opposing views to DNA barcoding are largely based on resistance to change (Robba et al. 2006). These concerns, however, have not deterred taxonomists from using these techniques and DNA barcoding has been useful in delimiting species where morphological differences are lacking (Wolf et al. 2011), supporting the consensus that molecular systematics is vital for species delimitation (Leliaert & De Clerck 2017).

Uncovering hidden diversity

Numerous studies have identified cryptic and pseudo-cryptic species in the largely underestimated biodiversity of the red algae (Maggs et al. 2007). Algae often lack morphological features which clearly differentiate between species, and are subject to high rates of convergent evolution, both of which can conceal cryptic and pseudo-cryptic species (Robba et al. 2006). It is impossible to distinguish cryptic species based on morphology alone while pseudo-cryptic species require the identification of appropriate defining characters (Maggs et al. 2007). Phenotypic characters in some cases evolve slower than DNA, and some organisms, especially algae, have very simple morphologies. Single-locus studies and morphological studies have therefore proved inefficient in defining species boundaries for taxa with complexities regarding speciation, recent divergence or lack of diagnostic phenotypes (Leliaert & De Clerck 2017).

Phenotypic expression is so closely linked to life-history, evolution or environmental factors that it seems prudent to use an array of species delimitation methods and to sample from as much of the geographical range as possible. Combining methods and finding congruence yields the best, most objective results. Common practice currently is to use multi-locus DNA data coupled with other distinguishing morphological features (Robba et al. 2006; Leliaert & De Clerck 2017).

Exploring new habitats at depth and at remote locations has resulted in descriptions of many new taxa (Wynne & Schneider 2010), but with the introduction of molecular techniques hundreds of new

species of algae have been identified, showing that taxonomists cannot simply use phenotypic characters when estimating algal biodiversity. Two problems arise with DNA analysis, however, with either too many lineages being grouped into one species, or the opposite, where intra-specific variation could be misinterpreted as multiple species (Leliaert et al. 2014).

Despite these problems, molecular tools have allowed for some clarification of previously confused taxa (Vázquez-Delfín et al. 2016). When used in conjunction with morphological analysis, DNA is very valuable in revealing diversity that was previously unknown, and in identifying entities that are more realistic (Robba et al. 2006; Leliaert et al. 2014). While DNA is useful in confirming species boundaries that were initially based on morphological analyses, in many cases it also reveals new taxonomic assemblages at all levels of classification, not just at the species level (Leliaert et al. 2014; Wynne & Schneider 2010).

Aims of the present study

The taxonomic and phylogenetic data pertaining to South African *Hypnea* currently consist of only 3 sequenced specimens on GenBank (all with *cox1* and *rbcL* genes sequenced) and brief descriptions of *Hypnea* species in floras on South African algae (Stegenga et al. 1997, De Clerck et al. 2005, Anderson et al. 2016). The genus has never been evaluated using DNA data in this country, some of the species recorded are doubtful and the limits of others are unclear. The aims of this study were therefore to analyse an extensive collection of *Hypnea* specimens by using a combination of multi-locus molecular data and morphological analyses to investigate the species diversity of South African *Hypnea* and their phylogenetic relationships at both the regional and the global scale.

These aims were achieved by combining phylogenetic analyses of the chloroplastic *rbcL* and mitochondrial *cox1* gene regions with the use of traditional morphological characters for new extensive collections of South African *Hypnea.*

Materials and Methods

Field Collections and specimen preservation

There are currently 11 listed species in the genus *Hypnea* in South Africa of which there are pressed specimens of only 8 in the Bolus Herbarium (BOL) at the University of Cape Town. Some of the herbarium specimens do not fit the type descriptions, *e.g*. *Hypnea arenaria* (Kylin 1938), and there is much taxonomic confusion within this genus so, on the shore, every *Hypnea*-like alga was collected to ensure specimens of as many South African species as possible. Sampling was done sporadically from 2009 to 2015, both as part of general macro-algal surveys, and specifically for this study by the student from 2014-2015. Collections were made at 23 sites along the coast of South Africa and additional opportunistic samples were obtained from Madagascar, Mozambique and Europa Island (Éparses Islands). As far as possible, whole specimens were photographed using a Zeiss DV4 dissecting microscope linked to a Canon G5 camera. A subsample of as many specimens as possible was preserved in silica gel for later DNA analysis, and some tissue was preserved in a 5% formalin in seawater mixture for later microscopic examination. The remaining specimens were pressed as Herbarium vouchers to be housed at the Bolus Herbarium at the University of Cape Town, South Africa (BOL). The study was conducted on only those specimens that had been pressed, dried in silica gel and preserved in 5% formalin and seawater.

Morphological and anatomical analyses

Specimens preserved in 5% formalin/seawater were used for morpho-anatomical analysis as formalin preserves the shape, size and cell structure of the samples. Hand-sections through the main axes and reproductive structures (when present) were made and photographed using a Leica DMLS compound microscope and a Leica MC120 HD camera. Microscope photographs were used in morpho-anatomical analysis and aspects such as the diameter of the axes, diameter and number of cells were recorded for the cortex, medulla and central filament (if visible). Pressed specimens were arranged into morphologically similar groups and presence or absence of hooks, and branching pattern was recorded. These were provisionally identified where possible using original diagnoses, seaweed catalogues and field guides (e.g. Lamouroux 1813; Kützing 1849; J. G. Agardh 1852; Kylin 1938; Papenfuss 1947; Stegenga et al. 1997; De Clerck et al. 2005).

DNA extraction, amplification and sequencing

The protocol for DNA extraction followed methods successfully used in previous studies on red algae (Freshwater & Rueness 1994; Saunders 2005; Gurgel & Fredericq 2004; Gulbransen et al. 2012). DNA was extracted from tissue samples preserved in silica gel, using the NucleoSpin 96 Plant II kits

(MACHEREY-NAGEL, Düren Germany) and following the manufacturer's instructions. For *rbcL* analyses the chloroplast DNA *rbcL* region was targeted using primer pairs from Freshwater & Rueness (1994): F*rbcL*-start to R753, F557 to R1150, F993 to R*rbcS*-start. For *cox1* analyses the mitochondrial DNA *cox1* region was targeted using the primer pair from Gulbransen et al. (2012): GazF1 and GazR1. The protocol from Lin et al. (2001) was followed for gene amplification of these regions and ExoSAP-IT (GE Healthcare, Picataway, NJ, USA) was used to clean PCR products following the manufacturer's instructions. The Big Dye Terminator chemistry (Applied Biosystem, Carlsbad, CA, USA) was used to conduct forward and reverse sequencing reactions. After a preliminary extraction and amplification conducted by the student in South Africa on a subsample of *Hypnea* tissue, the remaining extraction, amplification and sequencing was outsourced to the Australian Genome Research Facility, Adelaide node, South Australia in order to save on project running costs.

Sequence alignments

Hypnea rbcL and *cox1* alignments - In addition to sequences newly obtained for the specimens from South Africa, Mozambique, Madagascar and the Europa Islands (which form our collection), a relevant selection of sequences was downloaded from those published on Genbank to represent as many *Hypnea* species and localities as available. Sequences for *Calliblepharis ciliata* (Hudson) Kutzing, *Rhodophyllis volans* Harvey and *Craspedocarpus venosus* (Kützing) Min-Thein & Womersley were chosen as outgroup for the *Hypnea rbcL* analysis and sequences for *Gracilaria vermiculophylla* (Ohmi) Papenfuss and *Gracilariopsis chorda* (Holmes) Ohmi were chosen as outgroups for the *cox1* analysis following Geraldino et al. (2010).

Cystocloniaceae and Gigartinales *rbcL* alignments - Following preliminary analyses that raised concerns about the generic position of some species, it was decided to run additional analyses to clarify the position of *Hypnea* and especially the numbered clades F1, F2 and E, within the family Cystocloniaceae and within the order Gigartinales. The *rbcL* gene was used for this, as it evolves slower and is more useful for generic delimitation than *cox1*. Different genera within the Cystocloniaceae were analysed using sequences from our collection and additional GenBank sequences choosing *Solieria filiformis* (Kutzing) P.W. Gabrielson (Solieriaceae) as the outgroup. The positions of clades F1, F2 and E were further investigated within the order Gigartinales to determine whether they fit genetically within the Cystocloniaceae. Sequences representing different families within the order Gigartinales were downloaded from GenBank and used with sequences from our own collection for this analysis. Sequences of *Gracilariopsis mclachlanii* Buriyo, Bellorin & M.C. Oliveira and *Gracilaria vermiculophylla* (Gracilariales) were used as outgroups.

Phylogenetic analyses

Bayesian and Maximum likelihood analyses

The nucleotide sequences were edited and assembled in the Staden Package (Staden et al. 2003) and aligned by eye in BioEdit (Hall 1999). Identical sequences were identified using RaxML-HPC BlackBox (Stamatakis et al. 2008) through the CIPRES Science Gateway (Miller et al. 2010) and removed from the analysis. An appropriate model for the data was selected based on the hierarchical likelihood ratio test implemented by the software Modeltest version 3.06 (Posada & Crandall 1998). This test was run on both *rbcL* and *cox1* alignments for *Hypnea* and for the Cystocloniaceae and Gigartinales *rbcL* alignments. The chosen model for all alignments was a GTR model and it was used in Bayesian phylogenetic inference performed in MrBayes on XSEDE Version 3.2.6 (Huelsenbeck & Ronquist 2001) through the CIPRES Science Gateway (Miller et al. 2010).

For the Bayesian analysis two simultaneous runs with four chains (three heated and one cold chain) of the MarkovChain MonteCarlo (MCMC) algorithm were made with the temperature set at the default 0.02 and a burn-in of 10%. The *Hypnea rbcL* and *cox1* analyses were run for 5 000 000 generations with the sampling frequency set at 500 *i.e.* Markov chains were sampled every 500th generation. Due to the increased diversity of the sequences in the following two analyses, the Cystocloniaceae analysis was run for 10 000 000 generations at a sampling frequency of 1000, while the Gigartinales analysis was run for 20 000 000 generations with a sampling frequency of 1000.

ML analysis was performed using RaxML-HPC BlackBox (Stamatakis et al. 2008) through the CIPRES Science Gateway (Miller et al. 2010). Nodal support was achieved using 1000 bootstraps.

Outputs from MrBayes and RaxML were viewed in Figtree (Rambaut n.d.). Adobe Illustrator was used to edit the Bayesian and Maximum likelihood trees to display both the probability and likelihood support values on one tree. Bayesian probability (BP) and maximum likelihood (ML) support values >95 and >0.95, >75 and >0.75, <75 and <0.75 were considered strong, good, and poorly supported clades respectively. Values below 0.5 for the Bayesian analyses and below 50 for the ML analyses were not considered or displayed on the trees.

Hypnea rbcL analyses

The total alignment included 124 *rbcL* sequences (appendix 1&2): 33 *rbcL* sequences were newly obtained for specimens from South Africa, Mozambique, Madagascar and Europa Island, and 88 global sequences and 3 outgroup species were downloaded from GenBank. The final alignment was 1477 base pairs (bp) long, including gaps. A NJ tree was constructed with 1000 bootstraps to view an initial layout of the sequences and to test whether the alignment was sound. Identical sequences

were identified and removed using RaxML-HPC Blackbox (Stamatakis et al. 2008) through the CIPRES Science Gateway (Miller et al. 2010). The MrBayes and ML analyses were run on the remaining 97 sequences (29 of our new specimens, 65 *Hypnea* from GenBank, and 3 outgroups) and maximum likelihood values and Bayesian probabilities were displayed on the Bayes tree (figure 2).

Hypnea cox1 analyses

The total alignment included 123 *cox1* sequences: 58 *cox1* sequences were newly obtained during this study from our samples from South Africa, Mozambique, Madagascar and Europa Island, and 63 global sequences and 2 outgroups were downloaded from the GenBank (appendix 1&2). The final alignment was 546 bp long, including gaps. A NJ analysis was conducted using 1000 bootstraps to check the quality of the data and to get an initial idea of the arrangement. Identical sequences were identified and removed using RaxML-HPC Blackbox (Stamatakis et al. 2008) through the CIPRES Science Gateway (Miller et al. 2010). The MrBayes and ML analyses were run on the remaining 59 sequences (30 of our new sequences, 27 *Hypnea* from Genbank, and 2 outgroups) and likelihood values and Bayesian probabilities were displayed on the Bayesian cladogram (figure 3).

Cystocloniaceae rbcL analysis

The total alignment included 28 *rbcL* sequences: a subselection of 11 *rbcL* sequences from South Africa (this study), 5 previously published *Hypnea* sequences, 11 sequences from genera within the Cystocloniaceae and 1 outgroup all downloaded from GenBank. The final alignment was 1477 bp long, including gaps. The maximum likelihood values and Bayesian probabilities were displayed on the Bayes cladogram (figure 4).

Gigartinales rbcL analysis

The total alignment included 106 *rbcL* sequences: a subselection of 11 *rbcL* sequences from South Africa (this study), 5 *Hypnea* GenBank sequences*,* 88 GenBank sequences representing as many different families within the Gigartinales and 2 outgroups from the Genbank. The final alignment was 1317 bp long, including gaps. A NJ analysis was conducted using 1000 bootstraps to assess the data quality, the MrBayes and ML analyses were run and likelihood values and Bayesian probabilities were displayed on the Bayes cladogram (figure 5).

Genetic Distance Matrix

Genetic Distance (GD) matrices for the *rbcL* and *cox1 Hypnea* analyses were created using PAUP* (Swofford 2002). Percent GD between and within clades was calculated by hand.

Genetic distance matrix for Hypnea rbcL

A genetic distance (GD) matrix was calculated for the 97 sequences and average percent distance between and within clades was calculated (appendix 3). In recent literature, the intraspecific GD values range between 0.2 and 1.8% for *rbcL* while interspecific GD values range between 1.2 – 7.3% (Yamagishi & Masuda 2000; Yamagishi et al. 2003; Nauer et al. 2014b; de Jesus et al. 2016). These values served as reference for our study.

Genetic distance matrix for Hypnea cox1

A GD matrix was calculated for the 59 sequences and average percent distance within and between clades was calculated (appendix 4). In a recent study on *Hypnea* in South America intra-specific GD for *cox1* was between 0 and 4% while inter-specific GD was between 5.3 and 15.7% (de Jesus et al. 2016). These values were used as a reference in the present study.

Results:

Each phylogenetic analysis is described individually and molecular clades assigned alphanumeric codes. These clades were then identified where possible to species level classification based on evidence from anatomical and morphological analyses. Possibly misidentified sequences downloaded from GenBank are denoted with a *. Intraspecific genetic distance (<1,7% for *rbcL*, <6% for *cox1*) and intergeneric genetic distance (>8% for *rbcL*, >13% for *cox1*) were chosen based on results and literature.

Analysis of the *Hypnea rbcL* phylogeny (Figure 2)

Phylogenetic reconstruction:

The *rbcL* analysis (Fig. 2) generated six main clades labelled A-F, and these letters were combined with numbers to further designate the 26 subclades. The main clades A, B and C formed one branch (ML: 83, BP: 0.68) while D formed another (84, 0.87), and E (100, 1) and F (100, 1) formed two separate clades. Sequences were grouped into 26 clades based on *rbcL* data. Nine of these clades contained only South African specimens, two contained sequences newly obtained from Mozambique, Madagascar and Europa Island, and fifteen contained previously published sequences from GenBank. None of the South African sequences grouped with any foreign sequences.

Clade A

Molecular analysis grouped the specimens identified as *H. rosea* with GenBank sequences of *H. cervicornis* and 2 possibly misidentified sequences of *H. japonica** and *H. tenuis** into four subclades within clade A (99, 0.99) (Fig. 2). The GenBank sequences of *H. cervicornis* and one *H. japonica** in clade A clustered in their own subclade, A4 (83, 1) and were sister to one South African *H. rosea** and the GenBank *H. tenuis*,* A3 (100, 1). The South African sequences of *H. rosea* clustered in two separate subclades: A1 (78, 0.69) & A2 (100, 1). The genetic distance (GD) between A1 and A2 was at the interspecific limit of 1.7% and the GD between A3 and A4 was lower at 1.4%, while the GD between A1+A2 and A3+A4 was >2.5%. Clade A therefore is made up of 3 molecular species, clade A1 *(Hypnea rosea sp.1)*, clade A2 *(Hypnea rosea sp.2),* and clade A3+A4 *(Hypnea cervicornis)*.

Clade B

Sequences from GenBank which are representative of the type species, *H. musciformis,* clustered together: B3 (100, 1), sister to the recently described *H. pseudomusciformis*: B4(100, 1). No South African specimens were grouped in clade B.

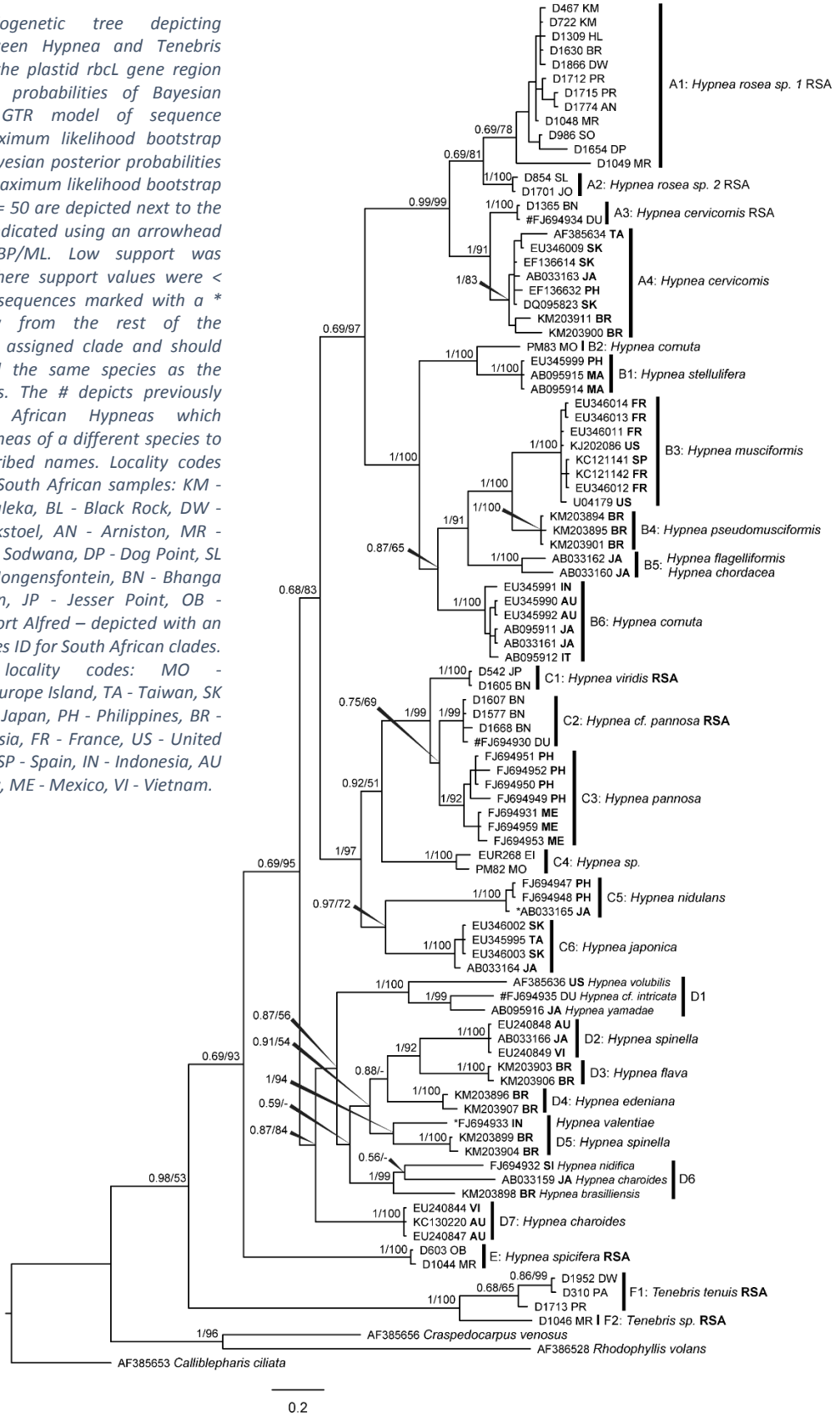

*Figure 2. Phylogenetic tree depicting relationships between Hypnea and Tenebris species based on the plastid rbcL gene region showing posterior probabilities of Bayesian inference under GTR model of sequence evolution and maximum likelihood bootstrap proportions. All Bayesian posterior probabilities (BP) >= 0.50 and maximum likelihood bootstrap proportions (ML) >= 50 are depicted next to the relevant node or indicated using an arrowhead in the format: BP/ML. Low support was indicated by '-' where support values were < 0.5/50. Individual sequences marked with a * differ significantly from the rest of the sequences in their assigned clade and should not be considered the same species as the majority sequences. The # depicts previously published South African Hypneas which clustered with Hypneas of a different species to their previous ascribed names. Locality codes are as follows for South African samples: KM - Kei Mouth, H - Hluleka, BL - Black Rock, DW - Dwesa, PR - Preekstoel, AN - Arniston, MR - Mission Rocks, SO - Sodwana, DP - Dog Point, SL - Saint Lucia, JO - Jongensfontein, BN - Bhanga Nek, DU - Durban, JP - Jesser Point, OB - Olifantsbos, PA - Port Alfred – depicted with an RSA after the species ID for South African clades. Other country locality codes: MO - Mozambique, EI - Europe Island, TA - Taiwan, SK - South Korea, JA - Japan, PH - Philippines, BR - Brazil, MA - Malaysia, FR - France, US - United States of America, SP - Spain, IN - Indonesia, AU - Australia, IT - Italy, ME - Mexico, VI - Vietnam.*

Clade C

Clade C grouped two *Hypnea* specimens from South Africa in a well-supported clade with a sequence identified as *H. pannosa* from South Africa and an *H. viridis* sequence from GenBank: C2 (99, 1). These grouped sister to a well-supported clade of previously published *H. pannosa* sequences: C3 (92, 1). *Hypnea viridis,* C1 (100, 1) from our own collection was sister to these two clades. The GD between C1 and C2+C3 was 1.7% while the GD between C2 and C3 was 1.4%. Clade C2 was therefore identified as a South African *H. cf. pannosa.*

Clade E

Specimens identified as *H. spicifera* clustered together in a well-supported clade: E (100, 1) and separate from the remaining *Hypnea* species (clades A-D) with a GD between 6 and 9%.

Clade F

The sequences from specimens that fitted the morphological description of "*H. tenuis"* grouped in a fully supported clade F (ML: 100, BP: 1) separate from the rest of the *Hypnea* specimens, with a GD of over 10% from all other *Hypnea* (appendix 3). This clade was further split into 2 subclades (F1 and F2) and showed a distribution ranging from the South Coast to Northern KZN. Clade F1 contained 3 specimens with GD <1.21% between them and differed from F2 with a GD of 3.15%. We ended up considering clade F as a new genus, see later for more detail.

Analysis of the *Hypnea cox1* phylogeny (Figure 3)

Phylogenetic reconstruction:

Although the *cox1* and *rbcL* datasets did not match completely and contained some sequences from different specimens*,* the analyses produced similar clades. The *cox1* clades were therefore labelled with the same codes as the corresponding *rbcL* clades, with clades added or excluded depending on presence or absence of the corresponding specimens. There were no *cox1* sequences available for specimens identified as *H. tenuis* and *H. spicifera* from our South African collection and so clades E and F are not shown in the *cox1* analysis. In total, the *cox1* analysis generated 7 main clades and 20 subclades with 23 apparent taxa (Fig. 3). Sequences were grouped into 20 clades based on *cox1* data. Three of these clades contained only South African specimens, four contained specimens from South Africa, Mozambique, Madagascar and Europa Island, ten clades contained GenBank specimens, and 3 clades each contained sets of 2 sequences distinct from all other sequences in the analysis.

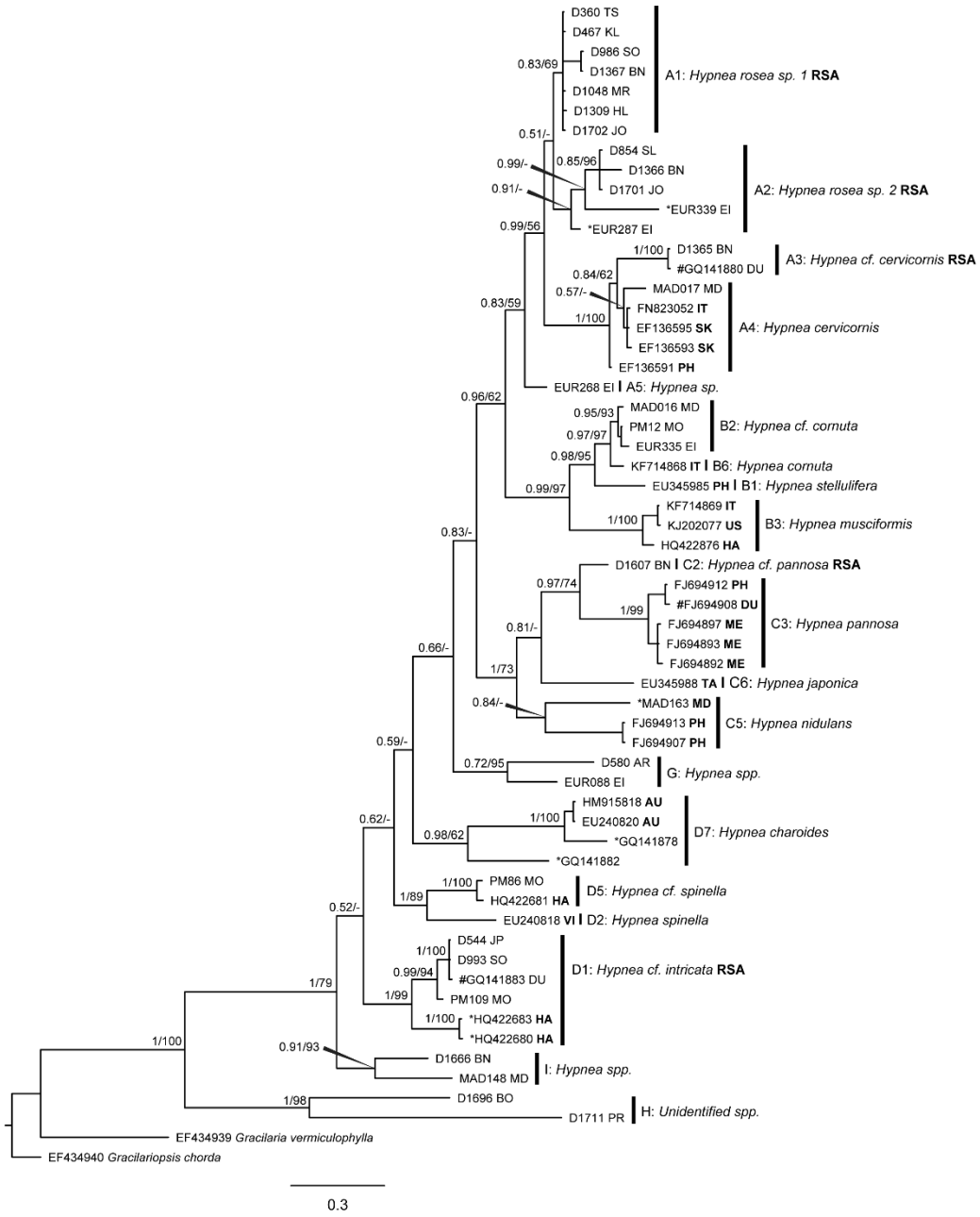

*Figure 3 Phylogenetic tree depicting relationships between Hypnea species based on the mitochondrial cox1 gene region showing posterior probabilities of Bayesian inference under GTR model of sequence evolution and maximum likelihood bootstrap proportions. All Bayesian posterior probabilities (BP) >= 0.50 and maximum likelihood bootstrap proportions (ML) >= 50 are depicted next to the relevant node or indicated using an arrowhead in the format: BP/ML. Low support was indicated by '-' where support values were < 0.5/50. Individual sequences marked with a * differ significantly from the rest of the sequences in their assigned clade and should not be considered the same species as the majority sequences. The # depicts previously published South African Hypnea species which clustered with Hypnea species of a different species to their previous ascribed names. Locality codes are as follows for South African samples: TS - Three sisters, KL - Kleinemonde, MR - Mission Rocks, JO - Jongensfontein, SO - Sodwana, BN - Bhanga Nek, HL - Hluleka, SL - Saint Lucia, AR - Antons Reef, JP - Jesser Point, BO - Bordjiesdrif – depicted with an RSA after the species ID for South African clades. Other country locality codes: MD - Madagascar, MO - Mozambique, EI - Europa Island, IT - Italy, SK - South Korea, PH - Philippines, US - USA, HA - Hawaii, ME - Mexico, TA - Taiwan, VI – Vietnam.*

Clade A

Molecular analysis grouped into five subclades within Clade A (-, 0.83), which comprised the specimens identified as *H. rosea,* together with GenBank sequences of *H. cervicornis* and two

possibly misidentified sequences of *H. valentiae** and *H. tenuis**. The GenBank sequences of *H. cervicornis* and one *H. valentiae** clustered in their own subclade A4 (-, 0.57), sister to one South African *H. rosea** and the GenBank *H. tenuis** A3 (100, 1) with a GD of 5.93% between them. The South African sequences of *H. rosea* clustered in two subclades A1 (69, 0.83) & A2 (96, 0.85) with a GD of 6.18% between them. Clade A therefore is made up of 3 molecular species, clade A1 *(Hypnea rosea sp.1)*, clade A2 *(Hypnea rosea sp.2),* and clade A3+A4 *(Hypnea cervicornis)*.

Clade B

There were none of our new South African sequences in clade B3 (100, 1) which contained previously published sequences of the type species *H. musciformis* with specimens collected from the type locality (Italy), Hawaii and the USA.

Clade C

The South African sequences for specimens morphologically identified as *H. pannosa* clustered (C2) sister to one previously published *H. viridis* and previously published sequences of *H. pannosa:* C3 (99, 1) with a GD of 7.56%. Clade C2 was tentatively identified as a new record for South Africa of *H. cf. pannosa.*

Clade D

Clade D1 (99, 1) also contained samples from some specimens that had been tentatively identified as *H. rosea*,* as well as sequences from unidentified specimens in our South African collection, a previously published *H. rosea** sequence, a sequence we identified as *H. rosea** from Mozambique and previously published sequences of an *H. musciformis** and an *H. valentiae** from Hawaii. Excluding the *H. musciformis** and the *H. valentiae*,* the sequences in D1 had a GD of <2,15% amongst them and has been tentatively identified as *Hypnea intricata* (see later morphological analysis).

Clades G, H, and I

Clades G, H and I are 3 clades which each contain two sequences. All 6 of these sequences corresponded to distinct taxa each representing a potential different species. The 2 sequences in clade G had a GD of >10.5% from each other and from all other samples in the analysis. The 2 sequences in clade I had a GD of >12.5% from each other and from all other samples in the analysis. The 2 sequences in clade H had a GD of over 17% from each other and all other *Hypnea* and therefore the two taxa in clade H were identified as not belonging to the genus *Hypnea*.

Analysis of the Cystocloniaceae *rbcL* phylogeny (Figure 4)

The tree was divided into 3 well-supported clades which further subdivided into 6 sub-clades with 3 sequences not grouped in any sub-clades.

The *Hypnea* sequences clustered together in a well-supported clade (93, 1). Sequences for *H. spicifera* clustered together (100, 1) and were sister to the rest of the *Hypnea* species (100, 1). Sequences for clades F1 and F2 also clustered as a well-supported clade (100, 1) as a sister clade to the rest of the *Hypnea* species.

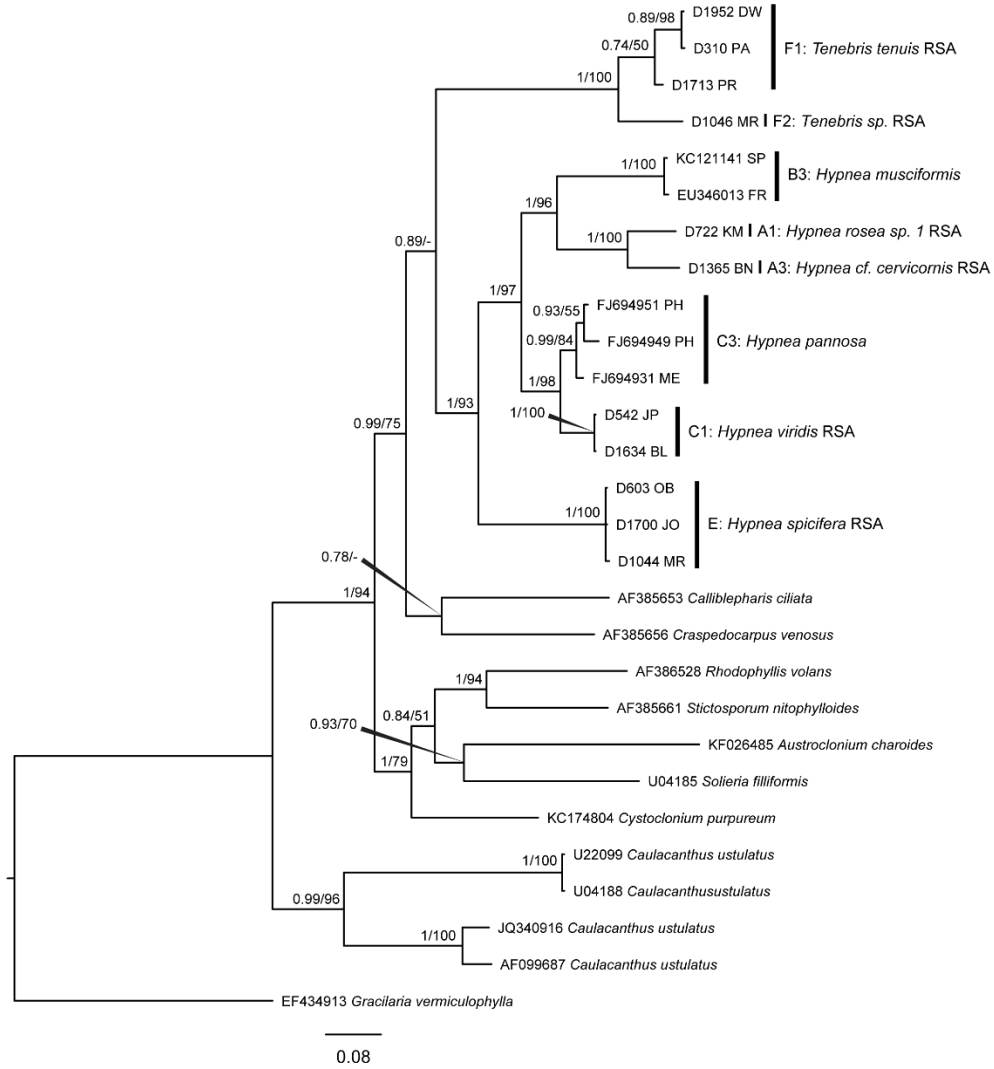

Figure 4 Phylogenetic tree depicting relationships between selected species in the Cystocloniaceae based on plastid rbcL gene sequences showing posterior probabilities of Bayesian inference under GTR model of sequence evolution and maximum likelihood bootstrap proportions. All Bayesian posterior probabilities (BP) >= 0.50 and maximum likelihood bootstrap proportions (ML) >= 50 are depicted next to the relevant node or indicated using an arrowhead in the format: BP/ML. Low support was indicated by '-' where support values were < 0.5/50. Locality codes for South African samples are as follows: PA - Port Alfred, PR - Preekstoel, DW - Dwesa, BL - Black Rock, OB - Olifantsbos, JO - Jongensfontein, MR - Mission Rocks.

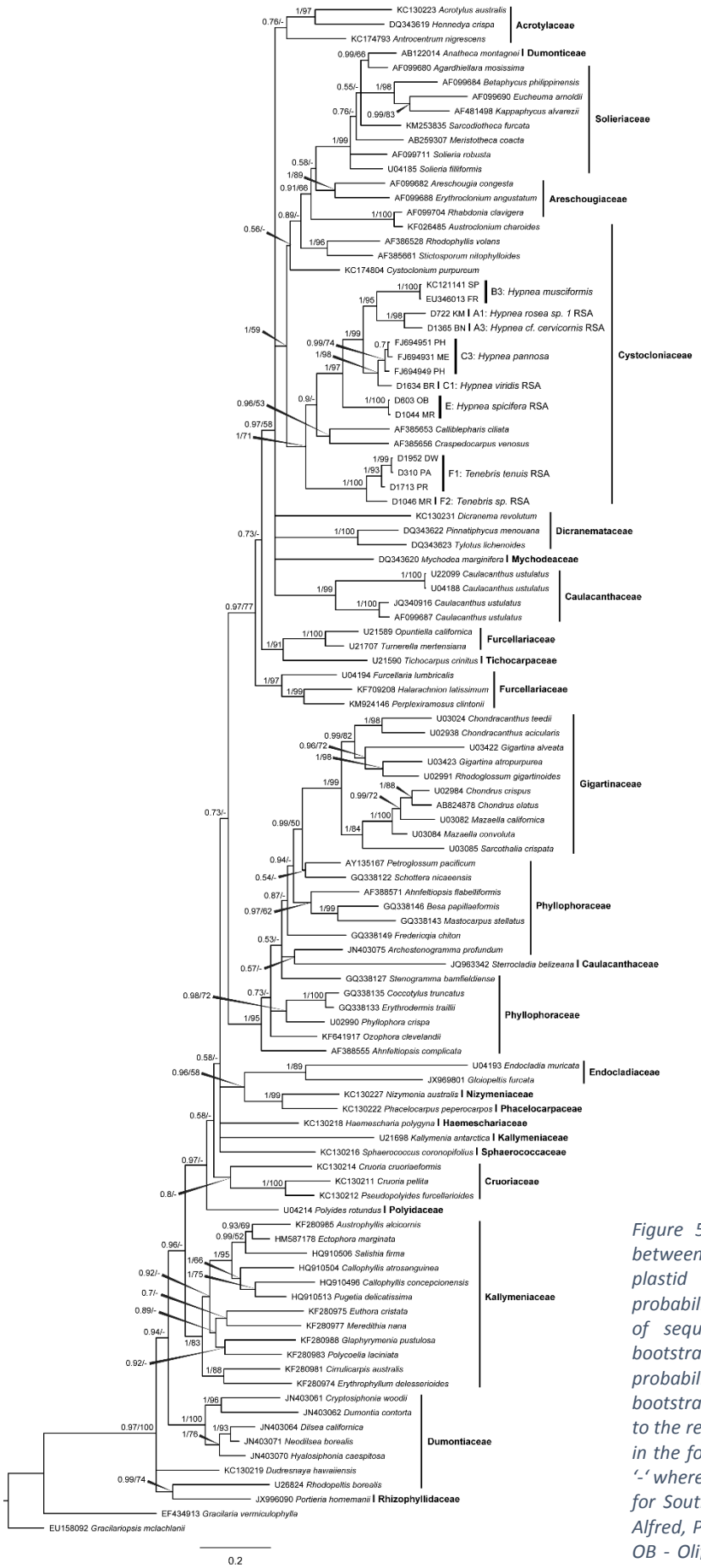

Figure 5 Phylogenetic tree depicting relationships between families within the Gigartinales based on plastid rbcL gene sequences showing posterior probabilities of Bayesian inference under GTR model of sequence evolution and maximum likelihood bootstrap proportions. All Bayesian posterior probabilities (BP) >= 0.50 and maximum likelihood bootstrap proportions (ML) >= 50 are depicted next to the relevant node or indicated using an arrowhead in the format: BP/ML. Low support was indicated by '-' where support values were < 0.5/50. Locality codes for South African samples are as follows: PA - Port Alfred, PR - Preekstoel, DW - Dwesa, BL - Black Rock, OB - Olifantsbos, JO - Jongensfontein, MR - Mission

Analysis of the Gigartinales *rbcL* phylogeny (Figure 5)

The topology represented is not always very well supported. Most families appear polyphyletic and the Cystocloniaceae is no exception. The one big well supported clade (1, 71) of interest to this study contains all of the South African sequences of presumed *Hypnea* species, the F1 and F2 clades and GenBank sequences for *Calliblepharis ciliata* and *Craspedocarpus venosus.* Clade F1+F2 form a well-supported clade (1, 100) distinct to the rest of the *Hypnea* species. *Hypnea spicifera* sequences grouped in a well-supported clade (100, 1) as sister taxa to the rest of the *Hypnea* species which all clustered together in their own well-supported clade (100, 1).

Morphological Description

Morphology of Clade A

Clade A1 (plate 1, Figs. 1,4,7,8,10) and Clade A2 (plate 1, Figs. 2,3,5,6) – habit Fig. 9

Thalli mostly caespitose, comprising several terete main axes with thin branches and occasional hooked apices. Diameters of main axes between 600 and 1100µm; central filaments about 60µm in diameter, clearly visible; surrounding medullary cells about 180µm in diameter. Cortex single-layered, cells averaging 25µm high in cross section. Tetrasporangia about 50µm long, scattered sub-terminally on swollen tips of fertile branchlets. These specimens fitted the morphological descriptions of *H. rosea* (type description: Papenfuss 1947) and in some part the descriptions of *H. musciformis* in South African seaweed floras (Stegenga et al. 1997, Anderson et al. 2016). There was no morphological data available for clades A3 and A4 as they were all sequences downloaded from GenBank apart from one South African sample which had no material available for morphological analysis.

Morphology of Clade C

Clade C1 (plate 2, Figs. 1,2,3)

Thalli iridescent blue when fresh, with a cushion-like habit. Axes terete, bearing short, spiky branchlets; hooked apices absent. Main axes about 900µm diameter; central filament distinct, about 80µm in diameter, surrounded by medullary cells of about 120µm diameter. Anatomy and appearance corresponding to descriptions of *H. viridis* (type description: Papenfuss 1947; and in De Clerck et al. 2005). There were no morphological data available for the remaining subclades of Clade C as some were sequences downloaded from GenBank and the South African samples did not have any material available for morphological analysis.

Morphology of Clade G

Clade G specimen D580 (plate 3, Fig. 1,2)

The specimen D580, identified in the field as *H. cf. pannosa*,* did not fit the type description of *H. pannosa* (plate 3, Fig. 3 – photo of true *H. pannosa,* J. Agardh 1847; Coppejans et al. 2010). Thalli terete, stiff cushion-like growth, iridescent blue when submerged – all traits of true *H. pannosa* however the central filament (diam. 90µm), medullary cells (diam. 140µm), and cortical cells (diam. 8µm) do not correspond with the *H. pannosa* description. This taxon therefore remained unidentified.

Morphology of Clade E

Clade E (plate 4, short morphotype: Figs. 2,4,5,7,9, long morphotype: Figs, 1,3,6,8)

Thalli variable in size and appearance, from small 2-3cm bright green turfs to plants growing in dark purple stands up to 60cm long. Plants erect, usually in dense tufts. Uprights arising from a rhizomatous base, stiff, irregularly branched, terete, up to 3mm in diameter, with short spines arising from distal portions; hooked apices absent. Anatomical examination of both the short and the long morphotypes of this species revealed no central filament in either, but rather a parenchyma of similar-sized cells surrounded by a single layer of cortical cells (ca 14µm diameter). Short, green morphotype with main axis about 1500µm in diameter and with parenchyma cells 65µm in diameter. Long, dark green/purple morphotype with main axis about 1700µm in diameter, parenchyma cells 100µm in diameter. These measurements corresponded with the current descriptions of the morphologically variable *H. spicifera* in South African seaweed floras (Stegenga et al. 1997, De Clerck et al. 2005, Anderson et al. 2016).

Morphology of Clade F

Clade F1 (plate 5, Figs. 3,5,6) and F2 (plate 5, Figs. 4,7) – habit Fig. 1,2

Thalli caespitose, thin, dark red and loosely tangled around other algae; up to 5cm long. Multiple main axes with hooked apices. Clade F1 main axes diameter ca. 200µm, with conspicuous central filament (diameter 25µm) with surrounding medullary cells (ca. 50µm diameter), surrounded by single layer of cortical cells (ca. 20µm high). Clade F2 main axis diameter ca. 180µm; conspicuous central filament (30µm diameter) with surrounding medullary cells (ca. 30µm diameter), surrounded by single layer of cortical cells (ca. 14µm high). While Clade F1 fitted the description of "*H. tenuis"* (Stegenga et al. 1997, De Clerck et al. 2005, Anderson et al. 2016) the anatomy of Clade F2 was slightly smaller than "*H. tenuis"* as described, and more closely fitted the very brief type description of "*H. arenaria",* which was described as having an axis diameter of 180µm (Kylin 1938). A new genus is proposed for this clade (see later for description).

Morphology of Clade D

Clade D1 (plate 6, Figs. 1-6)

Thalli creeping, no clear percurrent axis, irregularly branched, dark red and growing up to 3cm tall, conspicuous central filaments (50µm diam.), 6 pericentral cells (100 – 300µm diam.), single layer of cortical cells (55µm length), and axes variable between specimens ranging from 500 to 1000µm

diameter. Based on the brief diagnosis of *H. intricata* (Kylin 1938), these specimens were identified as *H. cf. intricata*.

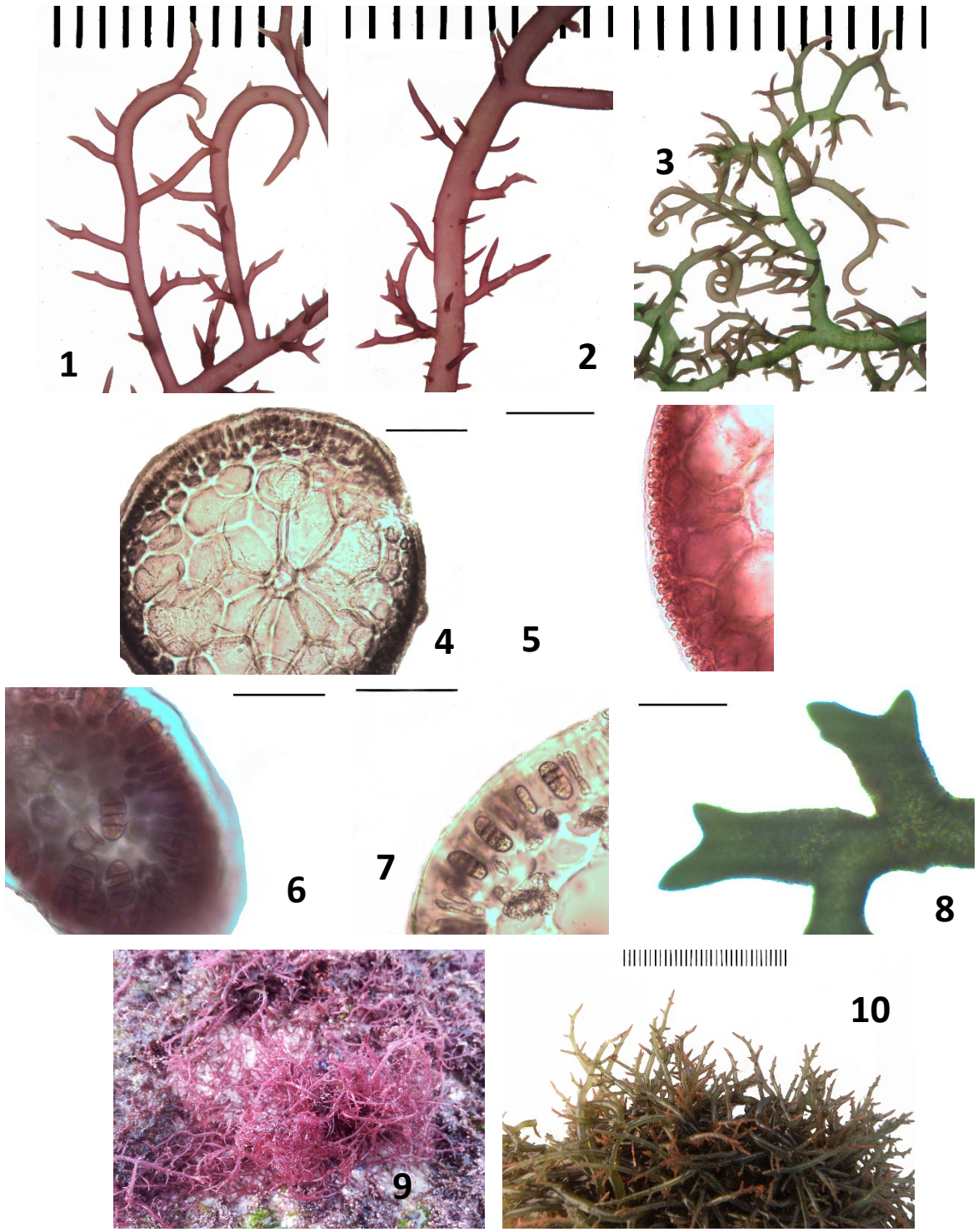

Plate 1. Hypnea rosea complex (clade A1 & A2) ***1-3*** *Dissecting microscope photographs.* ***1,*** *hooked tips and branches (A1);* ***2,*** *main axis and branches (A2);* ***3,*** *hooked apices, variable colour (A2).* ***4-8.*** *Compound microscope photographs* ***4,*** *cross section of main axis (A1);* ***5,*** *cross section showing cortical cells (A2);* ***6,*** *cross section showing tetrasporangia (A2);* ***7,*** *cross section showing tetrasporangia (A1);* ***8,*** *swollen apices containing tetrasporangia (A1);* ***9,*** *photograph showing habit;* ***10,*** *photograph showing thallus, variable colour (A1) – Scale: 1-3,10 = 1mm, 4 = 200um, 5-7 = 100um, 8 = 500um.*

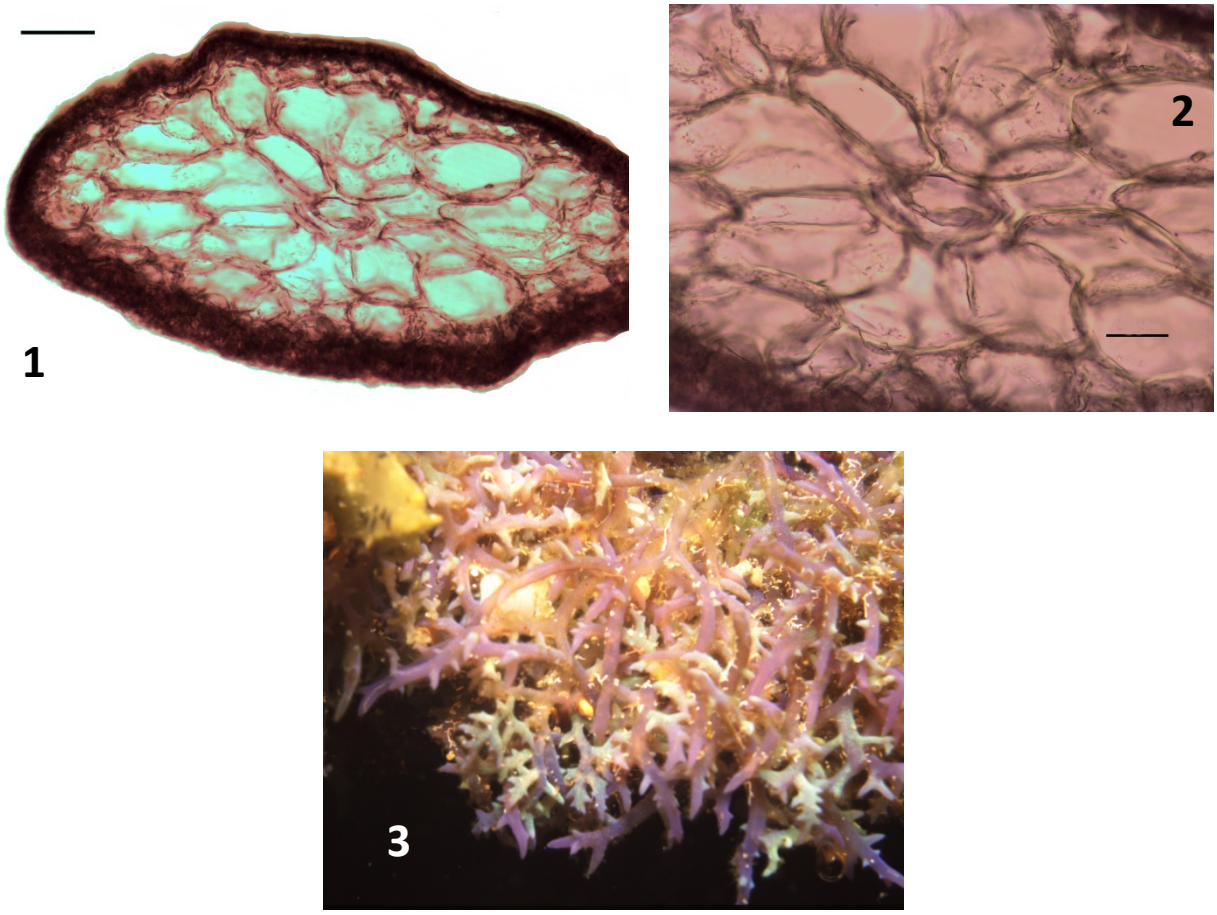

Plate 2. ***1-2.*** *compound microscope photographs of rehydrated specimens of Hypnea viridis (clade C);* ***1,*** *cross-section showing thallus and central filament* ***2,*** *central filament and medulla.* ***3,*** *photograph showing habit of H. viridis. Scale: 1 = 100um, 2 = 50um.*

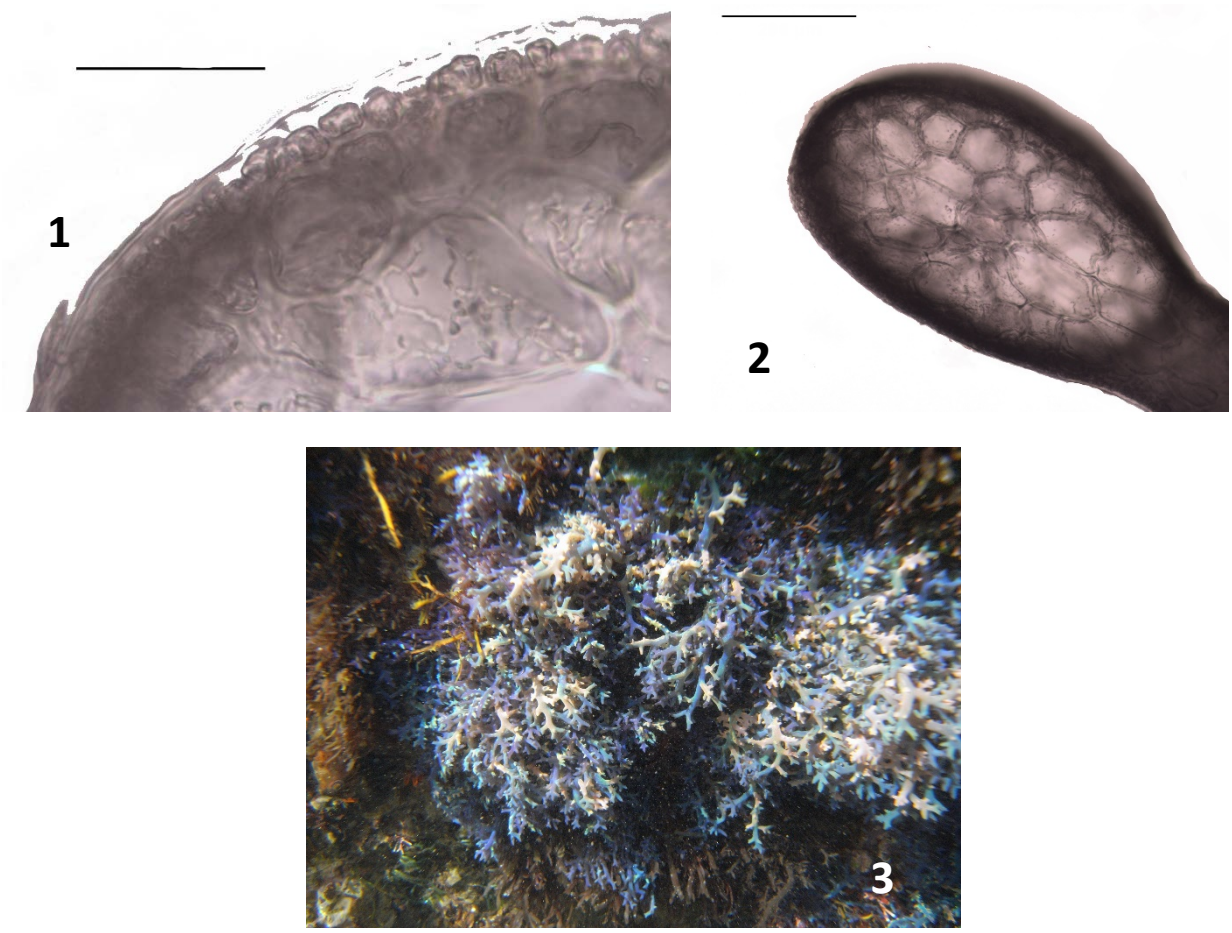

Plate 3. 1-2 compound microscope photographs of Unidentified sp. clade G. 1, cross-section showing compressed thallus and central filament (D580) 2, cortex and medulla (D580). 3, Photograph of true H. pannosa. Scale: 1 = 50um, 2 = 200um.

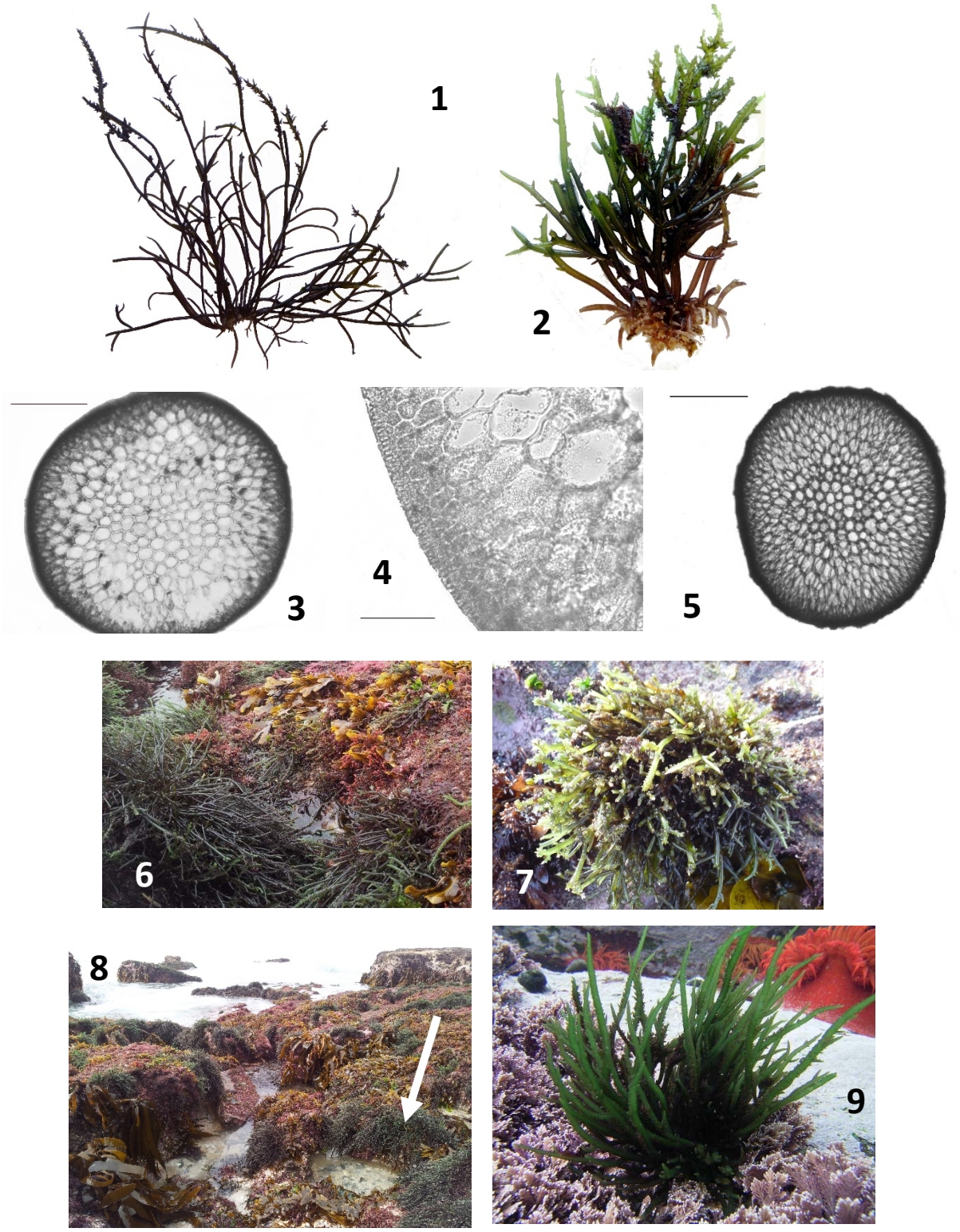

Plate 4. Hypnea spicifera (clade E); ***1-2*** *Photographs* ***1,*** *long morphotype thallus (length - 25cm);* ***2,*** *short morphotype thallus (length – 7cm);* ***3-5*** *Compound microscope photographs.* ***3,*** *cross-section of long morphotype;* ***4,*** *section showing cortex (D1700);* ***5,*** *cross section of short morphotype.* ***6-9*** *Photographs of habit* ***6,*** *long morphotype;* ***7,*** *short morphotype;* ***8,*** *long morphotype in situ (De Hoop Nature Reserve);* ***9, s****hort morphotype in situ (Arniston). Scale: 3,5 = 500um, 1 = 100um.*

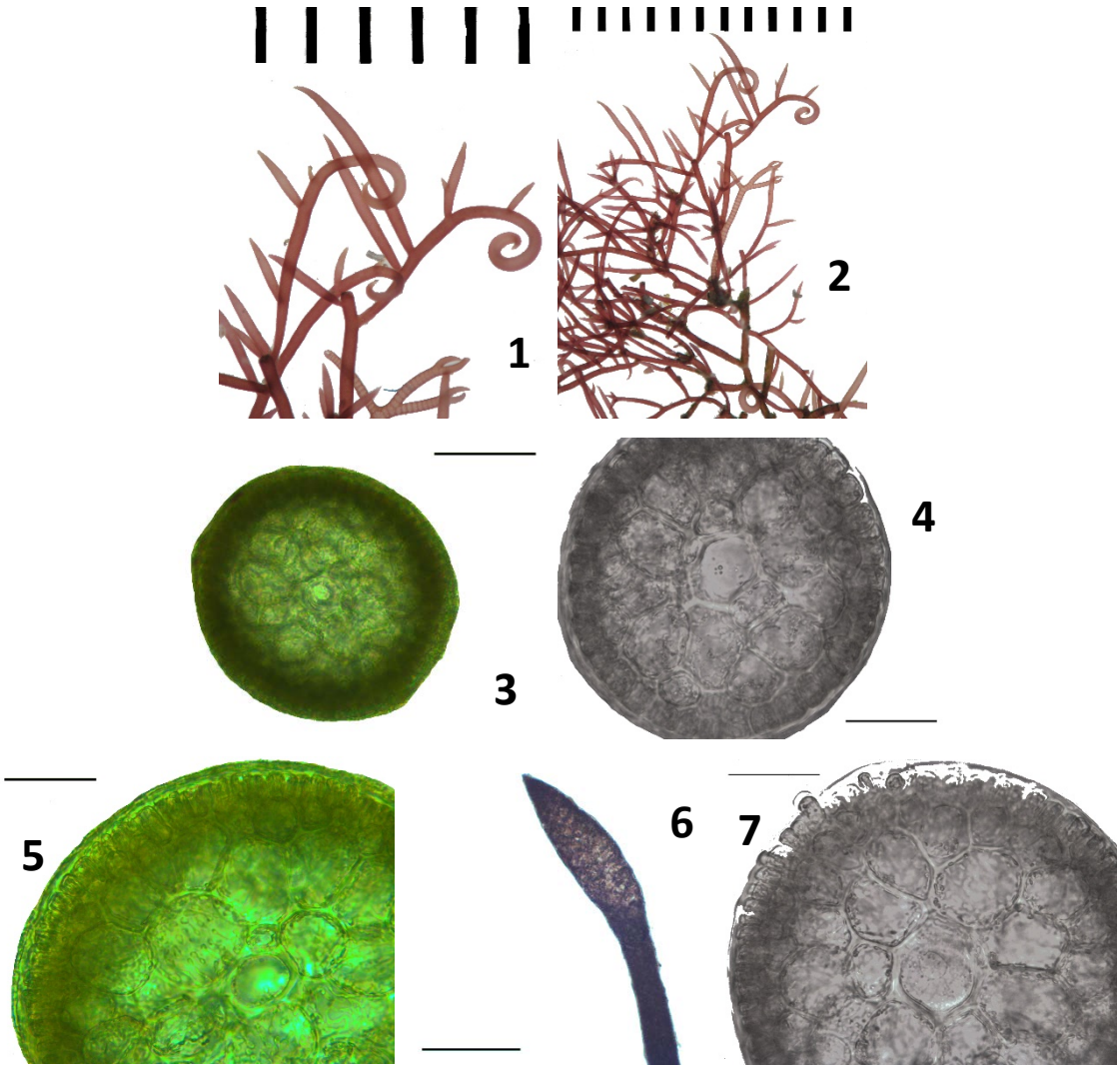

Plate 5. Clade F1 & F2. ***1-2,*** *dissecting microscope photographs of habit showing branches, branchlets and hooked apices.* ***3-7*** *Compound microscope photographs.* ***3,*** *cross section showing central filament (F1);* ***4,*** *cross section showing central filament (F2);* ***5,*** *cross section showing medulla and cortex (F1);* ***6,*** *reproductive tissue containing tetrasporangia (F1);* ***7,*** *cross section showing medulla and cortex (F2). Scale: 1,2 = 1mm divisions; 3-5,7 = 50um; 6 = 500um*

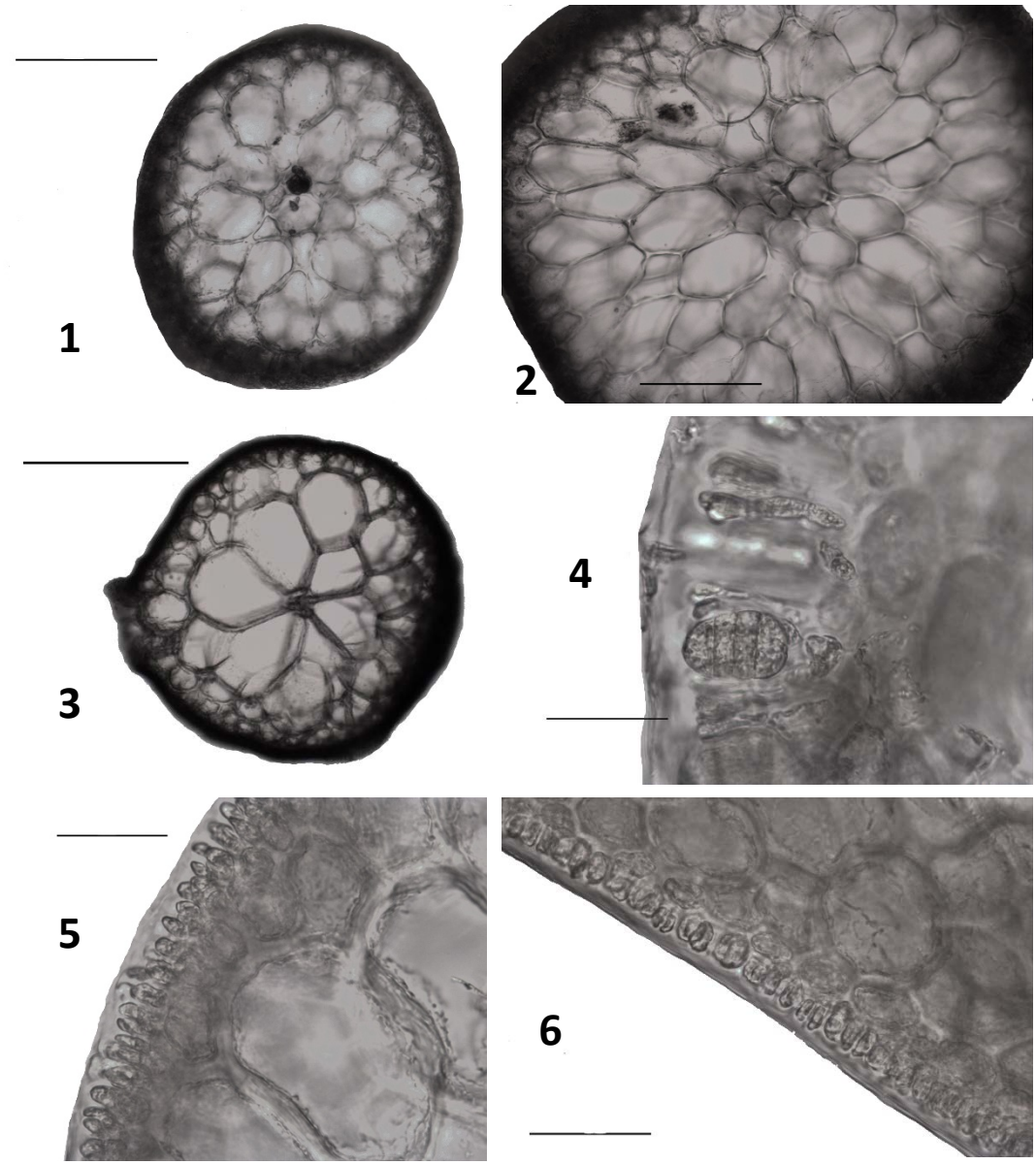

Plate 6. Clade D1. ***1-6*** *Compound microscope pictures of clade D1;* ***1-3,*** *cross sections showing central filament and medulla* ***1:*** *(D557),* ***2:*** *(D1045),* ***3:*** *(D993);* ***4,*** *section showing tetrasporangia (D557);* ***5,*** *section showing cortical cells (D993);* ***6,*** *section showing cortical cells (D1045). Scale: 1,2 = 200um, 3 = 500um, 4-6 = 50um.*

Discussion

This study was the first to examine the diversity of the genus *Hypnea* in South Africa using molecular data. Previous taxonomic work on this genus in South Africa recorded 11 *Hypnea* species, 8 of which appeared in recent floras and catalogues (Seagrief 1984, Silva et al. 1996, Stegenga et al. 1997, De Clerck et al. 2005, Anderson et al. 2016).

Diversity of SA Hypnea and phylogenetic relationships

I used a combination of multi-locus molecular and morphological approaches to investigate the species diversity of South African *Hypnea* and their phylogenetic relationships at both the regional and the global scale. The gene regions analysed were the chloroplastic *rbcL* and the mitochondrial *cox1* which have both been shown to be adequate markers for species identification in *Hypnea* and in other Rhodophyta (e.g. Geraldino et al. 2010; Yamagishi & Masuda 2000; Yamagishi et al. 2003; Nauer et al. 2014a,b; de Jesus et al. 2016; Le Gall & Saunders 2010; Milstein & Saunders 2012).

Intraspecific genetic distance (<1,7% for *rbcL*, <6% for *cox1*) and intergeneric genetic distance (>8% for *rbcL*, >13% for *cox1*) were chosen based on results and literature (e.g. Yamagishi & Masuda 2000; Yamagishi et al. 2003; Robba et al. 2006; Geraldino et al. 2009; Le Gall & Saunders 2010; Geraldino et al. 2010; Nauer et al. 2014a,b). The results of the present study identified 13 molecularly distinct taxa from South Africa. Of these 13, 7 were *Hypnea* clades identified to species level: *Hypnea cf. cerviconis* (clade A3)*, H. cf. intricata* (clade D1)*, H. cf. pannosa* (clade C2)*, H. rosea sp. 1* (clade A1)*, H. rosea sp. 2* (clade A2)*, H. spicifera* (clade E)*,* and *H. viridis* (clade C1), 2 taxa were represented by single sequences and remain unidentified (in clade G and I), 2 were also represented by single sequences, remain unidentified, and are genetically distinct enough to be assigned to a different genus, while for the remaining 2 we propose a new genus, *Tenebris gen. nov.* to accommodate the species previously known as *Hypnea tenuis* now *Tenebris tenuis* (clade F1) and second species that may represent the entity formerly known as *Hypnea arenaria* – here denoted as *Tenebris sp.* (clade F2).

Hypnea rosea complex and H. cervicornis (Clade A)

Field identifications and morphological data indicated that clades A1 and A2 had no clear phenotypic differences: specimens from both clades matched morphological criteria for *H. rosea* (table 1; Papenfuss 1947; Stegenga et al. 1997) and the specimens had the same distribution range – however the GD indicated that they were possibly separate taxa. For clades A3 & A4 there were no morphological data available as they were sequences downloaded from GenBank and it was necessary to rely on the GenBank identification of these sequences, and there was no available

material for sectioning D1365. When the sequence for D1365 (clade A3) was blasted through the NCBI website - *H. flexicaulis* (a known synonym for *H. cervicornis*) was returned with 100% similarity. This blast result, coupled with clade A3 and A4 grouping sister to each other with a low GD, indicated that clade A3 was a possible new South African record of *H. cervicornis* – current name for *H. flexicaulis* and *H. boergesenii*.

We therefore propose that without the ability to tell them apart morphologically clades A1 and A2 form an *H. rosea* species-complex of two species denoted as *H. rosea sp. 1* and *H. rosea sp. 2*, and clade A3 is *H. cf. cervicornis*. The South African distribution of the two species in the *H. rosea* complex and *H. cf. cervicornis* can only be known after further DNA work is done. The data also confirmed the findings of de Jesus et al. (2016) that separated *H. cervicornis* (clade A4) from the newly described *H. brasiliensis* (clade D6).

No Hypnea musciformis species in South Africa

Hypnea musciformis, the type of this genus, is reported in floras and catalogues of South African algae (Silva et al. 1996, Stegenga et al. 1997, Anderson et al. 2016, Guiry & Guiry 2017) and in preliminarily identifications in our own collection. However as evidenced from the combined morphological and molecular data, this species does not occur in South Africa. No published sequences – which were from all over the world including the type locality (Trieste, Italy) – clustered with any of our new South African sequences (Clade B3).

Nauer et al. (2014b) described a new species, *H. pseudomusciformis* (fig 2: clade B4), which was commonly misidentified as *H. musciformis* in Brazil. The South African description of *H. musciformis* was very similar to that of *H. rosea* which would have resulted in the past in the misidentification of members of that complex as *H. musciformis*. The specimens previously identified as *H. musciformis* in South Africa appeared not closely related to *H. musciformis* (unlike Brazilian *H. pseudomusciformis:* see Nauer et al. (2014b)). Samples which in any way resembled descriptions of *H. musciformis* in the South African collection genetically clustered within the *H. rosea* complex. Given the morphological similarity of some of our specimens to *H. musciformis* but the clear molecular evidence, it is concluded that this species does not occur in South Africa.

Evidence for Hypnea viridis and Hypnea pannosa link

Molecular data from this study supported the link between *Hypnea viridis* and *H. pannosa* which was hypothesised in De Clerck et al. (2005). *Hypnea viridis* (clade C1) was only present in the *rbcL* analysis but clustered sister to clade C2 with a GD of 1.7%. A link between *H. viridis* and the tropical *H. pannosa* was supported by our results. The *rbcL* and *cox1* analyses both had the South African clade

C2 clustered with *H. pannosa* (C3). These sister clades C2 & C3 contained GenBank sequences of *H. pannosa* (C3) from Mexico (type locality) and the Philippines, and South African sequences either unidentified or tentatively identified as *H. cf. pannosa* (C2) from Northern KwaZulu-Natal. There was one GenBank sequence named *H. viridis** from South Africa which clustered in C2 (*H. cf. pannosa*) in the *rbcL* analysis and in C3 (*H. pannosa*) in the *cox1* analysis. Given the low GD (*rbcL*: 1.4%; *cox1*: 7.56%) between C2 & C3 and that the *H. viridis** sample was not partial to either clade in both analyses, clade C3 is a clade of *H. pannosa* and clade C2 is a South African *H. cf. pannosa.*

The true South African *Hypnea viridis* (C1) was closely related to the two *H. pannosa* subclades (C2 and C3) and it was evident from the *rbcL* analysis (figure 2, appendix 3) that *H. pannosa* and *H. viridis* are closely related sister taxa. While *H. pannosa* has a wide distribution spanning in all three oceans between the tropics (Pacific, Indian and Atlantic), the South African distribution of these two species may be along the East coast from Durban to Sodwana, however, the morphological data were lacking and further investigation into these two species is required.

Hypnea cf. intricata in clade D1

The very brief type description of *Hypnea intricata* (Kylin 1938) makes it nearly impossible to ascribe this name to any samples in the field. Clade D1 contained samples that were either unidentified or tentatively identified as *Hypnea rosea.* With GD in the *cox1* analysis of <2,2% between the sequences we confidently assigned them to the same species. The results of the morphological analysis almost fit the brief type description with some variation in thallus measurements. The thallus measurement variation could be due to the position along the axis from where the section was taken, or due to size variation in different aged plants. The localities of the South African samples in the *cox1* tree suggest a warm water *Hypnea.* The name *H. cf. intricata* was therefore assigned to clade D1 based on the limited description and the distribution range.

Hypnea spicifera: one morphologically variable species

H. spicifera specimens were morphologically variable but genetically very similar (GD: 0 - 0.068%). This was the most morphologically variable species within this genus in South Africa, with some morphs present as small bright green tufts a few centimetres high to others ranging all the way to long dark purple thalli up to 60cm long (Stegenga et al. 1997; pers. obs.). The results here showed that *H. spicifera is* one species despite the range of morphologies. However, it could be asked whether it belongs in the genus *Hypnea*. The GD of *H. spicifera* from the other *Hypnea* species was larger than any other species within the genus (between 6 and 9%). *Hypnea spicifera* could perhaps be classified in a new genus within the Cystocloniaeae. However, without *cox1* data and considering

that it grouped separately, but sister to the other *Hypnea* species in the Gigartinales cladogram, caution is required.

Clade G, H and I – unidentified samples

The 6 individual sequences paired in these three clades represent molecularly distinct taxa which were each represented by a single *cox1* sequence. Only 4 of these sequences were South African while the other two were from Europa Island (clade G) and Madagascar (clade H). They returned no conclusive result from a nucleotide blast and therefore remained unidentified. The sequences in clade H had a GD >17% from all other sequences indicating that they are not *Hypnea* species at all – and with an inconclusive nucleotide blast it is unclear in what genus or family these taxa belong. This further emphasizes the fact that some *Hypnea* species are very difficult to identify based on morphology alone and often samples are collected which may not be *Hypnea* at all. In such cases, distinction may only be possible by using DNA evidence.

Hypnea ecklonii

The identity of *Hypnea ecklonii* remained a problem. Although Stegenga et al. (1997) stated it to be a west coast species, the type specimen is from Algoa Bay on the south coast. It is also recorded from Mauritania, Senegal and Namibia (Guiry & Guiry 2017). There are no DNA analyses for this species, and none of the specimens we collected fitted the description. It appears that if this species exists, we did not collect it.

"Hypnea tenuis" and "Hypnea arenaria" – now in genus Tenebris

Specimens tentatively identified as *Hypnea tenuis* clustered in two separate clades (F1 and F2) and were anatomically distinct from each other with slight differences in cell size and axis diameter. Clade F1 fitted the morphological description of "*Hypnea tenuis",* while clade F2 could fit the morphological description of "*H. arenaria".* What we knew to be "*Hypnea tenuis"* was recognisable based on descriptions in the literature and field guides (Kylin 1938; Stegenga et al. 1997; De Clerck et al. 2005). It possessed morphological features typical of other *Hypnea* species*,* such as the apical hooks, short tapering branchlets, tetrasporangia in the reproductive tissue, a terete thallus, a very conspicuous central filament surrounded by medullary cells of a similar size and a cortex of two cell layers. The medullary cells in the *H. rosea* complex were three times larger than the central filament whereas the medullary cells in samples from both clade F1 and F2 were the same size or only slightly larger than the central filament. The difference in cell size ratios coupled with the large GD between them and the rest of the *Hypnea* species (GD: 8 – 13%, *rbcL* intergeneric limit >8%) indicated that these species comprise a separate genus.

Their GD from the rest of the *Hypnea* species was the same as the GD of the 3 outgroups from the other *Hypnea* species. The closest relatives of clade F1 and clade F2, based on the GD matrix, was *Cystoclonium purpureum* (Hudson) Batters, the type species of the Cystocloniaceae. The next closest relatives were *H. viridis* and *H. pannosa*. These results indicated that the new genus should should remain within the Cystocloniaceae, although the family does seem polyphyletic (Fig. 5) and should be reassessed using additional molecular markers. The GD of *C. purpureum*, *H. viridis* and *H. pannosa* from clade F1 and clade F2 was between 8% and 11% which indicated that they should not be grouped in the same genus as their nearest relatives. The GD between clade F1 and clade F2 is between 2.7 and 3.4% which indicated that they belong to the same genus. As there was only one sequence available for clade F2 this requires further investigation. Nevertheless, a new genus is proposed to accommodate these taxa. – *Tenebris,* including the new species *Tenebris tenuis* and a second species *Tenebris sp.* which may represent the taxon described as *Hypnea arenaria.*

Taxonomy

Description of Tenebris – a proposed new genus containing two species

Tenebris V.J. Johnson, J.J. Bolton, L. Mattio, & R.J. Anderson, ***gen. nov****.*

Description: Thalli thin, caespitose, terete, no percurrent axis, multiple main axes, irregular branching pattern, main axes mostly with hooked apices, dark red to brown in colour, small (up to 5cm long), main axes <20µm in diameter, conspicuous central filament 20–30µm diam. surrounded by equal size periaxial cells (20-30µm), single cell-layer of elongated cortical cells ca. 14µm in length, zonate tetrasporangia in swollen apices of fertile branchlets. Intertidal, reported either loosely tangled around other algae in rock pools or on outer edges of rocks partially covered in sand. Distinguished from the genus *Hypnea* based on molecular evidence (8-13% GD) while morphologically distinguishing features include diameter ratio of central filament:pericentral cell (equal diam. in *Tenebris*, yet medulla significantly larger than central filament in other *Hypnea*) and diameter of thallus (ca. 300µm narrower than smallest *Hypnea*).

Type species: *Tenebris tenuis* (Kylin) V. Johnson, J. Bolton, L. Mattio, R. Anderson gen. nov.

Basionym: *Hypnea tenuis* Kylin

Holotype: D1713 – collected by V. Johnson, J. Bolton & R. Anderson 2 March 2014 in the intertidal zone at Preekstoel, Still Bay, Western Cape, South Africa.

Etymology: *Tenebris* - Latin word for dark.

Single marker barcoding vs multi-locus marker and morphological data

In light of this new genus description, it's important to note that there has been a flood of studies identifying new taxa over the past few decades, indicating that we are far from having completely discovered and described all the algal species (Leliaert et al. 2014). Revision of taxa has resulted in either descriptions of new genera, or reverting to the names of genera which were previously recognised but have since been subsumed into other groups (Wynne & Schneider 2010; Sutherland et al. 2011). The rate at which species are being discovered based on molecular data, however, is much faster than phycologists are formally describing species and there are increasingly more conflicts being uncovered between morphological and molecular species boundaries (Belton et al. 2014).

Definitions of extant species rely on DNA and not strictly morphology, as with the morphologically indistinguishable species of the *H. rosea* complex. In some cases, DNA evidence has revealed ancient cryptic lineages previously grouped together due to a lack of morphological differences or convergent evolution (Leliaert et al. 2014). The use of multi-locus gene sequences coupled with morphological data was invaluable in revealing the organisation of the genera and species in this study.

Molecular data are increasingly clarifying relationships within the Florideophytes at higher taxonomic levels, revealing paraphyletic clades and resulting in the proposal of new subclasses based on single- and multi-gene phylogenies (Verbruggen et al. 2010).

Florideophytes phylogeny

There is no existing comprehensive phylogeny of the Florideophytes (Verbruggen et al. 2010) despite recent attempts to develop a method of achieving this (Sherwood et al. 2010; Kim et al. 2014; Yang et al. 2015). In the current study, a Gigartinales phylogeny was attempted in which a few families appear to be polyphyletic, suggesting that more genera within the Gigartinales should be assessed with additional markers (eg. SSU rDNA in Saunders et al. 2004) molecularly to establish their place within this Order and within the Florideophytes as a whole.

Species delimitation based on molecular systematics

Disagreements over fundamentals of species delimitation are rife, but this has not deterred biologists from systematic and taxonomic studies which separate life into what is widely accepted as species components (Leliaert et al. 2014; Leliaert & De Clerck 2017). Molecular analyses have, over the past few decades, become the norm and are providing researchers with fast and effective tools for species delimitation (Wolf et al. 2011; Leliaert & De Clerck 2017).

Evolution and speciation events are continuous and constantly occurring which means we are studying an ever-changing concept requiring periodic reassessment and evaluation (Leliaert et al. 2014). Along with the evolution of the organisms we study, the methods we use are improving as information is becoming more readily available and new comparative models and tools are being developed. Due to the high volume of molecular studies, there is a range of accepted intra- and interspecific cut off values for many organisms. These can be used in comparison with your data from the same gene region when setting taxonomic boundaries.

Increasing loss of biodiversity

Biodiversity loss is increasing rapidly, driving the need to identify and describe species before they disappear (Robba et al. 2006). Degradation of the environment and loss of species far exceeds the speed at which adequate management and conservation plans can be implemented, necessitating the current drive to quickly and concisely describe and analyse species populations and their dynamic natural patterns and processes (Amado Filho et al. 2006). Red algae fall within the category identified as severely under-studied, because most studies focus on organisms of a more conspicuous nature such as birds, mammals and flowering plants (Verbruggen et al. 2010).

Under the target set by the IUCN (1992) 20% of the world's coastline should be conserved. To help determine which sections of coast should be protected, a comprehensive and detailed account of marine algal diversity is needed. Observations and studies have shown a general increase in sea surface temperature which has had notable side effects on marine and estuarine organisms (Pecl et al. 2017). Assessing the effect of climate change in the future will be assisted by reliable data on algal distributions, data which are also useful in conservation planning and delimiting Marine Protected Areas (Anderson et al. 2009).

Conclusion

The diversity of the genus *Hypnea* in South Africa is somewhat different from what has historically been recorded. *Hypnea rosea* is made up of two molecularly distinct entities indistinguishable based on morphology, forming an *H. rosea* complex. There is also a possible molecular link between *Hypnea rosea* and *H. cervicornis*. *Hypnea viridis* is very closely linked to the tropical *Hypnea pannosa, Hypnea spicifera* is confirmed to be one morphologically variable species, and it is possible that *Hypnea intricata* is a valid species, although more evidence for this is needed. *Hypnea musciformis* (the type species of the genus) does not seem to occur in South Africa*. Hypnea ecklonii* requires further study, starting with a search for specimens fitting the type description. The present study indicated the possible presence of two species not previously recorded from South Africa: *Hypnea*

pannosa and *Hypnea cervicornis*, but these require further study. The entity previously known as *Hypnea tenuis* (and possibly that described as *H. arenaria*) is molecularly distinct and is described as a new genus, *Tenebris*, with *Tenebris tenuis* as the type species and containing two species, from molecular evidence.

Current species number for confirmed species of *Hypnea* in South Africa is therefore 4 (*Hypnea rosea sp. 1, H. rosea sp. 2, H. spicifera* and *H. viridis*), 2 species require further study (*H. cervicornis, H. pannosa*), 2 remain unidentified (clade G & I), while 2 fall into and new genus, *Tenebris* (*Tenebris tenuis* and *Tenebris sp.*)

While the present study goes some way to clarifying the diversity of South African *Hypnea* species, several taxa remain incompletely resolved, illustrating a need for further studies. Such studies should be based on more comprehensive collections and a combination of molecular and morphological techniques, as currently used in this and other taxonomic investigations.

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Appendix 1

Table of collected specimens from South African, Madagascar, Europa Islands and Mozambique.

Specimen Number	Collection site	Date of Collection	Identification	RbcL	Cox1	Clade
D467	Kleinemonde	2009-07-08	*H. rosea sp. 1*	X	X	A1
D722	Kei Mouth	2010-07-11	*H. rosea sp. 1*	X	X	A1
D1309	Hluleka	2013-08-20	*H. rosea sp. 1*	X	X	A1
D1630	Black Rock	2013-10-05	*H. rosea sp. 1*	X	X	A1
D1866	Dwesa	2014-05-13	*H. rosea sp. 1*	X	X	A1
D1914	Dwesa	2014-05-14	*H. rosea sp. 1*	X	X	A1
D1915	Dwesa	2014-05-14	*H. rosea sp. 1*	X	X	A1
D1918	Dwesa	2014-05-14	*H. rosea sp. 1*	X	X	A1
D1950	Dwesa	2014-05-14	*H. rosea sp. 1*	X	X	A1
D1712	Preekstoel	2014-03-02	*H. rosea sp. 1*	X	X	A1
D1720	Morris Point	2014-03-03	*H. rosea sp. 1*	X	X	A1
D1702	Jongensfontein	2014-03-01	*H. rosea sp. 1*	X		A1
D1715	Preekstoel	2014-03-02	*H. rosea sp. 1*	X	X	A1
D1774	Arniston	2014-03-15	*H. rosea sp. 1*	X		A1
D1714	Preekstoel	2014-03-02	*H. rosea sp. 1*	X	X	A1
D1048	Mission Rocks	2011-09-26	*H. rosea sp. 1*	X	X	A1
D1951	Dwesa	2014-05-15	*H. rosea sp. 1*	X	X	A1
D986	Sodwana	2011-03-22	*H. rosea sp. 1*	X	X	A1
D1367	Bhanga	2013-10-03	*H. rosea sp. 1*	X	X	A1
D1654	Dog Point	2013-10-06	*H. rosea sp. 1*	X	X	A1
D1049	Mission Rocks	2011-09-26	*H. rosea sp. 1*	X	X	A1
D854	Cape St. Lucia	2010-09-11	*H. rosea sp. 2*	X	X	A2
D1701	Jongensfontein	2014-03-01	*H. rosea sp. 2*	X	X	A2
D1776	Arniston	2014-03-15	*H. rosea sp. 2*	X	X	A2
D1837	De Hoop	2014-04-29	*H. rosea sp. 2*	X	X	A2
D1365	Bhanga	2013-10-03	*H. cervicornis*	X	X	A3
PM12	Pemba	2012-05-08	*H. cornuta*	X	X	B2
PM83	Pemba	2012-05-08	*H. cornuta*	X	X	B2
D1607	Bhanga	2013-10-04	*H. cf. pannosa*	X	X	C2
D1577	Bhanga	2013-10-04	*H. cf. pannosa*	X		C2
D1668	Bhanga Rocks	2013-10-07	*H. cf. pannosa*	X	X	C2
D542	Jesser Point	2009-10-03	*H. viridis*	X		C1
D1634	Black Rock	2013-10-05	*H. viridis*	X		C1
D1605	Bhanga	2013-10-05	*H. viridis*	X		C1
EUR268	Europa Islands	2011-11-10	*H. sp.*	X	X	C4
PM82	Pemba	2012-05-08	*H. sp.*	X		C4
D544	Jesser Point	2009-10-03	*H. cf. intricata*	X	X	D1
D603	Olifantsbos	2009-11-17	*H. spicifera*	X		E
D1700	Jongensfontein	2014-03-01	*H. spicifera*	X		E
D1044	Mission Rocks	2011-09-26	*H. spicifera*	X		E
D637	Olifantsbos	2009-11-17	*H. spicifera*	X		E
D1952	Dwesa	2014-05-15	*Tenebris tenuis*	X		F1
D310	Port Alfred	2009-03-09	*T. tenuis*	X		F1
D1713	Preekstoel	2014-03-02	*T. tenuis*	X		F1

D1046	Mission Rocks	2011-09-26	*T. sp.*	X		F2
D1696	Bordjies	2014-01-19	*Unidentified sp.*		X	H
D1711	Preekstoel	2014-03-02	*Unidentified sp.*		X	H
D1666	Bhanga Rocks	2013-10-07	*H. cf. intricata*		X	D1
MAD148	Madagascar	2012-05-25	*H. cf. intricata*		X	D1
PM109	Pemba	2012-05-10	*H. cf. intricata*		X	D1
D993	Sodwana	2011-03-23	*H. cf. intricata*		X	D1
PM86	Pemba	2012-05-08	*H. cf. spinella*		X	D5
MAD017	Madagascar	2012-05-18	*H. sp.*		X	A4
EUR287	Europa Islands	2011-11-11	*H. sp.*		X	A2
EUR339	Europa Islands	2011-11-12	*H. sp.*		X	A2
D1366	Bhanga	2013-10-03	*H. rosea sp. 2*		X	A2
D360	3 Sisters	2009-03-11	*H. rosea sp. 1*		X	A1
D1702	Jongensfontein	2014-03-01	*H. rosea sp. 1*		X	A1
MAD016	Madagascar	2012-05-18	*H. cf. cornuta*		X	B2
EUR335	Europa Islands	2011-11-12	*H. cf. cornuta*		X	B2
MAD163	Madagascar	2012-05-26	*H. sp.*		X	C5
EUR088	Europa Islands	2011-11-08	*H. sp.*		X	G
D580	Anton's Reef	2009-10-05	*H. sp.*		X	G
D1047	Mission Rocks	2011-09-26	*H. rosea sp. 1*		X	A1
D1347	Hluleka	2013-08-24	*H. rosea sp. 1*		X	A1
D1703	Jongensfontein	2014-03-01	*H. rosea sp. 1*		X	A1
D1704	Jongensfontein	2014-03-01	*H. rosea sp. 1*		X	A1
D1820	De Hoop	2014-04-28	*H. rosea sp. 1*		X	A1
D1831	De Hoop	2014-04-29	*H. rosea sp. 1*		X	A1
D1721	Morris Point	2014-03-03	*H. rosea sp. 2*		X	A2
D1775	Arniston	2014-03-15	*H. rosea sp. 2*		X	A2
D577	Jesser Point	2009-10-04	*H. cf. intricata*		X	D1
D1045	Mission Rocks	2011-09-26	*H. cf. intricata*		X	D1
D1611	Khosi Bay	2013-10-05	*H. cf. intricata*		X	D1

Appendix 2

*Table 3: GenBank sequences, Hypnea and Outgroups. Possible misidentification marked with **

GenBank Number	Species name	Collection site	Reference	Clade
AF385634	*H. cervicornis*	Long Tung Park, NE Taiwan	Hommersand & Fredericq (2002)	A4
EU346009	*H. cervicornis*	Jeju, Korea	Geraldino *et al*. (2009)	A4
EU345994	*H. cervicornis*	Jeju, Korea	Geraldino *et al*. (2009)	A4
EF136612	*H. cervicornis*	Keelung, Taiwan	Geraldino *et al*. (2009)	A4
EF136614	*H. cervicornis*	Daejeon, Korea	Geraldino *et al*. (unpub.)	A4
EF136613	*H. cervicornis*	Daejeon, Korea	Geraldino *et al*. (unpub.)	A4
DQ095823	*H. japonica**	Tonggumi, Korea	Kim *et al*. (unpub.)	A4
KM203911	*H. cervicornis*	Sao Paulo, Brazil	Nauer *et al*. (2014)	A4
KM203900	*H. cervicornis*	Rio de Janeiro, Brazil	Nauer *et al*. (2014)	A4
FJ694934	*H. tenuis**	Durban, South Africa	Geraldino *et al*. (2010)	A3
EU345999	*H. stellulifera*	Panglao, Phillipines	Geraldino *et al*. (2009)	B1
AB095913	*H. stellulifera*	Pulau Besar, Malaysia	Geraldino *et al*. (2009)	B1
AB095915	*H. stellulifera*	Pulau Sipadan, Malaysia	Geraldino *et al*. (2009)	B1
AB095914	*H. stellulifera*	Pulau Sipadan, Malaysia	Geraldino *et al*. (2009)	B1
EU346004	*H. stellulifera*	Panglao, Phillipines	Geraldino *et al*. (2009)	B1
EU346014	*H. musciformis*	Antibes, France	Geraldino *et al*. (2009)	B3
EU346013	*H. musciformis*	Antibes, France	Geraldino *et al*. (2009)	B3
EU346011	*H. musciformis*	Villefranche, France	Geraldino *et al*. (2009)	B3
KJ202086	*H. musciformis*	North Carolina, USA	Freshwater *et al*. (unpub.)	B3
KC121141	*H. musciformis*	Cadiz, Spain	Diaz-Tapia *et al*. (unpub.)	B3
KC121142	*H. musciformis*	Marsella, France	Diaz-Tapia *et al*. (unpub.)	B3
EU346012	*H. musciformis*	Cannes, France	Geraldino *et al*. (2009)	B3
U04179	*H. musciformis*	Fort Fisher, NC, USA	Hommersand & Fredericq (2002)	B3
KM203894	*H. pseudomusciformis*	Sao Paulo, Brazil	Nauer *et al*. (2014)	B4
KM203895	*H. pseudomusciformis*	Sao Paulo, Brazil	Nauer *et al*. (2014)	B4
KM203901	*H. pseudomusciformis*	Sao Paulo, Brazil	Nauer *et al*. (2014)	B4
AB033162	*H. flagelliformis*	Fukaura, Japan	Geraldino *et al*. (2009)	B5
AB033160	*H. chordacea*	Shizuoka, Japan	Geraldino *et al*. (2009)	B5
EU345991	*H. cornuta*	Bali, Indonesia	Geraldino *et al*. (2009)	B6
EU345990	*H. cornuta*	Perth, Australia	Geraldino *et al*. (2009)	B6
EU345992	*H. cornuta*	Perth, Australia	Geraldino *et al*. (2009)	B6
AB095911	*H. cornuta*	Okinawa, Japan	Geraldino *et al*. (2009)	B6
AB033161	*H. cornuta*	Nagasaki, Japan	Geraldino *et al*. (2009)	B6
AB095912	*H. cornuta*	Taranto, Italy	Geraldino *et al*. (2009)	B6
FJ694931	*H. pannosa*	El Sargento, Mexico	Geraldino *et al*. (2010)	C3
FJ694959	*H. pannosa*	El Sargento, Mexico	Geraldino *et al*. (2010)	C3
FJ694958	*H. pannosa*	El Sargento, Mexico	Geraldino *et al*. (2010)	C3
FJ694957	*H. pannosa*	Oaxaca, Mexico	Geraldino *et al*. (2010)	C3
FJ694956	*H. pannosa*	Oaxaca, Mexico	Geraldino *et al*. (2010)	C3
FJ694955	*H. pannosa*	El Sargento, Mexico	Geraldino *et al*. (2010)	C3
FJ694954	*H. pannosa*	El Sargento, Mexico	Geraldino *et al*. (2010)	C3
FJ694953	*H. pannosa*	El Sargento, Mexico	Geraldino *et al*. (2010)	C3
FJ694951	*H. pannosa*	Bohol, Philippines	Geraldino *et al*. (2010)	C3
FJ694952	*H. pannosa*	Ilocos Norte, Philippines	Geraldino *et al*. (2010)	C3
FJ694950	*H. pannosa*	Bohol, Philippines	Geraldino *et al*. (2010)	C3
FJ694949	*H. pannosa*	Bohol, Philippines	Geraldino *et al*. (2010)	C3
FJ694930	*H. viridis**	Durban, South Africa	Geraldino *et al*. (2010)	C2
EU346002	*H. japonica*	Gyeongju, Korea	Geraldino *et al*. (2009)	C6
EU345995	*H. japonica*	Keelung, Taiwan	Geraldino *et al*. (2009)	C6
EU346003	*H. japonica*	Gyeongju, Korea	Geraldino *et al*. (2009)	C6
EU345996	*H. japonica*	Keelung, Taiwan	Geraldino *et al*. (2009)	C6
EU346003	*H. japonica*	Gyeongju, Korea	Geraldino *et al*. (2009)	C6
AB033164	*H. japonica*	Kagoshima, Japan	Geraldino *et al*. (2009)	C6
FJ694947	*H. nidulans*	Ilocoso Norte, Philippines	Geraldino *et al*. (2010)	C5

FJ694946	*H. nidulans*	Bohol, Philippines	Geraldino *et al*. (2010)	C5
FJ694948	*H. nidulans*	Surigao del Norte, Philippines	Geraldino *et al*. (2010)	C5
AB033165	*H. pannosa**	Okinawa Prefecture, Japan	Geraldino *et al*. (2009)	C5
EU240848	*H. spinella*	Panang Bay, Vietnam	Geraldino *et al*. (2009)	D2
AB033166	*H. spinella*	Okinawa Prefecture, Japan	Geraldino *et al*. (2009)	D2
EU240849	*H. spinella*	Panang Bay, Vietnam	Geraldino *et al*. (2009)	D2
KM203903	*H. flava*	Sao Paulo, Brazil	Nauer *et al*. (2014)	D3
KM203906	*H. flava*	Espirito Santo, Brazil	Nauer *et al*. (2014)	D3
KM203896	*H. edeniana*	Sao Paulo, Brazil	Nauer *et al*. (2014)	D4
KM203907	*H. edeniana*	Espirito Santo, Brazil	Nauer *et al*. (2014)	D4
KM203899	*H. spinella*	Sao Paulo, Brazil	Nauer *et al*. (2014)	D5
KM203904	*H. spinella*	Sao Paulo, Brazil	Nauer *et al*. (2014)	D5
KM203905	*H. spinella*	Espirito Santo, Brazil	Nauer *et al*. (2014)	D5
FJ694933	*H. valentiae*	Bali, Indonesia	Geraldino *et al*. (2010)	D5
KM203898	*H. brasiliensis*	Sao Paulo, Brazil	Nauer *et al*. (2014)	D6
KM203897	*H. brasiliensis*	Sao Paulo, Brazil	Nauer *et al*. (2014)	D6
KM203909	*H. brasiliensis*	Sao Paulo, Brazil	Nauer *et al*. (2014)	D6
KM203908	*H. brasiliensis*	Sao Paulo, Brazil	Nauer *et al*. (2014)	D6
KM203910	*H. brasiliensis*	Espirito Santo, Brazil	Nauer *et al*. (2014)	D6
FJ694932	*H. nidifica*	Sentosa Island, Singapore	Geraldino *et al*. (2010)	D6
AB033159	*H. charoides*	Shimoda, Japan	Yamagishi & Masuda (2000)	D6
FJ694935	*H. rosea**	Durban, South Africa	Geraldino *et al*. (2010)	D1
AB095916	*H. yamadae*	Nagasaki Prefecture, Japan	Geraldino *et al*. (2009)	D1
AF385636	*H. volubilis*	Sonier Banks, Louisiana	Hommersand & Fredericq (2002)	D1
EU240844	*H. charoides*	Perth, Australia	Geraldino *et al*. (2009)	D7
EU240843	*H. charoides*	Perth, Australia	Geraldino *et al*. (2009)	D7
KC130220	*H. charoides*	Pinnaroo Point, Australia	Barbara *et al*. (2013)	D7
EU240847	*H. charoides*	Perth, Australia	Geraldino *et al*. (2009)	D7
EU240846	*H. charoides*	Perth, Australia	Geraldino *et al*. (2009)	D7
EU240845	*H. charoides*	Perth, Australia	Geraldino *et al*. (2009)	D7
EF136593	*H. cervicornis*	Gyeongju, Korea	Geraldino *et al*. (unpub.)	A4
EF136592	*H. cervicornis*	Yeosu, Korea	Geraldino *et al*. (unpub.)	A4
HQ422901	*H. valentiae**	Maui, Kauai	Sherwood *et al*. (2010)	A4
EF136594	*H. cervicornis*	Gyeongju, Korea	Geraldino *et al*. (unpub.)	A4
EF136595	*H. cervicornis*	Gyeongju, Korea	Geraldino *et al*. (unpub.)	A4
FN823052	*H. cervicornis*	Venice, Italy	Wolf *et al*. (2011)	A4
GQ141880	*H. tenuis**	Durban, South Africa	Geraldino *et al*. (unpub.)	A3
EF136591	*H. cervicornis*	Bulusan, Phillipines	Geraldino *et al*. (2009)	A5
KF714868	*H. cornuta*	Messina, Italy	Manghisi *et al*. (2010)	B6
KF714867	*H. cornuta*	Messina, Italy	Manghisi *et al*. (2010)	B6
KF714866	*H. cornuta*	Messina, Italy	Manghisi *et al*. (2010)	B6
KF714865	*H. cornuta*	Messina, Italy	Manghisi *et al*. (2010)	B6
KF714864	*H. cornuta*	Messina, Italy	Manghisi *et al*. (2010)	B6
KF714863	*H. cornuta*	Messina, Italy	Manghisi *et al*. (2010)	B6
KF714862	*H. cornuta*	Messina, Italy	Manghisi *et al*. (2010)	B6
KF714861	*H. cornuta*	Messina, Italy	Manghisi *et al*. (2010)	B6
KF714860	*H. cornuta*	Messina, Italy	Manghisi *et al*. (2010)	B6
KF714859	*H. cornuta*	Messina, Italy	Manghisi *et al*. (2010)	B6
KF714858	*H. cornuta*	Messina, Italy	Manghisi *et al*. (2010)	B6
EU345985	*H. stellulifera*	Bohol, Philippines	Geraldino *et al*. (2009)	B1
EU345984	*H. stellulifera*	Bohol, Philippines	Geraldino *et al*. (2009)	B1
HQ422876	*H. musciformis*	Hawaii	Sherwood *et al*. (2010)	B3
HQ422646	*H. musciformis*	Hawaii	Sherwood *et al*. (2010)	B3
HQ422630	*H. musciformis*	Hawaii	Sherwood *et al*. (2010)	B3
HQ422612	*H. musciformis*	Hawaii	Sherwood *et al*. (2010)	B3
KF714869	*H. musciformis*	Messina, Italy	Manghisi *et al*. (2010)	B3
KJ202077	*H. musciformis*	Fort Fisher, North Carolina	Freshwater *et al*. (unpub)	B3
FJ694897	*H. pannosa*	El Sargento, Mexico	Geraldino *et al*. (2010)	C3
FJ694896	*H. pannosa*	El Sargento, Mexico	Geraldino *et al*. (2010)	C3
FJ694895	*H. pannosa*	El Sargento, Mexico	Geraldino *et al*. (2010)	C3

FJ694894	*H. pannosa*	El Sargento, Mexico	Geraldino *et al*. (2010)	C3
FJ694893	*H. pannosa*	Oaxaca, Mexico	Geraldino *et al*. (2010)	C3
FJ694892	*H. pannosa*	Oaxaca, Mexico	Geraldino *et al*. (2010)	C3
FJ694912	*H. pannosa*	Bohol, Philippines	Geraldino *et al*. (2010)	C3
FJ694910	*H. pannosa*	Bohol, Philippines	Geraldino *et al*. (2010)	C3
FJ694909	*H. pannosa*	Bohol, Philippines	Geraldino *et al*. (2010)	C3
FJ694908	*H. viridis**	Durban, South Africa	Geraldino *et al*. (2010)	C3
EU345988	*H. japonica*	Keelung, Taiwan	Geraldino *et al*. (2009)	C6
EU345987	*H. japonica*	Gyeongju, Korea	Geraldino *et al*. (2009)	C6
EU345986	*H. japonica*	Gyeongju, Korea	Geraldino *et al*. (2009)	C6
EU345989	*H. japonica*	Keelung, Taiwan	Geraldino *et al*. (2009)	C6
FJ694913	*H. nidulans*	Bohol, Philippines	Geraldino *et al*. (2010)	C5
FJ694907	*H. nidulans*	Ilocos Norte, Philippines	Geraldino *et al*. (2010)	C5
FJ694900	*H. nidulans*	Surigao del Norte, Philippines	Geraldino *et al*. (2010)	C5
GQ141883	*H. rosea**	Durban, South Africa	Geraldino *et al*. (unpub.)	D1
HQ422683	*H. musciformis**	Hawaii	Sherwood *et al*. (2010)	D1
HQ422680	*H. valentiae**	Hawaii	Sherwood *et al*. (2010)	D1
HQ422681	*H. spinella*	Hawaii	Sherwood *et al*. (2010)	D5
EU240818	*H. spinella*	Panang Bay, Vietnam	Geraldino *et al*. (2009)	D2
EU240817	*H. spinella*	Panang Bay, Vietnam	Geraldino *et al*. (2009)	D2
HM915818	*H. charoides*	Pinnaroo Point, Australia	Barbara *et al*. (2013)	D7
EU240823	*H. charoides*	Perth, Australia	Geraldino *et al*. (2009)	D7
EU240822	*H. charoides*	Perth, Australia	Geraldino *et al*. (2009)	D7
EU240821	*H. charoides*	Perth, Australia	Geraldino *et al*. (2009)	D7
EU240820	*H. charoides*	Perth, Australia	Geraldino *et al*. (2009)	D7
EU240819	*H. charoides*	Perth, Australia	Geraldino *et al*. (2009)	D7
GQ141878	*H. cornuta*	Unknown	Geraldino *et al*. (unpub.)	D7
GQ141882	*H. valentiae*	Unknown	Geraldino *et al*. (unpub.)	D7
AF385656	*Craspedocarpus venosus*	Port Macdonnell, Australia	Hommersand & Fredericq (2002)	O
AF386528	*Rhodophyllis volans*	Port Macdonnell, Australia	Hommersand & Fredericq (2002)	O
AF385653	*Calliblepharis ciliata*	Brittany, France	Hommersand & Fredericq (2002)	O
EF434939	*Gracilaria vermiculophylla*	Oki Island, Japan	Yang *et al.* (2008)	O
EF434940	*Gracilariopsis longissima*	Roscoff, France	Yang *et al.* (2008)	O

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