

Systematics of the *Laurencia* complex (Rhodomelaceae, Rhodophyta) in southern Africa

By

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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 and Dr Lydiane Mattio of the Department of Biological Sciences, University of Cape Town and Associate Professor RJ Anderson of Department of Agriculture, Forestry and Fisheries and the Department of Biological Sciences, University of Cape Town.

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ABSTRACT

The diversity, systematics and distribution of the red algal *Laurencia* complex (Rhodomelaceae, Rhodophyta) of South Africa were investigated, being generally poorly understood and taxonomically understudied. Prior to this study, ten currently recognised species in the *Laurencia* complex were recorded from South Africa: all were ascribed to the genus *Laurencia* J.V. Lamouroux (*Laurencia sensu stricto*). However, the diversity and distribution of the complex in South Africa, and the larger South Western Indian Ocean (SWIO), have not yet been reassessed following the numerous taxonomic changes in this group published over the last two decades.

The taxonomy, phylogeny and biogeography of the *Laurencia* complex in South Africa and a part of the SWIO were reassessed by examining external morphology, vegetative anatomy (including *corps en cerise* counts) and analysing the plastid-encoded *rbcL* gene sequence data of recent collections. The collection encompassed more than 250 specimens, were primarily from the coastline of South Africa and to a lesser extent from Madagascar, Mozambique, Reunion, Mauritius and the Europa and Glorioso Islands in the SWIO. In addition, a few new collections were included from Western Australia and Japan. The genetic analyses were done under Bayesian inference using the GTR + I + G model, from which phylogenetic hypotheses were deduced and pairwise sequence divergences were calculated.

The phylogenetic analyses provided support for the monophyly of the currently recognised six genera of the *Laurencia* complex as well as providing early molecular evidence for two new genera, one of which would be restricted to the SWIO. Nine of the previously recorded ten South African species of *Laurencia sensu stricto* species were validated through molecular and morpho-anatomical evidence. One of the nine species, *Laurencia stegengae* nom. nov. was renamed after the Dutch phycologist Dr. Herre Stegenga who first described

the species, following invalidation of the original species *L. peninsularis* Stegenga, Bolton and Anderson which had been used previously for a Californian species, *L. peninsularis* Taylor. The tenth species, *Laurencia obtusa* (Hudson) Lamouroux, was poorly-defined as a species, globally, and molecular analyses supported the exclusion of *Laurencia obtusa* from the flora of South Africa. Five new species were described from South Africa (*Laurencia dehoopiensis* sp. nov., *L. dichotoma* sp. nov., *L. digitata* sp. nov., *L. multiclavata* sp. nov. and *L. sodwaniensis* sp. nov).

New records of three other *Laurencia* complex genera, *Chondrophyucus*, *Laurenciella* and *Palisada*, were reported from South Africa for the first time, and together with the aforementioned *Laurencia sensu stricto* species the *Laurencia* complex in South Africa now stands at 19 species. The diversity is likely greater, with six additional unidentified lineages found in this study and awaiting more study. Diversity within *Laurencia sensu stricto* in South Africa increases from west to east i.e. cool-to-warm-temperate, with higher endemism in the warm-temperate regions of the south coast (5 spp.). Species common on the east coast (KwaZulu-Natal), *Laurencia complanata*, *L. natalensis* and *L. multiclavata* sp. nov., were shared with Madagascar and Glorioso Island. *Laurencia natalensis* appears as one of the most widely distributed species in the SWIO, alongside *L. multiclavata* sp. nov. and the undescribed lineage, *L. sp.* ‘morphotype K’. Species of the genus *Palisada* appeared to have a narrower distribution range in the SWIO, restricted either to the Mozambique Channel or around the Mascarene Islands.

The presence of *Laurenciella marilzae* on the south coast of South Africa, which prior to this study was recorded from Brazil, the Mexican Caribbean and Canary Islands in the north and central Atlantic Ocean only, provided early evidence supporting the Hommersand (1986) hypothesis which suggested South Africa as a gateway for Indian Ocean taxa to the Atlantic Ocean. This study highlighted the importance of incorporating South African and SWIO

floras when assessing global diversity in the *Laurencia* complex, and the significance of the South African flora when discussing distribution patterns and biogeographic relationships within this diverse assemblage of red algae.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Seaweeds

The algae are an assemblage of oxygen-producing, photosynthetic organisms united superficially on the basis of their ecological similarity (Graham and Wilcox 2000) - this is as a result of convergent evolution; the independent development of congruent features within taxa sharing a similar function, habitat and/or environment (De Clerck *et al.* 2005). Algae are found in most aquatic environments i.e. marine, estuarine or freshwater, but they also inhabit sub-aerial environments such as tree bark as well as harsh environments such as desert soils and hot springs (e.g. van den Hoek *et al.* 1995; Graham and Wilcox 2000; Barsanti and Gaultieri 2006; and Lee 2008). In the marine environment, the microalgae – single-celled organisms which range from 0.2µm to a few hundred micrometres, and macroalgae (also called seaweeds) – multicellular organisms that can reach up to 45 metres such as the giant kelp, *Macrocystis pyrifera* (Linnaeus) C. Agardh (van den Hoek *et al.* 1995) can be separated into three major groups of algae are the Chlorophyta, the Phaeophyceae in the Heteronkontophyta and the Rhodophyta commonly referred to as the green, brown and red algae respectively (Lee 2008).

From the macroalgal perspective the Rhodophyta are a highly diverse group present in marine environments throughout the world from polar, through temperate to tropical waters (Maggs *et al.* 2007, Robba *et al.* 2006). With more than 6,000 currently recognised seaweed species in the division, the rhodophytan seaweeds are significantly higher in number than either the Chlorophyta or the Phaeophyceae (Butterfield 2000, Guiry and Guiry 2014).

Evolutionarily speaking they represent one of the major radiations of eukaryotes (Ragan *et al.*

1994, Robba *et al.* 2006) and the emergence of red algae is considered the most ancient event detected so far in the evolution of all eukaryotic organisms (Hori and Osawa 1987, Xiao *et al.* 1998, Yoon *et al.* 2004; Robba *et al.* 2006).

1.2 Economic and chemical importance of seaweeds:

In many coastal countries marine resources form a fundamental part of the economy (Kildow and McIlgrom 2009). Marine macroalgae have long formed a significant part of these resources and are in fact a worldwide multi-billion dollar industry (Smit 2004, Dhargalkar and Verlecar 2009, FAO 2012) with the vast majority of the seaweed industry found in Asia. Edible seaweeds such as kombu (*Saccharina japonica* (Areschoug) C.E.Lane, C.Mayes, Druehl & G.W.Saunders), wakame (*Undaria pinnatifida* (Harvey) Suringar) and nori (*Pyropia* spp.) account for roughly 98.9% of the seaweed industry, grossing US\$ 5.7 billion per annum (FAO 2012). Phycocolloids derived from red algae account for most of the remaining US\$ 1 billion with carrageenan grossing US\$ 240 million and agar around US\$ 132 million, while alginates from brown algae contribute around US\$ 213 million (FAO 2004).

In South Africa, seaweeds are utilized as feed in the abalone industry. On average 5000 tons of kelp is harvested (Bolton *et al.* 2013), mainly *Ecklonia maxima* (Osbeck) Papenfuss, with some *Laminaria pallida* Greville (Troell *et al.* 2006, Anderson *et al.* 2007) as well as 2000t of *Ulva* spp. (Bolton *et al.* 2013) grown in aquaculture systems (Robertson-Andersson *et al.* 2008, Bolton *et al.* 2009). There is no phycocolloid extraction in South Africa, but roughly 500 tons of beach-cast kelp (*Ecklonia maxima* and *Laminaria pallida*) is collected, dried and exported annually for alginate extraction (Anderson *et al.* 2007) and around 80-100t of red algal species of the genus *Gelidium* is harvested for agar extraction (Anderson *et al.* 2003, DAFF Annual Report 2013). Smit (2004) stated that only in the last thirty years has the

commercial exploration of seaweed secondary metabolites increased significantly. This is not surprising as seaweed utilization shifts from edible seaweed species to those which produce compounds useful in industry (Dhargalkar and Verlecar 2009). Such metabolites within the Ceramiales (the order that includes *Laurencia* Lamouroux) are defined as “structurally elaborate halogenated natural products” (Gil-Rodriguez *et al.* 2009) and several studies both large-scale (Fuller *et al.* 1992, de S.F.-Tischer *et al.* 2006, Grünewald *et al.* 2009, Wang *et al.* 2009) and small-scale (Knott *et al.* 2005, Mann *et al.* 2007, Saravanakumar *et al.* 2008) have isolated a number of biochemical compounds primarily produced by red algal species.

A recent phytochemical study by Knott *et al.* (2005) into *Plocamium corallorhiza* (Turner) J. Hooker & Harvey, a red alga common on South African shores, revealed four compounds with cytotoxic effects on oesophageal cancer cells. Mann *et al.* (2007) screened *P. corallorhiza* from Kenton-on-Sea on the south coast of South Africa and discovered that it produced a number of unstable halogenated monoterpene aldehydes not found in west coast collections of the same species. In a recent review of the chemistry of the Rhodomelaceae by Wang *et al.* (2013) the chemistry of *Laurencia sensu stricto* species formed the basis on which almost all of the classes of organic molecules were reviewed. The genus contains over 700 halogenated organic molecules also called secondary metabolites (see Erickson 1983, Gil-Rodriguez *et al.* 2009) ranging from diterpenes to sesquiterpenes, non-terpenoid C₁₅ acetogenins, indoles as well as other organic molecules and a number of these molecules have been tested for their bioactivity as anti-bacterial, anti-fungal and anti-viral agents (Erickson 1983, Wang *et al.* 2013). The occurrence of these secondary metabolites in members of the genus *Laurencia sensu stricto*, their chemistry and the application thereof has been the focus of several publications over the last decade or more (e.g. Takahashi *et al.* 1998, 2002; Suzuki *et al.* 2005, Jung *et al.* 2008, Chatter *et al.* 2009, 2011; Stein *et al.* 2011,

Alarif *et al.* 2012, Campos *et al.* 2012, Wang *et al.* 2013). For example metabolites isolated from *Laurencia undulata* Yamada (= *Chondrophycus undulatus* (Yamada) Garbary & Harper) proved, in laboratory tests, to have anti-asthmatic properties (Jung *et al.* 2008), while *Laurencia glandulifera* (Kützing) Kützing (= *Chondrophycus glandulifer* (Kützing) Lipkin & P.C.Silva) produces a brominated diterpene with analgesic properties (Chatter *et al.* 2009). Potential for similar chemical studies exist in South Africa; for example *Laurencia brongniartii* J. Agardh, a species recorded from the east coast of South Africa, was reported to have anti-bacterial bioactivity by Horikawa *et al.* (1999). A firm taxonomic grounding is necessary to provide reliable identifications of species which might have interesting chemical properties.

1.3 General biology and taxonomy of Rhodophyta

Red algae are distinguished from the other lineages by the presence of several biochemical and ultrastructural features: they lack flagella, store food reserves as floridean starch, possess a combination of unique photosynthetic pigments as well as chloroplasts with non-aggregated thylakoids and lack an external endoplasmic reticulum (Woelkerling 1990, Graham and Wilcox 2000, Harper and Saunders 2001, Maggs *et al.* 2007).

The absence of flagella, and therefore motility, was instrumental in the development of a unique complement of reproductive structures in aid of sexual reproduction and spore dispersal by red algae (Saunders and Hommersand 2004, Maggs *et al.* 2007). Male gametes have extracellular mucilaginous appendages which alter their hydrodynamic properties i.e. directly affect sperm transportation, and they contain species-specific cell recognition proteins. One of these proteins is rhodobindin, which attaches to the sessile female gametes, the carpogonia (Broadwater *et al.* 1991, Kim *et al.* 1996, Delivopoulos 2000, Kim *et al.* 2005). Similarly female reproductive morphology (i.e. structures of the carpogonium and the

carpogonial branch) and post-fertilization development (i.e. presence and fate of the cells, orientation of the auxiliary cell(s) and pattern of zygote amplification) as described by Schmitz (1892) and refined by Kylin (1956) were crucial in red algal classification before DNA data became available and formed the basis for the taxonomic placement at ordinal rank in the most diverse rhodophyta class – the Florideophyceae (Maggs *et al.* 2007).

Life History

Florideophyte life-history phases can either be heteromorphic (different from one another) or isomorphic (similar to one another) (Hawkes 1990), but ultimately most of the red algae in this taxonomic group follow the same tri-phasic pattern. The Florideophyte life-history phases are depicted in Figure 1.1 using the genus *Laurencia sensu stricto* as an example. While all the genera in the *Laurencia* complex (of which *Laurencia sensu stricto* is a member) have isomorphic life-histories, each genus has a unique combination of reproductive structures, type of reproductive structure development and production of spores, be they carpospores or tetraspores (Martin-Lescanne *et al.* 2010). The life history of *Laurencia sensu stricto* is depicted in Figure 1.1. The features of the other genera are discussed in detail in section 1.4 of this chapter. *Laurencia* and the other genera of the *Laurencia* complex reproduce sexually and have typical tri-phasic isomorphic life histories i.e. their gametophyte, carposporophyte and tetrasporophyte life phases are similar in form (Cassano *et al.* 2009). While it has not been noted in literature published on the genus, collections of *Laurencia sensu stricto* on the coast of South Africa suggest that male and female plants are generally much rarer than sporophyte plants.

Laurencia s.s. Life Cycle

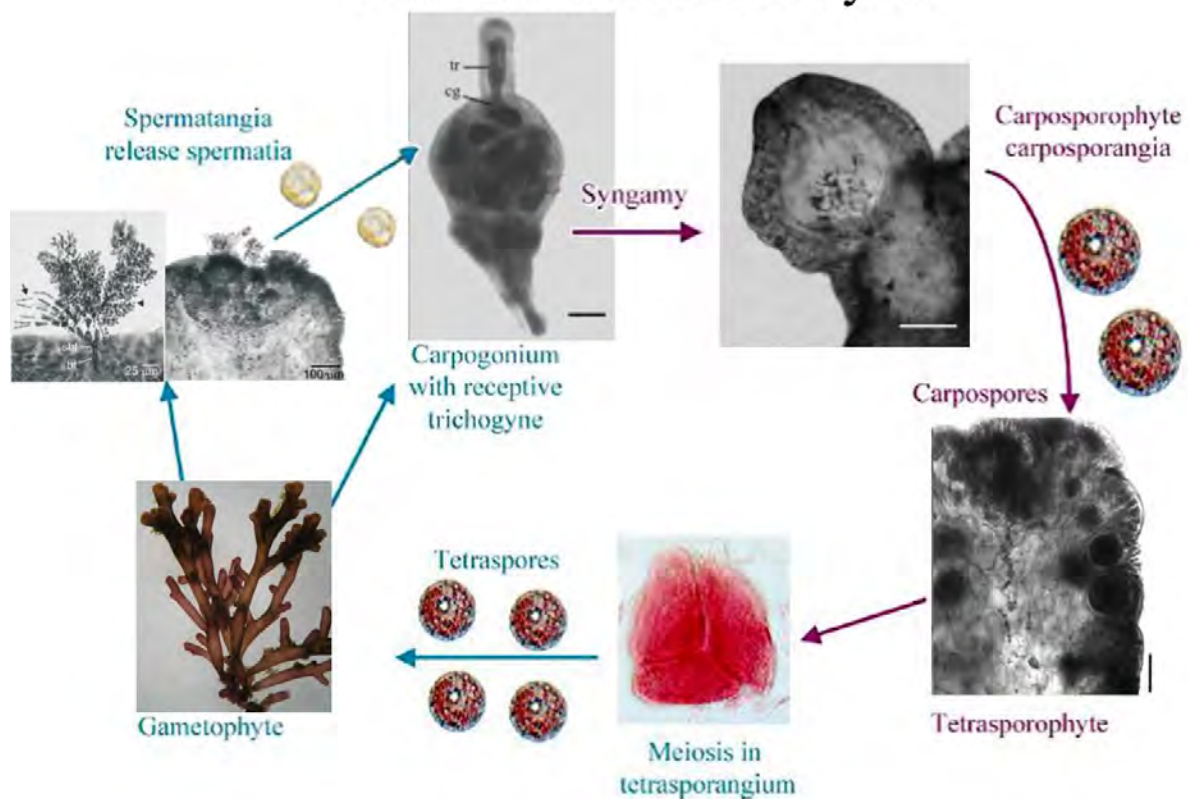


Figure 1.1: An illustration of the *Laurencia sensu stricto* life history. Purple and Blue text and arrows represents the diploid and haploid life stages, respectively. In the carpogonium with trichogyne image tr= trichogyne and cg = carpogonium. (Figure modified from Saunders and Harper 2004, using images from Gil-Rodriguez *et al.* (2009), Fujii *et al.* (2011) and RJ Anderson)

Rhodophyte systematics: continuous improvement with technological advancement

Perhaps the most significant anatomical feature in the red algae is the pre- and post-fertilization characteristics of female reproductive structures, particularly that of the carpogonial branch in the Florideophyceae, reported by Kylin (1956), which form the foundation of ordinal taxonomic placement in this highly diverse class (Maggs *et al.* 2007).

Systematics is defined by Simpson (2010) as “a science that includes and encompasses traditional taxonomy, description, identification, nomenclature and classification of organisms and that has as its primary goal the reconstruction of a phylogeny, or evolutionary history, of life.” The author goes on to describe systematics as an evolving science which improves as our knowledge; methods (for example extensive sampling campaigns, SCUBA, deep-sea exploration) and tools for investigating biological diversity (for example microscopy, biochemistry, and molecular techniques) develop as well. This development is apparent in higher level (i.e. ordinal rank and above) red algal systematics which has undergone and will likely continue to undergo major changes as the relationships between the classes and orders are better understood (Garbary and Gabrielson 1990). Classification, which ideally is a reflection of the evolutionary relationships between groups of organisms (Yoon *et al.* 2006, 2010), will therefore undergo similar changes because taxonomic descriptions are dynamic in nature and based on the data available at the time (Garbary and Gabrielson 1990, Barsanti and Gualtieri 2006), the number of taxa sampled (Kuhner and Felsenstein 1994, Nylander 2001) and sometimes the methods of analyses applied to these data types e.g. model- versus non-model-based approaches (Pickett and Randle 2005, Wortley and Scotland 2006, Rindal and Brower 2011).

Since the early 1990s molecular studies have been widely used to re-assess the traditional systematic system. Using either nuclear or chloroplastic markers a number of studies have suggested a somewhat different phylogenetic placement of classes within the Rhodophyta than was traditionally recognised (Maggs *et al.* 2007). Saunders and Hommersand (2004) proposed three subphyla, namely *Eurhodophytina*, *Rhodellophytina* and *Metarhodophytina*, and a separate division including Cyanidiophyceae. But Yoon *et al.* (2006) proposed only two subphyla: *Rhodophytina* and *Cyanidiophytina*, while Le Gall and Saunders (2007) stated

that the Rhodophyta should be divided into six classes: Stylonematophyceae, Porphyridiophyceae, Rhodellophyceae, Compsopogonophyceae, Bangiophyceae and Florideophyceae. Class-level phylogenetic relationships in the Rhodophyta have been examined from both the morphological and molecular perspective. Of the six classes mentioned above, the two most extensively studied are the simple-structured Bangiophyceae and the morphologically complex Florideophyceae (Graham and Wilcox 2000, Harper and Saunders 2001). Ragan *et al.* (1994) stated that the key ‘traditional’, i.e. morphological characters used to distinguish these two classes (for example plastid number, pattern of cell division or thallus complexity) are not taxonomically stable or absolute. Indeed, using molecular markers, Yoon *et al.* (2010) identified seven lineages (classes in this instance), including the Bangiophyceae and the monophyletic Florideophyceae previously identified by Yoon *et al.* (2006). The smaller of the two classes, Bangiophyceae, contains four orders which are often described as structurally and reproductively simple seaweeds (for example the sheet-like genus *Porphyra*) (Maggs *et al.* 2007). Much of their description was determined by the absence of features associated with the Florideophytes (e.g. secondary pit connections between cells) or the presence of characters only found in some of the classes (e.g. single star-shaped plastids), i.e. there is a lack of positive synapomorphic (shared derived) traits amongst these orders (Freshwater *et al.* 1994, Ragan *et al.* 1994, Maggs *et al.* 2007). Interestingly, Dixon (1963) discarded the absence of the secondary pit connections as a valid taxonomic distinction between Bangiophycean and Florideophycean algae as three genera (*Rhodochaete*, *Compsopogon* and *Bangia*) within the Bangiophyceae displayed pit connections, though of a less complex nature. It is generally accepted that poor taxonomic understanding of the Bangiophycean algae coupled with low taxon sampling has been reflected in the phylogenetically distant relationships between orders of this class as well as

their apparent polyphyly (Freshwater *et al.* 1994, Ragan *et al.* 1994, Graham and Wilcox 2000, Saunders and Hommersand 2004 and Maggs *et al.* 2007).

Unlike the Bangiophyceae, the orders within the Florideophycean algae have a strong monophyletic origin and it is postulated that they evolved much later than the Bangiophyceae (Freshwater *et al.* 1994, Ragan *et al.* 1994, Graham and Wilcox 2000, Harper and Saunders 2001 and Maggs *et al.* 2007). The Florideophyceae encompass the vast majority of the species diversity within red seaweeds and are often morphologically complex and diverse. Nearly all of the 24 orders (according to the system of Harper and Saunders 2001) within this class are multicellular and have developed unique reproductive structures; for example tetrasporangia and gonimoblasts. Female reproductive anatomy before and after fertilization became the foundation of alpha taxonomic descriptions for the orders within the Florideophyta (see Kylin 1956).

Rhodophyte taxonomy: from ultrastructure to DNA-based investigations

The next major step in elucidating red algal taxonomy was the study of pit-plug ultrastructure within the Florideophyta (Pueschel and Cole (1982). Two features of these structures (presence and absence of the inner and outer cap layers and morphology of the outer cap) in combination proved taxonomically significant, changing the face of high level systematics within the florideophytes from the early 1980's to the year 2000 (Maggs *et al.* 2007). From the mid 1990's until the present the use of molecular DNA in algal taxonomy and phylogenetics in general has gained popularity (Maggs *et al.* 2007) and for the red algae the works of Freshwater *et al.* (1994), Ragan *et al.* (1994), Saunders and Hommersand (2004) and Yoon *et al.* (2006) are important assessments of the taxonomic relationships of the red algae.

Rhodomelaceae: highly diverse and morphologically advanced red algae

According to Saunders & Hommersand (2004) and Maggs *et al.* (2007) the Ceramiales is the most advanced order in the subclass Rhodymeniophycidae by virtue of its complex female reproductive structures and is the most diverse order in the Rhodophyta (Stegenga *et al.* 1997). Nine families make up the Ceramiales, including the species-rich Rhodomelaceae with over 960 species and around 150 genera (Guiry and Guiry 2014).

Studies on the systematics of the Florideophyceae and the other families in the Ceramiales have shown the Rhodomelaceae to be a well-supported, monophyletic clade (Philips *et al.* 2000, Choi *et al.* 2002, Zuccarello *et al.* 2002 and Abbott *et al.* 2010). The Rhodomelaceae have varied morphological and anatomical features some of which include the thallus form - branched filamentous types, those with high levels of cortication and foliose types; the number of pericentral cells – four to twenty-four; the presence of trichoblasts (hair-like branches) in most species; spermatangia associated with the trichoblasts; cystocarps with a distinct pericarp and tetrasporangia generally borne on the pericentral cells (Stegenga *et al.* 1997). A notable exception to the last rhodomelacean characteristic is the genus *Laurencia* Lamouroux in which tetrasporophytes are held in the thallus and according to Stegenga *et al.* (1997) “seemingly inserted on the cortical cells...” The second-largest genus in the Rhodomelaceae, *Laurencia* Lamouroux, has 130 species occurring world-wide excluding the poles (Stegenga *et al.* 1997, Guiry and Guiry 2014).

1.4 Systematics of the genus *Laurencia* and the *Laurencia* complex

The name *Laurencia* was first used in 1813 by the French botanist J.V. Lamouroux to describe a diverse group of eight red seaweeds in the order he called *Floridées* on the basis of their coralloid organisation and purple to reddish colouration (Lamouroux 1813). Lamouroux (1813) failed to designate a type for the genus, but this was later rectified by Schmitz (1889)

who formed the tribe *Laurenciae* and formally assigned the type species *Laurencia obtusa* (Hudson) Lamouroux to the genus. It must be noted that around the same time as Lamouroux, the English botanist John Stackhouse proposed the genera *Osmundea* (1809) and *Pinnatifida* (1816) both of which shared some morphological similarities with *Laurencia*. *Osmundea* was based on the type species *O. expansa* Stackhouse nom. illeg., which was later synonymised with *Laurencia osmunda* (S.G. Gmelin) Maggs & Hommersand (Silva 1952), while *Pinnatifida vulgaris* Stackhouse, the type species of *Pinnatifida*, is considered a synonym of *Laurencia pinnatifida* (Hudson) Lamouroux 1813. Both Stackhouse's genera were rejected by Papenfuss (1947) - *Osmundea* as an earlier heterotypic synonym of *Laurencia*, and *Pinnatifida* as a later synonym of *Osmundea* - and Lamouroux's '*Laurencia*' was proposed for conservation by Papenfuss (1947) (Nam *et al.* 1994).

Between the work of Lamouroux (1813) and the designation of the type by Schmitz (1889) several species were added to the genus by various authors including Gaillon (1828), Greville (1830), J. Agardh (1841), Sonder (1845), J.D. Hooker & Harvey (1847), Kützing (1849, 1865), Harvey (1855), Zanardini ex Fraudenfeld (1855), P.L.Crouan & H.M.Crouan in Schramm & Mazé (1865) and Martens (1871). Following this, authors such as Kylin (1923, 1928), Yamada (1931), Saito (1967), Saito and Womersley (1974) and Garbary and Harper (1998) were instrumental in the development of key morphological (and later anatomical) features for the *Laurencia* classification. For example Saito (1967) and Saito and Womersley (1974) first noted the significance of the presence or absence of secondary pit connections between epidermal cells and described the subgenera *Laurencia* and *Chondrophyucus* (J.Tokida & Y.Saito) Garbary & Harper to accommodate these features by moving all *Laurencia* species without secondary pit-connections into subgenus *Chondrophyucus* while those with secondary pit-connections would be placed in subgenus *Laurencia*. These works

formed the foundations upon which later studies expanded the understanding of *Laurencia* as a genus, and then more recently as a complex of six genera (*Laurencia* complex).

Laurencia is part of the tribe Laurenciae within the family Rhodomelaceae. The Laurenciae includes the *Laurencia* complex and the parasitic genus, *Janczewskia* Solms Laubach. The Rhodomelaceae includes ten other tribes including the tribe Chondriaceae based on the genus *Chondria*. The genus *Chondria* C. Agardh is phylogenetically closely related to the *Laurencia* complex (Nam and Choi 2001, Diaz-Larrea *et al.* 2007), so much so that some *Laurencia* species were previously ascribed to *Chondria*. For example, the South African east coast species *Laurencia complanata* (Suhr) Kützing was known as *Chondria complanata* Suhr prior to its transfer to the genus *Laurencia* (Silva *et al.* 1996).

The advent of molecular methods in species delineation and their application to *Laurencia* taxonomy has lent strong support to the anatomical distinctions highlighted by the above authors and contributed significantly to the further characterisation and taxonomic understanding of the *Laurencia* complex. This is seen most clearly in the transition of the complex from three genera (*Laurencia sensu stricto*, *Chondrophyucus* and *Osmundea*) proposed by Garbary and Harper (1998) and supported by the plastid molecular phylogeny of Abe *et al.* (2006), to four genera (*Laurencia sensu stricto*, *Chondrophyucus*, *Osmundea* and *Palisada* Nam) supported by the chloroplastidic large subunit of the ribulose-bisphosphate carboxylase gene (*rbcL*) phylogenetic analyses of Nam (2006) and Diaz-Larrea *et al.* (2007). The complex was then increased to five genera with the addition of *Yuzurua* (Nam) Martin-Lescanne as recognised by Martin-Lescanne *et al.* (2010) on the basis of the chloroplast *rbcL* gene and a combination of shared vegetative features such as non-palisade epidermal cells shared within the overall *Laurencia* complex (except *Palisada*) and the presence of secondary pit connections as in *Laurencia sensu stricto* and *Osmundea*. Most recently the complex was

increased to six genera with the addition of *Laurenciella* Cassano, Gil-Rodríguez, Senties, Díaz-Larrea, Oliveira & Fujii, supported by the plastid-based phylogenetic analyses of Cassano *et al.* (2012). Following these revisions, each genus in the *Laurencia* complex is now well circumscribed morphologically and phylogenetically (Figure 1.2). With the exception of Lewis *et al.* (2008) and Sherwood *et al.* (2010), literature on the molecular systematics of the *Laurencia* complex is based on the plastid marker, *rbcL*. *RbcL* has been widely used in the Rhodophyta to answer phylogenetic questions and has been shown in several studies (Freshwater and Rueness 1994, Hommersand *et al.* 1994, Fredericq and Ramirez 1996, Gurgel and Fredericq 2004, Abe *et al.* 2006, Martin-Lescanne *et al.* 2010, Cassano *et al.* 2012) to provide a large proportion of sequence data with a high number of phylogenetically-informative sites owing to its relatively higher rate of mutation in comparison to the nuclear small subunit ribosomal marker (SSU) (Bailey and Freshwater 1998). The usefulness of this marker has been exemplified in the *Laurencia* complex where it has been shown to provide good resolution at the genus and species level (Nam *et al.* 2000, Abe *et al.* 2006, Gil-Rodríguez *et al.* 2009, Martin-Lescanne *et al.* 2010, Cassano *et al.* 2012).

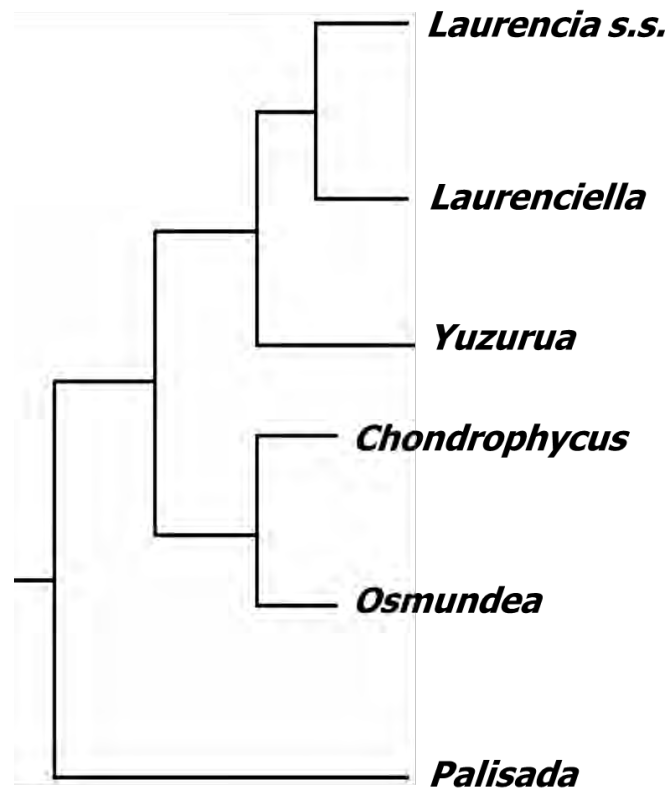


Figure 1.2 Simplified phylogeny of the *Laurencia* complex, based on the *rbcL* gene region
 (Adapted from Cassano *et al.* 2012).

General Classification (Guiry and Guiry 2014)

Division: Rhodophyta

Subdivision: Eurhodophytina

Class: Florideophyceae

Subclass: Rhodymeniophycidae G.W. Saunders & Hommersand 2004

Order: Ceramiales Oltmanns, 1904: 683

Family: Rhodomelaceae Areschoug (1847: 260)

Tribe: Laurencieae Schmitz (1889: 447)

Genus complex: *Laurencia* Lamouroux 1813: 130 (= *Laurencia sensu stricto*)

Chondrophyucus (Tokida & Saito) Garbary & Harper, 1998: 194

Osmundea Stackhouse 1809: 56, 79, 80

Palisada Nam 2007: 53

Yuzurua (Nam) Martin-Lescanne 2010: 59

Laurenciella Cassano, Gil-Rodríguez, Senties, Díaz-Larrea, Oliveira & Fujii, 2012: 354

Unifying features of the genera in the Laurencia complex

Nam *et al.* (1994) and later Garbary and Harper (1998) defined key vegetative and reproductive features representative of the genus *Laurencia* and two additional genera namely *Osmundea* Stackhouse (resurrected by Nam *et al.* 1994) and *Chondrophyucus* (raised to genus level by Garbary and Harper 1998). The genus *Osmundea* is closely related to *Chondrophyucus*, sharing some anatomical similarity outlined in further detail below (Garbary and Harper 1998). Several studies have focused on vegetative and to some extent reproductive anatomical reviews of the genera within the *Laurencia* complex (sometimes as subgenera within the genus *Laurencia*) and have delivered suitable additional generic morphological delimitations (Saito 1967, Nam *et al.* 1994, Garbary and Harper 1998, Nam *et al.* 1998, Masuda and Kogame 1998, Nam 2006, Martin-Lescanne *et al.* 2010, Cassano *et al.* 2012). The six genera of the *Laurencia* complex share a typical Rhodomelacean morphology i.e. they have apical cells sunk in apical pits at the apices of branchlets, a central cell row that is recognisable only near the apical cell and an extensive cortex (Nam and Choi 2001). In

addition to these vegetative features, the procarp-bearing segments in the female reproductive structures of each genus generally have five pericentral cells, and the spermatangial branch pit is cup-shaped, though it is noteworthy that there are instances of pocket-shaped pits in some species of *Osmundea* (Martin-Lescanne *et al.* 2010). The individual features of the genera as described by the authors listed above are outlined in Table 1.1.

1.5 Geographical distribution of the *Laurencia* complex

Relatively little is known about the distribution of the *Laurencia* complex. *Laurencia sensu stricto* is a genus with a cosmopolitan distribution in temperate and tropical regions, with the bulk of the species occurring in the Southern Hemisphere (McDermid 1988). The other genera have distinct biogeographical distributions (Nam 2006). *Osmundea* has so far been demonstrated to have a disjunct distribution, with 17 species (Guiry and Guiry 2014) present in Pacific North America, Brazil, Atlantic Europe, the Mediterranean Sea and India (Nam *et al.* 2000, Furnari *et al.* 2004) and quite likely in Australia and Northern Africa as well (Nam 2006). *Chondrophyucus* has 17 currently accepted species distributed in the Indo-West Pacific marine province (Nam 1999, Guiry and Guiry 2014). The genus *Palisada* has 22 species (Guiry and Guiry 2014), the vast majority of which occur within the Pacific (Nam 2007) with three of these species also present on islands in the Indian Ocean (Guiry and Guiry 2014).

The distribution of the single species in the genus *Yuzurua*, *Y. poiteaui* (J.V. Lamouroux) K.W. Nam, and the variety *Y. poiteaui* var. *gemmaifera* (Harvey) M.J. Wynne, is similar to that of *Palisada* i.e. both *Y. poiteaui* and the aforementioned variety have been recorded in the Mexican Caribbean Sea, Western Atlantic, the Atlantic Islands, and the Indo-West Pacific (Guiry and Guiry 2014).

Table 1.1: Distinguishing characters used to identify the different genera in the *Laurencia* complex following Martin-Lescanne *et al.* (2010)

Abbreviations: EC: epidermal cells; NA: not applicable; NPa: non-palisadic; Pa: palisadic; PC: pericentral cells; SPC: sterile pericentral cells;

STL: development of spermatangial branches from two laterals on suprabasal cell of trichoblast; SOL: development of spermatangial branches

from one of the two laterals on suprabasal cell of trichoblast; ?: Unknown.

Genus	<u>Vegetative structure</u>				<u>Male reproductive structure: spermatangial branch</u>			<u>Female reproductive structure</u>		
	PC	Secondary pit connections	<i>Corps en cerise</i>	Position of the 1 st PC relative to the TB.	EC arrangement	Development	Production	Pit-shape	Auxiliary cell-timing	Pro-carp bearing segment
<i>Laurencia sensu stricto</i>	Four ^{c,e}	Present ^a	Present ^{b,e}	Underneath ^g	NPa ^f	Trichoblast type ^d	SOL ^g	Cup ^d	Normal ^{e,g}	5 th PC ^d
<i>Chondrophycus</i>	Two ^{c,e}	Absent ^a	Absent ^e	Side ^g	NPa ^f	Trichoblast type ^d	STL ^g	Cup ^d	Delayed ^{e,g}	5 th PC ^d
<i>Osmundea</i>	Two ^{c,e}	Pres./Abs. ^a	Absent ^{b,d}	Side ^g	NPa ^f	Filament type ^d	Absente ^g	Pocket/Cup ^d	Normal ^{e,g}	5 th /6 th PC ^d
<i>Palisada</i>	Two ^f	Absent ^f	Absent ^f	Underneath ^h	P ^{a,h}	Trichoblast type ^d	SOL ^g	Cup ^g	Normal ^{e,g}	4 th /5 th PC ^g
<i>Yuzurua</i>	Two ^f	Absent ^f	Absent ^f	?	NPa ^f	Trichoblast type ^f	?	Cup ^g	?	5 th PC ^g
<i>Laurenciella</i>	Four ⁱ	Present ⁱ	Present ⁱ	Underneath ⁱ	NPa ⁱ	Trichoblast type ⁱ	SOL ⁱ	Cup ⁱ	?	5 th PC ⁱ

Table 1.1(cont.): Distinguishing characters used to identify the different genera in the *Laurencia* complex following Martin-Lescanne *et al.* (2010)

Genus	<u>Tetrasporangia</u>							
	Origin	PC position	Orientation	Arrangement of tetrasporangia	Presporangial cover cell arrangement	Tetrasporangia axis	Fertility on the 2 nd PC	Additional tetrasporangial PC
<i>Laurencia sensu stricto</i>	Particular PC ^d	3 rd , 4 th ^c	Abaxial ^d	Parallel ^a	Transverse ^d	2/3 SPC ^h	No ^g	No ^g
<i>Chondrophycus</i>	PC ^d	Additional PC ^d	Abaxial ^d	Right-angle ^a	Transverse ^d	2 SPC ^g	No ^g	Yes ^d
<i>Osmundea</i>	Epidermal ^d	Random Epidermal ^d	Lateral ^d	Parallel ^a	Parallel ^d	NA ^g	NA ^g	NA ^g
<i>Palisada</i>	PC ^g	Additional PC ^f	Abaxial ^f	Right-angle ^f	Transverse ^f	1 SPC ^g	Yes ^g	Yes ^d
<i>Yuzurua</i>	PC ^g	Additional PC ^f	Abaxial ^f	Right-angle ^f	Transverse ^f	1 SPC ^g	Yes ^g	Yes ^g
<i>Laurenciella</i>	Particular PC ⁱ	3 rd , 4 th ⁱ	Abaxial ⁱ	Right-angle ⁱ	Transverse ⁱ	2 SPC ⁱ	No ⁱ	No ⁱ

^a Saito (1967); ^b McDermid (1988); ^c Nam & Saito (1991); ^d Nam *et al.* (1994); ^e Garbary & Harper (1998); ^f Nam (1999); ^g Nam (2006), ^h

Martin-Lescanne *et al.* (2010); ⁱ Cassano *et al.* (2012)

Laurenciella, first described from the Canary Islands in the northeast Atlantic, has now been recorded from the western Atlantic as well and appears so far to be restricted to the Atlantic Ocean.

Since the splitting of the *Laurencia* complex into a number of distinct genera within a complex, a fairly substantial body of work has been done in various marine regions to revise several species in accordance with the new generic circumscriptions (See Nam and Choi 2001, Abe *et al.* 2006, Nam 1999, Nam 2006, Diaz-Larrea *et al.* 2007, Martine-Lescanne *et al.* 2010) but very few studies, if any, seem to have focussed on revising regional diversities. Furnari *et al.* (2001) is the closest to this scale of study in their assessment of the *Laurencia* complex in the Mediterranean Sea. Beyond this, most accounts of the *Laurencia* complex in regional studies are as part of marine benthic checklists, for e.g. Haroun *et al.* (2002), Wynne (2011). Silva *et al.* (1996) produced a catalogue of the marine benthic algae of the Indian Ocean including much of South Africa, but this catalogue was published before most of the major taxonomic changes in the *Laurencia* complex occurred. No work has been done since to determine which species are in which genera, limiting our understanding of distribution patterns of the complex in the Indian Ocean. To date there has been no regional study of the *Laurencia* complex that includes South Africa and/or the rest of the South-Western Indian Ocean and none that used molecular data either.

1.6 South Africa and *Laurencia*

South African marine biogeography

The coastline of South Africa has a wide range of temperature conditions, from cool temperate as a result of the Benguela upwelling system in the west to tropical in the extreme northeast (Bolton *et al.* 2004, Smit *et al.* 2013). Several studies using different taxa have

supported the existence of at least three distinguishable major biogeographic regions along the coastline of South Africa (see Stephenson & Stephenson 1972, Brown & Jarman 1978, Emanuel *et al.* 1992, Stegenga & Bolton 1992, Bustamante & Branch 1996, Bolton & Anderson 1997, Turpie *et al.* 2000, Bolton *et al.* 2004, Sink *et al.* 2005). The system described by Bolton & Anderson (1997) was used in this study to define the marine biogeographic regions of South Africa using seaweed distributions, and is illustrated in Figure 1.2.

South Africa has a highly diverse community of shallow-water species, on a global scale, and this diversity is reflected in the seaweed floras present along its coastline – three of the four major seaweed floras of sub-Saharan Africa are represented in South Africa (Bolton and Stegenga 2002, Bolton *et al.* 2004 and De Clerck *et al.* 2005). The west coast of South Africa has a cool-temperate flora which is shared with Namibia (Stegenga *et al.* 1997, Bolton and Stegenga 2002, De Clerck *et al.* 2005). The south coast has a unique warm-temperate seaweed flora with many endemic species (Stegenga & Bolton 1992, De Clerck *et al.* 2005, Anderson *et al.* 2009). The east coast is divided into the warm-temperate region of overlap on the southern and central east coast and the tropical Indo-West Pacific flora of the norther-east coast (Anderson & Bolton 1997). In terms of the distribution of diversity in the *Laurencia* complex, a general increase in species diversity from the west coast to the east coast is deduced from literature (Stegenga *et al.* 1997, De Clerck *et al.* 2005) which corresponds to the general Rhodophyta pattern outlined in Bolton and Stegenga (2002). *Laurencia glomerata* is the only species of the *Laurencia* complex present on the west coast proper while others namely *L. flexuosa*, *L. natalensis*, *L. obtusa* and *L. peninsularis* nom. illeg. occur in the western transition zone according to Stegenga *et al.* (1997). The east coast boasts five species (*Laurencia brongniartii*, *L. complanata*, *L. flexuosa*, *L. natalensis* and *L. pumila*), which with the exception of *L. brongniartii* also occur on the south coast of South Africa. At least three

of the South African *Laurencia* species (*L. complanata*, *L. natalensis* and *L. pumila*) are known to occur in other coastal regions of the South West Indian Ocean; all three species are reported to occur Mozambique (Silva *et al.* 1996), while *L. natalensis* is also recorded in Kenya (Silva *et al.* 1996, Bolton *et al.* 2007) and Mauritius (Silva *et al.* 1996). While we are limited in our knowledge of the *Laurencia* complex in the rest of the South West Indian Ocean, several taxonomic accounts of seaweeds for SWIO nations such as Mauritius (Børgesen 1945), Mozambique (Isaac 1958, Isaac and Chamberlain 1958) and Tanzania (Jaasund 1975, Oliveira *et al.* 2005) provide a platform from which we can begin to assess the distribution of species in the South West Indian Ocean. The catalogue of benthic algal diversity in the Indian Ocean by Silva *et al.* (1996) provides a general concept of *Laurencia* complex diversity in the SWIO, which is highest in the tropics with fourteen species recorded from Mauritius, Tanzania and the Seychelles, but decreases into the temperate regions with six species recorded in Mozambique and ten species in South Africa.

No studies using molecular markers have yet re-assessed species of *Laurencia* in the South West Indian Ocean or in South Africa. Currently available records are limited to *Laurencia sensu stricto* (= subgenus *Laurencia* in Stegenga *et al.* 1997). With the exception of the record of *Laurencia glomerata* from several locations along the west coast as far north as McDougall Bay, Port Nolloth (29°16'23"S, 16°52'59") as mentioned in Stegenga *et al.* (1997), the distribution of South African *Laurencia* spp. extends from the southern Cape Peninsula to the northernmost section of the East Coast in Kwa-Zulu Natal (Figure 1.3). The dominant species change as seawater temperatures become progressively warmer eastwards (De Clerck *et al.* 2005, Smit *et al.* 2013). It is noteworthy that Namibia, which forms part of the same marine system as the west coast of South Africa, has no records of *Laurencia* species (Engledow 1998, Lluich 2002).

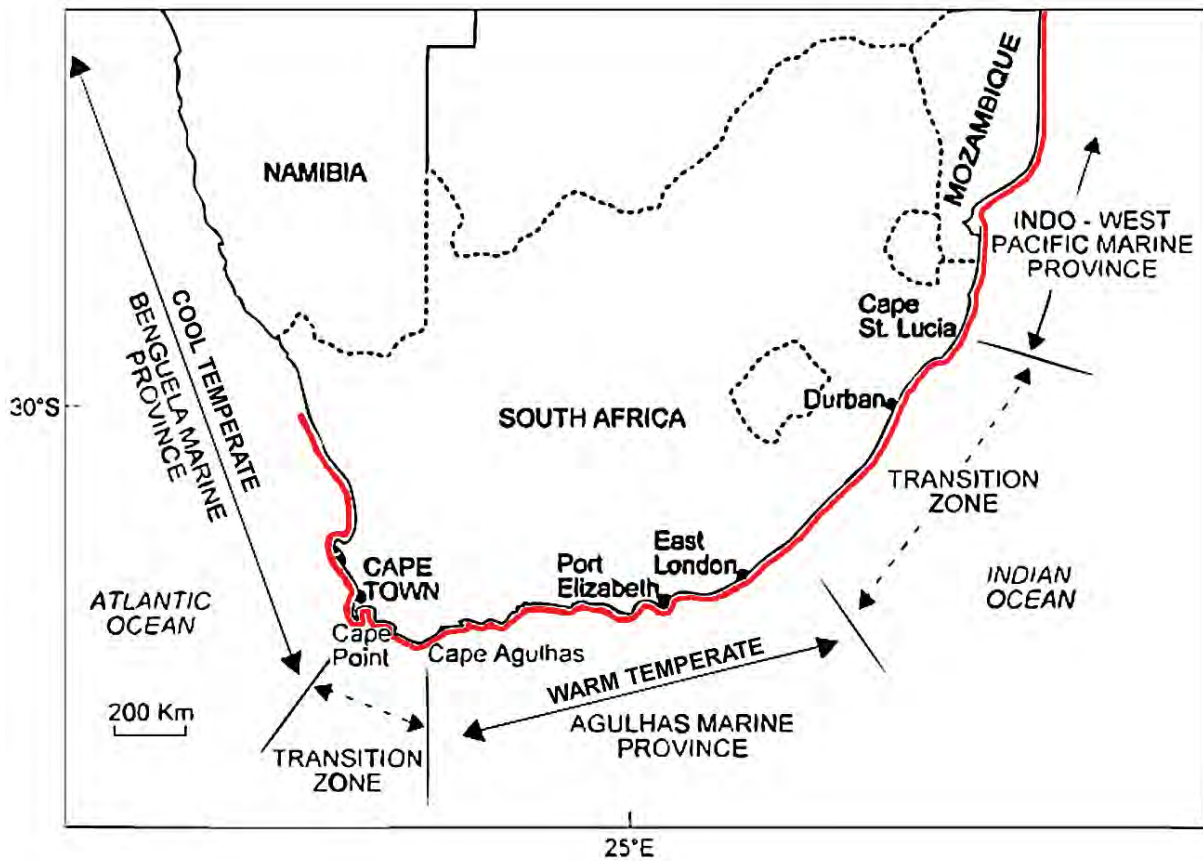


Figure 1.3: Known distribution of *Laurencia sensu stricto* (red outline) along the southern African coast. Cool-temperate, warm-temperate and tropical (Indo-West Pacific) seaweed floras and their overlapping regions in South Africa are shown respectively.

South African Laurencia in the literature

The first account of *Laurencia* in South Africa was by the German phycologist Friedrich T. Kützing (1849) who described *Laurencia flexuosa* Kützing and renamed two earlier species *Chondria glomerata* Kützing 1847 and *C. complanata* Suhr 1846 as *Laurencia glomerata* (Kützing) Kützing and *L. complanata* (Suhr) Kützing, respectively. In 1852, Kützing added another species, *Laurencia corymbosa* Kützing, to the list. This was followed 15 years later by the addition of *L. pumila* (Grunow) Papenfuss (as *L. flexuosa* var. *pumila*) by the German-Austrian phycologist Albert Grunow (1867).

Between 1867 and 2005 only another five species were added to the South African *Laurencia* species list, namely *Laurencia elata* (C. Agardh) Hooker and Harvey, *L. obtusa* (Hudson) Lamouroux (Barton 1893), *L. natalensis* Kylin (1938), *L. peninsularis* Stegenga, Bolton & Anderson nom. illeg. (Stegenga *et al.* 1997) and *L. brongniartii* J. Agardh by De Clerck *et al.* (2005). Together this makes up a total of 10 species of *Laurencia sensu stricto* currently known for South Africa among which three are considered endemic and seven have their type locality in South Africa (Table 1.2).

Members of *Laurencia sensu stricto* are a significant and often dominant component of the intertidal seaweed vegetation along the South African south and east coast shores (Bolton and Anderson 1997, De Clerck *et al.* 2005).

The taxonomy of *Laurencia* in South Africa has been little studied and many species are therefore difficult to identify. Stegenga *et al.* (1997) reviewed the anatomy of west coast *Laurencia* species and remarked that the five species occurring on the west coast and in the western transition zone belonged to *Laurencia* subgenus *Laurencia* (= *Laurencia sensu stricto*) as described by Saito (1967) & Saito and Womersley (1974). De Clerck *et al.* (2005) recorded five species of *Laurencia* for the KwaZulu-Natal coast (two of these in common with the west coast) making a total of eight species. However, these studies were non-comprehensive and are outdated in the context of the taxonomic changes which have occurred in the genus over the last two decades. Moreover, there is no descriptive work available for the rest of the coast of South Africa and there has never been a record of any of the other genera of the complex. Similarly, the most recent taxonomic accounts for the *Laurencia* complex in the South-Western Indian Ocean (SWIO) are Jaasund (1969-1979) and Oliviera *et al.* (2005) for Tanzania, Børgesen (1945) for Mauritius and De Clerck *et al.* (2004) for Rodrigues Island.

Table 1.2: *Laurencia* complex species recorded from South Africa prior to this study (from Seagrief 1984, Silva *et al.* 1996, Stegenga *et al.* 1997 and De Clerck *et al.* 2005) showing whether or not they have been sequenced with the plastid gene, *RbcL*.

Species Name	Type Locality	Sequence Reference	<i>rbcL</i> sequence & locality
<i>Laurencia</i> cf. <i>brongniartii</i> J. Agardh	Martinique, West Indies	Fujii <i>et al.</i> (2006)	Taiwan & Australia
<i>Laurencia complanata</i> (Suhr) Kützing	Natal Bay, South Africa	Fujii <i>et al.</i> (2006)	South Africa
<i>Laurencia corymbosa</i> J. Agardh	Cape of Good Hope, South Africa	-	No
<i>Laurencia elata</i> (C. Agardh) J. Hooker & Harvey	King Island, Bass Strait, Australia	-	No
<i>Laurencia flexuosa</i> Kützing	“Ad Caput Bonae Spei”, South Africa	Fujii <i>et al.</i> (2006)	South Africa
<i>Laurencia glomerata</i> (Kützing) Kützing	“Cap” [South Africa]		No
<i>Laurencia natalensis</i> Kylin	Isipingo Beach, near Durban, South Africa	Fujii <i>et al.</i> (2006)	South Africa
<i>Laurencia obtusa</i> (Hudson) Lamouroux	Lectotype: unspecified [presumably Devon or Sussex, England]	Fujii <i>et al.</i> (2006) Nam <i>et al.</i> (2000)	Venezuela & Guadeloupe Ireland
<i>Laurencia pumila</i> (Grunow) Papenfuss	Port Natal [Durban], South Africa	-	No
<i>Laurencia peninularis</i> Stegenga, Bolton & Anderson nom. illeg.	Clovelly, Cape Peninsula, South Africa	-	No

The remaining coasts and islands have only been partially surveyed and neither publications on taxonomy nor checklists of species are available. No phylogenetic information has ever been produced for the genus complex in southern Africa or in the South-Western Indian Ocean region.

Aims and hypotheses of the study

With the exception of three plastid *rbcL* sequences deposited in Genbank and brief descriptions of known species in two South African seaweed floras (Stegenga *et al.* 1997, De Clerck *et al.* 2005) very little has been done on the taxonomy and the phylogeny of *Laurencia* in South Africa, and its diversity is currently most likely underestimated. Considering the significant changes that have taken place in the broader classification of the genus (and sister genera) in the last decade and the importance of *Laurencia* both ecologically and chemically, the main goal of the PhD was to reassess the diversity, systematics and biogeography of the South African *Laurencia* complex.

To address this main goal, the project was focussed around three aims:

1. Revising the South African *Laurencia* diversity and systematics using a combined analysis of the chloroplastidic *rbcL* DNA marker and morpho-anatomical characters based on new extensive collections along the coasts of all South African marine regions [**Chapter 2**]
2. Finding accurate morpho-anatomical characters to delineate species, and providing precise descriptions, illustrations and a key to identify South African species of the *Laurencia* complex. [**Chapter 3**]

3. Assessing the biogeographical relationships of South African species of *Laurencia* in the South West Indian Ocean region using a rbcL-based phylogeny produced from new collections in the region. **[Chapter 4]**

Questions/hypotheses which will be assessed in the thesis include:

1. What is the diversity of the *Laurencia* complex in South Africa, and are all species referable to *Laurencia sensu stricto*, as reported in the literature?
2. Are the 10 species reported in the literature to occur in South Africa supportable taxonomic entities, and are they distinct from other described species?
3. Do the reported phylogenetic patterns and relationships between genera in the complex hold true following the addition of South African and South Western Indian Ocean specimens to the analyses.
4. Do South African species have wider occurrence in the South Western Indian Ocean, and what are the phylogenetic affinities and biogeographic relationships of South African taxa?

CHAPTER 2

MOLECULAR SYSTEMATICS OF THE LAURENCIA COMPLEX (RHODOMELACEAE, RHODOPHYTA) IN SOUTH AFRICA

2.1 Introduction

The taxonomic history of the *Laurencia* complex started with a genus of eight species, *Laurencia* J.V. Lamouroux, described just over 200 years ago (Lamouroux 1813, pg. 131-132). It was subsequently divided into four sections based on morphological and anatomical features (Saito 1967 citing Yamada 1931b), then into two subgenera distinguished by the presence (or absence) of secondary pit-connections between epidermal cells (Saito 1967, Saito and Womersley 1974), and later into a three-genus complex (*Laurencia*, *Chondrophyucus* and *Osmundea*) identified by vegetative and reproductive anatomy (Garbary and Harper 1998, Nam 1999). Based on molecular data, Nam *et al.* (2000), Martin-Lescanne *et al.* (2010), Fujii *et al.* (2011) and Cassano *et al.* (2012) proposed further taxonomic revisions supporting and expanding upon the previous taxonomic changes. The *Laurencia* complex is currently accepted as comprising six genera: *Laurencia* J.V. Lamouroux (*Laurencia sensu stricto*), *Chondrophyucus* (J. Tokida & Y. Saito) Garbary & J.T. Harper, *Osmundea* Stackhouse, *Palisada* (Yamada) KW Nam, *Yuzurua* (KW Nam) Martin-Lescanne and *Laurenciella* V. Cassano, Gil-Rodríguez, Senties, Díaz-Larrea, M.C. Oliveira & M.T. Fujii. The most diverse of the six genera is *Laurencia sensu stricto*, which comprises 130 currently recognised species distributed in temperate and tropical regions of all oceans (Guiry and Guiry 2014). Members of *Laurencia sensu stricto* are well known as they produce structurally elaborate halogenated natural products (Gil-Rodríguez *et al.* 2009) that have become the focus of many chemical studies (e.g. Masuda *et al.* 1996, Abe *et al.* 1999, Chatter *et al.* 2009, Jung *et al.* 2009, Stein *et al.* 2011, Campos *et al.* 2012). This genus forms a

significant and ecologically important part of cool-to-warm temperate and tropical shore ecosystems throughout the world (McDermid 1988).

The taxonomy of the *Laurencia* complex has been significantly improved by the combined use of anatomy and the plastid molecular marker RuBisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase) large sub-unit (*rbcL*), but it is still hampered by a lack of clearly defined morphological traits for species delineation. These identification difficulties might represent a major stumbling block considering the growing number of studies on *Laurencia*'s natural products and chemical properties (Jung *et al.* 2008, Chatter *et al.* 2009, Campos *et al.* 2012, Alarif *et al.* 2012). Molecular markers have proven essential to revise and discover new (cryptic) diversity in several studies (Gil-Rodriguez *et al.* 2009, Martin-Lescanne *et al.* 2010, Cassano *et al.* 2009). With the exception of Lewis *et al.* (2008) who used the nuclear marker *ITS1* and the plastid spacer *rbcLS*, all of the molecular studies on the *Laurencia* complex have used the chloroplast marker *rbcL*. Those studies have focussed chiefly on global phylogenies of the *Laurencia* complex (Martin-Lescanne *et al.* 2010) or on one of its genera (Nam *et al.* 2000, Machin-Sanchez *et al.* 2012) or, as is more often the case, a single species and the subsequent taxonomic implications (Nam 1999, 2006, Furnari *et al.* 2004, Díaz-Larrea *et al.* 2007, Rocha-Jorge *et al.* 2010, Cassano *et al.* 2012). With the exception of two studies, one in the Mediterranean (Furnari *et al.* 2001) and another in Brazil (Fujii *et al.* 2011), authors have seldom revised the species diversity of one region in particular and most of the molecular data available to date are focussed on species from North Atlantic regions.

South Africa is an intriguing locality from the perspective of *Laurencia* taxonomy as out of a total of ten species, seven have their type locality in South Africa and, with the exception of *Laurencia corymbosa* J. Agardh and *L. glomerata* (Kützinger) Kützinger, five are considered South African or east and southern African endemics: *L. complanata* (Suhr) Kützinger, *L. flexuosa* Kützinger, *L. natalensis* Kylin, *L. pumila* (Grunow) Papenfuss, and *L. peninsularis*

Stegenga, Bolton & R.J. Anderson. nom. illeg. (Seagrief 1984, Silva *et al.* 1996, Stegenga *et al.* 1997, De Clerck *et al.* 2005). Based on morphological and anatomical examinations, some authors have discussed the presence or taxonomic status of several of these species (Papenfuss 1952, Saito and Womersley 1974, Womersley 2003), but no phylogenetic studies to date have examined the diversity of the *Laurencia* complex in South Africa and *rbcL* sequences for only three species, *Laurencia natalensis*, *L. flexuosa* and *L. complanata*, have been published (Fujii *et al.* 2006).

The aim of this chapter was to contribute to the growing work on taxonomy and systematics of the *Laurencia* complex by revising the South African *Laurencia* diversity and systematics. To reach this aim, guided by previous work on the *Laurencia* complex, I analysed the chloroplastic *rbcL* sequences obtained from new extensive collections along the coastlines of all South African marine ecoregions.

2.2 Materials and Methods

Taxon sampling

The present study analyses data from 155 specimens (Appendix: Table A1) collected along almost the entire coastline of South Africa (Figure 2.1 and 2.2) with an effort to collect from type localities. The coast of South Africa can be divided into three seaweed marine provinces, with transition zones between them: (i) the west coast (Benguela Marine Province) with a cool-temperate seaweed flora, (ii) the south coast (Agulhas Marine Province) with a warm-temperate seaweed flora and, (iii) the northern part of the east coast (Indo-West Pacific Marine Province) with a tropical seaweed flora (Bolton and Anderson 1997; Bolton *et al.*, 2004; Anderson *et al.*, 2009). The *Laurencia* complex is rare on the west coast, with only a few populations of what Stegenga *et al.* (1997), with considerable doubt, referred to as *Laurencia glomerata*, but along the south and east coasts the complex is represented by ten

species (Stegenga *et al.* 1996, De Clerck *et al.* 2005). Collections were made from all of these marine provinces and their transition zones which are outlined in detail in Chapter 4.

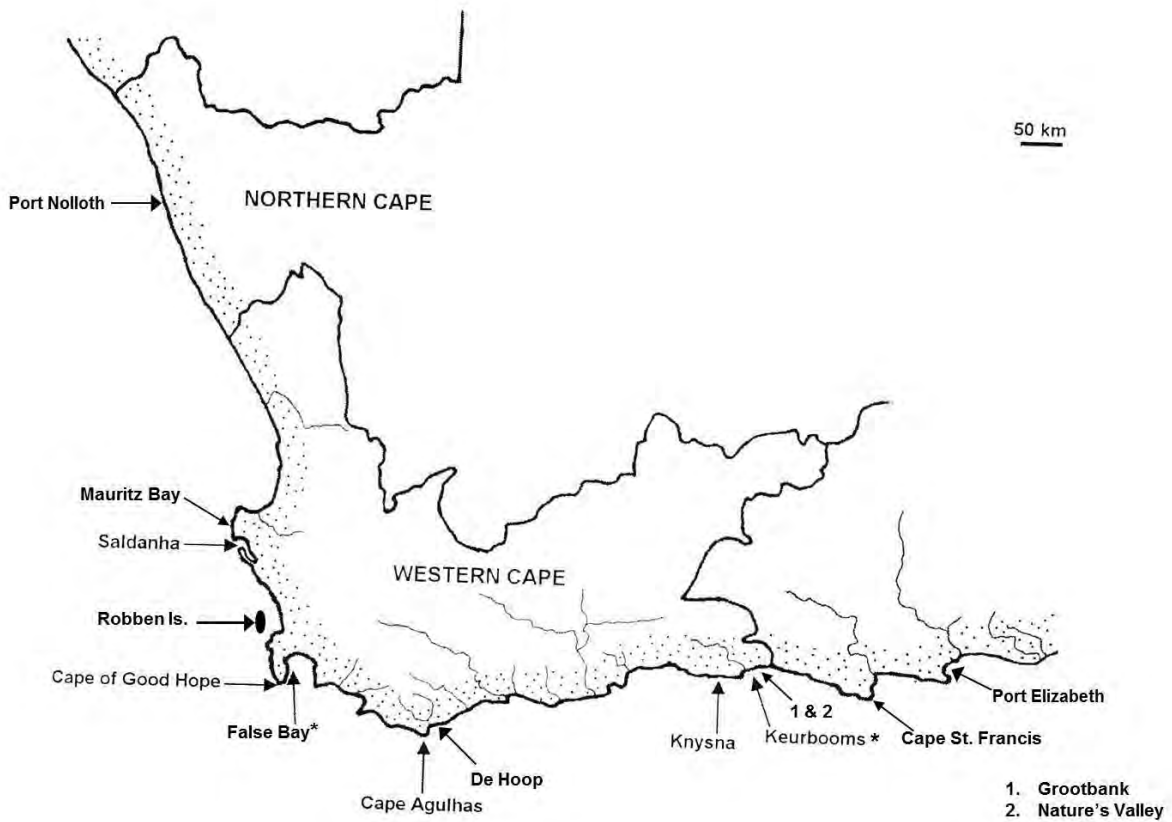


Figure 2.1: Sampling sites on the west coast and the western & central portion of the south coast. ($29^{\circ}40'S$, $24^{\circ}12'E$ to $33^{\circ}36'S$, $26^{\circ}55'E$). False Bay* includes Clovelly and Buffels Bay; Keurbooms includes Die Eiland and Platbank; De Hoop includes Koppie Alleen and Noetsie.

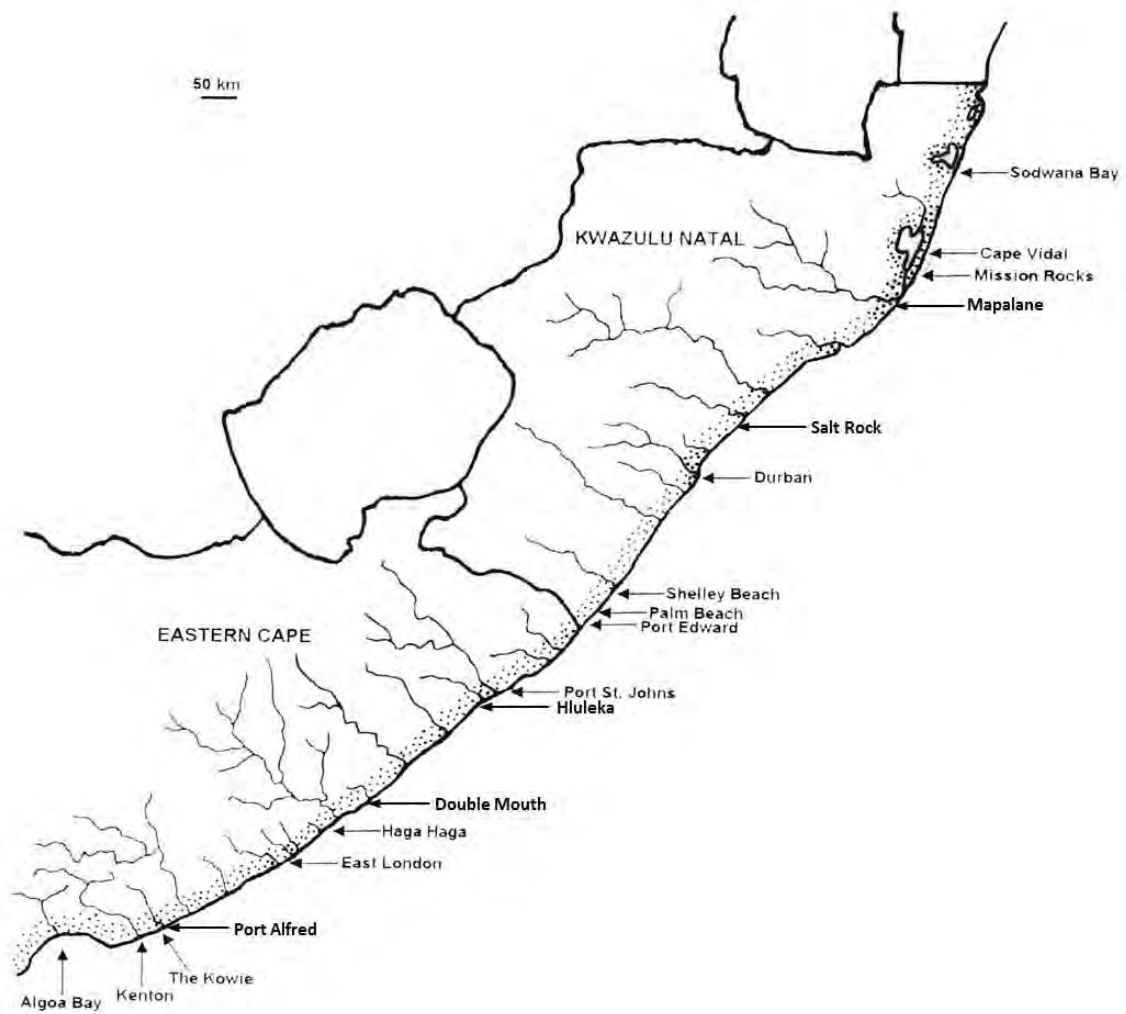


Figure 2.2: Sampling sites on the eastern portion of the south coast, and the east coast (33°58'S, 25°40'E to 27°0'00"S, 32°50'00"E).

Sample processing & morphotype identification

Subsamples of each specimen were (i) dried in silica granules for later DNA isolation, (ii) preserved in 4% formalin/seawater for later morpho-anatomical observations, and (ii) unpreserved material pressed dried on herbarium paper to serve as voucher. The voucher specimens were deposited in Bolus Herbarium (BOL) at the University of Cape Town.

Formalin preserved samples were used for morphological and anatomical examination and

formed the basis for morphotypes discrimination. A new morphotype was considered when all morpho-anatomical characters were distinct for that entity. Fresh material was used to examine the presence and number of *corps en cerise* (following Fujii *et al.* 2012) which were photographed with an Olympus D50 digital camera mounted on a Leitz Diaplan compound microscope or by holding a digital camera up against the eyepiece of a compound microscope with a solar (mirror) reflector in collection localities without access to electricity.

Morphotypes were initially identified to species using morphological characters, which currently include branching pattern, order of branching, axis width and other characters as described in Stegenga *et al.* (1997), De Clerck *et al.* (2005b) (for known South African species) and Gil-Rodriguez *et al.* (2009). For more detail on the morpho-anatomical analysis and species identification refer to Chapter 3.

DNA extraction, PCR and Sequencing

Total genomic DNA was isolated after automated grinding of silica-dried samples with the Mixer Mill MM400TM (Retsch GmbH, Haan, Germany) using the Qiagen Plant Mini Kit (Qiagen, Valencia, California, USA) following the manufacturers' instructions. The plastid RuBisCO Large sub-unit (*rbcL*) was PCR amplified in three sections of approximately 500 bp (base pair) each using primers published by Freshwater and Rueness (1994) to increase the likelihood of sequence fidelity through multiple regions of overlap. The PCRs were run in 50 μ l volumes using the same mix as in Nam *et al.* (2000) and a Kapa *Taq* DNA polymerase (Kapa Biosystems). The PCR profile had an initial denaturation phase of four minutes at 94°C, followed by 35 cycles of 60 seconds of denaturation at 94°C, 60 seconds of annealing at 40°C, 90 seconds of extension at 72°C and 10 minutes of final extension at 72°C. The products of the PCR were run on a 1% agarose gel stained with ethidium bromide to evaluate

the success of the PCR amplification and the size of bands was determined by comparison to the 100 bp DNA ladder (Biolabs™).

Successfully amplified PCR products were purified and sequenced in both directions by the Central Analytical Facility, DNA Sequencer of Stellenbosch University (South Africa) using the BigDye Terminator method.

Phylogenetic analysis

A total of 155 new *rbcL* sequences were generated in this study. Only specimens with more than 75% of the total expected sequence length (where total sequence length equals 1467 base pairs) were retained and gaps coded as missing data. The final DNA matrix including data downloaded from the GenBank included 219 *rbcL* sequences. Sequences for *Chondria dasyphylla* (Woodward) C Agardh, *Bostrychia radicans* (Montagne) Montagne and *Spyridia cupressina* Kützing together with six undescribed South African *Chondria* species were used as outgroups, the latter being South African samples sequenced in this study. Outgroups were chosen in a similar manner as outgroups in publications such as Abe *et al.* (2006), Gil-Rodriguez *et al.* (2009) and Rocha-Jorge *et al.* (2010). *Bostrychia* and *Chondria* were chosen because they are closely related to the *Laurencia* complex and within the Rhodomelaceae; *Spyridia* was chosen because it is a genus distantly related to the *Laurencia* complex but still within the order Ceramiales.

All sequences were edited and assembled using the Staden Package (Staden *et al.* 2003).

Multiple sequence alignments were performed using BioEdit v7.1.11 (Hall 1999) using the CLUSTAL W algorithm (Thompson *et al.* 1994) and double-checked by eye. The phylogenetic relationships were inferred using Bayesian statistical inference method performed in MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003) on the CIPRES Science Gateway (Millar *et al.* 2010).

The model used in the Bayesian analysis (GTR+I+G) was selected based on the maximum likelihood ratio tests implemented in *jModeltest* version 3.3 (Posada and Crandall 1998) with a significance level of 0.01 by the Akaike Information Criterion. Four chains of the Markov Chain Monte Carlo (three heated and one cold chain) were set, sampling one tree every 1000 generations for seven million generations and starting with a random tree. Calculation of posterior probabilities (PP) was performed after discarding 70,000 trees sampled during the ‘burn-in period’. A 50% majority-rule consensus tree was determined after the burn-in phase. The range of *rbcL* pair-wise divergence values within and among species was computed using uncorrected ‘p’ distances and their standard error using MEGA version 6.05 (Tamura *et al.* 2013). Standard error was estimated by the bootstrap method. A thousand replicates were tested with a random starting tree.

2.3 Results

Morphotype identification

Twenty-five morphotypes were distinguished from our collections in South Africa. Nineteen of these morphotypes belonged to *Laurencia sensu stricto*, one morphotype to *Chondrophyucus*, two morphotypes to *Laurenciella* and three morphotypes to *Palisada*. No morphotypes belonged to the remaining genera, *Yuzurua* and *Osmundea*. In *Laurencia sensu stricto* nine morphotypes were tentatively identified to the nine of the ten species previously recorded for South Africa: *Laurencia brongniartii* J. Agardh, *L. complanata*, *L. cf. corymbosa*, *L. cf. elata*, *L. flexuosa*, *L. glomerata*, *L. natalensis*, *L. obtusa* (Hudson) Lamouroux, *L. peninsularis* Stegenga, Bolton & R.J. Anderson nom. illeg., and *L. pumila* (Grunow) Papenfuss. Ten *Laurencia sensu stricto* morphotypes (A-J) remained unidentified as to our knowledge, no current species description fitted them; among these, five were represented only by one or two specimens (A & G-J) (Appendix: Table A1). *Laurencia cf. corymbosa* was represented by a

complex of specimens showing wide morphological variations. Within this complex I identified six “sub-morphotypes” (K-P).

Phylogenetic reconstruction

The *Laurencia* complex formed a strongly supported group which was subdivided into six strongly supported subgroups representing the six genera: *Chondrophyucus*, *Laurencia sensu stricto*, *Laurenciella*, *Osmundea*, *Palisada* and *Yuzurua*. Five of the six genera, *Chondrophyucus*, *Osmundea*, *Palisada*, *Yuzurua* and *Laurenciella* formed monophyletic clades and with the exception of the sister relationship between *Chondrophyucus* and *Osmundea* their phylogenetic relationships were similarly well-supported ($PP \geq 0.95$) (Figure 2.3). *Laurencia sensu stricto* formed a fully supported clade if *L. flexilis* was excluded, as suggested by Abe *et al.* (2006) because this species shares morpho-anatomical features with both *Laurencia sensu stricto* and *Palisada*.

Out of the 158 South African sequences included in the present study (155 produced in this study and 3 from Genbank), 145 grouped within the *Laurencia sensu stricto* clade, while 13 grouped in three other clades in the *Laurencia* complex as follows: eight in *Laurenciella*, four in *Palisada* and a single sequence in the *Chondrophyucus* clade. *Chondrophyucus* and *Osmundea* were resolved as sister genera with a PP (Posterior Probability) of 0.99, while *Palisada* formed the most basal group in the *Laurencia* complex. Each genus in this basal group was monophyletic (with 1.0 PP each). No South African sequences were recovered in the *Yuzurua* and *Osmundea* clades.

Within the *Laurencia sensu stricto* clade there were 14 well-supported subclades ($PP \geq 0.95$) of South African specimens, nine of which corresponded to nine of the ten species identified above. Intraspecific sequence divergence for these nine clades was low at less than 1% each of the nine species (see Table 2.1), within the levels reported for other studies on the

complex. The morphotype tentatively identified as *Laurencia obtusa*, *sensu* Stegenga *et al.* (1997), from South Africa appeared polyphyletic with sequences spread throughout the tree (identified by # symbols in Figure 2.3). None of the South African sequences identified to this morphotype grouped with the Genbank sequence from Ireland, the only available proxy to the type locality which was unspecified but presumed to be Devon or Sussex in England (Silva *et al.* 1996).

Laurencia cf. corymbosa was represented by a well-supported group of specimens (submorphotypes K to P) showing wide morphological variation, but the appropriate level of within-species sequence divergence (less than 1%, see Table 2.1). The remaining five subclades (at least two sequences each) were spread throughout the tree and represented morphotypes B to F (Figure 2.3).

Of the 62 *rbcL* sequences downloaded from Genbank representing the *Laurencia* complex, 29 grouped within the *Laurencia sensu stricto* clade and represented 17 species distributed throughout the tree. The three Genbank sequences available for *Laurencia flexuosa*, *L. natalensis* and *L. complanata* from South Africa grouped with newly obtained sequences for specimens morpho-anatomically identified to the same species (Appendix: Table A1). With the exception of two sequences from New Caledonia for specimens identified to *Laurencia cf. kuetzingii* and *L. cf. nidifica*, which appeared as the closest relatives to morphotypes E and F, no other published sequences clustered significantly with the sequences newly obtained in the present study.

Thirty-two of the remaining 33 Genbank sequences formed the core of the other genera in the complex, i.e. *Chondrophyucus*, *Laurenciella*, *Osmundea*, *Palisada* and *Yuzurua*, while the single *Laurencia flexilis* sequence available from the Philippines resolved as sister to the genus *Palisada*.

Genetic Distances

The intergeneric sequence divergence levels of the *Laurencia* complex ranged between 7.2% and 16.9% (Table 2.1). Excluding the comparison between *Laurencia pumila* and *L. dehoopiensis* sp. nov. which was markedly low (0.4-0.8%), the interspecific sequence divergence levels for each of the taxa in the *Laurencia sensu stricto* lineage ranged from 1.3% to 8.4%. In *Palisada* it was between 3.6–9.2%, in *Chondrophyucus* between 1.2–5.6% and *Osmundea* ranged from 2.0% to 8.9% (Table 2.1). *Yuzurua* and *Laurenciella* are monospecific genera and sequence divergence levels within each genus were less than 1% (Table 2.1). The intragenic divergence levels obtained in this study for the *Laurencia* complex were comparable with those reported by other authors for *Osmundea*, *Laurencia* and *Palisada* (5–9%, Nam *et al.* 2000a; 2–9%, McIvor *et al.* 2002; 6–9%, Díaz-Larrea *et al.* 2007; 3–10%, Cassano *et al.* 2009 and 1.3–9.7%, Rocha-Jorge *et al.* 2010). Sequence divergence between the Genbank sequence for *Laurencia flexilis* and sequences for species of *Laurencia sensu stricto* (7.9%-10.8%) was comparable to the intergeneric sequence divergence levels calculated in the present study. Intraspecific sequence divergence was less than 0.8% for *Laurencia sensu stricto*, *Chondrophyucus*, *Osmundea* and *Palisada* (Table 2.1).

Table 2.1: The intergeneric, interspecific and intraspecific divergence values obtained for *rbcL* sequences of the *Laurencia* complex in this study (*comparison between *L. dehoopiensis* sp. nov. and *L. pumila* excluded)

Taxa	Divergence values for <i>rbcL</i> sequences (%)
<u>Intergeneric</u>	
<i>Laurencia</i> – <i>Chondrophyucus</i>	8.4 - 9.2
<i>Laurencia</i> – <i>Palisada</i>	9.6 - 14.5
<i>Laurencia</i> – <i>Osmundea</i>	8.0 - 16.1

<i>Laurencia – Laurenciella</i>	7.2 - 13.3
<i>Laurencia – Yuzurua</i>	10.4 - 11.6
<i>Chondrophycus – Palisada</i>	10.4 - 13.3
<i>Chondrophycus – Yuzurua</i>	10.8 - 11.6
<i>Chondrophycus – Osmundea</i>	11.2 - 14.9
<i>Chondrophycus – Laurenciella</i>	10.4 - 11.6
<i>Palisada - Yuzurua</i>	10.0 - 14.1
<i>Palisada – Osmundea</i>	12.9 - 16.5
<i>Palisada – Laurenciella</i>	11.2 - 14.9
<i>Osmundea – Yuzurua</i>	14.1 - 16.9
<i>Osmundea – Laurenciella</i>	12.0 - 15.7
<i>Laurenciella – Yuzurua</i>	10.8 - 11.2
<u>Interspecific</u>	
<i>Laurencia</i>	1.3 - 8.4*
<i>Palisada</i>	3.6 - 9.2
<i>Osmundea</i>	2.0 – 8.9
<i>Chondrophycus</i>	1.2 - 5.6
<i>Yuzurua</i>	n/a
<i>Laurenciella</i>	n/a
<u>Intraspecific</u>	
<i>Laurencia complex</i>	<0.8%

Final species identification

A total of 14 out of the 25 South African morphotypes distinguished above based on morpho-anatomical characters were confirmed by the molecular analyses. They all formed strongly supported clades with intraspecific and interspecific variations comparable to those reported previously. One of the morphotypes (*L. obtusa*) appeared polyphyletic.

Nine of the morphotypes were identified to species previously recorded for South Africa: *Laurencia brongniartii*, *L. complanata*, *L. cf. corymbosa*, *L. cf. elata*, *L. flexuosa*, *L. glomerata*, *L. natalensis*, *L. peninsularis* nom. illeg. and *L. pumila*, while five represented unknown species which are considered to be new. They are described further in Chapter 3 as *Laurencia dehoopiensis* sp. nov., *L. dichotoma* sp. nov., *L. digitata* sp. nov., *L. multiclavata* sp. nov. and *L. sodwaniensis* sp. nov. Despite the considerable variation in morphology observed between submorphotypes K-P, *Laurencia cf. corymbosa* is included in the above list because it formed a well-supported group in the phylogenetic analysis and showed low levels of sequence divergence (less than 1% between morphotypes). Molecular and morpho-anatomical data for five morphotypes A and G to J were insufficient (only one specimen and/or one sequence each) to confirm them as distinct new lineages and more collections are needed to further assess their status. The specimens identified as *Laurencia obtusa* (see # in Figure 2.3) appeared polyphyletic in phylogenetic analyses; one specimen was representative of an undescribed species in the genus *Chondria*, another clustered with other sequences identified to *Laurencia cf. corymbosa* and the last specimen clustered with other sequences of the newly described species *L. dehoopiensis* sp. nov.

Because *Laurencia peninsularis* Stegenga, Bolton and Anderson nom. illeg. described from the Cape Peninsula (Stegenga *et al.* 1997) is a latter homonym of *Laurencia peninsularis* Taylor (1945) we propose the new name *Laurencia stegengae* (Stegenga, Bolton and Anderson) Francis, Bolton, Anderson and Mattio nom. nov. (see Chapter 3).

2.4 Discussion

Diversity of the *Laurencia* complex in South Africa

The revised list resulting from this study records a total of 20 species for the *Laurencia* complex in South Africa. Nine of the ten previous records (Stegenga *et al.* 1997, De Clerck *et al.* 2005) were confirmed, five new species are proposed (Chapter 3) and six represent new records for South Africa. Our results further indicate that this diversity is underestimated: five additional morphotypes were distinguished, but more morphological and molecular data is required before they can be confirmed as distinct and new entities.

Besides *Laurencia cf. elata*, which needs to be confirmed by the analysis of additional specimens (ideally from the type locality and the type specimen), *L. obtusa* represents the only previous record not confirmed in this study. The morphotype initially tentatively identified as *Laurencia obtusa* was revealed to be polyphyletic and to include at least three entities: a representative of a new putative species of *Laurencia sensu stricto*, a specimen later identified as part of the *Laurencia corymbosa* species complex, another representative of a newly described species *L. dehoopiensis* and an entity belonging to the genus *Chondria* (Figure 2.3). These results highlight the lack of clear species boundaries delineating *Laurencia obtusa*. While Stegenga *et al.* (1997) provided a brief description for this species on the South African west coast; the authors noted its dubious taxonomic status worldwide and stated that variation amongst descriptions of this species is so large that it is unlikely to represent a single species: this has been borne out by the current study. For South African “*Laurencia obtusa*” there was no conceptual framework to distinguish it from other *Laurencia* taxa beyond the notion of ‘any relatively small *Laurencia* taxon that is sparsely branched.’ The type species of *Laurencia obtusa* is unspecified, but is presumed to be from Devon or Sussex, England (Silva *et al.* 1996). The closest proxy to this geographic location is the Genbank-sourced *Laurencia obtusa* sequence from Ireland (Figure 2.3). None of the

South African taxa identified during the present study were closely related to this taxon in Bayesian analyses, suggesting that *L. obtusa* is not present in South Africa.

One of the most widely distributed *Laurencia* species in South Africa is *Laurencia flexuosa*. It is easily recognized by the characteristic flexuous main axis and regularly-alternate branching pattern. A second similar species with an Australia type, *Laurencia elata*, is significantly alike *L. flexuosa* morphologically. It was listed by Barton (1893). However, Womersley (2003) mentioned that records of this species for Indian Ocean localities other than Australia need verification. Saito and Womersley (1974) noted that the taxon *L. elata* f. *flexuosa* from South Africa, which they attribute to Yamada (1931, p. 242), ‘appears closely related to *L. elata*, but requires a detailed study to establish its relationships’. *Laurencia elata* was not cited again in the South African literature and its presence in South Africa has remained questionable. The diagnosis of *L. elata* f. *flexuosa* by Yamada (1931) could not be accessed during the present study and is listed neither in Algaebase™ nor in the *Index Nominum Algarum*. We believe the validity of *L. elata* f. *flexuosa* is doubtful and we do not recognise it here. Our analysis demonstrated that two South African morphotypes clearly corresponding morphologically to the diagnoses of both *L. flexuosa* and *L. elata* appeared in different and well supported clades (Figure 2.3) with a between-species pairwise sequence divergence level (2.7-3.1%) equivalent to that recognised in previously published works for *Laurencia* (Nam *et al.* 2000a, McIvor *et al.* 2002, Diaz-Larrea *et al.* 2007, Cassano *et al.* 2009, Rocha-Jorge *et al.* 2010). During the present study, the identification of South African specimens as *L. flexuosa* was confirmed morphologically by direct comparison to the type specimen; however the sequences produced may not be from the type locality (Cape of Good Hope, South Africa) as, historically, this locality encompasses the entire Cape Colony and not the Cape of Good Hope as it is known in present times. Identification to *Laurencia* cf. *elata* could only rely on comparison with specimens from Western Australia identified by Harvey

(TCD0015185,6). We noted that South-African specimens of *Laurencia* cf. *elata* can easily be mistaken for *L. flexuosa* and a clearer description of these species can be found in Chapter 3. A direct comparison with the type specimen of *Laurencia elata* together with a sequence from an Australian specimen is necessary to confidently confirm the identification of the South African *L. cf. elata*.

Laurencia corymbosa was described by J. Agardh (1852) from a specimen collected at the Cape of Good Hope in South Africa. The exact location of J. Agardh's type specimen for this species is difficult to determine because the Cape of Good Hope at the time of description meant the Cape Colony and not the region of the Cape peninsula now referred to as the Cape of Good Hope. With this in mind from the collections, six "submorphotypes" (K-P) initially distinguished corresponding to the general morphology of the species as described by J. Agardh (1852). The eight specimens shared anatomical traits such as the number of *corps en cerise* and the shape of the epidermal cell and formed a well-supported clade with sequence divergence below 1%. This is within the range for within-species variation reported in other studies of the *Laurencia* complex as calculated during the present study (Table 2.1) and previously published, as discussed above. These results indicate that despite the wide morphological range initially observed, *L. cf. corymbosa* represents a well-supported species in South Africa.

CHAPTER 3:
DESCRIPTIONS OF AND KEY TO SOUTH AFRICAN SPECIES IN THE
LAURENCIA COMPLEX, WITH THE ADDITION OF FIVE NEW SPECIES

3.1 Introduction

The genus *Laurencia* was described by J.V. Lamouroux in 1813 and included eight species (*L. pinnatifida*, *L. obtusa*, *L. gelatinosa*, *L. cyanosperma*, *L. lutea*, *L. caespitosa*, *L. intricata* and *L. versicolor*) of which Schmitz (1889) designated *L. obtusa* as the type. The current understanding of *Laurencia* is a complex of six genera known as the *Laurencia* complex (Garbary & Harper 1998, Nam 1994 and 2007, Martin-Lescanne *et al.* 2010 and Cassano *et al.* 2012) of which *Laurencia sensu stricto* represents the largest genus with 130 currently accepted species (Guiry and Guiry 2014). The remaining five genera in the complex, arranged in descending order of number of species, are *Palisada* (22 species), *Osmundea* (18 species), *Chondrophyucus* (17 species), *Laurenciella* (one species) and *Yuzurua* (one species).

Laurencia sensu stricto is anatomically distinguished from other genera in the complex (with the exception of *Laurenciella*) by having four pericentral cells and cellular inclusions termed *corps en cerise* as well as other traits described by several authors, most notably Garbary and Harper (1998), Nam (1999, 2006), and Nam and Choi (2001). *Laurenciella* shares many traits with *Laurencia sensu stricto* and is distinguishable from the latter primarily by molecular data and the presence of *corps en cerise* in all cells of the thallus. Since its description, only a single species, distributed in the tropical and warm temperate Atlantic, has been formally assigned to it: *Laurenciella marilzae* (Gil-Rodríguez, Senties, Díaz-Larrea, Cassano *et M.T. Fujii*) Gil-Rodríguez, Senties, Diaz-Larrea, Cassano *et M.T. Fujii*, although two more putative species were distinguished based on molecular data which did not present with *corps*

en cerise throughout their thalli (Cassano *et al.* 2012). *Chondrophyucus* is chiefly distributed in the tropical Indo-West Pacific and is distinguished from *Laurencia sensu stricto* by the presence of two pericentral cells and the absence of *corps en cerise* and secondary-pit connections between outermost cortical cells. The genus *Osmundea* has a disjunct distribution and is recorded from Pacific North America, Brazil, Atlantic Europe, the Mediterranean Sea and India (Nam *et al.* 2000a, McIvor *et al.* 2002, and Furnari *et al.* 2004) and probably from Australia (Nam 2006). *Osmundea* shares the general anatomical features of *Chondrophyucus* but differs from it in male reproductive anatomy. *Chondrophyucus* has trichoblast-type spermatangial development (like that of *Laurencia sensu stricto*) while *Osmundea* has filament-type spermatangial development. *Palisada* is characterised by palisade-like outermost cortical cells and also has trichoblast-type spermatangial development. Tetrasporangial development in *Osmundea* only occurs from particular cortical cells as opposed to any of the pericentral cells in all other genera of the *Laurencia* complex. The two taxa (one species and one variety) attributed to *Yuzurua* are found in tropical regions of all oceans. *Yuzurua* shares several anatomical traits with *Palisada*, but it lacks the characteristic palisade-like outermost cortical cells of *Palisada* and has a procarp that bears five pericentral cells as opposed to the four found in *Palisada*. Interestingly, *Yuzurua* is the only other genus in the complex to exhibit secondary pit-connections as seen in *Laurencia sensu stricto*.

Taxonomic accounts, including species descriptions, are available for *Laurencia sensu stricto* species in a number of world regions, including southern Australia (Saito & Womersley 1974, Womersley 2003), Japan (Yamada 1931, Saito 1967), Hawaii & the Philippines (Saito 1969), Tanzania (Jaasund 1976), Britain (Maggs and Hommersand 1993) and parts of South Africa (Stegenga *et al.* 1997 and De Clerck *et al.* 2005).

Many of the diagnostic characteristics for the complex were developed at the genus level in a series of papers by Nam & Saito (1990, 1991a, b), Nam *et al.* (1994), Garbary and Harper (1998), Nam (1999, 2006), Martin-Lescanne *et al.* (2010) and Cassano *et al.* (2012). These papers focussed strongly on reproductive and vegetative anatomy as a means of distinguishing between the genera, and used characters that include the position of the first pericentral cell relative to the trichoblast, absence/presence of fertility of the second pericentral cells and number of sterile cells in the tetrasporangial axis, spermatangial branches produced from one or two laterals on the suprabasal cell of trichoblast or not, presence/absence of additional tetrasporangium-bearing pericentral cells, the position of pericentral cells bearing tetrasporangia, the number of pericentral cells of the procarp-bearing segment and trichoblasts versus filament-type spermatangial development, the shape of the outermost cortical cells, the number of pericentral cells and the presence/absence of *corps en cerise* in the outermost cortical cells and/or the whole thallus.

The earliest record of *Laurencia sensu stricto* in South Africa was of *L. complanata* (Suhr) Kützing (as *Chondria complanata* Suhr) by Suhr in Krauss (1846) based on material from Durban [Natal Bay], KwaZulu-Natal. Kützing (1849) added two species to the list: *Laurencia flexuosa* and *Laurencia glomerata*, with type localities around the Cape Peninsula (near Cape Town). Thereafter, seven more species were attributed to the genus in South Africa, namely *Laurencia corymbosa* J. Agardh, *L. elata* (C. Agardh) Kützing, *L. natalensis* Kylin, *L. pumila* (Grunow) Papenfuss, *L. obtusa* (Hudson) Lamouroux, *L. peninsularis* Stegenga, Bolton *et Anderson nom. illeg.* and *L. brongniartii* J. Agardh (Seagrief 1984, Silva *et al.* 1996, Stegenga *et al.* 1997 and De Clerck *et al.* 2005) (See Table 1.2; pg. 24), making up a total of 10 species of which three are South African or southern African endemics (Guiry & Guiry 2014). The most recent accounts of the seaweed flora in South Africa (Stegenga *et al.* 1997, De Clerck *et al.* 2005), covering half of the South African coast, however did not list

Laurencia elata nor *L. corymbosa*. As mentioned in Chapter 1, the epithets *Laurencia corymbosa* and *L. elata* and the remaining eight species were only been briefly described by the aforementioned authors.

Results of the molecular studies presented in Chapter 2 suggested that the diversity of the *Laurencia* complex in South Africa had previously been underestimated and that at least five more species should be added to the list. Morpho-anatomical observations confirmed nine of the 10 *Laurencia sensu stricto* species known for South Africa in the literature while five did not correspond to any described species known to us and five represented new records for SA, including species belonging to other genera of the *Laurencia* complex. Results further highlighted the presence of six possible additional species which remained to be confirmed. The primary aim of the present chapter was to provide a detailed taxonomic account of species comprising South African *Laurencia sensu stricto* including morphological descriptions, illustrations and a dichotomous key for facilitated identification. A secondary aim was to provide provisional descriptions for the remaining unidentified morphotypes and new records of the other genera of the *Laurencia* complex as a baseline for future studies.

3.2 Material and Methods

Taxon sampling; sample preservation & preparation

A total of 211 specimens were collected from around the coast of South Africa (see Chapter 2 for details of sampling locations) in the period 2008 to 2013 and 155 of them were pressed as vouchers on herbarium sheets and housed in the Bolus Herbarium (University of Cape Town) (Appendix: Table A1) . When possible a small subsample of fresh material, approximately 1cm in length, was ‘squashed’ between two glass slides for the observation of *corps en cerise*. A drop of water was added to the specimen and a cover slip was put on before mounting on the glass slide. If *corps en cerise* (a French term meaning ‘cherry body’) were present they were counted and photographed with an Olympus D50 digital camera

mounted on a Leitz Diaplan compound microscope, or sometimes (in remote localities with no electricity) with a hand-held digital camera through the eyepiece of a small portable compound microscope.

Subsamples of the specimens were preserved in 4% formaldehyde/seawater for anatomical analyses. Liquid-preserved subsamples were sectioned by hand or with a freezing microtome. Samples were embedded in a clear glue solution prior to freezing microtome section. Sections were generally ca. 5 µm thick and mounted on glass slides in a 40% Karo solution. For some specimens staining with 1% aniline blue was necessary to aid clearer visualization of vegetative structures. Photographs were taken with an Olympus D50 digital camera mounted on a Leitz Diaplan compound microscope or Leica Wild M10 stereo microscope.

In addition to specimens collected over the course of this project specimens from the Bolus Herbarium, University of Cape Town were examined where available to produce the species descriptions for South African *Laurencia sensu stricto* species. Usually five or more specimens were examined for morphological and anatomical description; however for some of the new species discovered specimens were limited.

Morpho-anatomical characters analysis

The features used to describe species were a combination of those currently used by taxonomists who have studied the *Laurencia* complex or genera within the *Laurencia* complex: Saito and Womersley (1974), Nam and Sohn (1994), Nam *et al.* (1994) Nam and Saito (1995), Garbary and Harper (1998), Womersley (2003) and Gil-Rodriguez *et al.* (2009). The morphological features include branching pattern, order of branching, length intervals between branches on the axis, axis width, extent of thallus compression, branch basal constriction, and number of *corps en cerise* in the outermost cortical cells.

3.3 Results & Discussion

As a result of the present study, fourteen *Laurencia sensu stricto* species were described and illustrated below, including five new to science together with a dichotomous key to assist in identification. Further notes and morpho-anatomical details, excluding sexual reproductive structures as there was no fertile material in the study collection, are provided for the remaining unidentified morphotypes as well as for the rest of the *Laurencia* complex species, all representing new records for SA. The revised list of species for the South African *Laurencia* complex is as follows:

South African *Laurencia sensu stricto* species and new records of other members of the *Laurencia* complex based on evidence presented in this dissertation.

Division: Rhodophyta

Subdivision: Eurhodophytina

Class: Florideophyceae

Subclass: Rhodymeniophycidae

Order: Ceramiales

Family: Rhodomelaceae

Tribe: Laurencieae Schmitz (1889: 447)

Genus: *Laurencia* Lamouroux 1813: 130 (= *Laurencia sensu stricto*)

Laurencia brongniartii J. Agardh 1841: 20-21

Laurencia complanata (Suhr) Kützing 1849: 857

Laurencia cf. *corymbosa* J. Agardh 1852: 747

Laurencia cf. *elata* (C. Agardh) Hooker & Harvey 1847:401

Laurencia flexuosa Kützing 1849:856

Laurencia glomerata Kützing 1849:857

Laurencia natalensis Kylin 1938:24

Laurencia stegengae Francis, Bolton, Mattio & Anderson submitted

Laurencia pumila (Grunow) Papenfuss 1943: 91-92

Laurencia dehoopiensis Francis, Bolton, Mattio & Anderson submitted

Laurencia dichotoma Francis, Bolton, Mattio & Anderson submitted

Laurencia digitata Francis, Bolton, Mattio & Anderson submitted

Laurencia multiclavata Francis, Bolton, Mattio & Anderson submitted

Laurencia sodwaniensis Francis, Bolton, Mattio & Anderson submitted

Laurenciella Cassano *et al.* 2012: 349-357

Laurenciella marilzae (Gil-Rodríguez, Senties, Díaz-Larrea, Cassano *et* M.T. Fujii) Gil-Rodríguez, Senties, Díaz-Larrea, Cassano *et* M.T. Fujii

Palisada Nam 2007: 53

Palisada sp. 1

Palisada sp. 2

Palisada cf. *corallopsis* (Montagne) Senties, M.T. Fujii *et* Díaz-Larrea

Chondrophyucus (Tokida *et* Saito) Garbary *et* Harper 1998: 194

Chondrophyucus sp. 1

Morphological descriptions & illustrations of South African *Laurencia sensu stricto* species in South Africa

Laurencia brongniartii J. Agardh 1841: 20-21

Synonyms:

Laurencia concinna Montagne 1842a: 6

Laurencia grevilleana Harvey 1855b: 545

Misapplied name:

Laurencia distichophylla – Harvey 1855b:545 (according to Saito and Womersley 1974)

Type Locality: Martinique, West Indies (Agardh 1841: 20-21)

Etymology: '*brongniartii*' named for the French naturalist, Adolphe Brongniart

Habitat: Marine epilithic species present on subtidal reefs from a few to at least 37 metres depth (De Clerck *et al.* 2005)

Specimens examined:

KwaZulu-Natal: Saxon Reef, north of Bhanga Nek (Coppejans *et al.* 14.viii.1999: BOL 21990); Aliwal Reef, 50km south of Durban (Coppejans *et al.* 4.viii.1999: BOL 21989); Aliwal Shoal (Anderson 04.viii.1999: BOL21988), 2 Mile Reef, Sodwana Bay (this study, 22.ii.2011: D978)

Description:

External morphology:

(Figure 3.1A): Plants dark red, fleshy, between (2.7-) 3 and 5 cm high attached by discoid holdfast, with one to several percurrent axes slightly bent toward the substrate, complanately and regularly pinnately branched up to 2 (-4) orders inserted every 0.2-1 mm; axes strongly compressed, (1.5-) 2 to 3 mm wide and 0.5 – 1 mm thick, decreasing to short ultimate branchlets 0.5-0.7 mm wide near the base, with truncated apices; branches on the adaxial sides much more developed than those on the abaxial sides of the axes; branch basal constriction absent. First order branches inserted at regular intervals (2-5 mm), branch length increasing in length towards the holdfast to 2-10 mm. Secondary and higher order branches much shorter.

Internal structure:

In cross-section axial and pericentral cells not distinguishable. Medullary cells often with lenticular thickenings; cortical cells with secondary pit connections in the longitudinal

direction; medullary and innermost cortical cells larger than outermost cortical cells (Figure 3.1B); outermost cortical cells with 2 *corps en cerise* (Figure 3.1C).

Distribution:

South Africa: Protea Banks southern KwaZulu-Natal to Saxon Reef near the Mozambican border (De Clerck *et al.* 2005); Australia, Indonesia (Flores), Madagascar, & Sri Lanka (Silva *et al.* 1996)

Notes

Laurencia brongniartii was placed in synonymy with *L. concinna* Montagne by Seagrief (1984) based on a record of Barton (1893). However, Papenfuss (1943a) considered *Laurencia concinna* to be representative of the morphologically much larger *L. complanata*. The description of *Laurencia brongniartii* from Australia by Saito and Womersley (1974) suggests specimens significantly larger than the South African and Japanese (or Pacific) forms and is more in line with the description of *L. complanata* of which it could represent a misidentification.

Laurencia complanata (Suhr) Kützing 1849: 857

Basionym: *Chondria complanata* Suhr 1846: 211

Misapplied name: *Laurencia concinna* – Barton 1893:174 (according to Papenfuss 1943a)

Type Locality: Durban ‘Natal Bay’, KwaZulu-Natal, South Africa (Suhr in Krauss 1846: 211)

Etymology: *Complanata*: Adjective (Latin), meaning flattened out, usually in one plane (Stearn 1973)

Habitat: epilithic species present in intertidal pools and the shallow subtidal

Specimens examined:

Eastern Cape: Mzamba (Stegenga, 21.viii.2005: BOL 9435); Hluleka (Stegenga, 14.ix.1983: BOL 21405); Mkambati (Stegenga, 08.x.2002: BOL 21994).

KwaZulu-Natal: Ramsgate (Simons, 23.ix.1960: BOL 21999); Palm Beach (De Clerck 21.xii.1999: BOL 21992), Salt Rock (this study, 09.xii.2010: D859), Port Edward (this study, 28.ix.2011: D1053)

Description:

External morphology:

(Figure 3.2A) Plants dark red, between (5-) 6 and 20 (-22) cm high attached by discoid holdfast, with one to several axes, complanately and regularly pinnately branched up to 3 (-4) orders; axes strongly compressed, (1-)2 to 3.5 mm wide and 0.5 – 1mm thick, decreasing to short ultimate branchlets 0.5-2.2 (-3) mm wide near the base, with truncated apices; branches on the adaxial and abaxial sides of the axes equally developed; branch basal constriction absent. First order branches inserted at regular intervals (2-5mm), increasing in branch length towards the holdfast between (1-) 2 and 22 (-35mm). Secondary and higher order branches much shorter.

Internal structure:

In cross-section axial and pericentral cells not distinguishable: Medullary cells sometimes with lenticular thickenings; cortical cells isodiametric to higher than wide in cross-section 40-50µm and 25-40µm with secondary pit connection in the longitudinal direction (Figure 3.2B); medullary and innermost cortical cells larger than outermost cells (Figure 3.2C); outermost cortical cells 15-30µm across, visibly darker than cortical and medullary cells, polygon-shaped and contain 1 to 2 *corps en cerise* (Figure 3.2D).

Distribution:

South Africa: From Hluleka, Eastern Cape Province (Bolton & Stegenga 1987) northward, to Inhaca Is., southern Mozambique (Isaac 1957); southern Madagascar (see next Chapter)

Notes

Laurencia complanata is distinguished from the other pinnate South African species *L. brongniartii* by its relatively large size and largely lower-intertidal ecological niche in the warm temperate to tropical waters of South Africa. *Laurencia brongniartii* is similar to *L. complanata* in appearance and number of *corps en cerise* (generally two), but has only been recorded subtidally on shallow to deep reef systems in tropical waters in northern KwaZulu-Natal (De Clerck *et al.* 2005). A polytomy between between *Laurencia complanata* and *L. brongniartii* was recovered in the phylogenetic analyses in Chapter 2 (PP=0.99) showing poor resolution of the relationship between these two species. However, there is an 8% genetic distance between the two species in the *rbcL* gene region and this, together with the circular shape of the outermost cortical cells at surface view (versus distinctively polygonal in *L. brongniartii*) supports recognition of *L. complanata* as a distinct species.

Laurencia cf. corymbosa J. Agardh (1852: 747)

Misapplied name (*vide* Papenfuss notes in Silva *et al.* 1996):

Laurencia virgata – Delf & Mitchell (1921: 211)

Type Locality: Cape of Good Hope, South Africa (J. Agardh 1852: 747)

Etymology: *Corymbosa*: Adjective (Latin), meaning corymbose (Stearn 1973)

Habitat: Marine epilithic species present in the intertidal and subtidal (this study)

Specimens examined:

Western Cape: Koppie Alleen, De Hoop Marine Protected Area (this study, 19.xii.2008:

D164; 17.ii.2011: D903); East of Koppie Alleen, De Hoop Marine Protected Area (this study,

18.ii.2011: D926)

Eastern Cape: Double Mouth (this study, 14.vii.2010: D768); Port Elizabeth (this study, 14.xii.2012: D1181, D1188); Port Alfred (this study, 07.vii.2008: D31); Three Sister (this study, 27.ii.2013: D1257)

Description:

External morphology:

Plants maroon-red to sometimes dark purple when fresh, fleshy, between (5.5) -6 and 14 (-15.4) cm high (Figure 3.3A), attached to substrata by discoid holdfast, generally percurrent axes irregularly alternately branched up to 2 (-4) orders, higher order laterals suboppositely branched; axes terete, up to 1.5 mm wide, ultimate branchlets subverticillate with truncated apices; branch basal constriction absent. First order branches inserted at irregular intervals, branch length increasing in length towards the holdfast to 2-14 mm for some. Secondary and higher order branches much shorter and typically corymbose when fertile (Figure 3.3B).

Internal structure :

In cross-section axial and pericentral cells not distinguishable. Outermost cortical cells dome shaped 15-47 μm x 9-35 with one *corps en cerise* per cell (Figure 3.3D). Cortical cells with secondary pit connections in the longitudinal direction; medullary and innermost cortical cells larger than outermost cortical cells with spaces evident between the cells (Figure 3.3C);

Distribution:

South Africa: De Hoop to Port Alfred (this study); Seychelles (Kalugina-Gutnik *et al.* 1992); Vietnam (Pham-Hoàng 1969); Fiji (N'Yeurt *et al.* 1996, South & Skelton 2003).

Notes

Laurencia cf. corymbosa is represented by eight specimens that exhibit wide morphological variation and habitat (Figure A1). Shared traits include the irregularly alternate (sometimes polystichous) branching pattern and single *corps en cerise* in the dome-shaped outermost cortical cell. At least three of these specimens [*Laurencia* sp. (D164, D903, D926)] were

collected from around the Cape Colony, the broad type locality of the *Laurencia corymbosa*. Morphological comparisons to the original material of *Laurencia corymbosa* (BM000774817 & BM000774816, Natural History Museum (BM)) revealed similarities in branching pattern and axis width. The type specimens also exhibit the characteristic corymbose appearance of the secondary laterals for which the species was named. However, in South African specimens the corymbose nature of the branches seems to be evident only when the specimens are fertile. When non-fertile, specimens tend to remain sparsely branched and are easily mistaken for *Laurencia obtusa*. While the morphology of the specimens varies significantly, their DNA sequence divergence levels are low (< 1%) and there is full molecular support for this clade (Chapter 2: Figure 2). It is proposed that the specimens be referred to as *Laurencia cf. corymbosa* pending further investigation.

Laurencia dehoopiensis Francis, Bolton, Mattio *et* Anderson (Francis *et al.* submitted)

Holotype: BOL150571, East of Koppie Alleen, De Hoop Marine Protected Area, Western Cape, South Africa (34°26'03"S 20°32'52" E)

Etymology: “*dehoopiensis*” - after the type locality, De Hoop Marine Protected Area, Western Cape, South Africa,

Habitat: subtidal, epiphytic on the brown alga *Phloiocaulon suhrrii* (J.Agardh) P.C. Silva

Specimens examined:

Western Cape: Koppie Alleen, De Hoop Marine Protected Area (this study, 18.xiii.2008: D139; 19.xiii.2008: D154)

Eastern Cape: Saltvlei, Port Alfred (this study, 25.ii.2013: D1213); Three Sister (this study, 27.ii.2013: D1253)

Description:

External morphology:

(Figure 3.4A): Plants epiphytic, up to 9 (-14) cm high, reddish, attached by discoid holdfast, with several percurrent axes; axes terete, up to 1.5 mm in diameter; branching polystichous and up to 3 orders, second order lateral arrangement can vary from pinnate to opposite to alternate (Figure 3.4B), length of lateral increases in a proximal direction away from apices; ultimate laterals very short, wart-like towards apices and branching pattern is either alternate or subverticillate; ultimate branchlets longer than wide, apices sunken, branch basal constriction absent.

Internal structure:

Axial and pericentral cells visible in cross section(Figure 3.4C) ; Innermost cortical and medullary cells at least two times larger than outermost cortical cells; Outermost cortical cells longer than they are wide, approximately 18-25 μm in width (Figure 3.4D) and having a single *corps en cerise* in each outermost cortical cell (Figure 3.4E).

Distribution: So far found only on the south coast of South Africa at Koppie Alleen, De Hoop Nature Research and the Port Alfred.

Notes

Laurencia dehoopiensis forms a sister relationships in the *rbcL* analysis of the South African *Laurencia* complex alongside *L. pumila*. The latter species is found in the intertidal; however, *Laurencia dehoopiensis* occupies a different ecological niche and is found in the subtidal.

Laurencia dehoopiensis is much larger than *L. pumila* and thus far found only on the south coast of South Africa. The proposal for the new species *Laurencia dehoopiensis* sp. nov. is further substantiated by the ecological niche this species occupies as an epiphyte on the brown seaweed *Phloiocaulon suhrii* (J.Agardh) P.C. Silva in Silva *et al.* (1996) in the shallow subtidal warm temperate waters at De Hoop, on the South African south coast, while *L. pumila* and *L. digita* are found in warmer waters.

Laurencia dichotoma Francis, Bolton, Mattio *et* Anderson (Francis *et al.* submitted)

Holotype: BOL 150568 South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Point (27°31'59"S, 32°40'59"E), intertidal. Date collected 22.iii.2010.

Etymology: Adjective (Latin) – split in half. Named for the distinctive equal splitting of second order branches in this species.

Habitat: mid to low intertidal in northern KwaZulu-Natal.

Specimens examined:

KwaZulu-Natal: Jesser Point, Sodwana Bay (Francis *et al.* submitted, 23.iii.2010:

BOL57729); Bhanga Rock, Bhanga Nek (Bolton, Mattio & Anderson, 4.x.2013: D1583)

Description:

External morphology:

(Figure 3.5A): Plants caespitose, small (up to 4 cm tall), light brown, cartilaginous, with several percurrent axes; main axes terete, up to 0.7 mm in diameter, with dichotomous branching evident in second order branches (Figure 3.5B). Thallus sparsely branched, ultimate branches blunt with sunken apices, branch basal constriction absent;

Internal structure:

Axial and pericentral cells distinguishable in cross section (Figure 3.5E); Innermost cortical and medullary cells two to three times larger than outermost cortical cells; outermost cortical cells as long as broad, approximately 15-25 µm in width, darker (Figure 3.5C) and containing a single *corps en cerise* in both the outermost cortical and trichoblast cells (Figure 3.5D).

Distribution: collected from Sodwana Bay and Bhanga Nek in northern Kwazulu-Natal.

Notes:

Laurencia dichotoma is the only cartilaginous species in the South African *Laurencia* flora and appears distinct molecularly from its nearest relative, an undescribed *Laurencia* species, by 4.7-5%. The species exhibits distinctive dichotomous branching at the tips, unlike any other South African species in the genus, is a distinctly yellow-brown colour and is generally sparsely branched.

Laurencia digitata Francis, Bolton, Mattio *et* Anderson, sp. nov. (Francis *et al.* submitted)

Holotype: BOL150572 South Africa, KwaZulu-Natal, Cape Vidal. Date collected: 20.iii.2011.

Etymology: Adjective (Latin) – having fingers. This species has finger-like laterals below apices.

Habitat: mid to low intertidal in exposed situations such as the edges of rocky overhangs

Specimens examined:

Western Cape: Nature's Valley (Francis *et al.* submitted, 08.iv.2008: BOL57730); East of Koppie Alleen, De Hoop Marine Protected Area (this study, 18.ii.2011: D930, D932); Swartvlei near Sedgfield (this study, 15.xii.2012: D1195)

Eastern Cape: Storms River, Tsitsikamma National Park (this study, 13.xii.2012: D1174)

KwaZulu-Natal: Cape Vidal (this study, 25.ix.2011: D1027); Bhanga Rock, Bhanga Nek (this study, Bolton, Mattio & Anderson 07.x.2013: D1669)

Description:

External morphology:

(Figure 3.6A): Plants epilithic, bushy yet small (up to 4 cm in height), turf-like in appearance, thallus reddish brown, several percurrent axes attached by small tangled, rhizoidal holdfast; 2 sometimes 3 orders of branching, branching throughout thallus generally alternate but some branches appear subopposite and/or subverticillate at the tips (Figure 3.6B), finger-like

laterals below apices; axes terete, up to 0.5 mm wide with truncated apices; ultimate branches with sunken apices, branch basal constriction absent.

Internal structure:

Axial and pericentral cells distinguishable in cross section (Figure 3.6 E); Innermost cortical and medullary cells at least two times larger than outermost cortical cells; outermost cortical cells approximately 18-22 μm across, darker (because of pigmentation) and containing a single *corps en cerise* per cell (Figure 3.6 C and D); intercellular spaces not evident in cortex.

Distribution: South African endemic: South coast (from De Hoop) extending eastward into northern KwaZulu-Natal (as far as Bhanga Nek for the present study).

Notes:

At first glance this species appears similar to *Laurencia pumila* in both morphology, presence of a single *corps en cerise* per outermost cortical cell and habitat, however *Laurencia digitata* is entirely reddish brown, branched throughout the thallus and the laterals are longer (4-9 mm) than those of *L. pumila* (2-6 mm). Molecular analyses places this species in a sister-relationship with *L. cf. kuetzingii* (Kuetzing) Millar (with a genetic difference of 2.1-2.9%), a species collected from New Caledonia.

Laurencia cf. elata (C. Agardh) Hooker & Harvey 1847:401

Basionym: *Chondria pinnatifida* var. *elata* C. Agardh 1822: 340

Homotypic synonyms:

Chondria pinnatifida var. *elata* C. Agardh 1822: 340; 1824:202

Laurencia pinnatifida var. *elata* (C. Agardh) Sonder 1846:177

Heterotypic Synonyms:

Laurencia elata var. *luxurians* Harvey 1863

Laurencia luxurians (Harvey) J. Agardh 1876:659

Laurencia pinnatifida sensu Sonder 1880:30

Laurencia elata f. *luxurians* (Harvey) Yamada 1931

Type Locality: King Island, Bass Strait, Australia (C. Agardh 1822:340)

Etymology: Adjective (Latin), tall

Specimens examined

Western Cape: Grootbank, near Keurboomstrand (this study, 04.x.2008: D55)

Eastern Cape: Double Mouth, (this study, 14.vii.2010: D767); Cape St Francis, (this study,

29.iii.2010: D686); Kowie, (Becker 26.xi.1895, BOL21453); Port Alfred, Piano Rocks,

(Stegenga 21.x.1987; BOL21886); Port Alfred, Piano Rocks, (Stegenga 03.xi.1987;

BOL21887); Port Alfred, Shark Bay, (Stegenga 03.xi.1987; BOL21885); Saltvlei, Port Alfred

(this study, 25.ii.2013: D1214, D1215)

Habitat: Epilithic species occurring from just below low tide level.

Description:

External morphology:

(Figure 3.7A): Plants medium to dark red, between (9-)10 and 29 (-40) cm high with one to several erect axes, attached by stoloniferous holdfast. Axes compressed, (1-2) (-3) mm wide and 0.5 – 1 mm thick, complanate and alternately branched up to 2-3 (-4) orders, distance between first order branches on the same side of main axis highly variable; ultimate branchlets wider (1-2.5 (-4) mm) than they are long (1-1.2 (-2) mm), with truncated apices; branches slightly constricted at the base.

Internal structure:

In cross-section axial and pericentral cells not distinguishable. Innermost cortical cells more extensive laterally with secondary pit connection in the longitudinal direction; medullary and innermost cortical cells larger than outermost cortical (Figure 3.7B); outermost cortical cells 10-60 μm across, visibly darker than innermost cortical and medullary cells, contain 5 or 6 small *corps en cerise* (Figure 3.7C).

Distribution:

South Africa: Grootbank to Port Alfred (South Coast) (this study); New Zealand and Australia (Saito and Womersley 1974)

Notes

It was suggested by Saito and Womersley (1974) and Womersley (2003) that the inclusion of South Africa (and other Indian Ocean localities) in the distribution of *Laurencia elata* would require further investigation. Limited knowledge pertaining to the diagnostic features of *Laurencia elata* as well as the high degree of morphological similarity and partial overlap in habitat between *L. flexuosa* (a South African endemic) and *L. elata* had previously masked the presence of the latter on the coasts of South Africa. In the absence of an Australian

specimen of *Laurencia elata* to verify the number of *corps en cerise* this study tentatively calls the clade of South African specimens *Laurencia cf. elata* on the basis of the morphological and anatomical (i.e. dimensions of the cell layers) similarities between South African specimens and the description of Saito and Womersley (1974). In addition the phylogenetic arrangement and genetic distance between *Laurencia flexuosa* and *L. cf. elata* discussed in Chapter 2 provides further evidence for the distinction of these clades. The high degree of morphological similarity between *L. flexuosa* and *L. cf. elata* may be indicative of convergent evolution in a response to environmental conditions on the warm temperate south coast of South Africa.

Laurencia flexuosa Kützing 1849: 856

Synonym: none

Misapplied names – (see Seagrief 1984: 38-39):

Chondria pinnatifida – Suhr 1834:733

Laurencia pinnatifida – Barton 1893:174. – Delf & Michell 1921:113

Chondria pinnatifida (Hudson) C. Agardh var. *angusta*. – Krauss 1846:211

Laurencia elata – Delf & Michell 1921:113

Type Locality: Cape of Good Hope, South Africa (Kützing 1849: 856)

Etymology: *flexuosa*: Adjective (Latin), zigzag, refers to alternating branching pattern (Stearn 1973).

Specimens examined:

Western Cape: Kalk Bay, (Bolton, 02.v.1985: BOL 21462); Kalk Bay, (Stegenga, 07.xii.1984: BOL 21476); Muizenberg-St. James, (Stegenga, 22.xi.1984: BOL 21474); St.

James, (Stegenga, 26.iii.1983: BOL 21473); Natures Valley (this study, 04.iv.2008: D57; 12.xii.2012: D1157); Koppie Alleen, De Hoop Marine Protected Area (this study, 18.viii.2008: D140; 17.ii.2011: D904); the Eiland near Keurboomstrand, (this study, 22.ix.2010: D862); East of Koppie Alleen, De Hoop Marine Protected Area (this study, 18.ii.2011: D928); Knysna Heads (this study, 12.xi.2012: D1133), Swartvlei near Sedgefield (this study, 15.xii.2012: D1192)

Eastern Cape: Port St Johns, (Simons, 27.vii.1965: BOL 21465); Haga Haga (this study, 22.xi.2012: D1120); Storms River, Tsitsikamma National Park (this study, 13.xii.2012: D1167); Port Elizabeth (this study, 14.xii.2012: D1191); Kenton-on-Sea (this study, 26.ii.2013: D1237); Three Sisters (this study, 27.ii.2013: D1252); Hluleka Nature Reserve (this study, 21.viii.2013: D1332; 23.viii.2013: D1336)

KwaZulu-Natal: Mabibi, intertidal (Engeldow, 11.viii.1999: BOL 21458); Zinkwazi Main Beach & Black Rock Park, (Coppejans *et al.* 30.viii.1999: BOL 2160); Mission Rocks (this study, 19.iii.2011: D958; 26.ix.2011: D1036); Palm Beach (this study, 28.ix.2011: D1063, D1057)

Habitat: Mid-to-low intertidal of wave-exposed coasts, forming stands as turfs or isolated tufts.

Description:

External morphology:

(Figure 3.8A): Plants dark red, between (3-) 5 and 12 cm high attached by stoloniferous holdfast, with one to several axes, flexuous, complanate and irregularly alternately branching in up to 3 orders; axes slightly compressed, (0.5-) 1 to 1.5 mm wide and 0.5 – 1 mm thick, short ultimate branchlets 0.5-2 (-3) mm wide near the base, with truncated apices; branch basal constriction absent. Distance between first order branches on same side of main axis

(1.5-) 2 – 5 (-10) mm; laterals increasing in length towards the holdfast to between (4-) 5 and 20 (-40) mm. Secondary and higher order branches much shorter (Figure 3.8B).

Internal structure:

In cross-section axial and pericentral cells not distinguishable: medullary cells without lenticular thickenings, innermost cortical cells taller than wide in cross-section 22-64 µm long and 18-34 µm wide, with secondary pit connections in the longitudinal direction; medullary and innermost cortical cells larger than outermost cortical cells (Figure 3.8C); outermost cortical cells 16.5-20 µm across, visibly darker than innermost cortical and medullary cells, usually containing 1 to 3 small *corps en cerise* but (Figure 3.8D).

Trichoblasts with single *corps en cerise* per cell (Figure 3.8E).

Distribution:

South Africa: False Bay and around the whole south coast, eastward into KwaZulu-Natal at least up to Mabibi; South African endemic.

Notes

Stegenga *et al.* (1997) compared *L. flexuosa* to *L. elata* – an Australian species with similar morphological features. The description of *L. flexuosa* in Stegenga *et al.* (1997) closely resembles that of *L. elata* as described by Saito & Womersley (1974:837-838) and Womersley (2003:475-477). On the other hand, Saito and Womersley (1974) and Womersley (2003) had hinted that the inclusion of South Africa (and other Indian Ocean localities) in the distribution of *Laurencia elata* required further investigation. The results of the phylogenetic analyses presented in Chapter 2 pointed to a genetic distance of 2.9% to 3.5% between the two South African morphotypes identified to *L. flexuosa* and *L. cf. elata*. The distinctiveness of the two species was further confirmed by anatomical differences, particularly that *L. flexuosa* has 1-3 *corps en cerise* (while *L. cf. elata* has 5-6 *corps en cerise*) and the outermost

cortical cells of *L. flexuosa* are smaller than those of *L. cf. elata*. As a result, *Laurencia flexuosa* must be considered to be distinct from *L. cf. elata*.

Laurencia glomerata Kützing 1849: 857

Basionym: *Chondria glomerata* Kützing 1847: 2

Heterotypic Synonym(s):

Chondria obtusa var. *virgata* C.Agardh 1822

Laurencia virgata (C.Agardh) J.Agardh 1852

Misapplied names:

Laurencia obtusa var. *pyramidalis* Harvey 1849: 83

Laurencia papillosa Barton 1893: 174

Type Locality: Cape Peninsula, South Africa (Kützing 1847a:2)

Etymology: *glomerata* Adjective (Latin), clustered in a round mass.

Specimens examined

Western Cape: Mauritz Bay (Rothman *et al.*, 02.03.2011: D1003, D1005), Stillbaai, (Stegenga, 18.x.2001: BOL21937), Goukamma Marine Reserve, (Stegenga, 13.x.2001: BOL 21939); Grootbank, near Keurboomstrand (this study, 04.x.2008: D56); Koppie Alleen, De Hoop Marine Protected Area (this study, 18.viii.2008: D125; 17.ii.2011: D902, D908, D909, D910); the Eiland near Keurbooms (this study, 22.ix.2010: D863); Nature's Valley (this study, 12.xii.2012: D1161, D1163, D1164, D1164, D1165, D1166)

Eastern Cape: Haga Haga (Stegenga, 26.x.1999: BOL 21942); Hluleka (Bolton, 26.vi.1983, BOL21952); Port Alfred (this study, 03.ix.2009: D317); Cape St. Francis (this study, 29.iii.2010: D685); Storms River, Tsitsikamma National Park (this study, 13.xii.2012: D1178, D1179a); Port Elizabeth (this study, 14.xii.2012: D1190); Saltvlei, Port Alfred (this study, 25.ii.2013: D1211, D1212); Three Sisters (this study, 27.ii.2013: D1251)

KwaZulu-Natal: Island Rock, (Coppejans *et al.* 17.viii.1999, BOL21944)

Habitat: Epilithic species present in the lower intertidal and shallow subtidal in exposed habitats.

Description:

External morphology:

(Figure 3.9A): Plants dark red, between 6 and 15 (-40) cm high, bushy with several erect axes, epilithic and attached by a basal coralloid holdfast. Axes terete, 1 to 3 mm wide, branched up to 3 orders, branching pattern varied between orders: first order branching spiral or polystichous; second and higher order branches much shorter and branching distichous, subopposite or subverticillate (Figure 3.9B), plants pyramidal in outline; first order branches increasing in length basipetally; ultimate branchlets approximately 0.5 mm wide and 1 mm long, with truncated apices; branch basal constrictions absent.

Internal structure:

In cross-section axial and pericentral cells not distinguishable: some medullary cells with lenticular thickenings, innermost cortical cells wider than high with secondary pit connection in the longitudinal direction; medullary and innermost cortical cells larger than outermost cortical cells (Figure 3.9C); outermost cortical cells longer than wide and containing 1 (rarely 2) *corps en cerise* per cell (Figure 3.9D).

Distribution:

South Africa: From Port Nolloth on the west coast into northern KwaZulu-Natal (this study)

Notes

Laurencia glomerata is the only species of the genus which occurs on the west coast of South Africa. West coast specimens (from Mauritz Bay) were much larger in size than specimens of the south and east coasts, but a genetic difference of only 0.1 to 0.3% confirmed them as *L.*

glomerata. West coast specimens tend to grow subtidally in rather sheltered bays. De Clerck *et al.* (2005) noted that fertile and bushy *L. flexuosa* may be confused with *L. glomerata* on the east coast, however the latter species is more crowded in higher order branches and branching often appears whorled. Phylogenetically, *L. glomerata* emerges as a distinct species.

Laurencia multiclavata Francis, Bolton, Mattio *et* Anderson, sp. nov. (Francis *et al.*, submitted)

Holotype: BOL 150569 South Africa, Western Cape, De Hoop, Koppie Alleen (34°26'03"S 20°32'52" E), intertidal Date collected 17.ii.2011

Etymology: Adjective (Latin) – ‘many studs’. This species is named for the several short, stud-like ultimate ramuli present on higher-order branches.

Specimens examined:

Western Cape: East of Koppie Alleen, De Hoop Marine Protected Area (Francis *et al.* submitted, 18.ii.2011: BOL 57723); Koppie Alleen, De Hoop Marine Protected Area (this study, 18.viii.2008: D127); Knysna Heads (this study, 12.xi.2012: D1135); Nature’s Valley (this study 12.xii.2012: D1159); Swartvlei, Port Alfred (this study, 15.xii.2012: D1194)

Eastern Cape: Cape St. Francis (this study, 29.iii.2010: D687); Port Elizabeth (this study, 14.xii.2012: D1185); Kenton-on-Sea (this study, 26.ii.2013: D1239); Hluleka, (this study, 21.viii.2013: D1335)

KwaZulu-Natal: Cape Vidal (Francis *et al.* submitted, 20.iii.2011: BOL 57724; 25.ix.2011: D1024); Jesser Point, Sodwana Bay (Francis *et al.* submitted, 22.iii.2011: BOL 57725;

D981); Mission Rocks (this study, 19.iii.2011: D960); Bhanga Rock, Bhanga Nek (this study, 4.x.2013: 1602)

Habitat: mid to low intertidal on exposed ledges

Description:

External morphology:

(Figure 3.10A): Plants epilithic, caespitose to erect, green with purple to pale-pink apices, up to 6 (-8) cm high, attached by a stoloniferous holdfast, with several primary axes, branches polystichously arranged, rarely up to 3 orders, branching subopposite to subverticillate with highest order short and wart-like; axes terete, 0.5 - 1 mm wide, short ultimate branchlets half the width of the main axes, with truncated apices; branch basal constriction absent.

Internal structure:

(Figure 3.10B) In cross-section axial and pericentral cells easily distinguishable. Innermost cortical and medullary cells two to three times larger than outermost cortical cells; innermost cortical cells ovoid with intercellular spaces visible. Lenticular cell wall thickenings not observed. Outermost cortical cells generally containing on average 3, but sometimes 2 or 4 *corps en cerise* per cell (Figure 3.10C); trichoblasts with 2 (sometimes 3) *corps en cerise* per cell (Figure 3.10D).

Distribution: South African endemic: south coast (from De Hoop extending eastward into northern KwaZulu-Natal).

Notes:

Laurencia multiclavata is superficially similar in morphology to *L. natalensis* Kylin and is therefore can be mistaken for the latter species in the field. Closer inspection has shown that *L. multiclavata* has a generally wider, polystichous branching pattern as opposed to the narrow, radial branching pattern of *L. natalensis*. Most notably *Laurencia multiclavata* lacks

the pyramidal outline and has fewer orders of branching than *L. natalensis*. The number of *corps en cerise* per outermost cortical cell (2 to 4) is also different to that of *Laurencia natalensis* (only 1 *corps en cerise*). Molecularly, *Laurencia multiclavata* is distinct and closely related to a sequence of *Laurencia cf. nidifica* J. Agardh from New Caledonia (with genetic distance 1.6-2.0%) and not to *Laurencia natalensis* (differing genetically by 3.3-5.8%).

Laurencia natalensis Kylin 1938: 24

Homotypic Synonym(s):

Laurencia obtusa var. *natalensis* (Kylin) Børgesen 1945

Misapplied names:

Chondria obtusa var. *gracilis* Suhr 1834:177 (*fide* Papenfuss notes in Silva *et al.* 1996)

Laurencia obtusa Hohenacker 1862: no 569 (*fide* Papenfuss notes in Silva *et al.* 1996)

Laurencia hybrida Barton 1893: 174 – Delf & Mitchell 1921: 113 (*fide* Papenfuss notes)

Type Locality: Isipingo, KwaZulu-Natal, South Africa (Kylin 1938: 24)

Etymology: (Adjective) Latin: from the (then) Natal Province

Specimens examined:

Western Cape: Knysna Estuary (this study, 04.vii.2008: D50); Swartvlei near Sedgfield (this study, 15.xii.2012: D1193); Nature's Valley, (Stegenga, 19.vi.1987: BOL21738; this study, 12.xii.2012: D1155)

Eastern Cape: Tsitsikamma, (Stegenga, 17.x.1997: BOL 21746); Port Alfred (Stegenga, 31.vii.1997: BOL 21736; this study, 03.ix.2009: D316); Port Alfred, Piano Rocks, (Stegenga, 04.xi.1997: BOL21735); Port Elizabeth (this study, 14.xii.2012: D1186); Kenton-on-Sea (this study, 26.ii.2013: D1238)

KwaZulu-Natal: Zinkwazi, (Coppejans *et al.*, 30.viii.1999: BOL 21734); Jesser Point, Sodwana Bay (this study, 10.vi.2009: D587; 09.xi.2010 : D800; 09.ix.2010: D820); Maphelane (this study, 09.xi.2010: D836, D853, D857); Mission Rocks (this study, 19.iii.2011: D960), Cape Vidal (this study, 25.ix.2011: D1022); Bhanga Rock, Bhanga Neck (this study 04.x.2013: D1603)

Habitat: Epilithic in mid to low intertidal zones in warm-temperate to tropical waters, often associated with sand-affected rock.

Description:

External morphology:

(Figure 3.11A): Plants epilithic, caespitose to erect, green with bright orange-red apices, up to 6 (-8) cm high, attached by stoloniferous holdfast, with several primary axes, branches radially arranged, branching up to 3 orders with higher order branching subopposite and/or subverticillate giving the thallus a pyramidal outline (Figure 3.11B); axes terete, 0.5 - 1 mm wide, laterals inserted at regular intervals of 1-3(-5) mm, distance between laterals increasing basipetally; short ultimate branchlets half the width of the main axes, with truncated apices; branch basal constriction absent.

Internal structure:

In cross-section axial and pericentral cells easily distinguishable: some medullary cells have lenticular thickenings, innermost cortical cells more extensive laterally with secondary pit connection in the longitudinal direction; medullary and innermost cortical cells larger than outermost cortical cells; outermost cortical cells wider than they are long and containing one *corps en cerise* per cell (Figure 3.11C). Trichoblasts with 1 *corps en cerise* per cell (Figure 3.11D)

Distribution: South Africa: From Pearly Beach eastward (Stegenga *et al.* 1997), along the whole south and east coasts, extending into southern Mozambique (Isaac 1958, Isaac & Chamberlain 1958, this study); Kenya, southern Madagascar (this study), Mauritius.

Notes

Laurencia natalensis is readily recognised by its bright orange-red apices and radial branching that lends the thallus a pyramidal outline. Amongst South African *Laurencia* species, *Laurencia natalensis* is significantly narrower than all other taxa except *L. sodwaniensis*. Molecularly, *Laurencia natalensis* appeared sister to the clade including *Laurencia glomerata* Kützinger, *Laurencia venusta* Yamada, *Laurencia dichotoma* sp. nov. and a single specimen referred to as *Laurencia* sp. morphotype A (Figure 2, Chapter 2). It is molecularly distinct from the species mentioned previously, differing genetically by 1.8-4.1%.

Laurencia pumila (Grunow) Papenfuss 1943: 91-92

Basionym: *Laurencia flexuosa* var. *pumila* Grunow 1867: 87-88

Type Locality: Port Natal (Durban), South Africa (Grunow 1867: 87-88)

Etymology: *Pumila* - Adjective (Latin), dwarf.

Specimens:

Eastern Cape: Mzamba, (Simons, 29.vi.1962: BOL21796)

KwaZulu-Natal: Ramsgate (Simons, 23.ix.1960: BOL21794); Jesser Point, Sodwana Bay (this study, 10.xi.2009: D588; 06.xi.2010: D803; 09.ix.2010: D822); Cape Vidal (this study, 25.ix.2011: D1028); Bhanga Rock, Bhanga Neck (this study, 04.x.2013: D1604; 07.x.2013: D1665)

Habitat: Epilithic in mid-intertidal regions of wave exposed coasts

Description:

External morphology:

(Figure 3.12A) Plants green with purple apices, up to 2.5 cm high, attached by small rhizoidal holdfast, with several axes that have club-shaped tips, unbranched when young, mature thalli branched up to 2 orders (Figure 3.12B); axes terete, 1-1.5 mm wide; short ultimate branchlets 0.5-2.2 (-3) mm wide near the base, with truncated apices; branch basal constriction absent.

Internal structure:

(Figure 3.12C) In cross-section axial and pericentral cells indistinguishable. Innermost cortical and medullary cells two to three times larger than outermost cortical cells; Innermost cortical cells round with visible intercellular spaces. Lenticular cell wall thickenings not observed. Outermost cortical cells generally containing 1 *corps en cerise* per cell (Figure 3.12D).

Distribution: South Africa: From Tsitsikamma eastward into northern KwaZulu-Natal (De Clerck *et al.* 2005); southern Mozambique (this study – see Chapter 4)

Notes

Laurencia pumila (Grunow) Papenfuss is morphologically distinguished from all other South African *Laurencia sensu stricto* species by the distinctive club-shaped apices of its axes and generally sparsely-branched thallus. Molecular analyses place *Laurencia pumila* in a sister-relationship with *Laurencia dehoopiensis* sp. nov. (Figure 2.3, Chapter 2) with a genetic distance of 0.4-0.8%. While the genetic differentiation between these two species is low, they occupy two different habitats – *Laurencia pumila* is common on the intertidal, while *L. dehoopiensis* sp. nov. is found subtidally as an epiphyte on *Phloiocaulon suhrii*. *Laurencia pumila* and *L. dehoopiensis* are also geographically separated with the former being distributed along the east coast and the latter on the south coast. The habitat differentiation and geographical separation alongside the significant morphological difference and molecular

evidence presented in Chapter 2 supports the designation of *Laurencia pumila* and *L. dehoopiensis* sp. nov. as distinct species in the South African flora.

Laurencia sodwaniensis Francis, Bolton, Mattio *et* Anderson, sp. nov. (Francis *et al.* submitted)

Holotype: BOL150570 South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Point (27°31'59"S, 32°40'59"E), intertidal. Date collected 20.iii.2011

Etymology: “*sodwaniensis*” – after the type locality, Sodwana Bay (KwaZulu-Natal)

Habitat: Mid to low intertidal of the northern KwaZulu-Natal coast.

Description:

External morphology:

(Figure 3.13A): Plants epilithic, small, turf-like in appearance, up to 5 cm in height, attached by a discoid holdfast, thallus light pink in colour; main axes terete, 0.5 – 1 mm in diameter, laterals radially arranged and inserted at regular intervals, laterals increasing in length basipetally giving thalli a pyramidal outline (Figure 3.13B) tending to curve toward main axis; well-branched in higher orders, branching pattern from subopposite to subverticillate and truncated; branch basal constriction absent.

Internal structure:

Axial and pericentral cells distinguishable in cross section (Figure 3.13 E); innermost cortical and medullary cells at least two times larger than outermost cortical cells; outermost cortical cells longer than broad, approximately 18-25 µm in width, darker (Figure 3.13C) and containing on average 3, but sometimes 2 or 4 *corps en cerise* per outermost cortical cell (Figure 3.13D).

Distribution: South African endemic: Sodwana Bay, Northern KwaZulu-Natal

Notes:

This species was only collected from Northern KwaZulu-Natal. Turf-like, with a light pink thallus, it shares certain morphological features with *Laurencia natalensis* and *L. glomerata*, such as a pyramidal outline as a result of their radial branching with branch length increasing basipetally. Unlike *L. natalensis* and *L. glomerata*, the branching pattern of *L. sodwaniensis* is not as neat and the ultimate laterals are not as distinctly wart-like as in *L. natalensis*. While sharing some morphological traits with the aforementioned species, molecularly this species is distinct and not closely-related to either of them (differing genetically by 5.6-7.4%). The closest *rbcL* relative (with genetic distance 1.6-1.9%) is a sequence of *Laurencia dendroidea* from Spain.

Laurencia stegengae Francis, Bolton, Mattio *et* Anderson, nom. nov. (Francis *et al.* submitted)

Basionym: *Laurencia peninsularis* Stegenga, Bolton *et* R.J. Anderson *nom. illeg.*, Contributions from the Bolus Herbarium, 18: 538. 1987.

Holotype: BOL150062/G190 South Africa, Western Cape, Cape Peninsula, Clovelly, intertidal. Date collected 13.x.2000

Etymology of new name “*stegengae*” – after Dr. Herre Stegenga, Dutch phycologist who has made enormous contributions to our knowledge of South African seaweeds.

Specimens examined:

Western Cape: Stilbaai (Stegenga *et al.*, 18.x.2001: BOL21788); Nuwebaai (Stegenga *et al.*, 16.x.2001: BOL21787); Koppie Alleen, De Hoop Marine Protected Area (this study, 18.viii.2008: D126, D159; 17.ii.2011: D900, D901); Buffels Bay (this study, 17.ix.2008: D181); Clovelly (this study, 18.iii.2010: D680); Platbank Keurboomstrand (this study,

23.ix.2010: D872); Langebaan Leentjies (this study, 26.iii.2012: D1073, D1074); Knysna Heads (this study, 12.xi.2012: D1134); Nature's Valley (this study, 12.xii.2012: D1156)
Eastern Cape: Cape Padrone (Stegenga *et al.*, 26.x.2003: BOL21785); Storms River, Tsitsikamma National Park (this study, 13.xii.2012: D1170); Three Sister (this study, 27.ii.2013: D1254)

Habitat: Wave-exposed situations in the mid to lower intertidal.

Description:

External morphology:

(Figure 3.14A) Plants generally caespitose to sometimes erect, olive green to greyish violet, up to 4 cm high, epilithic, attached by tangled holdfast, with one to several axes, first order branches polystichously arranged; 2 orders of branching, secondary laterals very short, ultimate branchlets small, wart-like and crowded (Figure 3.14B); axes terete, up to 1.5mm in diameter, apices truncated; slight constriction at the base of branches.

Internal structures:

Axial and pericentral cells not observed. Medullary and innermost cortical cells up to two times larger than outermost cortical cells (Figure 3.14C); innermost cortical cells ovoid; medullary cells without lenticular cell wall thickenings; outermost cortical cells containing a single *corps en cerise* per cell (Figure 3.14D).

Distribution: South African endemic; from False Bay to East London (Stegenga *et al.* 1997; this study)

Notes

This endemic South African species was originally described by Stegenga *et al.* (1997) and named *Laurencia peninsularis*. However, this name had been used by Taylor (1945) for a different taxon which rendered the epithet of the South African species illegitimate. We

propose the new name *Laurencia stegengae*. Molecular analyses in this study have shown this *Laurencia sensu stricto* species to be genetically distinct; it differed from its closest relative, *Laurencia* cf. *corymbosa*, by 3.3% to 3.5%. There may be some geographic genetic distinction between populations on the southwest and south coasts, but more studies are needed to confirm the observed pattern.

Taxonomic Key to the South African species of *Laurencia sensu stricto*

- 1. Axes and laterals slightly to strongly compressed2
 - Axes and laterals terete5
- 2. Axes oppositely branched.....3
 - Axes alternately branched.....4
- 3. Three to four orders of branching, thallus between (5-) 6 and 20 (-22) cm in length
 - *Laurencia complanata*
 - Two to three orders of branching, thallus between (2.7-) 3 and 5 cm in length
 - *Laurencia brongniartii*
- 4. Irregularly alternate branching pattern, outermost cortical cells with 1-3 *corps en cerise* *Laurencia flexuosa*
 - Alternate branching pattern, outermost cortical cells with 5-6 *corps en cerise*
 - *Laurencia* cf. *elata*
- 5. Thalli usually larger than 7cm in length with coralloid holdfasts6
 - Thalli no larger than 7cm in length with discoid, rhizoidal or stoloniferous holdfasts
 - 8

6. Primary laterals spirally arranged, whorled or alternate branching in higher order
 laterals *Laurencia glomerata*
 Plants with polystichous branching pattern, alternate to opposite in higher order
 laterals7
7. Plants epiphytic on *Phloiocaulon sp.* in the subtidal, ultimate branchlets very short,
 wart-like *Laurencia dehoopiensis sp. nov.*
 Plants epilithic, forming turfs in the intertidal, ultimate branchlets exceptionally
 corymbose at times *Laurencia cf. corymbosa*
8. Thallus cartilaginous *Laurencia dichotoma sp. nov.*
 Thallus fleshy9
9. Axis width up to 1.5 mm10
 Axis width equal to or less than 1 mm11
10. Plants olive-brown, generally caespitose with short, closely-branched primary laterals
 bearing wart-like ultimate branchlets *Laurencia stegengae nom. nov.*
 Plants olive with purple distal ends, erect with club-shaped apices; unbranched when
 young, distal end of branchlet with subverticillate branching in mature plant
 *Laurencia pumila*
11. Laterals mostly adaxially arranged on axes, branching pattern alternate to
 subverticillate *Laurencia digitata sp. nov*
 Laterals radially arranged on axes, branching subopposite to subverticillate in higher
 orders 12
12. Plants epilithic, erect with pink thalli, laterals tend to curve strongly up toward central
 axis *Laurencia sodwaniensis sp. nov.*
 Plants epilithic, often caespitose with bi-coloured thalli, laterals with very little to no
 curvature towards central axis 13

13. Pyramidal outline, usually 3 orders of branching, 1 *corps en cerise* per outermost cortical cell

Laurencia natalensis

Bushy, rarely 3 orders of branching, 2-4 *corps en cerise* per outermost cortical cell

..... ***Laurencia multiclavata sp. nov***

New records for the *Laurencia* complex and additional notes

In addition to the fourteen *Laurencia sensu stricto* species described above, five specimens corresponding to five morphotypes could not be identified to any known species and additional specimens are needed to confirm them as different and new species. These are *Laurencia* sp. morphotype A (D991), *Laurencia* sp. morphotype G (D821), *Laurencia* sp. morphotype H (D1240& D1255), *Laurencia* sp. morphotype I (D1337) and *Laurencia* sp. morphotype J (D1339). Five new records, three for the genus *Palisada* (*Palisada* sp. 1 D819, *Palisada* sp. 2 D1361 & D1669 and *Palisada* cf. *corallopsis*), two for *Laurenciella* (*Laurenciella marilzae* and *Laurenciella* sp. D1077 & D1160) and one for *Chondrophycus* (*Chondrophycus* sp. D802) were also recorded (see Chapter 2).

While the above taxa are mostly not identified to species level nor described in text above, a table of morphological and anatomical features as well as representative pictures (as far as possible) are provided for reference (See Table 3.1 and Figure 3.15). Images of the sections for *Laurencia* sp. ‘morphotype H’ and *Palisada* sp. 2 and *P. cf. corallopsis* are not supplied

Table 3.1: Morphology and anatomy of additional undescribed South African taxa in the *Laurencia* complex Abbreviations: u.t.: up to, M:

Marked constriction, S: Slight constriction, N: No constriction, PC: Pericentral cell, A: Absent, P: Present, CeC: *Corps en Cerise*, Co: Cortical, Me: Medullary S: Subtidal, I: Intertidal. Distribution abbreviations: SDW: Sodwana Bay, DM: Double Mouth, DH: De Hoop, PA: Port Alfred, PE: Port Elizabeth, TS: Three Sisters, HLU: Hluleka, BN: Bhanga Nek, KoS: Kenton-on-Sea, BR: Bordjiesrif.

Taxa	Characters									
	Substratum	Size of mature thallus (cm)	Thallus colour	Holdfast	Axes terete or flattened	Main Axis width (mm)	Branch basal constriction (M/S/N)	General branching pattern	Branching pattern 2 nd Order Laterals	PC No.
<i>Laurencia</i> sp. A	Epilithic	u.t. 3	Pink-red	Discoid	Terete	u.t. 1	N	Polystichous	Scattered	4
<i>Laurencia</i> sp. G	Epilithic	u.t. 4	Olive green, pink tips	?	Terete	u.t. 0.7	N	Polystichous to alternate	Scattered, tips subverticillate	4
<i>Laurencia</i> sp. H	Epilithic	u.t. 3	Dark red-purple	Discoid	Terete	u.t. 1.2	N	Polystichous	(Sub)opposite to alternate	?
<i>Laurencia</i> sp. I	Epilithic	u.t. 3	Dark red-purple	?	Terete	u.t. 0.5	N	Alternate	Opposite	4
<i>Laurencia</i> sp. J	Epilithic	u.t. 3	Dark purple	?	Terete	u.t. 1	N	Alt. to sub-opp.	Opposite	4
<i>Palisada</i> sp. 1	Epilithic	2	Dark Red	Discoid	Terete	u.t.0.9	M	Polystichous	scattered	2
<i>Palisada</i> sp. 2	Epilithic	4.9-7.1	Dark Red	Discoid	Terete	u.t. 2	S	Pinnate	opposite	?
<i>Palisada</i> cf. <i>corallopsis</i>	Epilithic	u.t 10.9	Dark Red	Discoid	Terete	u.t. 1.5	S	Dichotomous	Dichotomous	?
<i>Chondrophyucus</i> sp.	Epilithic	1.5	Dark Red	?	Terete	u.t. 3	N	Polystichous	Dichotomous/wartlike	2
<i>Laurenciella marilzae</i>	Epilithic	u.t. 7	Red with light orange tips	Discoid	Terete	u.t. 0.8	N	Irreg. alternate to opposite	(sub-)opposite	4

Table 3.1: Morphology and anatomy of additional undescribed South African taxa in the *Laurencia* complex (continued): Abbreviations: u.t.: up to, M: Marked constriction, S: Slight constriction, N: No constriction, PC: Pericentral cell, A: Absent, P: Present, *CeC*: *Corps en Cerise*, Co: Cortical, Me: Medullary S: Subtidal, I: Intertidal. Distribution abbreviations: SDW: Sodwana Bay, DM: Double Mouth, DH: De Hoop, PA: Port Alfred, PE: Port Elizabeth, TS: Three Sisters, HLU: Hluleka, BN: Bhanga Nek, KoS: Kenton-on-Sea, BR: Bordjiesrif.

Taxa	Characters								
	<i>Corps en cerise</i>	No. of <i>CeC</i>	Outermost cortical cell shape	Cell Size (μm)		Space between Co & Me cells	2 nd Pit conn.	Distribution	Habitat
				Outermost cortical cell Length x Width	Innermost Cortical Length x Width				
<i>Laurencia</i> sp. A	P	1	Dome	26.5-41x11-29	38-50x35-70	Yes	P	SDW	S
<i>Laurencia</i> sp. G	P	2-3	Dome	23.5-41x21-29	41-67.6x53-85	Yes	P	SDW	I
<i>Laurencia</i> sp. H	?	?	?	?	?	?	?	HLU	I
<i>Laurencia</i> sp. I	P	1	Dome	26-50x9-20.5	47-76x23.5-50	Yes	P	KoS-TS	I
<i>Laurencia</i> sp. J	P	1	Dome	20.5-29x23-35	44-73.5x47-79	Yes	P	HLU	I
<i>Palisada</i> sp. 1	A	-	Elongated, Palisade	17.6-110x9-21	?	No	A	SDW	I
<i>Palisada</i> sp. 2	-	-	?	?	?	?	?	BN	I
<i>Palisada</i> cf. <i>corallopsis</i>	-	-	?	?	?	?	?	TS	I
<i>Chondrophycus</i> sp.	A	-	Round-Oval	18-26.5x9-23.5	?	No	A	SDW	I
<i>Laurenciella marilzae</i>	P	1	Oval	29-47x29-61	?	No	A	BR-KoS	I

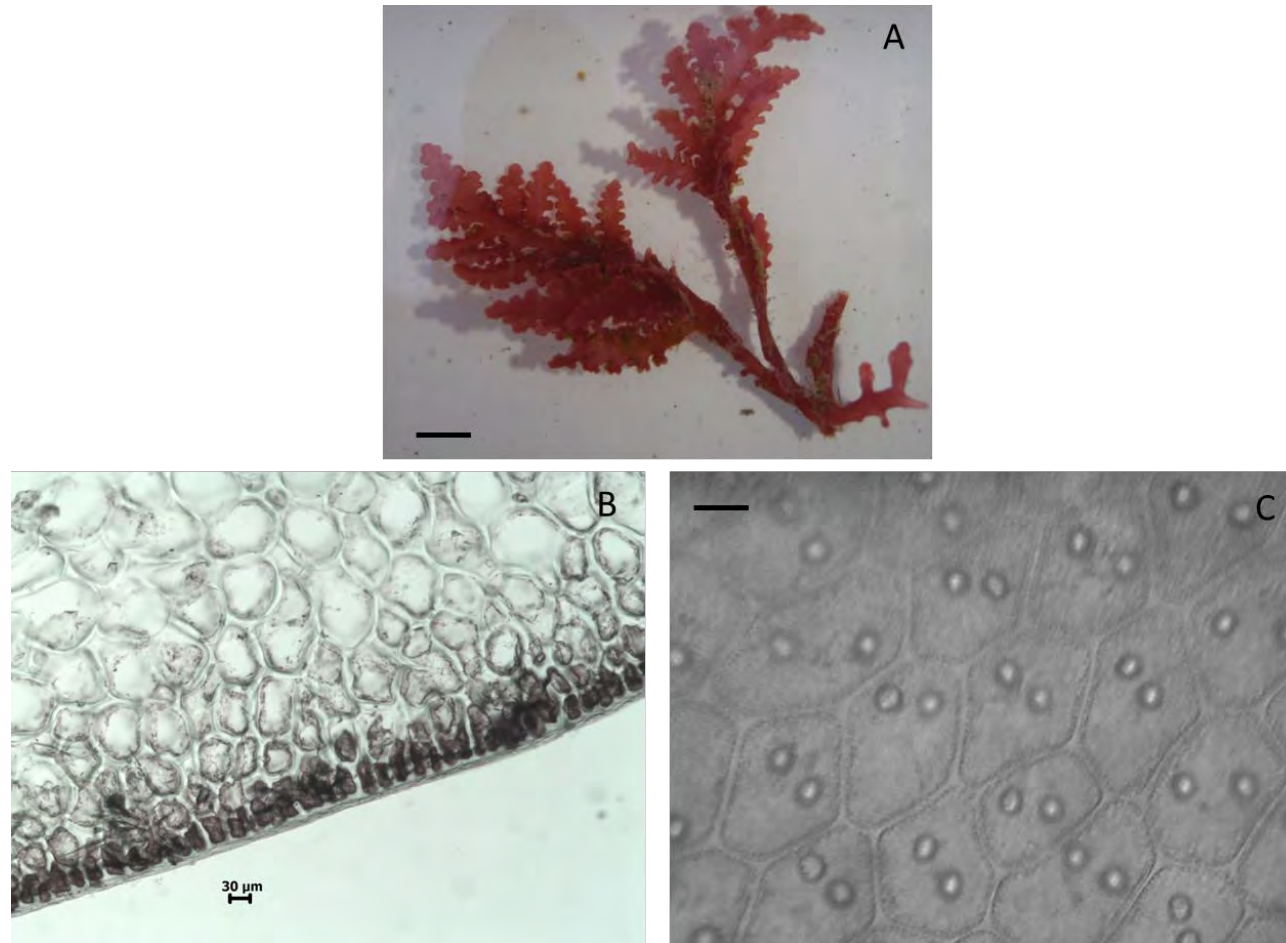


Figure 3.1: *Laurencia brongniartii*. A) Habit. (1x) Scale Bar: 1cm = 8.2mm B) Cross section of the thallus at (40x) C) Two *corps en cerise* in each outermost cortical cell (40x) Scale bar: 1cm=30µm

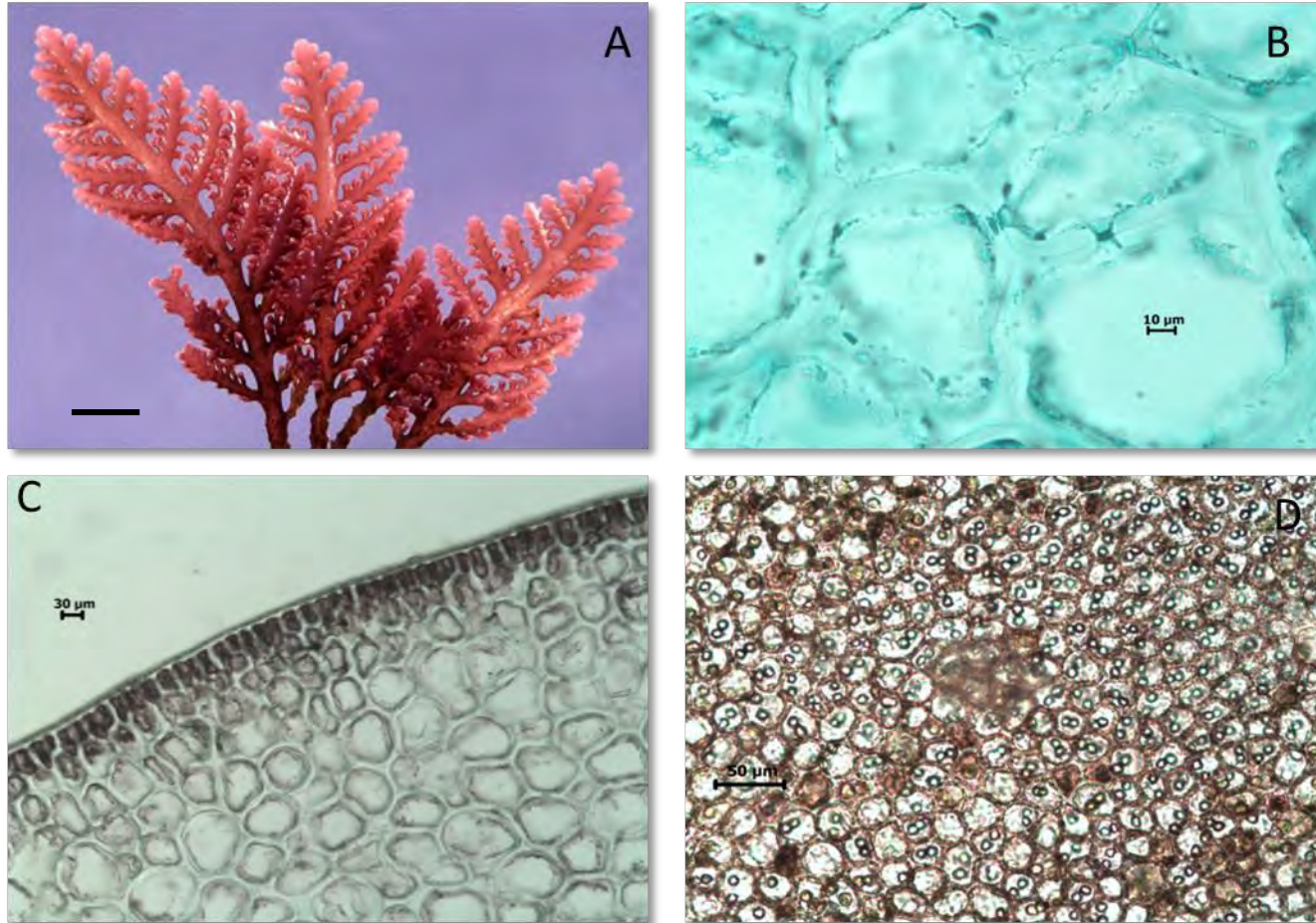


Figure 3.2: *Laurencia complanata*: A) Habit (1x) Scale 1 cm= 5 mm B) Secondary pit-connections between cortical cells (40x). C) Transverse section of thallus showing outermost cortical and cortical cells (20x). D) One to two *corps en cerise* per outermost cortical cell (20x)

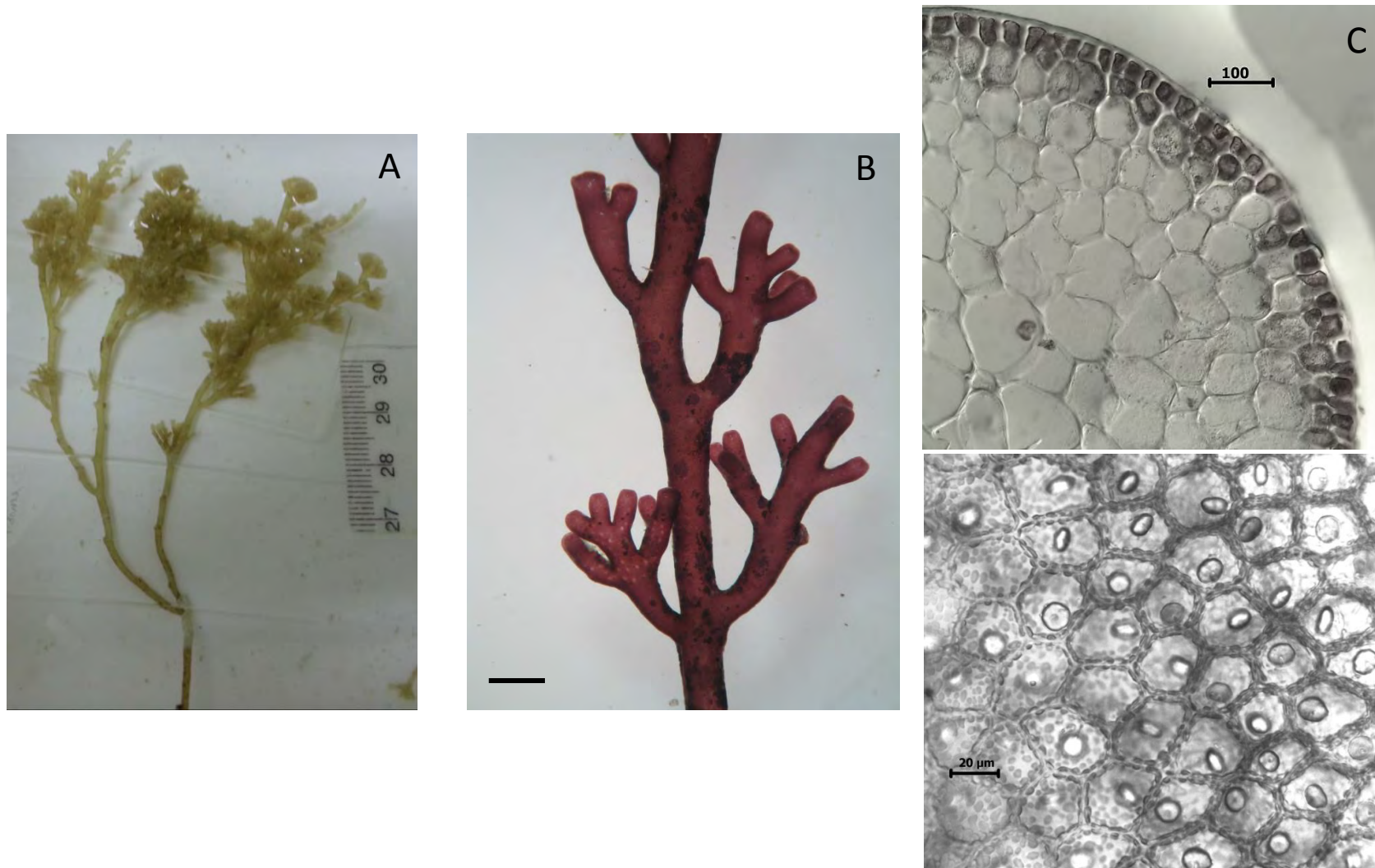


Figure 3.3: *Laurencia* cf. *corymbosa* morphotype ‘M’: A) Habit (1x) Scale 1 division = 1mm B) Branching pattern with corymbose second order laterals (8x) Scale bar: 1cm = 360µm C) Cross section through thallus showing outermost cortical cells and spaces between medullary and cortical cells D) One *corps en cerise* per cell at (40x)

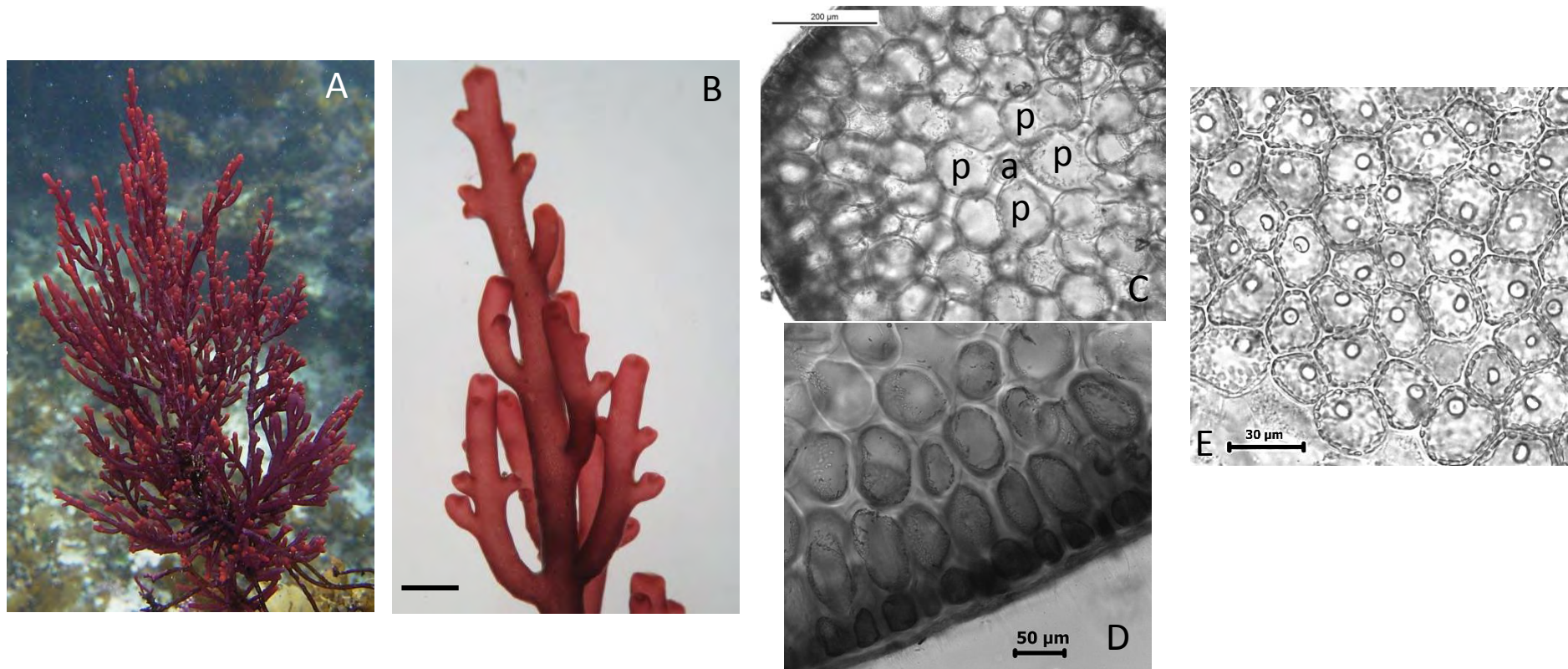


Figure 3.4: *Laurencia dehoopiensis* sp. nov. A) Habit. B) Polystichous branching pattern (8x). Scale: 1 cm = 360 μ m. C) Pericentral (p) and axial (a) cells (20x) D) Outermost cortical and cortical cells (20x). E) A single *corps en cerise* per cell (40x)

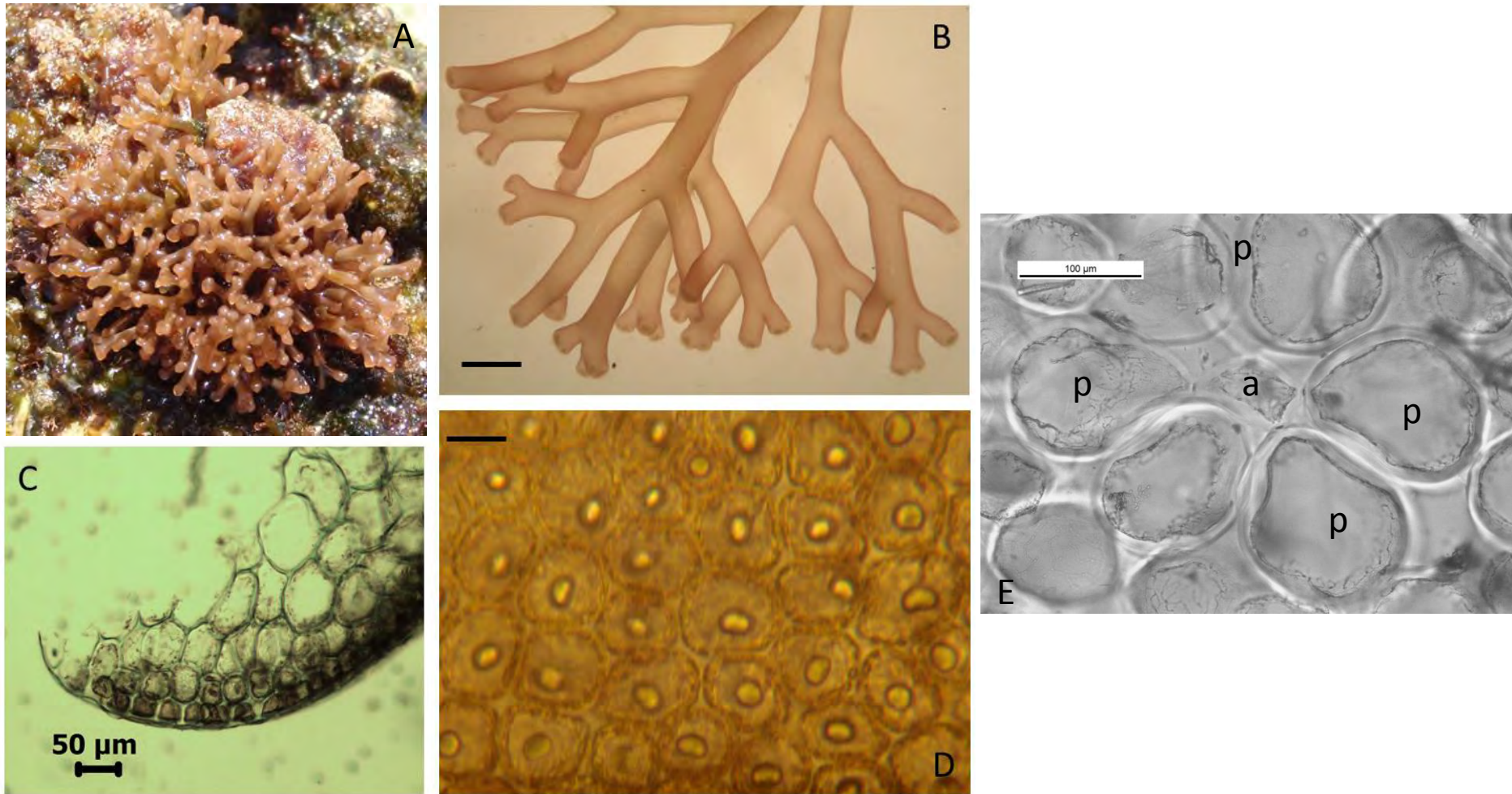


Figure 3.5: *Laurencia dichotoma* sp. nov.: A) Habit. B) Branching pattern with dichotomous branching in second order laterals (8x) Scale : 1cm = 85 μ m C) Outermost cortical and cortical cells (20x) D) One *corps en cerise* per outermost cortical cell. (40x) Scale : 1cm = 20 μ m E) Axial (a) and pericentral cells (p) (40x)

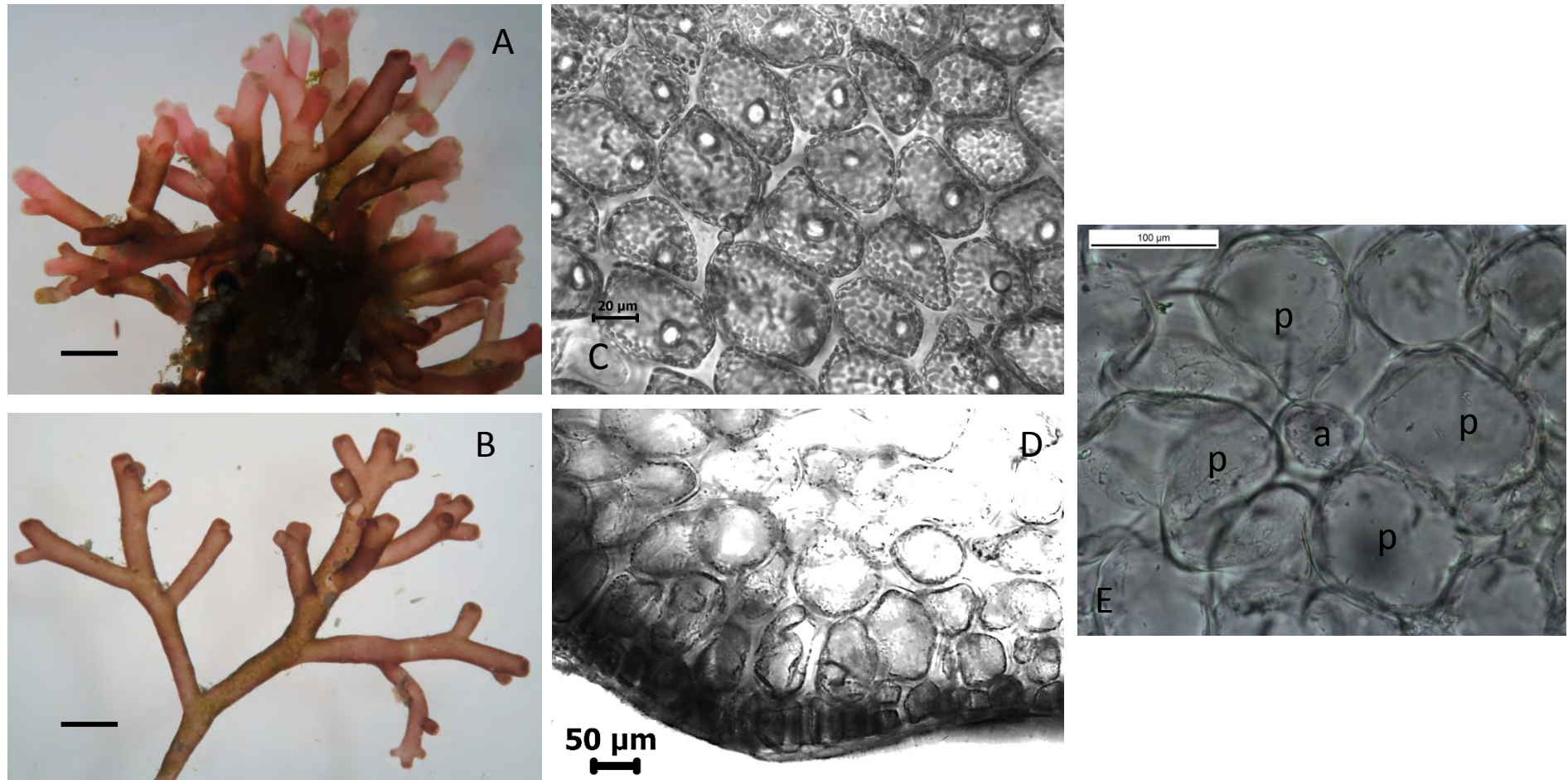


Figure 3.6: *Laurencia digitata* sp. nov. : A) Habit (8x) Scale: 1cm = 62µm. B) Subverticillate branching pattern at apices (8x) Scale: 1cm = 62µm C) A single *corpus en cerise* per cell (40x). D) Cross section of thallus showing outermost cortical and cortical cells (20x) E) Axial (a) and pericentral cells (p) (40x)

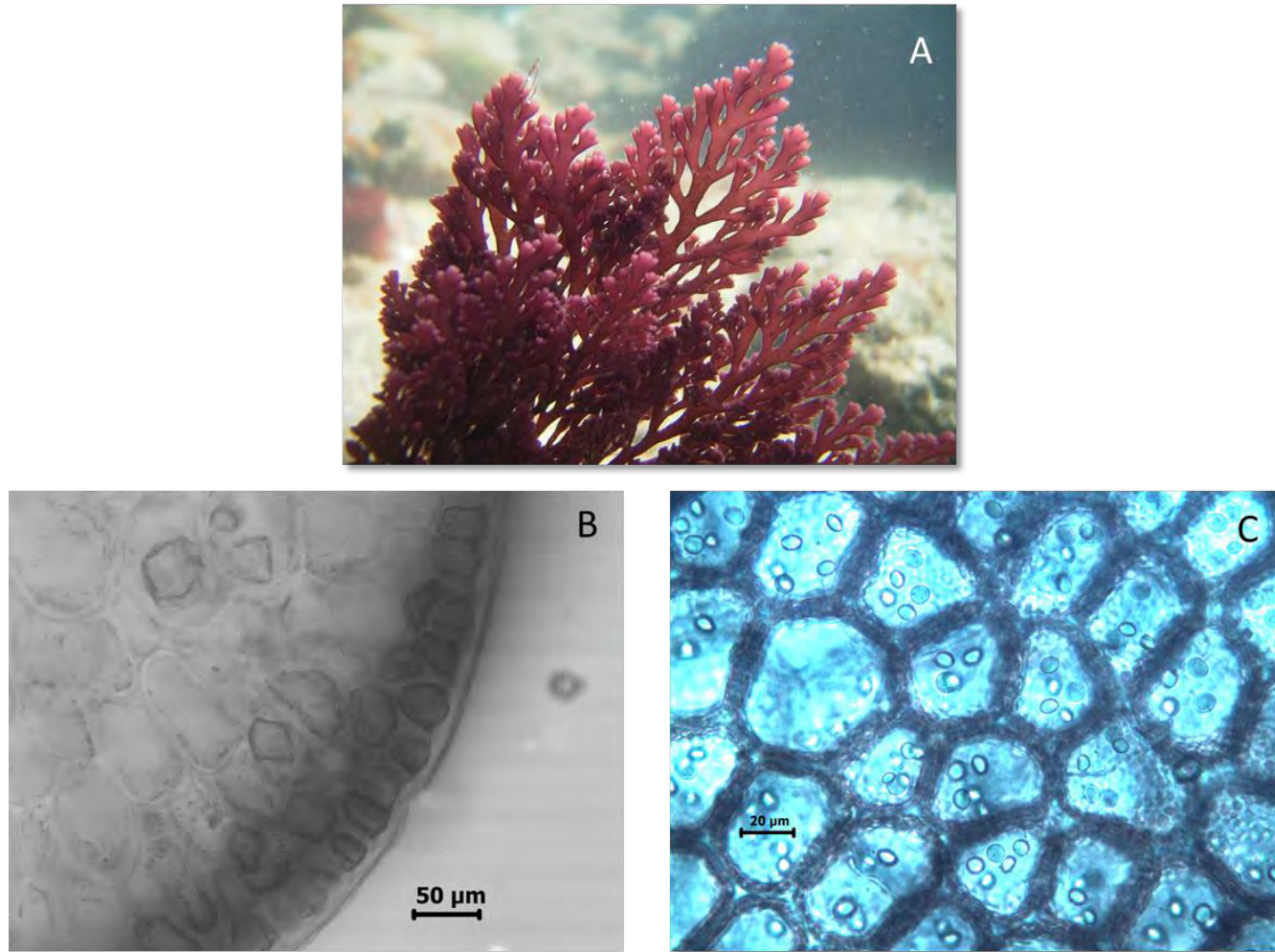


Figure 3.7: *Laurencia* cf. *elata*: A) Habit. B) Cross section through the thallus showing outermost cortical cells and elongated medullary cells (40x) C) 5-6 *corps en cerise* per outermost cortical cell (40x)

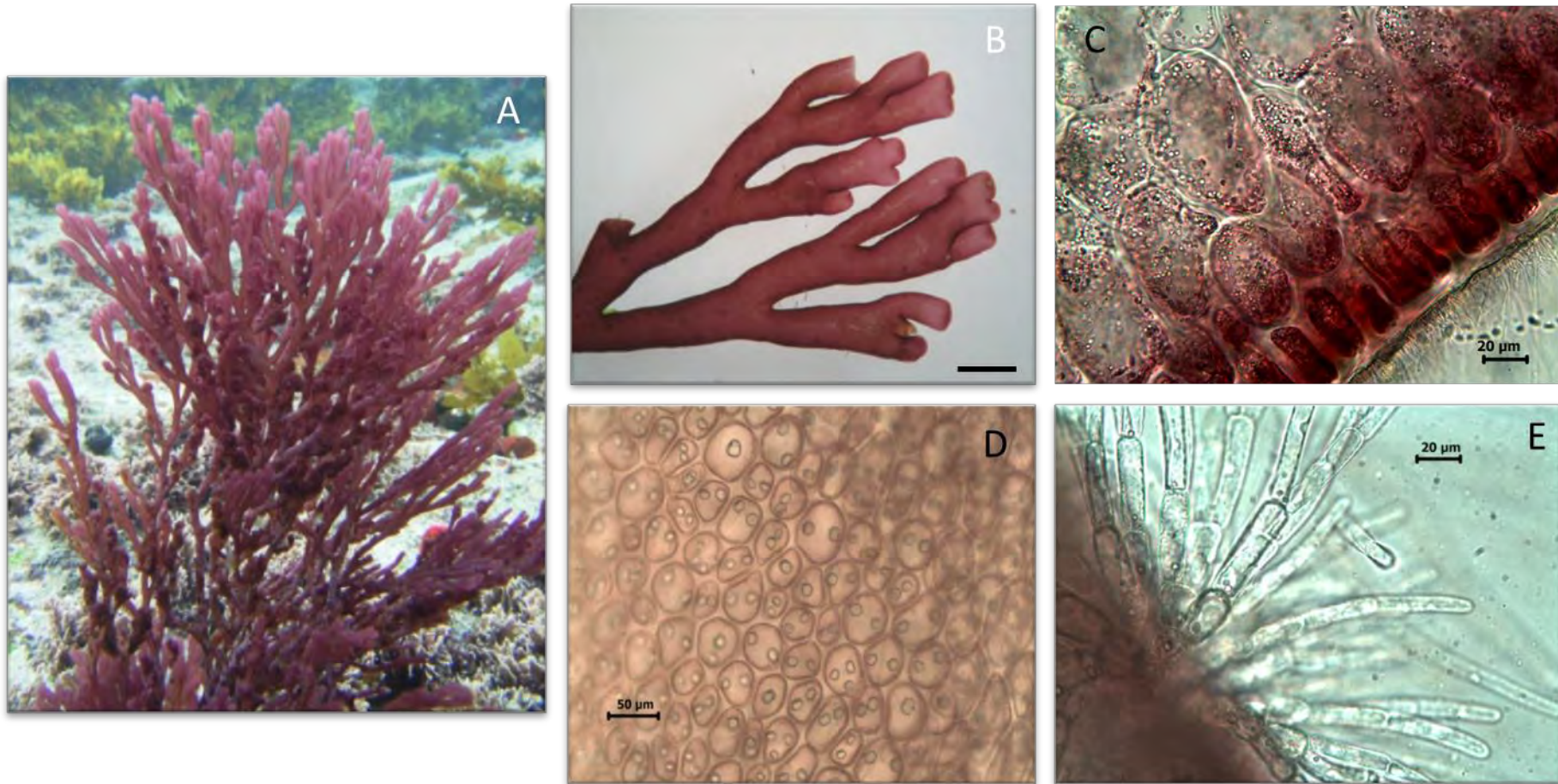


Figure 3.8: *Laurencia flexuosa*: A) Habit. B) Short higher order branching (x8). Scale bar 1cm = 360μm C) Transverse section through thallus (40x). D) One to three *corps en cerise* per outermost cortical cell (20x) E) Trichoblasts with a single *corps en cerise*. (40x)

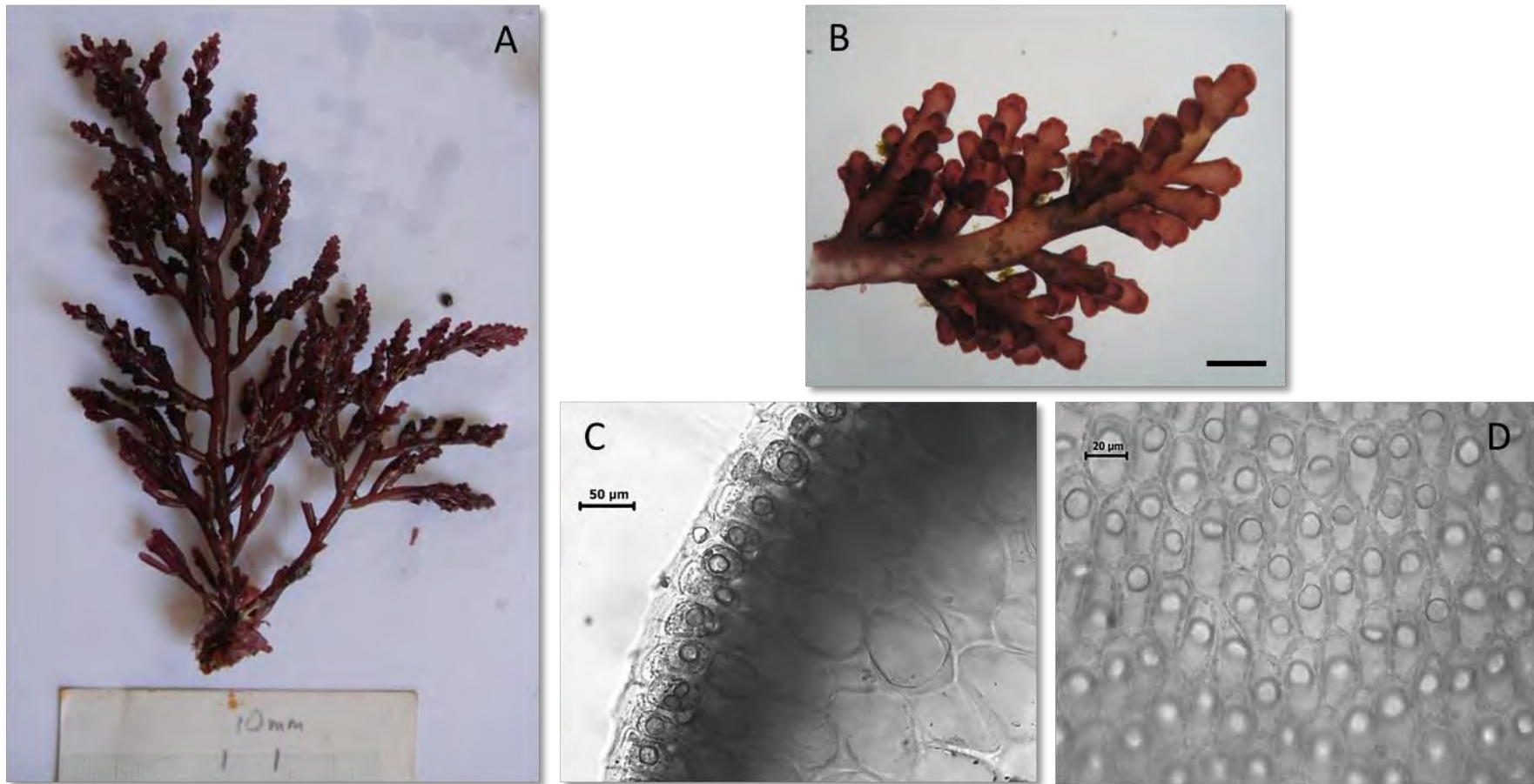


Figure 3.9: *Laurencia glomerata*: A) Habit. B) Branching pattern (x8). Scale bar: 1cm=360μm C) Transverse section of thallus showing outermost cortical cells (with *corps en cerise*) and cortical cells (20x) D) One *corps en cerise* per outermost cortical cell (40x)

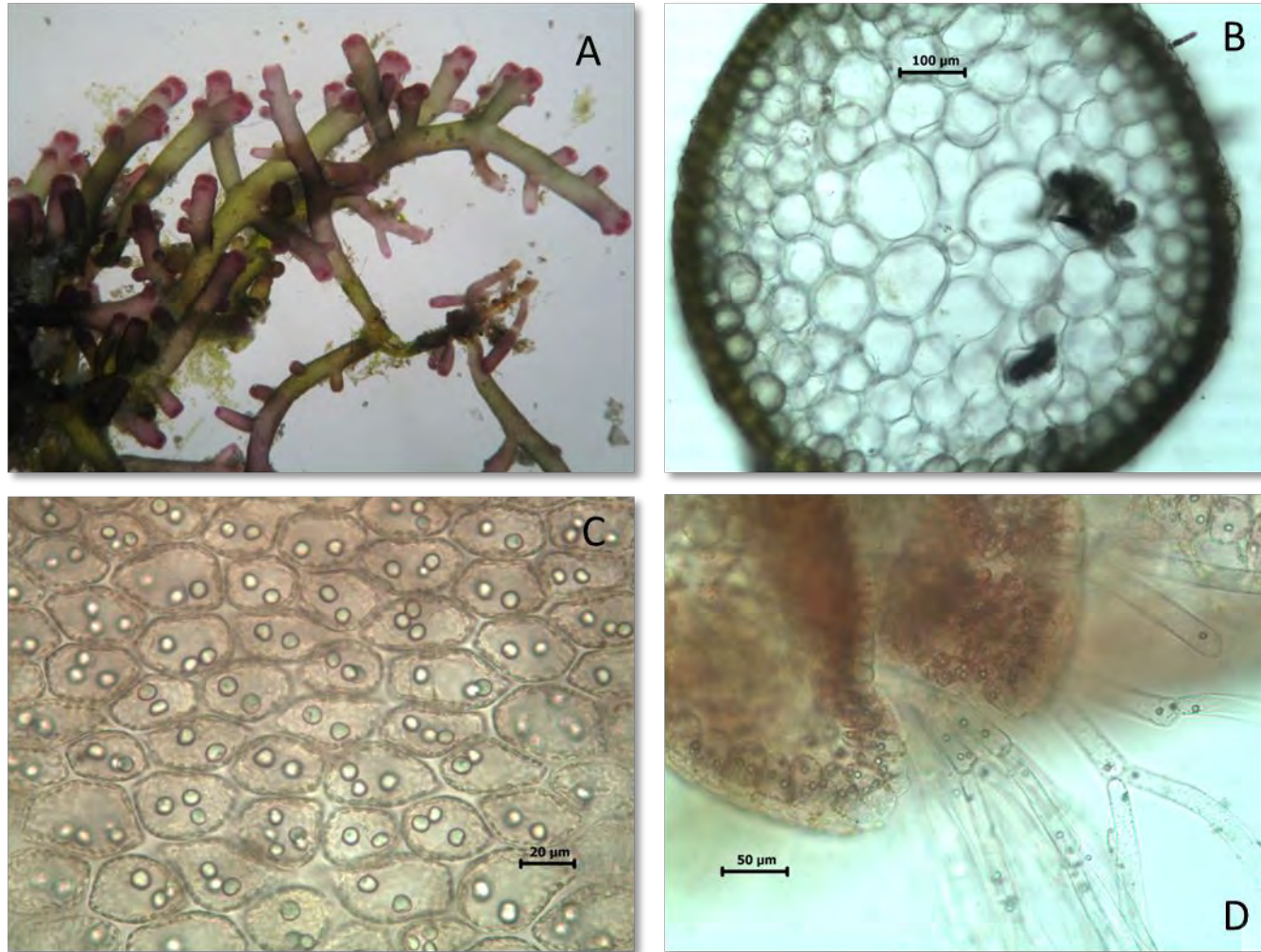


Figure 3.10: *Laurencia multiclavata* sp. nov. : A) Thallus; showing dark pink tips (8x) Scale: 1 cm = 240µm. B) Transverse section of thallus (10x). C) 2-3 *corps en cerise* per outermost cortical cell (40x). D) Trichoblasts with two *corps en cerise* per cell (20x)

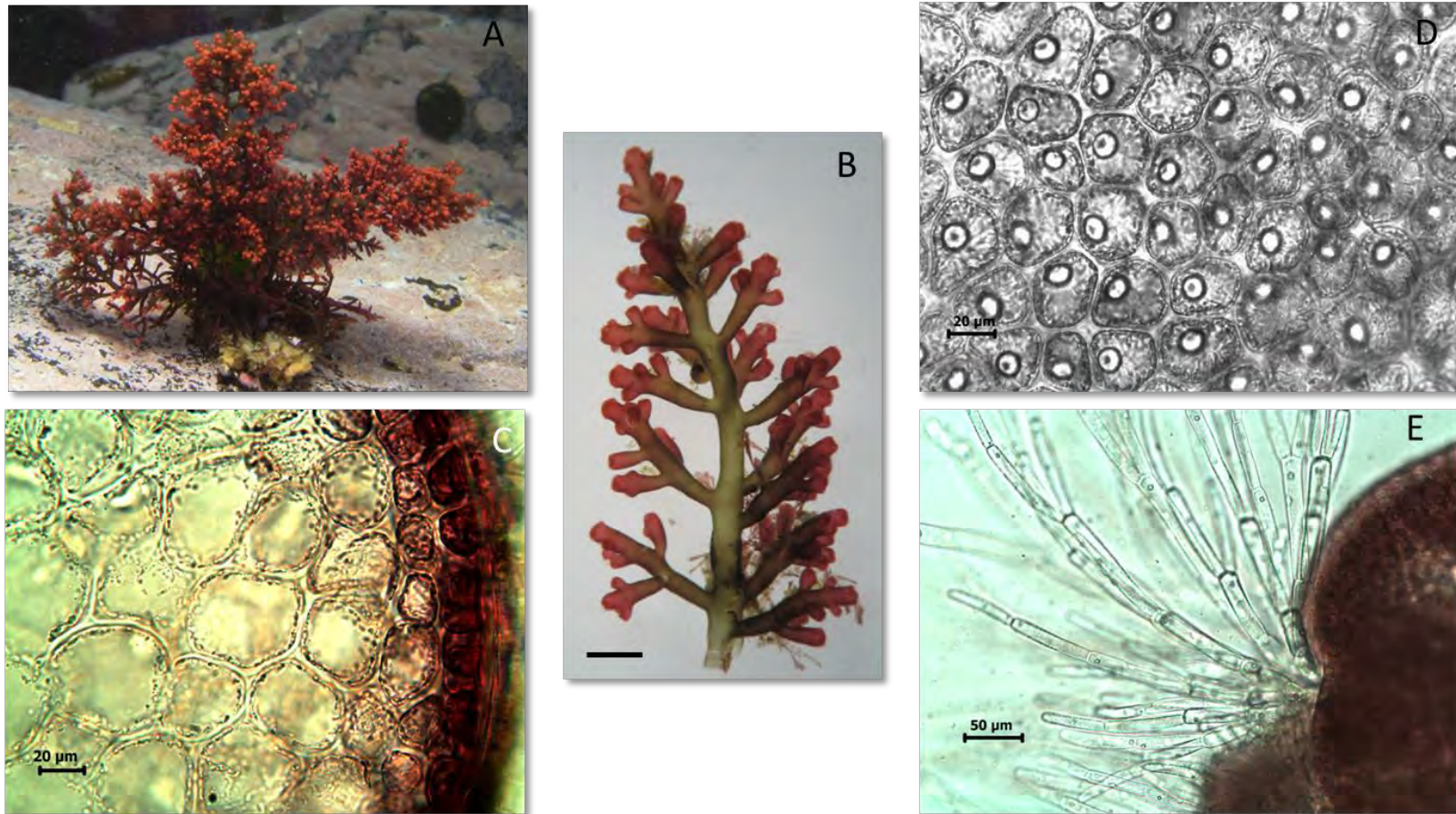


Figure 3.11: *Laurencia natalensis*: A) Habit. B) Subopposite to subverticillate branching pattern (x8). Scale bar: 1cm = 200μm C) Transverse section through thallus showing outermost cortical and cortical cells. (40x) D) One *corps en cerise* per outermost cortical cell (40x) E) Trichoblasts with single *corps en cerise* per cell (20x)

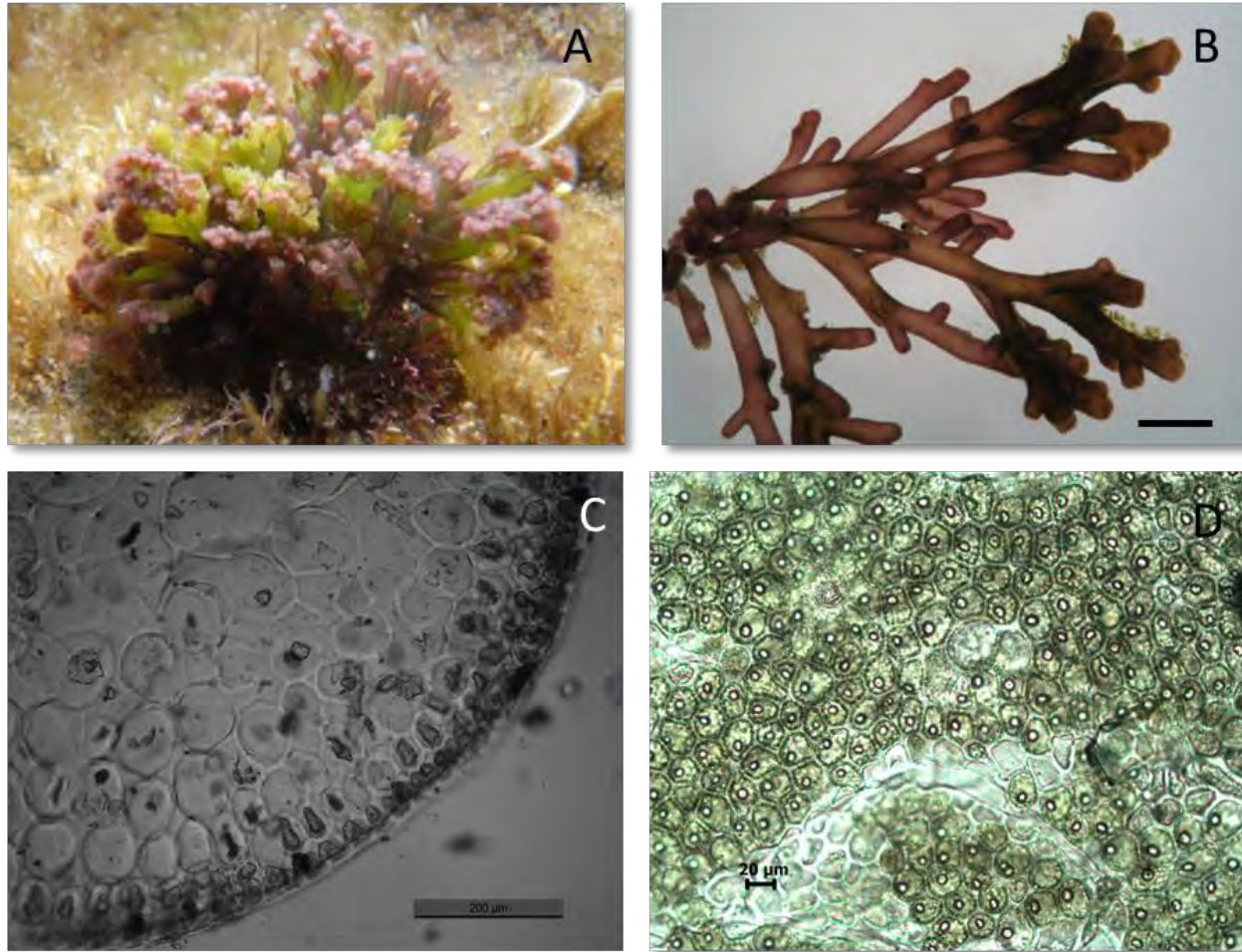


Figure 3.12: *Laurencia pumila*: A) Habit. B) Club-shaped apices on ultimate branchlets (8x). Scale bar: 1 cm = 220µm C) Cross section through the thallus showing outermost cortical cell as well as cortical and medullary cells (20x) D) One *corps en cerise* per outermost cortical cell (40x)

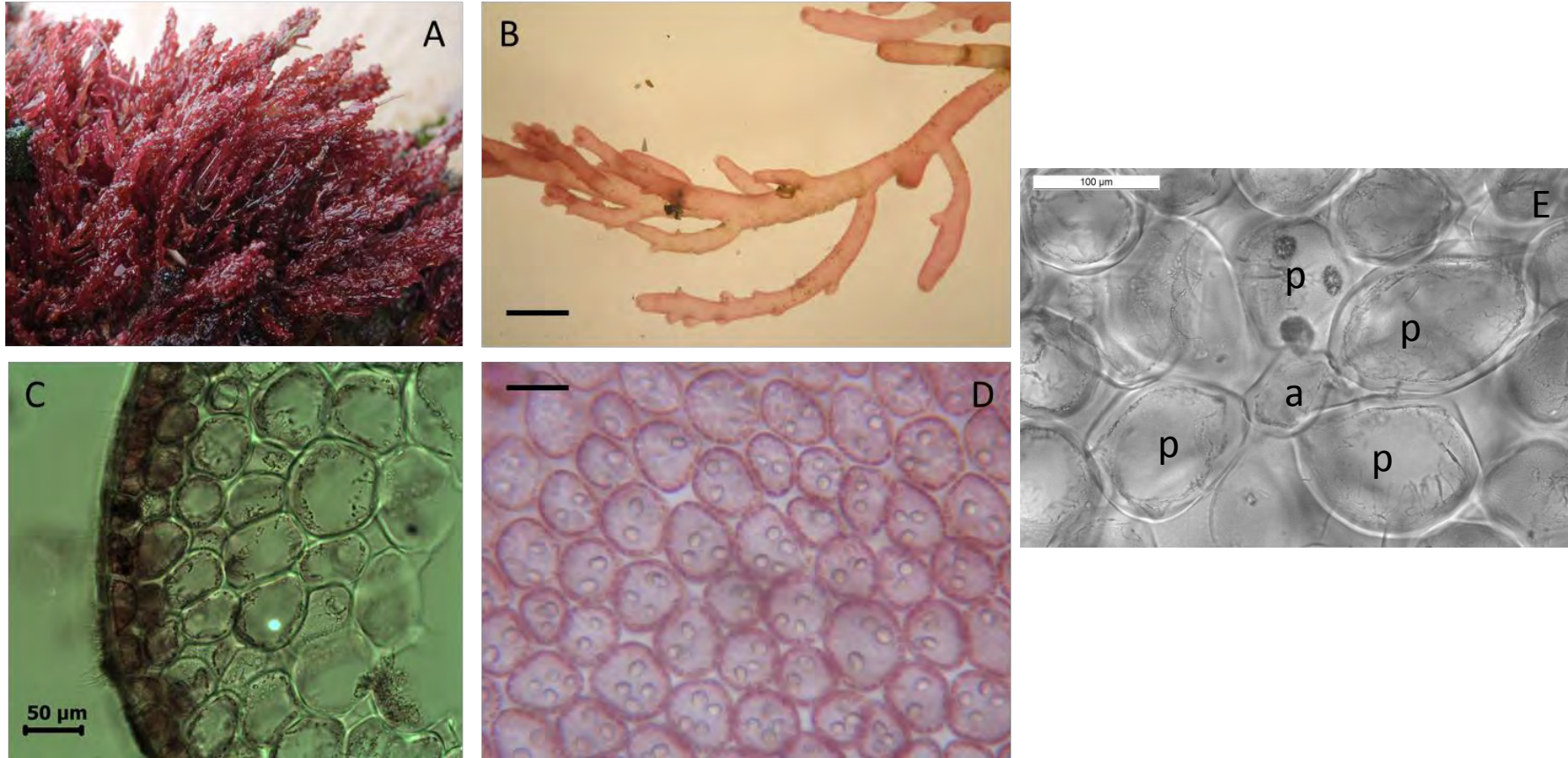


Figure 3.13: *Laurencia sodwaniensis* sp. nov.: A) Habit. B) Branching pattern (8x) Scale : 1cm = 240μm C) Outermost cortical and cortical cells (20x) D) 2-4 *Corps en cerise* per cell (40x) Scale 1cm = 20μm. E) Axial (a) and pericentral (p) cells (40x)

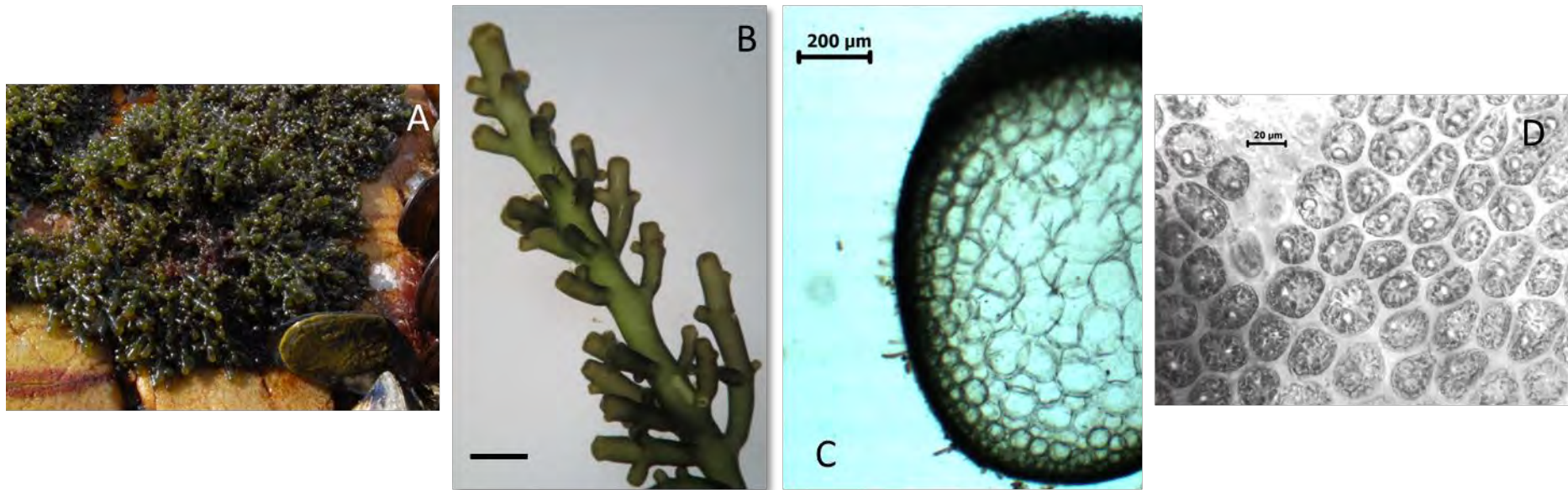


Figure 3.14: *Laurencia stegengae* nom. nov.: A) Habit B) Polystichous branching with wart-like higher order branching (x8). Scale bar: 1 cm = 200µm C) Cross section of thallus showing outermost cortical and cortical cells (20x) D) One *corps en cerise* per outermost cortical cell (40x)

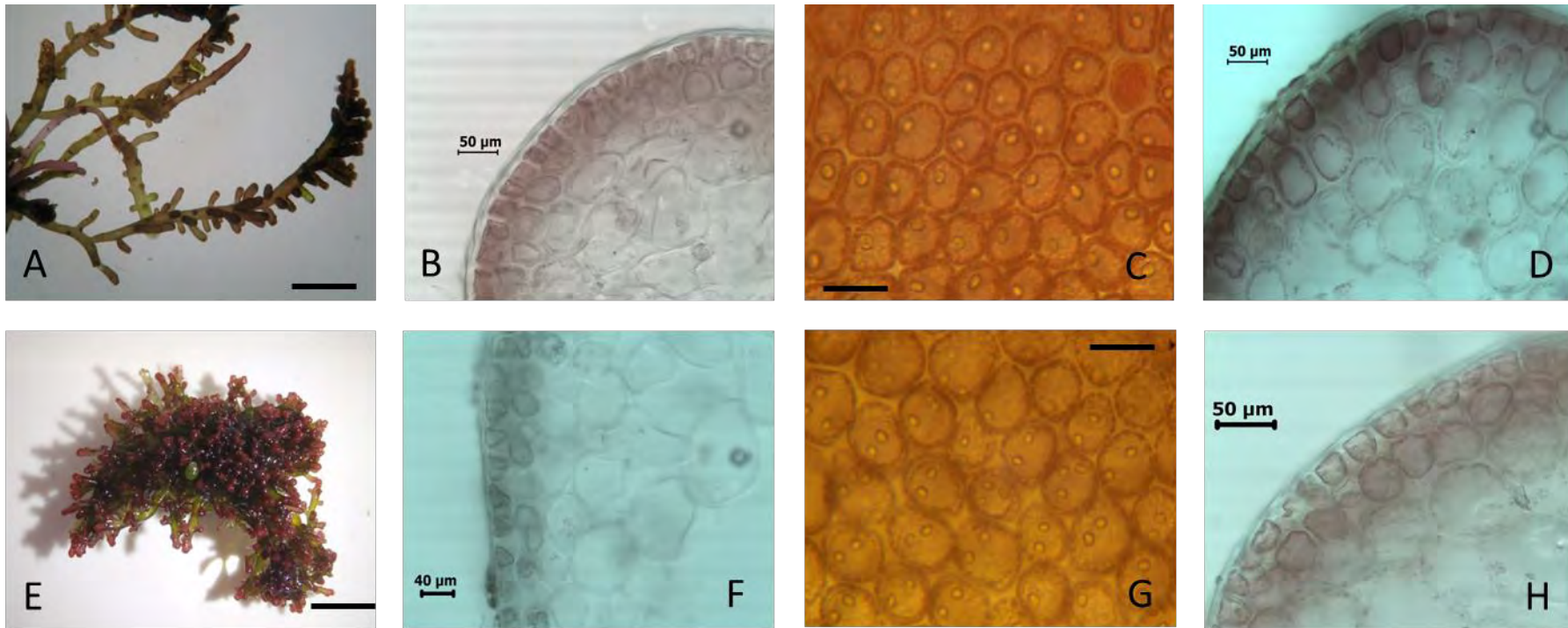


Figure 3.15: Undescribed morphotypes of the *Laurencia* complex. A-C: *Laurencia* sp. ‘morphotype A’ –A) Habit (8x) Scale Bar: 1cm=120μm; B) Cross section of thallus (20x); C) One *corps en cerise* per outermost cortical cell (20x). Scale Bar: 1cm = 30μm. D) *Laurencia* sp.’morphotype I’: Cross section of thallus (20x); E-G: *Laurencia* sp. ‘morphotype G’: E) Habit (8x) Scale Bar: 1cm = 360μm; F) Cross section of thallus (20x); G) two to three *corps en cerise* per outermost cortical cell Scale bar: 1cm = 30μm. H) *Laurencia* sp. ‘morphotype J’: Cross section of thallus (20x)

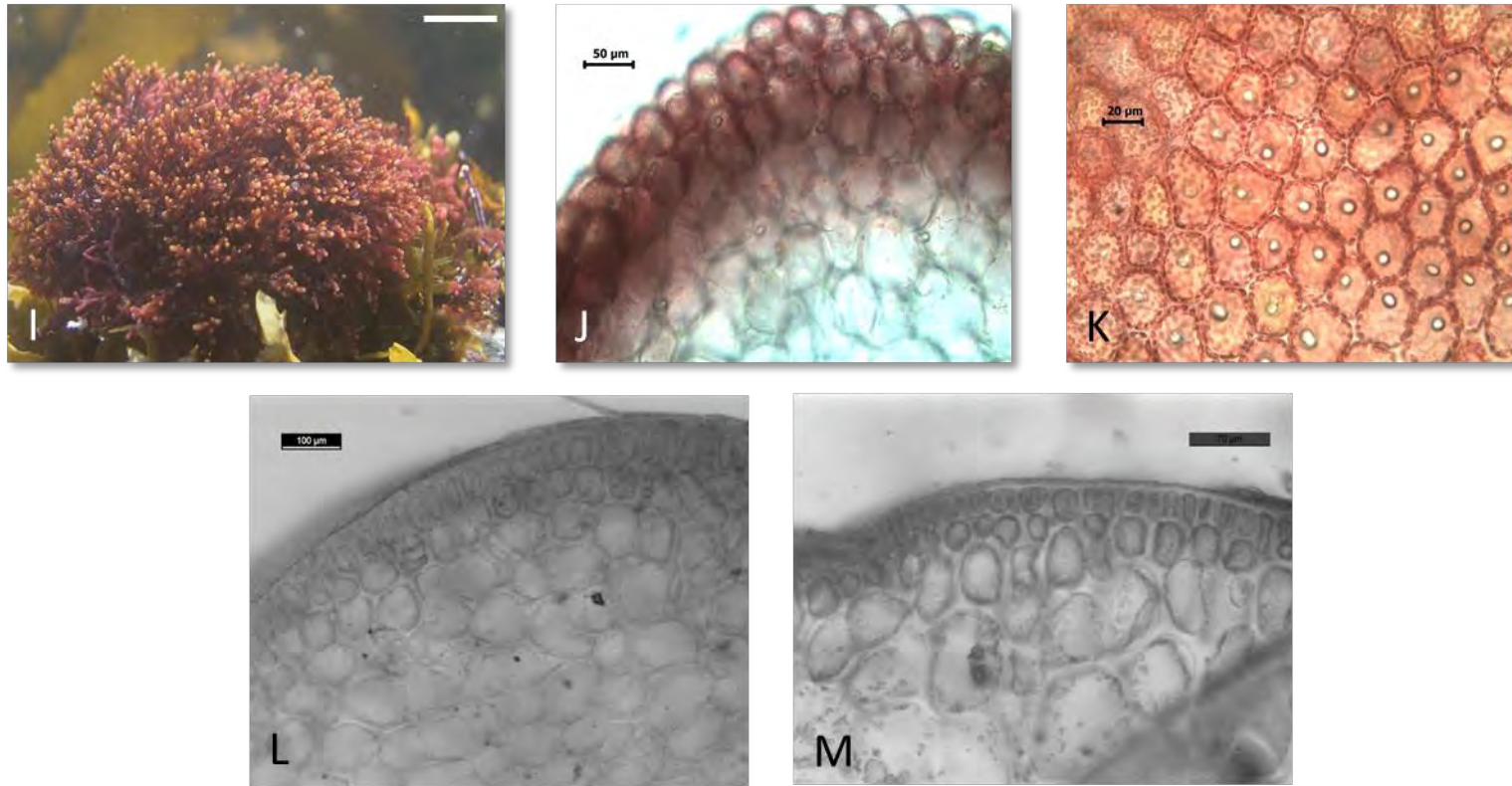


Figure 3.15: Undescribed morphotypes of the *Laurencia* complex. I-K: *Laurenciella marilzae*: I) Habit (x2) Scale 1cm = 20mm; J) Cross section of thallus showing *corps en cerise* in cortical and medullary cells (20x); K) One *corps en cerise* per outermost cortical cell (40x). L) *Chondrophycus* sp. 1: Thallus cross section (20x); M) *Palisada* sp. 1: Thallus cross section (20x)

CHAPTER 4:

BIOGEOGRAPHY AND PHYLOGENETIC DIVERSITY OF THE LAURENCIA COMPLEX, WITH EMPHASIS ON THE SOUTH WEST INDIAN OCEAN

4.1 Introduction:

The South Western Indian Ocean (SWIO) defined as “the waters bounded by the eastern coast of Africa between Kenya and South Africa and as far east as 65°E longitude” by Muths *et al.* (2011) includes island nations such as The Mascarene Islands (Mauritius, Reunion), Madagascar, the Comoros, and the Seychelles as well as the French Scattered Islands. The SWIO is considered to be one of the potential marine biodiversity hotspots in the world (Reaka and Lombardi 2011, Hoareau *et al.* 2013). In comparison to some other regions, marine biodiversity in the SWIO is not as well known (Wafar *et al.* 2011), yet based on high endemism for reef fishes and corals on the east coast of Africa and the Mascarene Islands, as well as species diversity in reef-building corals in the Western Indian Ocean, three biodiversity hotspots have been identified within the SWIO alone (Roberts *et al.* 2002, Obura 2012). Studies such as these highlight the importance of this region of the Indian Ocean for marine biodiversity and the evolutionary processes that bring about this diversity.

Geographical shifts in species distributions in response to factors such as changes in water temperature, ocean acidification, extreme climatic events or changes in local and global ocean circulation patterns (which are a few of the known consequences of anthropogenically-exacerbated climate change) have been the focus of several studies in ocean regions of the world (for example Kaustav *et al.* 2001, Perry *et al.* 2005, Lima *et al.* 2007, Sorte *et al.* 2010, Doney *et al.* 2012, Smale and Wernberg 2013). Being able to describe biogeographic patterns, knowing the present day distributions of species and being able predict shifts in the distribution of plant and animal species under changing climate conditions is important for

developing priorities in marine conservation (Lourie and Vincent 2004). Bolton *et al.* (2004) proposed that seaweeds are ideal organisms to use for biogeographic studies in shallow, marine rocky environments because they are ubiquitous, benthic, easy to collect, represent three major phyla, and have “relatively similar species numbers in any one large region from temperate to tropical regions”.

With the exception of Mauritius, which has the best known seaweed flora in the tropical Indian Ocean (Bolton *et al.* 2012) and South Africa (Stegenga *et al.* 1997, De Clerck *et al.* 2002, 2005), the diversity of the seaweeds in the SWIO is still in need of further investigation, and most taxonomic investigations, checklists and records predate molecular taxonomic methods (e.g. Kylin, 1938, Børgesen 1940 - 1954a&b; Isaacs 1967, 1968, 1971; Jaasund 1969, 1970a-c, 1976, 1977a-d, 1979; Silva *et al.* 1996; Stegenga *et al.* 1997, De Clerck *et al.* 2005, Bolton *et al.* 2007).

The rhodomelacean *Laurencia* complex is a closely-related assemblage of seaweed genera with the most speciose genus *Laurencia sensu stricto* distributed widely across temperate and tropical waters primarily in the southern hemisphere (Guiry and Guiry 2014). In terms of the *Laurencia* complex, the SWIO is home to a total of 41 species (Guiry and Guiry 2014) representing just under a quarter of all the *Laurencia* complex species in the world, making it a relatively high-diversity region. However, with the exception of a few genus name changes for Tanzanian *Laurencia* species in Oliveira *et al.* (2005), and the taxonomic revision of the South African species presented in Chapters 2 and 3, the rest of the SWIO has not yet been re-assessed under the revised and phylogenetically-based taxonomic system for the *Laurencia* complex. As a consequence of the outdated taxonomy and confusing identification based on morpho-anatomical characters, accurate assessments of the biogeographic ranges and affinities for the species and genera in the SWIO of this complex are lacking. The most recent publication to mention biogeography of *Laurencia* species linked to the SWIO is that of De

Clerck *et al.* (2005), in which the authors briefly mention the distributions of at least three of the South African *Laurencia* species (*Laurencia complanata*, *Laurencia natalensis* and *Laurencia pumila*) dominant on the east coast, which extend into Mozambique. The most comprehensive account of the *Laurencia* complex in terms of distribution is the Silva *et al.* (1996) catalogue of benthic marine algae of the Indian Ocean. The authors recorded 39 *Laurencia* species (now divided amongst four genera: *Laurencia sensu stricto*: 28, *Chondrophyucus*: 5, *Palisada*: 5 and *Yuzurua*: 1) most of which have multiple collection localities in the SWIO. Some are, however, endemic to island nations (for example: *Laurencia verruculosa* Børgesen in Mauritius, *Palisada surculigera* (Tseng) Nam and *Chondrophyucus articulatus* (Tseng) Nam in the Seychelles) or continental nations (for example *Laurencia stegengae* nom. nov. Francis *et al.* (in prep.) in South Africa).

The highly diverse and ecologically important *Laurencia* complex provides an ideal study group from which a clearer understanding of the evolutionary biogeography of the SWIO can begin to develop. The abundant molecular data produced in Chapter 2 for South Africa together with new significant collections for several localities of the tropical SWIO provide material to produce the first oceanic-region phylogeny of the *Laurencia* complex. The growing amount of molecular data available for the complex globally on Genbank (nearly 100 sequences available for *rbcL*) and additional collections included here from Japan and Western Australia provide material for the construction of a global phylogeny in which to position the SWIO diversity.

The principal aim of the present chapter was to assess the diversity and biogeography of the *Laurencia* complex in the SWIO region and analyse its relationships to other oceanic regions. To reach this aim, the largest (to date) *Laurencia* complex phylogenies were produced. The distribution of the diversity revealed by phylogenetic analyses was analysed in detail for the

SWIO region, and globally. One expected result is a preliminary understanding of the global biogeography of the *Laurencia* complex.

4.2 Materials & Methods:

Two studies were conducted to address the questions on the phylogenetic relationships and biogeography of the *Laurencia* complex in the South Western Indian Ocean (SWIO). One study analyses specimens of the *Laurencia* complex of the South Western Indian Ocean specifically, while the other analyses the complex in a broader global context. All newly collected samples were sequenced according to the methods presented in Chapter 2.

Taxon sampling and species identification

South Western Indian Ocean

The SWIO study analysed *rbcL* sequence data from a reduced, yet representative, set of 108 specimens covering all South African species studied in Chapter 2 (and collected along almost the entire coastline) alongside 35 sequences from specimens newly collected from the following localities in the SWIO: Scattered Islands (Europa Island, Glorioso Island), Madagascar, Mauritius, Mayotte, Mozambique and Reunion (Figure 4.1). Sampling was not comprehensive for each locality – Madagascar, Mauritius and Mozambique had five or fewer sampling localities while the most extensively sampled localities in the SWIO were the Scattered Islands (Appendix: Table A2).

Samples for DNA extraction were mostly preserved in silica-gel, with a few in ethanol. Those preserved in silica-gel extracted and sequenced well, while those in stored in ethanol were not as successful resulting in a limited number of available sequences even for localities that were comprehensively sampled (e.g. Europa Is., Glorioso Is., Mayotte and Reunion). No DNA sequences were obtained from Mayotte specimens. In addition to sequences produced in Chapter 2 and here, 35 sequences were included from Genbank which either were

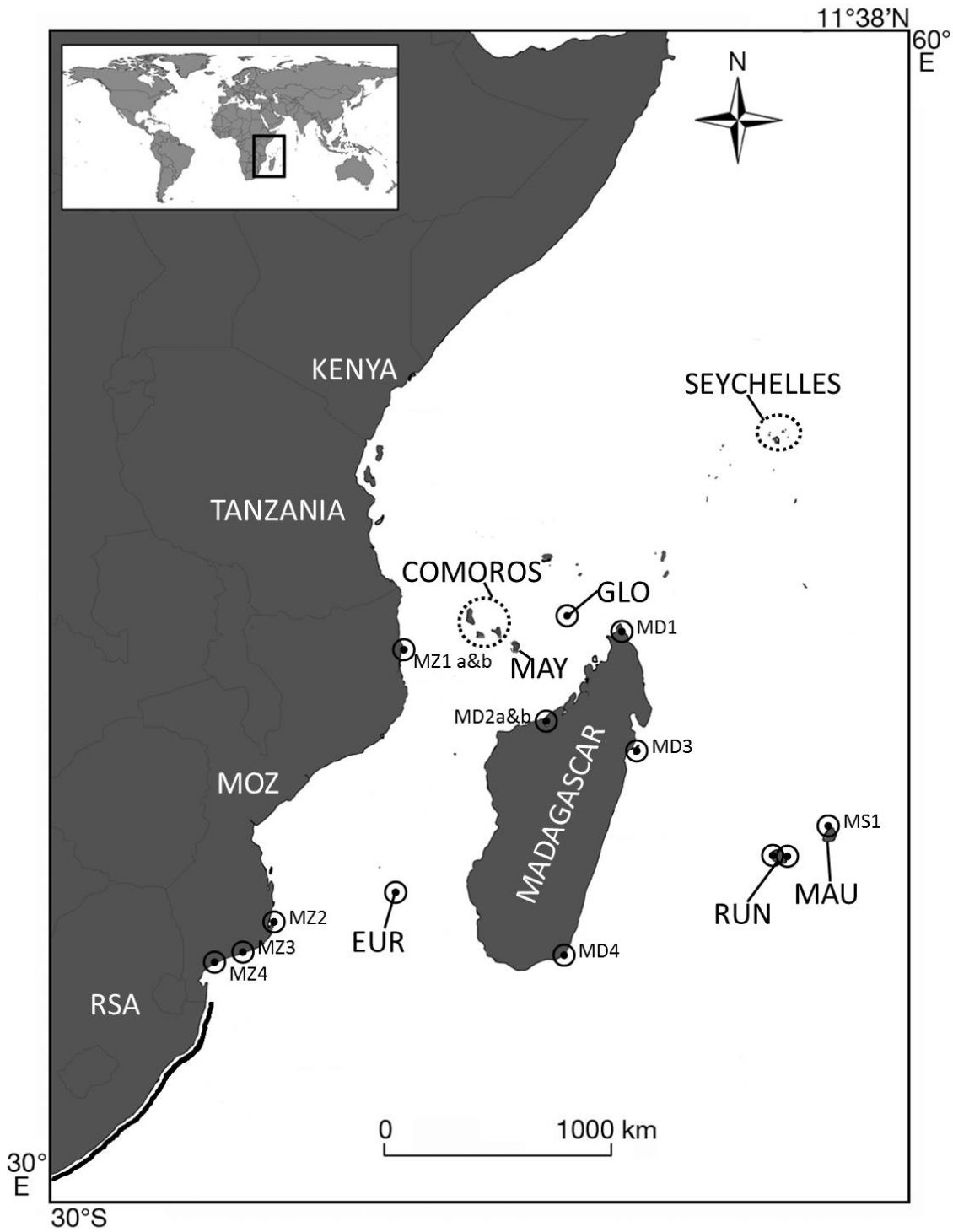


Figure 4.1: Map of the South Western Indian Ocean displaying collection localities (check marks). (Adapted from Muths *et al.* 2011) RSA: South Africa, EUR: Europa Island, GLO: Glorioso Islands, MAY: Mayotte, MAU: Mauritius, RUN: Reunion. South African collection sites are outlined in Chapter 2.

collected from localities in the SWIO or were representatives of the various genera in the *Laurencia* complex.

A total of 178 specimens were used in the SWIO analysis (Appendix: Table A1 and A2).

With the exception of those specimens collected in South Africa for which detailed morphological data were available, the SWIO specimens were assigned to the *Laurencia* complex on the basis of limited morphological data: a combination of having either only pressed material available, or no formalin-preserved specimens for anatomical analysis, and/or no assessment of the number of *corps en cerise* upon collection. As such the identification of the SWIO specimens was achieved based on molecular analysis and the findings corroborated using the available morphological information when possible.

In contrast with the studied region of the SWIO, South African collections were extensive, with 47 sites from along the coastline being sampled (see Chapter 2 for details).

Global (oceanic region) analysis

The global phylogeny dataset was designed to contain sequences for as many species and localities worldwide as possible. The SWIO dataset described above was used as a base and complemented by sequences obtained from new collections in Japan (14 - Hokkaido & Okinawa) and Western Australia (16 – Exmouth to Peron, and White Rock near Perth) as well as a selection of 35 sequences downloaded from the Genbank (Table A2 and A3). The full dataset included a total of 213 sequences representing 19 different countries and 8 different oceanic regions (Table 4.1).

Table 4.1: World oceanic regions and the specimen localities/nations which defined them for the purposes of this study

Ocean Regions	Locations/Nations
SWIO: South Western Indian Ocean	Europa Is., Glorioso Is., Madagascar, Mauritius, Mozambique, Reunion, South Africa
SEIO: South Eastern Indian Ocean	Western Australia
NWPO: North Western Pacific Ocean	Japan (Hokkaido and Okinawa Is.)
SWPO: South Western Pacific Ocean	New Caledonia
NEPO: North Eastern Pacific Ocean	USA
SEAO: South Eastern Atlantic Ocean	South Africa
NEAO: North Eastern Atlantic Ocean	Canary Islands, Spain, France
NWAO: North Western Atlantic Ocean	Cuba, Guadeloupe, Mexico, USA, Venezuela

No voucher specimens were kept for the Australian specimens and all morphological verifications had to be conducted based only on photographs of specimens captured before preservation for DNA isolation. Samples from Western Australia (SEIO) were donated to this study by Dr H. Verbruggen and Miss J. Costa (University of Melbourne, Australia). This limited genus-level identification extended to most of the Japanese specimens as well because accurate identification to species requires more detailed morpho-anatomical analyses.

Japanese specimens were identified to genus level by determining the presence or absence of *corps en cerise* as well as assessing the habit and gross morphology. Gross morphology included traits such as the branching pattern, orders of branching and whether the specimen was cartilaginous or fleshy.

Molecular procedure

DNA extraction, PCR and Sequencing

The procedure for DNA extraction, PCR and sequencing is the same as outlined in Chapter 2.

Phylogenetic analysis

A total 65 new *rbcL* sequences (35 for the SWIO, 14 for Japan and 16 for Western Australia) were newly generated in this study. Only specimens with more than 75% of the total expected sequence length (at least 1100 base pairs) were retained and gaps coded as missing data. The final DNA matrices, including data downloaded from GenBank, totalled 178 *rbcL* sequences for the SWIO analysis and 230 *rbcL* sequences for the global oceanic region analysis.

Outgroups for each analysis differed slightly. For the SWIO analysis, *Chondria dasyphylla* (Woodward) C Agardh from the USA and *Chondria capensis* (Harvey) Askenasy were used as outgroups, the latter being a South African specimen sequenced in this study. Specimens in the genus *Chondria* were chosen as outgroup for the SWIO analysis to minimise genetic distance between the ingroup (members of the *Laurencia* complex) and the outgroup. This was expected to highlight the diversity and phylogenetic relationships of taxa within the *Laurencia* complex for the SWIO. The global (oceanic region) analysis used the same outgroups as in Chapter 2, namely species of *Bostrychia* and *Chondria* in the Rhodomelaceae and *Spyridia* in the Ceramiaceae. This selection process for outgroups follows that of other publications focussed on the *Laurencia* complex as referenced in Chapter 2.

All sequences were edited and assembled using Staden Package (Staden *et al.* 2003). Multiple sequence alignments were performed using BioEdit v7.1.11 (Hall 1999) using the CLUSTAL W algorithm (Thompson *et al.* 1994) and double-checked by eye. The phylogenetic relationships were inferred using the Bayesian statistical inference method performed in

MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003) on the CIPRES Science Gateway (Millar *et al.* 2010). The model used in the Bayesian analysis for both datasets (GTR+I+G) was selected based on the maximum likelihood ratio tests implemented in *jModeltest* version 3.3 (Posada and Crandall 1998) with a significance level of 0.01 by the Akaike Information Criterion. Four chains of the Markov Chain Monte Carlo (three heated and one cold chain) were set, sampling one tree every 1000 generations for seven million generations and starting with a random tree. Calculation of posterior probabilities (PP) was performed after discarding 70,000 trees sampled during the ‘burn-in period’. A 50% majority-rule consensus tree was determined after the burn-in phase.

4.3 Results:

South Western Indian Ocean Analysis

The Bayesian SWIO phylogeny (Figure 4.2) supports of five clades fully (PP=1.00) which corresponds to five of the six genera in the *Laurencia* complex namely, *Chondrophyucus*, *Laurencia*, *Laurenciella*, *Osmundea* and *Yuzurua*. The remaining genus *Palisada* had moderate support (PP=0.89), and was nested within a larger, weakly-supported clade alongside several specimens tentatively identified as *Laurencia flexilis* Setchell, *Laurencia* sp. and *Chondrophyucus* sp. (Figure 4.2). These specimens grouped in two well supported subclades hereafter referred to as *Laurencia flexilis* subclades 1 and 2. *Laurencia flexilis* and its questionable placement in the genus *Laurencia* was discussed earlier (Chapter 2).

Out of the 149 SWIO sequences included in the present study (115 from South Africa, 31 from the rest of the SWIO and 3 from Genbank), 110 grouped within the *Laurencia sensu stricto* clade, while 21 grouped in five other clades in the *Laurencia* complex: Seven sequences each in *Laurenciella* and *Palisada*, three sequences in *Laurencia flexilis* subclade

2, and two each in the *Chondrophyucus* and *Laurencia flexilis* subclade 1. *Chondrophyucus* and *Osmundea* were resolved as sister genera with a PP of 1.00 and at the deeper node the genera of the *Laurencia* complex collapsed into a polytomy (Figure 4.2). The remaining 12 SWIO sequences produced in this study grouped with the outgroup genus *Chondria*, six of which were tentatively identified as *Laurencia* complex morphotypes prior to the molecular analysis. No SWIO sequences were recovered in the *Yuzurua* and *Osmundea* clades.

Within the *Laurencia sensu stricto* clade there were 19 well-supported subclades, 8 of which were restricted to South Africa while Reunion and Madagascar had one endemic sub-clade each. Eight lineages of *Laurencia sensu stricto* were included specimens from multiple localities in the SWIO and other oceanic regions. Fourteen of the *Laurencia sensu stricto* subclades corresponded to the South African species identified in Chapter 2. While the South African morphotypes G and J, represented by only a single sequence in Chapter 2 (Figure 4.2), formed well-supported subclades with specimens from the rest of the SWIO.

Of the 35 *Laurencia* complex *rbcL* sequences downloaded from Genbank, 6 sequences belonged to the genus *Laurencia sensu stricto* and represented 5 species distributed throughout the tree. One specimen from New Caledonia, *Laurencia* cf. *kuetzingii* was the closest relative to *Laurencia digitata* together with a specimen from Glorioso Island (GLO-315, Appendix: Table A2) sequenced in this study. Twenty-seven of the remaining 29 Genbank sequences formed the core of the other genera in the complex, i.e. *Chondrophyucus*, *Laurenciella*, *Osmundea*, *Palisada* and *Yuzurua*, while the single *Laurencia flexilis* sequence from the Philippines resolved within the *L. flexilis* sub-clade 1 (with two *Laurencia* specimens from the SWIO) as sister to the genus *Palisada*.

Six of the SWIO specimens grouped within sub-clades of South African *Laurencia sensu stricto* species or morphotypes identified in Chapter 2 and 3, namely *Laurencia complanata*,

L. multiclavata, *L. natalensis*, *L. pumila*, *L. sp.* morphotype G and morphotype K. Direct morphological comparisons with South African specimens supported conspecificity of the SWIO specimens. A *Laurencia sensu stricto* specimen from Glorioso Island (GLO-315, Appendix: Table A2 resolved in a well-supported subclade with *Laurencia cf. kuetzingii* from New Caledonia (Figure 4.2) but further morpho-anatomical details are required for confirmation of the specimen as *Laurencia kuetzingii*.

In addition, it should be noted that Metti *et al.* (2013) placed *Laurencia kuetzingii* in synonymy of *L. dendroidea*, but one of the analyses presented in Chapter 2 (Figure 2.4) did not support this synonymy and the original specimen name should be conserved.

The remaining SWIO specimens in *Laurencia sensu stricto* and the other genera of the *Laurencia* complex which formed unique sub-clades could not be identified and were labelled as unidentified taxa (sp.) of their respective genera. Where more than one distinct sub-clade occurred in a particular locality, they were labelled with consecutive numbers to distinguish these taxa from each other (Figure 4.2).

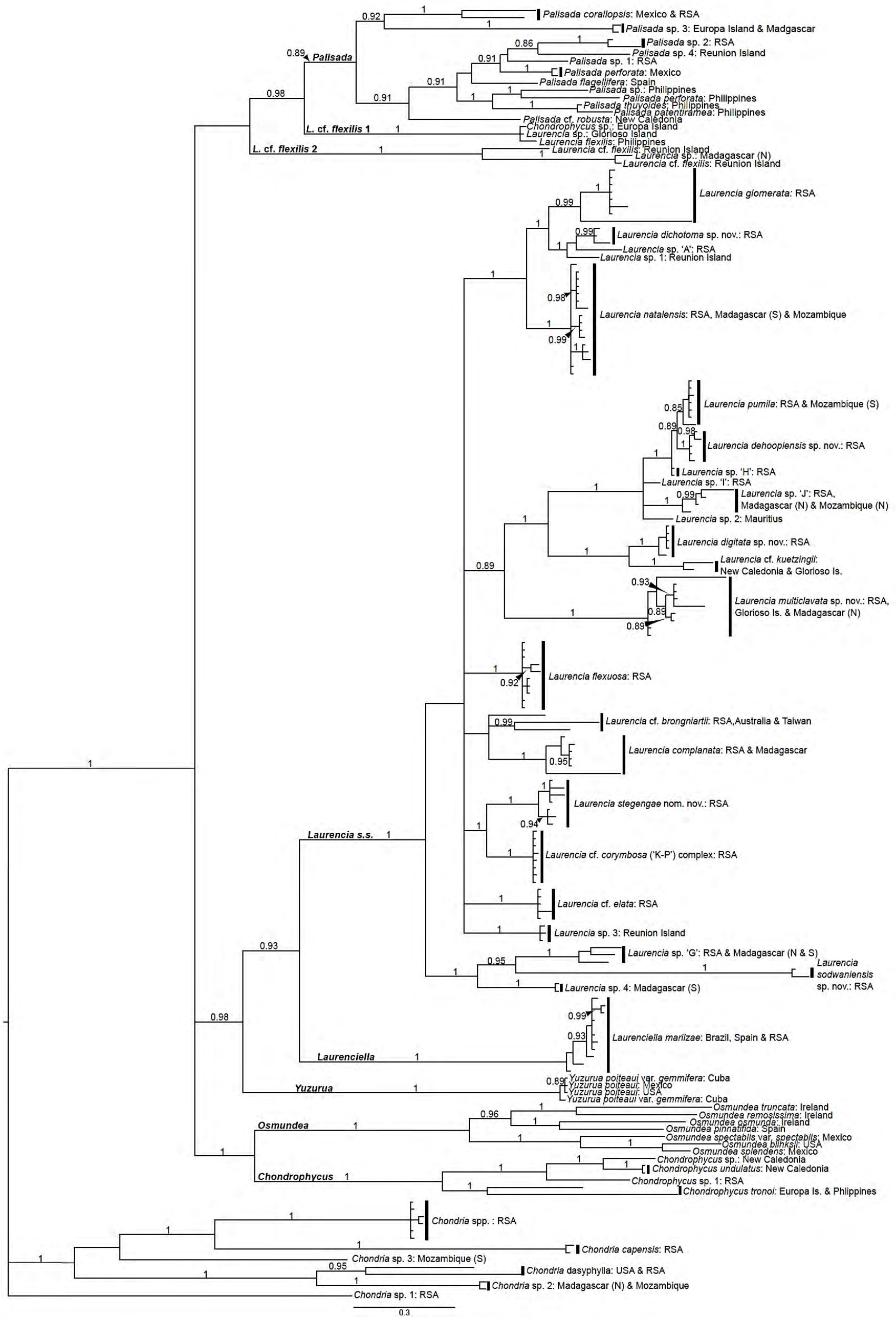


Figure 4.2: The 50% majority consensus Bayesian phylogeny of the *Laurencia* complex in the SWIO inferred from the plastid marker *rbcL*. RSA: South Africa; USA: United States of America, N: Northern; S: Southern

Genetic Distances

The uncorrected-p pairwise distance calculation of *Laurencia sensu stricto* taxa included in this study (Table 4.2) exhibited marked nucleotide sequence variation between morphotypes (sequence divergence: 1.2-9.4%). Within-morphotype sequence divergence was less than 0.9% and the intergeneric sequence divergence levels of the *Laurencia* complex ranged between 7.2% and 14.2%. The *Laurencia flexilis* sub-clades 1 and 2 showed levels of sequence divergence between one another (9.4-11.3%) and also between each other and *Laurencia sensu stricto* (sub-clade 1: 9.1-12.7%; sub-clade 2: 8.9-13.0%) comparable to intergeneric sequence divergence in this study (Table 4.2).

Table 4.2: The intergeneric and interspecific divergence values obtained for *rbcL* sequences of the *Laurencia* complex with emphasis on the SWIO in this study. (*: based on 1 species set; ‡: no multiples of one species in dataset+)

Taxa	Divergence values for <i>rbcL</i> sequences (%)
<u>Intergeneric</u>	
<i>Laurencia</i> – <i>Chondrophyucus</i>	9.2 – 12.9
<i>Laurencia</i> – <i>Palisada</i>	8.1 – 13.8
<i>Laurencia</i> – <i>Osmundea</i>	9.2 – 14.2
<i>Laurencia</i> – <i>Laurenciella</i>	8.1 – 11.7
<i>Laurencia</i> – <i>Yuzurua</i>	9.1 – 12.1
<i>Laurencia</i> – <i>L. flexilis</i> sub-clade 1	9.1 – 12.7
<i>Laurencia</i> – <i>L. flexilis</i> sub-clade 2	8.9 – 13.0
<i>Chondrophyucus</i> – <i>Palisada</i>	9.5 – 11.5
<i>Chondrophyucus</i> – <i>Yuzurua</i>	10.5 – 11.0

<i>Chondrophyucus – Osmundea</i>	9.2 – 12.2
<i>Chondrophyucus – Laurenciella</i>	10.9 – 11.9
<i>Chondrophyucus - L. flexilis</i> sub-clade 1	10.5 – 11.7
<i>Chondrophyucus - L. flexilis</i> sub-clade 2	9.9 – 11.3
<i>Palisada - Yuzurua</i>	9.4 – 11.8
<i>Palisada – Osmundea</i>	9.6 – 13.0
<i>Palisada – Laurenciella</i>	8.9 – 11.6
<i>Palisada – L. flexilis</i> sub-clade 1	7.8 – 11.5
<i>Palisada – L. flexilis</i> sub-clade 2	7.2 – 11.4
<i>Osmundea – Yuzurua</i>	10.0 - 12.9
<i>Osmundea – Laurenciella</i>	11.4 – 13.2
<i>Osmundea – L. flexilis</i> sub-clade 1	11.8 – 13.1
<i>Osmundea – L. flexilis</i> sub-clade 2	10.7 – 12.8
<i>Laurenciella – Yuzurua</i>	10.1 – 10.7
<i>Laurenciella – L. flexilis</i> sub-clade 1	10.0 – 11.8
<i>Laurenciella – L. flexilis</i> sub-clade 2	10.6 – 12.1
<i>Yuzurua – L. flexilis</i> sub-clade 1	9.7 – 10.7
<i>Yuzurua – L. flexilis</i> sub-clade 2	10.4 – 12.0
<i>L. flexilis</i> sub-clade 1 – <i>L. flexilis</i> sub-clade 2	9.4 — 11.3

Interspecific

<i>Laurencia sensu stricto</i>	1.2 – 9.4
<i>Palisada</i>	1.2 – 7.7
<i>Osmundea</i>	2.4 - 12.0
<i>Chondrophyucus</i>	1.2 - 5.6
<i>Yuzurua</i>	n/a
<i>Laurenciella</i>	n/a
<i>L. flexilis</i> sub-clade 1	n/a
<i>L. flexilis</i> sub-clade 2	1.7 – 4.4

Intraspecific

<i>Laurencia sensu stricto</i>	0.0 - 0.9
<i>Chondrophyucus</i>	0.0*
<i>Osmundea</i>	n/a‡
<i>Palisada</i>	0.1 – 0.6
<i>Yuzurua</i>	0.0 – 0.1
<i>Laurenciella</i>	0.0 – 1.0
<i>L. flexilis</i> sub-clade 1	0.0
<i>L. flexilis</i> sub-clade 2	0.4

Global Oceanic Region Analysis

Terminal nodes for all subclades within *Laurencia sensu stricto* have been collapsed and locality expressed only in terms of the oceanic regions for those specimens (see list of abbreviations in Materials and Methods above). The terminal nodes in the other genera in the

Laurencia complex, except *Laurenciella*, have been fully collapsed to aid better viewing of the tree which would otherwise be too large (Figure 4.3).

Four of the six genera in the *Laurencia* complex were fully supported (PP=1.00) in the Bayesian analysis, the exceptions being *Laurencia* and *Palisada* (Figure 4.3). The two sub-clades of *Laurencia flexilis*, a species which has previously been pointed out by Abe *et al.* (2006) as independent entity in the *Laurencia* complex, were also fully supported. Support for the relationships between genera at the deeper nodes lack support, and the resolution within *Laurencia sensu stricto* was poor (Figure 4.3). Out of the 230 sequences included in the present study (175 produced in this study – Chapter 2 and 55 from Genbank), 150 grouped within the *Laurencia sensu stricto* clade, while 62 grouped in five other clades in the *Laurencia* complex as follows: Twenty-six sequences in *Palisada*, eleven in *Laurenciella*, eight in *Chondrophyucus*, seven in *Osmundea*, four in *Yuzurua* and three each in the *Laurencia flexilis* sub-clades 1 and 2. *Chondrophyucus* and *Osmundea* were resolved as sister genera with a PP of 1.00, and at the deeper node the genera of the *Laurencia* complex collapsed into a polytomy (Figure 4.3). None of the sequences produced in this study belonged to the *Yuzurua* and *Osmundea* clades.

Within the *Laurencia sensu stricto* clade there were 28 well-supported sub-clades, 21 of which were found in the SWIO and two of these sub-clades were shared with Western Australia (temperate SEIO) (*L. sp.* morphotype A and *L. sodwaniensis*) and a further two subclades (*L. glomerata* and *L. stegengae*) occur west of Cape Agulhas in the SEAO, though most of their distribution is in the SWIO. Sixteen of the subclades in *Laurencia sensu stricto* were restricted to one oceanic region and almost 82% of these subclades occurred in the SWIO (13 of 16 subclades) especially along the South African coast (six of the 13 SWIO

subclades) (Figure 4.3). *Laurencia brongniartii* recorded from South Africa, the United States of America and Taiwan was the only *Laurencia sensu stricto* taxon recorded from more than two oceanic regions, namely the SWIO, NWPO & NEPO (Figure 4.3).

Three of the other genera in the *Laurencia* complex were also recorded from multiple oceanic regions, namely *Laurenciella* (NEAO, SEAO, SWAO & SWIO), *Palisada* (WPO, SWIO, SEIO & NWAO) and *Chondrophycus* (WPO & SWIO). Similarly, *Laurencia flexilis* sub-clade 1 was recorded from the NWPO and SWIO. *Yuzurua* and *Osmundea* were restricted to the Atlantic Ocean, although the latter genus is recorded in the literature) to occur on the Pacific coast of the United States (in Abbott and Hollenberg 1976 as *Laurencia* sp., www.ucjeps.berkeley.edu/californiaseaweeds.html) and the Mediterranean Sea (Serio *et al.* 1999).

The Australian specimens could not be assigned a species name based on the examination of photographs which were the only available morphological information. In the case of the Japanese specimens, only those specimens collected from Sapporo Prefecture in northern Japan were identified by local phycologists (Dr. Kazuhiro Kogame, Hokkaido University and Dr. Shinya Uwai, Niigata University) while those collected in Okinawa were identified to genus level only and further anatomical analyses are required for species assignment.

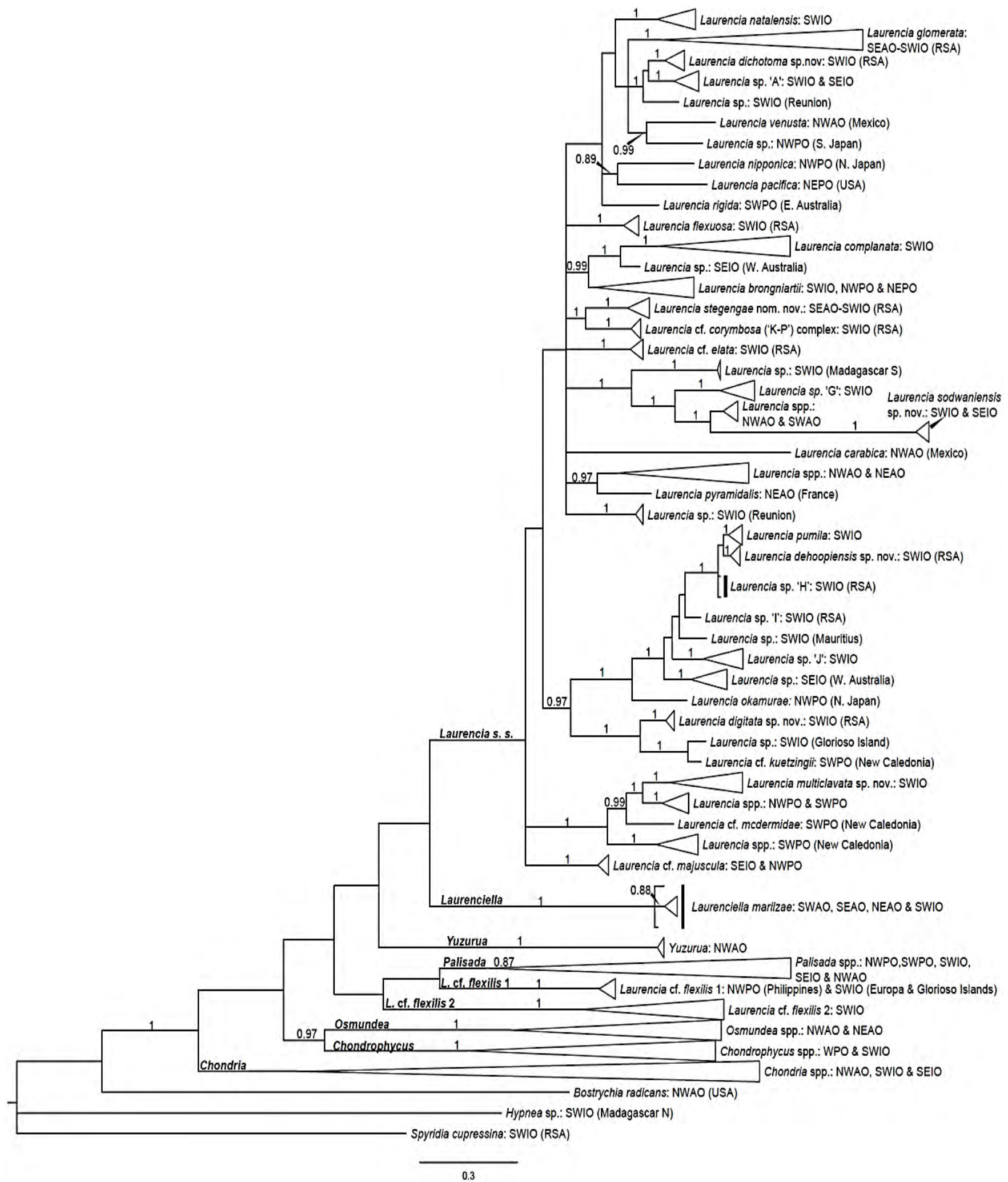


Figure 4.3: The 50% majority consensus Bayesian phylogeny of the *Laurencia* complex in the ocean regions of the world inferred from the plastid marker *rbcL*. RSA: South Africa; USA: United States of America; N: Northern; S: Southern

4.4 Discussion:

Diversity and distribution of the *Laurencia* complex in the SWIO

This study is the first to investigate the phylogenetic diversity of the *Laurencia* complex in the SWIO. Results highlighted the fourteen South African *Laurencia sensu stricto* species identified in Chapter 2, five additional entities from other localities in the southern SWIO namely Glorioso Island, Mauritius and Madagascar (one each) and Reunion (two; though one is represented by a single specimen), as well as five *Palisada* species, two *Chondrophycus* species and *Laurenciella marilzae* for a total of 30 *Laurencia* complex species in the region. The remaining three entities belong to the two subclades of *Laurencia flexilis* - sub-clade one contains one while sub-clade two has two and is thus far confined to the SWIO. Our results further indicate that this diversity is underestimated: five additional *Laurencia sensu stricto* morphotypes (A, G-J) were distinguished from South African data (see Chapter 2), two of which (morphotype G and J) were also found in Madagascar (G& J) and Mozambique (J). More morphological and molecular data are required before these entities can be confirmed as distinct and new species.

The highest diversity of the *Laurencia* complex was found in South Africa with 19 species belonging to 4 genera, followed by Madagascar with 3 genera and 8 species. It should be noted that the current analysis of *Laurencia* complex diversity in the SWIO, with the exception of South Africa, does not represent a full reflection of the phylogenetic diversity in the region. This is particularly true for Mauritius. Børgesen (1945, 1952-1954) reported a total of 11 *Laurencia* complex species, nine of which belong to *Laurencia sensu stricto* and one each to *Palisada* and *Chondrophycus*, but the current study only produced a single Mauritian *Laurencia sensu stricto* sequence (Figure 4.2). That being said, the species and generic diversity found in this study places South Africa above Mauritius as the most speciose locality in terms of *Laurencia* complex diversity in the region.

The most diverse region of the South African coastline with respect to the *Laurencia* complex is the warm temperate south coast with 13 species (of the total of 19). In contrast 9 of the 19 species occur in the tropical extreme northeast of South Africa (*sensu* Bolton *et al.* 2004) and only six of those species occur in the rest of the studied section of the tropical SWIO. Of the nine species with tropical distributions five (*Laurencia digitata*, *Laurencia flexuosa*, *Laurencia natalensis*, *Laurencia multiclavata* and *Laurencia pumila*) were also recorded from the south coast of South Africa. The results from this study suggest that the species diversity of the *Laurencia* complex decreases from the warm-temperate south coast into the tropical northeast coast of South Africa and the rest of the studied region of the SWIO. The study identified South Africa as the most diverse region (with four genera) and the only locality in the SWIO to have the recently described genus *Laurenciella* recorded. Comparatively, Europa Island and Reunion, the best represented localities (on the basis of sequences produced vs. numbers of specimens) outside of South Africa, each have three genera of the *Laurencia* complex present on their shores. However, patterns of diversity for species and genera in the SWIO are difficult to describe confidently because collections in significant floristic regions such as Mauritius and Madagascar are limited, and it is therefore not possible to identify hotspots of diversity in the SWIO at present.

This study identified 11 *Laurencia sensu stricto* sub-clades restricted to a single locality within the studied region of the SWIO (Figure 4.2). Nine of the eleven sub-clades are South African and represent 9 species (*Laurencia* cf. *corymbosa*, *L. dehoopiensis*, *L. dichotoma*, *L. digitata*, *L. cf. elata*, *L. flexuosa*, *L. glomerata*, *L. sodwaniensis* and *L. stegengae*). Thus far, five of the nine species are endemic to South Africa (*L. dehoopiensis*, *L. dichotoma*, *L. digitata*, *L. sodwaniensis*, *L. stegengae*), and at least two species, *L. dehoopiensis* and *L. stegengae*, can be confirmed as endemic to the warm temperate south coast of South Africa, and in the case of *L. stegengae* to the southwest transition zone (see Figure 1.3, Chapter 1).

Laurencia dichotoma and *L. sodwaniensis* are endemic to the warm waters of north eastern KwaZulu-Natal. *Laurencia digitata* has the widest distribution of the South African endemic species, being recorded from the warm temperate south coast to the tropical east coast of KwaZulu-Natal (see Chapter 3 for distribution details). Literature on the *Laurencia* complex indicated that the four remaining South African species have wider distributions than represented in this study and were reported from localities outside of the SWIO including, amongst others, Indonesia (*L. glomerata* – Amadtja and Prud’homme van Reine 2012), Mauritania (*L. flexuosa* – John *et al.* 1994), Fiji (*L. corymbosa* – N’Yeurt and Keats 1996, South and Skelton 2003) and Australia (*L. elata* – Saito and Womersley 1974, Womersley 2003). Putative species from Reunion (*Laurencia* sp. 3) and Madagascar (1 – *Laurencia* sp. 4) constitute the remaining two subclades (of the total eleven for *Laurencia sensu stricto*) and these entities appear endemic to their respective island.

The remaining eight sub-clades in *Laurencia sensu stricto*, which represent six species (*L. brongniartii*, *L. complanata*, *L. cf. kuetzingii*, *L. natalensis*, *L. multiclavata* and *L. pumila*) and two putative species (*L. sp. ‘G’* and *L. sp. ‘J’*) that are comparatively widely distributed, reveal some interesting patterns of distribution. The present study confirmed the distribution of *Laurencia natalensis* into Mozambique as reported by Isaac (1958) and Isaac and Chamberlain (1958) and is also the first record of this species from Madagascar (Figure 4.2). *Laurencia natalensis* and *L. multiclavata*, which are morphologically similar species, have interesting distribution patterns. Both species share a similar pattern of distribution within South Africa, i.e. from the temperate south coast into the tropical north east coast (see chapter 2), while in the rest of the SWIO their distribution differs. Both are present in the southern end of the Mozambican channel (Mozambique and Madagascar) together with *Laurencia natalensis*, while *L. multiclavata* is also recorded from the northern reaches of the channel at Glorioso Island and in northern Madagascar. Considering the limited collections available

from Mozambique and Madagascar for the present study, it must however be noted that the sampling in SWIO is not comprehensive and the observed pattern may be an artefact, especially considering that Lawson (1980) has recorded *L. natalensis* from Kenya.

Putative species of *Palisada* – *P.* sp. 1 and 2, were found along the warm temperate south coast and the tropical northeast coast of South Africa, respectively and the latter tropical species *Palisada* sp. 2, shares an affinity with a tropical *Palisada* sp. 4 (specimen #R69 – Table A2) from Reunion (Figure 4.2). While sequence representation is poor (1-2 sequences per putative species) at present all three putative species are endemic to the SWIO (Figure 4.2). These results represent a range expansion for *Palisada* as before this study the furthest south the genus *Palisada* had been recorded was Madagascar (Silva *et al.*, 1996). Similarly, a single putative species of *Chondrophycus* (represented by one DNA sequence each) was recorded from each of South Africa and Europa Island (Figure 4.2). While further morpho-anatomical details are needed to identify these taxa to species level, both taxa are discussed briefly later in this chapter.

New insights into the taxonomy of the *Laurencia* complex

The phylogeny of the *Laurencia* complex in the SWIO produced in this study showed full support (PP=1) for all of the genera except *Palisada* (Figure 4.2). *Palisada* formed a group with two *Laurencia* lineages, *Laurencia flexilis* subclades 1 and 2, each of which were fully-supported (PP=1) and had genetic differences ranging in the intergeneric level (see Table 4.2) supporting them as potential distinct genera. *Laurencia flexilis* was considered by Abe *et al.* (2006) to have intermediate morphology between *Laurencia* and *Chondrophycus*, exhibiting the diagnostic lack of secondary pit-connections of *Chondrophycus* and the four pericentral cells of *Laurencia*. Molecularly, *Chondrophycus* was separated into two distinct clades with

high bootstrap support in the *rbcL* analysis by Abe *et al.* (2006); the first clade contained the generitype of *Chondrophyucus*, *C. cartilagneus* (Yamada) Garbary & J.T. Harper, while the second contained a collection of sequences from mostly tropical localities in the Philippines, Brazil and Japan. Subsequent work by Nam (2006, 2007) has shown this second *Chondrophyucus* clade of Abe *et al.* (2006) - to which a sequence of *Laurencia flexilis* (AF489860) from the Philippines was most closely related - to be a distinct genus which they described and named *Palisada*. In the present study, the aforementioned Philippines *Laurencia flexilis* sequence clustered with two sequences from Europa Island and Glorioso Island (subclade 1, Figure 4.2) which appeared closely related to the *Palisada* subclade (Figure 4.2). A second subclade (subclade 2, Figure 4.2) including sequences from Reunion and northern Madagascar, tentatively identified as *Laurencia flexilis*, also grouped together (PP=1.00) and formed a weakly-supported clade (PP not shown) with subclade 1 and the *Palisada* subclade (Figure 4.2). Subclade 2 is the most basal group in the clade and the branch lengths in Figure 4.2 indicate this subclade is limited in sequence similarity to even its closest relatives - subclade 1 and the *Palisada* subclade. Comparing the genetic distance between each of the subclades (i.e. 1, 2 and *Palisada*) shows that the level of divergence is similar to those of *Palisada* and any of the other known genera in the *Laurencia* complex in this study (Table 2.1). In fact, the genetic distance between *Laurencia flexilis* subclade 1 and subclade 2 (9.4%-11.3%) is even higher on average than the distance between both sub-clade and *Palisada* (7.8%-11.5% and 7.2%-11.4% for subclades 1 and 2, respectively). These results provides early molecular evidence for two additional genera in the *Laurencia* complex, represented here as the two *Laurencia flexilis* subclades 1 and 2.

Global biogeography of the *Laurencia* complex

The global phylogeny produced in this chapter is the largest one to date and brings new insights into global distribution patterns of the sequenced *Laurencia* complex diversity.

Results from the phylogenetic analysis of a subset of taxa of the *Laurencia* complex in the oceanic regions of the world have revealed the following: *Laurencia sensu stricto* is recorded from both tropical and temperate waters in the Atlantic, Indian and Pacific Oceans with 30 of the 38 subclades of the genus (some of which represent groups of species) occurring in the southern hemisphere (Figure 4.3). In this analysis *Laurencia sensu stricto* comprises predominantly SWIO taxa (21 of the 28 subclades) and most of these are recorded from South Africa with a strong affinity for the temperate south coast flora (Agulhas marine province *sensu* Spalding *et al.* 2007). The endemism of *Laurencia* complex species in the South African flora was discussed above, though this analysis highlights that a few species (including *L. multiclavata* and putative species such as *L. sp. 'G'* and *L. sp. 'J'* [Figure 4.3]) are common to studied regions of the SWIO. Two predominantly east coast species, *Laurencia complanata* and *Laurencia sodwaniensis*, are closely related (i.e. in the same subclade) to specimens from temperate Western Australia (SEIO in Figure 4.3) which supports the proposal of Hommersand (1986) that the flora of KwaZulu-Natal shares affinities with that of the west (and south) coast(s) of Australia. The remaining seven subclades of *Laurencia sensu stricto* were spread across ecoregions in the Pacific and Atlantic Oceans and have affinities with temperate and tropical floras for both oceans (Figure 4.3). Taxa distributed in the Pacific have predominantly tropical affinities as a result of sampling efforts, even outside this study, being focussed in tropical habits (for e.g. Taiwan and New Caledonia) although a few are exclusively temperate such as *Laurencia nipponica* and *L. okamurae* from northern Japan (Figure 4.3). Conversely, Atlantic Ocean taxa are predominantly temperate in their floristic association with only two species from Atlantic Mexico, *Laurencia caraibica* and *L. venusta*, having tropical affinities (Figure 4.3).

The other five genera are not as common as *Laurencia sensu stricto*, however some patterns did arise. In *Palisada*, the second largest genus in the complex (22 species), the 18 taxa (for

which sequences are available) analysed in this study were distributed largely in the Pacific (number), with only six taxa in the Indian Ocean and two in the Atlantic. The species of the genus are predominantly recorded from warm-temperate and tropical shores (distributional data from Guiry and Guiry 2014), and in). In the present analysis the subclades (representing individual species of *Palisada* from the Pacific) had largely tropical affinities (see more details in Figure 4.2). Indian Ocean species often appeared to have affinities with species from the temperate floristic regions on the west coast of Australia and south coast of South Africa. The present study records *Palisada* for the first time in South Africa and so expands the distribution of the genus to the temperate Indian Ocean.

Species of the genus *Chondrophyucus* occur along the temperate and tropical shores of the Indo-West Pacific (Guiry and Guiry 2014). Prior to this study the known distribution of *Chondrophyucus* in the SWIO region was limited to the records of *Chondrophyucus columellaris* (Børgesen) E. Coppejans et A.J.K. Millar in Tanzania and Reunion (Guiry and Guiry 2014). The results of the present study expanded the distribution of the genus with two undescribed *Chondrophyucus* entities: one from Europa Island which appeared closely related to *C. tranoi* (E. Gazon-Fortes) K.W. Nam from the Philippines, and the second one collected from Sodwana Bay in the north-east of KwaZulu-Natal, South Africa.

The monospecific genus *Laurenciella* is recorded from tropical (Brazil and Mexico) and temperate (Tenerife and other islands of the Canary Islands, Spain and Portugal) floristic regions in the Atlantic Ocean (Guiry and Guiry 2014). *Laurenciella marilzae* was recorded in Chapter 2 for the first time on shores of the Indian Ocean from the temperate south coast of South Africa (Agulhas ecoregion viz. Spalding *et al.* 2010) (Chapter 2, Figure 4.2 and 4.3). *Laurenciella marilzae* is distributed in the warm temperate (Indian Ocean) region of the South African coast and is virtually indistinguishable from the specimens from the eastern

temperate and western tropical floras of the Atlantic Ocean. *Laurenciella* had never been recorded outside of the Atlantic Ocean before the present study.

Biogeographical hypotheses

In his development of a theoretical framework to explain the biogeography of South African red algae Hommersand (1986) stated that the *Laurenciae* amongst other families and tribes could have a primary distribution and origin in the Tethys Ocean. The Tethys was a tropical ocean (Poulsen *et al.* 1998) and therefore, accepting Hommersand's deductions, the *Laurenciae* originated in warm water. Hommersand (1986) proposed two assemblages within the Tethyan flora: a southern Tethyan Ocean assemblage with a centre of diversity in present day west and South Australia and a Pacific Ocean assemblage with a present centre of diversity in Tasmania, South Australia and New Zealand. Saito and Womersley (1974) and Womersley (2003) recorded 16 species (of a total 61 for Australia according to Duretto 2014) for temperate Australia, making this floristic region an area of significant diversity for the *Laurencia* complex, fitting well with the model proposed by Hommersand (1986). Given the large number of South African specimens in the global data set for the *Laurencia* complex, and the fact that the warm-temperate flora of South Africa is potentially derived from multiple sources including Western Australia and Japan (Hommersand 1986), it could be useful to investigate these temperate phylogeographic connections.

In the present study *Laurencia flexuosa* is one of the most widely distributed species within South Africa, occurring from False Bay on the temperate southwest coast eastward into KwaZulu-Natal. According to John *et al.* (2004) it is also part of the tropical floras on the shores of Mauritania, West Africa. In contrast, the morphologically similar *Laurencia* cf. *elata* was restricted to Cape St. Francis and other localities within a 200 km stretch of coastline (See Figure 2.2 in Chapter 2); it was not found elsewhere in the SWIO, yet

Laurencia elata is widely distributed along the temperate West and South coasts of Australia (see Saito and Womersley 1974 for distribution details). Although the molecular analysis does not include an Australian specimen, the morphology and anatomy of the SWIO specimens that were included, very closely matches the descriptions in Saito and Womersley (1974). Given the restricted range of South African *Laurencia cf. elata* it would be interesting to (1) confirm the South African specimens as *Laurencia elata* and (2) assess the degree of sequence divergence between these geographic populations given their contrasting distribution patterns and ultimately investigate if these populations fit the model time-frames proposed by Hommersand (1986).

In *Laurencia sensu stricto* there were no links between Japan and South Africa (Figure 4.3), which is contrary to the expectations of Hommersand (1986) who postulated that the evidence for linkages between these two temperate floras may increase with our knowledge of the flora. However, the analysis did resolve a relationship between two temperate North Pacific taxa (*Laurencia pacifica* from the US and an undescribed Japanese *Laurencia* species) which were the most basal taxa in their clade together with the temperate south-east Australian species *Laurencia rigida* (Figure 4.3) and this provides evidence for a link between these temperate Pacific floras. While Hommersand (1986) focussed on the potential pathways for the development of the South African flora, the Austral Pacific Ocean migratory path (see Figure 3 in Hommersand (1986)) could be an explanation for the relatedness evident between the high latitude Northern Pacific taxa and the south-east Australian *Laurencia rigida*. Also in Figure 4.3, *Laurencia cf. majuscula* is shared between temperate Western Australia (a single specimen from Coral Bay: Paradise Beach) and Okinawa, the largest of the Ryukyu Islands (several specimens from around Okinawa – see Table A2 for details). Sequence divergence levels for these specimens are less than 0.5% which is on par with the within-species divergence levels for confirmed species in the *Laurencia* complex,

and while the marine flora of Okinawa is tropical rather than mixed temperate-tropical such as in Western Australia, a link between this island and Western Australia was shown for echinoderms (Benzie 1999).

The results of the global phylogeny suggest that there is still a significant amount of work to be done on the *Laurencia* complex. As more sequences from the Indo-Pacific are included in the global phylogeny the biogeography of the *Laurencia* complex will be better understood and the hypotheses proposed by Hommersand (1986) can be further tested.

CHAPTER 5:
GENERAL DISCUSSION

This study is the first to re-assess the diversity of the *Laurencia* complex species in South Africa and the South West Indian Ocean (SWIO) using molecular markers and to assess its biogeography globally. Literature on the *Laurencia* complex prior to this study reported ten *Laurencia sensu stricto* species occurring along the South African coast (Stegenga *et al.* 1997, De Clerck *et al.* 2005), only three of which (*L. complanata*, *L. flexuosa* and *L. natalensis*) had been sequenced (DNA sequences available on Genbank). The SWIO was comparatively much poorer; with the most comprehensive taxonomic accounts of *Laurencia* species for most locations being several decades old (e.g. Børgesen 1945, Isaac and Chamberlain 1958, Jaasund 1970, 1976) with no DNA sequences available.

In terms of South African diversity, Chapter 2 examined the validity of the *Laurencia* complex taxa collected using morpho-anatomical and DNA sequence data, the combination of which confirmed that nine of the ten species (see Table 1.2, Chapter 1) were distinct in the genus *Laurencia sensu stricto*. The only species not confirmed was *Laurencia obtusa* which was found to be polyphyletic and highly variable in its morphology, and therefore unlikely to represent a single species as suggested previously with South African specimens by Stegenga *et al.* (1997). This study produced the first phylogeny of the *Laurencia* complex incorporating South African specimens, which represented most (67%) of the sequences analysed. Results of the analyses increased the number of known South African *Laurencia sensu stricto* species to fourteen, including five species newly described: *Laurencia dehoopiensis*, *L. dichotoma*, *L. digitata*, *L. multiclavata* and *L. sodwaniensis*. A further five *Laurencia sensu stricto* taxa representing putative new species were found, although

additional specimens are required to confirm them as distinct entities. In addition, the study identified five new records for South Africa in three other genera of the *Laurencia* complex, namely *Laurenciella* (1) *Palisada* (3) and *Chondrophycus* (1). The updated list for the *Laurencia* complex in South Africa thus includes 19 (potentially 24) species in total.

In Chapter 3, the fourteen South African *Laurencia sensu stricto* species highlighted in Chapter 2 were fully described using morpho-anatomical features which for the first time incorporated the number of *corps en cerise* per epidermal cell. The *corps en cerise* proved to be a consistent taxonomic character, particularly useful in distinguishing species. For example, this study found two species in what had been considered the wide-spread and well-known species, *Laurencia flexuosa* Kützing; one species had 2-3 *corps en cerise* (*L. flexuosa*) per epidermal cell while the other had 5-6 *corps en cerise* (*L. cf. elata*). The first taxonomic key to the South African *Laurencia sensu stricto* species was produced, following on the work of Stegenga *et al.* (1997) who had produced a key to the *Laurencia* species of the west coast and south-west transition zone of South Africa. The remaining morphotypes (five *Laurencia sensu stricto* taxa) identified in this study for the *Laurencia* complex in South Africa remain formally undescribed, but morphological and anatomical data were captured for future study.

In Chapter 4 the inclusion of SWIO specimens in the phylogenetic analyses produced a number of interesting results. For instance, specimens from Europa Island, Glorioso Island, Madagascar and Reunion, together with a sequence downloaded from Genbank (*Laurencia flexilis* AF489860, from the Philippines), were found to represent two putative new genera based on the level of sequence divergence between these specimens and all other taxa in the *Laurencia* complex. The distributions of South African *Laurencia* complex species were described (it should be noted that species distributions for *Chondrophycus* and *Palisada* were

inferred from relatively few and scattered specimens, see Chapter 4). The inclusion of specimens from the localities of the SWIO extended the distribution of several previously known, as well as newly-described South African *Laurencia sensu stricto* species. For example *Laurencia natalensis* and *L. multiclavata* were both recorded from Madagascar, while the latter species was also recorded from Glorioso Island. Similarly, *Laurencia complanata* which was recorded from South Africa and Mozambique before this study, has now also been recorded on the island of Madagascar.

A proper assessment of the global diversity of the *Laurencia* complex would require a broad oceanic perspective. In Chapter 4 an assessment on this scale was produced for the first time incorporating sequences for taxa from as many oceanic regions as possible. Results from this analysis, while preliminary, identified links between Western Australian and South African/SWIO *Laurencia sensu stricto* taxa (see Figure 4.3, Chapter 4). The floristic affinities between South Africa and Western Australia fits well with the hypotheses of Hommersand (1986) who outlined three potential mechanisms which could explain the relationship between the taxa of these regions. The mechanisms suggested by Hommersand (1986) were:

- (1) They represent vicariant relics of a previously continuous distribution along the coast of Gondwanaland at a time when the connection between the coastlines of Africa and Australia were relatively continuous as far back as the Cretaceous period,
- (2) Their relatedness is a result of the separate evolution of species that share a common Tethyan or Indian Ocean (i.e. warm-water) ancestry that have evolved similar morphological adaptations in response to corresponding changes in climate,
- (3) Species clusters that evolved initially in Western and Southern Australia produced offspring that migrated through the Indian Ocean via the North Equatorial Current to

South Africa during periods of major global cooling corresponding to times of maximal glaciation in Antarctica as in, for example, the Pleistocene glaciation.

Of the three hypotheses proposed by Hommersand (1986) the results of this study so far seem to support the third scenario (favoured by Hommersand himself) with some Western Australian taxa being closely-related to temperate and tropical South Africa taxa (namely *Laurencia complanata*, *L. sodwaniensis* and *L. sp. 'morphotype A'*) and tropical species in the SWIO (*Laurencia sp. 'morphotype J'*) (Figure 4.3 in Chapter 4). These results, as mentioned previously, are preliminary and further sampling (alongside additional analyses discussed further below) is required to verify these findings.

A connection between the floras of South Africa and Atlantic North and Central America was also evident. At the species level the presence of *Laurencia natalensis*, which was recorded in Venezuela by Garcia-Soto & Lopez-Bautista (2013), fits in well with the hypothesis of Hommersand (1986) that South Africa would be a gateway for taxa to move from the Indian into the Atlantic Ocean (particularly north America and Europe), as prior to the work of the aforementioned authors this species had not been recorded outside of the Indian Ocean. At the generic level finding the monospecific genus *Laurenciella* on the south-west and south (Indian Ocean) coast of South Africa when it had only been recorded before from the western Atlantic Ocean (Senties *et al.* 2011) and the Canary Islands also support the migration/South African gateway hypothesis proposed by Hommersand (1986).

Future Perspectives

Additional markers

The plastid marker *rbcL* has been highly successful in delineating genera and species in the *Laurencia* complex; however, additional molecular markers are needed to confirm the phylogenetic relationships determined using this marker. Lewis *et al.* (2008) successfully amplified the plastid *Rubisco* spacer, nuclear internal transcribed spacers (*ITS-1* and *ITS-2*) and rDNA (5.8S) for the *Laurencia* complex. They found that non-coding plastid (*Rubisco*) and nuclear (*ITS*) spacer sequence data were fairly successful at distinguishing species, while the coding nuclear marker 5.8S was generally only good at distinguishing between different genera. Despite its reported usefulness at identifying rhodophyte species (Hu *et al.* 2009), there have been no publications since Lewis *et al.* (2008) using nuclear *ITS* as a marker when analysing the *Laurencia* complex.

Other markers to explore include the main barcoding marker for red algae, the 5' region of cytochrome oxidase 1 (COI-5P), which has already been used successfully in analyses of two *Laurencia* complex species – *Laurencia pyramidalis* and *Laurenciella marilzae*, as well as cytochrome oxidase 2-3 spacer (*cox 2-3*) which has proven to be a useful marker for phylogeographic studies in red algae (Zuccarello and West 2002, Provan *et al.* 2005, Andreakis *et al.* 2007, Bolton *et al.* 2011, Paiano and Necchi 2013). The latter mitochondrial marker (*cox 2-3* spacer) was suggested as the next marker to be sequenced for the *Laurencia* complex, after *rbcL*, by the International *Laurencia* complex Working Group which formed at the International Phycological Congress (IPC-10) in Orlando, Florida, USA in August 2013.

The importance of sequencing barcoding makers as the next step for the *Laurencia* complex becomes apparent when assessing the representation of the group on the barcoding website

Barcode of Life Data Systems (BOLD; www.boldsystems.org). In the genus *Laurencia sensu stricto* there are only 101 records, representing 26 species. Fifty-four of the records are plastid *rbcL* sequences sourced from GenBank™ (www.ncbi.nlm.nih.gov) and 47 are from Universidad de la Laguna (Canary Islands, Spain). The current records are from Spain (31), Portugal (22), Brazil (17), Mexico (11), Unspecified (*7), South Africa (4), Sri Lanka (3), United States (3), Cuba (2) and France (1). Similarly there are only 17 records of *Palisada*, all but two of those being *rbcL* sequences sourced from Genbank™.

This poor and biased representation of the *Laurencia* complex gives a good idea of the paucity of genetic work on the complex in general, despite the success of *rbcL* in phylogenetic analyses.

Molecular phylogenetic dating

In light of some of the evidence found in this study which tentatively supports the Hommersand (1986) hypotheses for the origins of the South Africa flora, including the possible movement of South African Indian Ocean taxa into the Atlantic, the next step would be to test these hypotheses using molecular dating techniques for the *Laurencia* complex. These techniques would not only address biogeographical hypothesis testing but also evolutionary questions in regards to the species diversity of the *Laurencia* complex. Fleshy seaweeds, like those of the *Laurencia* complex, have very few fossil records against which calibration points for the molecular clock can be set; the clock, alongside rates of molecular change, are necessary for accurate dating of phylogenies. In their analysis of the *Bostrychia calliptera* - *B. pinnata* species complex, Zuccarello and West (2002) produced a dated molecular phylogeny which has formed the basis of later diversity and phylogeographic studies for the red algae (Andreakis *et al.* 2007, Payo *et al.* 2013). Perhaps the most notable of these is Payo *et al.* (2013) who found that the widely-distributed *Portieria hornemanii*

(Lyngbye) P.C. Silva previously believed to be the only species of the genus present throughout the Indo-Pacific was in fact representative of 21 cryptic species within the Philippines alone. Similar molecular techniques can be applied to the *Laurencia* complex to address questions of speciation particularly on a local scale when comparing species/molecular lineages that are genetically similar - as well as testing the if the evolutionary patterns of the *Laurencia* complex match the hypotheses proposed by Hommersand (1986).

Sampling strategies

A more comprehensive sampling in the SWIO is necessary for future study with the inclusion of specimens from Kenya, Tanzania, the Seychelles, and the Comoros, as well as more detailed collections in Madagascar, Mozambique and the Mascarene Islands which are likely to reveal more diversity than that currently known. Particularly important areas, alongside the SWIO localities mentioned above, are the northern Indian Ocean, especially the shores of India which has around 430 rhodophyte species (Kaladharan and Jayasankar 2003), twenty of which belong to the *Laurencia* complex (Guiry and Guiry 2014), but also the highly diverse Coral Triangle and much of Australia. Filling in these floristic gaps for the *Laurencia* complex will also prove useful for biogeographic studies and could test hypotheses for the origins (i.e. Tethyan or Indian Ocean) and pathways of distribution of the tropical and warm-temperate floras of South Africa and the shores of East Africa, such as those proposed by Hommersand (1986).

Chemotaxonomy

In addition to DNA markers, chemotaxonomic separation of species has been well documented in the *Laurencia* complex, primarily from the genus *Laurencia sensu stricto*.

This started with the study of Fenical and Norris (1975) and has spanned almost 40 years of research (e.g. Caccamese *et al.* 1979, Howard *et al.* 1980, Masuda *et al.* 1996, Pietra 2002, Gil-Rodriguez *et al.* 2009, Manchin-Sanchez *et al.* 2014) showing that *Laurencia* complex species can be distinguished on the basis of their secondary metabolites. For example Caccamese *et al.* (1979) found that using gas chromatography-mass spectrophotometry (GC-MS) they were able to characterise the six *Laurencia* species used in their study on the basis of compounds unique to each species. Similarly, Masuda *et al.* (1996) reported that alongside detailed morpho-anatomical study, *Laurencia composita* and *L. okamurae* could be distinguished from one another on the basis of their chemical constituents; *L. composita* produces chamigrane-type sesquiterpenoids, whereas *L. okamurae* produces cyclolaurane-type sesquiterpenoids. The most recent work using chemical analyses to discern species of the *Laurencia* complex is that of Machin-Sanchez *et al.* (2014) who, using capillary electrophoresis and mass spectrophotometry (CE-MS), were able to separate 28 species from Macaraonesia in the *Laurencia* complex, representing four of the six genera, from one another. From a South African perspective some preliminary evidence for species-level separation in *Laurencia sensu stricto* by means of gas-chromatography and nuclear magnetic resonance (NMR) spectroscopy was shown by Knott (2013, pers. comm.), though this was based on a limited number of specimens. The relatively high diversity of *Laurencia sensu stricto* in South Africa, the potential limitations of morphology and anatomy in distinguishing species, and the value of an additional source of evidence for species delimitation coupled with the bio-prospecting potential of *Laurencia sensu stricto* warrants further research into the chemistry of these species. A chemotaxonomic review on the Brazilian species of the *Laurencia* complex (Fujii *et al.* 2011) identified two of the other genera in the complex, *Palisada* and *Laurenciella* (as *Laurencia marilzae*), and they also produced secondary

metabolites which displayed some bioactivity. Both of these genera occur in South Africa and should be included in future chemical analyses of the South African *Laurencia* complex.

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Table A1: Collection details for specimens from South Africa sequenced in this study together with Genbank-sourced sequences (- : sequence not stored in Genbank yet)

Samples	Collection data/reference	GenBank accession numbers
<u>Outgroups</u>		
<i>Bostrychia radicans</i> (Montagne) Montagne	USA, Mississippi, St Louis Bay, leg. CFD Gurgel, 11 Feb. 1998	AS259497
<i>Chondria capensis</i> (Harvey) Falkenberg	South Africa, Western Cape, Mauritz Bay leg. M Rothman 02, Mar. 2011 (This study - #1004)	-
<i>C. capensis</i>	South Africa, Western Cape, Cape of Good Hope (pool) leg. RJ Anderson & JJ Bolton 18 Nov. 2009 (This study - #633)	-
<i>C. dasyphylla</i> (Woodward) C Agardh	USA, North Carolina, New Hanover County, Wrightsville Beach	U04021
<i>C. cf. dasyphylla</i>	South Africa, Western Cape, Natures Valley. leg CM Francis 12 Dec. 2012 (This study - #1162)	-
<i>Chondria</i> sp.	South Africa, Western Cape, De Hoop leg. RJ Anderson & JJ Bolton 19 Aug. 2008 (This study - #166)	-
<i>Chondria</i> sp.	South Africa, Western Cape, De Hoop, leg. RJ Anderson & JJ Bolton 18 Aug. 2008 (This study - #117)	-
<i>Chondria</i> sp.	South Africa, Western Cape, De Hoop, leg. RJ Anderson & JJ Bolton 18 Aug. 2008 (This study - #118)	-
<i>Chondria</i> sp.	South Africa, Western Cape, De Hoop, leg. RJ Anderson & JJ Bolton 20 Aug. 2009 (This study -	-

	#180)	
<i>Chondria</i> sp.	South Africa, Eastern Cape, Kenton-On-Sea leg. RJ Anderson & JJ Bolton 03 Oct. 2009 (This study - #335)	-
<i>Chondria</i> sp. (collected as <i>Laurencia obtusa</i>)	South Africa, Eastern Cape, Waterloo Bay leg. RJ Anderson & JJ Bolton 07 Sep. 2009 (This study - #479)	-
<i>Chondria</i> sp.1	South Africa, KwaZulu-Natal, Bhanga Nek, Rabbit Rock leg RJ Anderson (This study - #1688)	-
<i>Spyridia cupressina</i> Kützing	South Africa, Eastern Cape, Port Alfred, leg. RJ Anderson, JJ Bolton, 07 Jul. 2008 (This study - #25)	-
 <u><i>Chondrophyucus</i></u> (Tokida & Saito) Garbary & Harper		
<i>C. undulatus</i> (Yamada) Garbary & Harper	New Caledonia, Loyalty Island, Maré leg. C Payri 22 Mar. 2005 (Martin-Lescanne et al. 2010)	FJ785307
<i>C. undulatus</i>	New Caledonia, Loyalty Island, Maré leg. C Payri 22 Mar. 2005 (Martin-Lescanne et al. 2010)	FJ785308
<i>C. tranoi</i> (E. Gazon-Fortes)KW Nam [as <i>L. tranoi</i>]	Philippines. AO Lluisma (Unpub.)	AF489864
<i>Chondrophyucus</i> sp.	New Caledonia, Loyalty Island, Lifou leg. C Payri 26 Mar. 2005 (Martin-Lescanne et al. 2010)	FJ785309
<i>Chondrophyucus</i> sp. 1	South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Pt., leg. RJ Anderson & JJ Bolton 09 Jun. 2010 (This Study - #802)	-

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<i>L. cf. brongniartii</i> J. Agardh	Australia, Tarcoola Beach, leg S Fredericq, 1993 (unpub.)	EF061654
<i>L. cf. brongniartii</i>	Taiwan, Makang Harbout. S. Fredericq, 11 Jul. 1993 (Fujii et al. 2006)	AF465814
<i>L. cf. brongniartii</i>	South Africa, KwaZulu-Natal, Sodwana Bay, 2 Mile Reef leg. RJ Anderson & JJ Bolton 22 Mar. 2011 (This study - #978)	-
<i>L. cartilaginea</i> Yamada	Philippines. AO Lluisma (Unpub.)	AF489859
<i>L. complanata</i> (Suhr) Kützing	South Africa, KwaZulu-Natal, Port Edward, leg. S Fredericq & O De Clerck, 8 Jul. 2001 (Fujii et al. 2006)	AF465813
<i>L. complanata</i>	South Africa, KwaZulu-Natal, Salt Rock, leg. RJ Anderson & JJ Bolton 09 Dec 2010 (This study - #859)	-
<i>L. complanata</i>	South Africa, KwaZulu-Natal, Port Edward leg. CM Francis 28 Sep 2011 (This study - #1053)	-
<i>L. cf. corymbosa</i> J. Agardh	South Africa, Eastern Cape, Three Sister leg. RJ Anderson & JJ Bolton 27 Feb. 2013 (This study - #1257)	-
<i>L. cf. corymbosa</i>	South Africa, Eastern Cape, Double Mouth leg. RJ Anderson & JJ Bolton 14 Jul. 2010 (This study - #768)	-
<i>L. cf. corymbosa</i>	South Africa, Eastern Cape, Port Alfred leg. RJ Anderson & JJ Bolton 07 Jul. 2008 (This study - #31)	-
<i>L. cf. corymbosa</i>	South Africa, Western Cape, De Hoop, Koppie Alleen leg. RJ Anderson & JJ Bolton 17 Feb. 2011 (This study - #903)	-

<i>L. cf. corymbosa</i>	South Africa, Western Cape, De Hoop, East of Koppie Alleen leg. RJ Anderson & JJ Bolton 18 Feb. 2011 (This study - #926)	-
<i>L. cf. corymbosa</i>	South Africa, Western Cape, De Hoop leg. RJ Anderson & JJ Bolton 19 Aug. 2008 (This study - #164)	-
<i>L. dehoopiensis</i> sp. nov. Francis, Bolton, Anderson & Mattio [morphotype D]	South Africa, Western Cape, De Hoop leg. RJ Anderson & JJ Bolton 18 Aug. 2008 (This study - #139)	-
<i>L. dehoopiensis</i> sp. nov. [morphotype D]	South Africa, Western Cape, De Hoop leg. RJ Anderson & JJ Bolton 19 Aug. 2008 (This study - #154)	-
<i>L. dehoopiensis</i> sp. nov. [morphotype D]	South Africa, Western Cape, De Hoop, East of Koppie Alleen leg. CM Francis, RJ Anderson & JJ Bolton 18 Feb. 2011 (This study - #922/BOL150571)	-
<i>L. dendroidea</i> J Agardh	Brazil, Rio de Janeiro, Rio das Ostras, Areias Negras leg. V Cassano 2005	GU330237
<i>L. dendroidea</i>	Brazil, Sao Paulo, Ubatuba, Praia de Felix leg. MT Fujii 31 Aug. 2000 (Fujii et al. 2006)	AF465810
<i>L. dendroidea</i> [as <i>L. majuscula</i> (Harvey) A.H.S. Lucas]	Spain, Canary Islands, Tenerife, Punta del Hidalgo, Roca Negro leg. MC Gil-Rodriguez, 12 Jul. 2006 (Gil-Rodriguez et al. 2009)*	EF686000
<i>L. dendroidea</i> [as <i>L. cf. majuscula</i>]	New Caledonia, Ile de Pins. Leg C Payri 2 Dec. 2005 (Martin-Lescanne et al. 2010)	FJ785312
<i>L. dendroidea</i> (as <i>L. obtusa</i> (Hudson) Lamouroux)	Guadeloupe, Pointe de la Verdure, leg. S Fredericq 20 Mar. 1994 (Fujii et al. 2006)	AF465811
<i>L. dendroidea</i> (as <i>L. obtusa</i>)	Venezuela, Isla Pelone, leg. C.F. Gurgel 26 Jun. 1999 (Fujii et al. 2006)	AF465812

<i>L. dichotoma</i> sp. nov. Francis, Bolton, Anderson & Mattio [morphotype B]	South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Pt. leg. JJ Bolton, RJ Anderson & CM Francis 22 Mar. 2011 (This study - #977/BOL150568)	-
<i>L. dichotoma</i> sp. nov. [morphotype B]	South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Pt. leg. JJ Bolton, RJ Anderson & CM Francis 22 Mar. 2011 (This study - #989/57729)	-
<i>L. dichotoma</i> sp. nov. [morphotype B]	South Africa, KwaZulu-Natal, Bhanga Nek, Bhanga Rock leg. JJ Bolton, RJ Anderson 04 Oct. 2010 (This study - #1583)	-
<i>L. digitata</i> sp. nov. Francis, Bolton, Anderson & Mattio [morphotype E]	South Africa, Western Cape, Natures Valley leg. RJ Anderson & JJ Bolton 04 Jul. 2008 (This study - #52)	-
<i>L. digitata</i> sp. nov. [morphotype E]	South Africa, Western Cape, De Hoop, East of Koppie Alleen leg. CM, Francis, RJ Anderson & JJ Bolton 18 Feb. 2011 (This study - #930)	-
<i>L. digitata</i> sp. nov. [morphotype E]	South Africa, De Hoop, East of Koppie Alleen leg. CM Francis, RJ Anderson & JJ Bolton 18 Feb. 2011 (This study - #932)	-
<i>L. digitata</i> sp. nov. [morphotype E]	South Africa, KwaZulu-Natal, Cape Vidal leg. CM Francis, RJ Anderson & JJ Bolton 20 Mar. 2011 (This study - #971/BOL150572)	-
<i>L. digitata</i> sp. nov. [morphotype E]	South Africa, KwaZulu-Natal, Bhanga Nek, Bhanga Rock leg. CM Francis, RJ Anderson & JJ Bolton 18 Feb. 2011 (This study - #1669)	-
<i>L. digitata</i> sp. nov. [morphotype E]	South Africa, KwaZulu-natal, Cape Vidal leg. CM Francis, RJ	-

	Anderson & JJ Bolton 18 Feb. 2011 (This study - #1027)	
<i>L. digitata</i> sp. nov. [morphotype E]	South Africa, Eastern Cape, Storms River leg. CM Francis, RJ Anderson & JJ Bolton 18 Feb. 2011 (This study - #1174)	-
<i>L. cf. elata</i> (C Agardh) Hooker & Harvey	South Africa, Eastern Cape, Cape St Francis leg. RJ Anderson 29 Mar. 2010 (This study - #686)	-
<i>L. cf. elata</i>	South Africa, Eastern Cape, Double Mouth leg. RJ Anderson & JJ Bolton 14 Jul. 2010 (This study - #767)	-
<i>L. cf. elata</i>	South Africa, Western Cape, Grootbank, leg. RJ Anderson & JJ Bolton 04 Jul. 2008 (This study - #55)	-
<i>L. cf. elata</i>	South Africa, Eastern Cape, Port Alfred, Saltvlei leg CM Francis 25. Feb. 2013 (This study - #1214)	-
<i>L. cf. elata</i>	South Africa, Eastern Cape, Port Alfred, Saltvlei leg CM Francis 25 Feb. 2013 (This study - #1214)	-
<i>L. flexuosa</i> Kützing	South Africa, KwaZulu-Natal, Palm Beach, leg. S Fredericq & O De Clerck, 7 Feb. 2001 (Fujii et al. 2006)	AF465815
<i>L. flexuosa</i>	South Africa, Western Cape, De Hoop leg. RJ Anderson & JJ Bolton 18 Aug. 2008 (This study - #140)	-
<i>L. flexuosa</i>	South Africa, Western Cape, De Hoop, Koppie Alleen leg. RJ Anderson & JJ Bolton 17 Feb. 2011 (This study - #904)	-
<i>L. flexuosa</i>	South Africa, Western Cape, De Hoop, East of Koppie Alleen leg. RJ Anderson & JJ Bolton 18 Feb.	-

	2011 (This study - #928)	
<i>L. flexuosa</i>	South Africa, KwaZulu-Natal, Palm Beach leg. RJ Anderson & JJ Bolton 28 Sep. 2012 (This study - #1063)	-
<i>L. flexuosa</i>	South Africa, KwaZulu-Natal, Palm Beach leg. RJ Anderson & JJ Bolton 28 Sep. 2012 (This study - #1057)	-
<i>L. flexuosa</i>	South Africa, KwaZulu-Natal, Port Edward leg. RJ Anderson & JJ Bolton 28 Sep. 2012 (This study - #1053)	-
<i>L. flexuosa</i>	South Africa, Eastern Cape, Storms River leg. RJ Anderson & JJ Bolton 12 Dec. 2012 (This study - #1167)	-
<i>L. flexuosa</i>	South Africa, Western Cape, Natures Valley leg. RJ Anderson & JJ Bolton 13 Dec. 2012 (This study - #1157)	-
<i>L. flexuosa</i>	South Africa, Eastern Cape, Haga Haga leg. RJ Anderson & JJ Bolton 22 Jun. 2011 (This study - #1120)	-
<i>L. flexuosa</i>	South Africa, Western Cape, Knysna Heads 11 Dec. 2012 leg. RJ Anderson & JJ Bolton 18 Feb. 2011 (This study - #1133)	-
<i>L. flexuosa</i>	South Africa, Eastern Cape, Port Elizabeth leg. RJ Anderson & JJ Bolton 14 Dec. 2012 (This study - #1191)	-
<i>L. flexuosa</i>	South Africa, Western Cape, Sedgefield, Swartvlei leg. RJ Anderson & JJ Bolton 15 Dec. 2012 (This study - #1192)	-

<i>L. flexuosa</i>	South Africa, Eastern Cape, Kenton-on-Sea leg. RJ Anderson & JJ Bolton 26 Feb. 2013 (This study - #1237)	-
<i>L. flexuosa</i>	South Africa, Eastern Cape, Three Sisters. RJ Anderson & JJ Bolton 27 Feb. 2013 (This study - #1252)	-
<i>L. flexuosa</i>	South Africa, Eastern Cape, Hluleka leg. RJ Anderson & JJ Bolton 23 Aug. 2013 (This study - #1336)	-
<i>L. flexuosa</i>	South Africa, Western Cape, De Hoop, East of Koppie Alleen leg. RJ Anderson & JJ Bolton 18 Feb. 2011 (This study - #1332)	-
<i>L. flexuosa</i>	South Africa, KwaZulu-Natal, Mission Rocks leg. RJ Anderson & JJ Bolton 19 Mar. 2011 (This study - #958)	-
<i>L. flexuosa</i>	South Africa, Western Cape, Nature's Valley leg. RJ Anderson & JJ Bolton 04 Apr. 2008 (This study - #57)	-
<i>L. flexilis</i> Setchell	Philippines. AO Lluisma (Unpub.)	AF489860
<i>L. glomerata</i> Kützing	South Africa, Western Cape, De Hoop Koppie Alleen leg. RJ Anderson & JJ Bolton 17 Feb 2011 (This study - #902)	-
<i>L. glomerata</i>	South Africa, Western Cape, De Hoop, Koppie Alleen leg. RJ Anderson & JJ Bolton 17 Feb. 2011 (This study - #908)	-
<i>L. glomerata</i>	South Africa, Western Cape, De Hoop, Koppie Alleen leg. RJ Anderson & JJ Bolton 17 Feb. 2011 (This study - #909)	-
<i>L. glomerata</i>	South Africa, Western Cape, De	-

	Hoop, Koppie Alleen leg. RJ Anderson & JJ Bolton 17 Feb. 2011 (This study - #910)	
<i>L. glomerata</i>	South Africa, Western Cape, De Hoop, Koppie Alleen leg. RJ Anderson & JJ Bolton 18 Feb. 2011 (This study - #927)	-
<i>L. glomerata</i>	South Africa, Western Cape, De Hoop, Noetsie leg. RJ Anderson & JJ Bolton 19 Feb. 2011 (This study - #943)	-
<i>L. glomerata</i>	South Africa, Western Cape, Grootbank leg. RJ Anderson & JJ Bolton 04 Jul. 2008 (This study - #56)	-
<i>L. glomerata</i>	South Africa, Western Cape, De Hoop leg. RJ Anderson & JJ Bolton 18 Aug. 2008 (This study - #125)	-
<i>L. glomerata</i>	South Africa, Eastern Cape, Port Alfred leg. RJ Anderson & JJ Bolton 03 Mar. 2009 (This study - #317)	-
<i>L. glomerata</i>	South Africa, Eastern Cape, Cape St Francis leg. RJ Anderson 29 Mar. 2010 (This study - #685)	-
<i>L. glomerata</i>	South Africa, Western Cape, Keurbooms, Die Eiland 22 Sep. 2010 (This study - #863)	-
<i>L. glomerata</i>	South Africa, Western Cape, Mauritz Bay leg. M. Rothmann, C. Boothroyd & F. Kemp 02 Mar. 2011 (This study - #1003)	-
<i>L. glomerata</i>	South Africa, Western Cape, Mauritz Bay leg. M. Rothmann, C. Boothroyd & F. Kemp 02 Mar. 2011 (This study - #1005)	-
<i>L. glomerata</i>	South Africa, Western Cape,	-

	Natures Valley leg. CM Francis 12 Dec. 2012 (This study - #1161)	
<i>L. glomerata</i>	South Africa, Western Cape, Natures Valley leg. CM Francis 12 Dec. 2012 (This study - #1163)	-
<i>L. glomerata</i>	South Africa, Western Cape, Natures Valley leg. CM Francis 12 Dec. 2012 (This study - #1164)	-
<i>L. glomerata</i>	South Africa, Western Cape, Natures Valley leg. CM Francis 12 Dec. 2012 (This study - #1166)	-
<i>L. glomerata</i>	South Africa, Eastern Cape, Port Elizabeth leg. CM Francis 14 Dec. 2012 (This study - #1190)	-
<i>L. glomerata</i>	South Africa, Eastern Cape, Storms River leg. CM Francis 13 Dec. 2012 (This study - #1178)	-
<i>L. glomerata</i>	South Africa, Eastern Cape, Storms River leg. CM Francis 13 Dec. 2012 (This study - #1179a)	-
<i>L. glomerata</i>	South Africa, Eastern Cape, Port Alfred leg. CM Francis 25 Feb. 2013 (This study - #1211)	-
<i>L. glomerata</i>	South Africa, Eastern Cape, Port Alfred leg. CM Francis 25 Feb. 2013 (This study - #1212)	-
<i>L. glomerata</i>	South Africa, Eastern Cape, Three Sister, leg CM Francis 27 Feb. 2013 (This study - #1251)	-
<i>L. cf. kuetzingii</i> A Millar	New Caledonia, Loyalty Island, Ouvéa leg C Payri. 31 Mar. 2005 (Martin-Lescanne et al. 2010)	FJ785322
<i>L. cf. mariannensis</i> Yamada	New Caledonia, Lagon Sud-Ouest, Ilot Lagènére leg C Payri 11 Jul. 2003 (Martin-Lescanne et al. 2010)	FJ785313

<i>L. cf. mcdermidae</i> IA Abbott	New Caledonia, Ile de Pins leg C Payri 29 Jun. 2005 (Martin-Lescanne et al. 2010)	FJ785314
<i>L. multiclavata</i> sp. nov. Francis, Bolton, Anderson & Mattio [morphotype F]	South Africa, Western Cape, De Hoop leg. RJ Anderson & JJ Bolton 18 Aug. 2008 (This study - #127)	-
<i>L. multiclavata</i> sp. nov. [morphotype F]	South Africa, Eastern Cape, Cape St Francis leg. RJ Anderson 29 Mar. 2010 (This study - #687)	-
<i>L. multiclavata</i> sp. nov. [morphotype F]	South Africa, Western Cape, De Hoop leg. CM Francis, RJ Anderson & JJ Bolton 17 Feb. 2011 (This study - #906/BOL150569)	-
<i>L. multiclavata</i> sp. nov. [morphotype F]	South Africa, KwaZulu-Natal, Cape Vidal leg. CM Francis, RJ Anderson & JJ Bolton 20 Mar. 2011 (This study - #969)	-
<i>L. multiclavata</i> sp. nov. [morphotype F]	South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Pt. leg. JJ Bolton, RJ Anderson & CM Francis 22 Mar. 2011 (This study - #979)	-
<i>L. multiclavata</i> sp. nov. [morphotype F]	South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Pt. leg. JJ Bolton, RJ Anderson & CM Francis 22 Mar. 2011 (This study - #981)	-
<i>L. multiclavata</i> sp. nov. [morphotype F]	South Africa, Eastern Cape, Hluleka. leg. JJ Bolton, RJ Anderson & CM Francis 23 Aug. 2013 (This study - #1335)	-
<i>L. multiclavata</i> sp. nov. [morphotype F]	South Africa, KwaZulu-Natal, Bhangane Nek, Bhangane Rock leg. JJ Bolton, RJ Anderson 04 Oct. 2013 (This study - #1602)	-
<i>L. multiclavata</i> sp. nov. [morphotype F]	South Africa, KwaZulu-Natal, Cape Vidal. leg. JJ Bolton, RJ Anderson 25 Sep. 2011 (This study - #1024)	-
<i>L. multiclavata</i> sp. nov. [morphotype F]	South Africa, Western Cape,	-

	Knysna Heads leg. JJ Bolton, RJ Anderson & CM Francis 11 Nov. 2012 (This study - #1135)	
<i>L. multiclavata</i> sp. nov. [morphotype F]	South Africa, Western Cape, Natures Valley leg. JJ Bolton, RJ Anderson & CM Francis 12 Dec. 2012 (This study - #1159)	-
<i>L. multiclavata</i> sp. nov. [morphotype F]	South Africa, Eastern Cape, Storms River, leg. JJ Bolton, RJ Anderson & CM Francis 13 Dec. 2012 (This study - #1171)	-
<i>L. multiclavata</i> sp. nov. [morphotype F]	South Africa, Eastern Cape, Port Elizabeth leg. JJ Bolton, RJ Anderson & CM Francis 14 Dec. 2012 (This study - #1185)	-
<i>L. multiclavata</i> sp. nov. [morphotype F]	South Africa, Western Cape, Sedgefield, Swartvlei. JJ Bolton, RJ Anderson & CM Francis 15 Dec. 2012 (This study - #1194)	-
<i>L. multiclavata</i> sp. nov. [morphotype F]	South Africa, Eastern Cape, Kenton-on-Sea leg. JJ Bolton, RJ Anderson & CM Francis 26 Feb. 2013 (This study - #1239)	-
<i>L. natalensis</i> Kylin	South Africa, KwaZulu-Natal, Palm Beach, leg. S Fredericq & O De Clerck, 7 Feb. 2001 (Fujii et al. 2006)	AF465816
<i>L. natalensis</i>	South Africa, Western Cape, Knysna Estuary leg. RJ Anderson & JJ Bolton 04 Jul. 2008 (This study - #50)	-
<i>L. natalensis</i>	South Africa, Eastern Cape, Port Alfred leg. RJ Anderson & JJ Bolton 03 Sept. 2009 (This study - #316)	-
<i>L. natalensis</i>	South Africa KwaZulu-Natal, Sodwana Bay, Jesser Pt. leg. RJ Anderson & JJ Bolton 10 Jun. 2009	-

	(This study - #587)	
<i>L. natalensis</i>	South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Pt. leg. RJ Anderson & JJ Bolton 09 Jun. 2010 (This study - #800)	-
<i>L. natalensis</i>	South Africa, KaZulu-Natal, Sodwana Bay, Jesser Pt. leg. RJ Anderson & JJ Bolton 09 Sep. 2010 (This study - #820)	-
<i>L. natalensis</i>	South Africa, KwaZulu-Natal, Mapalane leg. RJ Anderson & JJ Bolton 09 Nov. 2010 (This study - #836)	-
<i>L. natalensis</i>	South Africa, KwaZulu-Natal, Mission Rocks leg. RJ Anderson & JJ Bolton 19 Mar. 2011 (This study - #960)	-
<i>L. natalensis</i>	South Africa, KwaZulu-Natal, Cape Vidal leg. RJ Anderson & JJ Bolton 25 Sep. 2011 (This study - #1022)	-
<i>L. natalensis</i>	South Africa, Western Cape leg. RJ Anderson & JJ Bolton 12 Dec. 2012 (This study - #1155)	-
<i>L. natalensis</i>	South Africa, Eastern Cape, Port Elizabeth leg. RJ Anderson & JJ Bolton 14 Dec. 2012 (This study - #1186)	-
<i>L. natalensis</i>	South Africa, Western Cape, Sedgefield, leg. RJ Anderson & JJ Bolton 15 Dec. 2012 (This study - #1193)	-
<i>L. natalensis</i>	South Africa, Eastern Cape, Kenton-on-Sea leg. RJ Anderson & JJ Bolton 26 Feb. 2013 (This study - #1238)	-
<i>L. natalensis</i>	South Africa, KwaZulu-Natal, Bhanga Nek, Bhanga Rock. leg. RJ	-

	Anderson & JJ Bolton 4 Oct. 2013 (This study - #1603)	
<i>L. cf. nidifica</i> J Agardh	New Caledonia, Ile de Pins. leg C Payri 30 Nov. 2005 (Martin- Lescanne et al. 2010)	FJ785315
<i>L. obtusa</i> (Hudson) Lamouroux	Ireland, Donegal, Fanad Head. leg. C Maggs 06 Dec. 1998 (Nam et al. 2000)	AF281881
<i>L. pacifica</i> Kylin	USA, California, Moss Beach, Central Beach leg. S Fredericq 17 Feb. 1992	AY588411
<i>L. pumila</i> (Grunow) Papenfuss	South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Pt. leg. RJ Anderson & JJ Bolton 10 Jun. 2009 (This study - #588)	-
<i>L. pumila</i>	South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Pt. leg. RJ Anderson & JJ Bolton 09 Jun. 2010 (This study - #803)	-
<i>L. pumila</i>	South Africa, KwaZulu-Natal, Bhanga Neck, Bhanga Rock. leg. RJ Anderson & JJ Bolton 04 Oct. 2013 (This study - #1604)	-
<i>L. pumila</i>	South Africa, KwaZulu-Natal, Bhanga Neck, Bhanga Rock. leg. RJ Anderson & JJ Bolton 07 Oct. 2013 (This study - #1665)	-
<i>L. pumila</i>	South Africa, KwaZulu-Natal, Cape Vidal leg. RJ Anderson & JJ Bolton 25 Sep. 2011 (This study - #1028)	-
<i>L. pumila</i>	South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Pt. leg. RJ Anderson & JJ Bolton 09 Sep. 2010 (This study - #822)	-
<i>L. pyramidalis</i> Bory de Saint ex Kützing	France, Brittany, Roscoff leg F. Rosseau. 5 Dec. 2002 (Martin- Lescanne et al. 2010)	FJ785316

<i>L. rigida</i> J Agardh	Australia, New South Wales, Botany Bay (Unpub.)	AY920852
<i>L. sodwaniensis</i> sp. nov. Francis, Bolton, Anderson & Mattio [morphotype C]	South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Pt. leg. JJ Bolton, RJ Anderson & CM Francis 09 Sep. 2010 (This study - #818)	-
<i>L. sodwaniensis</i> sp. nov. [morphotype C]	South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Pt. leg. JJ Bolton, RJ Anderson & CM Francis 22 Mar. 2011 (This study - #987)	-
<i>L. sodwaniensis</i> sp. nov. [morphotype C]	South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Pt. leg. JJ Bolton, RJ Anderson & CM Francis 20 Mar. 2011 (This study - #968/BOL150570)	-
<i>Laurencia stegengae</i> nom. nov. (Stegenga, Bolton and Anderson) Francis, Bolton, Anderson & Mattio	South Africa, Western Cape, De Hoop leg. RJ Anderson & JJ Bolton 19 Aug. 2008 (This study - #126)	-
<i>L. stegengae</i> nom. nov.	South Africa, Western Cape, De Hoop, Koppie Alleen leg. RJ Anderson & JJ Bolton 17 Feb. 2011 (This study - #901)	-
<i>L. stegengae</i> nom. nov.	South Africa, Western Cape, Buffel's Bay leg. RJ Anderson & JJ Bolton 17/09/2008 (This study - #181)	-
<i>L. stegengae</i> nom. nov.	South Africa, Western Cape, De Hoop leg. RJ Anderson & JJ Bolton 19 Aug. 2008 (This study - #159)	-
<i>L. stegengae</i> nom. nov.	South Africa, Western Cape, False Bay, Clovelly leg. JJ Bolton 18 Mar. 2010 (This study - #680)	-
<i>L. stegengae</i> nom. nov.	South Africa, Western Cape, Keurbooms, Platbank leg. RJ Anderson & JJ Bolton 23 Sep. 2010 (This study - #872)	-
<i>L. stegengae</i> nom. nov.	South Africa, Western Cape,	-

	Keurbooms, Arch Rock leg. RJ Anderson & JJ Bolton 24 Sep. 2010 (This study - #880)	
<i>L. stegengae</i> nom. nov.	South Africa, Western Cape, De Hoop, Koppie Alleen leg. RJ Anderson & JJ Bolton 17 Feb. 2011 (This study - #900)	-
<i>L. stegengae</i> nom. nov.	South Africa, Western Cape, Langebaan leg. RJ Anderson & JJ Bolton 26 Mar. 2012 (This study - #1073)	-
<i>L. stegengae</i> nom. nov.	South Africa, Western Cape, Langebaan Koppie Alleen leg. RJ Anderson & JJ Bolton 26 Mar. 2012 (This study - #1074)	-
<i>L. stegengae</i> nom. nov.	South Africa, Western Cape, Knysna Heads leg. RJ Anderson & JJ Bolton 11 Dec 2012 (This study - #1134)	-
<i>L. stegengae</i> nom. nov.	South Africa, Western Cape, Natures Valley leg. RJ Anderson & JJ Bolton 12 Dec. 2012 (This study - #1156)	-
<i>L. stegengae</i> nom. nov.	South Africa, Eastern Cape, Storms River leg. RJ Anderson & JJ Bolton 13 Dec. 2012 (This study - #1170)	-
<i>L. stegengae</i> nom. nov.	South Africa, Eastern Cape, Three Sisters leg. RJ Anderson & JJ Bolton 27Feb. 2013 (This study - #1254)	-
<i>L. venusta</i> Yamada	Mexico, Quintana Roo, Puerto Morelos, Punta Brava, leg. JD Larrea and A Senties, 18 Apr. 2004	EF061655
<i>L. viridis</i> Gil-Rodriguez & Haroun	Spain, Canary Islands, Tenerife, Punta del Hidalgo, Roca Negro leg. MC Gil-Rodriguez, 6 Oct. 2005	EF685999
<i>L. viridis</i>	Spain, Canary Islands, Tenerife,	EF686004

	Playa Paraiso leg MC Gil-Rodriguez, A Senties & MT Fujii, 14 Jul. 2006 (Gil-Rodriguez et al. 2009)	
<i>Laurencia sp.</i> morphotype A	South Africa, KwaZulu-natal, Sodwana Bay, Jesser Pt. leg. JJ Bolton, RJ Anderson & CM Francis 22 Mar. 2011 (This study - #991)	-
<i>Laurencia sp.</i> morphotype G	South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Pt., leg. RJ Anderson & JJ Bolton 09 Sep. 2010 (This study - #821)	-
<i>Laurencia sp.</i> morphotype H	South Africa, Eastern Cape, Kenton-on-Sea & Three Sisters, leg. RJ Anderson & JJ Bolton 09 Sep. 2010 (This study - #1240 & #1255)	-
<i>Laurencia sp.</i> morphotype I	South Africa, Eastern Cape, Hluleka leg. RJ Anderson & JJ Bolton 09 Sep. 2010 (This study - #1337)	-
<i>Laurencia sp.</i> morphotype J	South Africa, Eastern Cape, Hluleka leg. RJ Anderson & JJ Bolton 09 Sep. 2010 (This study - #1339)	-
 <i>Laurenciella</i> V.Cassano, Gil-Rodríguez, Senties, Díaz-Larrea, M.C.Oliveira & M.T.Fujii		
<i>L. marilzae</i> (Gil-Rodriguez, Senties, Diaz-Larrea, Cassano & MT Fujii) Gil-Rodriguez, Senties, Diaz-Larrea, Cassano & MT Fujii (as <i>Laurencia marilzae</i>)	Spain, Canary Islands, Tenerife, Punta del Hidalgo, Roca Negro leg. MC Gil-Rodriguez, 12 Jul. 2006 (Gil-Rodriguez et al. 2009)	EF686002
<i>L. marilzae</i>	Spain, Canary Islands, Tenerife, Playa Paraiso leg MC Gil-Rodriguez, A Senties & MT Fujii, 14 Jul. 2006 (Gil-Rodriguez et al. 2009)	EF686001

<i>L. marilzae</i>	Spain, Canary Islands, Tenerife, Punta del Hidalgo, Bahía Izquierda Faro leg. MC Gil-Rodríguez, 6 Oct. 2005 (Gil-Rodríguez et al. 2009)	EF686003
<i>L. marilzae</i>	South Africa, Western Cape, De Hoop leg. RJ Anderson & JJ Bolton 19 Aug. 2008 (This study - #155)	-
<i>L. marilzae</i>	South Africa, Western Cape, De Hoop leg. RJ Anderson & JJ Bolton 19 Aug. 2008 (This study - #168)	-
<i>L. marilzae</i>	South Africa, Western Cape, De Hoop East of Koppie Alleen leg. RJ Anderson & JJ Bolton 18 Feb. 2011 (This study - #923)	-
<i>L. marilzae</i>	South Africa, Eastern Cape, Kenton-on-Sea leg. RJ Anderson & JJ Bolton 26 Feb. 2013 (This study - #1241)	-
<i>L. marilzae</i>	South Africa, Western Cape, Bortjiesrif leg. RJ Anderson & JJ Bolton 22 Jun. 2012 (This study - #1078)	-
<i>L. marilzae</i>	South Africa, Western Cape, Bortjiesrif leg. RJ Anderson & JJ Bolton 22 Jun. 2012 (This study - #1077)	-
<i>L. marilzae</i>	South Africa, Eastern Cape, Storms River leg. RJ Anderson & JJ Bolton 13 Dec. 2012 (This study - #1177)	-
<u>Palisada</u> Nam		
<i>P. coralloropsis</i> (Montagne) Senties, MT Fujii & Diaz-Larrea	Mexico, Quintana Roo, Cancún, Chaac-Mol Beach, leg. J. Díaz- Larrea & A. Senties, 21 Aug. 2005 (Díaz-Larrea et al. 2007)	EF061646
<i>P. cf. corallopsis</i>	South Africa, Eastern Cape, Three Sister leg. RJ Anderson & JJ Bolton	-

	27 Feb. 2013 (This study - #1247)	
<i>P. flagellifera</i> (Kützing) Cassano, Senties, Gil-Rodriguez & MT Fujii [As <i>Chondrophyucus thuyoides</i>]	Spain, Canary Islands, Tenerife, Playa Paraiso leg MC Gil-Rodriguez, A Senties & MT Fujii, 12 Jul. 2006 (Gil-Rodriguez et al. 2009)	EF685998
<i>P. thuyoides</i> (Kützing) Cassano, Senties, Gil-Rodríguez & M.T. Fujii [as <i>Laurencia paniculata</i> (C. Agardh) J. Agardh]	Philippines. AO Lluisma (Unpub.)	AF489863
<i>P. papillosa</i> (C Agardh) KW Nam [as <i>Laurencia papillosa</i>]	Philippines. AO Lluisma (Unpub.)	AF489861
<i>P. patentiramea</i> (Montagne) Cassano, Senties, Gil-Rodriguez & MT Fujii	Philippines. AO Lluisma (Unpub.)	AF489862
<i>P. perforata</i> (Bory) KW Nam	Spain, Canary Islands, Tenerife, Puerto de la Cruz, San Telmo leg. MC Gil-Rodriguez, A Senties & MT Fujii 13 Jul. 2006 (Cassano et al. 2009)	EU256329
<i>P. perforata</i>	Mexico, Isla Mujeres, Cancun, Quintana Roo, leg. A Senties & Gil-Rodriguez. 2007. (Cassano et al. 2009)	EF658641
<i>P. perforata</i>	Brazil, Rio de Janeiro, Rio das Ostras, Areias Negras leg. V Cassano & MBB Barreto 3 Aug. 2005 (Cassano et al. 2009)	EU256330
<i>P. cf. robusta</i> (Yamada) KW Nam	New Caledonia, Lifou, leg. C Payri, 23 Mar. 2005	FJ785321
<i>Palisada</i> sp. 1	South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Pt. leg. JJ Bolton, RJ Anderson & CM Francis 09 Sep. 2010 (This study - #819)	-
<i>Palisada</i> sp. 2	South Africa, KwaZulu-Natal, Bhanga Nek, BN3, 8m. leg RJ Anderson 03 Oct. 2013 (This study - #1361)	-

<i>Palisada</i> sp. 2	South Africa, KwaZulu-Natal, Bhangana Nek, Bhangana Rock. leg JJ Bolton, RJ Anderson 08 Oct. 2013 (This study - #1667)	-
<i>Osmundea</i> Stackhouse		
<i>O. blinksii</i> (Hollenberg & Abbott) Nam	USA, California, San Mateo County, Año Nuevo, Greyhound Rock leg. MH Hommersand 17 Jul. 1996 (McIvor et al. 2002)	AY172575
<i>O. hybrida</i> (AP de Candolle) Nam	Ireland, Donegal County, Fanad Head leg. CA Maggs 07 Nov. 1999 (McIvor et al. 2002)	AF281878
<i>O. osmunda</i> (SG Gmelin) KW Nam	France, Brittany, Roscoff leg. F. Rosseau, 05. Dec. 2002 (Martin-Lescanne et al. 2010)	FJ785318
<i>O. osmunda</i>	Ireland, Donegal County, St John's Point leg. CA Maggs 12.Oct. 1999 (McIvor et al. 2002)	AF281877
<i>O. pinnatifida</i> (Hudson) Stackhouse	Ireland, Donegal County, St John's Point leg. CA Maggs, 12 Oct. 1999 (McIvor et al. 2002)	AF281875
<i>O. pinnatifida</i>	Ireland, Donegal County, St John's Point, epiphytic on <i>Fucus serratus</i> leg. CA Maggs, 12 Oct. 1999 (McIvor et al. 2002)	AF281876
<i>O. pinnatifida</i>	Spain, Canary Islands, Tenerife, Puerto de la Cruz, San Telmo leg. MC Gil-Rodriguez, 07 Oct. 2005 (Cassano et al. 2009)	EF686005
<i>O. pinnatifida</i> [as <i>Laurencia pinnatifida</i>]	Lin et al. (2001, unpublished)	AF259495
<i>O. ramosissima</i> (Oeder) Athanasiadis	Ireland, Donegal County, St John's Point, epilithic leg. CA Maggs 12.Oct. 1999 (McIvor et al. 2002)	AF281880
<i>O. spectabilis</i> var. <i>spectabilis</i>	Mexico, Baja California, Punta Santa Thomas leg. MH Hommersand 02 Jul. 1996 (McIvor	AY172574

		et al. 2002)	
<i>O. splendens</i> (Hollenberg) Nam	Mexico, Baja California, Bahia Colnett, Drift leg. MH Hommersand & J Hughley 02 Jul. 1996 (McIvor et al. 2002)		AY172576
<i>O. truncata</i> (Kützing) Nam & Maggs	Ireland, Cork County, Lough Hyne leg CA Maggs 11 Nov. 1999 (McIvor et al. 2002)		AF281879
 <i>Yuzurua</i> (Nam) Martin-Lescanne			
<i>Y. poiteaui</i> var. <i>poiteaui</i> * (JV Lamouroux) Martin- Lescanne [As <i>Chondrophycus poiteaui</i>]	Mexico, Quintana Roo, Playa del Carmen, leg. J. Díaz-Larrea & A. Senties, 15 Mar. 2005 (Díaz-Larrea et al. 2007)		EF061653
<i>Y. poiteaui</i> var. <i>poiteaui</i> * [As <i>C. poiteaui</i>]	USA, Florida, Long Key, Ocean Side, leg. S. Fredericq (Díaz-Larrea et al. 2007)		EF061652
<i>Y. poiteaui</i> var. <i>gemmifera</i> (Harvey) MJ Wynne [As <i>Chondrophycus gemmiferus</i>]	Cuba, La Havana, Rincon de Guanabo, leg. J. Díaz-Larrea & A. Mallea, 29 Jul. 2005 (Díaz-Larrea et al. 2007)		EF061650
<i>Y. poiteaui</i> var. <i>gemmifera</i> [As <i>C. gemmiferus</i>]	Mexico, Yucatan, Cancun, Playa del Carmen, leg J Diaz-Larrea & A Senties. 2004. (Diaz-Larrea et al. 2007)		EF061649

Table A2: Foreign specimens examined in this study (S: South; N: North; W: Western; ?: indicates uncertainty regarding genus placement)

Locality	Accession	Field Identifications	Site	DNA
Europa Island	E010	<i>Palisada</i> cf. <i>parvipapillata</i>	Platier station 1	NO
Europa Island	E028	<i>Laurencia</i> complex	Plongée 1	NO
Europa Island	E046	<i>Laurencia</i> sp.	Platier station 2	YES

Europa Island	E047	<i>Laurencia</i> complex	Platier station 2	NO
Europa Island	E050	<i>Chondrophyucus</i> sp.	Platier station 2	YES
Europa Island	E051	<i>Laurencia</i> complex	Platier station 2	NO
Europa Island	E053	<i>Laurencia</i>	Platier station 2	NO
Europa Island	E059	<i>Laurencia</i> cf. <i>natalensis</i>	Platier station 2	NO
Europa Island	E067	<i>Laurencia</i> sp.	Plongée 2	NO
Europa Island	E095	<i>Palisada</i> cf. <i>parvipapillata</i>	Platier Station 3	NO
Europa Island	E290	<i>Laurencia</i> sp.	Plongée 2	NO
Europa Island	E298	<i>Palisada</i> cf. <i>parvipapillata</i>	Platier Station 1 Mangrove	NO
Europa Island	E299	<i>Palisada</i> cf. <i>parvipapillata</i>	Platier Station 1 Mangrove	NO
Europa Island	E313	<i>Laurencia</i> sp.	Plongée 1	NO
Europa Island	E319	<i>Laurencia</i> sp.	Lagune	NO
Europa Island	E328	<i>Palisada</i> cf. <i>parvipapillata</i>	Point Joseph Sud	NO
Europa Island	E337	<i>Laurencia</i> sp.	Point Joseph Sud	NO
Europa Island	E342	<i>Palisada</i> cf. <i>perforata</i>	Point Joseph Sud	NO
Europa Island	E343	<i>Laurencia</i> complex	Point Joseph Sud	NO
Europa Island	E345	<i>Laurencia</i> cf. <i>natalensis</i>	Point Joseph Sud	NO
Europa Island	E346	<i>Laurencia</i> complex	Point Joseph Sud	YES
Mozambique, Pemba	P02	<i>Palisada</i> <i>perforata</i>	Off Pemba, reef in front of CEPAM	NO
Mozambique, Pemba	P24	<i>Laurencia</i> sp.	Off Pemba, reef in front of CEPAM	NO

Mozambique, Pemba	P31	<i>Laurencia</i> sp.2	Off Pemba, reef in front of CEPAM	YES
Mozambique, Pemba	P102	<i>Laurencia</i> sp.	Londo, 3 to 5 m SCUBA dive	YES
Mozambique, Pemba	P103	<i>Laurencia</i> sp.	Londo, 3 to 5 m SCUBA dive	YES
Mozambique, Pemba	P120	<i>Palisada perforata</i>	Murrebue Mangrove	NO
Mozambique (S)	MOZ003	<i>Laurencia natalensis</i>	Xai-Xai Beach	NO
Mozambique (S)	MOZ008	<i>Laurencia natalensis</i>	Chidenguele	NO
Mozambique (S)	MOZ009	<i>Laurencia</i> sp. 1	Chidenguele	YES
Mozambique (S)	MOZ010	<i>Laurencia</i> sp. 2	Chidenguele	YES
Mozambique (S)	MOZ023	<i>Laurencia</i> cf. <i>columinaris</i>	Praia de Tofo	NO
Mozambique (S)	MOZ024	<i>Laurencia</i> sp.	Praia de Tofo	NO
Mozambique (S)	MOZ025	<i>Laurencia natalensis</i>	Praia de Tofo	NO
Madagascar (N)	MD014	<i>Laurencia</i> sp.	Diego Suarez, Mer d'Emeraude	YES
Madagascar (N)	MD024	<i>Laurencia</i> complex	Diego Suarez, Mer d'Emeraude	NO
Madagascar (N)	MD037	<i>Laurencia</i> sp.	Diego Suarez, Mer d'Emeraude	YES
Madagascar (N)	MD066	<i>Laurencia</i> cf. <i>decumbens</i>	Nosy be	NO
Madagascar (N)	MD110	<i>Palisada perforata</i>	Marovasa be	NO
Madagascar (N)	MD138	<i>Laurencia</i> ?	Majunga	YES

Madagascar (N)	MD139	<i>Chondria</i> sp.	Majunga	NO
Madagascar (N)	MD140	<i>Chondria?</i>	Majunga	NO
Madagascar (N)	MD155	<i>Chondrophycus</i> sp.	Sainte Marie	NO
Madagascar (N)	MD159	<i>Chondria</i>	Sainte Marie	YES
Madagascar (N)	MD164	<i>Chondrophycus</i> sp. 2	Sainte Marie	NO
Madagascar (N)	MD184	<i>Palisada perforata</i>	Sainte Marie	NO
Madagascar (N)	MD190	<i>Laurencia</i> sp. 1	Sainte Marie	YES
Madagascar (N)	MD191	<i>Laurencia</i> sp. 2	Sainte Marie	NO
Madagascar (N)	MD194	<i>Chondria</i> sp.	Sainte Marie	YES
Madagascar (N)	MD201	<i>Chondrophycus?</i>	Sainte Marie	NO
Madagascar (N)	MD205	<i>Chondrophycus?</i>	Sainte Marie	YES
Madagascar (S)	D780	<i>Laurencia natalensis</i>	Fort Dauphin	YES
Madagascar (S)	D781	<i>Laurencia complanata</i>	Fort Dauphin	YES
Madagascar (S)	D782	<i>Laurencia</i> sp. 1	Fort Dauphin	YES
Madagascar (S)	D783	<i>Laurencia natalensis</i>	Fort Dauphin	YES
Madagascar (S)	KS5	<i>Laurencia</i> sp. 1	Fort Dauphin	YES
Madagascar (S)	KS7	<i>Laurencia</i> sp. 2	Fort Dauphin	YES
Madagascar (S)	KS21	<i>Laurencia complanata</i>	Fort Dauphin	YES
Madagascar (S)	KS25	<i>Laurencia natalensis</i>	Fort Dauphin	YES
Mauritius	MS003	<i>Palisada perforata</i>	Pointe aux biches	NO
Mauritius	MS004	<i>Palisada perforata</i>	Trou aux biches	NO
Mauritius	MS006	<i>Laurencia</i> cf. <i>natalensis</i>	Poste Lafayette	NO
Mauritius	MS010	<i>Laurencia</i> sp.	Palmar public beach	NO
Mauritius	MS018	<i>Laurencia</i> sp.	Pointe aux cannoniers	YES
Mayotte	LYD10-	<i>Chondria?</i>	Passe Acua	NO

	190			
Mayotte	LYD10-	<i>Chondria?</i>	N'goudja inner barrier	NO
	230			
Mayotte	LYD10-	<i>Laurencia</i> sp.	Basse prévoyante	NO
	312			
Mayotte	LYD10-	<i>Laurencia</i> sp.	Passe bateaux, récif	NO
	376		ouest extérieur	
Mayotte	LYD10-	<i>Laurencia</i> sp.	Basse prévoyante	NO
	326			
Mayotte	LYD10-	<i>Laurencia</i> sp.	Banc du Boa	NO
	132			
Mayotte	LYD10-	<i>Laurencia</i> sp.	Bord passe Choazil	NO
	153		intérieur	
Mayotte	LYD10-	<i>Laurencia</i> sp.	Ilot Choazil, face au	NO
	344		banc de sable	
Reunion	R037	<i>Laurencia</i> cf. <i>flexilis</i>	Boucan Canot	YES
Reunion	R078	<i>Laurencia</i> cf. <i>flexilis</i>	Trois bassins	YES
Reunion	R079	<i>Palisada perforata</i>	Trois bassins	NO
Reunion	R163	<i>Chondrophyucus columellaris</i>	Cap La Houssaye	NO
Reunion	R169	<i>Laurencia</i> sp. 2	Cap La Houssaye	YES
Reunion	R170	<i>Laurencia</i> sp. 3	Cap La Houssaye	NO
Reunion	R171	<i>Palisada</i> sp.	Cap La Houssaye	NO
Reunion	R238	<i>Palisada perforata</i>	Trois bassins	NO
Reunion	R352	<i>Laurencia</i> sp.	Saint Gilles	NO
Reunion	R394	<i>Laurencia</i> complex	R3	NO

Reunion	R496	<i>Laurencia</i> sp. 1	Anse des cascades	NO
Reunion	R612	<i>Laurencia</i> sp. 2	coulee 1977	YES
Reunion	R730	<i>Laurencia</i> sp. 1	Cap La Houssaye	NO
Reunion	R731	<i>Laurencia</i> sp. 2	Cap La Houssaye	NO
Reunion	R732	<i>Chondrophyucus columellaris</i>	Cap La Houssaye	NO
Reunion	R733	<i>Laurencia</i> sp. 1	Cap La Houssaye	NO
Reunion	R734	<i>Palisada</i> sp.	Cap La Houssaye	NO
Reunion	R735	<i>Laurencia</i> sp. 3	Cap La Houssaye	NO
Reunion	R736	<i>Laurencia</i> cf. <i>flexilis</i>	Cap La Houssaye	YES
Reunion	R737	<i>Laurencia</i> sp. 1	Cap La Houssaye	NO
Reunion	R746	<i>Laurencia</i> sp. 1	Boucan Canot	NO
Reunion	R747	<i>Laurencia</i> sp. 2	Boucan Canot	NO
Reunion	R748	<i>Laurencia</i> sp. 3	Boucan Canot	NO
Reunion	R774	<i>Laurencia</i> sp. 1	Anse des cascades	NO
Reunion	R776	<i>Laurencia</i> sp. 2	Anse des cascades	YES
Reunion	R798	<i>Laurencia</i> complex	coulée 2007	NO
Reunion	R811	<i>Chondrophyucus collumellaris</i>	coulée 2007	NO
Reunion	R817	<i>Palisada</i> cf. <i>robusta</i>	coulée 2007	NO
Reunion	R818	<i>Palisada perforata</i>	coulée 2007	YES
Glorioso Island	GLO-024	<i>Laurencia</i> sp.	Station 3	NO
Glorioso Island	GLO-252	<i>Laurencia</i> sp.	Station 10	NO
Glorioso Island	GLO-258	<i>Laurencia</i> sp.	Station 11	NO
Glorioso Island	GLO-263	<i>Laurencia</i> sp. 1	Station 12	NO
Glorioso Island	GLO-264	<i>Laurencia</i> sp. 2	Station 12	NO
Glorioso Island	GLO-265	<i>Laurencia</i> sp. 3	Station 12	NO

Glorioso Island	GLO-266	<i>Laurencia</i> sp. 4	Station 12	NO
Glorioso Island	GLO-268	<i>Laurencia</i> sp. 5	Station 12	NO
Glorioso Island	GLO-284	<i>Laurencia</i> sp.	Station 13	YES
Glorioso Island	GLO-314	<i>Laurencia</i> sp. 1	Station 15	NO
Glorioso Island	GLO-315	<i>Laurencia</i> sp. 2	Station 15	YES
Glorioso Island	GLO-069	<i>Palisada</i> sp.	Station 7	NO
Glorioso Island	GLO-163	<i>Chondrophyucus</i> sp.	Station 2	YES
Glorioso Island	GLO-182	<i>Palisada</i> sp.	Plongée 3	NO
Japan (S)	OKI-001	<i>Palisada perforata</i>	Awase	NO
Japan (S)	OKI-008	<i>Chondrophyucus</i> sp.	Awase	NO
Japan (S)	OKI-029	<i>Laurencia</i> sp.	Hanashiro	NO
Japan (S)	OKI-030	<i>Palisada perforata</i>	Hanashiro	YES
Japan (S)	OKI-031	<i>Laurencia tropica</i>	Hanashiro	NO
Japan (S)	OKI-032	<i>Laurencia majuscula</i>	Onna	YES
Japan (S)	OKI-037	<i>Laurencia</i> sp.	Onna	YES
Japan (S)	OKI-039	<i>Laurencia tropica</i>	Bise	NO
Japan (S)	OKI-044	<i>Palisada perforata</i>	Bise	NO
Japan (S)	OKI-045	<i>Palisada cf. concreta</i>	Bise	NO
Japan (S)	OKI-059	<i>Palisada perforata</i>	Teniya	NO
Japan (S)	OKI-060	<i>Chondrophyucus</i> sp.	Teniya	YES
Japan (S)	OKI-064	<i>Laurencia</i> sp.	Teniya	YES
Japan (S)	OKI-069	<i>Laurencia majuscula</i>	Heshikiya	YES
Japan (S)	OKI-070	<i>Palisada</i> sp.	Oura	YES
Japan (S)	OKI-075	<i>Chondrophyucus</i> sp.	Oura	NO
Japan (S)	OKI-076	<i>Palisada perforata</i>	Oura	YES

Japan (S)	OKI-086	<i>Laurencia</i> sp.	Sumnide	YES
Japan (S)	OKI-087	<i>Laurencia</i> sp. 1	Sumnide	YES
Japan (S)	OKI-089	<i>Laurencia</i> sp. 2	Sumnide	YES
Japan (S)	OKI-090	<i>Palisada perforata</i>	Sumnide	NO
Japan (S)	OKI-091	<i>Palisada</i> sp.	Sumnide	NO
Japan (N)	JP-006	<i>Laurencia okamurae</i>	Sapporo	YES
Japan (N)	JP-007	<i>Palisada intermedia</i>	Sapporo	YES
Japan (N)	JP-008	<i>Laurencia nipponica</i>	Sapporo	YES
Japan (N)	JP-017	<i>Laurencia nipponica</i>	Sapporo	NO
Australia (W)	HV03577	<i>Laurencia</i> sp. 1	Coral Bay: Paradise Beach	NO
Australia (W)	HV03579	<i>Laurencia</i> sp. 2	Coral Bay: Paradise Beach	YES
Australia (W)	HV03590	<i>Laurencia</i> sp. 3	Coral Bay: Paradise Beach	NO
Australia (W)	HV03619	<i>Laurencia</i> sp. 1	Coral Bay: Lotties	NO
Australia (W)	HV03644	<i>Laurencia</i> sp. 2	Coral Bay: Lotties	NO
Australia (W)	HV03659	<i>Laurencia</i> sp. 1	Coral Bay	NO
Australia (W)	HV03660	<i>Laurencia</i> sp. 2	Coral Bay	NO
Australia (W)	HV03687	<i>Laurencia</i> sp. 1	Coral Bay: Five Fingers lagoon	YES
Australia (W)	HV03693	<i>Laurencia</i> sp. 2	Coral Bay: Five Fingers lagoon	YES
Australia (W)	HV03695	<i>Laurencia</i> sp. 3	Coral Bay: Five Fingers lagoon	NO

Australia (W)	HV03730	<i>Laurencia</i> sp. 4	Coral Bay: Five Fingers lagoon	NO
Australia (W)	HV03737	<i>Laurencia</i> sp. 5	Coral Bay: Five Fingers lagoon	NO
Australia (W)	HV03752	<i>Laurencia</i> sp. 1	Exmouth	NO
Australia (W)	HV03768	<i>Laurencia</i> sp. 2	Exmouth	NO
Australia (W)	HV03771	<i>Laurencia</i> sp. 3	Exmouth	YES
Australia (W)	JFC0020	<i>Laurencia</i> sp.	Shark Bay: Gladstone	YES
Australia (W)	JFC0023	<i>Laurencia</i> sp. 4	Coral Bay: Paradise Beach	NO
Australia (W)	JFC0032	<i>Laurencia</i> sp. 5	Coral Bay: Paradise Beach	NO
Australia (W)	JFC0097	<i>Laurencia</i> sp. 1	Coral Bay: Bills Bommie	NO
Australia (W)	JFC0099	<i>Laurencia</i> sp. 2	Coral Bay: Bills Bommie	NO
Australia (W)	JFC0100	<i>Laurencia</i> sp. 3	Coral Bay: Bills Bommie	NO
Australia (W)	JFC0207	<i>Laurencia</i> sp. 1	Peron: Cape Peron	NO
Australia (W)	JFC0274	<i>Laurencia</i> sp. 2	Peron: Cape Peron	YES
Australia (W)	JFC0285	<i>Laurencia</i> sp. 3	Peron: Cape Peron	YES
Australia (W)	JFC0288	<i>Laurencia</i> sp. 4	Peron: Cape Peron	NO
Australia (W)	JFC0290	<i>Laurencia</i> sp. 5	Peron: Cape Peron	YES
Australia (W)	JFC0293	<i>Laurencia</i> sp. 6	Peron: Cape Peron	NO
Australia (W)	JFC0294	<i>Laurencia</i> sp. 7	Peron: Cape Peron	NO

Australia (W)	JFC0295	<i>Laurencia</i> sp. 8	Peron: Cape Peron	YES
Australia (W)	JFC0296	<i>Laurencia</i> sp. 1	Peron: White Rock	YES
Australia (W)	JFC0297	<i>Laurencia</i> sp. 2	Peron: White Rock	YES
Australia (W)	JFC0298	<i>Laurencia</i> sp. 3	Peron: White Rock	YES
Australia (W)	JFC0299	<i>Laurencia</i> sp. 4	Peron: White Rock	NO
Australia (W)	JFC0301	<i>Laurencia</i> sp. 5	Peron: White Rock	NO
Australia (W)	JFC0302	<i>Laurencia</i> sp. 6	Peron: White Rock	YES
Australia (W)	JFC0303	<i>Laurencia</i> sp. 7	Peron: White Rock	YES
Australia (W)	JFC0321	<i>Laurencia</i> sp. 8	Peron: White Rock	NO
Australia (W)	JFC0322	<i>Laurencia</i> sp. 9	Peron: White Rock	YES
Australia (W)	JFC0330	<i>Laurencia</i> sp. 10	Peron: White Rock	YES

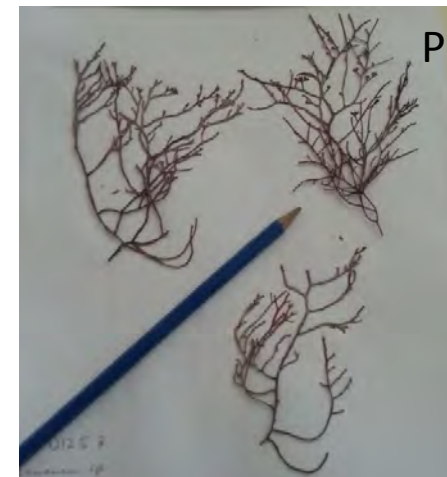


Figure A1: Submorphotypes of *Laurencia* cf. *corymbosa*. (1x) Scale for K-M, O and P (1cm = 1.7cm); Scale for N 1 division = 1mm.