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Actiniaria (Cnidaria: Anthozoa) of Port Phillip Bay, Victoria : including a taxonomic case study of *Oulactis muscosa* and *Oulactis mcmurrichi*

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Submitted by

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ABSTRACT

This study is the first dedicated survey cataloguing the actiniarian fauna of Port Phillip Bay, Victoria, Australia. There is a paucity of knowledge of Australian Actiniaria (true sea anemones) fauna. Therefore, Australian marine fauna surveys and most field guide books identify many sea anemones only to genus, if they are identified at all. Much of the knowledge of Australian actiniarian fauna is based upon preserved specimens, resulting from broad scale surveys, and sent to overseas actiniarian specialists for identification and description, and most species lack information on important *in-vivo* characteristics.

Surveys of Actiniaria fauna were conducted in new, and previously surveyed areas, of Port Phillip Bay. In addition, preserved Port Phillip Bay Actiniaria specimens housed in Museum Victoria, were examined to verify previous species identifications. Sixteen Actiniaria species, including *Oulactis muscosa*, are documented for Port Phillip Bay. In addition, four potentially new actiniarian species, that could not be verified during this study, were recorded.

Actiniarian taxonomy is a complex discipline, and problems of species identifications are exacerbated because important *in-vivo* characteristics are lost during the preservation process. In addition, poorly preserved material and lack of detailed information on characteristics of living actiniarians has meant that important data needed for distinguishing species is often absent. Therefore cryptic species and information on intraspecific regional variation of species is usually lacking.

Oulactis muscosa and *Oulactis mcmurrichi* are two morphologically similar actiniarian species that need taxonomic re-evaluation. Taxonomic keys for southern actiniarian fauna do not distinguish between the two species because of their similarity in appearance; and it may be the case that they constitute a single species. Accordingly, a taxonomic case study of the two Australian *Oulactis* species was conducted to determine if they should be synonymised. As genetics are increasingly being used in taxonomic studies, the use of ribosomal DNA as an aid in identifying actiniarian species was evaluated.

Oulactis muscosa and *O. mcmurrchi in-vivo* characteristics were documented from Australia, New Zealand and Argentina. In addition, morphological characteristics of *Oulactis* specimens from Australian Museums were documented together with newly collected specimens from Australia and New Zealand. The results from this study indicate that *Oulactis muscosa* and *Oulactis mcmurrichi* should remain as two separate species. Important *in-vivo* characterics are critical to the identification of these species. The regional intraspecific variation of these species is considerable, and therefore it is possible that some of these morphological variants represent potentially new species of *Oulactis.*

Further analyses of these species and other actiniarians examined in this study that incorporate genetic analyses may provide additional valuable information for resolving the taxonomic status of actiniarian species in Australia. It was found that ribosomal DNA may aid in distinguishing cryptic species, intraspecific regional variations, and identify juveniles and adults that have differences in appearance. This study has provided some important baseline data for future work on Port Phillip Bay Actiniaria and *Oulactis* species in Australia and New Zealand.

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Chapter 1 General Introduction

With their tentacles fully expanded, sea anemones are one of the most easily recognised animals in the marine environment, most notably on rocky shores and coral reef habitats. They are highly valued in the aquarium and tourism industries and in biomedical research (Mackie, 2002; Shuman et al., 2005). Ecologically important, they maintain many symbiotic relationships in the marine environment. Sea anemones have been under-studied in Australia and subsequently the fauna is poorly known. The gap in knowledge of Australian sea anemones may have occurred in part because, until recently, there has never been a resident taxonomist specialising in the group. Thus, overseas expertise was required for identifications and descriptions of anemones with material sent overseas or identified by visiting experts.

True sea anemones are classified within the Phylum Cnidaria, Class Anthozoa, Subclass Hexacorallia, Order Actiniaria. Sea anemones possess stinging cells called cnidae as do other cnidarians such as hydroids, corals, jellyfish, sea pens and sea whips (Fautin, 2007). Sea anemones are found in the deepest marine habitats to the highest intertidal zone (Friese, 1972; Fautin & Allen, 1997). They range in size from 0.5 cm to several metres across and exhibit a wide array of colours and patterns. Although predominantly considered to be a sessile animal of the seashore, some species may move rapidly to evade predators or unfavourable habitat conditions when required (Friese, 1972). Actiniarians are predominately opportunistic carnivorous feeders, capturing prey with their tentacles (Shick, 1991). In addition, some species of sea anemones have a symbiotic relationship with zooxanthellae (dinoflagellate microalgae) whereby some of their energy needs are met by translocation of photosynthate from the zooxanthellae (Davy & Turner, 2003).

There are approximately 1100 described species of actiniarian sea anemones worldwide (Daly et al., 2008). Australia and its Antarctic waters have 110 species of true sea anemones (Order Actiniaria) recorded (Fautin et al., 2008). Previously there were only 84 actiniarian species recorded for Australian territories (Crowther & Wallace, 2005). The increase in the number of recorded species is due in part to a dedicated project cataloguing sea anemones housed in Australian museums and a two week Australian Marine Sciences Association scientific workshop held on Stradbroke Island, Queensland (Crowther & Wallace, 2005;

Fautin et al., 2008). The results of that project highlight the need for further work to be undertaken on actiniarian fauna for the remaining Australian coastline and its waters.

Identification of sea anemones is complex as it relies on the animal as a whole rather than a single character (Stephenson, 1928; Häussermann, 2004). To identify preserved animals with a degree of certainty, actiniarian taxonomists advocate that experience and training are required to interpret key features correctly (Stephenson, 1928; Fautin, 2000). Oskar Carlgren's (1949) key to the Actiniaria, Corallimorpharia and Ptychodactiaria is still the primary reference used when identifying animals and is based around preserved characteristics. The disadvantage of this is that preserved specimens may have lost indicative living features, such as patterning and colour, during the preservation process (Häussermann, 2004). With the advent of modern digital imagery it is possible to now digitally document the animal's appearance before collection and accurately record indicative living features. These images may then be attached to registered specimens in museum databases to assist with future identifications. External and internal features in conjunction with cnidae composition are used in identification of species (Fautin, 1988; Häussermann, 2004). More often than not histological work is required to determine preserved features, especially on smaller animals and it can be a time-consuming process (Häussermann, 2004).

1.1 Actiniarian Anatomy

Sea anemones are solitary polyp invertebrates that are biradially symmetrical and have a well developed, diffuse, nerve net instead of a central nervous system (Barnes, 1987; Buchsbaum et al., 1987; Hand & Fautin, 1988; Shick, 1991). The body is tubular with an opening (mouth) at one end, fringed by rings of tentacles (Figure 1.1). At the opposite end of the mouth is a pedal disc that can attach to hard substrata. Some species have developed a physa in place of a pedal disc. The physa is a rounded ampullaceous aboral end, with the ability to swell, allowing sea anemones to anchor in soft sediments (Fautin, 2007). Cnidarian bodies are composed of two primary cell layers, the ectoderm and the endoderm (diploblastic), whereas most other animals have three cell layers (triploblastic) (Fautin, 2007). Between the ectoderm and endoderm is the mesoglea, a primarily structureless and sometimes acellular substance that contains a collagenous fibre system and amebocytes (Barnes, 1987; Harris, 1990).

Sea anemone musculature includes; parietobasilar, retractor (longitudinal) and sphincter muscles. The parietobasilar muscle stretches between the column wall and the pedal disc forming a triangular sheet (Stephenson, 1928). The longitudinal retractor muscle runs along the mesentery face, allowing the animal to contract down and in, while withdrawing the oral disc (Stephenson, 1928; Harris, 1990). The sphincter muscle found in the upper margin, allows the animal to withdraw the tentacles into the body (Harris, 1990). All three muscles vary in size, strength and shape (Stephenson, 1928).
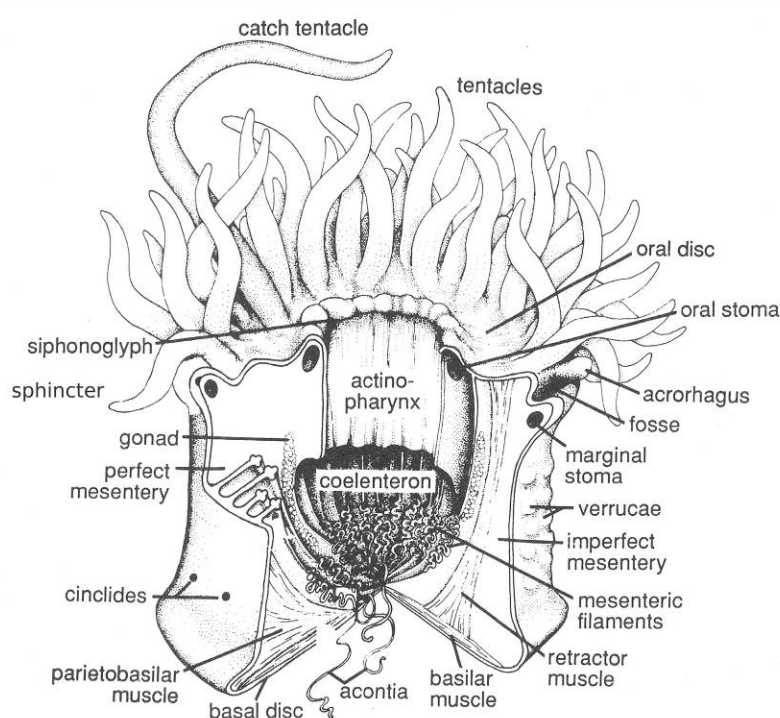
Figure 1.1 Representative chimeric diagram of a sea anemone (source: Shick, 1991).

The body cavity is radially divided by partitions called mesenteries that occur in pairs (Figure 1.2) (Harris, 1990). Mesenteries are referred to as either complete or incomplete; complete mesentery pairs reach and attach to the actinopharynx, whereas an incomplete pair do not reach the actinopharynx. Pairs of mesenteries in sea anemones are usually arranged in cycles of six (hexamerous), however they may occasionally occur in eights (octamerous) or tens (decamerous) (Stephenson, 1928; Carlgren, 1949). Depending on the order of the cycles of mesenteries, they are termed the primary (first), secondary (second) or tertiary (third) and so on (Stephenson, 1928). The directive mesentery pair occur in the primary cycle and establish the bilateral axis of symmetry and may be attached to the siphonoglyphs (Harris,

1990). Some elongated sea anemones, such as *Edwardsia,* have an imperfect mesentery arrangement. Perfect mesenteries are referred to as macronemes (mesenteries with strong retractors, gonads and filaments) and imperfect mesenteries as micronemes (lack strong retractors, gonads and filaments) (Carlgren, 1949). The internal compartments between mesenteries are termed endocoels (a complete mesentery pair enclosing a chamber) or exocoels (adjacent to an enclosed chamber) (Harris, 1990; Shick, 1991). When these compartments are filled with seawater they act as a hydrostatic skeleton for the sea anemone during movement (Harris, 1990). The actinopharynx (or throat) leads to the gastric cavity (coelenteron) and possesses one or more siphonoglyphs, which act as pumps to push seawater into the gastric cavity (Harris, 1990).
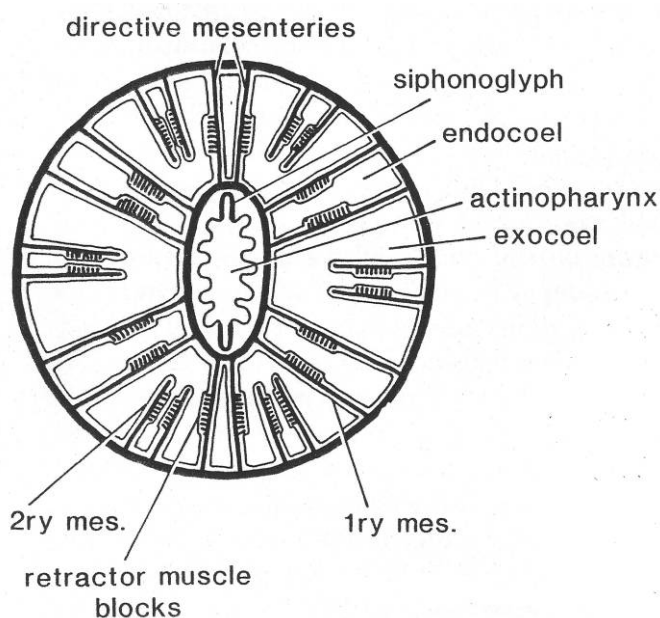
Figure 1.2 Cross section of a sea anemone (source: Harris, 1990).

1.2 Cnidae

Cnidae are the microscopic stinging capsules found in the tissue of cnidarian animals (Fautin, 2007). Cnidae are classified into 3 groups; spirocysts, nematocysts and ptychocysts (the latter are not present in Actiniaria) (Harris, 1990). Within these groups are 28 documented 'types' of cnidae with various functions (Kass-Simon & Scappaticci, 2002). Cnidae are used by cnidarians in food capture, for defence and aggression against predators

or competitors. The four basic functions of cnidae are to pierce, ensnare, adhere to prey, or adhere to substrata (Kass-Simon & Scappaticci, 2002).

Some cnidae contain toxins, and these cnidae release their toxins into the prey when ensnared (Kass-Simon & Scappaticci, 2002). In humans, cnidae stings from anthozoans, including sea anemones, can range from mild to very severe. Symptoms range from pain to weal formation, nausea, fever, headaches and in extreme cases kidney failure (Burke, 2002). Sea anemones have developed specialised structures heavily laden with nematocysts, such as acrorhagi, catch tentacles and acontia (Kass-Simon & Scappaticci, 2002). Acrorhagi (large spherules laden with holotrichs – a type of nematocyst), located on the upper margin of the column of some species, are used as weapons (Daly, 2003). If an anemone is threatened by another anemone they will repeatedly strike one another with the acrorhagi until one retreats (Kass-Simon & Scappaticci, 2002). Further details of the taxonomic importance of cnidae are provided in section 1.7.1.6

1.3 Actiniarian Life Cycle

The life cycle of sea anemones differs to that of some other cnidarians, as they lack a medusoid stage (Hand & Fautin, 1988; Shick, 1991). Instead, the planula larvae settle and metamorphose directly into juvenile polyps that resemble the adult form (Collins, 2002). There is evidence that some sea anemones are long lived: animals kept in aquaria have lived to be decades old, and age estimate studies calculate some to be centuries old (Fautin & Allen, 1997). However, anemones grow and shrink depending upon food availability, which makes it difficult to age an anemone by their size (Francis, 1988; Fautin & Allen, 1997).

1.4 Reproduction

Actiniarian reproduction can be sexual or asexual and within these categories there are several modes. Some actiniarian species are capable of using more than one reproductive strategy, such as *Anthothoe albocincta*, which uses asexual reproduction to maintain local populations and a sexual reproduction to establish widely dispersed colonies (Billingham & Ayre, 1997).

1.4.1 Sexual Reproduction

Sea anemones do not contain true sexual organs (Hand & Fautin, 1988). Instead, they possess groups of gametogenic material often referred to as gonads, which may be female or male (Stephenson, 1928; Fautin, 1999). In actiniarians the gametogenic material is found on the mesenteries (Stephenson, 1928). Some species such as *Oulactis muscosa* are sexually mature year-round while other species may be sexually mature at certain times of the year (Hunt & Ayre, 1989; Shick, 1991). Sexes are usually separate but occasionally animals may be hermaphroditic (Friese, 1972). It is possible for some hermaphroditic species to self-fertilise (Hand & Fautin, 1988). Sexual reproduction falls into two broad categories; broadcast spawning and brooding. External stimuli are required for the onset of spawning. Triggers may include the time of year, phase of moon or time of day and the specific triggers vary between species (Fautin Dunn, 1975; Fautin, 1999; Scott & Harrison, 2005; 2007; 2008).

1.4.2 Gametogenesis

Spermatogenesis

Spermatogonia arise in the mesenterial endoderm and then migrate into the mesoglea, where they form "vesicles", "cysts" or "packets" (Shick, 1991; Fautin, 1999; Harrison & Jamieson, 1999). As the packet enlarges the sperm mature from the centre outwards, with the periphery having the least mature sperm (Shick, 1991; Fautin, 1999). Once spermatozoa are mature the spermaries burst to release flagellated spermatozoa into the coelenteron (Shick, 1991; Fautin, 1999; Harrison & Jamieson, 1999; Scott & Harrison, 2008; 2009).

Oogenesis

Similar to the process of spermatogenesis, oogonia arise in the endoderm and migrate into the mesoglea for development and maturation (Shick, 1991; Fautin, 1999). The eggs (oocytes) gain nutrition from the coelenteron via a trophonema (funnel shaped structure). The trophonema is situated along the border of the mesentery, passing through the mesogloea, to the oocyte (Shick, 1991). The oocytes tend to mature asynchronously (Shick,

1991). Maturing oocytes remain near the gastric cavity for nutritional requirements (Fautin, 1999). Spawning involves oocytes moving from the mesogloea through the endoderm and into the coelenteron from which they may be ejected or remain for fertilisation depending on the mode of reproduction method used (Shick, 1991).

1.4.3 Sexual Reproduction Modes

Broadcast spawning

Broadcast spawners usually have separate sexes and release eggs and sperm into the water column where fertilisation takes place, and larvae then settle onto substrata (Fautin, 1999; Marshall et al., 2004; Scott & Harrison, 2005; 2008). Examples of broadcast spawners found in Australia are *Oulactis muscosa, Heteractis crispa* and *Entacmaea quadricolor* (Marshall et al., 2004; Scott & Harrison, 2005). Scott and Harrison's (2008) recent study showed that broadcast spawning and larval development for *H. crispa* and *E. quadricolor* takes approximately 2 weeks, with larvae settling between 4 and 10 days and juveniles developing tentacles between 5 and 17 days.

Brooding

There are two types of brooders; internal or external. Internal brooders may have embryos that develop for a short time in the gastrovascular cavity and are then released into the water column (Fautin, 1999). Alternatively, embryos may be retained for the entire developmental stage and are released as fully-developed juveniles, such as in *Actinia tenebrosa* (Ottaway & Kirby, 1975; Fautin, 1999). Some anemones may undertake "dribble" spawning whereby embryos are continually released at a certain developmental stage (Fautin, 1999); this infers that reproduction takes place over an extended period of time. External brooders release fertilised embryos that attach themselves to brood pouches located on the outside of the parent's column and develop into juveniles (Fautin Dunn, 1975). Once the embryos have the reached the juvenile stage they are released from the brood pouches (Fautin, 1999).

1.4.4 Asexual Reproduction

There are four modes of asexual reproduction and these comprise: cloning, fission, laceration, and budding. Fission may be longitudinal or less commonly transversal. Longitudinal fission is where the animal splits in half bisymmetrically. The animal will heal along the wound finally dividing at the sphincter, as in *Anthothoe albocincta* (Geller et al., 2005). Transverse fission occurs when the animal splits between the anterior and posterior halves (Fautin, 1999). The new anterior end will develop tentacles (Fautin, 1999) and the new posterior end will heal to form the new pedal disc. Laceration reproduction is when small fragments of the pedal disc break off, and the fragments then regenerate into whole animals (Fautin, 1999). Pedal laceration is commonly reliant upon the stretching of tissue as a catalyst, often induced by environmental factors (Geller et al., 2005). *Diadumene lineata* (synonym *Haliplanella luciae*), a marine invasive sea anemone, uses this mode of reproduction (Gollasch & Riemann-Zürneck, 1996; Fautin, 1999). Budding in sea anemones is the process whereby a tentacle piece will break off and form a new animal (Hand & Fautin, 1988), but it has only been observed in species within the Tribe Boloceroidaria (Shick, 1991). However, it is not budding in the strictest sense, as the developing offspring remain unattached anatomically to the parent during growth (Shick, 1991).

1.5 Ecology

Sea anemones are an important food source for some predators and are ecologically significant for the symbiont relationships they maintain.

1.5.1 Actiniarian Diet

The actiniarian diet consists primarily of small crustaceans (crabs and shrimp), however larger species can capture fish (Barnes, 1987). In captivity an anemonefish, *Amphiprion* sp. has been observed feeding their host anemone by capturing small mullet and dragging them into the anemone's tentacles (D. Bucher, 2009, pers. comm.). Mostly feeding opportunistically, sea anemones use their tentacles and cnidae to trap and immobilise prey (Barnes, 1987). However, some sea anemones obtain some or all of their energy requirement from a symbiotic relationship with zooxanthellae (refer to 1.5.3).

1.5.2 Predators of Sea Anemones

Predators of sea anemones include sea spiders (pycnogonids), sea stars, gastropods and some fish (Ottaway, 1977), and Hawksbill turtles have been observed feeding upon sea anemones (D. Bucher, 2009, pers. comm.). In addition they form part of the diet of a range of animals where the sea anemones are attached to the predator's primary food source, such as crabs (Ottaway, 1977). Sea anemones face predation not only in the marine environment, but from humans as well. They form part of the traditional diet in some countries, as in Sardinia, Italy, where fried sea anemone is part of the extensive seafood diet (Campanelli, 1997).

1.5.3 Symbiotic Associations

The most famous of the sea anemone symbiotic relationships is with anemonefish from the genera *Premnas* and *Amphiprion*, Family Pomacentridae. The interaction between these species is a prominent feature on coral reefs, and the focus of much study to understand the complexities of the relationship (Fautin & Allen, 1997). The anemonefish use the host sea anemone for shelter and protection, and the aggressive, territorial, nature of the fish aids in protection of the sea anemone from predatory fish (Arvedlund, 1998); and as observed earlier, they may also directly feed the anemone.

Some sea anemones have a mutualistic symbiosis with zooxanthellae (Shick, 1991). Zooxanthellae are photosynthetic dinoflagellates (golden brown algae) (Hand & Fautin, 1988). In the past zooxanthellae were classified as one species, *Symbiodinium microadriaticum*, however, genetic and morphological studies have now identified a range of different clades (Granados et al., 2008; Crabbe & Carlin, 2009). Studies have also indicated that the dinoflagellates have various ecological roles and composition of the symbiont population may vary with environmental conditions (Rodriguez-Lanetty et al., 2000). This was illustrated in a recent study by Venn et al., (2008) where two clades (A and B) of zooxanthellae were identified from the sea anemone *Condylactis gigantea*. Venn et al. (2008) found that the dominant clade (A or B) in sea anemones studied were influenced by environmental conditions and habitat depth. Clade A was dominant in the thermally variable inshore waters and Clade B dominant in offshore waters where thermal conditions were more uniform (Venn et al., 2008). In addition to their thermal tolerance the clades may also

have differed in their lightharvesting ability, hence the effect of depth. Some species of anemone such as *Anthopleura xanthogrammica* and *A. elegantissima* may also contain zoochlorellae (a unicellular green algae) (Shick, 1991; Davy & Turner, 2003).

Photosynthesis, undertaken by the zooxanthellae and zoochlorellae, provides energy-rich organic compounds to the host anemone and in some cases this constitutes the major source of energy for the sea anemone (Fautin & Allen, 1997). Zooxanthellae can be located in an anemone's tentacles and oral disc, however they may also be located in the mesenteric filaments or distributed heterogeneously through the anemone tissue (Shick, 1991; Fautin & Allen, 1997; Fautin & Smith, 1997). It is believed that sea anemones gain zooxanthellae either by maternal inheritance or uptake them from the surrounding seawater (Shick, 1991; Davy & Turner, 2003). Sea anemone species that have a symbiosis with zooxanthellae or zoochlorellae are generally limited to shallow depths (no greater than 50 m) in the marine environment where sufficient light is available for photosynthesis (Fautin & Allen, 1997).

Another common symbiosis is between crabs and sea anemones (Hand, 1975). The anemone attaches to the crab or hermit crab's shell, acting as camouflage and providing additional protection for the crab. The anemone benefits by receiving food scraps and added mobility (Ponder et al., 2002; Stevenson, 2006). Actiniarians have also been recorded attached to pycnogonids (King, 1973). There are numerous other documented associations of smaller invertebrates on sea anemones including shrimps, crabs and pycnogonids. Such relationships may be short or long term (Calado et al., 2007).

Not all symbiotic relationships are beneficial to the sea anemone. Pycnogonids have been documented as endoparasites in the sea anemone genera *Actinia* and *Urticinia* (Arnaud & Bamber, 1987; Miyazaki, 2002). Adult pycnogonids have been observed laying their eggs in the walls of host sea anemones. When the pycnogonids eggs hatch, the juveniles feed upon the walls of the host anemone (Arnaud & Bamber, 1987). Conversely, there are some anemones that are parasitic, such as *Edwardsia lineata,* which is a parasite of the ctenophore *Mnemiopsis leidyi* (Reitzel et al., 2007).

1.6 Human Use

Economically important to humans, sea anemones are used in biomedical research and are popular ornamentals in the aquarium trade (Mackie, 2002; Shuman et al., 2005). Sea anemones are also one of the most photographed animals on coral/rocky reefs (featuring prominently on postcards and as stock photographs, see www.oceanwideimages.com) and feature in marine eco-tourism (a popular attraction at dive/snorkel sites) (Gaskell et al., 2009). They also have a major role in education programs because of their recognisable forms in the intertidal environment.

1.6.1 Biomedical Research

Medical research is progressively exploring the marine environment as an untapped source of potential compounds and processes for use in pharmaceuticals, 'nutriceuticals', cosmetics and agricultural use (Hand & Fautin, 1988). For example, compounds contained in the toxin from the Caribbean sea anemone *Stichodactyla helianthus* may be useful in the treatment of multiple sclerosis (University Of California-Irvine, 21 November 2001; Mackie, 2002). Sea anemone toxins are also studied as a means to understand processes in vertebrates, such as the ability of proteins to target biological membranes (Poger, 2006).

1.6.2 Aquarium Trade

Some sea anemones are popular in home aquaria because of their brilliant colourings and the symbiotic relationship with clownfish. The supply of marine ornamentals for the aquarium trade is a booming industry with an estimated worth of US $24-40 million in 1985, rising to US$200-300 million in the 1990s (Shuman et al., 2005). Despite advances in aquaculture, the majority of 'marine ornamentals' are still caught in the wild as juveniles or adults by using destructive fishing practices (Fautin & Allen, 1997; Shuman et al., 2005). Studies have shown that unmonitored fishing of these marine ornamentals can lead to decreased numbers of sea anemones and anemonefish because of the unsustainable fishing practices (Shuman et al., 2005; Jones et al., 2008). Studies such as Scott and Harrison (2007; 2008) have demonstrated that as reproductive processes of these anemones become better understood

there is potential to produce marine ornamentals successfully via aquaculture thereby reducing the need of wild collections.

1.7 Taxonomy

1.7.1 Cnidarian Taxonomy in Australia

It is estimated that "some 95% of Australia's marine biodiversity is represented by the invertebrate phyla and the bulk of these are yet to be discovered or described" (Australian Marine Sciences Association, 2005). Marine invertebrates are little-studied compared with vertebrates and our overall knowledge of marine invertebrates with regard to biology, ecology and taxonomy is poor. This paucity of knowledge for some marine invertebrates was demonstrated in a case study conducted on ascidians by Davis et al. (1999) and applies to many marine invertebrates. In addition, there are few marine taxonomic experts in Australia despite the great diversity of fauna (Ponder et al., 2002).

Until recently there has never been a taxonomist specialising in actiniarians residing in Australia and as a consequence there has been little taxonomic work undertaken on the Australian fauna (Crowther & Wallace, 2005). Australian research on sea anemones has, until recently, dealt primarily with: population genetics (Hunt & Ayre, 1989; Billingham & Ayre, 1997), animal reproduction (Marshall et al., 2004; Scott & Harrison, 2005; 2008; 2009) and on a broader basis as components of ecological studies (Koss et al., 2005; Hidas et al., 2007). Surveys or research requiring taxonomic work on sea anemones in the past required specimens to be preserved and shipped overseas for experts to identify e.g. Haddon & Duerden (1896), Carlgren (1950b; 1950a; 1954) and Cutress (1971).

Due to the limited knowledge of Australian Actiniaria, many scientific surveys and marine fauna guides identify sea anemones to genus only or have to leave animals as unidentified (Edgar, 2001; Hidas et al., 2007; Gowlett-Holmes, 2008). It is estimated that the current 110 species recorded from Australia may only represent half of the total actiniarian species as smaller, cryptic or deeper water animals may have gone unnoticed (Fautin et al., 2008).

Actiniarian Taxonomy

Carlgren's (1949) comprehensive taxonomic work, *A Survey of the Ptychodactiaria, Corallimorpharia and Actiniaria,* still underpins modern taxonomic work and identification for those groups. In addition, Stephenson's (1928) comprehensive work on the biology of British Sea Anemones still provides a biological foundation for actiniarian taxonomists.

Carlgren's (1949) key and sea anemone taxonomy are based on the anatomy of preserved specimens. Species identification can be difficult if specimens are poorly preserved or lack colour and patterning notes. Stephenson (1928) was doubtful that species identification could be made from preserved material alone, and concluded that identification could only be made positively to the genus level. Actiniarian taxonomy requires a wide range of characters to be taken into consideration in addition to natural variation within those characters (Stephenson, 1928; Häussermann, 2004).

Using Carlgren's (1949) key it is possible to identify specimens to genus level, but there are few taxonomic keys available for individual genera hence papers detailing species descriptions are required for the final identification. Sea anemone identification is further complicated by the lack of standard terminology and definitions for some actiniarian features such as nematocysts, acrorhagi and marginal structures. Some papers have begun to address these issues, such as England's (1987) paper on nematocyst nomenclature, and Daly's (2003) review on acrorhagi and pseudoacrorhagi.

Actiniarian Taxonomic Characters

Carlgren's (1949) key to Actiniaria differentiates orders initially by the presence of ciliated tracks of filaments and presence of basilar muscles. Families and genera within these orders are separated by various characteristics such as: presence or absence of acontia, number of tentacles, the sphincter's shape and position if present, whether mesenteries are perfect or imperfect (macronemes or micronemes), and the number of mesentery pairs and their completeness.

1.7.1.1 Colour and Patterning

Colour can be an unreliable characteristic for actiniarian species identification as it may be influenced by food sources and locality and may vary greatly within a species (Stephenson, 1928; Hand & Fautin, 1988). Many wild anemones collected for aquaria lose colour after a period away from their normal environmental conditions. An example of this is *Actinia tenebrosa* kept in the touch tanks at Melbourne Aquarium. In the wild *A. tenebrosa* have a distinctive deep red colouring, whereas animals that are kept in the touch tanks became a dull green colour (M. Mitchell personal observation).

Stephenson (1928) noted that although colour may vary, pattern may be far more stable within a species. However, recent work has shown that patterning and colour may vary between adults and juveniles in some species of sea anemone (Scott & Harrison, 2008). This is an important consideration when describing animals and for identification purposes.

1.7.1.2 External Characteristics

External characteristics should be noted *in-vivo* and then again after preservation. External structures to observe include: tentacles, column, marginal structures and the pedal disc/physa. Observations of tentacles should include: positioning (if they are restricted to the margin or cover the entire oral disc), their length relative to each other, the shape and form of the tentacle, and whether or not they are they completely withdrawn into the body when the animal is contracted (Fautin, 1998).

Column features, though sometimes difficult to see in the wild, include: the attachment of detritus, which may indicate specialised structures present (e.g. verrucae), if the column is divided into regions (e.g. scapulus and scapus) (Fautin, 1998), whether the column is smooth or has specialised structures such as vesicles. Histology may sometimes be required to ascertain column structures such as verrucae.

The presence of a pedal disc or a physa is an important character (Fautin, 1998). The pedal disc or physa may be buried in sand or sediment, therefore collection of the animal is sometimes required if identifying an unfamiliar species in the region. Marginal structures are

also an important taxonomic feature, such as acrorhagi or frills. Structures may not be visible in the field as they can be hidden beneath tentacles or deep in the fosse and sometimes are only evident in the laboratory or after preservation.

1.7.1.3 Internal Characteristics

Internal structures are essential for accurate sea anemone identification (Fautin, 1998). Depending upon the condition of the preserved anemone most internal structures can be viewed by eye or under a stereo microscope. However, in some instances histological slides of muscle structures may be needed. The first internal structure to be observed should be the sphincter and its shape, as Carlgren's (1949) key uses the shape and position of the sphincter as a main character. To a lesser extent the parietobasilar and retractor muscles are used in the genus description. The number of mesentery pairs and their cyclic arrangement (if they are formed in sixes or eights) is an important characteristic. Sometimes fertility and the placement of gametogenic material on the mesenteries are indicative for species. Lastly, the composition of nematocysts in the various tissues is essential in some cases for the identification of a specimen (Fautin, 2007).

1.7.1.4 Cnidae

The importance of nematocysts in hexacorallian taxonomy was foretold by Weill (1929) and his subsequent (1934) publication became the basis for future nematocyst works. Weill's 1934 monograph included the classification of 16 nematocysts types within three fundamental groups (England, 1991). Since Weill's (1934) study there has been debate among researchers regarding the classification of cnidae and their importance to taxonomy. Early studies on nematocyst nomenclature include Stephenson (1929) and Carlgren (1940). Subsequent studies have introduced new categories or merged categories of nematocysts depending on the nuances of structure regarded as significant (see Cutress, 1955; Mariscal, 1974; England, 1991). Redefinition of nematocyst nomenclature, and assessment of their importance in anthozoan taxonomy still continues (Fautin, 1988; Williams, 2000; Ryland et al., 2004; Acuña et al., 2007b).

Spirocysts are thin single-walled capsules containing a tightly spiralled coiled thread (Figure 1.3A). Nematocysts are thick, double walled capsules containing a thread of varying armature and length. Nematocyst classification is based upon the structure and the appearance of the shaft, thread and presence of spines (Mariscal, 1974). Of the 28 types of nematocysts known, actiniarians possess only 6 (Fautin, 1988; Kass-Simon & Scappaticci, 2002). Nematocysts (Figure 1.3) are broadly classified as follows (England, 1991):

b-**Mastigophores** – have a terminal tubule and a tapered transition between the undischarged shaft and thread.

p-**Mastigophores** – have a terminal thread but are differentiated by the V-shaped funnel between the undischarged shaft and the thread.

Amastigophores – have a shaft only and no terminal thread.

Holotrichs – have a thread that is entirely covered by spines.

Basitrichs – have well developed spines at the base of the thread and no shaft.

Amastigophores, *p*-mastigophores and b-mastigophores may be further classified into the sub-categories of macro- or microbasic; the classification depends upon the length and width of the everted shaft in relation to the length of the capsule (England, 1991), and the classification will vary with the nomenclatural scheme being used. For instance Carlgren interpreted nematocysts as macrobasic *p*-mastigophores with a shaft that is less than 3 times the length of the capsule and microbasic *p*-mastigophores with a shaft 4 times the length of the capsule (England, 1991). To observe everted shafts in relation to capsule length, live material must be examined as once an animal is preserved the nematocysts no longer fire (Fautin, 2007).

The composition of cnidae in tissue and their capsule size is used as a taxonomic character in the identification of many anthozoans (Cutress, 1955; Kass-Simon & Scappaticci, 2002; Ryland et al., 2004). Taxonomic procedure is to measure unfired preserved capsules as the length of a cnidae capsule may change considerably after firing (Weill, 1929). The number of nematocyst measurements needed to provide a statistically sound baseline in determining

species has been queried. Various studies advocate that between 20 to 100 capsules should be measured (Stephenson, 1929; Williams, 2000; Acuña et al., 2007b). Current practise for actiniarians is to measure a minimum of 10 unfired capsules, of each type of cnidae, from preserved tissue including: tentacles, column, spherules (if present), mesenterial filaments, actinopharynx and acontia (if present) thus establish a range for each cnidae type.
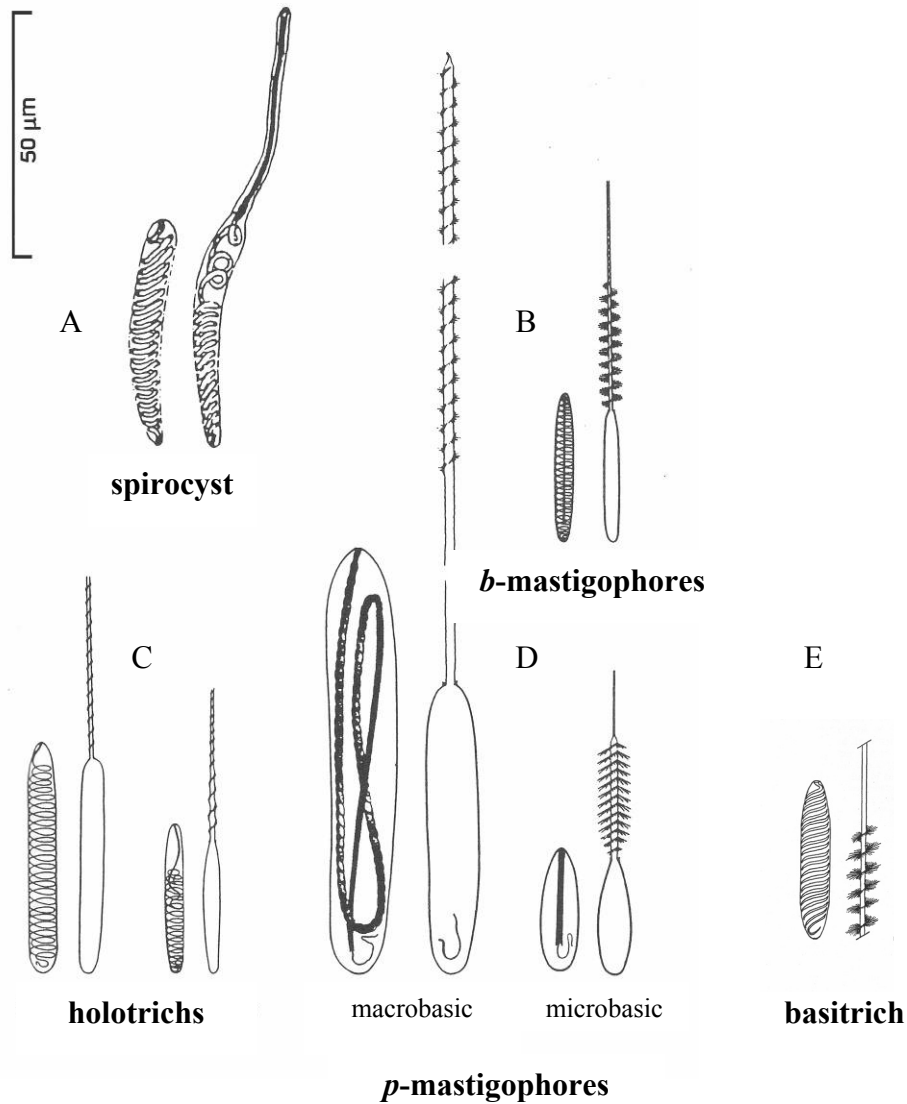
Figure 1.3 Diagrammatic illustration of cnidae: (A) spirocyst, (B) *b*-mastigophore, (C) holotrich, (D) *p*-mastigophore (macrobasic and microbasic) (source: Shick, 1991) (E) basitrich (source: Cutress, 1955).

1.7.2 Genetics in Taxonomy

Genetics is increasingly being incorporated into taxonomic projects (Hajibabaei et al., 2007). The establishment of DNA databases such as Genbank (http://www.ncbi.nlm.nih.gov/Genbank) and the Barcode of Life project (http://www.barcodinglife.org) allow specimens to be identified by comparing sample genetic sequences to known genetic sequences. The current global standard for genetic sequencing is a 650 base fragment from the 5' end of the COI (cytochrome c oxidase I) region (Hajibabaei et al., 2007). An alternative locus from the 18s region is suggested when COI cannot be sequenced successfully, or when further evidence for cryptic species is required (Hajibabaei et al., 2007).

1.7.2.1 Anthozoan Genetics

Anthozoans are relatively slowly evolving animals and mitochondrial divergence is much lower than in other invertebrates; as a consequence, markers such as COI are of limited use (Shearer et al., 2002). Accordingly, the use of genetics with actiniarians is still undergoing refinement and genetic work is now focusing on the use of alternative markers such as 18S rRNA and ITS regions of rDNA (Etnoyer et al., 2006; Acuña et al., 2007a). The use of genetic markers in some sea anemones may further be complicated by the presence of zooxanthellae in the animal tissue (Shearer et al., 2005).

1.8 Study Site - Port Phillip Bay, Victoria

1.8.1 Study Site Overview

Port Phillip Bay, Victoria, is situated on the temperate South East coast of Australia. Port Phillip Bay is a shallow marine embayment that covers a 1930 km^2 area with 260 km of coastline (Bird, 1993; Harris & Crossland, 1999). The coastline of Port Phillip Bay in the east is dominated by steep inclines and cliffs, rocky shores, quartzose sand and gravel beaches with intertidal and nearshore sand bars (Bird, 1993). The west coast between Melbourne and Geelong is predominately a low-lying plain composed of salt marsh areas,

mangroves and some shelly beaches (Bird, 1993). The south west of the bay, known as the Bellarine Peninsula, is higher and steeper than the eastern shore of the bay, with narrow beaches of sand and gravel (Bird, 1993).

The narrow entry at Port Phillip Heads means that there is a small tidal exchange with the Bass Strait, and residence time of water in the bay is around 1 year (Harris & Crossland, 1999). The tidal range in Port Phillip Bay is less than one metre during spring tides (Bird, 1993). Wave action in the bay is primarily determined by winds, and is generally stronger on the east coast which is exposed to prevailing westerly winds (Bird, 1993).

1.8.2 Human Impacts

Surrounding the bay is a heavily urbanised area encompassing the city of greater Melbourne and on the west coast Geelong. Melbourne's population reached 3.81 million in 2007 and continues to grow (Australian Bureau of Statistics, 2008). Since the area was settled it has been subject to extensive urban development and industrial use, and as a consequence the coastline has been heavily modified. Sea walls and boulder ramparts have been constructed to halt receding cliff faces, and artificial beaches have been placed in some areas (Bird, 1993). Several major marinas, boat ramps and numerous jetties occur along the shoreline along with sunken wrecks offshore.

Human use of the bay includes commercial and recreational fishing, shipping, tourism, swimming and recreational boating. The bay was subject to a prolific scallop dredging industry which was closed in 1997 after public debate concerning environmental effects of the dredging (Cohen et al., 2000).

Port Phillip Bay has a long history of international shipping (Hewitt et al., 2004). Around 3200 ship calls are made per year to the Port of Melbourne, handling approximately 38% of the Australian container trade (The Port of Melbourne Corporation, 2009). The impact of the shipping is evident by the marine invasive species present in the bay (Hewitt et al., 2004). Ballast water exchange and biofouling of ships are the two major contributors to the introduction of exotic species to ports (Lewis et al., 2003). Data suggest that Port Phillip Bay is the most invaded port by marine exotic species in Australia. One to two hundred exotic species have been recorded in the bay and an estimated three new invasive species establish

annually, however, only a small number are abundant enough to be considered pests (Australian Marine Sciences Association, 2007).

1.8.3 Scientific Research

Port Phillip Bay has been the subject of many large-scale scientific studies. This has created an extensive documented record of changes to the bay over time. The earliest survey was in 1840 (Hewitt et al., 2004). Meeting records from The Royal Society of Victoria show fauna survey results from 1888. Sea anemones collected by the society were sent to actiniarian taxonomists Haddon and Duerden for identification and the results were published in 1896. The next major survey was undertaken from 1957–1963. The anthozoans collected from that survey were sent to Charles Cutress for identification and he published *Corallimorpharia, Actiniaria and Zoanthidea of Port Phillip Bay* (1971). More recent work in Port Phillip Bay included a series of benthic fauna surveys conducted between 1969–1975 and 1994–1995 (Poore et al., 1975; Wilson et al., 1998). Together these studies of Port Phillip Bay resulted in a total of six new sea anemone species being described and more than 10 new records of actiniarians (Haddon & Duerden, 1896; Cutress, 1971; Poore et al., 1975; Wilson et al., 1998).

In addition to government funded research bodies, there are many active naturalist groups in Port Phillip Bay that collect and/or record species of sea anemones found there. Naturalist groups have collected many specimens that have been lodged with Museum Victoria in the Actiniaria collection. The museum's collection has relatively few identified specimens and numerous unidentified actiniarian specimens from the various surveys conducted by government funded research and naturalist groups.

1.9 Study Aims and Objectives

Although many surveys have been conducted in Port Phillip Bay, there has never been a survey devoted to assessing the sea anemone fauna. Sea anemone records until now have been components of general fauna and habitat surveys. Therefore, there is a need to reassess records from past studies and to confirm the identifications of sea anemones in Port Phillip

Bay, and to undertake a detailed survey to determine whether there are any other smaller or cryptic species of sea anemones present in the bay that may not have previously been recorded.

Accordingly the aims of this study are to:

- Verify actiniarian (true sea anemones) fauna present in Port Phillip Bay, Australia.
- Review the taxonomic identification of selected sea anemones using conventional and molecular techniques.

The specific objectives of this study are to:

- Review the species list of sea anemones in Port Phillip Bay intertidal areas and shallow subtidal areas from museum collections and to conduct surveys specifically targeting sea anemones in Port Phillip Bay.
- Produce a checklist and identification guide to the sea anemones of Port Phillip Bay.
- Review the taxonomic status and validity of *Oulactis mcmurrichi* (Lager, 1911).
- Undertake a pilot study to assess the utility of ribosomal DNA as an aid in Actiniarian species identification.

Chapter 2 Review, Checklist and Guide to Actiniaria of Port Phillip Bay, Victoria

2.1 Introduction

This is the first survey conducted in Port Phillip Bay that specifically focuses on Actiniaria (true sea anemones). Previously, broad scale fauna surveys have included Actiniaria without specifically targeting them. In those surveys a total of 16 species of true sea anemones were recorded for Port Phillip Bay. Of the 16 species, Port Phillip Bay is the type locality for six and the only known locality for *Isophellia stela, Anthothoe australiae* and *A. similis*. The most recent catalogue of Australian Actiniarian fauna lists 110 valid species. This number may only represent half of the species present in Australia and its territorial waters, as smaller or cryptic sea anemones present may have been overlooked (Fautin et al., 2008), and the same may be true for Port Phillip Bay actiniarian fauna.

The lack of a resident actiniarian specialist in Australia, until recently, may have contributed to our gap in knowledge of this fauna in general. Historically, preserved actiniarian specimens were sent to overseas taxonomists for identification and description. The disadvantage of identifying and describing actiniarians from preserved material only is that important *in-vivo* characteristics such as colour and patterning are lost (Stephenson, 1928; Häussermann, 2004).

Located on the temperate south east coast of Victoria, Port Phillip Bay is a 1930 km^2 semi-enclosed marine embayment with 263 km of coastline (Bird, 1993; Harris & Crossland, 1999; Walker, 1999). The bay is shallow, with an average depth of 14 m and a maximum depth of 24 m (Walker, 1999). The narrow entry to the bay at Port Phillip Heads means that there is restricted tidal exchange with the Bass Strait, and the residence time of water in the bay is approximately one year (Harris & Crossland, 1999).

The east and west coastline of Port Phillip Bay are geologically distinct from each other (Bowler, 1966). The eastern coastline of Port Phillip Bay is dominated by steep inclines and cliffs, rocky shores, quartzose sand and gravel beaches along which there are intertidal and nearshore sand bars (Bowler, 1966; Bird, 1993). The western coast of the bay between Melbourne and Geelong is predominately a low lying plain, composed of salt marshes, mangroves and shelly beaches (Bird, 1993). The south west of the Bay, known as the Bellarine Peninsula, is higher and steeper than the rest of the Port Phillip Bay coastline (Bird, 1993). The Peninsula is characterised by narrow beaches of sand and gravel, while the adjacent Swan Bay is bordered by salt marsh (Bird, 1993).

Port Phillip Bay is a highly disturbed marine environment subject to extensive industrial and recreational use. Surrounding the bay is major urban development including the suburbs of the state capital, Melbourne and the city of Geelong. Port Phillip Bay industry includes a major international shipping port, commercial and recreational fishing and urban development (such as marinas and residential canal estates) (Winstanley, 1996). Data suggest that the extensive shipping traffic in the bay contributes to the number of exotic marine invasive species, of which only a few are in a quantity significant enough to be considered a pest (Currie & Parry, 1999; Australian Marine Sciences Association, 2007).

Numerous scientific studies have been conducted over a long period in Port Phillip Bay. Port Phillip Bay is the type locality for over 200 species of marine invertebrates, of which 170 type specimens are stored in the invertebrate collection of 'Museum Victoria'. The earliest survey record of sea anemones in Port Phillip Bay was 1888. The survey was conducted by the Royal Society of Victoria, and survey data sheets recorded basic information for sea anemones including: location, colour and number observed (Lucas, 1890). Preserved specimens in conjunction with coloured drawings from the 1888 survey were sent to the overseas expert Alfred C. Haddon for identification and description. Haddon and Duerden (1896) described a new Actiniaria genus, *Mitactis,* and five new species including one corallimorpharian from the Port Phillip Bay material. These include *Actinioides spenceri*, now recognised as *Actiniogeton spenceri, Sargatia carlgreni* now called *Actinothoe carlgreni*, and *Corynactis australis,* which retains its original bionomen (Haddon & Duerden, 1896;

Fautin, 2009). The genus *Mitactis* has been synonymised with *Anthothoe* and the taxonomic validity of *A. australiae* and *A. similis* is unknown as there has been no known subsequent reference to them (Fautin, 2009). In addition, Haddon and Duerden (1896) listed one new Actiniaria record for Port Phillip Bay, *Phylctenactis tuberculosa* (previously *Cystiactis tuberculosa*) (Fautin, 2009).

The next major survey of Port Phillip Bay was conducted from 1957–1963 by the National Museum of Victoria (now known as Museum Victoria). Preserved survey material was sent to the overseas actiniarian specialist, Charles Cutress, for identification and description. Cutress (1971) confirmed 12 actiniaria records, described *Isophellia stela* and published three new records; *Cricophorous nutrix, Epiactis thompsoni* and *Bunodactis rubrofusca.* These three species were previously only known from New Zealand (Cutress, 1971).

The Victorian State government commissioned studies on the marine invertebrate benthos of Victorian bays and estuaries between 1969 and 1973 (Poore et al., 1975). The invertebrate material collected during this survey is stored in the Museum Victoria invertebrate collection (Poore, 1986). Of the actiniarian fauna collected during the benthos study of Port Phillip Bay, numerous burrowing sea anemones were found and identified as *Edwardsia* sp. by Dr Gary Poore (Museum Victoria) and S. F. Rainer. The third benthic survey was undertaken from 1994–1995, from which several *Edwardsia* sp. were found (Wilson et al., 1998). Identifications from that survey were made by Dr Robin Wilson (Museum Victoria) and Simon Heislers (then Department of Primary Industries). During Fisheries Victoria surveys of benthos surrounding blue mussel farms in Port Phillip Bay, burrowing sea anemones found were identified as *Edwardsia vivipara* (McKinnon et al., 2003).

In addition to the government-commissioned scientific studies, there are a number of naturalist groups in Victoria. These groups include the Field Naturalists – Marine Research Group (MRG), 'Friends of' groups that are dedicated to particular areas of interest such as the Marine Care Group of Ricketts Point Marine Park and Reefwatch Victoria, a non-for profit community program where divers and snorkellers monitor marine life at their preferred dive sites. These groups are dedicated to surveying and monitoring areas of Port Phillip Bay and members come from a broad cross-section of

the community. Some individuals in these groups have become experts in their chosen phyla (pers. obs). Field collections and research from these groups have contributed to the Museum Victoria invertebrate collection and knowledge of actiniarian distribution records around Port Phillip Bay.

The Museum Victoria Actiniaria collection is the result of broad ecological surveys and MRG field trips. There are few Actiniaria specimens in the collection identified to genus or species level. Specimens that have been identified to species level have been done so by specialists and non-specialists. Much of the sea anemone identification to date for Port Phillip Bay fauna has required shipment to overseas experts. Dr Daphne Fautin is the only known sea anemone specialist to have ever visited Museum Victoria. The visit was conducted as part of the Australian Biological Research Study (ABRS) grant to undertake a comprehensive catalogue of sea anemones in Australian museum collections. The cataloguing project required the use of foreign expertise as there is no resident expert in Australia (Crowther & Wallace, 2005).

The absence of a resident actiniarian specialist in Southern Australian has resulted in actiniarian fauna from the region being poorly known, with some species still undescribed (Thomas & Shepherd, 1982). Thomas & Shepherd (1982) created a simple key to the common species of Actiniaria from Southern Australian. They omitted some species from the key because of limited knowledge; *Bunodactis rubrofusca* and *Isophellia stela* were both excluded for this reason.

There is a need to address the gap in knowledge of the Port Phillip Bay sea anemone fauna, to collate records from disparate studies and confirm older records in order to produce a comprehensive checklist and guide to the anemones of the region.

Therefore the aims of this study are to:
- Review records of Actiniaria from Port Phillip Bay,
- Conduct surveys of new and previously surveyed areas of Port Phillip Bay to locate smaller or cryptic Actiniaria that may have been overlooked by more general benthic surveys,

- Confirm identifications of Actiniaria from Port Phillip Bay housed in the Melbourne Museum invertebrate collection, and
- Develop a checklist and guide to the Actiniaria of Port Phillip Bay.

2.2 Material and Methods

2.2.1 Materials

This study involved the use of both freshly collected material and preserved museum specimens. Fresh anemones were collected from sixteen survey sites around Port Phillip Bay (PPB) (Figure 2.1). Sites included; shallow sand bars, artificial structures (rock walls and jetties), small intertidal rock platforms and seagrass beds (see Table 2.1 for individual site descriptions). Collection areas were restricted to intertidal and shallow subtidal areas less than 1 metre in depth as these are the areas less intensively surveyed by past studies. In addition one site at the Annulus, at Pope's Eye Marine Park (38º 17' S 144º 41' E) was surveyed (observations only).

Surveys were conducted between 2005 and 2009, with initial site visits to establish the presence of sea anemone fauna and subsequent visit/s for collection or recollection purposes; therefore some sites were surveyed more than once (see Table 2.1 for survey dates). Each survey was conducted before, during and after low tide and search periods varied between 1–2 hours. Search methods included snorkelling, overturning of rocks and random search patterns of the site. On some occasions field assistants were present and enabled surveys of larger areas. In conjunction with the Marine Research Group, a survey was conducted at Holloway Bend, Brighton, where approximately 15 people were present for the marine fauna survey.

Sea anemones were photographed *in-situ* where possible using an Olympus C70-70 7.1 megapixel digital camera with underwater housing before collection. In some cases animals were removed from rocks using a knife, and a 'Yabbie pump' (a hand-operated suction pump sold commercially for collecting Callianasid shrimp as fishing bait) was used to extract burrowing anemones from sand. Specimens were then transported to the laboratory for observation and later preservation. Museum

registration numbers for specimens are recorded herein and have an 'F' prefix. Historical material was examined from the invertebrate collection of Museum Victoria (MV), which is stored in 70% ethanol. Some preserved specimens were also examined from the Australian Museum (AM) invertebrate collection, New South Wales.
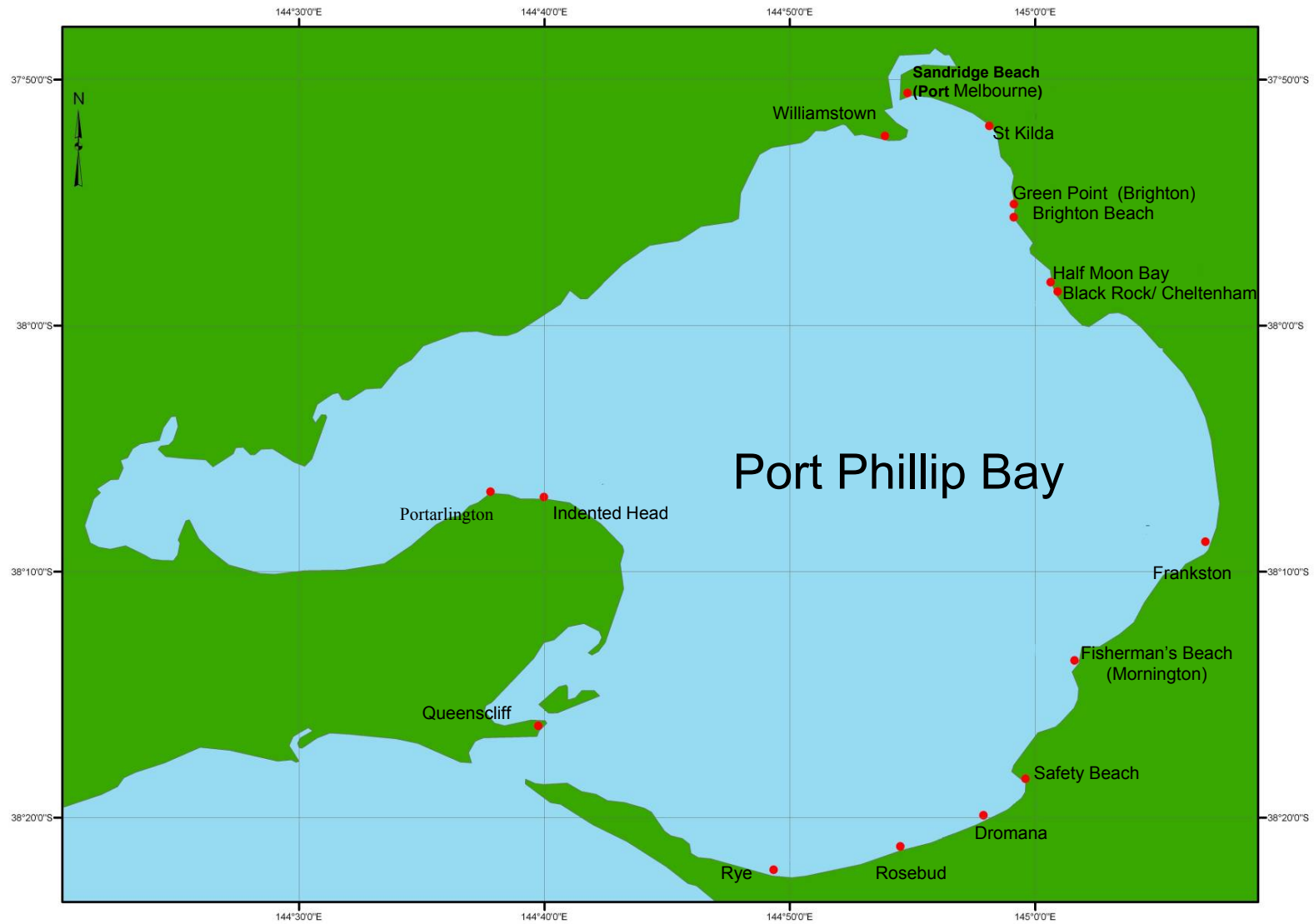
Figure 2.1 Port Phillip Bay Survey sites.

Table 2.1 Port Phillip Bay Survey Site Descriptions.

Site Location	Latitude/Longitude	Site Description	Survey Dates
Rye	37º 52' S 144º 54' E	Jetty with sandy beach and at the far end of the beach to the east a small breakwall with a stand of seagrass at the end.	01/11/2005 08/12/2006 22/05/2008 06/01/2009
Rosebud	38º 21' S 144º 54' E	Sandy Beach with a long jetty. Numerous sand bars at low tide	08/12/2006 06/01/2009 07/02/2009
Safety Beach	38º 19' S 144º 59' E	Site is next to Martha Cove Marina and in front of the sailing club. Shallow sandy area with small rocks.	31/10/2005 18/02/2006
Dromana	38º 20' S 144º 57' E	Jetty with flat sandy areas, deeper than other areas on same side of Mornington Peninsula	03/11/2005 18/02/2006
Mornington (Fisherman's Beach)	38º 13' S 145º 01' E	Area to the east of boat ramp, sand with considerable size boulders scattered over the area.	22/05/2008
Frankston	38º 07' S 145º 07' E	Frankston Jetty, flat sandy area with a fine silt covering the sand	06/04/2007
Sandringham	37º 57' S 145º 00' E	Beach at base of cliff, area has large underwater rocks interspersed with sandy flats. Small rock platform at the southern end of the beach.	09/04/2007
Half Moon Bay (Black Rock)	37º 58' S 145º 00' E	Located at the base of a bluff. Artificial structures include; car park, jetty and boat ramp. Sampling was undertaken on the rock platform to the west of the car park.	21/05/2007
Brighton	37º 54' S 144º 59' E	Flat sandy beach with small rock platforms further out from beach, visible at low tide only. To the north is an extensive stand of seagrass.	12/04/2007
Brighton Beach (Green Point)	37º 55' S 144º 59' E	Small artificial breakwall before a low lying level intertidal platform (visible only at low tide). Tapers off to sandy substrate with large boulders.	16/01/2006 26/01/2006 09/07/2006 11/04/2007 23/05/2008
St Kilda	37º 52' S 144º 58' E	Break wall next to marina and jetty, surrounded by sand.	09/07/2006 29/03/2007
Sandridge Beach, Port Melbourne	37º 50.5' S 144º 54' E	Beach area, at western end is an artificial breakwall surrounded by an industrial area on one side. Primarily sandy area with some algae	09/07/2006
Point Gellibrand, Williamstown	37º 52' S 144º 54' E	Low-lying intertidal area consisting of a boulder field. Sediment was silty, some areas of mud, with sparse seagrass and a lot of shell particles.	09/07/2006 21/05/2007
Queenscliff	38º 16' S 144º 39' E	Beach below a high cliff – completely submerged at high tide – small basalt rocks. Greater wave action than elsewhere in the bay.	31/05/2008
Portarlington	38º 07' S 144º 41' E	Artificial rubble groyne runs along the beach. Beach composed of pebbles, and there were some seagrass patches.	20/11/2007
Indented Head	38º 08' S 144º 43' E	Short jetty and boat ramp. Sandy substrata with very few rocks.	20/11/2007

2.2.2 Methods of Sample Analysis

Fresh Material

In the laboratory *in-vivo* characteristics of animals were recorded including: pattern and colour, body and tentacle measurements. Specimens with specialised structures, not visible in the field, were photographed in the laboratory. Where required cnidae squashes were made of live tissue to determine nematocyst types. Animals were then prepared for preservation by relaxing them in a solution of menthol crystals and seawater. In most cases 0.5 g menthol crystals: 500 ml sea water was sufficient to relax the animal within one hour. Once sea anemones showed no reaction to touch they were transferred to a solution of 10% formalin: 90% seawater for fixing and storage.

Preserved Material

External characteristics recorded from preserved specimens included: any distinguishing structures (e.g. verrucae), specimen dimensions, number and length of tentacles and body measurements. Internally: the number of mesenteries, the mesentery arrangement, the mesentery completeness and specimen fertility were all examined and recorded.

Cnidae slides prepared from preserved material and tissues examined included; tentacles, column, actinopharynx, acrorhagi (where present), acontia (where present) and mesenterial filament tissue. A minimum of ten unfired capsules of each type of cnidae were measured from each tissue type.

2.2.3 Taxonomic Information

Terminology and definitions of taxa used herein follows that of Carlgren (1949); in some cases there has been some rewording of definitions for ease of reading and consistency. Type specimen locality and synonymy is referenced from the 'Hexacorallians of the World' database (Fautin, 2009) unless otherwise stated. Basic nematocyst nomenclature follows that of England (1991).

Common taxonomic terms used can be found in Appendix I and additional taxonomic terms may be found online at http://www.nhm.ku.edu/tol/glossary/intro.html.

The field description and preserved description for each species is of Port Phillip Bay specimens where possible. Species cnidae tables are arranged so that capsule dimensions = length by width in microns (μm), n = total number of capsules measured and N = the proportion of the number of specimens examined that contain that type of cnidae. Systematic classification follows that currently accepted (Fautin, 2009). Until recently, Carlgren's (1949) systematics scheme was used, in which Tribes and Sub-Tribes were used in place of Infraorders and Superfamilies.

2.3 Results

A total of 16 actiniarian species and 4 other actiniarians that need further taxonomic study have been recorded in Port Phillip Bay (Table 2.2). The actiniarian species checklist developed here (Table 2.2) includes 8 species found during field surveys in this study, 14 species found after re-examination of the MV collection, and the 16 previous records for Port Phillip Bay. In addition to the checklist a taxonomic guide to each species has been collated for use in field work and working with preserved material.

As Port Phillip Bay is subject to a large thoroughfare of international shipping trade, and consequently a high number of recorded marine invasive species (Currie & Parry, 1999), field work included monitoring for the invasive sea anemone *Diadumene lineata,* formerly known as *Haliplanella luciae* (Gollasch & Riemann-Zürneck, 1996; Fautin, 2009); especially at the Port Melbourne site. No specimens of this species were found during this survey nor among the identified material in the MV invertebrate collections.

Some species included in the checklist still require further taxonomic analysis for verification including *Oulactis* cf. *muscosa*, and *Edwardsia vivipara*. Some species, including *Epiactis* sp., *Edwardsia* sp., *Bunodactis rubrofusca* and the unidentified burrowing anemone are still pending species identifications. Full identifications of these species could not be made due to the absence of material, poorly preserved material or only incomplete specimens being available.

Table 2.2 Actiniaria species recorded from published records, MV Invertebrate collection and new field surveys of Port Phillip Bay.

Species	Published records	MV Invertebrate Collection	Field Survey Sites Found
Edwardsia vivipara	✔	✔	
Edwardsia sp.		✔	
Actinia tenebrosa	✔	✔	8, 11, 13
Anthopleura aureoradiata	✔	✔	1, 2, 6, 10, 12*, 16
Aulactinia veratra	✔	✔	7, 9, 10, 13, 14
Bunodactis rubrofusca	✔		
Epiactis australiensis	✔	✔	
Epiactis thompsoni	✔	✔	
Epiactis sp.			
Isanemonia australis	✔	✔	17
Oulactis muscosa	✔		
Oulactis cf. *muscosa*		✔	1, 3, 5, 6, 9, 10, 11, 12
Phylctenactis tuberculosa	✔	✔	
Phylctenanthus australis	✔	✔	
Isophellia stela	✔	✔	
Cricophorus nutrix	✔	✔	1
Anthothoe albocincta	✔	✔	4, 7, 10, 11
Anthothoe australiae	✔		
Anthothoe similis	✔		
Unidentified (burrowing) Actiniaria			1, 2

Key to Site numbers: 1 – Rye, 2 – Rosebud, 3 – Safety Beach, 4 – Dromana, 5 – Fisherman's Beach, Mornington , 6 – Frankston , 7 – Sandringham, 8 – Half Moon Bay, Black Rock, 9 – Brighton , 10 – Green point, Brighton Beach, 11 – St Kilda, 12 – Sandridge Beach, Port Melbourne, 13 – Point Gellibrand, Williamstown, 14 – Queenscliff, 15 – Portarlington , 16 – Indented Head, 17 – Annulus, Pope's Eye Marine Park. *unconfirmed identification.

2.3.1 Systematic Checklist of Actiniaria from Port Phillip Bay

Order Actiniaria

    Suborder Nynantheae

    Infraorder Athenaria

        Family Edwardsiidae Andres, 1880

            Edwardsia vivipara (Carlgren, 1950)

            Edwardsia sp.

    Infraorder Thenaria

        Superfamily Endomyaria Stephenson, 1921

            Family Actiniidae Rafinesque, 1815

                Actinia tenebrosa Farqhaur, 1898

                Anthopleura aureoradiata (Stuckey, 1909)

                Aulactinia veratra (Drayton in Dana, 1846)

                Bunodactis rubrofusca Carlgren, 1924

                Epiactis australiensis Carlgren, 1950

                Epiactis thompsoni (Stuckey, 1909)

                Epiactis sp.

                Isanemonia australis Carlgren, 1950

                Oulactis muscosa (Drayton in Dana, 1846)

                Oulactis cf. *muscosa*.

                Phlyctenactis tuberculosa (Quoy & Gaimard, 1883)

                Phlyctenanthus australis Carlgren, 1950

        Superfamily Acontiaria Stephenson, 1935

            Family Isophellidae Stephenson, 1935

                Isophellia stela Cutress, 1971

            Family Hormathiidae Carlgren, 1932

                Cricophorus nutrix (Stuckey, 1909)

            Family Sagartiidae Gosse, 1858

                Anthothoe albocincta (Hutton, 1878)

                Anthothoe australiae (Haddon & Duerden, 1896)

                Anthothoe similis (Haddon & Duerden, 1896)

2.3.2 Guide to Actiniaria Species of Port Phillip Bay

This section contains the diagnosis for family, genus and species and other taxonomic information for currently recognised actiniarian species in Port Phillip Bay. Details of the material examined include, where possible; the museum registration number, the number of specimens in each lot, location, approximate latitude and longitude, date collected and who identified the specimen. Taxonomic information for the higher orders is taken from Carlgren (1949), unless otherwise stated; some grammar has been amended for consistency and readability. Bibliographic, type species, type locality and published species distribution is from the online database 'Hexacorallians of the World' Fautin (2009) unless otherwise stated. Where possible field description, cnidom and habitat is described from specimens found in the Port Phillip Bay area. All photographs were taken specifically for this study unless stated otherwise.

Infraorder Athenaria

Members of this infraorder lack basilar muscles. The body as a rule, is elongate, more or less vermiform and often divisible into different regions. The aboral end of body is usually rounded into a physa, which may sometimes adhere to small objects and will then become more or less flattened. A sphincter is generally absent, however if present, it may be either endodermal or mesogloeal. Tentacles and mesenteries are usually few, rarely more than 48, and cyclically arranged. Mesenteries are usually divisible into macro- and micronemes with retractors of the macronemes usually strongly restricted, reniform or circumscribed. The parietal part of the longitudinal mesenterial muscles is commonly differentiated from the retractors, forming a distinct parietal muscle together with the parietobasilar muscles. In the more differentiated genera acontia may appear.

Family *Edwardsiidae* Andres, 1880

These anemones have an elongate vermiform body that are usually divisible into at least two regions, a long scapus that is provided with a cuticle and a short upper scapulus. Often there is a rounded, naked physa at the aboral end and a very short, thin capitulum immediately below the tentacles. There is no sphincter or acontia present. The mesenteries are divisible into eight macronemes and at least four micronemes. Of the macronemes there are two pairs of directives and four pairs of lateral mesenteries, two on each side, whose retractor muscles

face the ventral directives. Retractor muscles are diffuse to strongly restricted and parietal muscles are always distinct.

Genus *Edwardsia* de Quatrefages, 1842

There are currently over 55 described species of *Edwardsia* making it the most species-rich actiniarian genus (Daly & Ljubenkov, 2008).

Diagnosis: The body is divisible into a physa, scapus, scapulus and capitulum; the physa is short and ampullaceous, the aboral part has no nemathybomes. The scapus is long with nemathybomes sunk into the mesogloea. There are at least 12 tentacles, and the inner tentacles are shorter than the outer. There is a weak ventral siphonoglyph. There are eight perfect macronemes and at least four imperfect, very weak ones, in the most distal part of the body. The retractor muscles are very strong and diffuse to restricted reniform; the parietal muscles are well developed. Only the eight perfect mesenteries are fertile and with filaments. Cnidom: Spirocysts, basitrichs, microbasic *b*- and *p*-mastigophores.

Type Species: *Edwardsia beautempsii* de Quatrefages, 1842.

Type Locality: Chausey, English Channel.

Edwardsia vivipara Carlgren, 1950
(Fig. 2.2; Table 2.3)

Synonymy:　*Edwardsia vivipara* Carlgren, 1950: 121–122 (original description).

　　　　　　Edwardsioides vivipara England, 1987: 218.

Material Examined: *Port Phillip Bay, Victoria, Australia.* **MV F118102**, 1, Corio Bay, 38º 06'S, 144º 22'E, 9 m, 28/08/1997; **MV F118103**, 1, Pt Henry Pier, Corio Bay, Geelong 38º 08'S, 144º 26'E, 4.5 m, 29/08/1997; **MV F118104**, Corio Bay, 38º 05'S, 144º 23'E, 6 m, 28/08/1997; **MV F188105**, 2, Pt Wilson, Geelong, 38º 06'S, 144º 32'E, 10 m, 29/08/1997; **MV F118106**, 3, Pt Wilson, Geelong, 36º 06'S, 144º 32'E, 10 m, 29/08/1997; **MV F118107**, 4, Pt Wilson, Geelong, 36º 06'S, 144º 32'E, 9 m, 29/08/1997.

Field Description: Light grey-brown colour, the oral disc a light grey and tentacles striped with dark grey bands, tentacles and oral disc lay on the sand and the entire column is buried (Gowlett-Holmes, 2008).

Preserved Description: The following preserved description is from Carlgren (1950b). Column up to 6 cm long, with approximately 12 tentacles. Nemathybomes of the scapus are scattered. The retractor muscles on the macronemes are diffuse.

Parietal muscles are triangular in outline in the uppermost part of the cnido-glandular tract. The endoderm has numerous zooxanthellae.

Cnidom: Cnidae measurements (Table 2.3) are from the original description provided by Carlgren (1950b).

Table 2.3 Distribution and size of cnidae of *Edwardsia vivipara*

Cnidae	Dimensions	n	N
Tentacles			
basitrichs	19.7–24 x about 3 μm	10	1/1
Mesenterial filaments			
basitrichs	29.6–38 x 4.2 μm	10	1/1
basitrichs	14–19.7 x 2.8 μm	10	1/1
microbasic *p*-mastigophores	28.2–29.6 x 4.2 μm	10	1/1
Capitulum			
nematocysts	7–11.3 x 2.2–2.5 μm	10	1/1
Nemathybomes			
nematocysts	26.8–38.1 x 3.5–4.2 μm	10	1/1

Note: dimensions of cnidae are length x width, n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae. Outlier capsule measurements are provided in brackets.

Habitat: Found on sand flats at 1–10 m depths (Carlgren, 1950b; Gowlett-Holmes, 2008).
Distribution: South Australia and Victoria, Australia (Wallace & Richards, 2009a). *Type Locality:* Outer Harbour near Port Adelaide, South Australia (Carlgren, 1950b).
Remarks: This species was not sighted during this survey and only museum material was examined. Due to the deteriorated condition of the specimens limited cnidae and anatomical data could be obtained. General taxonomic work on *E. vivipara* is required to establish a more comprehensive cnidae description than that of Carlgren's (1950b). In particular the nematocyst types for the capitulum and nemathybomes, which were absent from his description, need identification. In addition, the specimens identified as *E. vivipara* in the MV collection are small 3-10 mm, which is considerably smaller than the specimens Carlgren (1950b) examined.
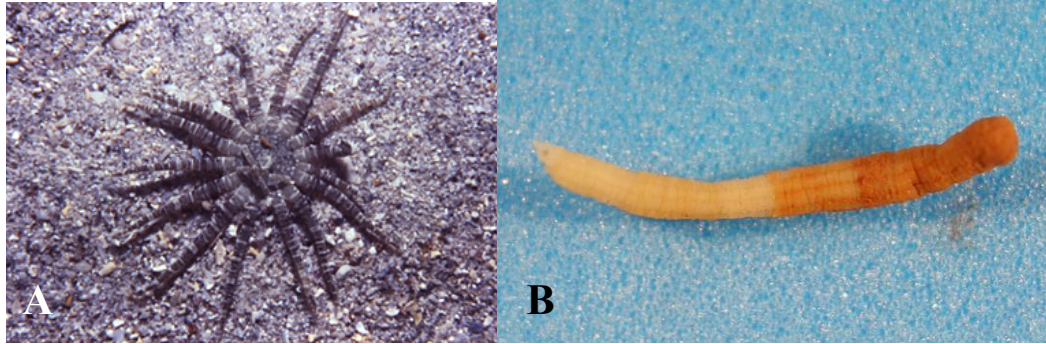
Figure 2.2 *Edwardsia vivipara* (A) *in-situ*, location unknown (photo N. Coleman source: Wallace & Richards, 2009a) (B) preserved specimen from Portland, Victoria (MV F117782).

Edwardsia sp.

(Fig 2.3; Table 2.4)

Material Examined: *Port Phillip Bay, Victoria, Australia.* Three specimens examined from MV Lot number: **Sp MOV1691**, Port Phillip Bay Benthic Survey (1969–1973).

Field Description: It is not known what this species looks like when alive. There are no notes associated with the MV specimens that relate to their appearance.

Preserved Description: The specimens are small and range in length from 2–15 mm. Tentacles are blunt at the tip and thin at the base, nine tentacles were counted on one specimen examined. Another specimen examined was fertile.

Cnidom: Cnidae measurements provided in table 2.4 are taken from a single specimen in lot number Sp: MOV1691.

Table 2.4 Distribution and Size of Cnidae of *Edwardsia* sp.

Cnidae	Dimensions	n	N
Tentacles			
spirocysts	(12.8) 18.4–21.6 (24) x 2.4–4 µm	10	1/1
basitrichs	17.6–21.6 x 2.4–2.4 µm	10	1/1
Nemathybomes/Column			
basitrichs	(31.2) 33.6–34.4 (38.4) µm	5	1/1
b-mastigophores	20.8 x 4 µm	1	1/1

Note: dimensions of cnidae are length x width, n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae. Outlier capsule measurements are provided in brackets.

Habitat: MV specimens were found in various depths, 2–24 m, in sandy and muddy substrates.

Distribution: Specimens from MV Lot Sp MOV1691 were collected from survey sites around Port Phillip Bay, Victoria including: off St Leonards, Corio Bay, Geelong Arm, off Werribee, central Port Phillip Bay.

Remarks: This species was not sighted during this survey and only museum material was examined. There are in excess of 700 specimens identified as *Edwardsia* sp. in the MV collection and they have been assigned to a MV lot number: Sp MOV 1691. Due to the number of individuals requiring identification and the specialised nature of the genus I have not identified this material to species level. It is possible that these specimens are *E. vivipara*, however specimens found in the collection are considerably smaller than the size described for *E. vivipara*. Additionally the specimen examined contained *b*-mastigophores which Carlgren (1950b) did not list from *E. vivipara*. To identify this *Edwardsia*, fresh material needs to be collected to determine their appearance when alive and many specimens in the MV collection are in poor condition, making them unsuitable for a full taxonomic examination.

Figure 2.3 Preserved specimen of *Edwardsia* sp. from Port Phillip Bay Benthic Survey (1969–1973). (MV Sp MOV1691).

Infraorder Thenaria

Members of this Infraorder are Nynantheae with basilar muscles. The aboral end is usually flattened and adherent, distinctly differentiated from the column. The column is variable in appearance, sometimes divisible into different regions; often with verrucae, marginal

spherules or pseudospherules, vesicles or other protuberances. The sphincter is usually endodermal or mesogloeal, but is sometimes absent. Tentacles and mesenteries are usually numerous, the former cyclically or radially arranged. Mesenteries are rarely differentiated into macro and micronemes. The retractor muscles are weak or strong and rarely circumscribed. Acontia may be present or absent.

Superfamily Endomyaria Stephenson, 1921

Thenaria without a sphincter, or with an endodermal one, which occasionally shows a strong tendency to be more or less mesogloeal. Acontia absent.

Family Actiniidae Rafinesque, 1815

This family contains 55 genera (Fautin et al., 2008). The column may be smooth or have various projections in the form of verrucae or vesicles. The upper margin of the column may possess spherules (acrorhagi) or pseudospherules. The sphincter may be absent or if present is endodermal and diffuse to circumscribed in shape. The simple tentacles are arranged in cycles with one tentacle per endocoel and exocoel. The mesenteries are not divisible into macronemes and micronemes. There are usually more than six pairs of perfect mesenteries and rarely only six.

Genus *Actinia* Linnaeus, 1767

There are 56 valid species in this genus, two of which are found in Australia, *Actinia tenebrosa* and *A. australiensis* (Fautin, 2009).

Diagnosis: Animals are characterised by a low, smooth column and wide pedal disc. Acrorhagi are located deep in the fosse. The sphincter is endodermal, weaker or stronger, diffuse and rarely with a tendency towards being mesoendodermal. There are numerous perfect mesenteries and all the stronger mesenteries are fertile, except for the directives. There are more mesenteries at the base than found at the margin. The retractor muscles are diffuse. Cnidom: Spirocysts, holotrichs, basitrichs, microbasic *p*-mastigophores.

Type Species: *Priapus equinus* Linnaeus, 1758.

Type Locality: North Sea.

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Actinia tenebrosa Farquhar, 1898

(Fig. 2.4, 2.5; Table 2.5)

</div>

Synonymy: *Actinia tenebrosa* Farquhar, 1898: 527, 535–536.

   Actinia c.f. *equina* Blackburn, 1937: 369.

Material Examined: *Port Phillip Bay, Victoria, Australia.* **MV F111179**, 1 disintegrated, Pope's Eye Annulus, 38º 16'S, 144º 42'E, 10/11/1957, Charles Cutress; **MV F111180**, 20, Observation Point, (Area 58, 1957–1963 PPB Survey), 38º 29'S, 145º 19'E, 18/01/1959, Charles Cutress; **MV F111181**, 7, Quarantine Jetty (Area 59, 1957–1963 PPB Survey), 38º 20'S, 144º 42'E, 11/1963, Charles Cutress; **MV F112735**, 6, Half Moon Bay, 38º 08'S, 144º 43'E, 21/05/1997, Michela Mitchell; **MV F112736**, 2, Half Moon Bay, 38º 08'S, 144º 43'E, 21/05/1997, Michela Mitchell.

Field Characteristics: Uniformly coloured and a bright to dark red anemone. Occasionally when the anemone is fully expanded the bright blue acrorhagi are visible however they are usually hidden deep in the fosse. The column is smooth in appearance and to touch.

Preserved Description: *Actinia tenebrosa* has a deep fosse, in which acrorhagi are enclosed. The column is smooth. Tentacles number approximately 121, are broad at the base and tapering. The pedal disc is 12 mm in diameter, column 12 mm and oral disc 20 mm expanded. The sphincter is endodermal and diffuse. Two siphonoglyphs are attached to directives. Forty eight mesentery pairs at the base, hexamerously arranged. The specimens examined were not fertile presumably as they had recently reproduced (see remarks). The reproduction strategy of *A. tenebrosa* is still not fully understood (Sherman et al., 2007).

Cnidom:

Table 2.5 Distribution and size of cnidae of *Actinia tenebrosa* (MV F112736).

Cnidae	Dimensions	n	N
Tentacles			
basitrichs	18.4–20.8 x 2.4–3.2 μm	10	1/1
spirocysts I	8.8–12.8 x 1.6–2.4 μm	10	1/1
spirocysts II	16.8–20 (28) x 2.4–3.2 μm	10	1/1
Acrorhagi			
holotrichs	(40.8) 46.4–52.8 x 3.2–4 μm	10	1/1
spirocysts	(13.6) 28–32 x 2.4–3.2 μm	10	1/1
basitrichs	13.6–18.4 x 2.4–3.2 μm	10	1/1
Actinopharynx			
basitrichs I	(20) 22.4–27.2 x 2.4–3.2 μm	10	1/1
basitrichs II	12.8–13.6 x 2.4–3.2 μm	10	1/1
p-mastigophores	19.2–21.6 x 4.8–5.6 μm	10	1/1
Column			
basitrichs	13.6–17.6 x 2.4–3.2 μm	10	1/1
Mesenterial Filament			
basitrichs I	9.6–14.4 x 2.4–2.4 μm	10	1/1
b-mastigophores	24–29.6 x 3.2–4 μm	10	1/1
p-mastigophores	16.8–22.4 x 4–4.8 μm	10	1/1

Note: dimensions of cnidae are length x width, n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae. Outlier capsule measurements are provided in brackets.

Habitat: Commonly found during low tide attached to rocks in sheltered areas in the high to mid intertidal zone. Animals contract completely during low tide and retain a considerable amount of seawater during contraction, commonly resembling blobs on the rocks.

Distribution: Found abundantly on the seashores of Australia from Heron Island, Queensland to Shark Bay, WA (Edgar, 2001). Also located in New Zealand and in offshore islands south of the Tropic of Capricorn (Ottaway, 1975b). *Type Locality:* near Wellington, New Zealand.

Remarks: *Actinia tenebrosa* were observed, but not collected, at St Kilda groyne and Point Gellibrand, Williamstown. The species is poorly represented in the Museum Victoria invertebrate collection with only seven lots registered, half of which are without locality detail. This is one of the most common sea anemones found along the Australian shoreline but it is under represented in most Australian museums (Fautin et al., 2008).

Actinia tenebrosa, however, is not the most common anemone found in Port Phillip Bay. This is presumably because it's habitat preference of sheltered rocky shore is limited. Large numbers were found at Half Moon Bay, which is characterised by sheltered rock enclaves. This animal was also found in man made 'rocky shores' of groynes and breakwalls. *Actinia tenebrosa* uses a brooding strategy for reproduction (Ottaway & Kirby, 1975). This was again confirmed in this study as two animals released live young after collection. Parry (1951) had limited cnidae measurements for *A. tenebrosa*, however they are similar to those listed here. Specimen MV F109405 from Point Cook is currently registered as *Actinia tenebrosa* in Museum Victoria records, however this identification is incorrect and the correct identification has yet to be determined.
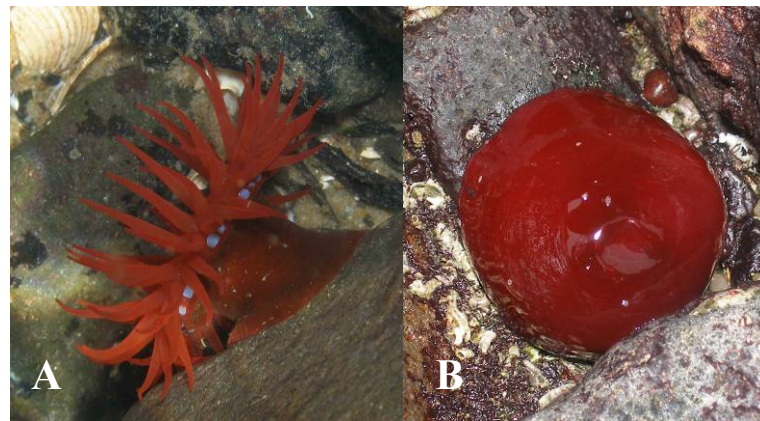
Figure 2.4 (A) Expanded *A. tenebrosa* displaying blue acrorhagi, found at St Kilda groyne. (B) Contracted *A. tenebrosa* from Half moon Bay.

Figure 2.5 Typical preserved specimen of *A. tenebrosa* (MV F111181).

Genus *Anthopleura* Duchassaing and Michelotti, 1860

A large genus currently consisting of 47 valid species (2009).

Diagnosis: Animals with well developed pedal discs. The column has adhesive verrucae arranged in more or less distinct longitudinal rows, especially in the upper part. Marginal acrorhagi are present. The sphincter is weak or strong and may be restricted to circumscribed. The tentacles are simple and are hexamerously or irregularly arranged. The longitudinal muscles of the tentacles are ectodermal or meso-ectodermal. There are numerous perfect mesenteries and all the stronger mesenteries are fertile. The retractor muscles of the stronger mesenteries are diffuse and sometimes restricted. The younger mesenteries grow from the basal disc upwards. Cnidom: spirocysts, basitrichs, microbasic *p*-mastigophores.

Type Species: *Anthopleura krebsi* Duchassaing and Michelotti, 1860.

Type Locality: St Thomas, Caribbean Sea.

Anthopleura aureoradiata (Stuckey, 1909)

(Fig. 2.6; Table 2.6)

Synonymy: *Bunodes aureo-radiata* Stuckey, 1909: 368–369.

Anthopleura aureo-radiata Carlgren, 1924: 208–211.

Anthopleura aureoradiata Parry, 1951: 88, 104–105.

Material Examined. *Port Phillip Bay, Victoria, Australia.* **MV F111182**, 11, North of Werribee River Mouth (Area 9, 1957–1963 PPB Survey), 37º 57'S, 144º 42'E, 28/03/1959, Charles Cutress; **MV F111183**, 9, Rosebud, 38º 22'S, 144º 52'E, 06/12/1958, Charles Cutress; **MV F111185**, 4, Port Phillip Bay, 38º 06'S, 144º 53'E, 21/02/1962, Charles Cutress; **MV F112717**, 2, Grassy Point, Indented Head, 38º 07'S, 144º 41'E, 22/11/2007, Michela Mitchell; **MV F112737**, 3, Rosebud Pier, 38º 22'S, 144º 53'E, 08/12/2006, Michela Mitchell; **MV F112738**, 2, N. of Rye Pier, 38º 23'S, 144º 50'E, 08/12/2006, Michela Mitchell.

Field Characteristics: The oral disc is clearly visible with 3 whorls of tentacles fringing the margin. The animal may have column partially buried under sand depending on substrata attached to. The oral disc, column and tentacles are light tan coloured, with tentacles speckled white and the oral disc striped with white.

Preserved Description: There are approximately 76 tentacles that taper to a blunt point arranged in three whorls. Inner tentacles (13 mm) are slightly longer than outer tentacles (11 mm). Column has verrucae on the upper two thirds, verrucae elevated on the margin.

Two siphonoglyphs are attached to two directives. Mesentery pairs number 32 and the first and second cycles are complete. Sphincter is endodermal, strong and circumscribed.

Cnidom:

Table 2.6 Distribution and size of cnidae of *Anthopleura aureoradiata* (MV F112737)

| Cnidae	Dimensions	n	N
Tentacles			
basitrichs	19.2–20.8 (24) x 2.4–4 µm	10	1/1
spirocysts	(15.2) 20.8–23.2 x 2.4–2.4 µm	10	1/1
Actinopharynx			
basitrichs	24–25.6 x 2.4–3.2 µm	10	1/1
p-mastigophores	20.8–22.4 x 4.8–4.8 µm	10	1/1
Column			
basitrichs	13.6–16 (21.6) x 1.6–2.4 µm	10	1/1
Mesenterial Filament			
basitrichs	12.8–20 x 1.6–2.4 µm	10	1/1
b-mastigophores	24.8–28.8 x 4–4.8 µm	10	1/1
p-mastigophores	16–22.4 x 3.2–5.6 µm	10	1/1

Note: dimensions of cnidae are length x width, n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae. Outlier capsule measurements are provided in brackets.

Distribution: Victoria, Australia and New Zealand (Schiel, 2006). *Type Locality:* Wellington Harbour, New Zealand (Stuckey, 1909).

Habitat: Smaller anemones that are generally found clustered together, commonly attached to pier pylons or buried substrata (mollusca at Rosebud), usually only the oral disc and tentacles are visible.

Remarks: This anemone is found in profusion on jetty pylons, especially around Rosebud and Rye, and it was also observed at Green Point, Brighton. There are two other species of *Anthopleura* occurring in Australia, *A. handi* and *A. buddermeieri*, but their distribution is further north in tropical to subtropical regions (Fautin et al., 2008).
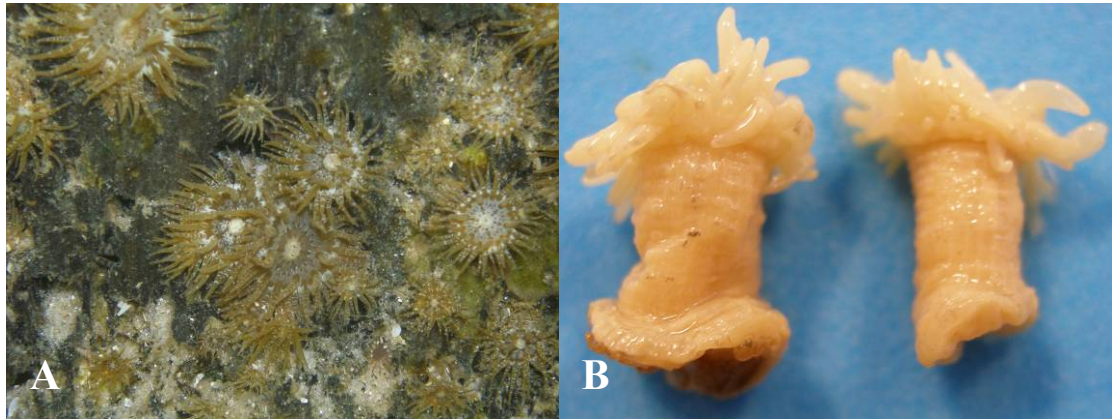
Figure 2.6 (A) *Anthopleura aureoradiata* found on pier pylons at Rosebud. (B) Preserved specimen of *A. aureoradiata* (MV F111183) from Rosebud.

Genus *Aulactinia* Verrill, 1864

Diagnosis: Sea anemone with a well developed pedal disc. The upper part of the column is covered with prominent verrucae in longitudinal rows. The uppermost verrucae are larger than the others and lobed. Foreign material may often be attached to the verrucae. Pseudoacrorhagi (pseudospherules) may be present. The sphincter is more or less circumscribed, sometimes circumscribed diffuse. The tentacles are numerous, simple and short. There are commonly two well developed siphonoglyphs. The mesenteries are numerous with two pairs of directives. The stronger mesenteries may be fertile however directives are sterile in some species. The retractor muscles are commonly strong and more or less restricted. The mesenteries grow from the proximal end and therefore may be more numerous proximally than distally. Cnidom: spirocysts, basitrichs, microbasic *p*-mastigophores

Type Species: *Aulactinia capitata* Verrill, 1864.

Type Locality: Charleston, South Carolina, USA.

Aulactinia veratra (Drayton in Dana, 1846)
(Fig. 2.7; Table 2.7)

Synonymy: *Actinia veratra* Drayton in Dana, 1846: 129–130.

Phymactis veratra Milne Edwards, 1857: 275.

Cribrina verruculata Lager, 1911: 233–234.

Bunodactis verruculata Carlgren, 1949: 65.

Cnidopus verater Carlgren, 1950: 124–125.

Cnidopus verater Ottaway, 1975: 53, 58–59.

Aulactinia veratra Edmands and Fautin, 1991: 59–68.

Material Examined. *Port Phillip Bay, Victoria, Australia.* **MV F111200**, 1, Sandridge Beach, Port Melbourne, 37º 50' 28''S, 144º 55' 5''E, 0 m, 30/04/2006, MRG; **MV F112722**, 1, Queenscliff, 38º 16' 3''S, 144º 39' 7''E, 0 m, 31/05/2008, Michela Mitchell.

Field Description: *Aulactinia veratra* is a large, dark green anemone with bright green numerous tentacles. Port Philip Bay specimens tend to be smaller than those of the east coast of Australia and predominately coloured brown with a red mouth. Sand and some larger particulate matter may be attached to this animal. The animals are jelly-like to touch at low tide as they retain a large amount of seawater during contraction.

Preserved Description: Tentacles (113) numerous, and inner tentacles (15 mm) are shorter than outer tentacles (20 mm). Column has verrucae or adhesive areas on at least the upper two thirds, if not covering entire column to the limbus. Specimens range in size from 2 cm in length to 4 cm in length. On the upper margin of the column the verrucae/adhesive areas may be elevated. Margin is distinct with a deep fosse. Two siphonoglyphs are attached to two pairs of directives. The sphincter is endodermal, strong and palmate circumscribed. Actinopharynx is strongly ribbed and cream coloured in preservation. Thirty-two pairs of mesenteries, arranged in four cycles. The first and second cycles of the mesenteries are complete. Specimens retain green colouring after preservation in 10% formalin: 90% seawater.

Cnidom: See Edmands and Fautin (1991) for full cnidae description. Table 2.7 provides cnidae data for a Port Phillip Bay specimen.

Table 2.7 Distribution and size of cnidae of *Aulactinia veratra* (MV F112722).

Cnidae	Dimensions	n	N
Tentacles			
basitrichs	20.8–26.4 x 2.4–3.2 μm	10	1/1
spirocysts	(12) 14.4–19.2 x 1.6–2.4 μm	10	1/1
Actinopharynx			
basitrichs	(18.4) 24–28 x 3.2–4 μm	10	1/1
Mid-Column		10	1/1
basitrichs	16–18.4 x 2.4–3.2 μm		
Lower Column			
basitrichs	18.4–22.4 x 3.2–3.2 μm	10	1/1
holotrichs	25.6–28.8 x 4.8–5.6 μm		
Mesenterial Filament			
b-mastigophores	(36.8) 44–50.4 (52.8) x 4–4.8 μm	10	1/1
p-mastigophores I	(23.2) 25.6–28.8 x 5.6–7.2 μm	10	1/1
p-mastigophores II	18.4–22.4 x 3.2–3.2 μm	10	1/1

Note: dimensions of cnidae are length x width, n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae. Outlier capsule measurements are provided in brackets.

Habitat: Generally found in crevices on rocky shores in the mid to low intertidal zone. Commonly found alongside *Oulactis muscosa* on the east coast of Australia, but not in Port Phillip Bay (personal observation).

Distribution: Rottnest Island, Western Australia to Southern Queensland, around Tasmania and New Zealand (Edmands & Fautin, 1991; Edgar, 2001). *Type Locality:* Wollongong, New South Wales, Australia.

Remarks: *Aulactinia veratra* was observed at Green Point, Brighton; St Kilda Pier groyne; boat ramp at the end of North road, Brighton; Holloway Bend, Brighton.

Though generally found in close proximity to *Oulactis muscosa* on the east coast of Australia it does not appear to be the case in Port Phillip Bay. The absence of this specimen in the MV collection is unusual, as it is a common anemone described in Victorian field guides and listed in the many studies conducted in Port Phillip Bay (Cutress, 1971; Marine Care Ricketts Point Inc., 2006; Phillips et al., 2006).
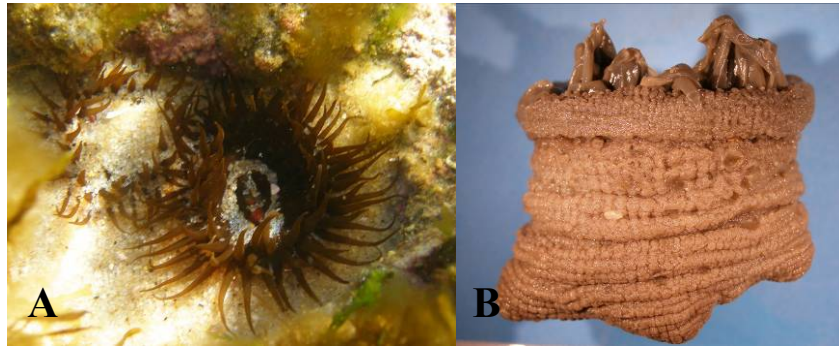
Figure 2.7 (A) Commonly found brown *A. veratra* in Port Phillip Bay. (C) Preserved *A. veratra* (MV F112722) from Queenscliff.

Genus *Bunodactis*

Diagnosis: Genus with well developed pedal disc. The whole or most part of the column with more or less distinctive adhesive verrucae, often simple but sometimes lobed in the distal part of the body. They may or may not be arranged in obvious vertical rows. Foreign bodies often attached to the verrucae. No acrorhagi are present but there may be pseudoacrorhagi. The sphincter is more or less circumscribed, sometimes circumscribed-diffuse. The tentacles are rather short and simple. Longitudinal muscles of the tentacles ectodermal or meso-ectodermal. Commonly there are two well developed siphonoglyphs. Pairs of mesenteries are usually numerous and usually two pairs of directives. All the stronger mesenteries are fertile, however sometimes the directives are sterile. Retractor muscles are commonly strong, more or less restricted. The younger mesenteries grow upwards from the proximal end and therefore the mesenteries are often more numerous in the proximal part than in the distal. Cnidom: spirocysts, basitrichs, microbasic *p*-mastigophores.

Type Species: *Actinia verrucosa* Pennant, 1777.

Type Locality: County Kerry, Ireland (Picton & Morrow, 2005).

Bunodactis rubrofusca Carlgren, 1924

(Table 2.8)

Synonymy: *Bunodactis rubro-fusca* Carlgren, 1924: 204–208.

             Bunodactis rubrofusca Parry, 1951: 88, 115.

Material Examined: None.

Field Characteristics: Unknown.

Preserved Description: The following description of preserved material is from Carlgren (1924). Pedal disc well developed. Column with small longitudinal rows of verrucae, especially distinct in the upper part of column. Deep fosse. The sphincter is weak and concentrated diffuse or palmate circumscript. Short cylindrical tentacles number 56 to 100, outer tentacles almost as long as inner, often irregularly arranged. One to three siphonoglyphs present. Mesenteries more numerous than tentacles (up to 122) and most are perfect. The parietobasilar muscles are strong forming a distinct fold inwards. Basilar muscles are strong. All stronger mesenteries are fertile except for the directives.

Cnidom: The measurements below were provided in the original description by Carlgren (1924).

Table 2.8 Distribution and size of cnidae of *Bunodactis rubrofusca* as provided by Carlgren (1924).

Cnidae	Dimensions
Tentacles	
unspecified nematocysts	19–29 x 1.5–2.5 µm
spirocysts	11–24 x almost 1–1.5 µm
Actinopharynx	
unspecified nematocyst	22–31 x 2–2.5 µm
unspecified nematocyst	24–26 x 4–5 µm
Column	
unspecified nematocyst s	15–22 x 1.5–1.5 µm

Note: dimensions of cnidae are length x width, n = total number of capsules measured.

Distribution: Port Phillip Bay (Cutress, 1971) and New Zealand (Carlgren, 1924). *Type Locality:* Bay of Islands**,** North Cape and Slipper Island, New Zealand (Carlgren, 1924).

Remarks: Cutress (1971) identified this anemone from the 1957–1963 PPB survey material. I was unable to locate the specimens Cutress (1971) referred to in the MV collection. The 1957–1963 PPB survey listed three specimens found off Indented Head (Black, 1971), which is the only record for Australia. There is no description of the living animal in the original description or subsequent checklists (Carlgren, 1924; Parry, 1951). I was not able to find any images of living *B. rubrofusca* nor do New Zealand fauna guide books contain images of it (S. Davey, 2009, pers. comm., 26 May). Until more specimens are found in Port Phillip Bay or the specimens originally identified by Cutress (1971) are located, this record cannot be verified.

Genus *Epiactis* Verrill, 1869

There are currently 18 valid species of *Epiactis* worldwide (Fautin, 2009).

Diagnosis: Animals with a well developed pedal disc and a smooth column that rarely has a cuticle. The margin and the fosse are distinct. The sphincter is often strong and circumscribed, rarely is it restricted. Tentacles are simple and short and not attenuated at the base. The longitudinal muscles of the tentacles and radial muscles of the oral disc are endodermal to more or less mesogloeal. The mesenteries are hexamerously arranged. The mesenteries grow from the base and therefore will be greater in number there than at the margin. At least 12 pairs of the mesenteries are perfect. The retractor muscles are diffuse to restricted and often very strong. There are gonads in all the stronger mesenteries. The embryos often develop in brood pouches or adhere to the column. Cnidom: spirocysts, basitrichs, microbasic *p*-mastigophores.

Type Species: *Epiactis prolifera* Verrill, 1869.

Type Locality: Puget Sound, Washington, United States of America.

Epiactis australiensis Carlgren, 1950

(Fig. 2.8; Table 2.9)

Synonymy: *Epiactis australiensis* Carlgren, 1950.

Material Examined: *Port Phillip Bay, Victoria, Australia.* **MV F108628**, 5, Off Mornington (Area 55, 1957–1963 PPB Survey), 38º 12'S, 145º 02'E, 06/03/1960, Charles Cutress.

Field Description: This distinctive anemone has white tentacles that end in vivid purple tips (Edgar, 2001). The column is red and white striped and the upper margin has a fluorescent green colouring (see Figure 2.8 A).

Preserved Description: The pedal disc is 20 mm in width and column 13 mm in length and 20 mm in width. Specimen examined partially contracted. Margin and fosse are distinct. Sphincter is endodermal, strong and circumscribed. Sixty two short tentacles (2 mm) in three whorls. The tentacles are stubby and round, terminating in a small nipple. Two well developed siphonoglyphs attached to two pairs of directive mesenteries. There are 24 pairs of mesenteries, of which at least the first cycle is complete. All mesenteries are fertile (male) on the specimens examined.

Cnidom:

Table 2.9 Distribution and size of cnidae of *Epiactis australiensis* (MV F108628).

| Cnidae	Dimensions	n	N
Tentacles			
basitrichs	29.6–36 x 2.4–3.2 µm	10	1/1
spirocysts	(19.2) 35.2–38.4 x 2.4–4 µm	10	1/1
Actinopharynx			
basitrichs	31.2–36 x 4–4.8 µm	10	1/1
Column		10	1/1
basitrichs	17.6–20.8 x 2.4–3.2 µm		
Mesenterial Filament			
b-mastigophores	(25.6) 35.2–41.6 x 4.8–6.4 µm	10	1/1
p-mastigophores I	22.4–28 x 5.6–6.4 µm	10	1/1
basitrichs	13.6–16.8 x 1.6–2.4 µm	10	1/1

Note: dimensions of cnidae are length x width, n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae. Outlier capsule measurements are provided in brackets.

Habitat: Found attached to rocks and buried in sand in depths up to 30 m (Edgar, 2001).

Distribution: South East Queensland to New South Wales (Fautin et al., 2008). *Type Locality:* Marine Rocks, South Australia (Carlgren, 1950b).

Remarks: This species was not found during surveys. There has been no taxonomic work on this species since it was established. The cnidom of the Port Phillip Bay specimen mostly agrees with Carlgren's (1950b) original description however he did not list spirocysts or *b*-mastigophores. Carlgren's (1950b) description also listed a small row of pseudospherules at the margin; I did not observe any spherules on the specimens from the MV collection. *Epiactis australiensis* is very similar to images of *E. thompsoni* published in Schiel (2006). The most notable difference is that *E. australiensis* is fluorescent green on the upper margin of the column, and tentacle tips are vivid purple.
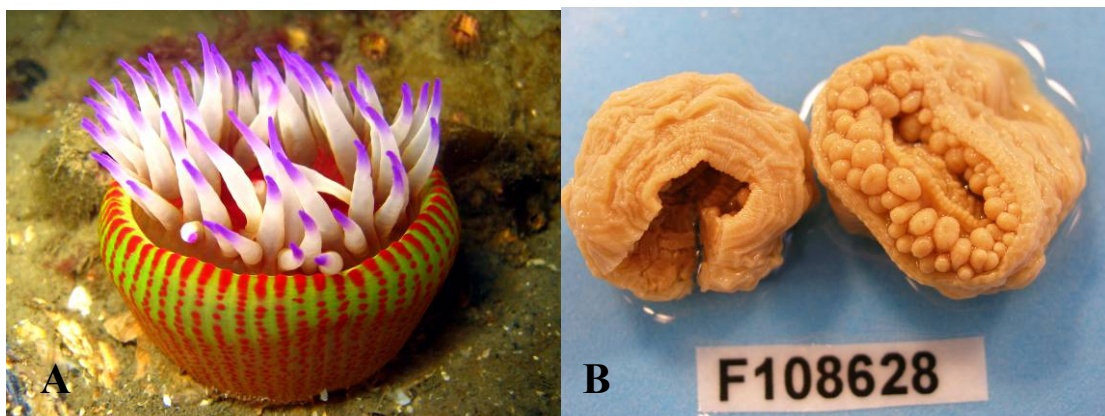
Figure 2.8 (A) Expanded *E. australiensis* in Port Phillip Bay (photo: J. Gaskell). (B) Preserved specimen of *E. australiensis* (MV F108628).

Epiactis thompsoni (Coughtrey, 1875)

(Fig. 2.9; Table 2.10)

Synonymy: *Actinia thompsoni* Coughtrey, 1875: 280.

Leiotealia thompsoni Seshaiya, 1909: 370–372.

Epiactis thomsoni Carlgren, 1949: 58; Cutress, 1971: 83, 84, 85;

Edgar, 2001: 129.

Material Examined: *Port Phillip Bay, Victoria, Australia.* **MV F108629**, 1, Corio Bay (Area 26, 1957–1963 PPB Survey), 38º 07'S, 144º 29'E, 16/02/1958, Charles Cutress; **MV F108630**, 1, Portsea, 38º 19'S, 144º 43'E, 15/09/1957, Charles Cutress; **MV F109273**, 6, Mentone (Area 23 & 14, 1957–1963 PPB Survey), 37º 60'S, 145º 02'E, 26/05/1957, Charles Cutress; **MV F109274**, 1, Mornington (Area 55, 1957–1963 PPB Survey), 38º 12'S, 145º 02'E, 20/10/1963, Charles Cutress.

Field Description: Descriptions of live specimens from Australia are lacking in detail. Edgar (2001) describes the species as having an extremely variable colour pattern with reds and pinks dominating and the tentacles generally have a stripe of unidentified colour on the upper surface.

Preserved Description: Tentacles of similar length and very short (5 mm), blunt in shape. Column smooth, approximately 8 mm in length and 16 mm in width. Pedal disc circular, 18 mm in length. No marginal structures and distinct fosse. Sphincter is circumscribed. Tentacles number between 46 and 62. Two siphonoglyphs attached to directives. Mesenteries pairs number 28–30 and the first, second and third cycles are complete. Gametogenic material is attached to mesenteries.

Cnidom:

Table 2.10 Distribution and size of cnidae of *Epiactis thompsoni* (MV F109273).

Cnidae	Dimensions	n	N
Tentacles			
basitrichs	33.6–41.6 x 2.4–3.2 µm	10	1/1
basitrichs II	19.2–24.8 x 2.4–4 µm	10	1/1
spirocysts	19.2–37.6 x 2..4–3.2 µm	10	1/1
Actinopharynx			
b-mastigophores I	6.4–12.8 (2.8) x 1.6–2.4 µm	10	1/1
b-mastigophores II	32.8–38.4 (45.6) x 4–4.8 µm	10	1/1
Column			
basitrichs	16–16.8 x 2.4–2.4 µm	10	1/1
basitrichs II	10.4–13.6 x 1.6–2.4 µm	10	1/1
Mesenterial Filament			
b-mastigophores	(35.2) 37.6–40.8 x 5.6–8 µm	10	1/1
p-mastigophores	25.6–29.6 x 4.8–6.4 µm	10	1/1
basitrichs	(11.2) 12.8–16 x 1.6–2.4 µm	10	1/1

Note: dimensions of cnidae are length x width, n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae. Outlier capsule measurements are provided in brackets.

Habitat: Mostly found under rocks in depths up to 10 m (Edgar, 2001).

Distribution: South Eastern Australia and New Zealand (Gowlett-Holmes, 2008). *Type Locality:* Deborah Bay, Port Chalmers**,** New Zealand (Coughtrey, 1875).

Remarks: This species was not found during field surveys. New Zealand specimens have been described as having longitudinal red and white stripes, occasionally with a pale green striped column (Schiel, 2006). Tentacles vary in colour and may be dull green, brown or grey with mauve or pink tipped tentacles (Schiel, 2006). As there is limited detail available for this species it needs to be described more completely and compared to New Zealand specimens to ensure they are the same species.

Over the years there has been some confusion with the species name; the 'p' has been dropped from *thompsoni* in Australian literature (Cutress, 1971; Thomas & Shepherd, 1982; Edgar, 2001). The 'p' was originally dropped in Carlgren (1949), so it may be a spelling error that has perpetuated throughout subsequent literature. The correct spelling is with the 'p', as the species was named after harbour master, Captain Thompson (Coughtrey, 1875). The correct species spelling was confirmed by Ottaway (1975a).
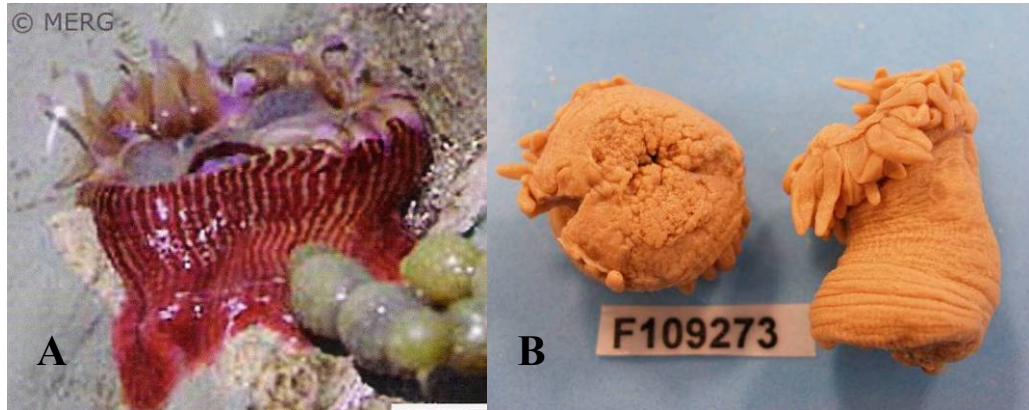
Figure 2.9 (A) *Epiactis thompsoni* from New Zealand (source: Schiel, 2006) (B) Preserved specimen of *E. thompsoni* (MV F109273).

Epiactis sp.

(Fig 2.10)

Material Examined: None examined.

Field Description: This description is from the photograph (Figure 2.10) provided by G. Greenwood and a verbal description from MRG members. The column is mottled red and white without the distinctive striping of *E. thompsoni*. Tentacles have a distinct W patterning of white, brown and yellow on all tentacles. Oral disc is striped brown-red and very light brown with concentric circles of white around the oral disc. Actinopharynx is red with two siphonoglyphs visible. Juveniles cream in colour lacking the distinctive red and white mottling on column, however they do have the white W pattern on the tentacles.

Preserved Description: Unknown.

Cnidom: Unknown.

Remarks: There is no registered material in the Museum Victoria collection matching the distinctive patterning of this anemone; MRG have been referring to this particular anemone as *E*. cf. *thompsoni*. I did not find this species during survey work. It is known from photographic evidence that this species is an internal brooding anemone. Specimens collected by Glenys Greenwood released juveniles from the gastrovascular cavity during captivity (Figure 2.10). This reproduction strategy differs from other *Epiactis* species that brood their young in pouches on the column (Fautin & Chia, 1986). There is little information on the brooding strategies used by *Epiactis* species in Australia; *E. australiensis* is commonly called the brooding anemone, however it is yet to be confirmed if it is an internal brooder (Wallace & Richards, 2009c). Without specimens matching the photograph,

identification to species level could not be made; this may be a new species of *Epiactis*. In addition, the photographic evidence showed a nudibranch, *Austraeolous ornata,* feeding upon the juveniles whilst in captivity.
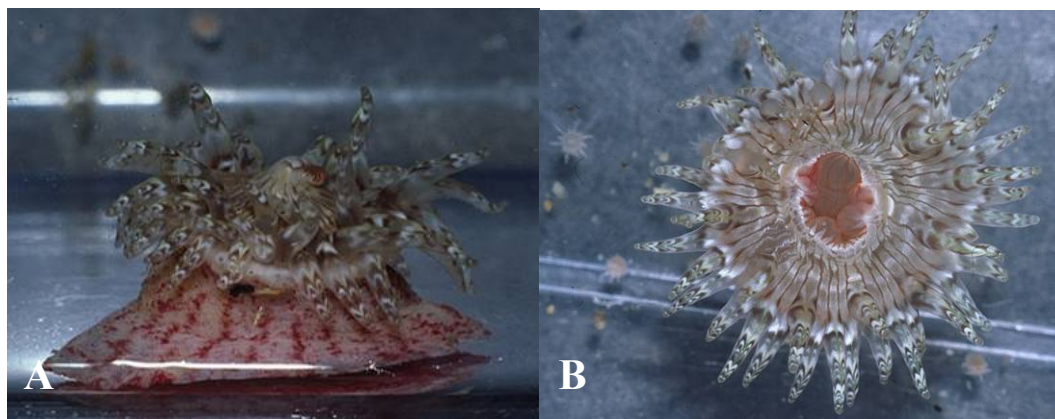
Figure 2.10 (A) *Epiactis* sp. in captivity (photo: G. Greenwood) (B) *Epiactis* sp. in captivity after juveniles released from the adult (photo: G. Greenwood).

Genus *Isanemonia* Carlgren, 1950

Diagnosis from Carlgren (1950b): The pedal disc is well developed and body cylindrical, the margin with perforated pseudospherules. The margin is distinct and fosse is broad. The sphincter is diffuse and elongate. There are numerous tentacles, moderate in length. Two well developed siphonoglyphs and two pairs of directive mesenteries. Numerous perfect mesentery pairs, the number of tentacles and number of mesenteries at the base are about the same. The retractor muscles are band-like, parietobasilar muscles strong and forming a distinct fold on the mesenteries. Most or all of the first cycle mesenteries are fertile. Cnidom: spirocysts, basitrichs, microbasic *p*-mastigophores and holotrichs, which were listed as atrichs in the original description.

Type Species: *Isanemonia australis* Carlgren 1950, by monotypy.

Type Locality: Port Willunga, South Australia (1950b).

***Isanemonia australis* Carlgren, 1950**

(Fig. 2.11; Table 2.11)

Synonymy: *Isanemonia australis* Carlgren, 1950.

Material Examined: *Port Phillip Bay, Victoria, Australia.* **MV F109278**, 1, Point Lonsdale, 38º 18'S, 144º 37'E, 19/11/1960, Charles Cutress. *Victoria, Australia.* **MV F112711**, 1, Venus Bay, Twin reefs, Inverloch, 38º 40'S, 145º 39'E, 02/03/1982, 0 m, Daphne Fautin.

Field Description: Tentacles are numerous, long, thin and dull green in colour. The animals were sighted between boulders at 0–1 m depth. Records of colour variation include: red or olive green with red, grey or green tentacles (Carlgren, 1950b; Carlgren, 1954).

Preserved Description: Tentacles number 123, are 10 mm in length, very broad at the base and tapering to a fine point. The specimens have a very deep fosse and the margin of the column may fold down during the preservation process. Both specimens lacked spherules and the sphincter is endodermal, elongated and diffuse. Column may be strongly contracted into folds, 3 cm in length with a broad pedal disc (2.5 cm). Two siphonoglyphs attached to two directive mesenteries. Specimen examined was not fertile. The retractor and parietobasilar muscles are diffuse and very weak. There are approximately 60 mesentery pairs with the first and second cycles complete.

Cnidom:

Table 2.11 Distribution and size of cnidae of *Isanemonia australis* taken from Cutress (1971) (MV F109278).

Cnidae	Dimensions
Tentacles	
spirocysts	30 x 2 μm
b-mastigophores	28–30 x 3 μm
Actinopharynx	
b-mastigophores	28–30 x 4.5 μm
p-mastigophores	24–25 x 5–6 μm
Mesenterial Filament	
b-mastigophores	35 x 5 μm
p-mastigophores	20 x 5 μm
Limbus	
holotrichs	25–26 x 5 μm
b-mastigophores	21 x 2.5 μm

Note: dimensions of cnidae are length x width.

Habitat: The species is found consistently under or among rocks in depths between 0–10 m (Gowlett-Holmes, 2008).

Distribution: From Western Australia to Victoria (Gowlett-Holmes, 2008). *Type Locality:* Port Willunga, South Australia (Carlgren, 1950b).

Remarks: There are only 5 registered Victorian specimens of *I. australis* in the MV invertebrate collection. This species was not collected in this survey; however was observed at Pope's Eye Marine Sanctuary. Of the three specimens examined by Cutress (1971) only one was found in the MV collection. The specimen is in poor condition, very hard and unmalleable. Unusually, Carlgren (1950b) omitted the type of sphincter from the original description and Cutress (1971) refers to it as entodermal [*sic*], however the specimens examined in MV have an endodermal sphincter. Cutress (1971) found the specimens he examined to be quite different to Carlgren's (1950b) because of a lack of pseudospherules, variation in tentacle number and tentacle length. There are discrepancies between the nematocyst descriptions of Cutress (1971) and Carlgren (1950b) in that Carlgren did not record *b*-mastigophores in South Australian specimens. The cnidom is incomplete in both Carlgren and Cutress' papers. This genus and species requires further taxonomic work to document the variability of characteristics within the species.

Figure 2.11 (A) *Isanemonia australis* in boulders at Pope's Eye Marine Sanctuary, Port Phillip Bay. (B) Preserved specimen of *I. australis* (F109278) identified by Cutress (1971).

Genus *Oulactis* Milne-Edwards and Haime, 1851

There are seven *Oulactis* species in the world and Australia has two, *O. muscosa* and *O. mcmurrichi* (Häussermann, 2003; Fautin, 2009)**.**

Diagnosis from Häussermann (2003): The animals in this genus are characterised by a wide pedal disc. The oral disc is wide and may be round to lobed. The column is smooth in the lowest part and covered with verrucae in the upper part. The verrucae become smaller towards the margin and may be compound or set on small lobes. On the upper margin of the

column is a marginal frill: a thin walled region of fronds. Acrorhagi may be present on the oral disc side of the marginal frill, the fosse is not distinct. The arrangement of mesenteries and tentacles are hexamerous. The majority of mesenteries are perfect and most mesenteries, except the youngest, are fertile. There are two well developed siphonoglyphs and two pairs of directive mesenteries. The sphincter is endodermal, diffuse and weak. The retractor muscles are diffuse to weak and strong and the parietobasilar muscles are strong. Cnidom: Spirocysts, basitrichs, microbasic *b*-mastigophores, microbasic *p*-mastigophores B, microbasic amastigophores A, holotrichs.

Type Species: *Metridium muscosum* (Drayton in Dana, 1846).

Type Locality: Illawarra, Wollongong, New South Wales.

Oulactis muscosa (Drayton in Dana, 1846)

(Fig 2.12; Table 2.12)

Synonymy: *Metridium muscosum* Drayton in Dana, 1846: 153–154.

Oulactis muscosa Milne Edwards and Haime, 1851: 12.

Oulactis plicatus Hutton, 1878: 311–312.

Cradactis plicatus Stuckey, 1909: 392–393.

(questionable synonymy) *Tealidium cinctum:* Stuckey 1909: 389–390.

Oulactis plicata Carlgren, 1949: 52.

Oulactis plumosa Carlgren, 1954: 572.

Oulactis muscosa Dawson, 1992: 38.

Material Examined: *New South Wales, Australia:* **AM G17437**, 1, Rocky Point, Balmoral, New South Wales, Australia, 33º 49' S, 151º 15' E, 09/09/2007, Michela Mitchell; **AM G17440**, 1, The Shallows, Shellharbour, New South Wales, Australia, 34º 32' S, 150º 51' E, 08/09/2007, Michela Mitchell. *Port Phillip Bay, Victoria, Australia.* **MV F111203**, 1, Ricketts point, 37º 59' S, 145º 2' E , 03/06/1967, L.E. Convey; **MV F111189**, 3, Williamstown (Area 5, 1957–1963 PPB Survey), 26/10/1963, Charles Cutress.

Field Characteristics: Distinguished in the field by the shell fragments that regularly adhere to the marginal ruff and column of the animal. The tentacles have a distinctive white bar pattern on a grey background. The oral disc may be a dark brown or dark purple with a bright green mouth, however they can lack the bright green mouth. Found in rock crevices in the mid to low intertidal zone with only the oral disc above ground.

Preserved Description: Verrucae on the column, a marginal frill and acrorhagi are the distinguishing characteristics of the species. The sphincter is diffuse and weak. Tentacles are

all approximately the same length and taper to a point. Mesenteries number 48 and the first, second and third cycles are complete. Mesenteries are generally fertile and sexes separate.

Cnidom:

Table 2.12 Distribution and size of cnidae of *Oulactis muscosa* (AM G17440).

Cnidae	Dimensions	n	N
Tentacles			
basitrichs I	(21.6) 24–28.8 (30.4) x 1.6–3.2 μm	10	1/1
basitrichs II	(16.8) 20–20.8 x 1.6–2.4 μm	10	1/1
spirocysts	(16) 23.2–27.2 x 2.4–3.2 μm	10	1/1
Acrorhagi			
holotrichs I	(29.6) 35.2–41.6 x 2.4–4 μm	10	1/1
holotrichs II	(46.4) 48–54.4 (60) x 5.6–8 μm	10	1/1
spirocysts	(13.6) 17.6–20 x 1.6–2.4 μm	10	1/1
Marginal ruff			
basitrichs	9.6–13.6 x 1.6–2.4 μm	10	1/1
Column			
basitrichs	14.4–19.2 x 2.4–2.4 μm	10	1/1
Actinopharynx			
basitrichs	(24.8) 30.4–36 x 3.2–4 μm	10	1/1
p-mastigophores	24–28 x 4.8–8 μm	10	1/1
Mesenterial Filament			
basitrichs	16–20.8 x 2.4–2.4 μm	10	1/1
b-mastigophores	50.4–57.6 x 5.6–8.8 μm	10	1/1
p-mastigophores	23.2–28 x 4.8–6.4 μm	10	1/1

Note: dimensions of cnidae are length x width, n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae. Outlier capsule measurements are provided in brackets.

Distribution: SE Queensland to Coffin Bay, South Australia, Tasmania and New Zealand (Edgar, 2001). *Original Type Locality:* Illawarra, Wollongong, New South Wales.

Remarks: No specimens of this particular colour morph were recorded during this survey. Häussermann (2003) stated that zooxanthellae are not present in the genus *Oulactis*, however they are present in specimens observed in Australia (see Chapter 3).
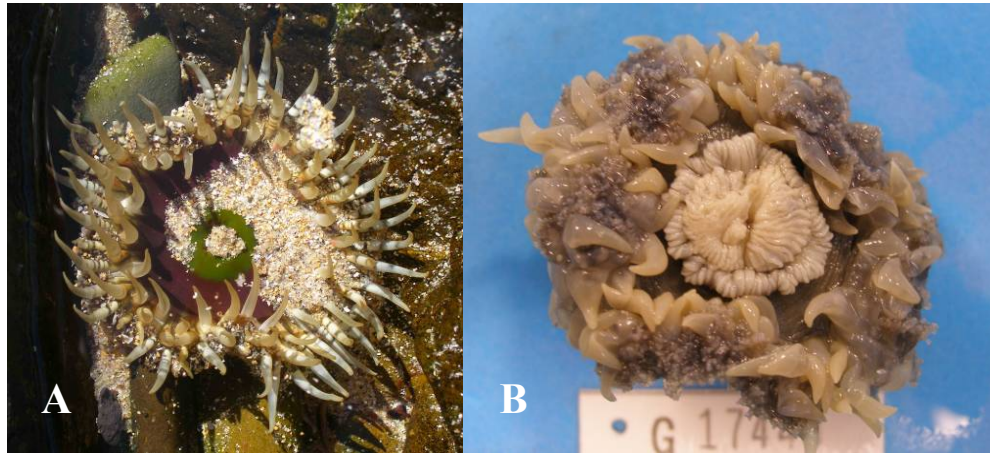
Figure 2.12 (A) *Oulactis muscosa* from The Shallows, Shellharbour, NSW (B) Preserved *O. muscosa*, The Shallows, Shellharbour, NSW (AM G17440).

Oulactis cf. *muscosa*

(Fig. 2.13; Table 2.13)

Material Examined: *Port Phillip Bay, Victoria, Australia.* **MV F109297**, 11, Safety Beach (Area 63, 1957–1963 PPB Survey), 38º 18'S, 144º 60'E, 22/09/1962, Charles Cutress; **MV F111186**, 1, Rosebud (Area 69, 1957–1963 PPB Survey), 38º 21'S, 144º 54'E, 06/12/1958, Charles Cutress; **MV F111188**, 1, 2 miles S. of Rosebud pier, 38º 21'S, 144º 54'E, 06/12/1958, Charles Cutress; **MV F112692**, 1, N. of Rye Pier, 38º 23'S, 144º 50'E, 1 m, 08/12/2006, Michela Mitchell; **MV F112715**, 1, Sandridge Beach, Port Melbourne, 37º 50' 28''S, 144º 55' 5''E, 23/05/2007, Michela Mitchell; **MV F112716**, 1, Safety Beach, 38º 18' 4''S, 144º 59' 6''E 18/02/2006, Michela Mitchell; **MV F112733**, 1, Queenscliff, 38º 16' 11''S, 144º 39' 20''E, 31/05/2008, Michela Mitchell.

Field Description: Distinctive anemones found buried in sand with only the tentacles visible and occasionally the oral disc. There is a pattern of white spots on the tentacles, which may be very faint in some cases. Tapered tentacles in three whorls, all the same length (10 mm) or longer depending on size of the animal. Colour morphs include: brown, white, bright green, bright yellow and white. The oral disc is the same colour as the tentacles and occasionally has a mottled white patterning.

Preserved Description: The oral and pedal discs are similar in size and the column is long with verrucae located on the upper half of column. Acrorhagi present on inner side of the marginal ruff. Sphincter is endodermal, weak and diffuse. Mesentery pairs number 48, are hexamerously arranged with the first and second cycles complete.

Two siphonoglyphs are attached to the directive mesenteries. Tentacles number 86 in the specimen examined, and measured 3 cm in preservation.

Cnidom:

Table 2.13 Distribution and size of cnidae of *Oulactis* cf. *muscosa* (MV F109297).

Cnidae	Dimensions	n	N
Tentacles			
basitrichs	(15.2) 20–25.6 x 1.6–2.4 μm	10	1/1
spirocysts	12.8–27.2 (28) x 2.4–4 μm	10	1/1
Acrorhagi			
holotrichs	(34.4) 39.2–44.8 x 4.8–6.4 μm	10	1/1
spirocysts	24–25.6 x 2.4–3.2 μm	10	1/1
Marginal ruff			
basitrichs	8.8–13.6 x 1.6–1.6 μm	10	1/1
Column			
basitrichs	9.6–18.4 x 1.6–2.4 μm	10	1/1
Actinopharynx			
basitrichs	5.6–14.4 (24) x1.6–3.2 μm	10	1/1
Mesenterial Filament			
basitrichs	(8.8) 19.2–22.4 x 2.4–3.2 μm	10	1/1
b-mastigophores	(16.8) 21.6–24.5 (45.1) x 3.2–4 (5.9) μm	10	1/1
p-mastigophores	19.2 –22.4 x 2.4–3.2 μm	10	1/1

Note: dimensions of cnidae are length x width, n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae. Outlier capsule measurements are provided in brackets.

Habitat: Predominately found attached individually to small rocks buried in sand flats, up to 20 cm under the surface in some cases. This species is located less frequently in rock crevices around Port Phillip Bay.

Distribution: Currently only known from Port Phillip Bay.

Remarks: These animals were also sighted at Green point, Brighton; under Rye pier; St Kilda Pier groyne; Frankston Pier; Sandridge beach, Port Melbourne; boat ramp at end of North Road, Brighton; Fisherman's Beach, Mornington. *Oulactis* cf. *muscosa* was not observed at some sites during initial surveys though observed subsequently. These animals are quite different in appearance to *O. muscosa* or *O. mcmurrichi*. It may be that this is a variant of *O. muscosa* however a complete taxonomic examination is required to determine this (see Chapter 3). Not only is the pattern and colouring different to that known for *O. muscosa,* there are differences in nematocyst size and composition in tissues.

Cutress (1971) identified animals from the same location as *O. muscosa,* however upon re-examination of those specimens they are similar to the specimens collected in this study so have been included here.
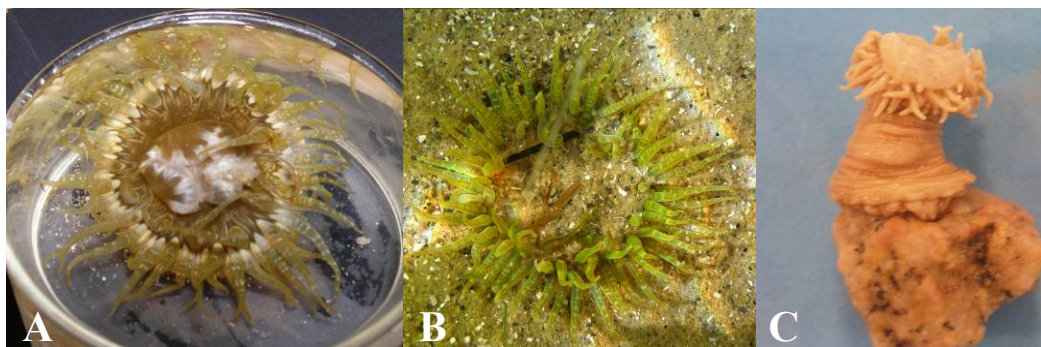
Figure 2.13 (A) Specimen of *Oulactis* cf. *muscosa* (MV F112692) after collection from Rye. (B) Specimen of *Oulactis* cf. *muscosa in-situ,* Safety Beach. (C) Preserved specimen *Oulactis* cf. *muscosa* (MV F109297).

Genus *Phlyctenactis* Stuckey, 1909

There are only two species found in this genus, *P. tuberculosa* and *P. morrisoni* (Fautin, 2009).

Diagnosis: Sea anemone characterised by a broad pedal disc. The column is covered entirely in large, oval vesicles that are closely set in irregular rows. The fosse is well developed and there are no acrorhagi or pseudoacrorhagi. The sphincter is broad and diffuse. The tentacles are rather short and numerous and the longitudinal muscles of the tentacles and radial muscles of the oral disc are mesogloeal. There are two broad siphonoglyphs. Mesenteries are numerous and hexamerously arranged, and there are more mesenteries at the base than at the margin. The first and second cycles of mesenteries may be sterile. Retractor muscles are diffuse and the parietobasilar muscles are well developed and basilar muscles are distinct. Cnidom: spirocysts, basitrichs, microbasic *p*-mastigophores.

Type Species: *Phlyctenactis retifera* Stuckey, 1909.

Type Locality: New Zealand (Stuckey, 1909).

<div align="center">

Phlyctenactis tuberculosa (Quoy & Gaimard, 1883)

(Fig. 2.14; Table 2.14)

</div>

Synonymy: *Actinecta tuberculosa* de Blainville, 1830: 285.

Actinia tuberculosa Quoy and Gaimard, 1833: 159–160.

Cereus tuberculosus Milne Edwards, 1857: 268.

Cystiactis tuberculosa Duerden, 1895: 213.

Phlyctenactis retifera Stuckey, 1922: 396.

Cystiactis retifera Stephenson, 1922: 286.

Phlyctenactis tuberculosa Carlgren, 1945: 13.

Material Examined: *Port Phillip Bay, Victoria, Australia.* **MV F67129**, 3, Southern Port Phillip Bay, 38º 17'S, 144º 41' 24''E, 7 m, 25/07/1987, Clarrie Handreck; **MV F109275**, 1, Portsea, 38º 19'S, 144º 42'E, 26/05/1963, J H (nee Black) MacPherson; **MV F109281**, 1, Portsea (Area 66, 1957–1963 PPB Survey), 38º 20'S, 144º 42'E, 15/09/1957, Charles Cutress; **MV F109295**, 1, Portsea (Area 59, Loc. 25, 1957–1963 PPB Survey), 38º 19'S, 144º 42'E, Charles Cutress; **MV F109289**, 1, offshore between Mornington and Frankston, 38º 11' 12''S, 145º 03' 30''E, 03/05/1963, Tim Stranks; **MV F109296**, 1, off Mornington (Area 47, Loc. 29, 1957–1963 PPB Survey), 38º 14'S, 145º 02'E, Charles Cutress; **MV F109294**, 2, Queenscliff, 38º 16'S, 144º 40'E, 17/10/1990, Michela Mitchell.

Field Description: Very large anemone. Column covered in large vesicles varying in colour from brown to orange (Gowlett-Holmes, 2008). The tentacles are very short in relation to the size of the animal.

Preserved Description: This species is easily distinguished by the large vesicles that cover the entire column. Vesicles retain a grey or brown colouring after preservation. The grey colouring indicates the presence of zooxanthellae in the vesicles. Specimens range in size from 2–8 cm in length and 2–10 cm in width. Tentacles are cream in colour, number over 360, short (inner 1.5 cm and outer 0.5 cm) and pointed. The fosse is distinct and shallow. The pedal disc is scalloped in shape and the actinopharynx is cream. The sphincter is endodermal, weak and diffuse. Two siphonoglyphs present however they are not obvious in some preserved specimens. Numerous mesenteries and at least the first and second cycles of mesenteries are complete. The specimen examined was female.

Cnidom:

Table 2.14 Distribution and size of cnidae of *Phlyctenactis tuberculosa* (MV F109288).

Cnidae	Dimensions	n	N
Tentacles			
basitrichs	21.6–23.2 x 2.4–3.2 µm	10	1/1
spirocysts	(24) 32–36 (48) x 2.4–3.2 µm	10	1/1
Vesicle			
basitrichs	18.4–22.4 x 2.4–3.2 µm	10	1/1
Column			
basitrichs	19.2–24.8 x 2.4–4 µm	10	1/1
Actinopharynx			
basitrichs	(11.2) 13.6–18.4 (22.4) µm x 2.4–3.2 µm	10	1/1
p-mastigophores	24–32 x 5.6–8 µm	10	1/1
spirocysts	(20) 30.4–32 (41.6) x 1.6–2.4 µm	10	1/1
Mesenterial Filament			
basitrichs	37.6–38.4 x 4–4.8 µm	10	1/1
basitrichs II	20–24 x 3.2–3.2 µm	10	1/1

Note: dimensions of cnidae are length x width, n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae. Outlier capsule measurements are provided in brackets.

Habitat: Commonly found contracted into a ball during the day and drifting with the current or attached to seaweed (Gowlett-Holmes, 2008). Primarily a nocturnal species, actively roaming at night to capture food (Edgar, 2001; Gowlett-Holmes, 2008). The species may be found at depths up to 35 m (Gowlett-Holmes, 2008).

Distribution: Southern Australia from south-west WA to Byron Bay, New South Wales and around Tasmania (Edgar, 2001) and New Zealand (Schiel, 2006). *Type Locality:* Bass Strait, Australia.

Remarks: This species is well represented in the MV collection, probably due to its very distinctive appearance. Though commonly seen by many people I did not find this animal during the survey work, but observed them in captivity at the Marine Discovery Centre, Queenscliff.
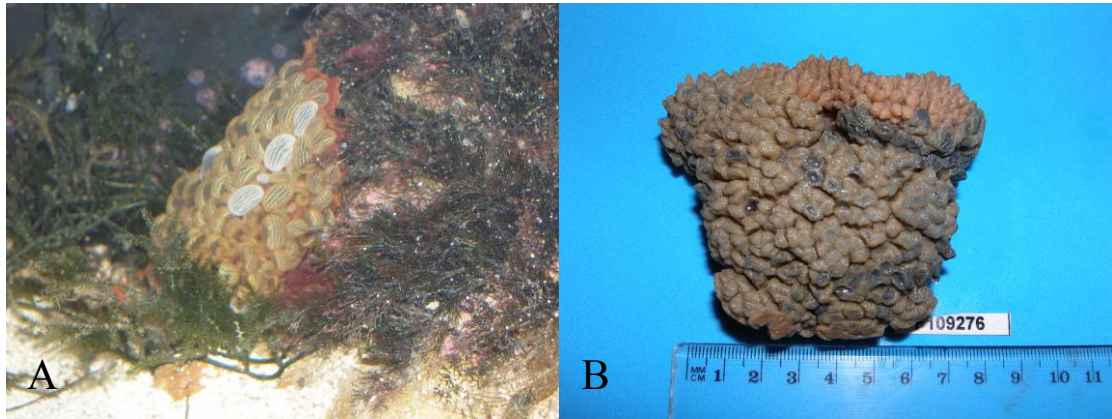
Figure 2.14 (A) *Phlyctenactis tuberculosa* in captivity at the Marine Discovery Centre, Queenscliff. (B) Preserved specimen of *P. tuberculosa* (MV F109276: Somers, Western Port, Victoria).

Genus *Phlyctenanthus* Carlgren, 1950

Diagnosis: The pedal disc is well developed and the column is entirely covered in simple vesicles that are very close set. The fosse is well developed and there are no marginal acrorhagi or pseudoacrorhagi. The sphincter is strong and decidedly circumscribed. The tentacles are rather short and number up to 96. The longitudinal muscles of the tentacles and the radial muscles of the oral disc are ectodermal. There are two broad siphonoglyphs and two pairs of mesenterial directives. There are 48 pairs of mesenteries, all seemingly perfect and fertile apart from the directives. The retractor muscles of the stronger mesenteries are diffuse and band like. The retractor muscles on the weaker mesenteries are more restricted. The parietobasilar and basilar muscles are strong. There are the same number of mesenteries proximally and distally. Cnidom: spirocysts, basitrichs, microbasic *p*-mastigophores.

Type Species: *Phlyctenanthus australis* Carlgren, 1950.

Type Locality: Sydney, Australia.

Phlyctenanthus australis Carlgren, 1950
(Fig. 2.15; Table 2.15)

Synonymy:　　*Phlyctenanthus australis* Carlgren, 1950: 61.

　　　　　　　　Phyctenanthus australis Rodriguez, López-Gonzánlez and Gili, 2007: 1891.

Material Examined: MV 109279, 1, between Point Lonsdale and Queenscliff (Area 58, 1957–1963 PPB Survey), 38º 16'S, 144º 40'E, 64 m, 08/04/1959, Charles Cutress.

Field Description: A large red anemone with column covered in light blue-grey vesicles, tentacles coloured red (Davey, 1998).

Preserved Description: Tentacles may number up to 100 (Edgar, 2001), the small specimen I examined had 81. The inner tentacles (10 mm) are slightly longer than the outer (8 mm). Vesicles on the column arranged in vertical rows. Sphincter is endodermal, strong and circumscribed. Mesenteries (46 pairs) arranged hexamerously with the first two cycles being complete. Two directive mesenteries pairs attached to two siphonoglyphs. The specimen examined was not fertile.

Cnidom:

Table 2.15 Distribution and size of cnidae of *Phlyctenanthus australis* (MV F109279).

Cnidae	Dimensions	n	N
Tentacles			
basitrichs	33.6–39.2 x 2.4–3.2 μm	10	1/1
spirocysts	(22.4) 28.8–30.4 (40)x 2.4–3.2 μm	10	1/1
Column			
basitrichs	(15.2) 17.6–20 x 2.4–2.4 μm	10	1/1
Actinopharynx			
basitrichs	32–36 x 4–4.8 μm	10	1/1
Mesenterial Filament			
basitrichs	12.8–16.8 x 2.4–2.4 μm	10	1/1
p-mastigophores	24–28.8 x 4.8–6.4 μm	10	1/1
b-mastigophores	(32.8) 36–38.4 x 4.8–8 μm	10	1/1

Note: dimensions of cnidae are length x width, n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae. Outlier capsule measurements are provided in brackets.

Habitat: Found often in the mid to low intertidal zone rocky platforms firmly attached to substrata (Davey, 1998).

Distribution: From Sydney, New South Wales around to the Great Australian Bight in South Australia, including Tasmania (Davey, 1998). *Type Locality:* Sydney, Australia.

Remarks: The genus has two species, *P. australis* and *P. regularis.* During field work in Sydney in 2007 a green colour morph of *P. australis* was collected.
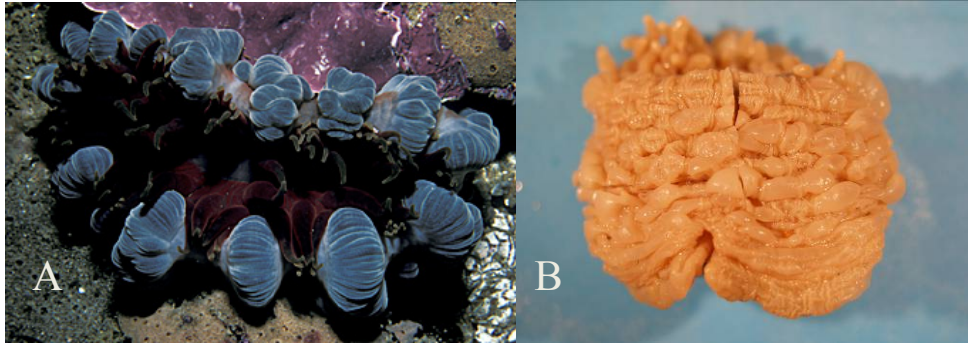
Figure 2.15 (A) *Phlyctenanthus australis* (photo K. Davey, source: Wallace & Richards) (B) Preserved specimen of *P. australis* from Eagle rock, Inverloch, Victoria (MV F112699).

Superfamily Acontiaria Stephenson, 1935

The diagnostic feature of this family is the presence of acontia. The sphincter is generally absent or if present endodermal, though more commonly mesogloeal.

Family *Isophellidae* Stephenson, 1935

This is a family of acontiate anemones, characterised by a mesogloeal sphincter. The mesenteries are divisible into macro- and micronemes, the older micronemes may, however, be provided with filaments and acontia. Cnidom: Acontia have basitrichs and microbasic amastigophores.

Genus *Isophellia* Carlgren, 1900

Diagnosis: Anemones with a small pedal disc and a cylindrical, elongate body. The column is divisible into a scapus and scapulus, the former with tenaculi and cinclides. The sphincter is mesogloeal and may be strong to fairly weak. The longitudinal muscles of the tentacles and radial muscles of the oral disc are ectodermal. Tentacles are short and the inner are longer than the outer. Tentacles are hexamerously arranged and are more numerous than the mesenteries at the base. The mesenteries are divisible into macro and micronemes. The mesenteries of the first cycle and at least half of the second are definite macronemes, the other half marked by micronemes, but with weaker retractors than the former. Each pair of the second cycle consists of a stronger and a weaker mesentery, all arranged in the same manner in relation to the directive plane. Mesenteries of the first cycle and at least the stronger ones of the second fertile. The retractor muscles of the macronemes are strong, restricted and with high folds.

The parietal muscles are weak to rather well developed and basilar muscles are distinct. There are two narrow siphonoglyphs. Cnidom: spirocysts, basitrichs, probably microbasic *p*-mastigophores and microbasic amastigophores.

Type Species: *Isophellia sabulosa* Carlgren, 1900.

Type Locality: Tumbatu, Kokotoni, Zanzibar, Tanzania, East Africa.

Isophellia stela Cutress, 1971

(Fig. 2.16; Table 2.16)

Synonymy: *Isophellia stela* Cutress, 1971.

Material Examined: *Type material. Port Phillip Bay, Victoria, Australia. Holotype:* **MV F41547**, 1, off Brighton (1957–1963 PPB Survey), 37º 54' 45''S, 144º 58' 30''E, 6.5 m, 22/05/1960 Charles Cutress. *Paratype:* **MV F41548**, 2, Off Brighton Bay (1957–1963 PPB Survey), 38º 05' 18''S, 144º 54' 12''E, 22 m, 19/12/1962, Charles Cutress; **MV F66954**, 1, off Brighton, 37º 54' 45''S, 144º 58' 30''E, 6.5 m, 22/05/1960, Charles Cutress.

Field Description: Unknown.

Preserved Description: The following description of preserved material is taken from Cutress (1971): refer to his paper for the full description. The column is divisible into a short distinct thin walled scapulus and a thick walled scapus bearing prominent tenaculi on its distal half. There are approximately 80 tentacles, thin and evenly tapered. Mesogloea sphincter is strong, stratified near the margin and becoming alveolar down the column. Twenty-four pairs of mesenteries, divisible into macro- and micronemes. Two pairs of directives and two prominent siphonoglyphs. The first, and mostly all of the second cycle of mesenteries, are perfect and fertile. Acontia are sparse and short.

Cnidom: The following measurements are from Cutress (1971).

Table 2.16 Distribution and size of cnidae of *Isophellia stela* type specimens as provided by Cutress (1971).

Cnidae	Dimensions
Tentacles	
spirocysts (numerous)	15–18 x 2.5 µm
microbasic *b*-mastigophores (common)	18–20 x 2–2.5 µm
microbasic *p*-mastigophores (few)	17 x 3 µm
Column	
microbasic *b*-mastigophore (few)	18 x 2.5 µm
microbasic *p*-mastigophores (few)	13 x 3 µm
Actinopharynx	
microbasic *b*-mastigophores (numerous)	30 x 3 µm
microbasic *p*-mastigophores (common)	16 x 4 µm
Mesenterial Filament	
microbasic *p*-mastigophores (few)	11–14 x 4 µm
Acontia	
microbasic *b*-mastigophores (numerous)	28–32 x 3–4 µm
microbasic *p*-mastigophores (numerous)	32 x 4 µm

Note: dimensions of cnidae are length x width, n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae. Outlier capsule measurements are provided in brackets.

Habitat: Holotype and paratypes were collected from a sandy substrate, found whilst SCUBA diving, at 6.5 m and 22 m depths (Black, 1971). The type specimens were attached to fibrous material thought to be a polychaete tube (Cutress, 1971).

Distribution: Known only from Port Phillip Bay (Fautin, 2009). *Type Locality:* off Middle Brighton, Port Phillip Bay, Victoria, Australia (Cutress, 1971).

Remarks: Additional, unregistered, MV material from the 1969–1973 benthic survey has been identified as *I. stela*. Further work needs to be undertaken on this material as it appears that some of the specimens may not be *I. stela*. These specimens have an extremely narrow elongated lower column and with very long and abundant acontia, which disagrees with Cutress' (1971) diagnosis of short, sparse acontia. In addition, the acontia contain basitrichs and *p*-mastigophores and no *b*-mastigophores as listed above for *I. stela*. Cutress (1971) cited 3 specimens in paratype lot number MV F41548 however this has been amended to 2 after examination by Dr Daphne Fautin.

Figure 2.16 Unregistered specimens of *I. stela* from Port Phillip Bay and housed in the MV collection.

<div align="center">Family Hormathiidae Carlgren 1925</div>

The family is characterised by a strong mesogloeal sphincter. There are usually six pairs of perfect mesenteries, sometimes more though never numerous. The perfect mesenteries are usually sterile and rarely fertile. There are only basitrichs found in the acontia.

<div align="center">Genus Cricophorus Carlgren, 1924</div>

Diagnosis: Animals have a broad pedal disc and the column is thin, smooth and without cinclides. The sphincter is mesogloeal and strong. Tentacles are short, numbering less than the mesenteries. The inner tentacles are considerably longer than the outer. Mesenteries are hexamerously arranged, more numerous proximally than distally. The longitudinal muscles of the tentacles and radial muscles of the oral disc are ectodermal. Six pairs of mesenteries are perfect and there are two pairs of directives. The six oldest pairs of mesenteries are sterile. Longitudinal muscles of the mesenteries are weak, forming no distinct pennons. Parietobasilar and basilar muscles are weak. The acontia is slender with very short nematocysts. Cnidom: spirocysts, basitrichs, microbasic *p*-mastigophores.

Type Species: *Sagartia nutrix* Stuckey, 1909.

Type Locality: Island Bay, Ohiro Bay, New Zealand (Stuckey, 1909).

<div align="center">Cricophorus nutrix (Stuckey, 1909)</div>

<div align="center">(Fig. 2.17; Table 2.17)</div>

Synonymy: *Sagartia nutrix* Stuckey, 1909: 382–383.

<div align="right" style="margin-right:30%">Cricophorus nutrix Carlgren, 1924: 252–258.</div>

Material Examined: *Port Phillip Bay, Victoria, Australia.* **MV F111192**, 1, Pope's Eye Annulus (Area 59, 1957–1963 PPB Survey), 38º 16'S, 144º 41'E, 16/04/1961, Charles Cutress; **MV F111193**, 8, Rosebud (Area 69, 1957–1963 PPB Survey), 38º 21'S, 144º 55'E, 06/12/1968, Charles Cutress; **MV F112721**, 4, N. of Rye Pier, 38º 23'S, 144º 41'E, 1 m, 08/12/2006, Michela Mitchell; **MV F112739**, 1, Rye Pier, 38º 23'S, 144º 50'E, 22/05/2008, Michela Mitchell.

Field Characteristics: Animals have a brown column and a brown oral disc, with a white radiating pattern from the mouth extending onto half of the oral disc. Tentacles brown with faint white stripes. Mouth is white with a yellow-brown actinopharynx. Pedal disc may have white stripes. *Cricophorus nutrix* is a small animal approximately 10–20 mm in height and 10 mm in diameter.

Preserved Description: Tentacles number between 77–98, arranged in three whorls. Tentacles are short, blunt and terminate in a nipple. Inner tentacles (10 mm) are longer than the outer (2 mm). There are 48 pairs of mesenteries hexamerously arranged; only the first cycle is complete. The specimen is fertile on the third and fourth mesentery pairs. The sphincter is mesogloeal, strong, large and rounded on the upper margin and tapering to a point on lower end, similar to that described by Stuckey (1909). The acontia is found internally. The column wall is exceptionally thin. The retractor and parietobasilar are muscles weak and diffuse. Cinclides are not visible on the column.

Cnidom:

Table 2.17 Distribution and size of cnidae of *Cricophorus nutrix* (MV F112721).

Cnidae	Dimensions	n	N
Tentacles			
basitrichs	(11.8) 19.6–22.5 x 2–2.9 μm	10	1/1
spirocysts	(17.6) 25.5–29.4 (33.3) μm	10	1/1
Column			
basitrichs	12.7–15.7 x 2–3.9 μm	10	1/1
Actinopharynx			
basitrichs	24.5–29.4 x 2.9–3.9 μm	10	1/1
p-mastigophores	20.6–24.5 x 3.9–4.9 μm	10	1/1
Mesenterial Filament			
p-mastigophores	17.6–23.5 x 3.9–4.9 μm	10	1/1
Acontia		10	1/1
basitrichs	18.6–20.6 x 2.9–3.9 μm	10	1/1

Note: dimensions of cnidae are length x width, n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae. Outlier capsule measurements are provided in brackets.

Habitat: Animals attach to seagrass stems (*Zostera* spp.) by their pedal disc in Port Phillip Bay.

Distribution: Victoria and New Zealand (Cutress, 1971).

Remarks: Little taxonomic work has been done on this species since its description. First recorded in Australia and Port Phillip Bay by Cutress (1971). Further investigation is required to determine if the species in Port Phillip Bay is a cryptic species. Some characteristics vary from New Zealand descriptions. No iridescence was evident on any of the animals collected from Port Phillip Bay, which was noted in New Zealand specimens by Stuckey (1909). The nematocyst data from this study are slightly larger than those listed by Carlgren (1924) and no *p*-mastigophores are recorded in New Zealand specimens. There is no mention of patterning on the pedal disc in the original description (Stuckey, 1909). This species was found in substantial numbers over summer sampling periods in a small patch of seagrass in Rye. A resurvey of the site during autumn recorded fewer *C. nutrix* present, and they had been replaced by mussels as the dominant species on the seagrass stand.

Figure 2.17 (A) *Cricophorus nutrix* pedal disc attached to seagrass from Rye. (B) *Cricophorus nutrix* oral disc. (C) Preserved specimen (MV F111193) collected at Rosebud during the 1957–1963 Port Phillip Bay survey.

Family Sagartiidae Gosse, 1858

Acontiate anemones with a mesogloeal sphincter. The mesenteries are not differentiated into macro and micronemes. The acontia contain microbasic amastigophores and basitrichs.

Genus *Anthothoe* Carlgren, 1938

There are currently 13 valid species in the genus (Fautin, 2009).

Diagnosis: Anemones with a well developed base. The column is smooth and has cinclides, sometimes indicated by small elevations and the margin is distinct. The sphincter is strong and mesogloeal, wholly separated from the endodermal muscles of the column. The tentacles are numerous; rather short and the inner are much longer than the outer. The longitudinal muscles of the tentacles and radial muscles of the oral disc are ectodermal. The oral disc is broad. There are typically two siphonoglyphs and two pairs of directives. Mesenteries are numerous and about the same number proximally and distally. There are at least three cycles of perfect mesenteries and at least the first cycle of mesenteries are sterile. The retractor muscles of the mesenteries are diffuse and band like. The parietobasilar muscles are weak and the basilar muscles are distinct. Acontia are numerous. Cnidom: basitrichs, spirocysts, *p*-mastigophores Acontia: basitrichs, *p*-mastigophores.

Type species: *Cereus stimpsonii* Verrill 1869.

Type locality: South Africa, Cape of Good Hope, False Bay.

Anthothoe albocincta (Hutton, 1878) (orange and white striped var.)

(Fig. 2.18; Table 2.18)

Synonymy: *Gregoria albocincta* Hutton, 1878: 312.

                  Sargatia albocincta Seshaiya, 1909: 372–373.

                  Actinothoë albocincta Carlgren, 1949: 103.

                  Anthothoë albocincta Carlgren, 1950:130–132.

                  Actinothoe albocincta Parry, 1951: 89–90.

                  Anthothoe albocincta Cutress, 1971: 83–84, 88.

Material Examined: *Port Phillip Bay, Victoria, Australia.* **MV F109298**, 6, Point Cook (Area 10 PPB Survey), 12/07/1959, Charles Cutress; **MV F109299**, 27, South Channel Fort (Area 61, PPB Survey), 38º 18' 22''S, 144º 47' 31''E; 11/1963, Charles Cutress; **MV F109300**, 2, Portsea Jetty, (1957–1963 PPB Survey), 38º 19'S, 144º 43'E, 15/09/1957, Charles Cutress; **MV F109301**, 9, Quarantine jetty (Area 59, 1957–1963 PPB Survey), Charles Cutress; **MV F109303**, 4, Portarlington (Area 29, 1957–1963 PPB Survey), 38º 06' 30''S, 144º39'E, 09/08/1959, 5 m, Charles Cutress; **MV F109304**, 7, Corio Bay (Area 25, 1957–1963 PPB Survey), 18/08/1960, Charles Cutress; **MV F109417**, 6, Appleton Dock, 37º 49'S, 144º 55'E, 6 m; **MV F109418**, 4, Werribee South Jetty, 37º 56'S, 144º 41'E, 22/09/1995, 0 m, **MV F109422**, 1, Werribee South Jetty, 37º 56'S, 144º 41'E, 22/09/1995, 1 m; **MV F109421**, 33, Werribee South, 37º 56'S, 144º 41'E, 19/03/1996, 2.5 m; **MV F109420**, 5, Altona Pier, 37º 52'S, 144º 49'E, 08/11/1995, 3.5 m; **MV F109423**, 4, Queenscliff Pier, 38º 16'S, 144º 40'E, 01/11/1195; **MV F109424**, 1, Point Cook, 37º 56'S, 144º 45'E, 18/03/1996, 3.5 m; **MV F109730**, 4, Point Ormond, 37º 54'S, 144º 57'E, 23/03/1996; **MV F112720**, 3, Off Green Point, Brighton, 37º 56'S, 144º 56'E, 11/04/2007, Michela Mitchell.

Field Description: This small anemone is easily recognised by the orange and white striped column. Oral disc is orange and fringed by numerous white tentacles, which are held erect when the anemone is expanded. The anemone may fire acontia from the mouth; however it takes a substantial amount of disturbance to prompt this reaction.

Preserved Description: The animal becomes translucent and loses all colouring during the preservation process. Tentacles number in excess of 100, arranged in five whorls, and taper to a point. Inner tentacles (7 mm) are considerably longer than the outer (2 mm). The oral disc is wider than the pedal disc. More mesenteries proximally than distally. There are approximately 93 pairs of mesenteries with at least the first, second and third cycles complete, with fourth and fifth cycles of mesenteries high up under the oral disc.

Two siphonoglyphs are attached to two directives mesentery pairs. The sphincter is mesogloeal and diffuse. The acontia may extrude from the mouth and cinclides on the column after preservation. The column wall is thin. The retractor and parietobasilar muscles are weak and diffuse.

Cnidom:

Table 2.18 Distribution and size of cnidae of *Anthothoe albocincta* (MV F112720).

Cnidae	Dimensions	n	N
Tentacles			
basitrichs	(14.4) 20–24.8 x 2.4–3.2 µm	10	1/1
spirocysts	(14.4) 20–24.8 x 2.4–4 µm	10	1/1
p-mastigophores	22.4–24.8 x 4–4.8 µm	10	1/1
Column			
basitrichs	9.6–10.4 x 1.6–2.4 µm	10	1/1
p-mastigophores	16–20 x 4–4.8 µm	10	1/1
Actinopharynx			
basitrichs	24–26.4 (29.6) x 2.4–3.2 µm	7	1/1
p-mastigophores	19.2–21.6 x 4–4.8 µm	6	1/1
Mesenterial Filament			
basitrichs	13.6–18.4 (26.4) x 1.6–2.4 µm	10	1/1
p-mastigophores	9.6–12 (26) x 4–5.6 µm	10	1/1
Acontia			
basitrichs	24.8–30.4 x 30.4 µm	10	1/1
p-mastigophores	62.4–68 (73.6) x 7.2–8.8 µm	10	1/1

Note: dimensions of cnidae are length x width, n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae. Outlier capsule measurements are provided in brackets.

Habitat: Tends to inhabit low intertidal areas under rock overhangs or on the vertical face of rocks; quite often attached so the oral disc hangs down.

Distribution: Ceduna, South Australia around to New South Wales, Tasmania and New Zealand (Edgar, 2001; Schiel, 2006). *Type Locality:* In rock pools near Dunedin, New Zealand (Hutton, 1878).

Remarks: Also sighted at St Kilda Pier groyne and Sandringham in Port Phillip Bay. This animal does not vary in appearance to that of New Zealand specimens.
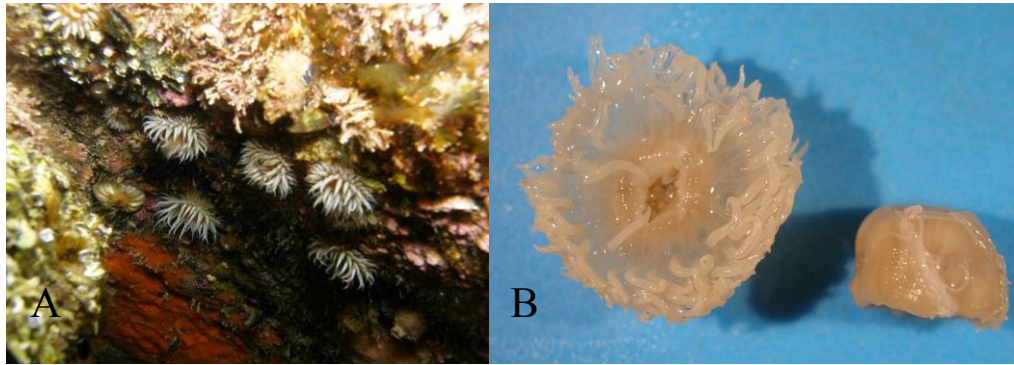
Figure 2.18 (A) Orange and white striped *A. albocincta* from Green Point, Brighton. (B) Preserved specimen of from *A. albocincta* (MV F112720).

Anthothoe albocincta (green var.)

(Fig. 2.19; Table 2.19)

Material Examined: *Port Phillip Bay, Victoria, Australia.* **MV F112719**, 2, Off Green Point, Brighton, 37º 56'S, 144º 56'E, 23/05/2008, 0.5 m, Michela Mitchell.

Field Description: The column is striped green and cream-green and the oral disc and tentacles are orange fringed with white tentacles. The pedal disc is far broader than that of the orange striped morph described above, and much lower in height. Little disturbance is required for the acontia to be ejected from the mouth and cinclides on the column.

Preserved Description: Tentacles number in excess of 130, arranged in five whorls. The tentacles are thin and taper to a point. The inner tentacles (10 mm) are considerably longer than the outer (2 mm). Numerous mesenteries, higher in number distally than proximally, and the directives, first, second and third cycles are complete. The fifth cycle of mesentery pairs occurs low down on the column. Two siphonoglyphs are attached to the directives. The sphincter is mesogloeal and diffuse. The acontia are long and thin and may exude from clearly visible cinclides on the column after preservation. The column wall and mesentery walls are very thin. The retractor and parietobasilar are muscles weak and diffuse.

Cnidom:

Table 2.19 Distribution and size of cnidae of *Anthothoe albocincta* green variation (MV F112719).

Cnidae	Dimensions	n	N
Tentacles			
basitrichs	17.6–24.8 x 2.4–3.2 μm	10	1/1
spirocysts	16.8–20.8 x 2.4–4 μm	10	1/1
p-mastigophores	20.8–24.8 x 4–4.8 μm	10	1/1
p-mastigophores II	12–16 x 3.2–4 μm	10	1/1
Column			
basitrichs	11.2–12 x 1.6–2.4 μm	10	1/1
p-mastigophores	16–17.6 x 3.2–4 μm	10	1/1
Actinopharynx			
basitrichs	24.8–28 x 2.4–3.2 μm	10	1/1
p-mastigophores	18.4–22.4 x 4–4.8 μm	10	1/1
Mesenterial Filament			
basitrichs	14.4–16 x 1.6–1.6 μm	10	1/1
p-mastigophores	9.6–14.4 (18.4) x 3.2–5.6 μm	10	1/1
Acontia			
basitrichs	(18.4) 21.6–24.8 (28) x 1.6–2.4 μm	10	1/1
p-mastigophores	(53.6) 64–71.2 (77.6) x 6.5–8 μm	10	1/1

Note: dimensions of cnidae are length x width, n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae. Outlier capsule measurements are provided in brackets.

Habitat: This animal is found further from shore and deeper than the orange and white stripe variety described previously. The animals attach to the surface of rocks so that the oral disc is orientated towards the water surface.

Distribution: Victoria and New South Wales.

Remarks: Notable differences between the orange and the green varieties of *A. albocincta* include: the green variation preferring deeper water, a second size of *p*-mastigophores on the tentacles, and the fourth and fifth cycles of mesenteries lower down the column towards the pedal disc. The green variety of this species was also observed at Sandringham. In addition, a study from New South Wales (Billingham & Ayre, 1997) and South Australian field guides (Gowlett-Holmes, 2008) list *A. albocincta* as possessing green oral discs and tentacles, but do not mention if the column is also green and cream. Conversely, the specimens found in this study had a green and cream column and the oral disc was orange and tentacles are white.

Figure 2.19 Green variety of *A. albocincta* at Green Point, Brighton.

Anthothoe australiae Haddon and Duerden, 1896

(Fig. 2.20)

Synonymy: *Mitactis australiae* Haddon and Duerden, 1896

Anthothoë australiae Carlgren, 1949

Material examined: None examined.

Field Characteristics: Unknown.

Preserved Description: The following description of preserved material is from Haddon and Duerden (1896). Column short, thick and smooth, the height of the column and the width of the oral disc are similar, while the pedal disc is somewhat narrower. Oral disc tentacles short, thick and pointed, arranged in three whorls. Mesogloeal sphincter, that extends for a considerable distance. Actinopharynx large and folded. Mesenteries may not be in multiples of six. Four cycles of mesenteries, the first and second complete. Single pair of sterile directives, second and third cycles fertile. Specimen examined was female and ovaries visible through the body wall as orange coloured masses.

Cnidom: Tentacles have small oval nematocysts, acontia have long nematocysts (Haddon & Duerden, 1896).

Habitat: Unknown.

Distribution: Port Phillip Bay. *Type Locality*: Port Phillip Bay, Victoria, Australia (Haddon & Duerden, 1896).

Remarks: The type specimen and associated slides for *A. australiae* are stored in the Museum of Zoology, Lund University (Fautin, 2009). No work has been completed on this species since it was described (Fautin, 2009), and it needs to be taxonomically reviewed as it may be a synonym of *A. albocincta* (Cutress, 1971).
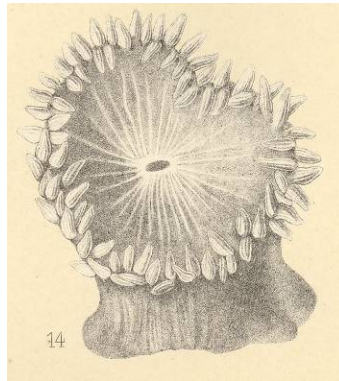
Figure 2.20 Illustration *A. australiae* from the original description (Source: Haddon & Duerden, 1896).

Anthothoe similis Haddon and Duerden, 1896

(Fig. 2.21)

Synonymy: *Mitactis similis* Haddon and Duerden, 1896

Anthothoë similis Carlgren, 1949

Material Examined: None examined.

Field Characteristics: Unknown.

Preserved Description: The following description of preserved material is from Haddon and Duerden (1896). Column is short and thick with oral disc and pedal disc wider than the column. Tentacles are short, thick and blunt, arranged in three or four cycles. An elongated, mesogloeal sphincter lies towards the endodermal side of mesogloea. Actinopharynx is ribbed with deep folds. Fifty-two pairs of mesenteries, of which 13 pairs are perfect, including two pairs of directives. The mesentery arrangement is irregular.

Cnidom: Numerous dark coloured nematocysts on tentacles.

Habitat: Unknown.

Distribution: Port Phillip Bay (Fautin, 2009). *Type Locality:* Port Phillip Bay, Victoria, Australia (Haddon & Duerden, 1896)

Remarks: Haddon and Duerden (1896) thought this species very similar externally to the previous species, *A. australiae*, however they decided that there was sufficient anatomical difference between the sphincter and retractor muscles to define it as a new species. The location of the type specimen of *A. similis* is unknown and no taxonomic work has been completed on this species since being described (Fautin, 2009). The species needs to be taxonomically reviewed as it may be also synonym of *A. albocincta* (Cutress, 1971).
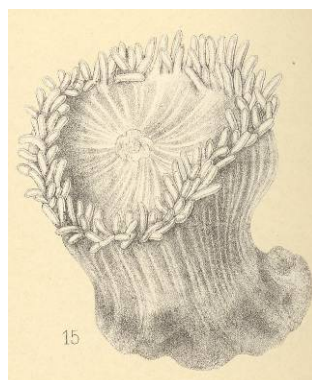
Figure 2.21 Illustration of *A. similis* from the original description (Source: Haddon & Duerden, 1896)

Unidentified Actiniaria

(Fig. 2.22; Table 2.20)

Due to the difficulty in collecting this actiniarian only the upper third of two specimens were obtained. To date I have found no similar material in the Museum Victoria. The characteristics obtained from the two incomplete specimens are provided below.

Material Examined: *Port Phillip Bay, Victoria, Australia.* **MV F112752**, 1, Rosebud Pier, 38º 21'S, 144º 54'E, 0.5 m, 08/12/2006, Michela Mitchell; **MV F112753**, 1, Rosebud Pier, 38º 21'S, 144º 54'E, 0.5 m, 07/02/2009, Michela Mitchell.

Field Characteristics: Found on sandy flats, this anemone camouflages well with its surroundings. Only the tentacles and oral disc are visible above the sand and the remainder of the column is buried. Tentacles are held erect and are translucent with a white V pattern on the tentacles. Column is cream in colour. Animals withdraw below the sand very quickly when disturbed. Over 30 specimens were observed *in-situ*, all of which were expanded and measured 2.5–3 cm in diameter.

Preserved Description: There are 24 tentacles, completely retracted below the margin. Tentacles are blunt and wide. Outer tentacles (6 mm) are longer than inner tentacles (2 mm). The retractor muscles are very strong. Upper column is 1.3 cm in width and smooth. Specimens had large oral disc stomata. Mesenteries are arranged in two cycles of six (12 mesentery pairs). A sphincter was not observed in the two specimens collected.

Cnidom:

Table 2.20 Distribution and size of cnidae of the unidentified burrowing anemone (MV F112752).

Cnidae	Dimensions	n	N
Tentacles			
basitrichs	2.4–3.2 x 20–2 μm	10	1/1
spirocysts	2.4–3.2 x (16) 20–23.2 μm	10	1/1
Column			
basitrichs	2.4–2.4 x (10.4) 16–16 μm	10	1/1
Actinopharynx			
basitrichs	3.2–4 x 20.8–24 μm	10	1/1
Mesenterial Filament			
basitrichs	2.4–3.2 x 20–24 μm	10	1/1
b-mastigophores	4.8–6.4 x 24–29.6 μm		
p-mastigophores	4.8–6.4 x 16–20.8 μm	10	1/1

Note: dimensions of cnidae are length x width, n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae. Outlier capsule measurements are provided in brackets.

Distribution: Port Phillip Bay, Victoria, Australia.

Remarks: Animals were also observed underneath Rye pier. Until a complete specimen can be collected of this animal it cannot be identified. In Gowlett-Holmes' (2008) marine invertebrate guide to South Australia there is an image of an unidentified species from the family Edwardsiidae that closely resembles the image shown here. However, the species cannot be formally identified until complete specimens have been collected and analysed.

Figure 2.22 Unidentified burrowing actiniarian found at Rosebud pier.

2.4 Discussion

Sixteen species of Actiniaria have previously been recorded in Port Phillip Bay, Victoria. Of the 16 species previously recorded, Museum Victoria has 12 represented in their invertebrate collection and seven species were located in the field during this study. In addition to the 16 known species there were four new actiniarian species/records found during this study. Of the four new species/records, three (*Edwardsia* sp., *Oulactis* cf. muscosa and *Epiactis* sp.) were resolved to genus level, and one burrowing species was resolved to order only. Port Phillip Bay is also the only known locality of *Isophellia stela*.

Three species could not be re-examined during this study; *Bunodactis rubrofusca, Anthothoe australiae* and *A. similis.* These species were identified and described by overseas taxonomists and specimens have been misplaced or are housed overseas. Specimens identified by Cutress (1971) as *B. rubrofusca* are missing from the MV collection (other material he examined has been returned and lodged in the MV collection), and this species was not located during current field surveys. The occurrence of this species in PPB is therefore unconfirmed.

Type material for *Anthothoe australiae* is located in the British museum of Natural History and the location of the type material for *A. similaris* is unknown (Fautin, 2009). There have been no published records of these two *Anthothoe* species since their description (Fautin, 2009). The taxonomic status of *A. australiae* and *A. similaris* needs resolution and it is possible that they are natural variations of *A. albocincta*. The descriptions by Haddon and Duerden (1896) for *A. australiae* and *A. similaris* are limited and have only minor differences.

It is possible that Haddon and Duerden examined the two colour morphs (green and orange) of *A. albocincta* found in Port Phillip Bay and erected the two new species. The colour morphs require further taxonomic examination and genetic sequencing to determine their true taxonomic status. Billingham & Ayre (1997) undertook a study into the genetic determination of colour patterns within *A. albocincta*, however they did not specify if they used both colour morphs or only specimens with variations in oral disc and tentacle colour.

Gowlett-Holmes (2008) also records colour variation of the oral disc and tentacles but does not refer to column variation. Both colour morphs of *A. albocincta* in Port Phillip Bay have orange oral discs and white tentacles but differ in their column colour. Other noticeable differences between the two colour morphs include body form and habitat. The green colour morph has a wider pedal disc than the orange colour morph, and the green morph is found further from shore in deeper water with the oral disc orientated upwards whereas the orange morph tends to hang downwards under rock crevices and ledges. These differences in morphology and habitat may indicate that these colour morphs are separate species.

Several previously recorded species were not located during the survey work for this study but are available in the MV invertebrate collection, including; *Epiactis thompsoni*, *E. australiensis*, *Isanemonia australis*, *Phylctenactis tuberculosa*, *Phylctenanthus australis* and *Isophellia stela*. *Edwardsia vivipara* was not found during the survey and there are few registered specimens in the MV collection, which were in poor condition and therefore could not be taxonomically verified.

The fewer number of Actiniaria species observed in the field during this study could be attributed to: site utilisation, seasonality, competition for habitat and habitat type. The field work season for Port Phillip Bay is traditionally in the warmer months between October and May therefore species that may be prevalent over the colder months may have been overlooked. Examples that illustrate the variability in locating actiniarian species at survey sites around Port Phillip Bay are *Oulactis* cf. *muscosa* and *Actinia tenebrosa*.

Oulactis cf. *muscosa* was not observed during summer sampling months at Green Point, Brighton, which coincides with peak beach activity. Subsequently the species was recorded during winter sampling at the same site when there was minimal beach activity. *O.* cf. *muscosa*, being a sand dweller, is quick to withdraw completely under the sand upon disturbance. Presumably due to the heavy usage of the beach during summer the animals had withdrawn therefore could not be observed until winter when there was minimal usage. Additionally, surveys recorded plentiful numbers of *A. tenebrosa* on the rock platform in Half Moon Bay whilst surveys conducted for Parks Victoria did not record any sea anemones at Half Moon Bay (Edmunds et al., 2004).

An example of variation in abundance of fauna found in Port Phillip Bay due to habitat competition is *Cricophorus nutrix*. Significant numbers of this species were found during autumn sampling months and they were the dominant fauna on the seagrass stand surveyed at Rye. During winter/spring sampling, *C. nutrix* was replaced as the dominant fauna by mussels on the same seagrass stand. The cause of this variation of fauna abundance is yet to be determined; possibilities may include seasonal or breeding cycles of species.

For logistical reasons, the sampling sites in this current study were restricted to intertidal and shallow water sites less than 1 m in depth and surveys were conducted during daylight hours. Various habitat substrata not included in this study include the muddy/silty environments and the less saline river environments. This meant that Actiniaria with different habitat requirements such as *P. tuberculosa*, *I. stela* and *E. vivipara* were not recorded during field work for this study.

To produce further comprehensive lists of the Actiniaria fauna in Port Phillip Bay, surveys need to encompass all habitat types and consider the nocturnal/diurnal habits of each species. In addition, a repetitive sampling regime is required to ensure that all fauna for an area is recorded. This is especially important as some sites within Port Phillip Bay are used by Parks Victoria as reference sites for ongoing monitoring studies of Marine Sanctuaries. Half Moon Bay, for instance, is the reference site for the Ricketts Point Marine Sanctuary (Edmunds et al., 2004).

Regional variation

Several actiniarian species in Port Phillip Bay have a shared distribution with New Zealand: *Cricophorus nutrix*, *A. tenebrosa*, *O. muscosa* and *A. albocincta*. While some species exactly match in appearance to those found in New Zealand, others such as *O. muscosa* and *C. nutrix* vary considerably. The possibility that some of the identified taxa in Port Phillip Bay are cryptic species should be explored.

Oulactis muscosa in New Zealand differs considerably in appearance from Australian specimens, especially in regards to patterning on the tentacles and oral disc. It is possible that they may be two different species (see Chapter 3). It has been suggested that because *O. muscosa* and *O. mcmurrichi* are so difficult to distinguish that they may constitute a single

species (Edgar, 2001). However, the *Oulactis* cf. *muscosa* located in Port Phillip Bay varies substantially in appearance and preferred habitat to that known for *O. muscosa* from the Australian east coast (Wallace & Richards, 2009b) and *O. mcmurrichi* from the Australian west coast (Edgar, 2001) and may prove to be a new species (see Chapter 3). Clearly a full taxonomic review of the *Oulactis* species in Australia and New Zealand is required to determine their taxonomic validity, and if there is sufficient taxonomic difference to establish *Oulactis* cf. *muscosa*, found in Port Phillip Bay, as a new species. This issue is discussed further in Chapter 3 using detailed morphological analyses, but genetics may required to determine the relationships between Australian and New Zealand *Oulactis* populations.

Comparison of *Epiactis* species descriptions between Australia and New Zealand specimens also raises taxonomic questions. The original description of *E. thompsoni* states that one colour variant collected had purple tips on the tentacles (Coughtrey, 1875), which would closely resemble *E. australiensis* (also with purple tips on tentacles). The description supplied by Schiel (2006) for *E. thompsoni* is also very similar to that of *E. australiensis*. Carlgren (1950b) thought that the type specimens of *E. australiensis* he examined were similar to that of *E. nova-zealandica*. However, due to the lack of information available at the time on *E. nova-zealandica*, Carlgren (1950b) preferred to erect a new species than unequivocally identify it as *E. nova-zealandica*. *Epiactis australiensis* requires a full redescription and comparison with *E. thompsoni* and *E. nova-zealandica* to determine their taxonomic validity. Preliminary data, from this study, show differences between *E. thompsoni* and *E. australiensis* in the cnidom, primarily in the actinopharynx.

This review of the Actiniaria fauna in Port Phillip Bay has demonstrated there is potential for the number of species recorded in the bay to increase once unresolved taxonomic issues identified in this study have been addressed. It is evident there is a considerable amount of taxonomic and survey work to be done on Port Phillip Bay and Australian actiniarian fauna in general. Many species lack complete taxonomic descriptions and there are still hundreds of specimens requiring full taxonomic identification in the MV invertebrate collection. Habitats not sampled during this study, in particular the muddy/silty environments and deeper water (requiring SCUBA) also need to be surveyed to provide a comprehensive list of the actiniarians present in Port Phillip Bay.

Chapter 3 Taxonomic review of *Oulactis muscosa* (Drayton in Dana, 1846) and *Oulactis mcmurrichi* (Lager, 1911)

3.1 Introduction

3.1.1 Background

There are currently seven valid species recognised in the genus *Oulactis* (Häussermann, 2003; Fautin, 2009). Two of these are found in Australia, *Oulactis muscosa* (Drayton in Dana, 1846) known as the Eastern Sand Anemone, and *O. mcmurrichi* (Lager, 1911) known as the Western Sand Anemone. Their identification causes confusion: they are so similar morphologically that Davey (1998) and Edgar (2001) questioned the taxonomic validity of the two species and suggested they may constitute a single species.

Both *O. muscosa* and *O. mcmurrichi* are similar in appearance (Figure 3.1), and have a distinctive bar pattern on the tentacles (usually white on a grey background) (Edgar, 2001). The oral disc may be brown with a green mouth or plain dark brown and reaches a maximum oral disc diameter of 80 mm (Edgar, 2001; Wallace & Richards, 2009b). Sand and shell fragments adhere to the column *via* specialized structures called verrucae (Thomas & Shepherd, 1982). On the upper margin of the column, below the tentacles, is a distinctive marginal frill (ruff) with acrorhagi attached on the inner, lower edge of the frill (Thomas & Shepherd, 1982). Not only are the two species similar in external morphology, they also share a common habitat niche. Both species prefer to establish in deep crevices along rock platforms, situated in the mid to low intertidal zone (Edgar, 2001).

Figure 3.1 (A) *Oulactis muscosa*, Tinderbox, Tasmania (source: Edgar, 2001) (B) *Oulactis mcmurrichi*, Marmion Lagoon, Western Australia (source: Edgar, 2001).

Field guides distinguish the two species in the field by the colour of the column (Davey, 1998; Edgar, 2001). *Oulactis muscosa* has a cream column with dark spots (Edgar, 2001), whereas *O. mcmurrichi* has a reddish-brown or green to light purple column giving it a much darker appearance (Davey, 2009). Ascertaining column colour in the field is difficult as the column is hidden in crevices or small footholds of the rocky platform. Furthermore, animals are quick to withdraw completely when disturbed. Additionally, colour is not a distinctive feature in sea anemones, as it may be erratic within a species and therefore not reliable as an identification tool (Stephenson, 1928). Patterning however may be a distinguishing feature and is more stable within a species than colour (Stephenson, 1928).

Original descriptions provide little detail of the appearance of these species in life. The illustration of *O. muscosa* from the original description (Figure 3.2 A) clearly shows the distinct bar patterning on the outer tentacles and stripes on the limbus. Conversely, the description for *O. mcmurrichi* lacks any pattern or colour notes as descriptions were based on preserved specimens (Lager, 1911). The paper by Lager (1911) contains only line drawings of preserved specimens (Figure 3.2 B) and briefly mentions a dark pigmentation on the upper column, especially around the frill, which is presumably from zooxanthellae.

Subsequent taxonomic works on the Australian species lack detail of their appearance when alive as they were based on preserved specimens and had limited associated *in-vivo* detail (Carlgren, 1950a; Carlgren, 1950b; Carlgren, 1954; Cutress, 1971).
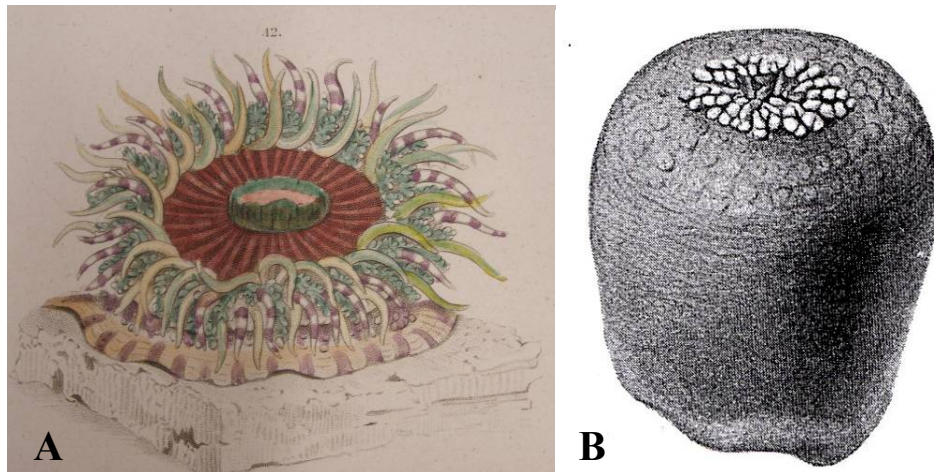
Figure 3.2 (A) Photograph of illustration of *O. muscosa*, Illawarra region, New South Wales (Dana, 1849) (Photo M. Mitchell). (B) *O. mcmurrichi* type specimen (Lager, 1911).

As a consequence of the similarity in appearance of the two Australian species, they are predominately differentiated by locality. The distribution of *O. muscosa* extends from Spencer Gulf, South Australia, around to southern Queensland including Tasmania (Thomas & Shepherd, 1982; Davie, 1998). The species' ranges (Figure 3.3) meet in South Australia where *Oulactis mcmurrichi*'s distribution extends from Coffin Bay, South Australia, to Perth, Western Australia (Davey, 1998; Edgar, 2001).

Figure 3.3 Australian ranges of *O. muscosa* (blue) and *O. mcmurrichi* (red) in Australia.

Oulactis muscosa is also recorded from New Zealand and Argentina (Edgar, 2001; Acuña et al., 2007b). The colour and pattern descriptions for New Zealand and Argentinian *O. muscosa* are considerably different to that of the Australian species. Parry's (1951) colour and pattern description of New Zealand *O. muscosa* states that the species has a wide range of colours. Tentacles have white bars and a line running along the tentacle. The inner tentacles are usually coloured a mottled brown while the outer tentacles are a transparent rosy pink. The oral disc may be one colour but is more likely to have a complex radiating pattern with an underlying colour ranging from red to brown. Alternatively, Morton & Miller (1968) described the oral disc pattern as zigzagged in brown, white and gold and the column brown, orange or green brown with cream or white verrucae. Parry's (1951) colour description is consistent with the colour schemes that are recognised for the species in New Zealand today (Schiel, 2006). Animals from Mar del Plata, Argentina, are characterised by various colours on the oral disc, either one half or a quarter of the oral disc with one colour (green or yellow) and the other half or quarter white. The column is white with verrucae on the upper half of the column (M. Zamponi, 2008, pers. comm.).

Previous taxonomic descriptions and revisions of *O. muscosa* and *O. mcmurrichi* for Australia have been based solely on preserved specimens sent to overseas taxonomists. This has meant potentially distinguishing *in-vivo* characteristics, such as patterning, have not been documented. Identification of preserved specimens of *O. muscosa* and *O. mcmurrichi* is difficult, as colours and patterns fade during the preservation process (Stephenson, 1928; Häussermann, 2004). Identification based solely on locality may be unreliable, especially if the specimen originates from the region where the distributions of the two species meet. The taxonomic descriptions available for *O. muscosa* and *O. mcmurrichi* do not contain enough detail to allow definitive identification and separation of the Australian specimens (Dana, 1846; Lager, 1911; Carlgren, 1950a; Carlgren, 1954).

In addition to the lack of detailed descriptions for *O. muscosa*, there are no known type specimens for *O. muscosa* (Häussermann, 2003) and consequently, none for the genus. The genus *Oulactis* was originally described by Milne-Edwards and Haime (1851), the type species being *Metridium muscosum*, now known as *O. muscosa* (Fautin, 2009). The type locality of *O. muscosa* is Wollongong, Illawarra region, New South Wales (Dana, 1846).

Oulactis mcmurrichi, as it is now known, was originally placed in the genus *Saccactis*. Lager (1911) described the genus *Saccactis* and 3 species in a paper detailing sea anemones collected on the 1905 Hamburg Research Expedition to Southwestern Australia; *S. mcmurrichi* (genus type species), *S. mcmurrichi* var., *S. musculosa*, and *S. australis* (Lager, 1911). Carlgren (1954) mistakenly believed that *Saccactis* type specimens had been destroyed during World War II, however syntypes of *S. mcmurrichi*, *S. australis*, and *S. musculosa* are housed in several museums. Some are in Hamburg, in poor condition (Riemann-Zürneck & Gallardo, 1990), Western Australia Museum has one small intact syntype, Humboldt University Museum of Berlin has six syntypes, and the Naturhistoriska Riksmuseet in Stockholm has four (Fautin, 2009). Riemann-Zürneck & Gallardo (1990) examined *Saccactis* syntypes during their study and found them to be in poor condition, and some of the specimens Lager (1911) wrote descriptions from were partial animals.

The syntype localities of *O. mcmurrichi* is Bunbury, Western Australia, located on the southwest coast, and Albany, located on the southern coast of Western Australia (Lager, 1911).

Lager (1911) dismissed the Western Australian specimens examined as belonging to the already established genera of *Cradactis* or *Asteractis*, both now synonymised with *Oulactis* (Fautin, 2009), and *Actinostella*. Those genera were characterised by a more circumscript sphincter and lacked acrorhagi (Lager, 1911). *Saccactis* was therefore established on the grounds that the sphincter is diffuse and animals possess randsäckchen (outside edge pouches) and blasenförmigen (bubble shaped excrescences). Here randsäckchen are defined as acrorhagi, and blasenförmigen as the marginal frill.

Since the establishment of *Saccactis* the validity and placement of species within the genus has been reviewed repeatedly. Initially Stephenson (1922) placed *Saccactis* in *Cradactis*, and Carlgren (1949; 1950a) then placed *Cradactis* in the genus *Oulactis*. Subsequently Carlgren (1954) synonymised three of Lager's species, *O. mcmurrichi* var., *O. australis* and *O. musculosa* with *O. mcmurrichi*, as he could find no substantial differences between species in Lager's (1911) descriptions. The genus *Saccactis* was resurrected by Riemann-Zürneck & Gallardo (1990) but placed back in synonymy with *Oulactis* by Häussermann (2003) because both genera shared characters which were thought to define *Oulactis*; adhesive verrucae on the column, frond-like papillae and the presence of acrorhagi.

Häussermann's (2003) review of *Oulactis* resulted in amendments to Carlgren's (1949) generic diagnosis (see Appendix II for the current description). Key characteristics include: column smooth in the lower part and verrucae on the proximal half, marginal ruff present, acrorhagi maybe present, mesenteries and tentacles arranged hexamerously, endodermal sphincter — weak to well developed. In addition to *O. muscosa* and *O. mcmurrichi*, current valid *Oulactis* species and their distribution are: *O. magna* (Stuckey, 1909), New Zealand; *O. orientalis* (Averincev, 1967), Posjet bay, Sea of Japan, Russia; *O. coliumensis* (Riemann-Zürneck & Gallardo, 1990), Chile; *O. concinnata* (Drayton in Dana,

1846), Chile; and the questionable synonymy of *O. (Tealidium) cinctum* (Stuckey, 1909), New Zealand (Häussermann, 2003; Fautin, 2009).

This study aims to determine if *O. mcmurrichi* is a valid species or if it should be synonymised with *O. muscosa*, the senior synonym. To achieve this, colour and pattern variations of *O. muscosa* and *O. mcmurrichi* are documented *in-vivo*. Morphological variation of fresh and preserved material of supposed *O. muscosa* and *O. mcmurrichi* is examined and published literature reviewed. In addition, some of the new material collected for this study will be assigned neotype and paratype status with details of specimens included in a preliminary redescription of *O. muscosa*.

3.2 Materials

Live specimens were collected from eight localities; Safety Beach, Port Phillip Bay, Victoria (38º 19' S, 144º 59' E), Rye Beach, Port Philip Bay, Victoria (38º 22' S, 144º 50' E), Flat Rock, Lennox Head, New South Wales (28º 48' S, 153 33.5' E), Shellharbour, New South Wales (34º 32' S, 150º 51' E), Wy-ar-gine Point, Balmoral, New South Wales (33º 49' S, 151º 15' E), Rocky Point, Balmoral, New South Wales (33º 49' S, 151º 15' E), Mudarup Rocks, Cottesloe, Western Australia (31º 59' S, 115º 45' E) and Seatoun, Wellington, New Zealand (41º 19' S, 174º 49' 59" E). No living *Oulactis* species were located during field work on the Fleurieu Peninsula, Spencer Gulf, in South Australia to include in the study. No field work could be undertaken at the syntype localities of *O. mcmurrichi*, at Bunbury and Albany, Western Australia. Appendix III contains the full listing of material examined during this study.

Specimens designated as the *O. muscosa* neotype and paratype have been deposited in the Australian Museum with the registration numbers of: G17437 (Rocky point, Balmoral, New South Wales) and G17440 (The Shallows, Shellharbour, New South Wales).

Included in the study is a specimen I identified as *Oulactis* sp. The specimen was collected by the Environmental Protection Authority, from a ships' ballast whilst harboured in Port Phillip Bay. The exact origin of the specimen is unknown as the ship had been dry-docked in Singapore in October 2005 after which it commenced a regular transit route between Devonport, Tasmania and Melbourne, Victoria. The specimen was included as an indication of the potential variability in morphological characteristics of *Oulactis* species.

Preserved material identified as *O. muscosa*, *O. mcmurrichi* and *Oulactis* sp. were borrowed from Australian museums; Museum Victoria (MV), the Australian Museum (AM), the South Australian Museum (SAM), Western Australian Museum (WAM) and the Tasmanian Museum and Art Gallery, Hobart (TMAG). The Museum and Art Gallery of the Northern Territory invertebrate collection holds no specimens identified as *Oulactis*. Two syntypes of *S. mcmurrichi* (Reg. No. C5321) were examined from the Zoological Institute and Zoological Museum of the University of Hamburg and one syntype (Reg. No. MNH 5440) from the Museum für Naturkunde - Leibniz Research Institute for Evolution and Biodiversity at the Humboldt University Berlin. Dr M. Zamponi had previously deposited *O. muscosa* specimens from Argentina in the Mar del Plata Museum of Argentina (2008, pers. comm.), however the museum holds no record of this material nor any other specimens identified as *Oulactis*.

3.3   Methods

3.3.1 Field and Laboratory

Oulactis specimens were located and photographed *in-situ* prior to collection. Specimens that resembled illustrations from the original description of *O. muscosa* were chosen as the neotype and paratype. Collected animals were transported to the laboratory for observation. Colour and pattern details of the live animals were recorded. Specimens were grouped according to live appearance and similarity of tentacle and oral disc patterning.

Prior to preservation, animals were relaxed in either a solution of 0.5 g menthol crystals: 500 ml seawater or an isotonic solution of magnesium chloride: seawater until they showed no reaction to touch. Relaxation periods varied between 30–120 minutes. The method of relaxation used was dependent upon the host laboratory's supplies. Specimens were then transferred to 10% formalin: 90% seawater for fixation. Interstate specimens remained in the formalin solution until shipment to MV. Museum Victoria specimens were fixed and stored in 10% formalin: 90% seawater. Interstate specimens were transferred to ethanol (24–80%), dependent upon the host institutions' loan policy then shipped to MV. Upon arrival at MV, specimens in 24% ethanol for transport were transferred to 70% ethanol. Specimens that did not survive during field collection were stored in 90–100% ethanol at the host institute for future DNA work.

3.3.2 Taxonomic Analysis

Examination of material (freshly collected and preserved) included analysis of external and internal morphological characteristics and cnidae measurements. Histological examination of the sphincter, retractor and parietobasilar muscles and verrucae was also undertaken.

Data collection on external morphology included: presence/absence of acrorhagi, the extent of verrucae coverage on the column, number of tentacles and the number of siphonoglyphs. The dimensions of each animal were recorded, together with the length of the inner and outer tentacles relative to each other. Internal morphology data included: mesentery cycle completeness, arrangement of mesentery pairs (hexamerously or otherwise), number of mesentery pairs, and the fertility/sex of the specimen.

Cnidae smears of preserved tissue from the mid-column, marginal frill, acrorhagi, inner and outer tentacles, actinopharynx and mesenterial filaments were prepared. Each smear was examined using a compound microscope (1000x oil magnification) and a minimum of 10 unfired capsules of each nematocyst type, were measured. Where possible, cnidae

smears were made from living tissue to further classify the types of nematocysts present. Nematocyst nomenclature follows that of England (1991). For the purpose of this study nematocyst terminology from earlier literature is defined as follows: Carlgren (1950a; 1954) and Parry's (1951) atrichs are referred to as holotrichs, Lager's (1911) thin walled capsules as spirocysts, and thick walled capsules as basitrichs.

Transverse and cross-sections of preserved specimen sections were embedded in paraffin wax. Permanent slides were prepared by cutting 8μm thick sections, mounting and staining slides with Mallory's Triple Stain. Slides are lodged with associated specimens in their host museum.

3.3.3 Statistical Analysis

Using a matrix of characters prepared in Microsoft Excel and the statistical package PRIMER 6 (Clarke & Gorley, 2006), data were analysed with a Principal Component Analysis (PCA) to determine potential characters useful in distinguishing between *Oulactis* species and subsequently Australian species.

The data matrix was prepared by assigning discrete numbers for freshly collected and preserved specimens character states including the following: number of tentacles, number of mesentery pairs, number of siphonoglyphs, number of tentacle whorls, number of complete cycles of mesentery pairs, length of inner and outer tentacles, oral disc length and pedal disc width. The presence (1) or absence (0) of: the acrorhagi, fighting tentacles and directives attached to actinopharynx was also included in the matrix. Characteristics that were missing or not specified in published descriptions were given a value of 3 (unknown). In addition, reproductive status of the specimen, if directive mesenteries possessed gametic material, and the types of cnidae in various tissue were also scored. A weighting number was assigned for the extent of verrucae coverage on a specimen (Table 3.1). In addition to the above characters, a second matrix was developed for freshly collected material including tentacle and oral disc patterning.

Table 3.1 Weighting assigned for verrucae coverage on the column of *Oulactis* specimens.

Weighting	Weighting Definition
1	Upper third of column covered in verrucae
2	Upper half of the column
3	Two thirds of the column
4	Entire column covered to limbus

Data from previously published descriptions were used to score the same characters states for *O. muscosa* and *O. mcmurrichi* and the remaining *Oulactis* species, to determine the levels of variation between *Oulactis* species. Published descriptions used in the analysis include; *O. muscosa* (Carlgren, 1950a), *O. mcmurrichi* (Lager, 1911), *O. magna* (Carlgren, 1924), *O. coliumensis* (Riemann-Zürneck & Gallardo, 1990), *O. concinnata* (Häussermann, 2003) and *O. orientalis* (Averincev, 1967).

Analysis in PRIMER 6 consisted of a pre-treatment of the data by square root transformation. Factor groupings for specimen data were added and based on tentacle and pattern similarity and preliminary specimen identifications. Data were then transformed by weightings of characters. Character weightings were determined from a review of literature and the relative variability of characters between and within species. Data were analysed using Principal Component Analysis (PCA) to determine characters distinguishing the species. The PCA was then generated again for specimens with *in-vivo* data, to determine the significance of *in-vivo* characteristics in distinguishing between species and specimens.

3.4 Results

3.4.1 Taxonomic Descriptions

External character variation between published descriptions of *O. muscosa* and *O. mcmurrichi* (Table 3.2) occurs in: the number of tentacles, the relative length of inner and outer tentacles and the extent of verrucae coverage. Descriptions of the sphincter muscles are in agreement, weak and diffuse (Lager, 1911; Carlgren, 1950a; Carlgren, 1954), with

two exceptions. Cutress (1971) described *O. muscosa* from Port Phillip Bay as having diffuse to circumscribed sphincters, and Parry (1951) described New Zealand specimens as having strong and diffuse sphincters. The fosse is shallow, the retractor muscles are strong and diffuse and parietobasilar muscles well developed and strong (Lager, 1911; Carlgren, 1950a; Carlgren, 1954). Lager (1911) had little to add for the variant of *O. mcmurrichi* she described, except that the specimen was a little larger. Carlgren (1954) had nothing more to contribute to Lager's (1911) description of the anatomy of *O. mcmurrichi* and included a comparison of drawn sphincters from Western Australia and South Australia, which were in agreement. These sphincter diagrams are also in agreement with those of Lager's (1911). The terminology from the original published descriptions has been retained throughout the taxonomic comparison.

Table 3.2 Character states for *Oulactis muscosa* and *Oulactis mcmurrichi* from published literature based on preserved material.

Tissue/Species	*Oulactis muscosa (NZ)*	*Oulactis muscosa*	*Oulactis mcmurrichi*	*Oulactis (australis) mcmurrichi*	*Oulactis (musculosa) mcmurrichi*
Author	Parry, 1951	Carlgren, 1950a	Lager, 1911	Lager, 1911	Lager, 1911
No. of tentacles	96 which occur in four cycles 12, 12, 24, 48	96	86 in 3 whorls	80 and arranged in 2 or 3 cycles on outer third of disc	24 on ¼ of animal
Tentacle shape	Short and do not differ in size, length 1–1.5 cm	Short conical tentacles	Inner longer than outer	Tentacle length 0.5 cm sharply pointed in distal end.	Tentacles similar to *Oulactis (australis) mcmurrichi*
No. of mesenteries and cycles	Generally 48, numerous perfect pairs of mesenteries	48 pairs mesenteries	48 (4 cycles, 6+6+12+24). First 3 cycles complete	Probably corresponds to formula 6+6+12+24+48 = 96 tentacles, 2 directives. First 2 complete and only some of the third	Mesenteries arranged in 6's, fewer mesenteries than other species examined (*O. mcmurrichi* and *O. australis*) (6+6+12 = 24). First cycle probably complete. Pairs of the third cycle are very small
Verrucae	Numerous verrucae in longitudinal rows, more numerous in the upper part of the column where the last few (about 12) are carried on a ridge	Lower part smooth. Largest verrucae being in the middle of the column, smallest in upper part	Strongly developed and mostly in top part of column. 2–5 verruca in each vertical row and of the 1–3 simple bubbles	Not recorded	Upper part has suction warts
Column	Straight	Low	Body cylindrical	Not recorded	Not recorded
Spherules (acrorhagi)	Orange or pink and usually number 24	Not recorded	Not recorded	Not recorded	Not recorded
Oral and marginal stomata	Not recorded	Not recorded	Oral stomata are little and marginal fairly big	Edge stoma is small – could not see oral stoma	Oral stomata not seen and edge stomata close to body-wound
Comments		Height 2.5 cm and breadth 5 cm			Pedal disc 1.8 cm and body 1.3–1.6 cm diameter 1.7 cm

Cutress (1971) contributed little additional information for nematocyst information except for the acrorhagi of *O. muscosa* from Port Phillip Bay: holotrichs (40–50 x 4–5 µm) and spirocysts (28 x 2 µm). Lager (1911) provide nematocyst measurements only for of *O. mcmurrichi* var. tissue for the oral, pedal disc and verrucae (and so has been excluded from the cnidom comparison in Table 3.3).

Table 3.3 Cnidom of *Oulactis muscosa* and *O. mcmurrichi* from published literature based on preserved material. Capsule dimension length x width in microns (µm)

Tissue/Species	Cnidae Type	*Oulactis muscosa*	*Oulactis muscosa*	*Oulactis muscosa*	*Oulactis mcmurrichi*	*Oulactis mcmurrichi*	*Oulactis mcmurrichi*
Author		(Acuña et al., 2007b)[#]	(Parry, 1951)	(Carlgren, 1950a)	(Carlgren, 1954)	(Carlgren, 1945)	(Lager, 1911)
Tentacles							
	Basitrichs	11–29 (abundant)	21–32 x 3	19.7–25.4 x 2.5–3.5 (common)	19.2–22.6 x 2.5–2.8 (17–21 (24) x 2.8–3 17–24 x 2.5–2.8)	14.8–19.7 x 2.1–2.5	17–22
	Spirocysts	14–33 (common)	24 x 2.5	Absent	Absent	18.5 x 3	22–26
	Microbasic *p*-mastigophore	Absent	16–24 x 2-2.5	Absent	Absent	Absent	Absent
Column							
	Basitrichs I	15–26 (abundant)	10–13 x 2.5	15.5–19.7 x 2.2–2.8, 22.6–29 x about 3.5 (very common)	14–18.3µ x 2–2.5* (14.8–18.3µ x 2.5, 14.8–19.7 x 2.5–2.8)	14–16 x 1.5–2.5 (fairly common)	Present
	Basitrichs II	8–16 (scarce)	Absent	Absent	Absent	Absent	Absent
	Holotrich	15–30	Absent	19.7–33.8 x 4.2–5.6	21–28.2 x 4.2–5 (18.3–21 x 4.5 (few), 24–31 x 3.5–4)	19.7–22.6 x 3.5–4.2 (few)	
Actinopharynx							
	Basitrichs	9–20 (abundant)	Not provided	27.5–32.4 x about 4 (common)	(19.7) 21–23.2 x 3–4 (… …, 21–31 x 3–4)	(8.5) 12.7–14 x 2	24–26
	Basitrichs	Absent	Not provided	Absent	Absent	21–25.4 x 3–3.5[+]	Absent
	Microbasic *p*-mastigophore	12–27 (scarce/present)	Not provided	21–26.8 x 4.2–6	Absent	Absent	Absent
	Microbasic *b*-mastigophore	21–37 (common)	Not provided	Absent	Absent	Absent	Absent
Marginal Ruff							
	Basitrichs	Not provided	Not provided	Not provided	Not provided	15.5–19.7 x 1.4–2.4	10–12
Acrorhagi							
	Basitrichs	12–29 (scare/present)	Absent	36.6–45 x 2.8 (numerous)	Absent	Absent	34–41
	Holotrich I	41–81 (common)	48–51 x 4–5.5	46.5–70.5 x about 7	33.3–43 x 4.2–7 (36.7–48 x 4.2–5.6 37–56 x 4.2–5.6)	31–43.7 x 4.2–5.6	Absent
	Holotrich II	42–73 (abundant)	Absent	Absent	Absent	Absent	Absent
	Spirocysts	17–41 (present)	Absent	Absent	Absent	Absent	Absent
Mesenterial Filaments	Microbasic *p*-mastigophore	14–28	Not provided	22.6–26.8 x 4.5–5.5	Partly 24–29.6 x 5.6–6.3 (… …, 21–25 x 4.2–5.6)	18.3–21 x 3.5–4.5	Not provided
	Basitrichs I	9–25 (common)	Not provided	15.5–18.3 x 2.2–2.5 38–48 x 5.6–7	Partly 33.8–43.7 x 5.6–7 (… …, 24–42.3 x 4.2–7)	29.6–38.1 x 4.2–5.6	Absent
	Basitrichs II	Absent	Not provided	Absent	Partly 19–25.4 x 2.5–2.8 (… …, 14–18.6 x 2–2.5)	12.7–14 x 1.5–2	Absent
	Microbasic *b*-mastigophore	23–52 (abundant)	Not provided	Absent	Absent	Absent	Absent

* (Lowest part of column), [+] Author not certain of cnidae identity, [#] Author did not provide capsule widths.

3.4.2 Defining Characters of *Oulactis* Species

External morphological characters of *Oulactis* species including; *O. orientalis*, *O. concinnata*, *O.* (*Tealidium*) *cinctum*, *O. coliumensis* and *O. magna* are listed in Table 3.4. The source of the species description has been listed in the Table and the terminology used by each author has been retained. It was found that external characters that primarily distinguish *Oulactis* species, excluding *O. muscosa* and *O. mcmurrichi*, were: the number, type, shape and length of tentacles, and the extent of verrucae coverage on the column.

Table 3.4 External morphology characteristics of *Oulactis* species.

Characteristic/Species	Oulactis orientalis	Oulactis concinnata	Oulactis (Tealidium) cinctum	Oulactis coliumensis	Oulactis magna
Author	(Averincev, 1967)	(Häussermann, 2003)	(Stuckey, 1909)	(Riemann-Zürneck & Gallardo, 1990)	(Carlgren, 1924)
Zooxanthellae present	Unknown	None present	Unknown	None present	Unknown
No. of tentacles	72	Up to 400 +	48	96	192
No. of tentacle whorls	4	3–4	4	4	4
Catch tentacles	Absent	Occasionally thread-like elongated fighting tentacles	Absent	Absent	Absent
Tentacle form	Tapered	Short, thick conical feeding tentacles with a small subterminal split	Unknown	Pointed	Short and conical
Tentacle length	Unknown	Unknown	16 mm	< 1 cm	Unknown
Tentacle relative to each other	Unknown	Unknown	Unknown	Similar length	Outer longer than inner tentacles
Fosse	Unknown	Not distinct	Unknown	Unknown	Unknown
No. of verrucae rows	24	96 + In longitudinal rows	Unknown	48	Unknown
Extent of verrucae coverage	Unknown	Verrucae cover entire column from limbus up	Upper column with verrucae and lower column smooth	Upper column	Upper two thirds of column
Acrorhagi	Present	Maybe present	Unknown	Maybe present	Unknown

Internal characteristics (Table 3.5) that vary between species include: the sphincter shape and position, number of mesentery pairs, cycles and mesentery completeness.

Table 3.5 Internal morphology of *Oulactis* species.

Characteristic/Species	O. orientalis	O. concinnata	O. (Tealidium) cinctum	O. coliumensis	O. magna
Author	(Averincev, 1967)	(Häussermann, 2003)	(Stuckey, 1909)	(Riemann-Zürneck & Gallardo, 1990)	(Carlgren, 1924)
Sphincter	Endodermal, circumscript and weak	Endodermal, diffuse and weak	Mesogloeal, diffuse	Endodermal, diffuse	Diffuse, weak and elongated
Number of mesenteries pairs	48	180–340 (Same number proximally and distally)	24	48	96 (More mesenteries in upper column than lower)
Number of mesentery cycles	4 cycles	6 – 7 cycles	Unknown	4 cycles	Unknown
Mesentery completeness	First and second cycles complete	Half or more perfect	All complete	First cycle only	First 4 cycles complete
Fertile	First 3 orders and some of the 4th order	Youngest mesenteries not fertile	Large masses of gonads between the mesenteries	First 3 cycles fertile	All mesenteries with ovaries
Parietobasilar muscles	Strong	Strong	Well developed	Weak	Strong

In addition to examining the variation of external and internal morphology between *Oulactis* species, the cnidom was also compared to determine which tissues' cnidom composition may be important in distinguishing the species of *Oulactis* in Australia. Parry's (1951) *O. magna* cnidom description has been used as it is more comprehensive than Carlgren's (1924) description, which was used in comparing morphological characteristics. *Oulactis* (*Tealidium*) *cinctum* was excluded as no published cnidom data for the species has been located. The composition of cnidae types (Table 3.6) in the tentacles (excluding the fighting tentacles, as they are present only in *O. concinnata*) and the marginal frill are consistent across *Oulactis* species. However, the cnidom of the column, acrorhagi, actinopharynx, and mesentery filaments varies considerably between species.

Table 3.6 Published distribution and cnidae size of *Oulactis* species. Capsule length x width in microns (µm) with outliers in brackets.

Tissue/Species	Cnidae Type	*O. orientalis*	*O. concinnata*	*O. coliumensis*	*O. magna*
Author		(Averincev, 1967)	(Häussermann, 2003)	(Riemann-Zürneck & Gallardo, 1990)	(Parry, 1951)
Fighting (catch) tentacle					
	microbasic *b*-mastigophore	Does not possess structure	22.5-31.5 x 3.2-5.0	Does not possess structure	Does not possess structure
Tentacle					
	Spirocyst	12–22 x 1.5-2	(14.4) 16.2-27 x 1.8-2.7	33 x 3-3.5	16-19 x 1.5-2.2
	Basitrichs	16-24 x 2-3	(15.3) 19.8-28.8 x 1.8-3.2	23-29 x 2-2.5	19-21 x 2.2-2.8
Column					
	Atrich	-	-	-	-
	Basitrichs I	25-29 x 3-3.5	(13.5) 15.3-21.6 (24.3) x 1.4-2.7	13-19 x 2-2.5	10.8-13.5 x 1.5
	Basitrichs II	14-24 x 2-2.5	-	-	-
	Holotrich	-	25.2-35.1 x 4.5-6.3	-	-
	Rod like Basitrichs	-	(22.5) 27-33.3 x 1.4-1.8	-	-
Acrorhagi					
	Spirocyst	16-24 x 2-2.5	-	23-25 x 1.5-2.5	-
	Atrich	51-63 x 3.5-4.5	-	-	40.5-54 x 5.4?
	Basitrichs	10-18 x 2	-	-	-
	Holotrich I	-	39.6-53.1 x 2.3-3.2	50-64 x 5-6.5	-
	Holotrich II	-	(36) 43.2-54.9 (60.3) x 4.5-8.1	32-43.5 x 3.5-4	-
Marginal ruff					
	Basitrichs	Not measured	11.7-15.3 (21.6) x 1.4-1.8	14.5-19 x 2-2.5	10.8 x 1.6-1.9
Actinopharynx					
	basitrichs	18-33 x 3	17.1-27 (30.6) x 1.8-3.2 (3.6)	26-33 x 3	27.5-32.4 x 3-3.5
	microbasic *b*-mastigophore	-	-	-	-
	microbasic *p*-mastigophore	25-27 x 2-2.5	-	-	24.4-26.8 x 6.3-7
	microbasic amastigophore A	-	21.7 x 4.1	-	-
Mesenterial Filaments					
	Basitrichs I	12-16 x 1.5-2	13.5-19.8 x 1.4-1.8 (2.3)	14.5-17.5 x 1.5-2.5	-
	Basitrichs II	29-35 x 2.5-3	-	-	-
	Basitrichs III	43-47 x 3.5-4.5	-	-	45-56.4 x 6.3-8.5
	Microbasic *b*-mastigophore	-	38.7-57.6 (62.1) x 5.4-9.9	35-46 (55) x 4.5-6.5	-
	Microbasic *p*-mastigophore	20-25 x 4	22.5-28.8 (34.2) x 2.7-4.1 (5.4)	17.5-20 x 3.5-4.5	18.3-24 x 3-3.5
	p-mastigophore? microbasic	-	-	20-25 x 3-4.5	-
	amastigophore A	-	18-22.5 x (3.6) 4.1-5.4	-	-
	Rod-like basitrichs	-	33-36 x 1.5-2	-	-
Pedal disc					
	Basitrichs	Not measured	(10.8) 15.3-21.6 x 2.3-2.7	17-20 x 2-2.5	Not measured
	Spirocyst		-	17-20 x 2-2.5	

3.4.3 Field Results

Nine distinct colour and pattern variations are grouped and documented (Table 3.7) for specimens observed *in-situ* and from photographs from QLD, NSW, VIC, WA, New Zealand. The colour and pattern description provided for Argentinean specimens (M. Zamponi, 2008, pers. comm.) has been included for comparison. The associated specimen numbers for each allocated grouping are given in Appendix III.

Table 3.7 Documented colour and patterns of *Oulactis mcmurrichi* and *Oulactis muscosa in-vivo* from Australia, New Zealand and Argentina.

Preliminary Identification	*Oulactis mcmurrichi*	*Oulactis muscosa*	*Oulactis* cf. *muscosa*	*Oulactis* cf. *muscosa*	*Oulactis* cf. *muscosa*	*Oulactis muscosa*	*Oulactis muscosa*	*Oulactis muscosa*	*Oulactis* sp.
Taxa Grouping	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Location collected	Mudarup, Cottesloe, WA	Flat Rock, Ballina NSW, Balmoral and Shellharbour, NSW	Balmoral, NSW	Port Phillip Bay, VIC	Port Phillip Bay, VIC	Seatoun, NZ	Seatoun, NZ	Mar del La Plata, Argentina	Unknown origin
Oral Disc	Rusty red/brown mouth. Oral disc brown/olive or dark brown and lightly striped towards the outer edge.	Bright green mouth surrounded by a dark purple oral disc, dark red purple, dark purple brown. May lack the bright green mouth and have uniformly coloured oral disc	Radiating reddish brown lines from the mouth graduating to prominent stripes on the outer edge oral disc.	Uniformly coloured mouth and oral, same as tentacle colour. Some animals may have may be heavily patterned on the oral disc.	Dark brown central oral disc, punctuated with light brown radiating stripes on outer disc. Inner mouth.	Unpatterned bright or dark red oral disc. Black concentric circle around base of tentacles	Orange mouth. Zigzag/radiating pattern on oral disc alternating in gold, light brown, dark brown and white.	Various colours, either ¼ to ½ of oral disc green or yellow and remaining oral disc white.	Green with radiating stripes of red, forming a solid block of red around the mouth.
Tentacle Colour	Translucent light to dark brown or olive green.	Translucent light grey. May be tinged green or brown by zooxanthellae in tentacles.	Translucent grey tentacles; fuchsia hue at base.	Various colours range from bright green to yellow, white, translucent white or brown.	Light khaki.	Translucent dark grey, outer whorl pale to vivid pink.	Inner tentacles translucent brown; vivid pink hue on outer whorl.	Tentacle colour is between white and yellow, occasionally grey.	Multicoloured tentacles; pink, orange and white.
Tentacle Patterning	Faint small white spots on inner side of tentacle or patterning absent.	White bars on inner side of tentacle on outer two whorls. Faint or not present on inner whorl. Bright pink patch or small stripe may be on base of tentacles.	White bars on inner side of tentacle on outer whorl. Patterning absent from inner whorl.	Small white spots and a faint vertical white stripe along length of inner side of all tentacles. Spots may also be absent.	White double or single small stripes on upper side of tentacle. Solid white blocking at the base of the tentacle.	Heavy white bands on inner side of all tentacles. Heavy white or reddish/white block at base of tentacles.	White bars and diamonds shapes with a dark grey stripe running along tentacle length. Faint patterning on the inner whorl of tentacles.	No patterning	White stripe running length of inner tentacles and outer whorl has scattered white spots.

Table 3.7 Continued.

Preliminary Identification	Oulactis mcmurrichi	Oulactis muscosa	Oulactis cf. muscosa	Oulactis cf. muscosa	Oulactis cf. muscosa	Oulactis muscosa	Oulactis muscosa	Oulactis muscosa	Oulactis sp.
Taxa Grouping	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Location collected	Mudarup, Cottesloe, WA	Flat Rock, Ballina NSW, Balmoral and Shellharbour, NSW	Balmoral, NSW	Port Phillip Bay, VIC	Port Phillip Bay, VIC	Seatoun, NZ	Seatoun, NZ	Mar del La Plata, Argentina	Unknown origin
Acrorhagi	Yellow to orange yellow	Light orange or white	Light Orange or white	White	Cream	White, stained pink.	Light grey	Unknown	White
Marginal Frill	Light brown to olive green	Light to dark grey or white.	Dark olive terminating in white tips.	Grey to dark grey, terminating frond maybe be white	White	White tips on end of frond with dark grey underneath frill	White	Unknown	Dark grey
Column	Olive or cream and olive striped	Cream, upper column shaded grey	Cream, upper column shaded grey	Light tan to cream, upper column dark to light grey	Cream	Dark red brown on upper column graduating to light brown and cream stripes upper mid column to the limbus column	Dark brown on upper two thirds of the column and light brown on lower .	Green, no indication of zooxanthellae	Light grey with dark grey verrucae
Verrucae	Very light brown	Olive green	Olive green	Olive green	Cream with centre olive green	Grey cream	Cream	Unknown	Dark Grey

Taxon group 2 displays the predominant oral disc colour/pattern (Figure 3.4 A) of *O. muscosa* and closely resembles that of Dana's (1849) illustrations. The geographic range of these colour morphs occurs from Mallacoota, far eastern Victoria, extending to Moreton Bay, Queensland (approximately 2000 km in distance). Specimens of *O. mcmurrichi* (Taxon group 1) from Western Australia (Figure 3.4 B) are uniformly coloured with tentacles and oral disc coloured the same. The tentacles are also patterned, although not as heavily as *O. muscosa* (Taxon group 2) and *O.* cf. *muscosa* (Taxon groups 3–7). These groups range in distribution from SE Queensland to Victoria and New Zealand.
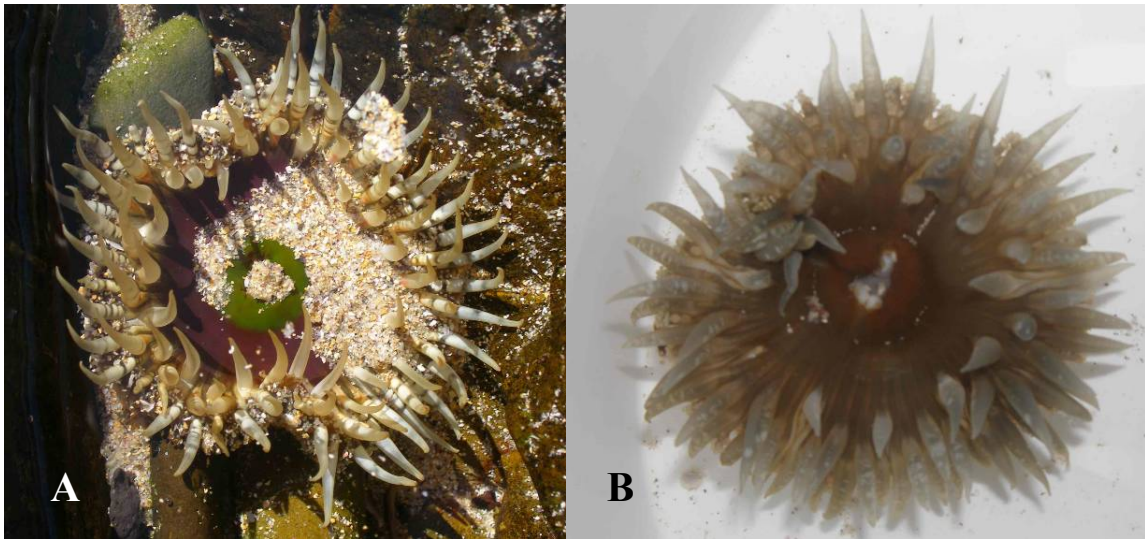
Figure 3.4 (A) *Oulactis muscosa* Shellharbour, New South Wales (photo: M. Mitchell). (B) *Oulactis mcmurrichi* Mudarup Rocks, Cottesloe, Western Australia (photo: M. Mitchell).

There was a second oral disc colour/pattern variation (Taxon group 3) found only at Balmoral, New South Wales, *Oulactis* cf. *muscosa* (Figure 3.5 A), which is considerably different to that of Dana's (1849) illustration. In Port Phillip Bay, Victoria, animals have been divided into two groups (Taxon groups 4 and 5). The commonly found colour morph (Taxon group 4) has numerous colour variations but a similar tentacle patterning (Figure 3.5 C & D), whereas the second colour morph (Figure 3.5 B) has a radiating patterning on the outer edge of the oral disc while the former colour morph may have an irregular patterning extending from the outer edge to the mouth.
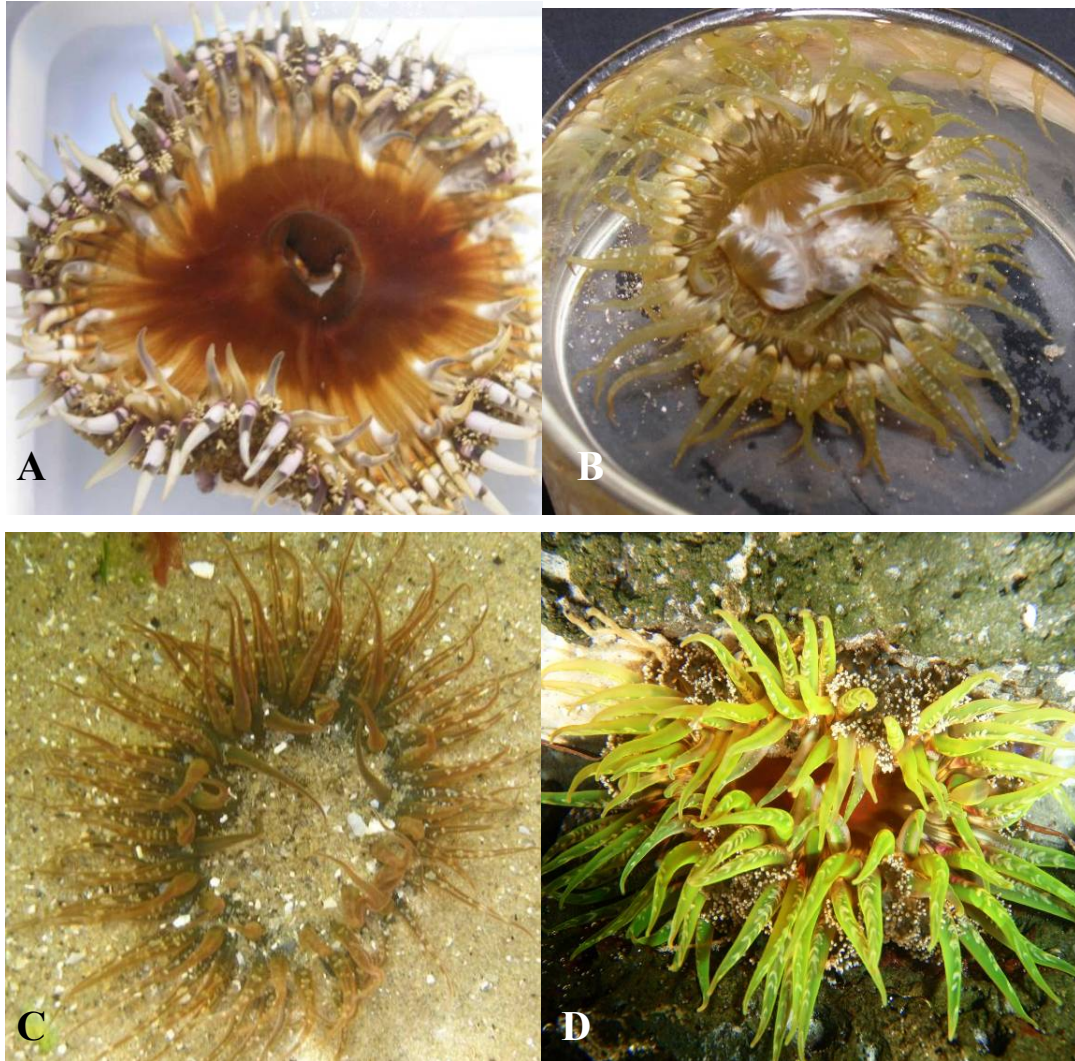
Figure 3.5 (A) *Oulactis* cf. *muscosa*, Wy-ar-gine Point, Balmoral, New South Wales (Taxon Group 3) (photo: M. Mitchell). (B) *O.* cf. *muscosa*, Safety Beach, Port Philip Bay, Victoria (Taxon Group 5) (photo: M. Mitchell). (C) Red colour of common form *O.* cf. *muscosa*, Port Melbourne, Port Philip Bay, Victoria (Taxon Group 4) (photo: M. Mitchell) (D) Green colour of common form *O.* cf. *muscosa*, Jaw Bone Marine Sanctuary, Williamstown, Port Philip Bay, Victoria (Taxon Group 4) (photo: A. Tew).

New Zealand specimens of *O. muscosa* have two distinct oral disc patterns in various colours and two distinct tentacle patterns (Figure 3.6 A and B). Both of the New Zealand patterns are quite different to those of Australian specimens.

Argentinean specimens have no tentacle patterning and a distinctive oral disc colouring; unfortunately, it was not possible to obtain a colour picture of specimens from Argentina.

Figure 3.6 (A) *O. muscosa* Seatoun, New Zealand (photo: J. Davy). (B) *O. muscosa* Seatoun, New Zealand (photo: J. Davy).

3.4.4 Laboratory Results

Limited data could be obtained from examination of *O. mcmurrichi* syntypes as the specimens are in very poor condition. Most of the ectoderm has disintegrated and all of the internal structures have collapsed.

External and internal characters (Table 3.8) from freshly collected and preserved material examined possessed verrucae, specimens were fertile (predominately male) and the directive mesentery pairs were sterile, and specimens had weak and diffuse sphincters (Figure 3.7 A and B). Cutress (1971) recorded Victorian specimens of *O. muscosa* as having diffuse to circumscribed sphincters. On re-examination of this material, the specimens with a circumscribed sphincter are actually *Aulactinia veratra*. Retractor muscles were predominately diffuse and parietobasilar muscles were diffuse and weak (Figure 3.8 A and B) with the exception of the New Zealand specimens. Retractor and parietobasilar muscles in New Zealand specimens were almost circumscribed in appearance.

The inner and outer tentacle cnidom was compared to determine if the patterning on the outer tentacles indicated specialist tentacles (such as catch tentacles). No difference was found in the cnidom (Table 3.9) between inner and outer tentacles on individual specimens. Cnidom (Table 3.9) variation was most pronounced in the actinopharynx and mesentery filaments. *Oulactis mcmurrichi* notably lacked *p*-mastigophores in the actinopharynx of the specimens examined.

Table 3.8 External and Internal characters of preserved *Oulactis* species examined from Australia and New Zealand.

Preliminary Identification	*Oulactis mcmurrichi*	*Oulactis muscosa*	*Oulactis* cf. muscosa	*Oulactis* cf. *muscosa*	*Oulactis* cf. *muscosa*	*Oulactis muscosa*	*Oulactis muscosa*	*Oulactis* sp.
Taxa Grouping	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 9
Location	Mudarup, Cottesloe, WA	Ballina NSW, to Shellharbour, NSW	Balmoral, NSW	Port Phillip Bay, VIC	Port Phillip Bay, VIC	Seatoun, NZ	Seatoun, NZ	Unknown origin
No of Tentacles	92-93	96-98	152-168	86	98	96	96-104	130
No of Tentacle Whorls	3	3	3	3	4	3	3	3
Tentacle form	Wide base and tapered point	Tapered	Tapered	Tapered	Wide base and tapering	Taper to a sharp point	Blunt	Tapered
Relative Tentacle length	Inner shorter than outer	Inner marginally shorter than outer	Inner shorter than outer	Inner marginally shorter than outer	Inner shorter than outer	Equal length	Equal length	Equal length
Fosse	Very shallow	Very shallow to none	Shallow	Indistinct	No	No	Indistinct	Deep
Verrucae coverage	Upper 2/3rds	Upper 2/3rds to a 1/2	Entire column to limbus	Upper 1/2	Upper 1/4	Entire column to limbus	Upper 2/3rds to 1/2	Entire column to limbus
Sphincter	Endodermal, diffuse and weak to strong	Endodermal, diffuse and weak	Endodermal, diffuse and weak	Endodermal, diffuse and weak	Endodermal, diffuse and weak	Endodermal, diffuse and strong	Endodermal, diffuse and weak	Endodermal, diffuse and weak
Number of Mesenteries Pairs	48	48-49	64-74	48	48	48	48	64
Mesentery arrangement	Hexamerous (6, 6, 12, 24)	Hexamerous (6, 6, 12, 24)	Octamerous (8, 8, 16, 32)	Hexamerous (6, 6, 12, 24)	Hexamerous (6, 6, 12, 24)	Hexamerous (6, 6, 12, 24)	Hexamerous (6, 6, 12, 24)	Octamerous (8, 8, 16, 32)
Cycles complete	1, 2, 3 and occasionally 4th	1, 2 and occasionally 3rd	1, 2 and occasionally 3rd	1, 2	1, 2 & some 3rd	1, 2	1, 2, 3	1, 2, 3
Parietobasilar muscles	Diffuse, weak	Diffuse, weak to strong	Diffuse, weak	Diffuse	Diffuse, strong, small	Circumscribed diffuse, strong	Diffuse, weak to strong	Diffuse, small
Retractor muscles	Diffuse to circumscribed, strong	Diffuse, strong, large	Diffuse, weak to strong,	Diffuse, long	Diffuse to circumscribed	Diffuse, strong, long	Diffuse, very strong	Diffuse, strong
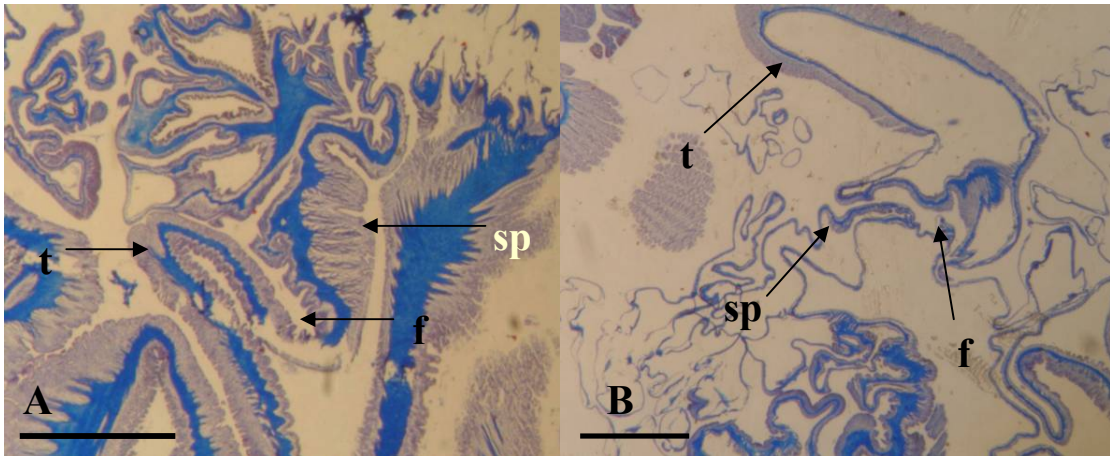
Figure 3.7 (A) Longitudinal section *Oulactis mcmurrichi* (WAM Z50032) Scale = 1 mm. (B) Transverse section of well expanded *O. muscosa* (AM G17740) Scale = 1 mm. Sphincter muscle (sp), tentacle (t) and fosse (f).
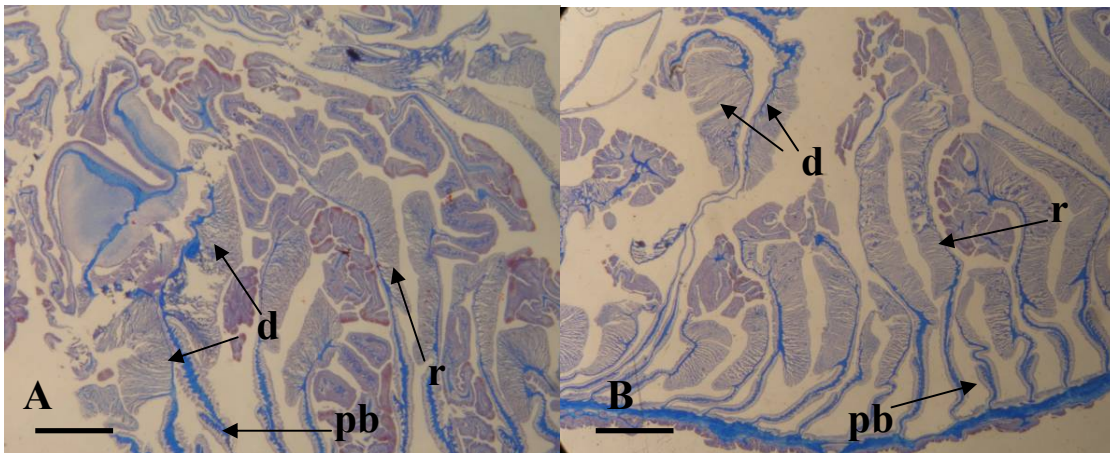
Figure 3.8 (A) Cross section of *Oulactis mcmurrichi* (WAM Z50032) Scale = 1 mm. (B) Cross Section of *O. muscosa* (AM G147740) Scale = 2 mm. Retractor (r), parietobasilar muscles (pb) and directives (d).

Table 3.9 Distribution and Size (in microns) of cnidae from *Oulactis* species, preserved and freshly collected, examined from Australia and New Zealand.

Preliminary Identification	*Oulactis mcmurrichi*	*Oulactis muscosa*	*Oulactis* cf. *muscosa*	*Oulactis* cf. *muscosa*	*Oulactis* cf. *muscosa*	*Oulactis muscosa*	*Oulactis muscosa*	*Oulactis* sp.
Taxa Grouping	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 9
Location	Bunbury, WA, Cottesloe, WA	Ballina to Shellharbour, NSW	Balmoral, NSW	Port Phillip Bay, VIC	Port Phillip Bay, VIC	Seatoun, NZ	Seatoun, NZ	Unknown origin
Tentacles								
Basitrichs I	(12) 16.7-24 2.4-3.2 n = 60, N = 4/4	18.6-28.8 (30.4) 1.6-3.2 n =70, N = 3/3	20-30.4 (32) 1.6-4 n = 40, N = 2/2	(15.2) 20-25.6 1.6-2.4 n = 25, N = 1/1	20-26.4 2.4-3.2 n = 20, N = 1/1	(16.7) 21.6-25.5 2-2.9 n = 20, N = 1/1	20-26.4 2.4-3.2 n = 40, N = 2/2	19.6-25.5 (27.4) 2-3.9 n = 10, N = 1/1
Basitrichs II	Absent	(16.8) 20-20.8 1.6-2.4 n = 10, N = 1/3	9.6-12 1.6-2.4 n = 10, N = 1/2	Absent	Absent	Absent	Absent	Absent
Spirocysts	7.8-30.4 1.5-3.2 n = 42, N = 4/4	(14.4) 15.7-29.6 1.6-3.2 n = 60, N = 3/3	(17.6) 22.4-28.8 1.6-3.2 n = 40, N = 2/2	12.8-28 1.6-2.4 n = 20, N= 1/1	(16) 19.2-30.4 2.4-3.2 n = 30, N = 1/1	(19.6) 22.5-27.4 2-2.9 n = 20, N = 1/1	17.6-28 2.4-3.2 n = 40, N = 2/2	19.6-25.5 2-2.9 n = 10, N = 1/1
Marginal Ruff								
Basitrichs	9.8-20.8 1.6-3.2 n = 30, N =3/3	8.8-19.5 0.9-2.4 n = 30, N = 3/3	8.8-16 1.6-2.4 n = 20, N = 2/2	8.8-13.6 1.6-1.6 n = 12, N = 1/1	14.4-18.4 2.4-2.4 n = 10, N = 1/1	11.8-15.7 2-2 n = 10, N = 1/1	10.4-16.8 1.6-3.2 n = 20, N = 2/2	9.8-12.7 (14.7) n = 10, N = 1/1
Basitrichs II	Absent	Absent	Absent	Absent	Absent	19.6-26.5 2-2.9 n = 10, N = 1/1	Absent	Absent
Spirocysts	Absent	Absent	Absent	Absent	Absent	17.6-27.4 2-2.9 n = 10, N = 1/1	Absent	Absent
Holotrichs	Absent	Absent	Absent	Absent	Absent	(42.1) 47-52.9 (55.9) 3.9-5.9 n = 10, N = 1/1	Absent	Absent
Column								
Basitrichs	11.8-24 (25.6) 1.6-4 n = 30, N = 3/3	(11.8) 14.4-20.6 2.9-4.9 n = 40, N = 3/3	(12.8) 17.6-22.4 1.6-3.2 n = 20, N = 2/2	9.6-18.4 1.6-2.4 n = 13, N = 1/1	12.8-18.4 2.4-2.4 n = 10, N = 1/1	14.7-20.6 2-2.9 n = 10, N = 1/1	12-17.6 1.6-2.4 n = 20, N = 2/2	15.7-25.5 2-3.9 n = 10, N = 1/1
Basitrichs II	20.8-24 3.2-4 n = 10, N = 1/3	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Acrorhagi								
Basitrichs	(12.8) 15.6-21.6 1.6-3.2 n = 20, N = 2/3	Absent	Absent	Absent	Absent	10.4-16.7 1.6-2.4 n = 5, N = 1/2	8-9.6 (13.6) 1.6-2.4 n = 5, N = 1/2	Absent
Spirocysts	14.7-29.6 2-3.9 n = 30, N = 3/3	(12) 14.4-20.6 (21.6) 1.6-3.2 n = 30, N = 3/3	(16.8) 21.6-25.6 1.6-3.2 n = 20, N = 2/2	24-25.6 2.4-3.2 n = 3, N = 1/1	33.6-47.2 4.8-5.6 n = 10, N = 1/1	15.7-26.4 2.4-3.2 n = 5, N = 1/1	24-30.4 2.4-4 n = 20, N = 2/2	(13.7) 17.6-25.5 2.9-2.9 n = 10, N = 1/1
Holotrichs I	33.6-45.6 3.9-6.4 n = 35, N = 3/3	(29.6) 32.3-47 (62.7) 2-6.9 n = 40, N = 3/3	(36) 40-53.6 (65.6) 32.8-8 n = 20, N = 2/2	(34.4) 39.2-44.8 4.8-6.4 n = 10, N = 1/1	33.6-47.2 4.8-5.6 n = 10, N = 1/1	(39.2) 46-54.4 (64) 4-5.9 n = 12, N = 1/1	(40) 41.6-59.2 (66.4) 4-7.2 n = 43, N = 2/2	(42.1) 51-53.9 (68.6) 2.9-6.9 n = 10, N = 1/1
Holotrichs II	Absent	44-60 (64) 5.6-8 n = 20, N = 2/3	Absent	Absent	Absent	Absent	Absent	Absent

Note: dimensions of cnidae are length x width (μm), n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae, Outliers in brackets. * excludes type specimens

Table 3.9 Continued.

Tissue /Specimens	*Oulactis mcmurrichi*	*Oulactis muscosa*	*Oulactis* cf. muscosa	*Oulactis* cf. *muscosa*	*Oulactis* cf. *muscosa*	*Oulactis muscosa*	*Oulactis muscosa*	*Oulactis* sp.
Taxa Grouping	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 9
Location	Bunbury, WA, Cottesloe, WA	Ballina NSW, to Shellharbour, NSW	Balmoral, NSW	Port Phillip Bay, VIC	Port Phillip Bay, VIC	Seatoun, NZ	Seatoun, NZ	Unknown origin
Actinopharynx								
Basitrichs I	(20) 24-28 2.9-4 n = 30, N = 3/3	(24.8) 25.5-36 3.2-4 n = 30, N = 3/3	22.4-30.4 3.2-4.8 n = 23, N = 2/2	5.6-14.4 (24) 1.6-3.2 n = 20, N = 1/1	(22.5) 28-32 2.9-3.2 n = 10, N = 1/1	29.4-34.3 2.9-3.9 n = 10, N = 1/1	23.2-33.6 2.4-5 n = 30, N = 2/2	(12.7) 25.5-29.4 2.9-3.9 n = 10, N = 1/1
Basitrichs II	(10.8) 15.7-17.6 2-2.9 n = 10, N = 1/3	12-17.6 1.6-2.4 n = 10, N = 1/3	Absent	Absent	11.2-14.4 1.6-2.4 n = 10, N = 1/1	Absent	Absent	Absent
p-Mastigophores	Absent	(19.2) 20.6-28 4-5.9 n = 30, N = 3/3	19.2-24 4.8-6.4 n = 20, N = 2/2	Absent	22.4-27.4 4.8-5.6 n = 10, N = 1/1	22.5-28.4 (2.9) 6.7-8.8 n = 10, N = 1/1	22.4-29.6 4.8-6.4 n = 20, N = 2/2	17.6-24.5 4.9-5.9 n = 10, N = 1/1
Spirocysts	Absent	Absent	Absent	Absent	Absent	21.6-27.4 2-2.9 n = 10, N = 1/1	Absent	Absent
Mesenterial filaments								
b-Mastigophores	(30.4) 34.4-41.6 5.6-8.8 n = 15, N = 2/2*	(32) 34.4-39.2 (41.6) 5.6-6.4 n = 19, N = 2/4	(21.6) 33.6-48 (4) 7.2-10.4 n = 20, N = 2/2	(16.8) 21.6-24.5 (45.1) 3.2-4 (5.9) n = 10, N = 1/1	(20.8) 29.6-36.8 4.8-6.4 n = 10, N = 1/1	(30.4) 39.2-43.1 (47) 3.9-5.9 n = 10, N = 1/1	(28) 36-44 (48) 4-6.4 n = 28, N = 2/2	20.6-24.5 3.9-5.9 n = 10, N = 1/1
b-Mastigophores II	Absent	16-22.4 2.4-4 n = 20, N = 1/4	Absent	Absent	Absent	16.7-20.6 2-3.9 n = 10, N = 1/1	17.6-19.2 2.4-3.2 n = 6, N = 1/2	Absent
b-Mastigophores III	Absent	50.4-57.6 5.6-8.8 n = 10, N = 1/4	Absent	Absent	Absent	Absent	Absent	Absent
Basitrichs	12.8-16.8 1.6-2.4 n = 20, N = 2/2*	12-20.8 2.4-2.9 n = 31, N = 3/3	(10.4) 12.8-21.6 (26.4) 1.6-2.4 n = 30, N = 2/2	(8.8) 19.2-22.4 2.4-3.2 n = 11, N = 1/1	9.6-12 (14.7) 1.6-2.4 n = 10, N = 1/1	(11.8) 18.6-25.5 2-2.9 n = 10, N = 1/1	11.2-13.6 (16.8) 1.6-2.4 n = 20, n = 2/2	12.7-21.6 2-2.9 n = 10, N = 1/1
Basitrichs II	Absent	Absent	5.6-8 0.8-1.6 10, 1/2	Absent	Absent	Absent	Absent	Absent
p-Mastigophores	24-28.8 4.8-6.4 (10.4) n = 20, N = 2/2*	20.6-28 4.8-6.4 n = 30, N = 3/3	19.2-24 (26.4) 4.5-5.6 n = 20, N = 2/2	19.2-22.4 2.4-3.2 n = 8, N = 1/1	24-27.2 4-5.6 n = 10, N = 1/1	22.5-26.5 4.9-7.8 n = 10, N = 1/1	(18.4) 20.8-24.8 4-6.4 n = 20, N = 2/2	(24.5) 42.4-47 (3.9) 5.9-8.8 n = 10, N = 1/1

Note: dimensions of cnidae are length x width (μm), n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae, Outliers in brackets. * excludes type specimens

Some loan material examined had similar morphology to *O. muscosa* and *O. mcmurrichi*, however there were significant character differences. The Tasmanian specimen of *O. muscosa* examined lacked acrorhagi, had 52 mesentery pairs and the actinopharynx lacked *p*-mastigophores. On this basis it was unclear if this specimen was *O. muscosa*, and this issue requires further investigation. Another specimen was from Barrow Island, Western Australia, which has a circumscribed sphincter yet retains *Oulactis* characteristics such as a frill, acrorhagi and verrucae. This specimen had no life appearance notes associated with it, and final identification is still pending for this specimen.

In addition, some loan material examined was previously incorrectly identified as *Oulactis* sp. or *O. muscosa*. Correct identifications include *Heteranthus verruculatus* and *Aulactinia veratra*. Appendix III lists all loaned specimens examined with the original and revised identifications.

3.4.5 Statistical Analysis Results

To determine the similarity of species within the genus *Oulactis* and the variation of specimens examined from Australia and New Zealand, a matrix of morphological characters was collated. Two sets of data were used in the statistical analysis: one containing the preserved specimen data of freshly collected specimens, and the second using the preserved specimens' data in conjunction with their *in-vivo* data: tentacle and oral disc pattern determined from taxa groupings.

Based upon their morphological differences, several species and specimens were expected to group separately from potential *O. muscosa* and *O. mcmurrichi* including; *O. orientalis*, *O. magna*, *Oulactis* cf. *muscosa* collected from Balmoral (Taxon group 3) and the ship's ballast specimen *Oulactis* sp. (Taxon group 9). Morphology of *Oulactis concinnata* is the least similar to other *Oulactis* species and was expected to be substantially different. The number of tentacles, length of tentacles, extent of verrucae

coverage and the cnidom of the actinopharynx, acrorhagi and mesenterial filaments were expected to be important in the separation of specimens.

Weighting variables (Table 3.10), based on the results of section 3.3.2, were assigned to morphological characters considered to be important in distinguishing Actiniaria and *Oulactis* species. Characters that clearly define *Oulactis* species were weighted 4 (most important) and for characters that showed little or no variation were weighted 1 (least important). Factor groupings were based on the taxa groupings according to similarity of tentacle and oral disc patterning and preliminary identifications assigned to freshly collected specimens (see section 3.4.3). Due to the lack of morphological characteristics available for *O. muscosa* from Argentina (Taxon Group 8) they were omitted from the statistical analysis.

Table 3.10 Assigned Weighting for *Oulactis* Morphological Characters.

Characters	Weighting
Number of tentacles, sphincter shape, inner and outer tentacle length, verrucae coverage, number of mesentery pairs, hexamerous arrangement of mesenteries, oral disc patterning, tentacle patterning.	4 = Most Important
Acrorhagi present, number of complete mesenteries, directives fertile, acrorhagi contains basitrichs.	3
Mesenterial filaments containing *p*-mastigophores, amastigophore-A's or a second or third size of basitrichs. Actinopharynx containing *b*- or *p*-mastigophores, amastigophore-A or a second size of basitrich.	2
Number of siphonoglyphs, fighting tentacles present, oral disc and pedal disc width, directives attached to siphonoglyph, number of tentacle whorls, inner and outer tentacles containing a second size of basitrich or spirocyst, acrorhagi containing two sizes of holotrichs and spirocysts, column containing holotrichs or a second size of basitrichs.	1 = Least Important

Data incorporating all preserved specimens examined, without *in-vivo* data, were
analysed using PCA. *Oulactis concinnata* returned a PCA score of 47.3, which was more
than twice the score of the next closest species *Oulactis magna* (17.7), and was therefore
removed from the analysis as it was too dissimilar from the other specimens to provide a
meaningful separation of the remaining specimens.

The PCA graph (Figure 3.9) generated for preserved *Oulactis* specimens without *in-vivo*
data distinguished several outlying groups. Particularly *O. magna*, *O. orientalis*, Taxon
groups 5 (Victoria), 3 (Balmoral, NSW) and 9 (Ship's ballast specimen). The PCA did
not clearly separate the remaining specimens (Taxon groups 1, 2, 4, 6 and 7) as either *O.
muscosa* or *O. mcmurrichi.* The species *O. coliumensis* appears to be the most similar to
O. muscosa and *O. mcmurrichi*.
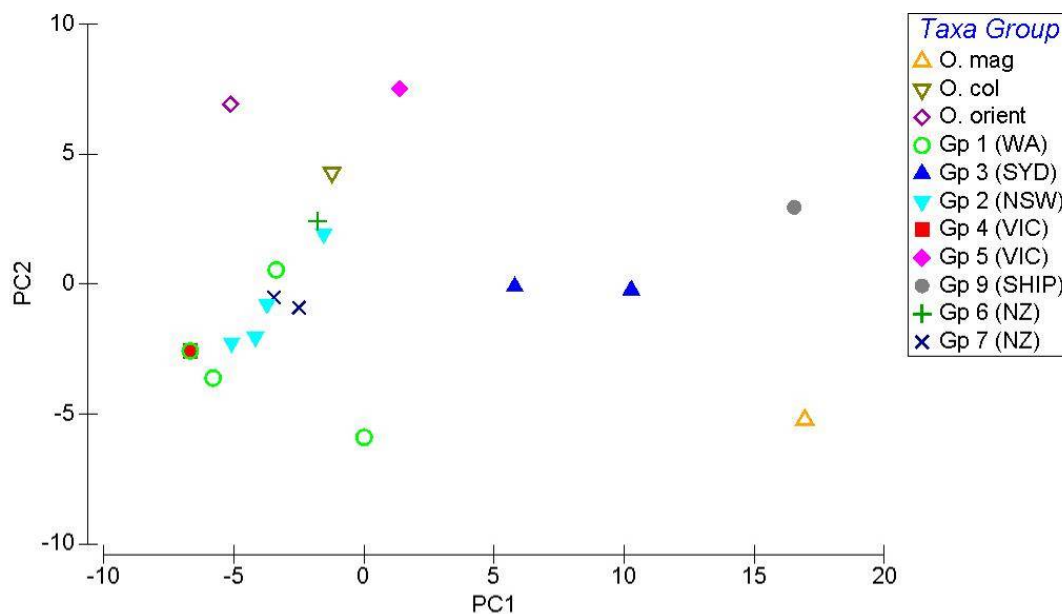
Figure 3.9 Graphic representation of Principal Component Analysis of preserved *Oulactis*
specimens without *in-vivo* data.

The PCA graph (Figure 3.10) generated for *Oulactis* specimens incorporating *in-vivo*
characteristics showed a clear grouping of most *O. muscosa* and *O. mcmurrichi*
specimens. Outlier Taxa groups which remained the same, with or without *in-vivo* data,

include; *O. magna*, *O. orientalis*, Taxon groups 5 (Victoria), 3 (Balmoral, NSW) and 9 (Ship's ballast specimen). *Oulactis coliumensis* also became a distinct outlier. Taxon groups 6 and 7 from New Zealand group together although were also grouped closely with *O. muscosa* (Taxon group 2).
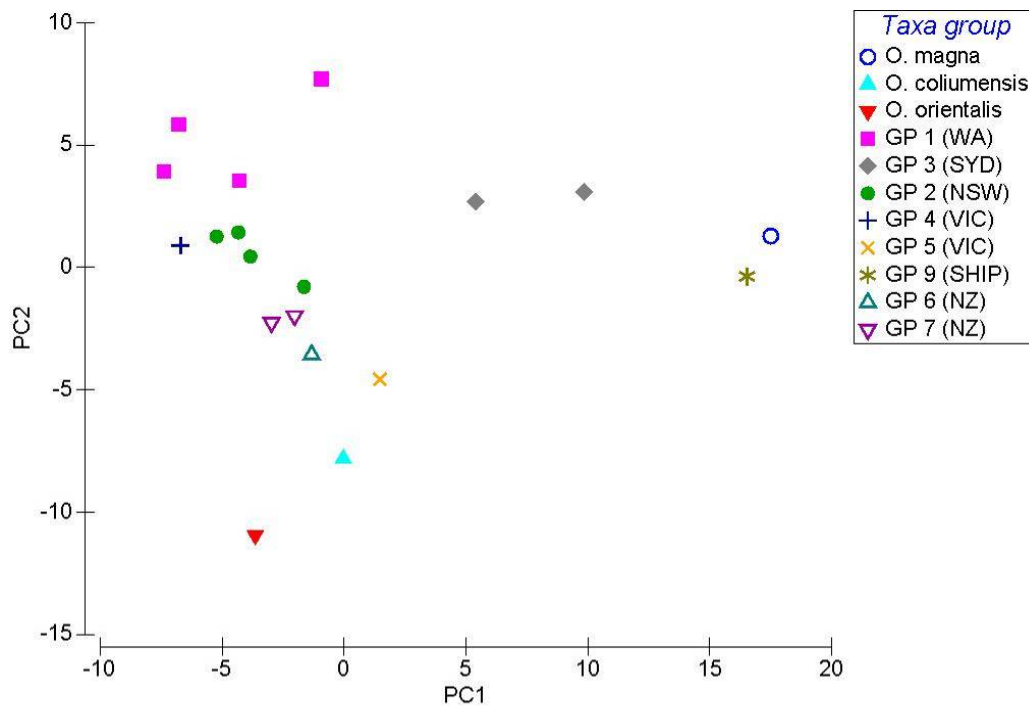
Figure 3.10 Graphic representation of Principal Component Analysis of preserved *Oulactis* specimens with *in-vivo* data.

Eigenvalues without *in-vivo* data (Table 3.11) and Eigenvalues incorporating *in-vivo* data (Table 3.12) have been limited to the six most important morphological characters that distinguish the specimens/species in the two sets of data. These morphological characters with high positive and negative eigenvalues were: the number of tentacles, the number of mesenteries (which are directly proportional to the number of tentacles), tentacle length and verrucae coverage. The addition of the *in-vivo* data changed the six most important morphological characters to include tentacle and oral disc patterning as important in distinguishing the groups of specimens into species.

Table 3.11 Results of Principal Components Analysis, including eigenvalues, eigenvectors and proportion contributing to variance for data with *in-vivo Oulactis* characteristics.

Component	1	2	3
Eigenvalues	52.4	13.3	4.79
Component Character Variable			
No. of tentacles	0.722	-0.382	0.091
Inner tentacle length (cm)	0.309	0.503	0.012
Outer tentacle length (cm)	0.380	0.666	-0.082
Verrucae coverage	0.054	-.0600	0.389
No. of mesentery pairs	0.462	-0.249	-0.154
Hexamerous arrangement	-0.104	0.035	-0.150
% Variation	62.6	15.9	5.7
Cum. % Variation	62.6	78.5	84.2

Table 3.12 Results of Principal Components Analysis, including eigenvalues, eigenvectors and proportion contributing to variance for data with *in-vivo Oulactis* characteristics.

Component	1	2	3
Eigenvalues	53.5	20.5	8.25
Component Character Variable			
Oral disc patterning	0.125	-0.403	0.351
Tentacle pattern grouping	0.111	-0.608	0.455
No. of tentacles	0.702	0.344	0.225
Inner tentacle length (cm)	0.318	-0.292	-0.393
Outer tentacle length (cm)	0.390	-0.371	-0.53
No. of mesentery pairs	0.450	0.208	0.206
% Variation	53.6	20.5	8.3
Cum. % Variation	53.6	74.2	82.4

3.5 *Discussion*

Oulactis muscosa (Dana in Drayton, 1846) and *O. mcmurrichi* (Lager, 1911) are the only two species of *Oulactis* that occur in Australia (Fautin, 2009). *Oulactis muscosa* was originally described in 1846 from specimens located in Wollongong, Illawarra, New South Wales (Dana, 1846) and is the type species of the genus. *Oulactis mcmurrichi* was described 65 years later from specimens collected from Bunbury and Albany, Western Australia (Lager, 1911).

Due to their similarity in appearance both Davey (1998) and Edgar (2001) question their taxonomic validity, and suggest that these may constitute a single species. Due to the lack of detailed descriptions for either species, identification has previously been based upon specimen locality i.e. designated as *O. mcmurrichi* if it occurs on the west coast of Australia, and *O. muscosa* on the east.

As published descriptions of both species lack detail, descriptions of other *Oulactis* species were tabulated to provide additional data in determining characters that may be significant in defining the two Australian species. In addition, detailed examination of the *in-vivo* characteristics of specimens has provided a set of characteristics which may define the two species. Matrices of character states sorted into taxon groupings were used to generate a Principal Component Analysis to provide an illustration of specimens examined in "species" groups and determine the level of importance of tentacle and oral disc patterning in defining both species.

As a result of this analysis several clear patterns emerged. Regional variation in appearance of *Oulactis* species from Australia and New Zealand is far more diverse than initially thought. Based upon the tentacle and oral disc patterns recorded, specimens deemed to be *Oulactis* or closely resembling *Oulactis* were ascribed to nine distinct groups. Specimens assigned as *O. muscosa* and *O. mcmurrichi* formed two discrete groups. Discernible differences in the patterning of the tentacles and oral disc, the number of tentacles and the cnidae composition of the actinopharynx supports the separation of the two species. In addition, these differences are consistent with characters currently used to distinguish other species within the genus *Oulactis*.

Oulactis muscosa can primarily be identified by the broad bar patterning on the tentacles and the plain coloured oral disc (with or without a bright, moss green coloured mouth). The tentacles of *O. mcmurrichi* are far lighter in patterning and more like spots than bars, or the patterning may even be absent. Characters possessed by both species and that are consistent with the genus diagnosis include; presence of acrorhagi, a marginal frill, a weak and diffuse endodermal sphincter and hexamerous arrangement of mesentery pairs.

Preliminary species descriptions for *O. muscosa* and *O. mcmurrichi* are as follows:

Oulactis muscosa

Distinct white or grey bars on translucent tentacles, oral disc lacks patterning and is dark purple with a bright green mouth, colouring around mouth may be absent. Tentacles number 96–98, arranged in three whorls. Mesentery pairs hexamerously arranged, normally numbering 48. Cnidom includes: basitrichs, holotrichs, spirocysts, *b*-mastigophores, *p*-mastigophores. Acroraghi contain holotrichs and spirocysts.

Oulactis mcmurrichi

Tentacles may be lightly patterned with white spots or patterning is absent, oral disc is unpatterned. Tentacles and oral disc uniformly coloured generally brown, but may have a rust coloured mouth. Tentacles number 80–93 in three whorls. Hexamerous arrangement of mesentery pairs, numbering 48. Cnidom: basitrichs, holotrichs, spirocysts, *b*-mastigophores and p-mastigophores: notably the actinopharynx lacks *p*-mastigophores. Acroraghi contain holotrichs, spirocysts and may have basitrichs.

A formal redescription of *O. mcmurrichi* is pending the collection of new specimens from the syntype locality regions of Bunbury and Albany, Western Australia. *Oulactis mcmurrichi* type specimens are in very poor condition, therefore representative specimens from the type locality need to be included in any redescription, and the morphological and *in-vivo* variability need to be documented. Additionally, the Western Australian coastline surrounding Albany has a rich and unique biodiversity because of environmental influences, and as such the marine fauna in the region has a high endemism (Alderslade, 2003). It is therefore important to include species variability from the Albany area in the redescription of

O. mcmurrichi, to ensure that a comprehensive range of regional characters are documented. Due to time and funding restrictions only specimens from Perth, the northern distribution limit of *O. mcmurrichi*, were examined in this study.

For the first time, *b*-mastigophore nematocysts are reported to occur in Australia and New Zealand specimens of *Oulactis*. Further work needs to be undertaken on the classification of these *b*- and *p*-mastigophores, as macro- or microbasic, in Australian and New Zealand material. Attempts to make the nematocysts fire to observe the shaft and thread from live material from Australia were unsuccessful. Only preserved New Zealand material was examined, and once material is preserved the nematocysts no longer have the ability to fire, therefore prohibiting detailed classification of cnidae.

In addition, Australian and New Zealand specimens examined have a dark grey colouring on the upper column and/or marginal frill indicating the presence of zooxanthellae. Preliminary genetic analysis of *O. muscosa* confirms the presence of zooxanthellae (*Symbiodinium* sp.) in Australian specimens (Worthington Wilmer and Mitchell, unpubl. data). Häussermann's (2003) updated *Oulactis* genus description states that there are no zooxanthellae present in the genus, therefore the diagnosis needs to be amended to include these new findings.

New Zealand specimens were separated into two groups, one displaying a complex radiating pattern and the other a zigzag pattern on the oral disc, a feature which is lacking in *O. muscosa*. Preliminary taxonomic data indicate that there are differences between the cnidom and verrucae coverage of *Oulactis* specimens examined from New Zealand. Whether these differences reflect natural variability among New Zealand specimens is yet to be resolved. It is worth noting here that Parry (1951) listed microbasic *p*-mastigophores as being present in *O. muscosa* tentacles from New Zealand, whereas none were present on specimens examined in this study from New Zealand and Australia nor in other studies of *O. muscosa* (Carlgren, 1950a; Carlgren, 1954).

Some other issues that need to be resolved, but which are beyond the scope of this study, include; additional analysis of *Oulactis* groups that display significant morphological variation that are potentially new species from New South Wales, Port Phillip Bay, Victoria, New Zealand and Argentina. Following the preliminary redescription of *O. muscosa*, the placement of *O. concinnata* and *O. (Tealidium) cinctum* in the genus should be reviewed.

This is because their morphological characteristics including sphincter shape, verrucae coverage and the presence of catch tentacles are inconsistent with other species in the genus.

In addition, the relationship of *O. orientalis* with other *Oulactis* species is complex because it has a circumscribed sphincter. In a recent publication in Russian, Sanamyan & Sanamyan (2008) have returned *O. orientalis* to the genus *Anthopleura* where it was originally assigned. Their findings are in agreement with the observations from this study.

In summary, the analysis from this study indicate that there are sufficient morphological differences in conjunction with *in-vivo* characteristics to retain *O. muscosa* and *O. mcmurrichi* as separate valid species. Future research on these species needs to include genetic analysis. Samples collected during this study are preserved in 100% ethanol for this purpose and future genetic analysis will further add information to better define the taxonomic status of these species. Specimens of *O. muscosa* need to be collected and *in-vivo* and morphological variation documented for Tasmania and South Australia, and a redescription of *O. mcmurrichi* is required once specimens from Bunbury and Albany, Western Australia and South Australia are collected and analysed.

Chapter 4 A Preliminary Investigation of the Utility of Ribosomal Genes for Species Identification of Sea Anemones (Actiniaria)

Accepted by the journal *Memoirs of the Queensland Museum*: *Nature* and published in December 2008; the published paper reprint is provided in Appendix V.

<u>Declaration of authorship:</u>

This research was initiated by M Mitchell, and contributed significantly to the other aspects of the study, in partial fulfilment for Master of Science (by Research). J Worthington Wilmer (Queensland Museum) undertook genetic analysis components and initiated the writing of the paper. P Davie (Queensland Museum), D Fautin (University of Kansas) and an anonymous reviewer reviewed the paper prior to publication. The relative contributions of the two authors to the manuscript are indicated below.

Conception of the study:	MM (100%)
Design of the Study:	MM (30%), JWW (70%)
Collection of Data:	MM (50%), JWW (50%)
Analysis of the data:	MM (20%), JWW (80%)
Conclusions:	MM (30%), JWW (70%)
Writing up of the Paper:	MM (50%), JWW (50%)

4.1 Introduction

In February 2005, the Australian Marine Sciences Association, SEQ Branch, hosted The Thirteenth International Marine Biological Workshop, The Marine Fauna and Flora of Moreton Bay, Queensland. Fieldwork was conducted over a period of three weeks and occurred in a variety of environments including off-shore reefs, small islands accessible only at low tide, piers, estuarine mouths and mud flats. One of the studies undertaken

during the workshop was research on the Actiniaria of the Moreton Bay region (Fautin et al., 2008). A taxonomic paper documenting the Actiniaria species found in the Moreton Bay region is presented by Fautin et al. (2008). Of the more than 20 species that are now known from Moreton Bay, we obtained tissue from the following six species of sea anemones to assess the usefulness of DNA in identification, and the potential for understanding phylogenetic relationships: *Heteractis malu*, ?*Anemonia* sp., *Stichodactyla haddoni*, *Entacmaea quadricolor*, *Macrodactyla doreensis* and *Oulactis muscosa*.

Species identification of sea anemones (Anthozoa: Actiniaria) can be difficult, especially in the field. The taxonomic key currently utilised, designed by Carlgren (1949), is based mainly on histological differences and therefore requires collection of whole animals, which may not always be practical. Furthermore, histological analysis of sea anemones is time consuming and requires considerable expertise as some closely related species are almost impossible for the non specialist to identify, often resulting in incorrect taxonomic assignment (Stephenson, 1928; Fautin, 2000; Häussermann, 2004).

Identification is further complicated by the fact that some species are virtually identical in appearance, distinguished by only one or two morphological features. For example, the two species *Heteractis malu* and *Heteractis crispa* are differentiated in the field on the basis of 1) column texture, which is firmer (leathery) in *H. crispa* than in *H. malu,* and 2) tentacle length, which is meant to be twice as long in *H. crispa* than in *H. malu* (Fautin & Allen, 1997). Both these characteristics can be misleading since the former is open to subjective interpretation if both species are not present side by side in the field, while the latter may not necessarily be useful as the tentacles can be contracted at the time of collection/observation.

Alternatively, delineation of some species may be quickly achieved using appropriate molecular genetic methods (e.g. the Barcoding of Life initiative Hebert et al., 2003). DNA barcoding is increasingly being viewed as a valuable tool in aiding the taxonomic workflow by identifying species requiring further thorough taxonomic analysis (Hajibabaei et al., 2007). However, previous genetic studies including sea anemone taxa

have either only focused on questions pertaining to higher order anthozoan relationships (Won et al., 2001; Daly et al., 2003; Daly et al., 2008) or intraspecific population structure (Hunt & Ayre, 1989; Chomsky et al., 2009). Numerous mitochondrial DNA genes such as COI (Fautin & Smith, 1997), COIII and 16S rDNA (Geller & Walton, 2001) have been used to infer phylogenetic relationships among the Actiniaria. However, mitochondrial gene sequence divergences within and among anthozoan families, including sea anemones, has been found to be significantly lower than for other marine invertebrate species (Shearer et al., 2002). Barcoding studies also revealed that mitochondrial DNA evolved too slowly in sea anemones and other cnidarians for mitochondrial DNA differences to be an informative indicator of species (Hebert et al., 2003). Interestingly, Shearer et al. (2002) found that, unlike other metazoan taxa studied, substitution rates in anthozoan nuclear genes are much higher than in mitochondrial genes and therefore may be of greater utility in terms of species identification. Indeed, a number of other studies have suggested that the nuclear ribosomal (rDNA) gene complex incorporating 18S, ITS1, 5.8S, ITS2 and 28S could be ideally suited to examining relationships below genus level within the Actiniidae (McCommas, 1991; Odorico & Miller, 1997; Daly, 2002). Recently Acuña et al. (2007a) used the ITS region of rDNA in addition to morphology to distinguish between three species within the Actiniaria genus *Aulactinia*.

Molecular studies of sea anemones can be potentially complicated by the presence of symbiotic algae or zooxanthellae in the anemone tissue (Shearer et al., 2005) and possibly tissue consistency (Pinto et al., 2000). For sea anemone species that possess zooxanthellae, these are generally found in the tentacles, oral disc or upper column, although in some species they can be distributed heterogeneously throughout their hosts, being rare in only the pedal disc region or mesenteric tissue layers (Fautin & Smith, 1997; Häussermann, 2004). Therefore DNA extractions can contain both the host and algal genomes, which may cause confounding results especially for sequence data generated using broadly conserved or "universal" primers (see Shearer et al., 2005). A study by Pinto et al. (2000) found that tissue consistency impinged on the success of extraction of DNA from sea anemones, due to the hardness of tissue that had

been preserved in ethanol. They concluded that a slow and gradual digestion method was optimal for extraction.

This is a preliminary study designed to examine the utility of the rDNA gene complex in the identification of sea anemone species, and to test whether a known universal primer pair is sufficient for such studies or whether anemone specific primers will be required. Furthermore, we use modern DNA extraction techniques, such as extraction kits, to determine if previous problems associated with sea anemone DNA extraction can be circumvented.

4.2 Material and Methods

4.2.1 Specimen and Tissue Collection

Collection techniques included; removing anemones from rocks by chisel and hammer, scraping animals off rocks, or taking a small tissue sample from the animal in the wild for genetic analysis if identification was 100% positive in the field. Tissues for analysis were collected from twelve species (based solely on field identifications). Of these, six samples representing an initial five species were used in the genetic analysis (Table 4.1). Additional samples of *Heteractis malu* were collected from Shag Rock subsequent to the Moreton Bay workshop.

In order to examine and minimise possible zooxanthellae contamination, small tissue samples of less than 5 mm in length were excised from either the lower column/pedal disc or, where possible, separate tissue samples from both the tentacles and pedal disc region of each species were taken. Where whole specimens were collected, tissue samples were taken after animals were relaxed in an isotonic magnesium chloride: seawater solution and before being preserved in 10% formalin: seawater. All samples for genetic analysis were stored in 100% ethanol at room temperature for the duration of the workshop and then stored at -20°C until genetic analyses were done. *Heteractis malu* specimens were stored in 100% ethanol and kept at room temperature (approximately 21°C).

Table 4.1 Sea anemone species collected from Moreton Bay used in the genetic analysis.

Collection Location	Latitude and Longitude	Field Identification	Laboratory Identification	Genetic Material Source	Queensland Museum Registration Number
Bird Island	27º 30' S 153º 23' E	*Entacmaea quadricolor* juvenile. [+][$]	?*Anemonia* sp.[$]	Whole animal	MTQ G58754
Flat Rock Bommie, East of Stradbroke Island	27º 24' S 153º 33' E	*Entacmaea quadricolor**[*]	Not available	Tentacle	Whole specimen not collected
Dunwich, sand flats directly in front of Moreton Bay Research Station	27º 30'S 153º 24'E	*Stichodactyla haddoni*[+][*]	Not available	Pedal disc and Tentacle sample	Whole specimen not collected
Shag Rock, E. of Stradbroke Island	27º 24.85'S 153º31.59'E	*Heteractis malu*[&]	*Heteractis malu*[$]	Pedal disc	MTQ G58749
Frenchmen's Beach	27º 25' S 153º 32' E	*Oulactis muscosa*[$]	*Oulactis muscosa*[$]	Pedal disc	MTQ G58756
Dunwich	27º 30' S 153º 24' E	*Macrodactyla doreensis*[^]	*Macrodactyla doreensis*[+][$]	Pedal disc and tentacle	MTQ G58748
Shag Rock, E. of Stradbroke Island (Collected Subsequent to Workshop)	27º 24.85'S 153º31.59'E	*Heteractis malu*[^]	*Heteractis malu*[*]	Pedal disc	Deposited in Museum Victoria and awaiting registration.

Species identifications by [*] M. Mitchell, [+] D. Fautin, [$]A. Crowther, [^]P. Davie & D. Potter, [&]C. Wallace.

4.2.2 DNA Extraction, PCR and Sequencing

To test to the usefulness of current DNA extraction kits with ethanol preserved sea anemone tissues, total genomic DNA was extracted from both tentacles and pedal disc tissues using DNeasy Tissue Kits (QIAGEN), as opposed to the far more labour intensive protocol of Pinto et al. (2000). Partial 18S rDNA, complete ITS1, 5.8S, ITS2 and partial 28S rDNA sequences were initially amplified using the primer pairs RA2 and ITS2.2 described by Wörheide (1998). RA2 is located in the flanking 3' end of the small subunit ribosomal gene (18S) and ITS2.2 in the 5' end of the large subunit ribosomal gene (28S). PCR amplifications were performed in 25 µl reaction volumes that contained a final concentration of: 1 x Taq polymerase buffer, 2.5 mM $MgCl_2$, 0.2 µM each primer, 0.8 mM dNTPs and 0.75 U of Taq polymerase. The use of the hot start polymerase HotMaster Taq (Eppendorf) required an initial denaturation at 94°C for 2 min prior to the commencement of the remaining cycle parameters; this was then followed by 35 cycles of 94 °C for 20 sec, 55–58 °C for 20 sec, 65 °C for 45 sec and a final extension 65 °C for 5 min.

PCR products were gel purified using 'Perfect Prep" gel cleanup kit (Eppendorf) and forward and reverse sequencing reactions were carried out according to standard ABI PRISM dye-deoxy terminator sequencing protocols using Big Dye Terminator versions 1.1 and 3.1. Chromatographs were checked and all sequences were aligned using Se-Al v2.0a10 (Rambaut, 1996). Estimates of sequence divergence including insertions (uncorrected p-distances) were calculated using the pairwise base distance function in PAUP* v4.0b10 (Swofford, 2002). We verified the origin of the amplified sequence data by conducting a BLAST search in GenBank thus determining the phylogenetic affinity with sequences from other actiniarian or anthozoan species. Sequences for this same region were also obtained from GenBank from two individuals of the species *Heteractis magnifica* (Accession no: AF050201 (*H. magnifica* 1) and AF050211 (*H. magnifica* 2)).

4.2.3 Sea Anemone Primers

Based on the sequence results obtained from four of the six study species using the above described 'universal' primers, one of the *H. magnifica* sequences plus contaminating zooxanthellae sequences from the remaining two species (*Heteractis malu* and *Macrodactyla doreensis* — see Results), we designed two new primers. These primers were designed to be specific to sea anemones and located in regions of identical sequence among the sea anemone species (for which we had data) but mismatched the zooxanthellae sequences at 45–50% of sites (see Figure 4.1). These two new primers *seaanem18S*: 5' TTA GTG AGG ACT CCT GAT GGC 3' and *seaanem28S*: 5' AGT CTC GCC TGA TCT GAG G 3' lie within 50 bp downstream from RA2 and ITS2.2 respectively. We tested the primers against the same six species used with the 'universal' primers. Amplification conditions, clean up and sequencing reactions with the new primers are identical to those described earlier.

A) 18S rDNA 3'end

```
                                  ┌────────────────────────────────────────────────────────────────────────────┐
                                  │                                 18S rDNA                                    │
                                  └────────────────────────────────────────────────────────────────────────────┘

                     10        20        30        40        50        60        70        80        90       100]

                              ─────  Seaanem18S  ─────

H. magnifica 1       -------AAT GGTTTAGTGA GGACTCCTGA TTGGCGCCGC CGCCCCGGCA ACGGAGCAGT GGACTTGCCG AAAAGTTGTT CAAACTTGAT CATTTAGAGG
S. haddoni           CCGATTG... .......... .......... .......... .......... .......... .......... .......... .......... ..........
E. quadricolor       CCGATTG... .......... .......... ........A. .........c .......... ....CC.... .G........ .......... ..........
O. muscosa           CCGATTG... .......... .......... ........A. .......... ....G..... .....-.... .......... .......... ..........
?Anemonia sp.        CCGATTG... .......... .......... .......... .......... .......... .....-.... .......... .......... ..........
Zooxan M. doreensis  -----TG.G. .A.CCG.... AT.A...G.. C..A...A.T GCT.AGCTTC TG.AC.TT.C ------.T.. G......TCA TG...C.T.. ..C.......
Zooxan H. malu       CCGATTG.G. .A.CCG.... AT.A.T.G.. C..A...A.T GCT.AGCTTC TG.AC.TT.C ------.T.. G......TCA TG...C.T.. ..C.......
```

```
                                  ┌───────────────────────────────────────────────────┐
                                  │                    18S rDNA                        │───────────────────────────────►
                                  └───────────────────────────────────────────────────┘                           ITS 1

                    110       120       130       140       150       160       170       180       190       200]

H. magnifica 1       AAGTAAAAGT CGTAACAAGG TTTCCGTAGG TGAACCTGCG GAAGGATCAT TACCGATCAG CCAAGTCGTT AACTCGACAA CACACCGCGA ACCGTCCCCG
S. haddoni           .......... .......... .......... .......... .......... .......... .......... C......... .......... ..........
E. quadricolor       .......... .......... .......... .......... .......... .........GT T.G..GACCG CCACA.TTCT .T.G.AC..C GAACCGTA..
O. muscosa           .......... .......... .......... .......... .......... .........C GTTCTA.ACG T.GGAC..CG AgA....TAT GGA.AG----
?Anemonia sp.        .......... .......... .......... .......... .......... .........GA GTTCT..T.G G.ACACCGCG --A....TAT GGACAG----
Zooxan M. doreensis  ...G.G.... .......... .......... .......... .......... .-.GC.CTGA TTC.A..T.G .CTA..TG.. .TTTGGCT.T GAG.GATG.T
Zooxan H. malu       ...G.G.... .......... .......... .......... .......... .-.GC.CTGA TTC.A.TT.G .CTA..TG.. TTTTGGCT.T GAG.GATG.T
```

B) 28S rDNA 5' end

```
                        ITS 2                                ┌──────────────────────────────────────────────────┐
◄───────────────────────────────────────────────────────    │                    28S rDNA                        │
                                                             └──────────────────────────────────────────────────┘

                     10        20        30        40        50        60        70        80        90       100

                                                                      ─────  Seaanem28S  ─────

H. magnifica 1       GCACGGCGTT GGACCGGACG GACG----GT GGTTCGG--- -CCCTATCTT TCCA-TCTTG ACCTCAGATC AGGCGAGACT ACCCGCTGAA TTTA------
S. haddoni           .......... .....C.... ....----.. .......--- -....G.... ....-..... .......... .......... .......---- ----------
O. muscosa           AGG.CT.TGC T.G..-.... .TGCGGAC.. .......CAG C....GC..A ...TTC.... .......... .........T. .......---- ....AGCATA
?Anemonia sp.        AGG..C..GC C.GT.-.... .C.CCGT-.C ...C.TCGG- ----CCYT.C .TA.C..... .......... .......... .......---- ----------
Zooxan M. doreensis  .TTTTA.T.G A.TGAC.CT. CT.ATGCTTG CAAC..CTGG GATGC.GG.G CATGCCTC.A G.A.G.AG.. ..A.A..TGA ......---- ----------
```

Figure 4.1 Location of sea anemone specific primers Seaanem18S and Seaanem28S relative to a partial alignment of four anemones and two algal symbionts at A) 18S and B) 28S rDNA genes. Intervening sequence between A) and B) not included. Sequence data for *H. magnifica* 1 were derived from GenBank (accession #AF050201).

4.3 Results

4.3.1 DNA Extraction

In contrast to Pinto et al. (2000) no problems were experienced extracting DNA from ethanol preserved sea anemone tissues using the DNeasy tissue kit. Prior treatment of the samples to remove ethanol was not required; nor did the tissues need to be homogenised in liquid nitrogen prior to the extraction process. Furthermore, total tissue digestion was completed within 1–3 hours at 55° C as recommended by the manufacture's protocol, as opposed to the 72 hour period at 37° C used by Pinto et al. (2000).

4.3.2 Universal Primers

An 800 bp (approximately) PCR fragment was successfully amplified from all six sea anemone species and all tissue types using the universal primers RA2 and ITS2.2. Readable sequence data of the fragment (including the 3' end of the 18S gene, full length ITS1, 5.8S gene and ITS2 and the 5' end of 28S gene) were obtained from only three of the six species (?*Anemonia* sp, *M. doreensis* and *S. haddoni*). Partial/non-overlapping sequences were obtained from the remaining three species (*E. quadricolor*, *H. malu* and *O. muscosa*). Not all tissue types generated readable sequence data. For example, sequences obtained from the pedal disc tissues of *H. malu* and *M. doreensis* were unreadable with evidence of multiple sequences present in the chromatograph (Table 4.2). This result was unexpected given that the amplified PCR product revealed a clear single band. However, readable sequence data were obtained from the tentacles of those same two species. BLAST searches of all readable sequences (either complete or partial) revealed strong matches (90–97% identity) with other sea anemone and/or anthozoan species in GenBank for only four of the six study species (Table 4.2). The sequence data obtained from the tentacles from *H. malu* and *M. doreensis* however, matched with almost 99% identity to other symbiotic algae sequences (e.g. *Symbiodinium* sp.) indicating preferential amplification of the zooxanthellae DNA in each of these species. Interestingly, the sequence data obtained from both the pedal disc and tentacles of *S. haddoni* were identical, and BLAST searches of these and that obtained from the tentacles of *E. quadricolor* revealed closest similarity to other anthozoan species

indicating that the host DNA had preferentially amplified and/or that zooxanthellae are either not present or in high enough density to mask the host DNA in both these species.

Table 4.2 PCR and sequence results obtained from anemone tissues using both the "universal" primers and sea anemone specific rDNA ITS primers. Presence (+) or absence (−) of product is indicated.

		Preferential Amplification and Sequence Obtained using "Universal" Primers		Preferential Amplification and Sequence Obtained using Sea Anemone Specific Primers	
Species	Tissue used in DNA extractions	Anemone DNA	Zooxanthellae DNA	Anemone DNA	Zooxanthellae DNA
?Anemonia sp.	Column / Pedal disc	+	−	+	−
Entacmaea quadricolor	Tentacle	+	−	+	−
Heteractis malu	Pedal disc	+	+	−	−
	Tentacle	−	+	−	−
Macrodactyla doreensis	Pedal disc	+	+	+	−
	Tentacle	−	+	−	−
Oulactis muscosa	Column / Pedal disc	+	−	+	−
Stichodactyla haddoni	Pedal disc	+	−	+	−
	Tentacle	+	−	+	−

4.3.3 Sea Anemone Primers

Amplification success using the primers *seaanem18S* and *seaanem28S* varied from that seen with the universal primers. Approximately 750 bp were obtained from five of the six anemone species; no PCR product amplified from *H. malu* regardless of tissue source (Table 4.2). For the three species for which either tentacle and/or pedal disc tissues were available, amplification success varied from species to species. No PCR product was obtained from *M.*

doreensis tentacle DNA; in contrast, product amplified from the tentacle DNA of *E. quadricolor* and both tissue types for *S. haddoni* (Table 4.2).

The lack of amplification success for *H. malu* was surprising given that sequence data from the congeneric species, *H. magnifica*, was used in the alignment from which the new primers were designed and that the regions of both the 18S and 28S genes where these primers are located are identical among all the actiniarian genera (except for one site in *O. muscosa*), for which sequence data were available. In order to determine if it was possible to amplify product for *H. malu* but avoid zooxanthellae DNA contamination, sea anemone primers were used in combination with previously successful universal primers; *seaanem18S* paired with ITS2.2 and *seaanem28S* paired with RA2. Successful amplification from *H. malu* DNA from both pedal disc and tentacles was obtained using *RA2/seaanem28S* only.

Sequences, either partial or complete, obtained from ?*Anemonia* sp., *O. muscosa* and *S. haddoni* using the new sea anemone primers were identical to those obtained using the universal primers, which had previously been confirmed as originating from host anemone DNA rather than their algal symbionts. BLAST searches of complete sequences from *M. doreensis* and *H. malu* obtained using anemone specific primers indicated greatest similarity to other anemones. Hence the anemone specific primers had been successful in circumventing the problems of zooxanthellae contamination. Curiously, *E. quadricolor* did not return readable sequence data suggesting that further optimisation of the sequencing reaction for this species and these primers may be required. For *H. malu*, sequence obtained with RA2 revealed no mismatches in the 3' region of the 18S rRNA gene where *seaanem18S* is located that would explain why this primer did not work on this species. Further experiments may be required to secure successful amplification with both anemone specific primers on this species.

In summary, complete or overlapping sequences of the 18S–28S fragment were obtained from only three species (?*Anemonia sp.* (724 bp), *H. malu* (670 bp) and *S. haddoni* (734 bp)). Although partial or non-overlapping sequences were obtained from *E. quadricolor* (480 bp from 18S), *M. doreensis* (523 bp: 300 bp from 18S and 223 bp from 28S) and *O. muscosa* (556 bp: 285 bp from 18s and 271 bp from 28S), they were excluded from subsequent analysis due to incompleteness.

4.3.4 Species Identification

Among the three species for which full sequences were obtained (including the two *H. magnifica* sequences obtained from GenBank) estimates of sequence divergence ranged from 0.1% within *H. magnifica* up to 25.1% between *H. magnifica* 1 and ?*Anemonia* sp. (Table 4.3). The average level of sequence divergence among species was 23.8% indicating that this region may indeed prove to be useful for species identification in sea anemones. The exception was the comparison between *H. magnifica* and *S. haddoni*, where the divergence averaged only 1.7 % (Table 4.3). This result was not anticipated given that it is significantly lower than the level of divergence found among the congeneric *H. magnifica* and *H. malu* sequences (ave 23.9%) and is therefore suggestive of possible taxonomic misidentifications or cryptic species. Considering that the *H. magnifica* or *S. haddoni* samples cannot be taxonomically verified for this study due to specimen/tissue unavailability, it highlights the importance of using genetics in conjunction with traditional histological methods.

The potential utility of this region for species identification is also evident from the example of ?*Anemonia* sp., which was initially identified in the field as a juvenile *Entacmaea quadricolor* collected from Bird Island (Table 4.1). While only partial sequences were obtained from the adult *E. quadricolor* collected off Stradbroke Island, comparison of the sequences between the two specimens clearly showed they were significantly different (approx 18% sequence divergence over 480 bp) and possibly therefore two different species. Later histological analysis revealed that the Bird Island specimen was not *E. quadricolor* as originally identified but, may be ?*Anemonia* sp., although the exact identity of this specimen still awaits final taxonomic confirmation.

Table 4.3 Estimates of sequence divergence among species for which the complete 18S–28S fragment was obtained (max 758 bp). Sequences for *H. magnifica* obtained from GenBank.

	?Anemonia sp.	*Heteractis magnifica* 1	*Heteractis magnifica* 2	*Heteractis malu*	*S. haddoni*
*?*Anemonia sp.	–				
H. magnifica 1	25.1%	–			
H. magnifica 2	24.9%	0.1%	–		
H. malu*	21.6%	23.8%	24.0%	–	
S. haddoni	24.4%	1.6%	1.8%	23.0%	–

* Specimen collected subsequent to the Stradbroke Island Workshop

4.4 Discussion

The ribosomal DNA gene complex has proved highly successful for species identification across an incredibly broad range of taxonomic groups including plants (Chase et al., 2005), fungi (Ristaino et al., 1998; Iwen et al., 2002), digenean parasites (Nolan & Cribb, 2005), and mosquitos (Collins & Paskewitz, 1996). It has even been used to identify commercial crustacean species from larvae collected in plankton surveys (Wang et al., 2006). This study investigated for the first time, the utility of this region for identification of sea anemone species, and the potential problems of using universal primers in species that contain algae symbionts.

While this study was preliminary, results showed high levels of sequence divergence among species using this region, compared with divergence estimates an order of magnitude lower within a species. This indicates that it may indeed be ideal for assisting with sea anemone species identification. The questions at what taxonomic level and how useful this region may be for resolving phylogenetic relationships among sea anemone species was not the focus of this study, but should certainly be investigated as more sequences become available. Acuña et al. (2007a) used phylogenetic tools rather than estimates of sequence divergence to distinguish between different *Aulactinia* species, and found extremely short branch lengths among individuals within a species compared to those between species.

The usefulness of conserved 'universal' primers clearly depends on the species and tissue type available for analysis. However, as shown by the results obtained from *H. malu* and *M.*

doreensis, extraction of "uncontaminated" host DNA from samples taken only from pedal disc tissues clearly should never be assumed. In order to guarantee that host DNA is amplified alone, use of primers specific to sea anemones are recommended, if not on their own then at least in combination with another universal primer. The extent to which the primers designed for this study will work across all the actiniarian order remains to be determined. Further preliminary PCR testing using *seaanem18S* and *seaanem28S* on another seven species collected from Moreton Bay, which represent another seven different actiniarian genera, proved highly successful with strong amplicons produced in all seven species. Only subsequent sequencing will confirm whether or not the host DNA has been successfully targeted.

Modern DNA extraction kits also seem highly useful for overcoming any difficulties associated with DNA extraction from ethanol preserved sea anemone tissues. It is not clear why the techniques used in this study experienced so few problems compared with previous work by Pinto et al. (2000). It may be partly attributed to the use of recently preserved tissue as opposed to tissue that had been stored in ethanol for an extended period of time.

Finally this study has reinforced the value of being able to combine histological analysis with genetic testing to irrefutably verify a species' identity, especially given the early developmental stage of developing genetic markers for this group. As demonstrated in the case of the supposed juvenile *E. quadricolor* sample, while the genetic data strongly indicated the possibility of incorrect field identification, correct species identification (*?Anemonia* sp.) can only be established following further histological analysis. Furthermore, the unexpected result showing much greater sequence divergence between the two *Heteractis* species than that detected between *Heteractis magnifica* and *Stichodactyla haddoni* cannot be resolved further. While again indicative of possible misidentifications or the presence of a cryptic species, the *S. haddoni* specimen cannot be histologically analysed because the whole animal was not collected from the field. The *H. magnifica* sequences available on GenBank are not associated with registered specimens and therefore identification cannot be verified.

Chapter 5 General Discussion

Despite the long history of marine faunal surveys in Port Phillip Bay, Victoria (Wilson, 1895; Cutress, 1971; Poore et al., 1975; Wilson et al., 1998), little attention has been given to the specific cataloguing of Actiniaria species for the region. This study has provided the first dedicated survey of the Actiniaria of Port Phillip Bay, and has resulted in a checklist of 20 species. Of these 20 species, four are potentially new to science including; *Epiactis* sp., *Oulactis* sp., *Edwardsia* sp. and an unidentified burrowing anemone. In addition, four species have been identified as requiring taxonomic review including *Epiactis australiensis, E. thompsoni*, *Edwardsia vivipara* and *Isanemonia australis*. Three previously published species records for Port Phillip Bay could not be verified in this study: *Bunodactis rubrofusca*, *Anthothoe similis*, and *Anthothoe australis*.

This study has demonstrated that *in-vivo* characteristics are important for correct species identification and in recognising new or potentially cryptic species. It was also demonstrated that analyses of ribosomal DNA may be beneficial when dealing with uncertain identifications of actiniarians. The taxonomic review of *Oulactis* in Australia concluded that *Oulactis muscosa* and *O. mcmurrichi* should remain as separate species at present, and that rDNA may be useful to further assist in delineating these two morphologically similar species. This study has provided an important baseline for further Victorian surveys, and highlighted the need for continued cataloguing of Actiniaria species in Australia and demonstrated the importance of having taxonomic specialists with local expertise.

Prior to this study only 16 species of Actiniaria were reported from Port Phillip Bay. This study has potentially increased that number to 20 species and raised additional taxonomic questions that, when addressed, may vary this number. A similar sea anemone-specific survey conducted during a workshop in Moreton Bay, southeast Queensland in 2005 produced an increase in species number from 8 to 19 for that location (Fautin et al., 2008).

The number of actiniarian species recorded in Port Phillip Bay is likely to increase in the future when other habitats that could not be sampled during this study are surveyed. There are still extensive coastline areas and habitats, such as muddy/silty environments, estuarine and deeper waters, that need to be surveyed specifically for Actiniaria in Port Phillip Bay and along the remaining Victorian coastline. With the exception of Moreton Bay in southeast Queensland, no other Australian location has received a similar level of attention with regard to its anemone fauna. Some sea anemones are subject to seasonality and urban pressures (see Chapter 3). Seasonality was observed at various sites around Port Phillip Bay, for example in relation to *Cricophorus nutrix*, and needs to be taken in account when conducting future surveys. Seasonality and habitat selection may also explain why some commonly known species in Port Phillip Bay, such as *Epiactis* spp., were not observed in the field during this study.

Several taxonomic issues were highlighted during this study that need to be addressed by future studies including: regional variation of species, genetic sequencing, new species descriptions, and verification of species records.

Regional Variation

Previous taxonomic studies for actiniarian species occurring in Australia have been conducted predominately on preserved specimens. Incorrect preservation rendered many of these specimens unidentifiable or resulted in poorly preserved material for taxonomists to complete comprehensive taxonomic descriptions. This is especially the case for Lager's (1911) *Oulactis mcmurrichi,* where type specimens are incomplete and are now in such a poor condition that taxonomists are unable to obtain further data from the specimens. Indeed, Stephenson (1928) proposed that it may be impossible to identify preserved actiniarian specimens below the level of genus. For genera where species have no discernible morphological differences or where identification relies soley upon *in-vivo* characteristics such as patterning/colour, which are lost through the preservation process; identification to species level for preserved specimens may not be possible.

Poor preservation and lack of documentation on living specimen characteristics has meant that important *in-vivo* diagnostic characteristics have been overlooked. Preserved specimens of a similar morphology have often been identified as the closest known species from the area, which is likely to have resulted in the failure to identify some cryptic species and document regional intraspecific variation.

The importance of regional variation and *in-vivo* characteristics being well documented was clearly demonstrated in the taxonomic case study of *Oulactis muscosa* and *O. mcmurrichi* (Chapter 3). Animals that have been identified and known locally in Port Phillip Bay as *O. muscosa* are substantially different in appearance, and habitat, to specimens from the type locality, and may represent a new species. This issue has arisen because much of the taxonomic work undertaken on this species has been based on preserved specimens sent to overseas experts, and important life characteristics were not documented at the time of collection for identification and description purposes. The problem has also been compounded by the fact that there is no existing type specimen for *O. muscosa* and only incomplete descriptions. The addition of *in-vivo* characteristics can be crucial in the identification of actiniarian species in the field and from collections of preserved animals; this issue was highlighted by Häussermann (2004) who concluded that *in-vivo* characteristics are of paramount importantance for the correct identification of actiniarian species.

In addition to the regional variation around Australia, many Actiniaria species recorded in Australia have also been recorded from New Zealand. Species in common with New Zealand include; *Anthothoe albocincta*, *Cricophorus nutrix*, *Phlyctenactis tuberculosa* and *Oulactis muscosa*. In some species, such as *Anthothoe albocincta,* the documented appearance of a species differs little between Australian and New Zealand locations, whereas other species, such as *Oulactis muscosa,* vary substantially between the two countries. Preliminary work in this study (see Chapter 3) has indicated that New Zealand specimens of *Oulactis muscosa* may potentially be a new species. Now that *in-vivo* characteristics have been documented for neotype and paratype specimens collected for *O. muscosa*, further taxonomic and genetic analysis of New Zealand specimens should be undertaken. Similarly, species varying substantially in appearance from the type specimens of a country, which share a common distribution with other

countries, require further examination to determine if they are separate species and to fully document regional variation.

The knowledge of local naturalists/researchers and community groups is invaluable in documenting these regional variations and for locating smaller and/or potentially cryptic species. The Victorian Field Naturalists — Marine Research Group have been conducting surveys around Port Phillip Bay and Victoria since 1957 and have extensive local and specialist knowledge. The Reefwatch project of Victoria is also beneficial for collating distribution data on smaller and unidentified sea anemones. It is through these two groups that evidence of a potential new species, *Epiactis* sp. was collected and its reproductive strategy documented.

Species Verification and Diagnosis

Hundreds of actiniarian specimens have been collected over many years during marine surveys, and preserved and placed in the Museum Victoria invertebrate collection. Due to the large volume of unidentified material it was not possible to identify all material during this study. Therefore, only material that had previously been identified was assessed to verify identifications. The review of the Museum material showed that there were a number of common identification errors and questionable identifications made by non-specialists.

An example of one common misdiagnosis found during this study was that of *Aulactinia veratra* identified as *Oulactis muscosa*, possibly because both species occur in the same habitat and have shell fragments adherent to the body. This common misdiagnosis appears to have occurred both prior to collection and after preservation. Additionally, misdiagnosis of this species has occurred in collections containing lots of specimens that are generally sorted by non-specialists from large amounts of survey material and preserved before sending to a specialist. These broadly sorted samples can contain a mixture of species if they are similar in appearance. If multiple specimens are then rapidly identified or there is an assumption by the specialist that they are all sorted correctly, identification errors such as noted in Cutress' (1971) may occur (see Chapter 3).

Alternatively, specimens that have distinctive characteristics, such as a marginal frill, may tentatively be identified as a species that is known to occur in the region with a similar characteristic, as was illustrated in the misdiagnosis of *Heteranthus verruculatus* tentatively identified as *Oulactis muscosa* (see Chapter 3). Keys of local actiniaria fauna in conjunction with images that document the diagnostic characteristics, *in-vivo* and preserved, are needed to reduce the problem of incorrect identification by non-specialists and will highlight specimens that need further taxonomic analysis by a specialist.

Misdiagnosis of fauna and flora has implications for incorrect biodiversity assessments and inappropriate management policies, and also poses a biosecurity risk where incorrect identifications may have substantial impacts on local fauna and industries (Rice, 2008). For example, it is imperative that invasive species are identified quickly and that government management strategies are established to minimise their impact on industry and the environment. Port Phillip Bay has a large shipping industry and it already has a record of numerous non-endemic invasive species being present and so continual monitoring is important (Currie & Parry, 1999). While this survey did not locate the invasive sea anemone *Diadumene lineata* (Gollasch & Riemann-Zürneck, 1996) at the survey sites, the potential presence of this species needs to be monitored during future surveys. *Diadumene lineata*, also known by the synonym *Haliplanella luciae* (Fautin, 2009), has the ability to tolerate extreme environmental conditions (Gollasch & Riemann-Zürneck, 1996), making it an ideal marine invasive species. Current distribution records for *D. lineata* include: Europe, North and South America, Asia and New Zealand, but there are no known published records of this species in Australia (Fautin, 2009).

Australia's lack of knowledge for many marine invertebrates and the dwindling number of active taxonomists make it difficult to document biodiversity and identify some coastal and marine fauna. This basic knowledge of fauna and flora is required to instigate better management practises and policies (Ponder et al., 2002), especially as the human population grows and heavy urbanisation of the Australian coastline continues.

Actiniarian Taxonomy and Genetics

There is an increasing trend to incorporate genetics into morphological studies (Australian Marine Sciences Association, 2005; Hajibabaei et al., 2007). However, taxonomists are essential for formally establishing species descriptions and systematics, and are also needed to confirm the source of associated material for DNA sequences lodged in genetic databases, such as Genbank. Therefore, genetics should be viewed as an aid to traditional taxonomy (Australian Marine Sciences Association, 2005; Hajibabaei et al., 2007; Smithsonian Tropical Research Institute, 2008) . In addition, genetic sequencing is an expensive process compared to traditional taxonomic methods and therefore it is impractical to use on thousands of specimens collected during large scale ecological studies (Australian Marine Sciences Association, 2005), or on small dedicated local studies where extensive funding may not available.

Establishment of DNA databases such as GenBank (http://www.ncbi.nlm.nih.gov/Genbank) or the Barcode of Life project (http://www.barcodinglife.org) enables comparison of genetic sequences with unknown samples. The sequences should have associated material and be taxonomically verified before being uploaded. This was demonstrated during genetic analysis comparing *Heteractis* material used in this study with the lodged GenBank sequence of another *Heteractis* species (see Chapter 4). The sequences were very dissimilar and there is no way to taxonomically verify the specimen from which the lodged GenBank sequence was acquired, as there is no associated material. Therefore, strict guidelines need to be put in place regarding the uploading of sequences and verification of the associated material for GenBank. It is counterproductive and potentially dangerous to rely on the identification of animals based solely upon online genetic databases – and if the original identification is incorrect this will create ongoing problems for future studies and biodiversity management.

Genetic sequence databases are oriented towards the global standard of COI sequencing. This is problematic as COI has been shown not to be suitable for Actiniarian genetic extraction (see Chapter 4). Alternate loci still need to be explored and a global standard agreed upon for species where COI has been proven unsuitable.

Many studies are now exploring the use of rDNA sequences as an alternative for COI. However, there is still much research needing to be undertaken on suitable loci and guidelines established for a standard extraction processes, especially as new genetic extraction products and equipment become available.

Future genetic research may help provide definitive answers to some of the taxonomic questions raised during this study. These include the delineation of potentially new *Oulactis* species, a definitive answer regarding the synonymising of *O. muscosa* and *O. mcmurrichi* and determining the significance of intraspecific regional variation, and in the detection of cryptic species.

Future Research

There is substantial further research that needs to be done in Port Phillip Bay and Australia as whole, in addition to the specific taxonomic questions raised during this study. Future research projects include but are not limited to;

- Actiniaria specific surveys of Port Phillip Bay's subtidal waters, estuarine environments and various habitats not included in this study such as muddy/silty environments.
- Actiniaria specific surveys of the Victorian and greater Australian coastline.
- Taxonomic/genetic studies to identify potentially cryptic species such as *Cricophorus nutrix*.
- Taxonomic revision of species including *Epiactis thompsoni, E. australiensis, Anthothoe australis* and *A. similis*.
- Verification of species records including *Bunodactis rubrofusca*.
- Identification and description of potentially new species.
- Genetic protocols and standards need to be developed for the extraction of rDNA from Actiniaria and sequences lodged in genetic databases.
- Taxonomic and genetic analysis undertaken for *Oulactis* species from Australia and New Zealand.
- Revision of species placement within *Oulactis*.

The gap in knowledge of actiniarian fauna in Australia has started to be addressed through studies such as Fautin et al. (2008) and this research. The large amount of taxonomic work to be completed on actiniarian material housed in Australian museums will take considerable time, funding and taxonomic resources. These morphologically simple animals are complex taxonomically, and therefore require specialist taxonomic training for correct identification. This study has provided some important baseline data for further work on Port Phillip Bay actiniarian fauna, and has highlighted taxonomic revision that is needed for some common species found throughout Australia and New Zealand.

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Appendix I – Glossary of Taxonomical Terms

Glossary definitions are taken from the online 'Illustrated Glossary of Sea Anemone Anatomy' by Brian McCloskey unless otherwise referenced. Additional definitions may be found at http://www.nhm.ku.edu/tol/glossary/intro.html

Acontia – Thin threads attached to the edge of mesenteries and heavily laden with nematocysts. The threads may be extruded through the mouth or in some cases through cinclides on the column wall of the anemone for the purpose of defence or predation.

Actinopharynx – Throat of the anemone, also referred to as the stomodaeum, aesophagus or pharynx. The actinopharynx leads from the mouth of the anemone to the coelenteron.

Acrorhagi (Spherules) – Outgrowths found on the margin of the column wall. The acrorhagi contain holotrich nematocysts as its defining feature (Daly, 2003) and they may be spherule, slightly branched or frondose in structure.

Pseudo-acrorhagi – As above except that the outgrowths only contain basitrichs (Daly, 2003).

Brood-pouches – Small evaginations or folds in the column wall whereby juveniles may be enfolded or attached to the parent until they reach a certain developmental stage and are released from the adult (Fautin Dunn, 1975).

Cinclides – Small apertures or soft spots on the column wall that rupture readily through which the acontia fires.

Cnidae – Stinging capsules, including spirocysts and nematocysts, which are located in the tissue of cnidaria. There are 28 types of nematocysts and include more commonly in the Actiniidae; holotrichs, basitrichs, *p*-mastigophores, *b*-mastigophores (Kass-Simon & Scappaticci, 2002).

Cnidoglandular tract – The thickened rim that runs along the free edge of a mesentery. On the lower part of the mesentery the filament may be simple and in the upper tract it may be a triple cord. The lateral bands or outer bands are called ciliated tracts and the median band is referred to as the cnidoglandular tract.

Directives – The pair of mesenteries which lay on the directive axis. The longitudinal muscles (retractors), on the outer side of the pair, are turned towards the exocoels. Alternatively the retractors of ordinary pairs have their retractors in the inner side facing the endocoel.

Endocoel – The space between two mesenteries belonging to the same pair.

Exocoel – The space between two mesenteries belonging to different pairs.

Fosse – The groove enclosed by a distinct fold, the parapet or collar, running around the column below the tentacles.

Mesenteries – Infoldings of the endoderm and mesogloea that extend from the column wall into the gastrovascular cavity. Some of the mesenteries reach the actinopharynx and divide the gastrovascular cavity. The mesenteries increase surface area for digestion and uptake of nutrients.

Mesentery arrangement – mesenteries are arranged in pairs, each consisting of two mesenteries adjacent to one another. Mesenteries may be referred to as the directives and ordinary pairs. **Perfect mesenteries** are pairs that are attached to the actinopharynx and **imperfect mesenteries** are pairs that do not extend to the actinopharynx. The mesentery pairs are arranged into six (hexamerous), eight (octamerous) or ten (decamerous) rays. These cycles may be irregular due to displacement or absence of directives, dislocation of tentacles or injury. Dependant upon the order of the growth, the mesentery pairs are termed the first, second and third cycles and son on. In some orders the mesenteries are differentiated into macronemes and micronemes. Macronemes have very strong retractor muscles, gonads and filaments where as micronemes lack those structures.

Nemathybomes – Spheroid invaginations of the column ectoderm extending into the mesogloea and are laden with nematocysts.

Physa – The aboral ampullaccous end of anemones from the Infraorder Athenaria. The physa appears rounded and swollen and acts as an anchor for anemones which burrow into sediments.

Scapus – In some cases the column may be divisible into regions. The most proximal region is the physa. The longest main region on the column is referred to as the scapus and the above the scapus is either a thick walled **scapulus** or a thin walled **capitulum**. In some cases a scapulus and a capitulum may be present.

Sphincter – Circular muscles located at the margin or near the margin, depending upon the placement of the muscles they are classified as endodermal or mesogloeal. The sphincter may further be classified by the way it is attached to the wall. If the muscle is elongated it is **diffuse** or if the muscle is confined in a circular grouping it is **circumscribed**.

Siphonoglyphs – Anatomically differentiated smooth grooves which run the length of the actinopharynx from the mouth down. Sometimes the siphonoglyphs are attached

to the directive mesenteries; in some taxa the siphonoglyphs will form a separate tube from the actinopharynx.

Stomata – just below the oral disc, close to the actinopharynx and along the margin are the stomata. These are openings that run through each mesentery allowing water to flow between the endocoel and exocoels (Friese, 1972).

Tenaculi – papillae that are more or less solid, situated in the column. The ectoderm of the papillae is partly chitinised and provide with an unusually strong cuticle to which grains of sand or detritus may adhere.

Verrucae –evaginations on the column and predominately simple, however they may be compound, and have a modified ectoderm. They lack nematocysts in the central part.

Vesicles – non adhesive outgrowths (resemble bubbles when inflated) on the column wall, laden with various types of nematocysts.

Appendix II – Genus diagnosis for *Oulactis*

Genus diagnosis for *Oulactis* as provided by Häussermann (2003). The bold type highlights Häussermann's amendments of Carlgren's (1949) diagnosis.

"Actiniidae with well developed pedal disc; **oral disc wide, round to lobed.** Column smooth in its lowest part, otherwise **covered with** longitudinal rows of **adhesive** verrucae **except in its most proximal part; verrucae becoming smaller towards the margin, compound and** set on small lobes. **Thin-walled marginal region carrying delicate frond-like papillae set on lobes and forming a ruff below the tentacles; one frond in each exo- and endocoel. No distinct fosse. Acrorhagi present or not, when present they are placed on the oral side of the fronds. Arrangement of tentacles and mesenteries** hexamerous, longitudinal muscles of tentacles ectodermal. Most of the mesenteries perfect, **reproductive material may appear on all mesenteries except youngest cycles (in some species** directive may be sterile). Two well-developed siphonoglyphs and two pairs of directives. **Endodermal** sphincter diffuse, **weak to well developed**. Retractors **diffuse to restricted, moderately strong to strong, parietobasilar muscles and basilar muscles strong. No zooxanthellae present.**
Cnidom: spirocysts **(in tentacles, may also be found in pedal disc, acrorhagi and oral disc),** basitrichs **(in all body parts except acrorhagi and fighting tentacles), microbasic *b*-mastigophores (in filaments and fighting tentacles), microbasic *p*-mastigophores B (may be found in filaments), microbasic amastigophores A (in filaments, may also be found in pharynx), holotrichs (in acrorhagi, may also be found in the column), in some species rod-like basitrichs (in filaments and column).**"

Appendix III — Full listing of *Oulactis* Material Examined and current identification status

Specimen location collected; latitude and longitude co-ordinates are approximate and were sourced from the Australian Geosciences Gazetteer, www.ga.gov.au, (except for NZ specimens).

Museums Abbreviations: AM = Australia Museum, MV = Museum Victoria, SAM = South Australian Museum, TMAG = Tasmanian Museum and Art Gallery, WAM = Western Australia Museum. International museums: MB.R = Naturkundemuseum of Humbolt University, Berlin, Germany, ZMH = Zoological Institute and Zoological Museum of the University of Hamburg.

Museum	Registration Number	Original Identification	Identifier	No. of specimens in Lot	Location	Latitude/Longitude	Assigned Taxa Group	Current Identification	Identifier
WAM	Z33600	*Oulactis* sp.	D Fautin	1	Rottnest Island, WA	32º 01' S, 115º 30' E	Not Assigned	*Heteranthus verruculatus*	M Mitchell/ D Fautin
WAM	Z33601	*Oulactis* sp.	Unknown	3	Rottnest Island, WA	32º 01' S, 115º 30' E	Not Assigned	*Heteranthus verruculatus*	M Mitchell/ D Fautin
MV	F111194	*Oulactis muscosa*	C E Cutress	8	Anglesea, Victoria	38º 24' S, 144º 11' E	Not Assigned	*Aulactinia veratra* (2 specimens) and *Oulactis* sp.	M Mitchell
WAM	Z33603	*Oulactis* sp.	L M Marsh	6	Barrow Island, Cape Dupey, WA	20º 40' S, 115º 26' E	Not Assigned	Actiniidae	M Mitchell
MB.R	MNH 5440	*Oulactis mcmurrichi*	E Lager	11 1/2	Bunbury Bay, North and East of Casuarina Point, WA	33º 20' S, 115º 38' E	1	*Oulactis mcmurrichi*	
ZMH	C5321	*Oulactis mcmurrichi*	E Lager	15 +	Bunbury Bay, North and East of Casuarina Point, WA	33º 20' S, 115º 38' E	1	*Oulactis mcmurrichi*	
WAM	Z50032	*Oulactis* sp.	N/A	1	Mudarup Rocks, Cottesloe, WA	31º 59' S, 115º 45' E	1	*Oulactis mcmurrichi*	M Mitchell
WAM	Z50034	*Oulactis* sp.	N/A	1	Mudarup Rocks, Cottesloe, WA	31º 59' S, 115º 45' E	1	*Oulactis mcmurrichi*	M Mitchell
WAM	Z50035	*Oulactis* sp.	N/A	1	Mudarup Rocks, Cottesloe, WA	31º 59' S, 115º 45' E	1	*Oulactis mcmurrichi*	M Mitchell
AM	G17437	*Oulactis muscosa*	M Mitchell	1	Rocky point, Balmoral, NSW	34º 32' S, 150º 51' E	2	*Oulactis muscosa*	M Mitchell
AM	G17440	*Oulactis muscosa*	M Mitchell	1	The Shallows, Shellharbour, NSW	33º 49' S, 151º 15' E	2	*Oulactis muscosa*	M Mitchell
MV	F112714	*Oulactis muscosa*	M Mitchell	1	Flat Rock, Lennox Head, NSW	28º 48' S, 153º 33' E	2	*Oulactis muscosa*	M Mitchell

Museum	Registration Number	Original Identification	Identifier	No. of specimens in Lot	Location	Latitude/Longitude	Assigned Taxa Group	Current Identification	Identifier
AM	G17436	*Oulactis* sp.	M Mitchell	1	Bass Point, Shellharbour, NSW	34º 32' S, 150º 51' E	3	Actiniidae	M Mitchell
AM	G17441	*Oulactis* sp.	M Mitchell	1	Wy-ar-gine Pt, Balmoral, NSW	33º 49' S, 151º 15' E	3	Actiniidae	M Mitchell
MV	F109297	*Oulactis muscosa*	C E Cutress	11	Safety Beach, VIC	38º 19' S, 144º 59' E	4	*Oulactis* cf. *muscosa*.	M Mitchell
MV	F112692	*Oulactis* sp.	M Mitchell	1	Rye (Pier), Port Phillip Bay, VIC	38º 22' S, 144º 49' E	5	*Oulactis* cf. *muscosa*.	M Mitchell
MV	F112730	*Oulactis muscosa*	J Davey	3	Seatoun, Wellington, New Zealand	41º 19' S, 174º 49' E	6	*Oulactis* cf. *muscosa*.	M Mitchell
MV	F112731	*Oulactis muscosa*	J Davey	2	Seatoun, Wellington, New Zealand	41º 19' S, 174º 49' E	7	*Oulactis* cf. *muscosa*.	M Mitchell
MV	F112732	*Oulactis muscosa*	J Davey	2	Seatoun, Wellington, New Zealand	41º 19' S, 174º 49' E	7	*Oulactis* cf. *muscosa*.	M Mitchell
MV	F112690	*Oulactis* sp.	M Mitchell/ D Fautin	1	Ships Ballast, Location Unknown	Unknown	9	Actiniidae	M Mitchell
SAM	H1526	*Oulactis muscosa*	D Fautin	1	Barker Inlet, in mud, Adelaide District, Gulf of St Vincent, SA	34º 45' S, 138º 31' E	Not Assigned	*Oulactis* sp.	M Mitchell
SAM	H1527	*Oulactis muscosa*	D Fautin	2	Reevesby Is (btw Home bay and Nicholas Bay), Sir Joseph Banks Group, Spencer Gulf, SA	34º 31' S, 136º 16' E	Not Assigned	*Oulactis* sp.	M Mitchell
SAM	H1531	*Oulactis muscosa*	M Mitchell	1	Port Noarlunga, SA	35º 08' S, 138º 28' E	Not Assigned	*Oulactis* sp.	M Mitchell
AM	G16974	*Oulactis* sp.	D Fautin	3	Remarkable Cave, SE TAS	43 º 11' S, 147 º 49'E	Not Assigned	*Oulactis* sp.	M Mitchell
AM	G10871	*Oulactis muscosa*	Unknown	2	Coogee, NSW	33 55' S, 151 º 16'E	Not Assigned	*Oulactis* sp.	M Mitchell
MV	F111205	*Oulactis muscosa*	M Mitchell	2	Hamelin Bay, WA	34º 11' S, 115º 01' E	Not Assigned	*Oulactis* sp.	M Mitchell
MV	F112718	*Oulactis* sp.	M Mitchell	1	Halloway Bend, Brighton, VIC	37º 55' S, 144º 59' E	Not Assigned	*Oulactis* sp.	M Mitchell
MV	F 112733	*Oulactis* sp.	M Mitchell	1	Queenscliff, VIC	38º 16' S, 144º 39' E	Not Assigned	*Oulactis* sp.	M Mitchell
TAS	K194	*Oulactis* sp.	A Crowther	1	Roches Beach, South TAS	42º 54' S, 147º 29' E	Not Assigned	*Oulactis* sp.	M Mitchell
WAM	Z33602	*Oulactis mcmurrichi*	D Fautin	2	Careening Bay, Garden Island, WA	32º 12' S, 115º 40' E	Not Assigned	*Oulactis* sp.	M Mitchell

Appendix IV – Preliminary redescription of *Oulactis muscosa*

Family **Actiniidae** Rafinesque, 1815

Genus *Oulactis* Milne Edwards& Haime, 1851

Synonymy

Metridium muscosum Drayton in Dana, 1846: 153–154.

Oulactis muscosa Milne Edwards and Haime, 1851: 12.

Oulactis plicatus Hutton, 1878: 311–312.

Cradactis plicatus Stuckey, 1909: 392–393.

(questionable synonymy) *Tealidium cinctum:* Stuckey 1909: 389–390.

Oulactis plicata Carlgren, 1949: 52.

Oulactis plumosa Carlgren, 1954: 572.

Oulactis muscosa Dawson, 1992: 38.

Material Examined

Specimens of *Oulactis muscosa* collected from the type locality are designated as a neotype and paratype and have been deposited in the Australian Museum (AM). Additional material is housed in Museum Victoria (MV).

Neotype Material: **New South Wales, Australia:** AM G17440, The Shallows, Shellharbour, 1 specimen, 34º 32' S, 150º 51' E, 08/09/2007.

Paratype material: **New South Wales, Australia:** AM G17437, 1 specimen, Rocky point, Balmoral, 33º 49' S, 151º 15' E, 09/09/2007.

Material: **New South Wales, Australia:** MV F112714, 1 specimen, Flat Rock, Lennox Head, 28º 48' S, 153 33.5' E, 1, 13/02/2007.

Description
(Fig 1-3; Table 1)

Oral disc and tentacles

Oral disc is a deep royal purple to dark purple brown, with or without a bright green mouth. The oral disc has a width of 1.2–4 cm in preservation. Marginal frill present on

upper column and acrorhagi always present on inner side of fronds and between tentacles. Acrorhagi size is not dependent upon the size of the animal. Tentacles placed on outer edge of oral disc and before the frill, arranged in three whorls, they number 96–98. Ninety six tentacles is the perfect number for the number of mesentery pairs present (i.e. one tentacle per endo- and exocoel). Tentacles have a distinctive white bar patterning on the outer whorl and background colouring is a translucent grey or white, barring is faint on the inner whorl if present at all. Outer tentacles are the same length or slightly longer than the inner tentacles, preserved length ranged from 2–8 mm. Tentacles are stout in shape and taper to a point. Tentacles are held erect in live specimens. There is no distinct fosse.

Appendix IV Figure 1 *Oulactis muscosa* from Shellharbour, New South Wales.

Column

The upper half to two thirds of the column is covered in verrucae and the lower part is smooth. The number of verrucae in each row alternate in number. Shell fragments and particulate matter may be attached to the column and sometimes to the marginal frill in live animals. The upper column is generally coloured grey due to the presence of zooxanthellae, while the lower column is light tan to cream with some specimens having faint olive stripes on the limbus. Verrucae are olive green or grey. Column is 1.2–1.7 cm in length and width is 2.2–2.5 cm at mid column in preserved animals.

Pedal disc

Pedal disc is slightly scalloped in shape and becomes more so in preservation. Preserved pedal disc width is 1.4–2.5 cm.

Internal anatomy

Sphincter is located at the base of the tentacles and is endodermal, diffuse and weak (Figure 2 A). Retractor muscles are strong and diffuse with parietobasilar muscles weak, diffuse and a small pennon visible (Figure 2 B). White, ribbed actinopharynx and two symmetrical siphonoglyphs, also white. Mesenteries arranged hexamerously and there are 48 pairs. The first, second and third cycles are perfect. Directives always attached to siphonoglyphs. All animals examined were fertile; the specimens examined were male. The first, second and third cycles fertile on upper edges of mesenterial filaments, directives never fertile. The oral stomata are large and the marginal stomata very small and not always visible. Zooxanthellae occasionally present internally on mesenteries.
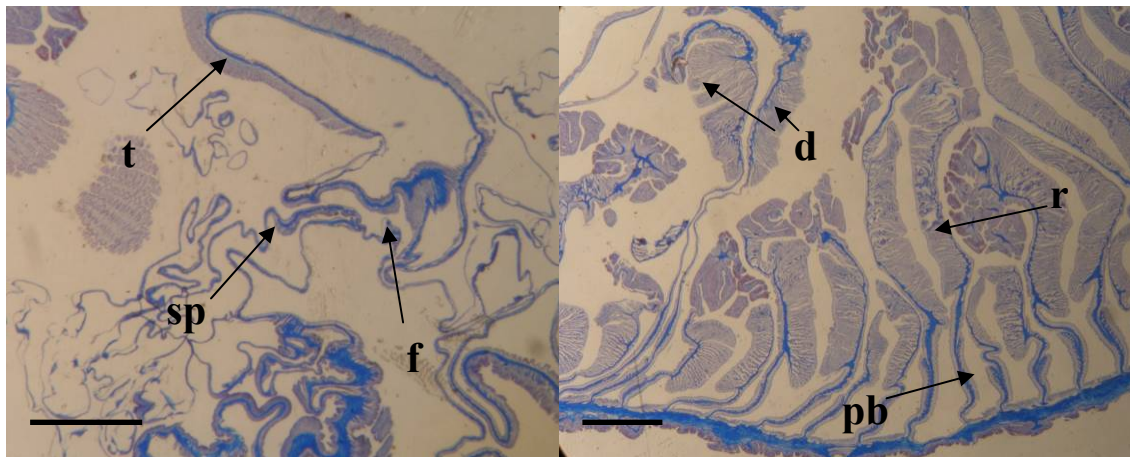
Appendix IV Figure 2 (A) Longitudinal section of well expanded *O. muscosa* (AM G17740) Scale = 1 mm. Sphincter muscle (sp), tentacle (t) and fosse (f). (B) Cross Section of *O. muscosa* (AM G147740) Scale = 2 mm. Retractor (r), parietobasilar muscles (pb) and directives (d).

Cnidom

Holotrichs, basitrichs, spirocysts, *p*-mastigophores and *b*-mastigophores. See Table 1. for distribution and range of cnidae and Figure 3 for images.

Appendix IV Table 1 Cnidae distribution and measurements for *Oulactis muscosa*.

Cnidae	Dimensions	n	N
Tentacles			
basitrichs I (A)	18.6–32 x 1.6–3.2 μm	60	3/3
basitrichs II	(16.8) 20–20.8 x 1.6–2.4 μm	10	1/1
spirocysts (B)	(14.4) 15.7–29.6 x 1.6–3.2 μm	60	3/3
Acrorhagi			
holotrichs I (E)	(29.6) 32.3–47 (62.7) x 2.4–6.9 μm	30	3/3
holotrichs II (F)	44–60 (64) x 5.6–8 μm	20	2/3
spirocysts (G)	(12) 14.1–21.6 x 1.6–2.9 μm	30	3/3
Marginal ruff			
basitrichs (C)	8.8–13.6 x 1.6–2.4 μm	30	3/3
Column			
basitrichs (D)	(11.8) 14.4–19.2 x 1.6–4.9 μm	30	3/3
Actinopharynx			
basitrichs (H)	(24.8) 25.5–36 x 3.2–4 μm	40	3/3
p-mastigophores (I)	(19.2) 22.4–28 x 4–8 μm	30	3/3
Mesenterial Filament			
basitrichs (J)	12–20.8 x 2.4–2.9 μm	30	3/3
b-mastigophores (K)	50.4–57.6 x 5.6–8.8 μm	10	1/3
p-mastigophores (L)	16–39.2 (42.1) x 2.4–6.4 μm	30	2/3

Note: dimensions of cnidae are length x width, n = total number of capsules measured, N = proportion of animals studies which contain that type of cnidae. Outlier capsule measurements are provided in brackets.
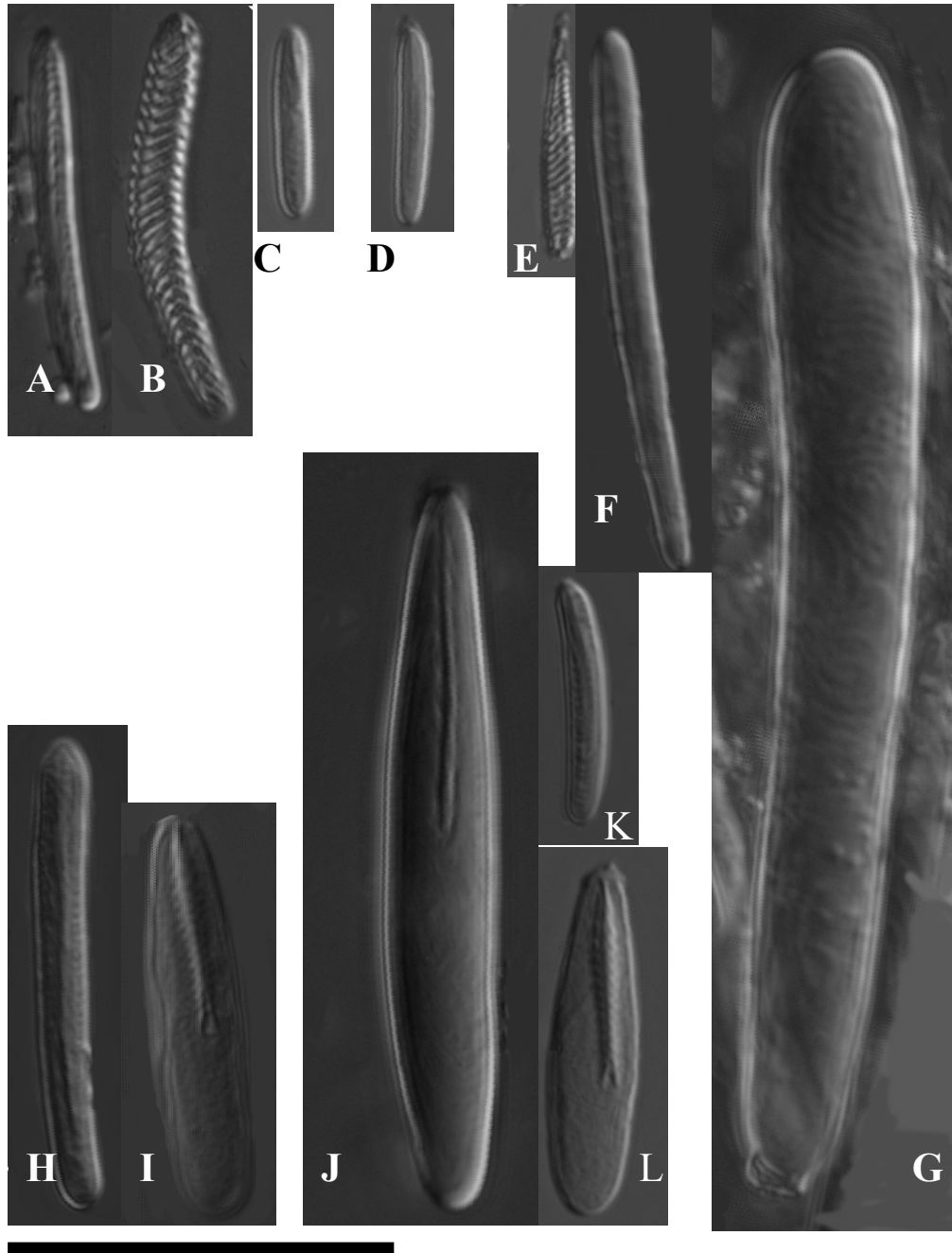
Scale = 30 μm

Appendix IV Figure 3 *Oulactis muscosa* cnidae (AM G17740). See Table 1 for key to letters.

Habitat and Geographic range

The known geographical range of *O. muscosa* in Australia extends from south eastern Queensland to Mallacoota, Victoria. *Oulactis muscosa* are located in rock crevices from the mid-tidal to low intertidal zone.

Appendix V - A preliminary investigation of the utility of ribosomal genes for species identification of Sea Anemones (Cnidaria: Actiniaria)

A preliminary investigation of the utility of ribosomal genes for species identification of Sea Anemones (Cnidaria: Actiniaria)

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ABSTRACT

The utility of the ribosomal DNA gene complex for species identification of Actiniaria was examined. The use of universal ribosomal PCR primers is problematic in this group due to the presence of algal symbionts. Universal primers were initially used to amplify a region containing partial 18S, complete ITS, 5.8S, ITS2, and partial 28S sequences from six sea anemone species. The development of two sea anemone specific primers for this region was necessary to avoid amplification of algal symbionts for a number of species. Complete sequences of the 18S–28S fragment were obtained from three species, ?*Anemonia* sp. (724 bp), *Heteractis malu* (670 bp) and *Stichodactyla haddoni* (734 bp); partial or non-overlapping sequences were obtained from *Entacmaea quadricolor* (480bp from 18S), *Macrodactyla doreensis* (523 bp: 300bp from 18S and 223bp from 28S) and *Oulactis muscosa* (556bp: 285bp from 18s and 271bp from 28S). Average sequence divergence among sea anemone species was approx. 24% indicating that this region may indeed be useful for species identification. However, unexpectedly low divergence recorded between two species in different genera, neither of which could be verified by histology due to specimen unavailability, indicated that traditional histological methods are still needed to confirm identification and certainly until such time that an rDNA database of sea anemone tissue has been established. ❑ *ribosomal DNA; sea anemone specific primers; universal primers*

In February 2005, the Australian Marine Sciences Association, SEQ Branch, hosted the Thirteenth International Marine Biological Workshop, The Marine Fauna and Flora of Moreton Bay, Queensland. Fieldwork was conducted over a period of three weeks and occurred in a variety of environments including off-shore reefs, small islands accessible only at low tide, piers, estuarine mouths and mud flats. A taxonomic paper documenting the species found is presented by Fautin *et al.* (2008, this volume). Of the more than 20 species that are now known from More-ton Bay, we obtained tissue from the following six species and genera of anemones to assess the usefulness of DNA in identification, and the potential for understanding phylogenetic relationships: ?*Anemonia* sp., *Heteractis malu*, *Stichodactyla haddoni*, *Entacmaea quadricolor*, *Macrodactyla doreensis* and *Oulactis muscosa*.

Species identification of sea anemones (Anthozoa: Actiniaria) can be difficult, especially in the field. The taxonomic key currently utilised, designed by Oskar Carlgren (1949), is based mainly on histological differences and therefore requires
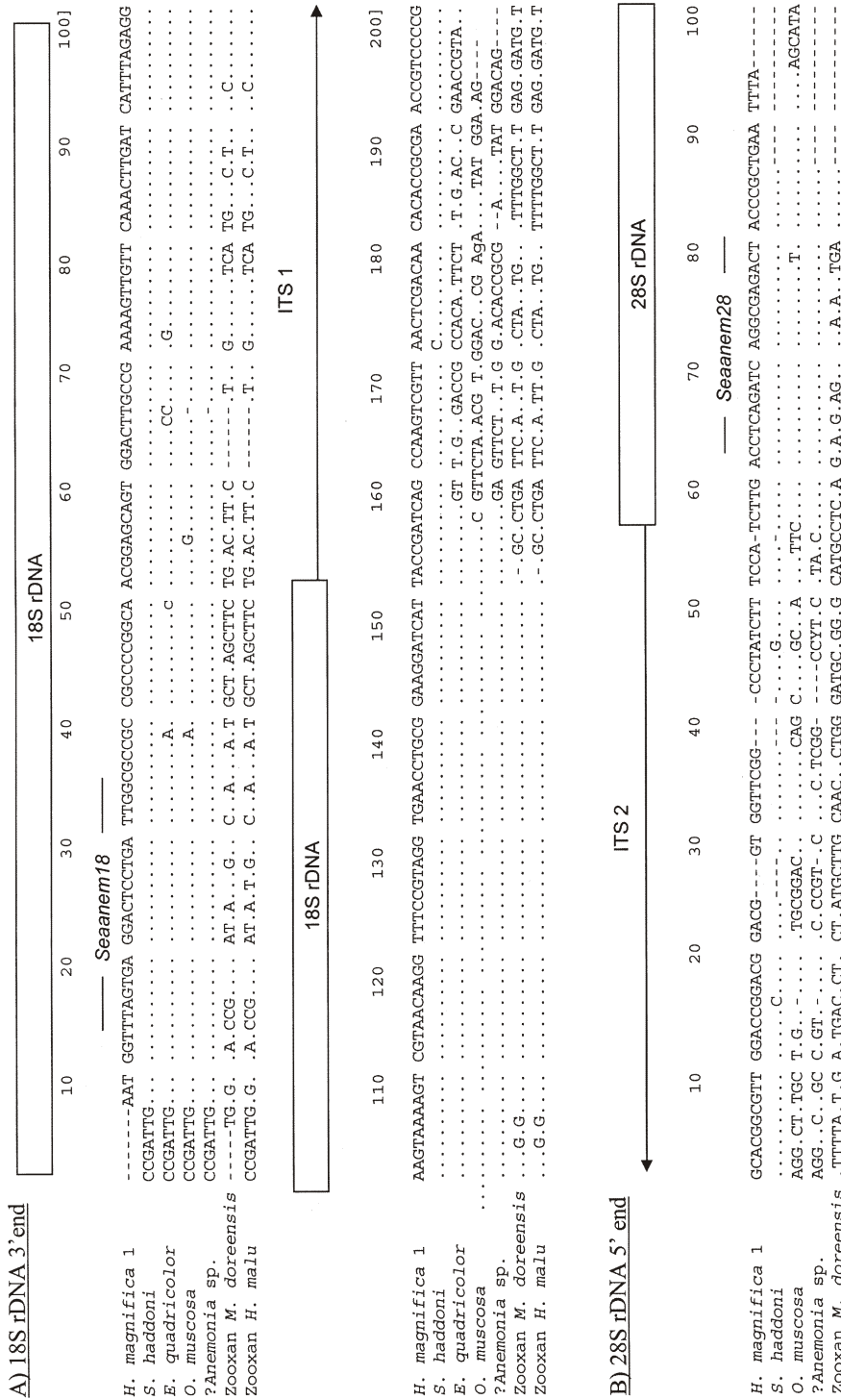
A) 18S rDNA 3' end

18S rDNA

— Seaanem18 —

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                 10          20          30          40          50          60          70          80          90          100]
H. magnifica 1   ------AAT GGTTTAGTGA GGACTCCTGA TTGGCGCCCGC CGCCCCGGCA ACGGAGCAGT GGACTTGCCG AAAAGTTGTT CAAACTTGAT CATTTAGAGG
S. haddoni       CCGATTG...  .........  .........  .........  .........  .........  ...CC....  ..........  ..........  .........
E. quadricolor   CCGATTG...  .........  .........  ...A....   ..C.......  .........  .G.......  ..........  ..........  .........
O. muscosa       CCGATTG...  .........  .........  ...A....   ...G......  .........  .........  ..........  ..........  .........
?Anemonia sp.    CCGATTG...  .........  .........  ......-..  .........  .........  --......  ..........  ..........  .........
Zooxan M. doreensis ----TG.G.  .A.CCG....  AT.A...G.  C..A...A.T GCT.AGCTTC TG.AC.TT.C  ------T. G........  TCA TG....C.T.  ..C......
Zooxan H. malu   CCGATTG.G.  .A.CCG....  AT.A.T.G.. C..A...A.T GCT.AGCTTC TG.AC.TT.C  ------T. G........  TCA TG...C.T.  ..C......
```

18S rDNA   ITS 1

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                 110         120         130         140         150         160         170         180         190         200]
H. magnifica 1   AAGTAAAAGT CGTAACAAGG TTTCCGTAGG TGAACCTGCG GAAGGATCAT TACCGATCAG CCAAGTCGTT AACTCGACAA CACACCGCGA ACCGTCCCG
S. haddoni       .........  .........  .........  .........  .........  .........  C......... ..........  ..........  ........
E. quadricolor   .........  .........  .........  .........  .........  ........GT T.G..GACCG CCACA.TTCT .T.G.AC..C GAACCGTA..
O. muscosa       .........  .........  .........  .........  .........  .........  C GTTCTA.ACG T.GGAC..CG AgA...TAT GGA.AG----
?Anemonia sp.    .........  .........  .........  .........  .........  .........  .GA GTTCT..T.G G.ACACCGCG --A...TAT GGACAG---
Zooxan M. doreensis ..G.G....  .........  .........  .........  .........  -.GC.CTGA TTC.A..T.G .CTA..TG.. .TTTGGCT.T GAG.GATG.T
Zooxan H. malu   ..G.G....  .........  .........  .........  .........  -.GC.CTGA TTC.A.TT.G .CTA..TG.. .TTTGGCT.T GAG.GATG.T
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B) 28S rDNA 5' end

ITS 2   28S rDNA

— Seaanem28 —

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                 10          20          30          40          50          60          70          80          90          100
H. magnifica 1   GCACGGCGTT GGACCGGACG GACG----GT GGTTCGG--- -CCCTATCTT TCCA-TCTTG ACCTCAGATC AGGCGAGACT ACCCGCTGAA TTTA------
S. haddoni       ....CT.TGC T.G...C... -........  ......G..  .........  .........  .........  ..........  .T....... ...........
O. muscosa       AGG.CT.TGC T.G..-..  TGCGGAC..  .....CAG C....GC..A ..TTC....  .........  ..........  ..T......  ...AGCATA
?Anemonia sp.    AGG..C..GC C.GT-....  .C.CCGT-.C ---CCYT.C .TA.C....  .........  .........  ..........  ---------  ---------
Zooxan M. doreensis .TTTTA.T.G A.TGAC.CT. CT.ATGCTTG CAAC..CTGG GATGC.GG.G CATGCCTC.A G.A.G.AG.. .A.A.TGA ..........  ---------
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Table 1. Moreton Bay sea anemone species used in the genetic analysis.

Collection Location	Latitude Longitude	Field Identification	Laboratory Identification	Genetic Source	Museum Reg. No.
Bird Island	27º 30′ S 153º 23′ E	*Entacmaea quadricolor* juv.	?*Anemonia* sp.	Whole animal	MTQ G58754
Flat Rock, N. Stradbroke I.	27º 24′ S 153º 33′ E	*Entacmaea quadricolor*	Not available	Tentacle	Whole spec. not coll.
Dunwich, flats in front of MBRS	27º 30′S 153º 24′E	*Stichodactyla haddoni*	Not available	Pedal disc & tentacle sample	Whole spec. not coll.
Shag Rock, N. Stradbroke I.	27º 24.85′S 153º31.59′E	*Heteractis malu*	*Heteractis malu*	Pedal disc & tentacle sample	MTQ G58749 QM Unreg.
Frenchmen's Beach	27º 25′ S 153º 32′ E	*Oulactis muscosa*	*Oulactis muscosa*	Pedal disc	MTQ G58756
Dunwich	27º 30′ S 153º 24′ E	*Macrodactyla doreensis*	*Macrodactyla doreensis*	Pedal disc & tentacle	MTQ G58748

collection of whole animals, which may not always be practical. Furthermore, histological analysis of sea anemones is time consuming and requires considerable expertise as some closely related species are almost impossible for the non specialist to identify, often resulting in incorrect taxonomic assignment (Stephenson 1928; Fautin 2000; Häussermann 2004).

Identification is further complicated by the fact that some species are virtually identical in appearance, distinguished by only one or two morphological features. For example, the two species *Heteractis malu* and *Heteractis crispa* are differentiated in the field on the basis of 1) column texture, which is firmer (leathery) in *H. crispa* than in *H. malu,* and 2) tentacle length, which is meant to be twice as long in *H. crispa* than *H. malu* (Fautin & Allen 1997). Both these characteristics can be misleading since the former is open to subjective interpretation if both species are not present side by side in the wild, while the latter may not necessarily be useful as the tentacles can be contracted at the time of collection/observation.

Alternatively, delineation of some species may be quickly achieved using appropriate molecular genetic methods (eg the Barcoding of Life initiative Hebert *et al.* 2003). However, previous genetic studies including sea anemone taxa have either only focused on questions pertaining to higher order anthozoan relationships (Won *et al.* 2001, Daly *et al.* 2003) or intraspecific population structure (Hunt & Ayre 1989). Numerous mitochondrial DNA

genes such as COI (Fautin & Smith 1997), COIII and 16S rDNA (Geller & Walton 2001) have been used to infer phylogenetic relationships among the Actiniaria. However, mitochondrial gene sequence divergences within and among anthozoan families, including sea anemones, has been found to be significantly lower than other marine invertebrate species (Shearer *et al.* 2002). Barcoding studies also discovered that mitochondrial DNA evolved too slowly in sea anemones and other cnidarians for mtDNA differences to be an informative indicator of species (Hebert *et al.* 2003). Interestingly, Shearer *et al.* (2002) also found that, unlike all other metazoan taxa, substitution rates in anthozoan nuclear genes are much higher than in mitochondrial genes and therefore may be of greater utility in terms of species identification. Indeed, a number of other studies have suggested that the nuclear ribosomal (rDNA) gene complex incorporating 18S, ITS1, 5.8S, ITS2 and 28S could be ideally suited to examining below genus level relationships within the Actiniidae (McCommas 1991; Odorico & Miller 1997). Most recently Acuña *et al.* (2007) used the ITS region of rDNA in addition to morphology to distinguish between three species within the genus *Aulactinia*.

Molecular studies of sea anemones can be potentially complicated by the presence of symbiotic algae or zooxanthellae in the anemone tissue (Shearer *et al.* 2005) and possibly tissue

consistency (Pinto *et al.* 2000). If species possess zooxanthellae they are generally found in the gastrodermal tissues (i.e. tentacles and oral discs), although in some species they can be distributed heterogeneously throughout their hosts, being rare in only the pedal disc region or mesenteric tissue layers (Fautin & Smith 1997; Häussermann 2004). Therefore DNA extractions can contain both the host and algal genomes, which may cause confounding results especially for sequence data generated using broadly conserved or `universal' primers (see Shearer *et al.* 2005). A study by Pinto *et al.* (2000) found tissue consistency to impinge on the success of extraction of DNA from sea anemones, due to hardness of tissue from being preserved in ethanol. They concluded that a slow and gradual digestion method was optimal for extraction.

Here we conduct a preliminary study to examine the utility of the rDNA gene complex in the identification of sea anemone species and test whether a known universal primer pair is sufficient for such studies or whether anemone specific primers will be required. Furthermore we use modern DNA extraction kits to see if previous problems associated with sea anemone DNA extraction can be circumvented.

MATERIALS AND METHODS

SPECIMEN AND TISSUE COLLECTION

Collection techniques included; removing anemones from rocks by chisel and hammer, scraping animals off rocks by fingernail or taking a small tissue sample from the animal in the wild for genetic analysis if identification was 100% positive in the field. Tissues for analysis were collected from twelve species (based solely on field identifications). Of these, six samples representing an initial five species were used in the genetic analysis (Table 1). Additional samples of *Heteractis malu* were collected from Shag Rock subsequent to the workshop.

In order to examine and minimise possible zooxanthellae contamination, small tissue samples of less than 5mm in length were excised from either the lower column/pedal disc or, where possible, separate tissue samples from both the tentacles and pedal disc region of each species were taken. All samples for genetic analysis were stored in 100% ethanol. Where whole specimens were collected, tissue samples were taken after animals were relaxed in magnesium chloride and before being preserved in 10% formalin: seawater. All ethanol preserved tissue samples were stored at −20°C until genetic analyses were performed. *Heteractis malu* specimens collected subsequent to the workshop were stored in 100% ethanol and kept at room temperature (approximately 21°C) only.

DNA EXTRACTION, PCR AND SEQUENCING

To test to the usefulness of modern DNA extraction kits with ethanol preserved sea anemone tissues, total genomic DNA was extracted from both tentacles and pedal disc tissues using DNeasy Tissue Kits (QIAGEN) as opposed to the far more labour intensive protocol of Pinto *et al.* (2000). Partial 18S rDNA, complete ITS1, 5.8S, ITS2 and partial 28S rDNA sequences were initially amplified using the primer pairs RA2 and ITS2.2 described by Wörheide (1998) RA2 is located in the flanking 3' end of the small subunit ribosomal gene (18S) and ITS2.2 in the 5' end of the large subunit ribosomal gene (28S). PCR amplifications were performed in 25 μl reaction volumes and contained to a final concentration: 1x Taq polymerase buffer, 2.5 mM MgCl$_2$, 0.2 μM each primer, 0.8 mM dNTPs and 0.75U of Taq polymerase. The use of the hot start polymerase HotMaster Taq (Eppendorf) required an initial denaturation at 94°C for 2 min prior to the commencement of the remaining cycle parameters; then followed 35 cycles of 94°C for 20 sec, 55–58°C for 20 sec, 65°C for 45 sec and a final extension 65°C for 5 min.

PCR products were gel purified using 'Perfect Prep' gel cleanup kit (Eppendorf) and forward and reverse sequencing reactions were carried out according to standard ABI PRISM dye-deoxy terminator sequencing protocols using Big Dye Terminator versions 1.1 and 3.1. Chromatographs were checked and all sequences were aligned using Se-Al v2.0a10 (Rambaut 1996). Estimates of sequence divergence including insertions (uncorrected p-distances) were calculated using the pairwise base distance function in PAUP* v4.0b10 (Swofford 2002). We verified the origin of the amplified sequence data by conducting a BLAST search in GenBank thus determining the phylogenetic affinity with sequences from other actiniarian or anthozoan species. Sequences

for this same region were also obtained from GenBank from two individuals of the species *Heteractis magnifica* (Accession no: AF050201 (*H. magnifica* 1) and AF050211 (*H. magnifica* 2)).

SEA ANEMONE PRIMERS

Based on the sequence results obtained from four of the six study species using the above described 'universal' primers and one of the *H. magnifica* sequences plus contaminating zooxanthellae sequences from the remaining two species (*Heteractis malu* and *Macrodactyla doreensis* — see Results), we designed two new primers. These primers were designed to be specific to sea anemones and located in regions of identical sequence among the sea anemone species (for which we had data) but mismatched the zooxanthellae sequences at 45–50% of sites (see FIG. 1). These two new primers *seaanem18S*: 5' TTA GTG AGG ACT CCT GAT TGG C 3' and *seaanem28S*: 5' AGT CTC GCC TGA TCT GAG G 3' lie within 50bp downstream from RA2 and ITS2.2 respectively. We tested the primers against the same six species used with the 'universal' primers. Amplification conditions, clean up and sequencing reactions with the new primers are identical to those described earlier.

RESULTS

DNA EXTRACTION

In contrast to Pinto *et al.* (2000) no problems were experienced extracting DNA from ethanol preserved sea anemone tissues using the DNeasy tissue kit. Prior treatment of the samples to remove ethanol was not required; nor did the tissues need to be homogenised in liquid nitrogen prior to the extraction process. Furthermore, total tissue digestion was completed within 1–3 hours at 55°C as recommended by the manufacture's protocol as opposed to the 72 hour period at 37°C used by Pinto *et al.* (2000).

UNIVERSAL PRIMERS

An 800bp (approximately) PCR fragment was successfully amplified from all six sea anemone species and all tissue types using the universal primers RA2 and ITS2.2. Readable sequence data of the fragment (including the 3' end of the 18S gene, full length ITS1, 5.8S gene and ITS2 and the 5' end of 28S gene) was obtained from only three of the six species (?*Anemonia* sp, *M. doreensis* and *S. haddoni*). Partial/non-overlapping

sequences were obtained from the remaining three species (*E. quadricolor*, *H. malu* and *O. muscosa*). Not all tissue types generated readable sequence data. For example, sequences obtained from the pedal disc tissues of *H. malu* and *M. doreensis* were unreadable with evidence of multiple sequences present in the chromatograph (Table 2). This result was unexpected given that the amplified PCR product revealed a clear single band. However, readable sequence data was obtained from the tentacles of those same two species. BLAST searches of all readable sequences (either complete or partial) revealed strong matches (90–97% identity) with other sea anemone and/or anthozoan species in GenBank for only four of the six study species (Table 2). The sequence data obtained from the tentacles from *H. malu* and *M. doreensis* however, matched with almost 99% identity to other symbiotic algae sequences (e.g. *Symbiodinium* sp.) indicating preferential amplification of the zooxanthellae DNA in each of these species. Interestingly, the sequence data obtained from both the pedal disc and tentacles of *S. haddoni* were identical and BLAST searches of these and that obtained from the tentacles of *E. quadricolor* revealed closest similarity to other anthozoan species indicating that the host DNA had preferentially amplified and/or that zooxanthellae are either not present or in high enough density to mask the host DNA in both these species.

SEA ANEMONE PRIMERS

Amplification success using our primers *seaanem18S* and *seaanem28S* varied from that seen with the universal primers. Approximately 750 bp were obtained from five of the six anemone species; no PCR product amplified from *H. malu* regardless of tissue source (Table 2). For the three species for which either tentacle and/or pedal disc tissues were available, amplification success varied from species to species. No PCR product was obtained from *M. doreensis* tentacle DNA; in contrast, product amplified from the tentacle DNA of *E. quadricolor* and both tissue types for *S. haddoni* (Table 2).

The lack of amplification success for *H. malu* was surprising given that sequence data from the congeneric species, *H. magnifica*, was used in the alignment from which the new primers were designed and that the regions of both the

Table 2. PCR and sequence results obtained from anemone tissues using both the `universal' primers and sea anemone specific rDNA ITS primers. Presence (+) or absence (–) of product is indicated.

Species	Tissue used in extractions	Preferential amplification and sequence obtained using `Universal' primers		Preferential amplification and sequence obtained using sea anemone specific primers	
		Anemone DNA	Zooxanthellae DNA	Anemone DNA	Zooxanthellae DNA
?*Anemonia* sp.	Column / Pedal disc	+	–	+	–
Entacmaea quadricolor	Tentacle	+	–	+	–
Heteractis malu	Pedal disc	+	+	–	–
	Tentacle	–	+	–	–
Macrodactyla doreensis	Pedal disc	+	+	+	–
	Tentacle	–	+	–	–
Oulactis muscosa	Column / Pedal disc	+	–	+	–
Stichodactyla haddoni	Pedal disc	+	–	+	–
	Tentacle	+	–	+	–

18S and 28S genes where these primers are located are identical among all the actiniarian genera (bar one site in *O. muscosa*), for which sequence data was available. In order to see if we could amplify a product for *H. malu* but avoid zooxanthellae DNA contamination, we tried the sea anemone primers in combination with the previously successful universal primers; using *seaanem 18S* paired with ITS2.2 and *seaanem28S* paired with RA2. Successful amplification from *H. malu* DNA from both pedal disc and tentacles was only obtained using *RA2/seaanem28S*.

Sequences, either partial or complete, obtained from ?*Anemonia* sp., *O. muscosa* and *S. haddoni* using the new sea anemone primers were identical to those obtained using the universal primers, which had previously been confirmed as originating from host anemone DNA rather than their algal symbionts. BLAST searches of complete sequences from *M. doreensis* and *H. malu* obtained using anemone specific primers indicated greatest similarity to other anemones. Hence the anemone specific primers had been successful in circumventing the problems of zooxanthellae contamination. Curiously, *E. quadricolor* did not return readable sequence data suggesting that further optimisation of the sequencing reaction for this species and these primers may be required. For

H. malu, sequence obtained with RA2 revealed no mismatches in the 3' region of the 18S rRNA gene where *seaanem18S* is located that would explain why this primer did not work on this species. Further experiments may be required to secure successful amplification with both anemone specific primers on this species.

In summary, complete or overlapping sequences of the 18S–28S fragment were obtained from only 3 species (?*Anemonia sp.* (724 bp), *H. malu* (670 bp) and *S. haddoni* (734 bp)). Although partial or non-overlapping sequences were obtained from *E. quadricolor* (480bp from 18S), *M. doreensis* (523 bp: 300bp from 18S and 223bp from 28S) and *O. muscosa* (556bp: 285bp from 18s and 271bp from 28S), they were excluded from subsequent analysis due to incompleteness.

SPECIES IDENTIFICATION

Among the three species for which full sequences were obtained (including the two *H. magnifica* sequences obtained from GenBank) estimates of sequence divergence ranged from 0.14% within *H. magnifica* up to 25.10% between *H. magnifica* 1 and ?*Anemonia sp.* (Table 3). The average level of sequence divergence among species was 23.84% indicating that this region may indeed prove to be useful for species identification in sea anemones. The exception

was the comparison between *H. magnifica* and *S. haddoni*, where the divergence averaged only 1.7% (Table 3). This result was somewhat unanticipated given that it is significantly lower than the level of divergence found among the congeneric *H. magnifica* and *H. malu* sequences (ave 23.92%) and is therefore suggestive of possible taxonomic misidentifications. Considering that *H. magnifica* or *S. haddoni* cannot be taxonomically verified for this study due to specimen/tissue unavailability, it highlights the importance of using genetics in conjunction with traditional taxonomic methods.

The potential utility of this region for species identification is also evident from the example of ?*Anemonia* sp., which was tentatively identified in the field as resembling a juvenile *Entacmaea quadricolor* collected from Bird Island (Table 1). While only partial sequences were obtained from the adult *E. quadricolor* collected off Stradbroke Island, comparison of the sequences between the two specimens clearly showed they were significantly different (approx. 18% sequence divergence over 480bp) and possibly therefore two different species. Later histological analysis revealed that the the Bird Island specimen was not *E. quadricolor* as originally identified but may be ?*Anemonia* sp., although the exact identity of this species still awaits final taxonomic confirmation.

DISCUSSION

The ribosomal DNA gene complex has proved highly successful for species identification across an incredibly broad range of taxonomic groups including plants (Chase *et al.* 2005), fungi (Ristaino *et al.* 1998; Iwen *et al.* 2002), digenean parasites (Nolan & Cribb 2005) and mosquitos

(Collins & Paskewitz 1996). It has even been used recently to identify commercial crustacean species from larvae collected in plankton surveys (Wang *et al.* 2006). In this study we investigated for the first time, the utility of this region for identification of sea anemone species and the potential problems of using universal primers in species, which contain algal symbionts.

While of a preliminary nature, our results showed high levels of sequence divergence among species using this region compared with divergence estimates an order of magnitude lower within a species indicating that it may indeed be ideal for assisting with sea anemone species identification. The questions at what taxonomic level and how useful this region may be for resolving phylogenetic relationships among sea anemone species was not the focus of this study but should certainly be investigated as more sequences become available. Acuña *et al.* (2007) used phylogenetic tools rather than estimates of sequence divergence to distinguish between different *Aulactinia* species and found extremely short branch lengths among individuals within a species compared to those between species.

The usefulness of conserved `universal' primers clearly depends on the species and tissue type available for analysis. However, as shown by the results obtained from *H. malu* and *M. doreensis*, extraction of `uncontaminated' host DNA from samples taken only from pedal disc tissues clearly should never be assumed. In order to guarantee that host DNA is amplified alone, use of primers specific to sea anemones are recommended; if not on their own then at least in combination with another universal primer. The extent to which the primers designed

Table 3. Estimates of sequence divergence among species for which the complete 18S–28S fragment was obtained (max 758bp). Sequences for *H. magnifica* obtained from GenBank. * Specimen collected subsequent to Workshop.

	?Anemonia sp.	*H. magnifica* 1	*H magnifica* 2	*H. malu*	*S. haddoni*
?*Anemonia* sp.	–				
H. magnifica 1	25.10%	–			
H. magnifica 2	24.93%	0.14%	–		
H. malu*	21.60%	23.84%	23.99%	–	
S. haddoni	24.42%	1.64%	1.77%	22.98%	–

for this study will work across all actiniarians remains to be seen. Further preliminary PCR testing using *seaanem18S* and *seaanem28S* on another seven species from Moreton Bay, and representing another seven actiniarian genera, proved highly successful with strong amplicons produced in all seven species. Only subsequent sequencing will confirm whether or not the host DNA has been successfully targeted.

Modern DNA extraction kits also seem highly useful for overcoming any difficulties associated with DNA extraction from ethanol preserved sea anemone tissues. Why we experienced so few problems compared with the earlier work of Pinto *et al.* (2000) is unclear. It may be that we were able to work with tissues from recently ethanol preserved specimens, rather than ones, that had been in ethanol for an extended time.

Finally, a number of aspects of this study reinforce the value of being able to combine histological analysis with genetic testing to irrefutably verify a species' identity, especially given the embryonic stage of developing genetic markers for this group. In the case of a supposed juvenile *E. quadricolor*, the genetic data strongly indicated an incorrect field identification, and a subsequent histological analysis proved this to be so, identifying it instead as a probable *Anemonia* species. Furthermore, the curious result showing much greater sequence divergence between the two *Heteractis* species than that detected between *Heteractis magnifica* and *Stichodactyla haddoni* cannot, frustratingly, be resolved further. While again indicative of possible misidentifications, the *H. magnifica* sequences available on GenBank are not associated with registered specimens and the *S. haddoni* cannot be analysed histologically as the whole animal was not collected from the field.

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