# A TAXONOMIC STUDY OF TROPICAL MARINE SPONGES (PORIFERA: DEMOSPONGIAE: HAPLOSCLERIDA AND PETROSIDA) USING MORPHOLOGICAL, CHEMICAL AND REPRODUCTIVE CHARACTER SETS

Thesis submitted by

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in July 1990

for the Degree of Doctor of Philosophy
in the Department of Marine Biology at
James Cook University of North Queensland

*Volume One of Two Volumes*

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J. Fromont

**26** July 1990

## ACKNOWLEDGEMENTS.

I thank my supervising committee particularly Professor P. Bergquist (Auckland University, New Zealand) for suggesting the topic and for critical comment and advice for the duration of the study. I thank Associate Professor C. Alexander (Dept. Marine Biology, J.C.U.) for constant advice and support, and comments on drafts of the thesis, and Dr. P. Murphy (Australian Institute of Marine Science) for guidance and comment on chemical aspects of the thesis. Special thanks to Associate Professor D. Hopley (Sir George Fisher Centre for Tropical Marine Studies) for generous provision of desk space and administrative support throughout the study. I also thank Dr. J. Baker (AIMS) for the initial organisation which facilitated this study.

Many scientists and fellow students provided assistance during this project. Staff and students of the Chemistry Department, Stanford University, California, gave help and friendship during my stay there. I thank Dr. C. Djerassi for providing funding for the visit. I particularly thank S. Kerr for expert guidance in the laboratory, for chemical analysis and sterol characterisations, and R. Kerr for critical comment and advice on sterol chemistry.

I thank the many dive buddies and field assistants who volunteered their time for this study especially Katrina Barratt, Anne Jackson, Kerrie Licastro, Yvette MacNeil and David Sutton. I am grateful to Orpheus Island Research Station personnel for use of the facilities, Zollie Florian for provision of microscopes and technical advice, Leigh Winsor for histological advice, and the Bioactivity Unit (AIMS) for some of the photographs used in the thesis.

Bill Williams, Brian McArdle and Martin Riddle provided advice and assistance with numerical analyses. David Sutton suggested many helpful comments on drafts of the thesis, Cathy Robinson advised on word processing and typed the tables, the proof reading team: John de Klerk, Ian Dight, Rundi Larsen, James Scandol, Jill St.John, David Sutton and Moya Tomlinson found the missing commas. Thankyou.

Finally, many thanks to staff and students of the Sir George Fisher Centre for their friendship and support.

**This thesis is dedicated to Muriel Fromont.**

## ABSTRACT.

This study was undertaken to determine whether the separation of sponge species (Phylum: Porifera, Class: Demospongiae, Subclass: Ceractinomorpha) into two orders, the Haplosclerida and Petrosida, was valid. To this end selected species from each order were collected from the Great Barrier Reef, Queensland, Australia. Their taxonomic position was compared using a number of character sets: morphological, chemical and reproductive.

The most useful morphological characters for taxonomic purposes were organisation of the internal sponge skeleton, its components and their quantities. These characters allowed separation of the sponge species examined into five families, and also distinguished sponges at the species level. Development of the ectosomal skeleton was found to differentiate genera, and microscleres were useful for distinguishing some species.

As a result of the morphological study four new species of Haplosclerida and three new species of Petrosida were described.

Analyses of sterol chemistry did not support clear separation of sponge species into two orders but were found to be useful at the species level. Sponge species were found to have both a qualitative and quantitative sterol fingerprint. Sterol complements of species remained remarkably constant with geographic locality and season. This character set provided evidence for a species complex within *Xestospongia muta*. Three specimens of this species contained significantly different sterol complements, and two of the specimens were more similar to other species in the data set (*Xestospongia n.sp.1* and *X. testudinaria*) than they were to each other. Results of this chemotaxonomic study suggest that most species currently classified in the same genus are generic complexes, as few species of the same genus had similar sterol complements. No cyclopropene or cyclopropane ring structures, previously thought to characterise species of the Petrosida, were found in the side chains of the fifty four sterols found from all species examined.

The reproductive biology of six species, three classified in the Haplosclerida and three in the Petrosida, was monitored for two to four years. The reproductive mode of these species clearly separated them into two orders. Species of the Haplosclerida were found to brood larvae which is the normal reproductive mode of the subclass Ceractinomorpha. Species of the Petrosida did not conform to the normal ceractinomorph pattern. The three species examined (*Xestospongia exigua, X. testudinaria* and *Xestospongia n.sp.1*) had separate sexes and broadcast eggs and sperm.

Reproductive characters also distinguished sponges at the species level. One species complex, in the Petrosida, was differentiated into two species (*X. testudinaria* and *Xestospongia n.sp.1*) when temporal isolation of spawning events was discovered. The spawning events of the two species were consistently separated by at least 15 days and diel timing of these events in both species was from 0700 hours. The mechanism, and possible environmental cues, whereby these species consistently separate their spawning events was not determined although *X. testudinaria* may have a semi-lunar periodicity. No lunar pattern was observed for *Xestospongia n.sp.1.* Larva of *Xestospongia n.sp.1* were seen in aquaria three days after the spawning event in 1989. This is the first reported observation of larva from a species of the Petrosida.

Two haplosclerid species found to brood female reproductive products did so in brood chambers situated basally in sponge individuals. This location may be a protective mechanism to avoid damage from turbulence or dehydration. Females of a third haplosclerid species, *Haliclona symbiotica*, had reproductive products aligned along the central axis of branches of adults. This location of sexual products is most likely a spatial confinement related to the symbiotic association this sponge maintains with the macroalga, *Ceratodictyon spongiosum*.

All species of both orders examined that were reproductively active released sexual products in spring and summer.

As a result of this study, one character set (reproductive mode) clearly supported the division of species into two orders. Sterol chemistry and morphological characters did not, but were useful at lower taxonomic levels. The discrepancies between higher taxonomic groupings, such as genus and family, suggests the taxonomy of these sponges is not currently resolved.

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# CHAPTER ONE.
# GENERAL INTRODUCTION.

Sponge taxonomy has traditionally placed great emphasis on skeletal structures and their organisation (Bergquist 1978), and a great diversity of skeletal types occurs within the Porifera (Simpson 1984). Of primary importance in demosponge taxonomy is architecture of the skeleton, for example, organised into axial, reticulate or plumose construction. There may be regional differentiation into specialised ectosomal (surface), choanosomal (internal), subectosomal (cortical) and basal components. Skeletons are constructed of either organic spongin fibre or of mineral elements, which are siliceous spicules or calcareous masses. Numerous elaborations of shape occur in structural spicules (megascleres) and smaller spicules (microscleres) that provide packing between megascleres or reinforcing of canal and surface membranes (Bergquist 1978).

A major problem in the taxonomy of the Porifera, as for other groups of marine invertebrates, is that the significance of skeletal differences is not clear. It is not known to what extent such differences are due to genotype or external factors (Simpson 1984). For instance, spicule geometry and distinctions in their size are characters which have been used in sponge taxonomy but spicule length and thickness may vary with environmental fluctuations. Palumbi (1986) found colonies of *Halichondria panicea* were stronger and stiffer in high wave force habitats because of increased spicule sizes and numbers. He suggests that these changes are structural engineering solutions to changing environmental stresses. Jones (1984) found spicule dimensions within species varied depending on differences in number of juvenile spicules in a sample, and several external factors such as amount of silica in the environment and seasonal variations in temperature and growth of sponge colonies. The shape of spicules is less variable than their size and both characters continue to be used in taxonomy. However, it is important to indicate the variation in size, within a species, when spicules are used as a taxonomic character (Bergquist 1978, de Weerdt 1985).

Few characters other than skeletal parameters have been found to be as stable within the Porifera, or as convenient for field identifications and species descriptions.

The taxonomy of the Order Haplosclerida (Porifera: Demospongiae) is made particularly difficult due to reduced spicule diversity and simplified skeletal structures (Wiedenmayer 1977a, Bergquist & Warne 1980, de Weerdt 1985). This order is defined by the presence of a reticulate skeleton that is always structurally simple and frequently isodictyal (triangular) in arrangement. There may be fibre and spicule development, and an ectosomal skeleton in addition to a choanosomal one, but no further differentiation of the skeleton into subectosomal or basal regions occurs. The spicules are geometrically simple, most frequently equiended and pointed (oxeote), without numerous elaborations of spines or knobs. There is no regional localization of different spicule

types or sizes, as is typical of other orders. Microscleres are absent or of one type (usually sigmas or toxas) and can be variable in occurrence and abundance in individuals (Bergquist & Sinclair 1973).

One consequence of the simple skeletal composition in this order is that there are differences of opinion regarding species delineations and relationships. This can be seen from a number of recent contributions to the taxonomy of the Haplosclerida which have established new families, resurrected or synonymised old ones, and provided information for establishment of a new order (Table 1.1).

A synopsis of changes to the taxonomy of the group since 1971 is outlined (Table 1.1). Wiedenmayer (1977b) examined palaeontological data and found a correspondence of morphological characters, including skeletal organisation, spicule types and ecological affinities, between fossil sponges of the family Heliospongiidae of late Palaeozoic age, the Devonian species, *Nepheliospongia avocensis*, and recent sponges such as *Petrosia* and related genera. He concluded that these three groups of sponges were an ancestral stock of the remainder of the Haplosclerida and established a new family, the Nepheliospongiidae, for them (Wiedenmayer 1977a). He located this family in the Haplosclerida, and included in it genera characterised by enhanced development of megascleres forming a dense skeletal architecture, and reduced spongin fibre development.

Bergquist (1980) raised the Nepheliospongiidae to ordinal status, the Nepheliospongida. This decision was based on evidence from palaeontological, chemical, reproductive and morphological data, working mainly with recent species. Bergquist's decision principally included the following factors. First, Wiedenmayer's findings showing separation of the Nepheliospongiidae from the remainder of the Haplosclerida since the Devonian. Second, the presence of novel sterols having cyclopropene rings in the side chain and found in three genera of the Nepheliospongida. Third, oviparity in *Xestospongia muta*, and finally, siliceous skeletons developed to the point where the sponges are hard and stony.

Following a different line of argument van Soest (1980) established three new families within the Haplosclerida, two of which, the Petrosiidae and Oceanapiidae, incorporated genera Wiedenmayer (1977a) had previously placed in the Nepheliospongiidae (Table 1.1). Van Soest refrained from using Wiedenmayer's family name as he considered the association of recent with fossil genera was made on insubstantial evidence, and concluded that they should not be incorporated into one family. Therefore the name Nepheliospongiidae, derived from a fossil genus, could not be used for a family of recent genera.

Van Soest (1980) did not support the ordinal status proposed by Bergquist (1980) for this group

of sponges. He felt if the reproductive character of ovipary was a primitive condition within the Demospongiae (Bergquist 1980) then it could not be used to define a group. His conclusion stems from a cladistic approach to taxonomy whereby monophyletic groups can only be defined by derived (advanced) character states (van Soest, in press). In addition, van Soest suggested there was no evidence that oceanapiids and petrosiids were more closely related to each other than to any other groups within the Haplosclerida. Therefore they were not sufficiently distinctive to be separated at the ordinal level.

Bergquist and Warne (1980) retained the order Nepheliospongida, with two families, the Nepheliospongiidae containing the same genera in van Soest's Petrosiidae, and the Oceanapiidae (Table 1.1).

Since 1980 there has been dichotomy in taxonomic works on the Haplosclerida, some authors following van Soest (1980) and maintaining all families within the Haplosclerida (van Soest & Sass 1981, Desqueyroux-Faundez 1981, de Weerdt 1985, de Weerdt & van Soest 1986, van Lent & de Weerdt 1986, Zea & van Soest 1986, Wiedenmayer 1989) while others follow Bergquist (1980) and use two orders, the Haplosclerida and Nepheliospongida (Hartman 1982, Desqueyroux-Faundez 1984, Desqueyroux-Faundez 1987a, b, Desqueyroux-Faundez in press, Kelly Borges & Bergquist 1988). Most of these publications were taxonomic descriptions and few offered reasons for selecting one or other of the classification systems.

Three authors (de Weerdt 1985, Hartman 1982 & Desqueyroux-Faundez 1987a, in press) have compared the two classification systems and have reached different conclusions.

Hartman (1982) and Desqueyroux-Faundez (1987a) uphold the separate order Nepheliospongida but call it Petrosida because of lack of resemblance between recent petrosiids and fossil nepheliospongids. Hartman includes only the family Petrosiidae within the Petrosida, leaving the Oceanapiidae within the Haplosclerida (Table 1.1). De Weerdt (1985) does not recognise the order Nepheliospongida principally for two reasons. This author considers ovipary is a primitive poriferan condition, and also that too few species have been examined for cyclopropene rings in sterol side chains for this character to distinguish the order.

Despite the studies, noted above, concerned with examination of the orders Haplosclerida and Nepheliospongida/Petrosida, no studies since Bergquist (1980) have looked at the relationship between the orders, or attempted to offer additional data to support, or refute, the ordinal separation. Therefore, the aim of this study, using species and genera from both orders, was to use a multidisciplinary approach incorporating diverse character sets to examine this taxonomic division.

The study was undertaken on fauna of the Great Barrier Reef, Australia, for two reasons. Firstly, the Haplosclerida, and Petrosida, constitute one of the most important and diverse orders of Demospongiae in tropical seas (Wiedenmayer 1977b), and consequently many species and genera are available for study. Secondly, species of these orders have been poorly described from this region, consequently, this provided the opportunity to describe a largely unknown fauna and contribute to the knowledge of sponge species found on the Great Barrier Reef. Some authors (Ridley 1884, Burton 1934, Pulitzer-Finali 1982) have incorporated haplosclerid species into general taxonomic works of the area, but none have looked in particular for species and genera from the Haplosclerida and Petrosida.

A multidisciplinary approach was selected for two reasons. The first of these, as noted above, is that few skeletal characters are available for taxonomic description of these groups, therefore few characters are available to provide new perspectives on the problem. The second reason is that Bergquist (1980) established the Nepheliospongida using a range of character sets which offered approaches for additional study. Three of these sets were included and developed further in this study. The four character sets examined were morphological and skeletal, chemical, reproductive, and enzyme electrophoresis.

For this study, the separation into two orders is followed. The second chapter describes morphological characters of species of Haplosclerida from three families, Haliclonidae (3 species), Niphatidae (8 species), and Callyspongiidae (3 species). The morphological character of primary importance is the organisation of the skeleton (Bergquist & Warne 1980) followed by description of spicule types and ratio of spicule to fibre in the skeleton.

The third chapter describes the same morphological characters for species of the Petrosida from two families, Petrosiidae (4 species) and Oceanapiidae (2 species).

In addition to these primary skeletal characters, other morphological characters, particularly useful for field identifications, are described. These are growth form, colour, surface appearance, texture, habitat description and species distribution.

The fourth chapter describes the sterol chemistry of twenty one species from both orders. Cyclopropene rings and derivative structures have previously been reported in the side chains of sterols of three genera *Calyx*, *Petrosia* and *Strongylophora* of the Petrosida (Bergquist 1980). In this study, the sterol complements of ten species of *Xestospongia*, two species of *Petrosia* (Petrosida) and Haplosclerid genera, *Haliclona* (2 species), *Niphates* (1 species), *Amphimedon* (2 species), *Gelliodes* (1 species), *Orina* (1 species) and *Callyspongia* (2 species), were compared. The sterol complement of each species was determined and the results analysed using presence/absence

and quantitative cluster analyses. The resulting dendrograms, and sterol structural parameters, were evaluated in relation to biological and morphological characters of the species. Sterol complements of replicate samples within some species were compared to determine variability of this character set within a species.

The fifth chapter addresses the reproductive biology of four species of Haplosclerida (two species of *Haliclona*, one of *Niphates* and one of *Amphimedon*), and three species of the genus *Xestospongia* in the Petrosida. The primary aim of this aspect of the study was to determine if further species of Petrosida were oviparous, as suggested by Bergquist (1980). As both these orders belong to a subclass that normally broods larvae (Lévi 1957), the Haplosclerida were examined to determine if they were viviparous (brood larvae). This character could not be treated in isolation. Although reproductive mode appears to be phylogenetically determined in sponges (Lévi 1957), few authors have reported reproduction in an environmental context or determined if other reasons exist for this process. These characteristics were monitored for one (*Amphimedon n.sp.2*), three (*Haliclona amboinensis*, *H. symbiotica*, *Niphates n.sp.* & *X. exigua*) or four (*Xestospongia n.sp.1* and *X. testudinaria*) years of the study.

A pilot study of two sympatric haplosclerid species, *Niphates n.sp.* and *H. amboinensis*, was undertaken to determine whether enzyme electrophoresis could be used to differentiate species and genera within this order. The results were favourable but time limitations prevented a thorough analysis using this technique. Preliminary data and analysis are included in Appendix II.

The final chapter is a discussion of the results of the three character sets, morphological, chemical and reproductive, and the implications for the taxonomy of the two orders Haplosclerida and Petrosida.

# CHAPTER TWO.
# SPECIES DESCRIPTIONS OF THE HAPLOSCLERIDA.

## INTRODUCTION.

This chapter addresses the taxonomy of the Haplosclerida and follows the classification system proposed for that order by van Soest (1980), but excludes the two families Oceanapiidae and Petrosiidae. Morphological characters of species from three families are described and presented sequentially: the Haliclonidae (3 species), Niphatidae (8 species) and Callyspongiidae (3 species).

The species descriptions here and in Chapter Three, on species of the Petrosida, emphasize morphological characters that can be recognised in the field, and describe the variability that could be expected in each species. The characters reported are skeletal organisation, spicule morphologies and sizes, growth form, colour, texture, surface appearance, habitat descriptions and species distributions.

The morphological characters of primary importance in this taxonomic treatment relate to the organisation of the skeleton. This provides the most readily observed distinctions between families, genera and species in the Haplosclerida and Petrosida (Bergquist & Warne 1980). The families of the Haplosclerida can be broadly defined using the following skeletal characters: architecture, spicule to spongin fibre ratio, regional differentiation, and type and size of spicules. The Haliclonidae have simple reticulate skeletons, usually isodictyal, a simple surface skeleton which may or may not be present, and megascleres of one type. The Niphatidae includes species with more robust fibre and spicule tracts than the Haliclonidae, the presence of a surface skeleton, and one type of megasclere. The Callyspongiidae have a less developed mineral skeleton, small or vestigial spicules, and an enhanced organic fibre skeleton with regional differentiation (see Glossary, Appendix I, for explanation of skeletal terms).

Large intraspecific variation in spicule sizes has been reported for sponges in the Haplosclerida, and an overlap in spicule size between closely related species may occur (Jones 1984). The form and size of spicules can help to describe a species if the extent of variability in size is reported. Consequently these characters were examined from as many specimens, from as many sites, as possible. Thinner forms of the principal spicules were found in many of the sponges investigated and are considered to be developmental stages of the principal forms. In sponges where they are common they were measured as a separate category, and therefore the mean size and range of the principal spicules is not skewed by the addition of measurements of less abundant thin forms.

Species of the Haplosclerida have few types of microsclere, but where they were present, they are described and the measurements provided. In this study microscleres are used as a character at the species level, and because of their uncommon occurrence, are not used to define generic and

family levels.

Sponge growth form is difficult to describe, but is useful for field identification. Therefore, photographs of all the species examined are provided in addition to descriptions. Some sponges have large variation in overall form, but can be easily recognised with complementary information on colour and consistency. Pigmentation may vary with microhabitat and depth, but the colour range of a species is often characteristic, and can be a useful guide to identification (Bergquist & Warne 1980).

Texture and consistency of sponges can be used for family allocation (Bergquist & Warne 1980), is relatively easy to describe, and in many cases is species specific (de Weerdt 1985). Consistency is a manifestation of the architectural components of the underlying skeleton. For example, callyspongiids with fibre skeletons are elastic, haliclonids with unispicular reticulation and light spongin development are fragile and compressible or brittle, and niphatids with multispicular tracts and enhanced fibre development are tough and elastic, or incompressible.

Appearance and texture of the surface may vary between geographic localities but usually a specific structural conformation can be recognised and is species specific. Smooth surfaces indicate the presence of a bounding membrane, while furry surfaces indicate the extension of vertical spicule brushes beyond the pinacodermal membrane.

Habitat descriptions and distributions are included as some species occur in specific environments while others have large geographic ranges and are found in many different habitats. The locality where a species occurs is described in general terms, such as fringing reef, reef front, depth and substrate type.

The data are presented in the following way. Definitions of each family and genus represented in the study are provided, and followed by a discussion of the species allocated to the family or genus. The species descriptions are followed by discussion of closely related species. A glossary of terms used in this Chapter and Chapter Three is presented in Appendix I.

## METHODS.

Sponges were collected on snorkel or SCUBA at various localities on the Great Barrier Reef, photographed in situ, and preserved in 70% ethanol.

Skeletal architecture and spicule morphology were examined using light microscopy. Spicules were prepared by boiling small pieces of sponge, including ectosome and choanosome, in concentrated nitric acid. This solution was centrifuged consecutively through two changes each of distilled water and absolute alcohol. The spicule extract was dried on a glass slide and mounted in a polystyrene

based mounting medium 'D.P.X.'.

The skeleton was prepared by cutting a representative section at right angles to the surface of the sponge, dehydrating it through an ascending ethanol series, clearing in toluene and infiltrating in paraffin wax at 56°C using an automatic tissue processor on a 9 hour cycle. The sponge tissue was infiltrated with paraffin under a vacuum of 635 mm Hg for 30 minutes prior to embedding in paraffin wax. Blocks were sectioned at 100 µm thickness with a Leitz base-sledge microtome, and section rolling was eliminated by placing filter paper, moistened with distilled water, on top of the block. Sections were placed on a glass slide smeared with egg albumin for adhesion, dried overnight at 60°C and decerated in two changes of xylene. They were mounted in either a low viscosity epoxy resin (Spurr's) polymerised overnight at 60°C, or more conveniently in D.P.X. and dried for 48 hours at 40°C. Liberal quantities of D.P.X. were required or air bubbles occurred in the sections. Sections of the surface skeleton were prepared in the same manner but cut parallel to the surface.

Illustrations of spicules and skeletons were drawn using an Olympus CH-2 microscope equipped with drawing apparatus, and measured with a calibrated micrometer eyepiece. Photographs of these characters were taken with an Olympus BHS microscope and an automatic camera system.

Type material and voucher specimens will be lodged in the North Queensland branch of the Queensland Museum, Townsville.

## SPECIES DESCRIPTIONS.

### ORDER HAPLOSCLERIDA TOPSENT 1928

**Definition. Demospongiae with a reticulate skeleton of primary tracts regularly connected by secondary tracts, both composed of spicules, spicule and fibre, or fibre. The skeletal pattern is characteristically isodictyal or rectangular. The megasclere complement is of one type, diactinal, usually oxeas. The microscleres, if present, are sigmas and/or toxas. There is no regional size differentiation of the spicules.**

### FAMILY HALICLONIDAE DE LAUBENFELS 1932

**Definition. Haplosclerida with a delicate skeletal organisation which is either a simple unispicular reticulation with spongin cementing the ends of the spicules, or a network of fibres in which spicules are incorporated. The primary tracts may become multispicular and emphasised but the reticulation of primary and secondary tracts is never as strongly developed nor as densely packed with spicules as in the family Niphatidae (cf. Fig. 2.3a,b with c-f). A dermal skeleton, if present, is a unispicular tangential reticulation. Megascleres are usually**

**oxeas and microscleres are sigmas and/or toxas.**

Microsclere-containing species are included in this family following Bergquist and Warne (1980) who described two species of the genus *Haliclona*, one with sigmas and one with toxas.

The design of the dermal skeleton is important because some authors recognise two closely related families, the Haliclonidae and Adociidae, which are distinguished by construction of the ectosomal skeleton. Species of the Adociidae, de Laubenfels 1936, have an ectosomal skeleton that is clearly distinct from the internal skeleton, and species belonging to the type genus *Adocia* contain extensive subdermal spaces which allow the surface skeleton to be easily detached (de Laubenfels 1950, Wiedenmayer 1977a, Bergquist & Warne 1980). These authors retained the Haliclonidae for species that lacked this special dermal skeleton. Bergquist and Warne (1980) retained the Adociidae because they found that by thorough examination of skeletons of the sponges they could distinguish a distinct dermal network in species of *Adocia*.

Wiedenmayer (1977a) maintained reservations about the separation of the Haliclonidae and Adociidae because the design and amount of surface specialisation between the families may intergrade, and this reservation is considered valid by van Soest (1980), Hartman (1982), de Weerdt (1985), and de Weerdt & van Soest (1986), who use only one family, the Haliclonidae, and include within it species that have surface specialisation as well as those that do not. Van Soest (1980) thought it doubtful that two genera as similar as *Haliclona* and *Adocia* could have developed separately in different families. He amended the diagnosis of the family Haliclonidae to include sponges which have an ectosomal skeleton and included in the family the genera *Adocia*, *Sigmadocia*, and *Gellius* among others.

No species of *Adocia* were examined in this study so no conclusion can be reached about the validity of this genus. Ectosomal skeletons are used as generic characters in other genera in this study, such as *Amphimedon* and *Niphates*, but are not considered to characterise families. Van Soest (1980) may therefore be correct in suggesting *Haliclona* and *Adocia* should not remain in separate families.

The Adociidae may prove to be a valid family, but the two species of *Sigmadocia* considered in this study lack the principal diagnostic feature of the family, a distinct ectosomal skeleton, and are placed in the Haliclonidae.

Genus *Haliclona* Grant 1835

**Definition. Sponges with a skeletal network which can be unispicular, spongin-reinforced or spicule reinforced, and which may have an ectosomal skeleton that is a tangential unispicular reticulation. Megascleres are usually oxeas and microscleres, if present, are sigmas or toxas.**

Type Species: *Spongia oculata* Linne, 1759

In this study two species previously located in the genus *Sigmadocia* are transferred to the genus *Haliclona* for three reasons. First, the presence of sigmas is not considered a generic character because of the variability in occurrence of microscleres in species of the order Haplosclerida. Second, the presence of a dermal skeleton in these species, which is an extension of the internal skeleton and not distinct from it, is not recognised as a generic character. Third, their choanosomal skeletons are isodictyal or rectangular, may be irregular, and do not differ from skeletons found in species of *Haliclona*.

The genus *Sigmadocia* de Laubenfels 1936, was established for species with a special dermal skeleton and sigmas as microscleres, but some species of this genus, for example *Sigmadocia caerulea*, have varied development of the tangential dermal skeleton such that in some parts of the sponge the tangential spicules are absent and the internal spicules project beyond the surface (Hechtel, 1965).

The genus *Sigmadocia*, following de Laubenfels 1936, has been located in the family Adociidae (Hechtel 1965, Wiedenmayer 1977a, Bergquist & Warne 1980) because of the presence of a special dermal skeleton. The type species of *Sigmadocia, Gellius texapatina*, should be examined to determine if the surface skeleton is distinct from the internal skeleton. If it is not distinct, like the species in this study, the genus should be transferred to the Haliclonidae and synonymised with *Haliclona*.

De Weerdt and van Soest (1986) synonymised the genera *Reniera, Adocia* and *Gellius* with the genus *Haliclona*. De Weerdt (1986) synonymised the above mentioned genera and *Toxadocia* and *Orina* with *Haliclona*. These synonymies are most likely related to the author's hypotheses that presence of an ectosomal skeleton and microscleres are primitive characters on which a phylogenetic classification cannot be based (de Weerdt 1985).

*Haliclona amboinensis* (Lévi 1961)

**Synonymy.**

*Gellius couchi* (Bowerbank) Topsent, 1897: p.471.
*Gellius amboinensis* Lévi, 1961: p.143; Desqueyroux-Faundez, 1981: p.49, fig.66.
*Sigmadocia amboinensis* Kelly Borges & Bergquist, 1988, p.146, pl.5e.

**Occurrence.** This species occurs in intertidal areas and reef flats around Magnetic Island (Fig. 2.1). It is found in cracks, crevices and beneath small boulders covered in *Sargassum* and occurs in sympatry with *Niphates n.sp.* As mentioned by Kelly Borges & Bergquist (1988), some specimens grow over *Psammaplysilla purpurea* which is a thin, slippery, brilliant yellow encrusting sponge that turns dark purple in alcohol often staining *H.amboinensis*.
**Description.** This is an encrusting species that may spread in solid ramose branches or as thick mats (Fig. 2.2a). Oscules are raised on small nodes or ridges on the upper surface.
**Colour.** Ice blue to sky blue changing to fawn internally, and fawn in alcohol with purple blotches if stained by *Psammaplysilla purpurea*.
**Texture.** Firm and incompressible. It is brittle and can be easily broken into chunks.
**Surface.** The surface is microscopically hispid and rough to the touch. It reflects light indicating some surface skeletal organisation. Oscules are up to 3 mm across with membranous tissue internally.
**Skeleton.** The skeleton is a regular to confused isodictyal reticulation with 6-10 spicules coaxially in the centre of the sponge. Towards the extremities the number of spicules decreases to a unispicular reticulation (Fig. 2.4b). The spicules are dense, the mesh spaces very small, up to 50 $\mu$m wide, although internal canals are 150-750 $\mu$m wide. There is no spongin fibre development in the specimens examined but Kelly Borges & Bergquist (1988) mention nodal spongin in their description. Sigmas occur throughout the membranes around the internal pores. The ectosomal skeleton is an extension of the choanosomal skeleton and is a single layer of spicules parallel to the surface with erect single spicules extending beyond them (Figs. 2.4c, 2.3a).
**Spicules.** (Table 2.1). Principal megascleres are thick sharply pointed oxeas, usually curved, occasionally straight. Strongylote and stylote modifications are found. Thin forms occur. Sigmas are abundant and are small c-shaped forms (Fig. 2.4d).
**Discussion.** The specimens from the Great Barrier Reef conform well to the redescription of the species by Kelly Borges and Bergquist (1988). *Haliclona amboinensis* has previously been placed in the genus *Gellius* by Lévi (1961) and Desqueyroux Faundez (1981), but this genus was considered poorly described and probably unrecognisable (de Laubenfels 1936), and has more recently been synonymised with *Haliclona* de Weerdt & van Soest (1986), de Weerdt (1986).
**Distribution.** Amboine, Vietnam, Papua New Guinea.

*Haliclona symbiotica* (Bergquist & Tizard 1967)

**Synonymy.**

*Sigmadocia symbiotica*, Bergquist & Tizard, 1967: p.183, fig.1, pl.4; Kelly Borges & Bergquist: 1988, p.146, pl.5a,b.

**Occurrence.** This species occurs intertidally and subtidally to 4 metres depth on inshore fringing reefs from Cape Tribulation to the Whitsunday Islands (Fig. 2.1). It is attached to rock platforms.

**Description.** (Fig. 2.2b) A ramose sponge with solid branches frequently anastomosing to form large spreading mats up to 1 metre across. The branches are up to 2 cm wide and the branching tips are usually bifurcate. The sponge is a thin encrustation that completely surrounds the red algae *Ceratodictyon spongiosum* in a symbiotic association (refer Price *et.al.* 1984). The bulk of the branches are algae tissue.

**Colour.** The sponge is dark green throughout and cream to fawn in alcohol.

**Texture.** Firm, incompressible and tough, but can be torn.

**Surface.** Microscopically hispid with the algal fronds visible just beneath the surface. One surface has small oscules up to 2.0 mm in diameter that are flush with the surface.

**Skeleton.** The skeleton of the sponge is between the algae fronds which form an anastomosing network of thalli 200-500 $\mu$m across (Fig. 2.5b). Between these thalli in spaces up to 300 $\mu$m wide is a spongin fibre reticulation. The fibres are 40-100 $\mu$m wide and cored centrally by 2-6 spicules. Between the fibres a unispicular isodictyal reticulation occurs and is also found at the surface.

**Spicules.** (Table 2.2). Thin curved and sharply pointed oxeas. Rare strongylote and stylote forms occur. Sigmas are abundant, c-shaped and centrangulate (Fig. 2.5c).

**Discussion.** Kelly Borges and Bergquist (1988) did not mention that sigmas in their specimens were centrangulate. In all other details these specimens conform with their description. This species may need to be synonymised with *Gellius cymiformis* (Esper) described by Vacelet and Vasseur (1971) as a sponge growing in symbiotic association with a red alga. The spicule characters of the two species are similar, and if the red alga associated with *G. cymiformis* is *Ceratodictyon spongiosum*, then it is likely that these sponge species are identical.

**Distribution.** New Caledonia, Torres Strait, Darwin, Great Barrier Reef, Australia, Papua New Guinea.

## Genus *Cladocroce* Topsent 1892

**Definition. Sponges with the primary tracts accentuated forming a dendritic skeleton composed of spongin encased spicules that occasionally anastomose. Between the primary tracts the remainder of the skeleton is an isodictyal reticulation typical of the family Haliclonidae (Fig.2.3b).**

Type Species: *Cladocroce fibrosa* Topsent 1892

Topsent (1892) did not include a description of the secondary skeleton in his definition of *Cladocroce*, but included the genus in the sub-family Renierinae because of the tendency of the skeleton to become renierid towards the surface of the sponge. The Renieridae has been synonymised with the family Haliclonidae (van Soest, 1980), and *Cladocroce* is subsequently placed in this family.

### *Cladocroce aculeata* Pulitzer-Finali 1982

**Synonymy.**
*Cladocroce aculeata* Pulitzer-Finali, 1982: p.109, fig.20,21.

**Occurrence.** This species occurs from Lizard Island (Cairns Section) to John Brewer Reef in the central section of the Great Barrier Reef (Fig. 2.1). It is found on midshelf islands and reefs in full light conditions and at depths greater than 10 metres. Localities where it was found are: reef front Palfrey Island, depth 12-15 metres, fringing reef North Point, 18 metres, fringing reef Coconut Beach, 12-15 metres, MacIlray reef, 19 metres (all sites near Lizard Island) and reef slope John Brewer Reef, 13 metres.

**Description.** An erect, hollow, tubular sponge with 6 to 8 tubes interconnected basally (Fig.2.2c). Large oscular cavities up to 30 mm in diameter are at the apex of the tubes, and the tube walls are 7 mm thick. A small ophiuroid was abundant over the external surfaces, and bases, of the sponges.

**Colour.** Salmon pink alive (Lizard Island) or mauve (John Brewer Reef) and fawn or cream in alcohol.

**Texture.** Firm, compressible and soft to the touch. Some specimens are more springy than others. The sponges are fragile and easily torn exposing skeletal fibres.

**Surface.** The tube walls are smooth, conulose and porous externally and smooth and porous internally. A thin membranous ectosome cannot be disconnected from the internal skeleton.

**Skeleton.** The ectosomal skeleton is a unispicular isodictyal reticulation that is an extension of the choanosomal skeleton. In the interior of the sponge the reticulation is formed by up to 6 parallel spicules (Fig. 2.6b), with spongin occurring at nodes of the reticulation. In addition, longitudinal primary spicule tracts encased in a thin layer of spongin extend from the base to the apex of the

tubes, and are visible when the sponge is torn. There is no reticulation of these tracts although they may anastomose and branch (Fig. 2.3b). They are 40-140 μm in diameter and cored by 7-35 spicules. Symbionts, probably cyanobacteria, are abundant in the choanosome.

**Spicules.** (Table 2.3.) Principal spicules are strongyloxeas constricted at the ends, occasionally pointed or with knobs or clubbed ends (Fig. 2.6c). Thin developmental forms occur and are less abundant than the principal forms.

**Discussion.** *Cladocroce aculeata* conforms well to the generic definition by having an isodictyal skeleton throughout the sponge strengthened by thicker primary tracts.

Five species of *Cladocroce* have been described worldwide, and the four species, other than *C.aculeata*, have been reported from the Northern Hemisphere in temperate waters such as the North Atlantic, Sea of Okhotsk and Sea of Japan.

**Distribution.** Great Barrier Reef, Australia.

## FAMILY NIPHATIDAE VAN SOEST 1980

**Definition. Haplosclerida with a multispicular reticulation of spongin fibres and spicule tracts and a dermal skeleton that consists of a reticulation of spongin fibres or spicule tracts. Megascleres are of one type, usually oxeas. Microscleres, if present, are sigmas or toxas.**

Van Soest (1980) established this family, removing the genera included in it from the Haliclonidae, because the sponges had a three dimensional (paratangential) ectosomal skeleton and a multispicular, fibrous choanosome.

The strengthening of the choanosomal reticulation to multispicular primary and secondary fibres with consistent and extensive development of spongin is an easily recognised character (Figs. 2.3c-f, 2.13a). The dense skeleton in the Niphatidae produces firm, incompressible or elastic sponges. Species of the Haliclonidae may have internal skeletons with multispicular primary tracts, for example *Cladocroce*, but they retain unispicular secondary tracts (Fig. 2.3a, b). This type of skeleton produces sponges that are softer and more compressible, or brittle. The development in the Niphatidae of a skeleton with increased spongin and mineral content, separates the Niphatidae from the Haliclonidae by extent of skeletal development.

Van Soest's description of the ectosomal skeleton as paratangential or three dimensional is difficult to visualise and interpret. Para means 'by the side of, beyond', and a tangential skeleton is one that is parallel to the surface. It is assumed that the three dimensions van Soest refers to are as follows: 1. spicules or tracts parallel to the surface, 2. tracts or spicules below the surface and at right angles to it, and 3. tracts or spicules beyond the surface and at right angles to it. In some species the ectosomal skeleton intergrades with the internal skeleton, for example in species of *Amphimedon* in this study (Figs. 2.3d-f, 2.13a). In this case a distinctive three dimensional ectosomal skeleton is difficult to distinguish from a tangential ectosomal skeleton intercepted by primary tracts of the internal skeleton. Therefore the generic definitions in this study refer to an ectosomal skeleton but do not attempt to describe it in three dimensions.

The principal character used to define the family Niphatidae in this study is the development, internally, of a fibre and spicule reticulate skeleton. Van Soest's use of this family is upheld, but no additional characters to further differentiate it from the Haliclonidae were found in the Great Barrier Reef sponges examined.

The two genera *Niphates* and *Amphimedon* are distinguished solely on the construction of their ectosomal skeletons. *Niphates* has thick primary tracts that extend beyond the surface and *Amphimedon* has primary fibres, less strongly developed at the surface than *Niphates*, that extend to the surface or slightly beyond it (cf. Fig. 2.3c-f, 2.13a). The difference between extension of primary tracts to the surface, or beyond it, as a generic trait could not be evaluated as only one species of *Niphates* was described in this study. More species of this genus need to be examined to evaluate this character.

Four of the eight species examined from this family belong to the genus *Amphimedon*, one to the genus *Niphates*, one to the genus *Gelliodes*, and two to the genus *Siphonodictyon*.

**Genus *Niphates* Duchassaing & Michelotti 1864**

**Definition. Sponges in which the ectosomal reticulation is dominated by erect tufts of well developed choanosomal primary tracts. Microscleres, if present, are sigmas.**

Type Species: *Niphates erecta* Duchassaing & Michelotti 1864

Rare sigmas have previously been included within the definition of this genus (Wiedenmayer, 1977a, van Soest, 1980) but the definition is here extended to include *Niphates n.sp.*, which consistently contains sigmas.

Previously, *Gelliodes* was the only genus in the family Niphatidae to contain sigmas, but this genus has a characteristic skeletal structure of accentuated primary and reduced secondary fibres (Fig. 2.13b). *Niphates n.sp.* does not have this specialised skeleton and therefore does not fit within the generic definition of *Gelliodes*. In this study, the skeletal character of reduced secondary tracts is considered diagnostic of *Gelliodes*. The presence or absence of sigmas is not considered a reliable generic character.

*Niphates n.sp.* is a difficult species to allocate to a genus. It has an internal skeleton with enhanced fibre and spicule development characteristic of the family Niphatidae and a well developed surface skeleton. The primary fibres supporting the surface skeleton are strongly developed and support a well developed tangential surface skeleton that is isodictyal and unispicular (Fig. 2.3c). This contrasts with the surface skeletons of species of *Amphimedon* (Fig. 2.3d-f, 2.13a) where the primary tracts and tangential surface reticulation are not strongly developed. This species is placed in the genus *Niphates* because of robust primary tracts at the surface supporting a well developed isodictyal skeleton. The ectosomal skeleton of this species produces a smooth surface, except surrounding the oscules, not characteristic of species of *Niphates*, which usually have hispid or roughened surfaces as a consequence of protruding primary fibres (van Soest 1980).

This may not be the correct generic allocation for this species, but it does not fit well in any other genus. It does not have slender supporting tracts at the surface seen in species of *Amphimedon*, or long and narrow internal meshes that characterise the related genus *Cribrochalina*. It may be necessary to establish a new genus for this species, but this option is not adopted here. Too few related species were examined to assess the reliability of this surface skeletal character as diagnostic for a genus.

*Niphates n.sp.*

**Occurrence.** Found in shallow waters to depths of 4 metres on fringing reef flat, on the sides or tops of coral outcrops beneath *Sargassum* (Fig. 2.2e). This species is abundant as small colonies in Geoffrey Bay, Magnetic Island (Fig. 2.1).

**Description.** A small ramose encrusting sponge, the colonies are up to 5 cm high and 7x7 cm in extent (Fig. 2.2d, e).

**Colour.** Green in life and fawn in alcohol.

**Texture.** The sponge is firm, compressible, elastic and tough.

**Surface.** Oscules 2-4 mm diameter are at the centre of small erect lobes 0.5-2.0 cm apart. The oscules have a raised collar 1 mm high of erect spicules. The surface is smooth and glossy (Fig. 2.2d).

**Skeleton.** The internal skeleton is a wide meshed fibrous reticulation with rounded or triangular meshes and primary tracts 100-500 µm apart (Fig. 2.3c, 2.7b). Primary fibres are 104-146 µm wide and cored by up to 5 spicules, the secondary fibres are 20-50 µm wide and cored by 2 or 3 spicules. Single oxeas and sigmas occur between the fibres without orientation. The primary fibres extend to the surface of the sponge, and beyond around oscules, and support a distinct surface skeleton which is an isodictyal reticulation (Fig. 2.7c).

**Spicules.** (Table 2.4). Short fat oxeas with occasional strongylote or stylote modifications, straight or curved centrally with pointed ends. Some of the spicules have central thickenings but this does not occur in all specimens (Fig. 2.7d). Less common thin oxeas occur, and microscleres are centrangulate sigmas.

**Discussion.** *Niphates n.sp.* is characterised by its thick encrusting habit, green, glossy surface, ectosomal isodictyal reticulation, and presence of centrangulate sigmas.

This species is similar to *Gelliodes carnosa* Dendy (1889) in colour and surface characteristics but *Niphates n.sp.* never achieves the size of *G. carnosa* and the flabelliform or tubular branching habit. *G. carnosa* is 11 to 17 cm tall (Desqueyroux-Faundez, 1984) while specimens of *Niphates n.sp.* are never greater than 5 cm and remain thickly encrusting. Both species have an isodictyal network of oxeas at the surface but *G. carnosa* has abundant multispicular primary fibres in the choanosome and reduced secondaries. *Niphates n.sp.* has thick primary fibres that are not accentuated and form part of a skeletal reticulation characteristic of the genus *Niphates*.

## Genus *Amphimedon* Duchassaing & Michelotti 1864

Synonym *Pachychalina* Schmidt 1868

**Definition. Sponges with an optically smooth surface due to a dermal membrane supported by slender choanosomal primaries that barely protrude the surface (Figs. 2.3d-f, 2.13a). The surface may be microscopically hispid if the primary tracts protrude beyond it. An ectosomal tangential reticulation is present. Microscleres, if present, are sigmas.**

Type Species: *Amphimedon compressa* Duchassaing & Michelotti 1864

The definition of this genus has been extended to include microscleres as *Amphimedon n.sp.3*, from the Great Barrier Reef, consistently contains sigmas and conforms skeletally to the definition of the genus *Amphimedon*. The presence of sigmas in this case is a good specific character.

### *Amphimedon viridis* Duchassaing & Michelottl 1864

**Synonymy.**

*Amphimedon viridis*, Duchassaing & Michelotti, 1864: p.81, pl.16, fig.2,3; van Soest, 1980: p.29, pl.4, fig.3, text.fig.10; Desqueyroux-Faundez, 1984: p.778, fig.4, 46 & 48.

*Haliclona (Amphimedon) viridis*, Wiedenmayer, 1977a: p.84, pl.12, fig.1,2, text.fig.113.

**Occurrence.** This species occurs from Lizard Island to the Whitsunday Islands (Fig.2.1). It is found in shallow water in lagoonal areas and on reef flats or slopes at depths of 3-4 metres. It grows firmly attached to hard substrata, usually limestone or dead coral. Colonies occur most on slightly silted fringing reefs, where there is a prevalence of soft corals, small *Porites* bommies and stands of the branching corals *Acropora* and *Montipora*. This species was found in the following localities: Palfrey Island, shallow reef flat to 2 metres depth, Blue Lagoon, Lizard Island to depths of 5 metres, Geoffrey Bay, Magnetic Island 3 metres, Brampton Island, 4 metres, Carlisle Island, 6 metres.

**Description.** A thickly encrusting to ramose sponge approximately 2 cm thick with slightly raised oscules 2-4 mm across occurring along the upper surface ridge (Fig. 2.2f, 2.8a) and spaced 1-1.5 cm apart.

**Colour.** The sponge is olive green alive and fawn in alcohol.

**Texture.** The specimens are very firm, slightly compressible and elastic.

**Surface.** The surface is microscopically hispid and porous and the ectosomal skeletal pattern is visible.

**Skeleton.** The choanosomal skeleton is a dense fibrous irregular reticulation with small mesh spaces 100-300 µm and occasional large internal pores 400-1000 µm (Figs. 2.3d, 2.9b). The primary fibres, cored by approximately 10 spicules, are 50-160 µm wide. The widest fibres are fasciculate. The secondary fibres are 20-50 µm wide and cored by 5-10 spicules. Many interstitial spicules occur and form a loose reticulation between the fibres and around pore areas. The reticulation becomes more regular at the surface and spicules of the primary fibres extend the sponge surface upwards and cause a faint hispid texture. Sub-dermal spaces are 20-500 µm across. Some spicules are tangential to the surface and form a reticulation (Fig. 2.9c), and others at right angles to the surface, extend slightly beyond it (Fig. 2.3d).

**Spicules.** (Table 2.5). Short fat oxeas with stepped or pointed ends. Less common thin developmental forms occur (Fig. 2.9d). The spicules vary considerably in dimensions between sponges collected from different depths and from different localities across the continental shelf.

**Discussion.** These specimens agree well with the descriptions of Wiedenmayer (1977a) and Desqueyroux-Faundez (1984) in surface detail, skeletal architecture and length of megascleres but the spicules of the Great Barrier Reef specimens are slightly thicker.

Wiedenmayer's comments of consistency in life as softly spongy and limp differs from Great Barrier Reef specimens which are firm and elastic. The Great Barrier Reef specimens contain more spongin fibre development than noted by Wiedenmayer or Desqueyroux-Faundez and this accounts for their firmer consistency. These differences may mean the Great Barrier Reef specimens do not belong to *A. viridis*. Until type material can be examined the specimens are assigned to this species because of similarities in skeletal details described above*.

**Distribution.** St. Thomas, Florida, Bimini, Bermuda, West-Central Pacific, Puerto Rico, Indian Ocean, New Caledonia.

* Type material of *A. viridis* (BM 1928.11.12.35a) was examined prior to final acceptance of this thesis. The specimens from the Great Barrier Reef do not conform to the Type material and will be given a new species name for publication.

*Amphimedon n.sp.1*

**Occurrence.** Found from Lizard Island in the north to the Whitsunday Islands in the south at depths of 10-15 metres on reef slopes, attached to rocky substratum (Fig. 2.1). This species is not abundant.
**Description.** An undulating spreading fan or erect lamellate sponge (Fig. 2.8b, c) 5 mm wide at the thickest point which is the short stem of attachment, and 2 mm thick at the uppermost edge of the fan.
**Colour.** Pale pink or mauve in full sunlight but parts of the sponge in shade are fawn.
**Texture.** Slightly compressible and spongy.
**Surface.** Reflects light and appears shiny and smooth but is faintly hispid. The internal skeletal reticulation is visible at the surface and produces a round meshed pattern. Small oscules 1 mm across are on slightly raised nodes at the centre of the sponge.
**Skeleton.** The internal skeleton is a regular spongin fibre reticulation that is rectangular or isodictyal and cored by 1-5 spicules (Fig. 2.10b). The mesh size is small, the primary and secondary fibres spaced 80-150 $\mu$m apart. The primary fibres are 20-35 $\mu$m wide, the secondaries have a greater range of widths and are 10-35 $\mu$m across. Spicules are also dispersed interstitially. Superficially the spicules of the primary tracts extend to, or slightly beyond, the surface, and the secondary fibres are parallel to the surface (Fig. 2.3e, 2.10c).
**Spicules.** (Table 2.6). Principal megascleres are thin curved oxeas with long tapering points (Fig. 2.10d). Rare stylote modifications occur. The specimen from inshore waters, Carlisle Island, had longer and thicker spicules than those from the specimen from mid-reef waters at Lizard Island (see Table 2.6).
**Discussion.** This species is characterised by its erect lamellate growth form and fine surface patterning. Its regular reticulate skeleton with small meshes and almost equally sized primary and secondary fibres are also characteristic of the species.
*Amphimedon n.sp.1* is similar in skeletal design to the Northern Hemisphere species *Haliclona simulans* but the two species differ considerably in external shape; encrusting or branching in *H. simulans*, and spreading or lamellate in *Amphimedon n.sp.1*. The oxeas of *H. simulans* are thicker than those in *Amphimedon n.sp.1.* and the secondary fibre is unispicular in *H. simulans* and multispicular in *Amphimedon n.sp.1.* The surface skeleton of the former is an isodictyal unispicular reticulation lacking the fibre development at the surface of *Amphimedon n.sp.1.*
Ridley (1884 p.410) describes a lamellate sponge from Torres Strait as *Reniera sp.* and this sponge is similar to *Amphimedon n.sp.1* in external morphology and skeletal detail. Differences between the species are the secondary fibres are unispicular in *Reniera sp.* and the oxeas are considerably larger (175 x 7.9 $\mu$m) than in *Amphimedon n.sp.1.*

*Amphimedon n.sp.2*

**Occurrence.** Mid-shelf reefs and islands from 10 to 20 metres depth in full light. This species has been found at the following localities: John Brewer Reef, back reef slope, 12-16 metres; Rib Reef, 15 metres; North Point, Lizard Island, back reef slope, 20 metres; MacIlray Reef, near Lizard Island, front and back reef slopes, 10-19 metres (Fig. 2.1).

**Description.** Tall erect lamellate and/or coalescing branches (Fig. 2.8d) up to 1 cm thick and 30 cm tall, sometimes looking like flattened organ pipes.

**Colour.** Red-brown alive with a thin maroon band approximately 1 mm thick inside the ectosome and a fawn interior. The maroon band is caused partially by a sponge pigment and partly by a cyanobacterial symbiont (Battershill, pers. comm.). When the sponge is dying it exudes a pink dye from this region. Cream throughout in alcohol.

**Texture.** Furry to the touch, slightly compressible and elastic.

**Surface.** Smooth but microscopically hispid surface which looks like down or fur. Small oscules up to 2 mm in diameter occur on sponge edges, raised ridges or nodes.

**Skeleton.** The internal skeleton is fibro-reticulate with densest fibre development in the centre of the sponge and dense mesohyl development everywhere except for subdermal spaces (Fig. 2.11b). The primary tracts cored by 4-8 spicules and 40-150 $\mu$m in diameter are sinuous and can be fasciculate. The secondary tracts, 20-150 $\mu$m in diameter, and cored by 2-4 spicules, may also be fasciculate. The mesh spaces are 200-450 $\mu$m wide. Primary tracts of spicules, in narrow fans, extend the sponge surface. The superficial skeleton is supported by primary spicule fans and by sparse tangential spicules (Fig. 2.3f).

**Spicules.** (Table 2.7). Long slender oxeas usually centrally curved, generally evenly tapering to long points but also with stylote and strongylote modifications. The axial canal is frequently visible (Fig. 2.11c).

**Discussion.** *Amphimedon n.sp.2* is characterised by its gross morphology of erect lamellate branches, red brown colour when alive, and dense mesohyl throughout its skeleton. No other described species of *Amphimedon* have these characters.

*Amphimedon n.sp.3*

**Occurrence.** Abundant small sponges that are found in a broad range of habitats, particularly on reef slopes in full light, but also under overhangs. The species is found on patch reefs, shallow reef crests on front and back reefs, and in lagoons. This species was seen at the following localities: Palfrey Island, 12 metres, North Point Reef, 9 metres, Hicks Reef, 12 metres, Eagle Island, 9 metres, Lizard Head, 14 metres, Blue Lagoon, Lizard Island, 10 metres, South Island, 3 metres, MacIlray Reef, 9 & 15 metres, all sites are near Lizard Island (Fig. 2.1).

**Description.** Small globular sponges that can be flat, pancake-shaped, or form small cushions up to 2 cm tall with a diameter of 1-2 cm (Fig. 2.8e, f).

**Colour.** Mauve alive, cream or fawn in alcohol.

**Texture.** The sponges are slightly compressible and elastic and can easily be torn.

**Surface.** Subsurface meandering parallel ridges which are interspersed with gaps give a convoluted or brain-like appearance to the surface. The ridges are hispid, the gaps have tiny oscules 1 mm in diameter, and a fine membrane connects the ridges (Fig. 2.8e).

**Skeleton.** The choanosome has a fibrous reticulate skeleton with dense plumo-reticulate fibre centrally (Fig. 2.12b, 2.13a). Primary fibres are 83 µm wide and cored by 2-4 spicules, and the secondaries, 20-45 µm across, are cored by 1 or 2 spicules. Choanosomal meshes are 100-200 µm and occasional internal cavities are up to 800 µm wide. Spicules also occur haphazardly throughout the choanosome. The primary fibres extend to the surface and spicule ends protrude slightly beyond a dermal membrane which spreads between the primary tracts and is supported by tangential spicules (Fig. 2.12b, 2.13a).

**Spicules.** Smooth straight or slightly curved oxeas with sharply pointed, faintly stepped ends (Fig. 2.12c). Mean: 139 x 4.5, Range: 122-153 x 3.0-5.3 µm. Less common thin oxeas occur, Mean: 126 x 2.3, Range: 94-140 x 1.6-2.6 µm. Microscleres are elongate C-shaped sigmas, Mean: 15.9, Range: 13.0-16.9 µm.

**Discussion.** This species is characterised by its external morphology, consistent external colour alive and intricate surface patterning. Internally it has a rectangular meshwork of primary and secondary fibres, and abundant sigmas.

*Amphimedon n.sp.3* is most similar to *Gelliodes incrustans* Dendy (1905) in external colour and spiculation, but differs in skeletal development and gross morphology of the sponge. *G. incrustans* is a thin encrustation or flat cushion (0.7 cm, Dendy, 1905; 0.8 cm, Wiedenmayer, 1989) while *Amphimedon n.sp.3* always forms small raised cushions 2 cm tall. A skeletal slide of the Type material (BM 1907.2.1.33a) clearly shows that the skeletons of the two species are distinctive. The Type material lacks the dense, central plumoreticulate skeleton found in the Great Barrier Reef species and has thinner primary and secondary fibres.

## Genus *Gelliodes* Ridley 1884

**Definition. Sponges with a skeletal organisation where multispicular primary fibres or tracts predominate and interconnecting secondary fibres are reduced in abundance (Fig. 2.13b). Between the tracts is a unispicular isodictyal or rectangular reticulation. Megascleres are usually oxeas, microscleres are sigmas. The dermal skeleton is dominated by protruding tufts of the primary and secondary tracts of the choanosome and the surface is hispid or spiny as a result.**

Type Species: *Gelliodes fibulata* (Carter 1881)

The genus *Gelliodes* is similar, in enhanced development of primary tracts, to the haliclonid genus *Cladocroce*. However, the skeleton of the former species has stronger development of the primary tracts, the texture is much coarser and the consistency incompressible.

### *Gelliodes fibulata* (Carter 1881)

**Synonymy.**

*Axos fibulata* Carter, 1881: p.383, pl.18,fig.4

*Gelliodes fibulata* Ridley, 1884, p.427, pl.XXXIX fig.1, pl.XLI fig.bb; Ridley & Dendy, 1887: pl.47, pl.12 fig.2; Topsent, 1897: p.470; 1932 p.114; Hentschel, 1912, p.393; Babic, 1922: p.234, pl.8, fig.4; Lévi, 1961: p.141. fig.16; Wiedenmayer, 1977a: p.95; Desqueyroux-Faundez, 1981: p.744, fig.56, 117; 1984: p.780, fig.6, 51, 52, 58 & 61; Kelly Borges & Bergquist, 1988: p.151 pl.6a.

**Occurrence.** On fringing reef, Sir Charles Hardy Islands, reef slope at 14 metres depth, growing on the sides of coral bommies, and at North-East Reef, Orpheus Island at a depth of 7 metres (Fig. 2.1).

**Description.** Erect or horizontally spreading solid branches (Fig. 2.14a) with a firm central core, that may anastomose. The Orpheus sponge was growing inside a *Millepora* colony.

**Colour.** Bright blue alive and fawn in alcohol.

**Texture.** Firm, tough and slightly compressible.

**Surface.** Prickly and hispid, covered with spines up to 10 mm long which have smaller spines extending at right angles. Oscules are at the bases of the spines and are not easily seen.

**Skeleton.** The choanosomal skeleton is fibro-reticulate with numerous spicules densely packed into primary fibres up to 400 µm in diameter. In the centre of the sponge the skeleton is plumo-reticulate and has a ladder-like reticulation peripheral to the primary fibres (Fig. 2.15b). Secondary tracts are 50-200 µm across. The primary tracts extend beyond the surface forming the long spines and secondary tracts form the smaller spines. Interstitial spicules are abundant, irregularly organised, and can form narrow longitudinal tracts of 3 to 5 spicules (Fig. 2.13b, 2.15b).

**Spicules.** (Table 2.8). Principal megascleres are long thin oxeas, thinner developmental forms occur and microscleres are sigmas (Fig. 2.15c).

**Discussion.** The Great Barrier Reef specimens conform to the redescription of the species by Kelly Borges & Bergquist (1988).

**Distribution.** Northern Australia, New Caledonia, Indonesia, Vietnam, Philippines.

## Genus *Siphonodictyon* Bergquist 1965

**Definition. Sponges with a cryptic habit always burrowing into calcified substrata, such as dead coral, and producing fistules which are the only part of the sponge visible in the field. Spicule tracts support the fistules; the choanosomal skeleton is isotropic and the texture is pulpy. Copious mucus is secreted by the endosome. Spicules are oxeas with mucronate, stepped or rough ends. There is reduced spongin fibre development.**

Type Species: *Siphonodictyon mucosa* Bergquist 1965

Rützler and Stone (1986) synonymised the genus *Siphonodictyon* with *Aka* de Laubenfels (1936) because of similarities between the spiculation of *Aka labyrinthica* Hancock and *S. obruta* Rützler. However, Bergquist (*in litteris*) retains the genus *Siphonodictyon* because the type species, *S. mucosa*, does not conform to the description of the genus *Aka*. The genus *Siphonodictyon* is retained in this study, following Bergquist, because the type material of *Aka, Acca insidiosa*, Johnson 1899, was not available for examination. Therefore the synonymy of the two genera cannot be addressed.

It is difficult to determine the correct family allocation of this genus on the basis of skeletal morphology. The skeleton of the fistules is reticulate, frequently round meshed, consisting of thick primary tracts of spicules that run parallel to the longest axis of the fistules and secondary tracts that extend toward the surface. The ectosomal skeleton of the fistules is an erect spicule palisade and/or a tangential reticulation (Figs. 2.13c,d, 2.16b, 2.17b). In contrast, the choanosomal skeleton is isotropic and unispicular (Figs. 2.13e, 2.16c, 2.17c). There may be some aggregation of spicules into tracts but these are not common in the choanosome.

Bergquist (1965) placed the genus in the Adociidae and reported that it was closely related to *Phloeodictyon (=Oceanapia)* except that it lacks a bark-like dermal region and spongin development, produces mucus, has a cryptic habit, and brushes of oxea in the dermal skeleton. Rützler (1971) followed Bergquist and retained this genus in the Adociidae.

Wiedenmayer (1977a) placed *Siphonodictyon* and *Oceanapia* in the family Nepheliospongiidae (= Petrosiidae & Oceanapiidae) agreeing with Bergquist (1965) that the genera were closely related. Hartman (1982) and Rützler and Stone (1986) follow Wiedenmayer and locate both genera in the family Oceanapiidae (refer Chapter Three).

The two genera *Siphonodictyon* and *Oceanapia* have pulpy choanosomes, produce fistules and have oxeas as megascleres (and strongyles in the case of *Oceanapia*). The fistulose skeletons are very similar, both consisting of a reticulation of spicule tracts which extend into a tangential skeleton at the surface with some supporting spicule brushes (cf. Figs. 2.12c, d, 3.2e). *S.mucosa* is distinctive among the four species of these genera, examined in this study, because of a superficial spicule palisade (Fig. 2.12c).

The principal differences between *Siphonodictyon* and *Oceanapia* are organisation of the choanosomal skeleton, development of spongin fibre and development of an ectosomal skeleton in species of *Oceanapia*, and an excavating habit in species of *Siphonodictyon*. Species of *Oceanapia* have extensive development of spicule tracts in the choanosome (Fig. 3.2f); parts of the skeleton may be poorly organised, producing a pulpy effect, but spicule tracts are always present as a structural support. These species have spongin fibre development not found in species of *Siphonodictyon*, and an ectosomal skeleton that is well developed, often forming a thick crust (Fig. 3.7a).

The difficulty with relating the genus *Siphonodictyon* to other genera is its cryptic growth habit. It is difficult to interpret which aspects in the design of the skeleton have been reduced because of habitat. The sponges of this genus occur inside coral heads and the fistules extend into the water column as the sole means of water exchange. The presence of fistules could be a response to survival in a cryptic habit and a polyphyletic character, not necessarily meaning that sponges which have fistules are closely related. The mucus production appears to be related to killing coral polyps and protecting the larvae during settlement (Rützler 1971). The lack of spongin development and pulpy interior may be related to the habitat of the sponge. It may not require a skeleton for support as it is enclosed and protected inside a coral head. In addition, it is not likely to contain any form of dermal skeleton, for protection or as a support system, when its surface tissue is not in direct contact with the external environment.

*Siphonodictyon* has been located in the Niphatidae by van Soest (1980), Kelly Borges & Bergquist (1988), and this study. Although it lacks the multispicular reticulation of spongin fibres and spicules in the choanosome characteristic of this family, these characters are found in the fistules. Study of additional characters, such as reproductive biology and biochemistry, is required to determine its correct familial position.

*Siphonodictyon mucosa* Bergquist 1965

**Synonymy.**

*Siphonodictyon mucosa* Bergquist, 1965: p.158,fig.20a,b, Rützler, 1971: p.2,fig.1,10a,pl.1, Kelly-Borges & Bergquist, 1988: p.154.

**Occurrence.** This species is found in shallow water to 2 metres depth in bays on the leeward side of Orpheus Island (Fig. 2.1).

**Description.** The sponge burrows into rock and coral and only erect black fistules are visible above the substratum. These are up to 5 cm high, 0.7 cm diameter and the fistule walls 1 mm thick. The choanosome is within a cavity 5 mm inside the coral head in most specimens examined (Fig. 2.14b). The interior of the sponge can only be collected by breaking open the coral head.

**Colour.** In life black throughout and in alcohol brown or black.

**Texture.** The fistules are firm and brittle, the choanosome soft and pulpy. Both parts of the sponge exude mucus when collected.

**Surface.** Very finely hispid.

**Skeleton.** The fistules contain a reticulate skeleton and have an erect spicule palisade at the surface (Figs. 2.13c, 2.16b). The choanosome has dense mesohyl with no regular organisation of the spicules (Fig. 2.16c).

**Spicules.** (Fig. 2.16d). Oxeas in two size categories, thick principal forms, Mean: 174 x 6.1, n=10, Range: 159-185 x 4.9-7.5 µm and thin forms, Mean: 165 x 2.2, n=10, Range: 156-179 x 1.3-3.9 µm. The axial canal is frequently visible.

**Discussion.** The spicules of the specimens examined are shorter than those in published descriptions but in all other characters the sponges are identical. This species is recognised by its burrowing habit, black coloration, erect fistules and production of mucus.

**Distribution.** Palau, Indonesia, Papua New Guinea.

***Siphonodictyon coralliphagum* Rützler 1971 *forma typica***

**Synonymy.**

*Siphonodictyon coralliphagum forma typica*. Rützler, 1971: p.5-6, fig.4,5,9,10, pl.3,4,8,9.

**Occurrence.** Pandora Reef, 10 metres depth on back fringing reef and Orpheus Island, Pioneer Bay, 20 metres on a back fringing reef (Fig. 2.1).

**Description.** The species is a boring sponge found within dead coral and visible only by the erect hollow tapering fistules 4 to 6 cm high extending above the substratum (Fig. 2.14c).

**Colour.** White fistules and cream sponge body both alive and after preservation in alcohol.

**Texture.** Very soft, moist and compressible internally, like dense wet bread. The fistules are compressible, brittle and easily broken.

**Surface.** The fistules are smooth and microscopically hispid.

**Skeleton.** The fistule walls consist of a reticulate skeleton composed of 30 to 60 spicules packed into tracts 100-300 µm wide (Figs. 2.13d, 2.17b). The tracts become thinner and more closely spaced towards the outer surface of the wall and at the surface make up a loose isodictyal reticulation of 1-6 spicules. The choanosomal skeleton is a very loose network of spicules (Figs. 2.13e, 2.17c) in vague tracts without orientation. These tracts are 50-125 µm across and form round meshes approximately 100 µm across. There is faint spongin fibre development.

**Spicules.** (Table 2.9). Oxeas with rounded or stepped ends and terminating in a short point (mammiform Fig. 2.17d). The axial filament through the centre of the spicules is frequently visible (Fig. 2.17d). Thinner forms occur but grade into the main size category of oxeas in the specimen from Pandora Reef.

**Discussion.** Rützler (1971) describes four forms of this species, two of which lack fistules, *forma obruta* and *incrustans*, and *typica* and *tubulosa* possess them. *Forma typica* has single conical chimneys or tubes and small oxeas (142.1-156.3 x 5.0-6.4 µm; 129-142.9 x 2.2-2.7 µm). *Forma tubulosa* has clusters of ectosomal tubes and oxeas with dimensions of 165.9-169.3 x 6.1-6.9 µm and 144.5-152.2 x 2.2-3.2 µm. The Great Barrier Reef specimens most resemble *forma typica* in having single fistules and similar sized oxeas. They differ slightly, in colour, from Rützler's description; their fistules were white alive and after preservation and their choanosome creamy yellow changing to fawn after preservation. Rützler describes the fistules of *S. coralliphagum* as deep yellow, lemon yellow to whitish yellow and the choanosome beige yellow to yellow tan with bright yellow embryos. This is the first record of this species from the Great Barrier Reef.

**Distribution.** Jamaica and West Indies.

## FAMILY CALLYSPONGIIDAE DE LAUBENFELS 1936

**Definition. Haplosclerida with an internal skeleton consisting of a reticulation of spicules and spongin fibre; the spongin is always well developed while the spicules may be sparse or absent. The megascleres are small, usually very thin or vestigial oxeas or strongyles that core the fibres and may occur interstitially. Microscleres are rare but if they occur are toxas. Some genera incorporate foreign detritus into the skeleton.**

### Genus *Callyspongia* Duchassaing & Michelotti 1864

Synonyms *Siphonochalina* Schmidt 1864; *Spinosella* Vosmaer 1885.

**Definition. Sponges with an internal skeleton formed of a reticulation of spongin fibres with a spicule axis. A specialised dermal skeleton may be of two forms, either a tangential reticulation of size differentiated primary, secondary and sometimes tertiary tracts with an organisation that is distinct from the internal skeleton, or a peripheral condensation where the skeletal organisation is an extension of the internal skeleton but the mesh is more compact. Megascleres are oxeas or strongyles. Microscleres are absent.**

Type Species: *Callyspongia fallax* Duchassaing & Michelotti 1864

Two genera, *Siphonochalina* and *Spinosella*, which are synonymised with *Callyspongia* in this study, have previously been synonymised or separated from *Callyspongia* depending on the characters considered relevant for generic diagnoses.

Dendy (1890) considered the genus *Siphonochalina* was for smooth tubular sponges and *Spinosella* for spiny tubular sponges but questioned whether this was a diagnostic character sufficient to separate two genera.

Wiedenmayer (1977a), using ectosomal skeletons as a distinguishing character, retained *Spinosella* as a genus but synonymised *Siphonochalina* with *Callyspongia*. He concluded that *Callyspongia* species had peripheral condensation at the surface, while species allocated to *Spinosella* had surface skeletons formed by a tangential reticulation of size differentiated fibres. In addition, these genera were distinguished by the organisation of their internal skeletons. *Callyspongia* species had a ladder-like reticulation without fascicules and species of *Spinosella* had a disorientated internal skeleton with fasciculate primary fibres.

Bergquist & Warne (1980) synonymised *Spinosella* and *Siphonochalina* with *Callyspongia* because they disagreed with separating these genera on account of differences in their surface skeletal organisation. They found that dermal skeletons often vary considerably and the two types of skeleton can be found in the same species or individual, depending on stage of growth or prevailing environmental conditions. They observed variable surface skeletons in two New Zealand species, *C. ramosa* and *C. fistulosa* and examination of the type species of *Callyspongia*, *C. fallax*, showed this species had a dermal skeleton intermediate between the two types of construction referred to by Wiedenmayer (1977a) (Bergquist & Warne 1980).

Van Soest (1980) concluded that differences in construction of dermal skeletons are of minor importance and did not use them as criteria for distinguishing genera. He divided the genus *Callyspongia* into two subgenera based on features of the internal skeleton previously suggested by Wiedenmayer. Van Soest (1980) recognised three types of skeletal organisation in contrast to Wiedenmayer's two. Van Soest used the subgenus *Callyspongia* for sponges with a regular choanosomal reticulation and the subgenus *Spinosella* for sponges with fasciculate primary fibres. He retained *Siphonochalina* at the generic level for sponges with an irregular internal skeleton in conjunction with an irregular surface tangential reticulation not divided into distinctive primary and secondary fibres.

De Weerdt & van Soest (1986) examined material of the type species of *Siphonochalina*, *S. coriacea*, and found that it conforms to the generic description of *Callyspongia*, therefore *Siphonochalina* was synonymised with *Callyspongia*.

In this study, the genus *Spinosella* is synonymised with *Callyspongia* for the following reasons. First, because the type material of *Callyspongia*, *C. fallax*, has a dermal skeleton that is intermediate between the two types considered diagnostic for *Siphonochalina* and *Spinosella* (Bergquist & Warne 1980). Second, the dermal skeleton of some species and individuals varies depending on stage of growth or prevailing environmental conditions (Bergquist & Warne,1980) and third, the two characters described by van Soest (1980), a regular choanosomal skeleton in *Callyspongia* compared with fasciculation of primary fibres in *Spinosella*, both occur in *Callyspongia aerizusa* (Table 2.10).

The genus *Siphonochalina* is synonymised with *Callyspongia* because the type species, *S. coriacea*, conforms to the generic description of *Callyspongia* (de Weerdt & van Soest 1986), and because the first two reasons given in the paragraph above also apply here.

The three species of *Callyspongia* described here from the Great Barrier Reef all have some fibre fasciculation in their internal skeletons although the extent of this character differs between species.

*C. confoederata* has fasciculate primary and secondary fibres, *C. pseudoreticulata* has fasciculate primary fibres and *C. aerizusa* has fasciculation only at the bases of the surface conules (Table 2.10, Fig. 2.13f, 2.19b, e). The choanosomal skeleton of *C. confoederata* is irregular while those of *C. aerizusa* and *C. pseudoreticulata* are regular (Fig. 2.13f, 2.19b, e). The dermal skeletons of the three species also differ. *C. confoederata* and *C. aerizusa* have tangential surface skeletons consisting of size differentiated fibres and *C. pseudoreticulata* has peripheral condensation (Fig. 2.19a. c. f).

***Callyspongia confoederata*** **(Ridley, 1884)**

**Synonymy.**

*Tuba confoederata*, Ridley, 1884: p.400.

*Siphonochalina confoederata*, Lendenfeld, 1887: p.803.

*Spinosella confoederata*, Topsent, 1897: p.479, pl.XIX, fig.20; Hentschel, 1912: p.400; Desqueyroux-Faundez, 1981: p.744, fig.57, 118.

*Callyspongia confoederata*, Burton, 1934: p.541; Lévi, 1961: p.144; Bergquist, 1969: p.65; Desqueyroux-Faundez, 1984: p.788, fig.10, 74-79.

**Occurrence.** Found on mid-shelf and outer reefs at the base of shallow reef slopes in depths of 7-10 metres. This species occurs in full light between *Porites* bommies and *Acropora* stands on sand flats. Rib Reef 7 metres, Davies Reef, 10 metres, Day Reef, 10 metres (Fig. 2.1).

**Description.** A large erect fan or tubular sponge up to 17 cm high by 13 cm across (Fig. 2.14d), the thickness of the sponge is 0.5 cm - 0.8 cm becoming thinner at the apex.

**Colour.** Maroon to brown alive; fawn in alcohol.

**Texture.** The sponge is coarse, firm, compressible and requires some force to tear.

**Surface.** The surfaces are microscopically hispid and differentiated with strong spines, 0.5 cm long, and pores on one side, and only pores on the other. Both surfaces feel rough. The internal fibres protrude longitudinally to form slightly raised ridges and extend beyond the top of the sponge in a palisade 0.5 cm tall (Fig. 2.14d). In tubular specimens the central oscular aperture is 0.5 cm across at its apex.

**Skeleton.** The skeleton is dense fasciculate fibres up to 650 μm across, forming meshes 300-700 μm apart, with the fibres centrally cored by up to 8 spicules (Fig. 2.18b). Thinner secondary fibres form a reticulation around the primary fibres and are 100-150 μm wide, fasciculate, and sparsely cored with 3 to 4 spicules. This secondary skeleton branches in a fine network of tertiary fibres, 10-50 μm across, forming meshes 100-150 μm wide with the fibres centrally cored by 1-2 spicules. The mesohyl is packed with dark brown pigment cells (Fig. 2.18b). The surface skeleton is a tangential reticulation of fibre differentiated into primary and secondary fibres and forming triangular, square or polygonal meshes (Fig. 2.19a). The primary fibres are 60-120 μm wide and the mesh spaces 400-500 μm wide. Secondary fibres are 30-60 μm across and tertiary fibres 15-30 μm. All fibres in the surface skeleton are sparsely cored by spicules.

**Spicules.** (Table 2.11). Very thin degenerate oxeas with blunt points and a wide axial canal (Fig. 2.18c).

**Discussion.** This species has most recently been redescribed, using specimens collected from New Caledonia, by Desqueyroux-Faundez (1984). Desqueyroux-Faundez, incorporating data from four authors, listed skeletal measurements for the species. The Great Barrier Reef specimens have thin spicules whose measurements conform to those of Bergquist (1969) and Desqueyroux-Faundez (1984), but are thinner than in the type material described by Ridley (1884). The tertiary superficial skeleton in the Great Barrier Reef specimens is fibrous without spicules, but is spiculose in the specimens described by Ridley (1884). In other respects the Great Barrier Reef specimens fit the species description.

**Distribution.** Amboine, Great Barrier Reef, Australia, New Caledonia, Torres Straits, West Australia.

***Callyspongia aerizusa*** **Desqueyroux-Faundez 1984.**

**Synonymy.**

*Callyspongia aerizusa*, Desqueyroux-Faundez, 1984: p.803, fig.18, 21, 129-136, 157-162.

**Occurrence.** Found in high sediment areas with fine silt at the base of reef slopes, Pioneer Bay, Orpheus Island at depths of 13-17 metres, and Thursday Island at 3 metres depth (Fig. 2.1). It is not abundant.

**Description.** An erect sponge, either a fan or tube with spines, 0.7 cm long, on one side of the fan or on the outside edge of the tube, and a smooth but porous surface internally in the tube or on the opposite surface of the fan (Fig. 2.14e).

**Colour.** Fawn or blue-green alive and fawn in alcohol.

**Texture.** The sponge is soft, compressible, spongy and easily torn.

**Surface.** Prickly where spines extend, otherwise smooth, transparent and shiny. The primary tracts of spicules are visible supporting the spines (Fig. 2.14f).

**Skeleton.** The skeleton is a regular, almost ladder-like reticulation of multi-spicular primary fibres interspersed with a reticulation of thinner secondary and tertiary fibres cored by spicules (Fig.2.19b). The primary fibres are 50-80 µm wide, packed with 10-20 spicules, and form meshes 200-400 µm wide (Fig. 2.20b). The primary tracts that support the surface spines may be fasciculate. The secondary skeletal fibres are 10-30 µm across, cored by 2-6 spicules, and form rectangular or triangular meshes 50-150 µm wide. The tertiary fibres are uni- or bispicular, 10-20 µm wide and form meshes of 30-70 µm. The surface skeleton is a compact reticulation of primary, secondary and tertiary spicule-cored fibres (Fig. 2.19c). Primary fibres, 50-80 µm wide and completely cored by spicules, radiate from the base of the surface spines and form triangular meshes 100-350 µm wide (Fig. 2.19c). Within these meshes the secondary fibres, 10-30 µm across with meshes 50-100 µm across, and numerous tertiary fibres, up to 10 µm wide, form a ladder-like reticulation.

**Spicules.** (Table 2.12). Principal megascleres are small hastate oxeas with abruptly constricted ends often terminating in sharp points (Figs. 2.19d, 2.20c).

**Discussion.** The spicules of the Thursday Island sponge are slightly but consistently thinner than the Orpheus Island sponges and the spines on the branches of the Thursday Island sponge are closer together (Fig. 2.14e).

The Great Barrier Reef specimens have thicker primary fibres cored by more spicules then a specimen examined from New Caledonia (MHNG 976/280). Desqueyroux-Faundez (1984) described spicules from New Caledonian specimens as strongyloxeas but one of her specimens (MHNG 976/280), loaned and examined for this study, had oxeote spicules not dissimilar from spicules of Great Barrier Reef specimens. The specimens from the Great Barrier Reef, in all other skeletal characters, conform to the description of *C. aerizusa*.

**Distribution.** New Caledonia.

***Callyspongia pseudoreticulata*** **Desqueyroux-Faundez 1984**

**Synonymy.**

*Callyspongia pseudoreticulata*, Desqueyroux-Faundez, 1984: p.809, fig.24, 175-181.

**Occurrence.** This species is common on reefs around Lizard Island (Fig. 2.1) occurring in lagoonal areas and front and back reef slopes with high densities of hard coral cover (see Fig. 3.1a). It is common at depths between 3-9 metres and less abundant in deeper water up to 15 metres.

**Description.** An erect sponge with bifurcating branches up to 30 cm tall and 2 cm wide (see Fig. 3.1a). Some branches are dorsoventrally flattened and are less than 1 cm thick in this plane.

**Colour.** Black or dark brown throughout alive; black or brown throughout in alcohol.

**Texture.** Soft, compressible and spongy. The sponge can be torn with minimum force.

**Surface.** The surface is flat, reflects light, and is microscopically hispid. Oscules are numerous, flush with the surface and 1-2 mm diameter. They occur on the edges of the branches and on one side of the branches that are dorsoventrally flattened.

**Skeleton.** The skeleton is a regular, ladder-like, spongin fibre reticulation with peripheral condensation at the surface, and thick primary fibres, that may be fasciculate, internally (Fig. 2.21b). The tangential surface mesh is formed by radiating secondary fibres, supported by internal primary fibres, and intersected by tertiary fibres (Fig. 2.19f). The surface secondary fibres are 20-50 µm wide and form meshes up to 500 µm across, the tertiary fibres are slightly thinner, up to 20 µm wide, and form meshes up to 130 µm across. Patches of pigment cells occur at the surface (Fig. 2.19f, 2.21c). The surface skeleton extends into the choanosome of the sponge in narrow parallel layers (Fig. 2.19e). Beneath this superficial region the mesh size is 50-360 µm across and the fibres are 10-50 µm wide. In the centre of the sponge the primary fibres become fasciculate, are up to 175 µm wide, and mesh sizes vary between 100-360 µm. Spicules are rare, where they do occur it is singly within all parts of the fibre skeleton.

**Spicules.** Spicules are very thin, vestigial, blunt strongyloxeas, mean 61 x 1.0 µm, range 57-65 x 0.8-1.3 µm (Fig. 2.21d).

**Discussion.** The Great Barrier Reef specimens differ from the description of *C. pseudoreticulata* in the following characters. They are black or very dark brown alive and not ochre to brown as described by Desqueyroux-Faundez (1984), and they are larger sponges with thicker branches, 10 mm diameter

compared with 3-5 mm in the New Caledonian specimens examined for this study (MHNG 977/686 & 976/282). The internal skeletons, in specimens from both localities, have identical architecture, but the fibres of the Great Barrier Reef sponges are thicker, possibly a structural modification to support the larger size of these sponges. In other skeletal characters, surface skeleton, spicule morphology and sizes, the Great Barrier Reef sponges conform to the species description.

**Distribution.** New Caledonia.

## DISCUSSION.

This study of species of the Haplosclerida revealed problems in the taxonomy of this order, the resolution of which are beyond the scope of the study, but should be addressed in future work on the group.

The validity of the two families Haliclonidae and Adociidae could not be addressed as no species of the type genus of *Adocia* were examined. Two species of *Sigmadocia* were examined and transferred to the genus *Haliclona* because the ectosomal skeletons were an extension of the internal skeleton and not distinct from it. This is the principal distinguishing character of the family Adociidae. Examination of material of the type species of *Adocia, Isodictya simulans*, Bowerbank 1866, would determine if a distinct ectosomal skeleton existed and, with examination of other species of *Adocia*, if this character should be used at the generic and family level.

The genera *Niphates* and *Amphimedon*, are not easily distinguished as the principal difference between them is in the construction of the ectosomal skeleton and whether the principal tracts extend above the surface. In this study, emphasis was placed on the development of the primary tracts at the surface as well as construction of the ectosomal skeleton. The new species of *Niphates* was placed in this genus because of the organisation of its primary tracts and ectosomal skeleton. It is important that additional characters are found to distinguish, and provide better definitions, of these genera.

The location of *Siphonodictyon* within the Niphatidae is questionable, but this genus does not fit well into any other family in the Haplosclerida. It shares a number of skeletal characters with *Oceanapia* in the Petrosida (see Chapter Three). Some of these characters, such as the supporting reticulate skeleton in the fistules and the pulpy choanosome, may be a result of its cryptic growth habit, rather than any close relationship with species of *Oceanapia*. More species of both genera should be examined, along with characters other than those of their skeletons, to resolve this problem.

# CHAPTER THREE.
# SPECIES DESCRIPTIONS OF THE PETROSIDA.

## INTRODUCTION.

This chapter addresses the taxonomy of the Petrosida, an order established by Bergquist (1980), but called a different name (Nepheliospongida), for a group of sponges previously located in the Haplosclerida (see Chapter Two). Bergquist used the name Nepheliospongida (following Wiedenmayer (1977a) who used the name Nepheliospongiidae for a new family) when she established this order. Wiedenmayer used the fossil genus, *Nepheliospongia*, as the type for the family, in which he included recent genera that had similar skeletal characters to *Nepheliospongia*. Van Soest (1980) concluded that the evidence for association of recent with fossil genera was not sufficient for these genera to be included in the same family and, with some changes to the genera placed in the family, renamed it Petrosiidae. In addition, van Soest reported that Wiedenmayer (*in litteris*) agreed with his proposal. Since that time, Hartman (1982) and Desqueyroux-Faundez (1987a & b) have used the name Petrosida for this order. For these reasons, the name Petrosida will be used in this study. Bergquist and Warne (1980) use Nepheliospongida and Bergquist (*in litteris*) still accepts the original name for the family, Nepheliospongiidae, and consequently the original name of the order.

A dichotomy has occurred among sponge workers regarding the establishment of this order (see Chapter One). Some (Hartman 1982, Desqueyroux Faundez 1987a & b) adopt the two order system, i.e. Haplosclerida and Petrosida, while others, for example van Soest (1980) and de Weerdt (1985), recognise only one order, the Haplosclerida. Furthermore, differences of opinion occur regarding the location of families in the orders. Bergquist & Warne (1980), and Desqueyroux Faundez (1987a & b) locate the two families, Petrosiidae and Oceanapiidae, in the Petrosida, Van Soest (1980) and de Weerdt (1985) retain the two families in the Haplosclerida, and Hartman (1982) locates the Petrosiidae in the Petrosida and Oceanapiidae in the Haplosclerida.

In this study the two families Petrosiidae and Oceanapiidae are located in the Petrosida. The Petrosiidae, in particular, have the stony consistency typical of the order. The Oceanapiidae have a dense ectosomal crust composed of spicules and, in some species, spongin fibre, while the interior of the sponge is frequently pulpy.

The morphological characters examined are the same as those used in species descriptions of the Haplosclerida (see Chapter Two). Primary importance is given to the organisation of the skeleton, the spicule to spongin fibre ratio, regional differentiation and the type and size of spicules. These characters are evaluated for their validity in defining orders, families and genera.

The methods used are the same as the methods described in Chapter Two. Presented below are definitions of the two families Petrosiidae and Oceanapiidae, three genera *Xestospongia*, *Petrosia*, and *Oceanapia*, and six species descriptions, four species belonging to the Petrosiidae and two species of the Oceanapiidae.

## SPECIES DESCRIPTIONS.

### ORDER PETROSIDA HARTMAN 1982

**Definition. Demospongiae with a reticulate skeleton that is characteristically isotropic or round meshed with enhanced spicule, or spicule and fibre development. The mineral skeleton may become so dense the architecture of the skeleton becomes obscured. The megasclere complement is diactinal, usually oxeas but strongyloxeas or strongyles can occur. Some genera, for example, *Petrosia*, have distinct size categories of megascleres. Microscleres, if present, are toxas, microxeas or microstrongyles. The Petrosida are characterised by a dominance of mineral skeleton and the sponges have a brittle stony texture (Bergquist & Warne 1980).**

### FAMILY PETROSIIDAE VAN SOEST 1980

**Definition. Sponges with a dominance of mineral skeleton over soft tissues and spongin elements hence the sponges all have a brittle or stony texture. The ectosomal skeleton is a tangential reticulation, frequently isotropic, as is the choanosomal skeleton. The latter skeleton varies depending on the genera examined. For instance, the genus *Xestospongia* has an isotropic skeleton with some development of spicule tracts and the genus *Petrosia* has a more organised round meshed reticulation. The spicules are always diactinal, usually oxeas or strongyles, that are one size category in *Xestospongia* or two or three categories in *Petrosia*. Microscleres may be present and are microxeas, microstrongyles, sigmas or toxas.**

## Genus *Xestospongia* de Laubenfels 1932

**Definition. Sponges with a choanosomal skeleton that is isotropic and without fibre e.g. *X. exigua*, coarsely organised into a reticulation with some fibre, e.g. *Xestospongia n.sp.1*, or a reticulate skeleton with fibre development e.g. *X. testudinaria*. Spicules are abundant, of a single size class, but occasionally with thinner, possibly developmental forms. The dermal skeleton is an unmodified extension of the choanosome and does not form a surface crust. No microscleres.**

Type Species: *Xestospongia diprosopia* de Laubenfels 1932

A slide of the Type Species of *Xestospongia, X. diprosopia* BMNH.29.8.22.59a, was examined and contained large oxeas (mean = 393 x 22 $\mu$m) as well as thinner forms (333 x 9.7 $\mu$m). The skeleton was an isotropic isodictyal arrangement of spicules in tracts with little or no binding spongin. *Xestospongia n.sp.1* and *X. diprosopia* have similar skeletal organisation and amount of spongin fibre development. The difference in spicule sizes between these species is a specific distinction (393 x 22 cf. 325 x 12.2 $\mu$m).

*X. testudinaria* has a skeleton structurally similar to *X. diprosopia* and *Xestospongia n.sp.1* but with enhanced spongin fibre development. The two Great Barrier Reef species, *Xestospongia n.sp.1* and *X. testudinaria*, on the basis of skeletal characters, are distinguished almost exclusively because of the enhanced spongin fibre development in the latter.

The inclusion of the three species, *X. testudinaria, Xestospongia n.sp.1*, and *X. exigua*, within the genus *Xestospongia* is not considered accurate in this study. These three species conform to the generic definition, and *X. testudinaria* and *Xestospongia n.sp.1* are similar to the type species in skeletal architecture. However, *X. exigua* has quite different skeletal characters, much smaller spicules (129 x 4.7 $\mu$m), a very compact and disorganised reticulation, extreme emphasis of the spicule component of the skeleton, and consequent brittle and crumbly texture. This species is most likely better located in another genus, closely related to *Xestospongia*, within the same family. More species with similar complements of skeletal characters, should be examined before such a step is taken.

***Xestospongia exigua* (Kirkpatrick 1900)**

**Synonymy.**

*Petrosia exigua* Kirkpatrick, 1900: p.139, pl.XII & XIII.

*Xestospongia exigua* Bergquist, 1965: p.149, fig.14.

Bergquist & Tizard, 1967: p.181.

*Xestospongia pacifica* Kelly Borges & Bergquist, 1988: p.155, pl.6c,d.

**Occurrence.** This species occurs in inshore waters near Darwin, from Sir Charles Hardy Islands to Magnetic Island on the Great Barrier Reef (see Fig. 2.1), and is common over a broad range of habitats. It is found in high sediment areas such as mainland fringing reefs, for example off Cape Tribulation, as well as clear waters on midshelf reefs and outer barrier reefs such as Hicks Reef. It occurs on reef flats and beneath overhangs in depths of 3-4 metres (Fig. 3.1b), and is also found on reef slopes to depths of 15 metres (Fig. 3.1c).

**Description.** Its morphology is variable, a 1-3 cm thick encrustation or an erect sponge with lobes or turrets up to 15 cm high. The latter morphology is more commonly found in deep water or sheltered areas beneath overhangs. Small oscules, 1 mm across, occur on raised surface areas at distances of 1-2 cms apart (Fig. 3.1b).

**Colour.** The specimens have a light ochre to medium or dark brown surface in life, with a yellowish to fawn interior. In alcohol they are uniform medium to dark chocolate brown.

**Texture.** The sponges are compressible and crumbly, characteristically sticky to touch when alive leaving some of the ectosome adhering to the fingers.

**Surface.** The exterior is smooth but microscopically hispid. A thin layer of tissue and pigmented cells cover most of the superficial spicules.

**Skeleton.** The ectosomal skeleton is a continuation of the dense and irregular, uni- or multispicular isodictyal reticulation that forms the internal skeleton of the sponge. The internal meshes are triangular or circular and compact, 58-168 µm wide (Fig. 3.3b). In the centre of the sponge the spicules occasionally form vertical parallel tracts (Fig. 3.2a). Dark staining symbionts are frequently visible in the mesohyl beneath the sponge surface in thin sections (<10 µm).

**Spicules.** (Table 3.1). The principal spicules are consistently oxeote but may have hastate or fusiform ends (Fig. 3.3c). Rare forms have double points at one end. There are thin, probably developmental, forms of the spicules as well as the principal megascleres, both are straight or gently curved.

**Discussion.** *Xestospongia exigua* has been well described and discussed by Bergquist (1965). The present description provides additional field characters for recognition of the species and emphasizes the variability of some characters.

Specimens from shallow water, e.g. Magnetic Island, 5 metres (Fig. 3.1b), generally are an ochre colour possibly as a response to high light conditions. They often have uneven "knobbly" surfaces with oscules on raised ridges (Fig. 3.1b). Specimens on mid-shelf and outer reefs in deep water, e.g. Lizard Island, 9 metres (Fig. 3.1c), are consistently darker in colour. The Lizard Island specimen is encrusting but lacks a knobbly surface. This sponge was photographed on exposed reef slope where a smooth encrusting shape, rather than forms with elevated ridges, may be less prone to damage by ocean turbulence.

The spicules of the sponges from shallow water e.g. specimens from Magnetic Island, Orpheus Island, and Darwin, were larger and thicker than spicules of specimens from deeper water and from mid-shelf reefs, i.e. Britomart Reef and North-east Reef (Table 3.1). The pronounced tracts of spicules observed by Bergquist (1965) were not prevalent in the Great Barrier Reef specimens but this may relate to an encrusting growth form lacking a requirement for an erect supporting skeleton.

Material of *X. pacifica* Kelly Borges and Bergquist (1988) was examined and found to conform to the description of *X. exigua* and has been synonymised with this species.

There are many sponges, in external morphological features, closely related to *X. exigua* but none are as common on the Great Barrier Reef. They have similar field characters, are shades of brown or black, sticky to handle, encrusting, massive or lobed, and are difficult to define and distinguish easily. A study throughout the Australian tropics could distinguish these species and molecular techniques, such as DNA sequencing, may be the only method of differentiating them.

**Distribution.** Christmas Island, Yap, Palau, Darwin, Australia.

***Xestospongia testudinaria*** **(Lamarck 1884)**

**Synonymy.**

*Reniera testudinaria* (Lamarck), Ridley, 1884: p.409, pl.39.

*Petrosia testudinaria* Dendy, 1889: p.77,pl.3; 1905: p.144, fig.1.

Hentschel, 1912: p.403; Topsent, 1933: p.40; Wilson, 1925: p.399,pl.40; Vacelet, Vasseur & Lévi, 1976: p.88,pl.10.

*Xestospongia testudinaria* Desqueyroux-Faundez, 1987a: p.198,pl.3,9.

**Occurrence.** This species occurs from Lizard Island in the Cairns Section of the Great Barrier Reef to the Whitsunday Islands in the Mackay/Capricorn Section of the Great Barrier Reef, and within this area, on inshore fringing reefs to midshelf reefs such as John Brewer and Davies Reefs and reefs adjacent to Lizard Island (see Fig. 2.1). Individuals have been found in lagoonal areas, on forereefs and backreefs at depths of 5-15 metres. The sponges occur in full light conditions on rock or dead coral substrata, and are common in silty areas inshore as well as less turbid mid-shelf areas.

**Description.** An erect cup-shaped sponge locally described as the "volcano" sponge with vertical flukes or ridges on its outer surface (Fig. 3.1d). These ridges may be very pronounced extending at right angles up to 5 cm from the surface, or flatter and forming rounded knobs. Individuals range in size from 15 cm to 1.5 metres, with the apical cup forming a central hollow up to one third of the overall height of the sponge.

**Colour.** The sponges are red-brown in life; in alcohol they are a uniform fawn.

**Texture.** The sponges are firm, springy and slightly compressible when alive. They can be torn by hand but this requires some force. The tissue is very compact and interspersed with canals 0.5 cm in diameter.

**Surface.** The exterior is microscopically granular and has a thin superficial skeleton firmly attached to the underlying choanosome.

**Skeleton.** The surface skeleton is an extension of the choanosomal one and consists of an isotropic isodictyal reticulation of spicule tracts forming small meshes 90-227 µm wide (Fig. 3.4b). The tracts are 2-6 spicules wide and 34-84 µm across. The internal skeleton is a multispicular reticulation with irregular oval meshes 360-640 µm across. Tracts forming the reticulation are 100-150 µm across and cored by 10-20 strongyloxeas (Fig. 3.4c). Thin spicules, more oxeote in shape, occur interstitially. Spongin fibre development occurs around the spicule tracts, especially at the nodes of the reticulation, and is emphasised when stained with haematoxylin-eosin (Fig. 3.2b).

**Spicules.** (Table 3.2). The principal spicules (Fig. 3.4d, e) are extremely variable in size and shape - many have strongylote endpoints but others have stepped ends or tapering oxeote ends. Occasionally, knob-like protuberances extend from near the end of the spicules. The shapes range from short squat to long thin forms and all combinations of length and width are found.

**Discussion.** For discussion of this species refer to the discussion on *Xestospongia n.sp.1.*
**Distribution:** Indo-Pacific.

***Xestospongia n.sp.1.***

**Occurrence.** This species occurs from Cape Tribulation in the Cairns Section of the Great Barrier Reef to the Whitsunday Islands in the Mackay/Capricorn Section of the Great Barrier Reef (see Fig. 2.1). It is found in sympatry with *Xestospongia testudinaria* on inshore fringing reefs but has not been found on mid-shelf reefs. Individuals occur in areas of high sediment load, in caves, as well as in full light conditions.

**Description.** An erect cup-shaped sponge with vertical flukes or ridges on its outer surface (Fig. 3.1e). These ridges may be very pronounced extending at right angles up to 5 cm from the surface, or flatter and forming rounded knobs. Individuals range in size from 5 cm to 1 metre, with the apical cup forming a central hollow up to one third of the overall height of the sponge.

**Colour.** In life the sponges are maroon to red-brown; in alcohol a uniform fawn.

**Texture.** They are firm and compressible when alive, do not spring back when compressed, like *Xestospongia testudinaria*, and can be pulled apart relatively easily. The sponge tissue is very compact and interspersed with canals 0.5 cm in diameter.

**Surface.** The exterior is smooth as there is a thin membrane firmly attached to the underlying choanosome.

**Skeleton.** The surface skeleton is an isotropic isodictyal reticulation of spicule tracts forming small meshes 100-210 µm wide (Fig. 3.5b). The tracts, 40-220 µm across, contain 2-6 loosely aligned spicules. Internally, the skeleton is a multispicular reticulation with irregular oval meshes 320-640 µm across, the tracts forming the reticulation are 100-200 µm across and cored by 6-12 oxeas (Figs. 3.2c, 3.5c). Thinner oxeote spicules occur interstitially.

**Spicules.** (Table 3.3). The principal spicules are extremely variable in size and shape, many have oxeote endpoints but others have stepped ends, strongylote ends or are stylote. Occasionally, knob-like protuberances extend from near the end of the spicules (Fig. 3.5d). The shapes range from short squat spicules to long thin forms and all combinations of length and width are found. The spicules are usually gently curved, some are centrally bent while others are undulating (Fig. 3.5e).

**Discussion.** *Xestospongia n.sp.1* is characterised by its more open skeleton and lack of spongin fibre development producing sponges that are not as elastic as *X. testudinaria*. In the field they can be positively identified by piercing or cutting the specimen. If this is relatively easy and does not require a sawing action or a lot of strength then the sponge is a specimen of *Xestospongia n.sp.1*. This species has only been found on inshore reefs.

*Xestospongia testudinaria* and *Xestospongia n.sp.1.* are similar in many morphological characters. *Xestospongia n.sp.1* differs from *X. testudinaria* in lacking pronounced spongin fibre around the spicule tracts and as a consequence being less robust, easier to tear, and more compressible. *X. testudinaria* has a more compact consistency in the live organisms while *Xestospongia n.sp.1* is more porous. *X. testudinaria* may have a thinner apical cup wall and is usually reddish-brown in colour in contrast to *Xestospongia n.sp.1* which can be reddish-brown or maroon. Neither of the latter two characters are consistent; all intergrading shapes and colours occur in both species. *X. testudinaria* has a broader cross-shelf distribution occurring on inshore fringing reefs with high sediment loadings as well as mid-shelf reefs.

The species are almost identical in spiculation, with strongyles and oxeas of similar size ranges, similar skeletal design and mesh sizes, superficial skeletons and gross morphology (Figs. 3.1d, e, 3.2b, c, 3.4, 3.5).

Most authors who have described specimens of *X. testudinaria* have commented on the variability of spicule types from strongyles to oxeas and the great range of spicule widths. Aspects of these species descriptions, such as spicule sizes, morphologies, and amount of spongin development, incorporate a sufficient range within each character that both *X. testudinaria* and *Xestospongia n.sp.1*, fit the descriptions. Consequently, specimens of both species have previously been referred to *X. testudinaria*. Only with continual observation of the sponges *in situ* and in sympatry has it become obvious that two morphologies exist and can be distinguished. Texture in life is the best field guide to species identification. A study of their reproductive biology (Appendix IV) disclosed a temporal isolation of their spawning events and their distinctively different sterol chemistry (Chapter Four) supports the conclusion that they are two species.

A difficult problem has been to resolve which of the species should receive Lamarck's name *X. testudinaria*. Lamarck's type specimen in the Paris Museum, supposedly from the seas of Europe, although this locality is queried by Ridley (1884), has been lost (Topsent 1933). A slide of this material in the British Museum, likely to be the only type material of this species remaining, cannot be borrowed. For the purposes of this study, S. Stone (British Museum) examined this material which consists of three sections (T.S. & surface). These show a loose skeletal network of oxeas without obvious fibre development, immature oxeas measuring 290 x 3 µm and structural spicules, oxeas and strongyles, measuring 430 x 12 µm (S. Stone pers.comm.).

Ridley (1884) examined Lamarck's material when he described a specimen from Cape Denison, a fringing reef area south of Bowen, Queensland, Australia (see Fig.2.1), as *X. testudinaria*. He mentioned stout, strong, multispicular skeleton-fibre in his description, and examination of his material

(BMNH 1881.10.21.266), stained with haematoxylin-eosin, showed spongin fibre development. Fibre development is easily visible when skeletal material is stained with haematoxylin-eosin but is not as apparent if the material is not stained.

There are two possible conclusions that could be drawn from the available material and descriptions of *X. testudinaria*. Either the type material lacks spongin development and Ridley assigned his sponge with fibre development incorrectly to *X. testudinaria*, or he correctly interpreted Lamarck's material, spongin was present, and *X. testudinaria* is a species with fibre development. In this study, the latter conclusion is adopted, and *X. testudinaria* is the species name used for sponges with fibre development.

Other authors have established names for species closely related to *X. testudinaria*. Vacelet *et al.* (1976) established the species *Reniera viridenigra* for specimens which they had previously referred to *X. testudinaria* (Vacelet & Vasseur, 1965: p.111, Vacelet & Vasseur, 1971: p.109). This species is not illustrated but from the spicule measurements (230-280 x 6-11 µm), the skeletal characters (irregular network of paucispicular fibre in the choanosome) and colour (green-black) it is not the new species from the Great Barrier Reef described here.

Wilson (1925) established *X. testudinaria variety fistulophora* for a sponge where the outer surface had fistular processes instead of vertical ridges but fistules have not been found in any Great Barrier Reef specimens. Wilson also described a new species, *Petrosia lignosa*, which is vase-shaped, has irregular external outgrowths and a little spongin in the skeleton. The type material (USNM 21283) was examined in this study and the spiculation is different from that of *Xestospongia n.sp.1.*

Genus *Petrosia* Vosmaer 1887

**Definition. Sponges with an ectosomal unispicular reticulation and an internal skeleton that is lamellate-isotropic or lamellate with round-meshes. Megascleres, with distinct size categories, are strongyles or oxeas.**

Type Species: *Reniera dura* (Schmidt 1862)

*Petrosia n.sp.*

**Occurrence.** Carlisle Island, Whitsunday Islands, at a depth of 12 metres in full light conditions on an inshore fringing reef (see Fig. 2.1).

**Description.** A massive sponge with short protuberances over the surface (Fig. 3.1f).

**Colour.** Dark maroon exterior with cream interior when alive, and in alcohol, a dark brown ectosome 1.7 mm thick and medium brown interior.

**Texture.** Very firm, slightly compressible and springy.

**Surface.** Smooth and microscopically hispid.

**Skeleton.** The ectosomal skeleton is an extension of the choanosomal skeleton with the addition of occasional fans of short spicules extending to, or just beyond, the surface (Fig. 3.6b). This superficial area, 1.7 mm deep, is covered with brown pigment cells. Immediately beneath the surface small subdermal spaces, 200-230 $\mu$m diameter, are separated by tracts 15-20 $\mu$m wide of 2 or 3 spicules (Figs.3.2d, 3.6b). Below this region are extensive spaces, up to 730 $\mu$m wide, supported by thick tracts, 500 $\mu$m across, containing 20-25 spicules. Internally, the skeleton is more compact containing tracts of 15 to 20 tightly packed spicules bound by spongin and forming a reticulate fibre skeleton. The fibres are 160-300 $\mu$m across and the circular or oval interstitial spaces 270-300 $\mu$m in diameter (Fig. 3.6c). Most of the large sizes of strongyles core the choanosomal fibres but all sizes of spicules occur throughout the sponge.

**Spicules.** (Table 3.4). The species has 4 sizes of strongyles that have smooth rounded ends (Fig. 3.6d). Thin oxeas occur but are not abundant.

**Discussion.** *Petrosia n.sp.* is characterised by its graduated skeleton which superficially is simple and multispicular and internally is a thick fibre reticulation cored with many spicules (Figs. 3.2d, 3.6b). Neither the massive shape or maroon colour are unique but, in conjunction with skeletal organisation and spicules, they distinguish the species.

Other species of *Petrosia* have been described with some characters similar to *Petrosia n.sp.* but the new species does not conform, in all characters, to any of these descriptions. For example, *P. spheroida* Tanita (1967) redescribed by Vacelet *et al.* (1976) has strongyles, microstrongyles and microxeas of similar sizes to *Petrosia n.sp.*, but has characteristic, prominent apical oscules. *P. canariensis* (de Weerdt & van Soest 1986), has much larger spicules (290 x 24 & 135 x 8 $\mu$m) than *Petrosia n.sp.*, and *P. volcano* Hoshino (1976), a red-brown massive sponge distinguished by three large oscules on the upper surface, has a characteristic growth form lacking in *Petrosia n.sp.* *P. solida* Hoshino (1981) is spheroidal in shape with oscules on the upper surface, and does not contain dense fibre and spicule tracts that occur in *Petrosia n.sp.* Desqueyroux-Faundez (1987a) describes *P. capsa* which has similar spicules and external morphology to *Petrosia n.sp.*, but is lemon yellow alive, exudes abundant mucus, and does not contain the dense fibre skeleton found in *Petrosia n.sp.*

## FAMILY OCEANAPIIDAE VAN SOEST 1980

**Definition. Sponges with an irregular tangential ectosomal skeleton which may form a crust reinforced by spongin. Fistules are common. The choanosomal skeleton is an isotropic unispicular reticulation interspersed with spicule tracts usually parallel to the surface. The weak organization of the interstitial skeleton produces a pulpy appearance after death. Megascleres are oxeas or strongyles. Microscleres may be present and are sigmas and/or toxas.**

### Genus *Oceanapia* Norman 1869

Synonyms *Rhizochalina* Schmidt 1870 *Phloeodictyon* Carter 1882

**Definition. Fistule-bearing sponges with walls supported by an irregular reticulation of spicule tracts bound by spongin. A spongin-reinforced tangential ectosomal crust covers the surface of the sponges. The choanosomal skeleton is a combination of spicule tracts and single spicules without apparent orientation. Megascleres are oxeas or strongyles and microscleres sigmas and/or toxas.**

Type Species: *Desmacidon jeffreysii* (Bowerbank 1866)

In some recent publications, Bergquist & Warne (1980), van Soest (1980), de Weerdt (1985), the two genera *Rhizochalina* and *Phloeodictyon* have been synonymised with *Oceanapia*. These three genera have a fistulose habit and a bark-like dermal crust.

The original descriptions of *Oceanapia* and *Phloeodictyon* differ in possession of microscleres (sigmas) in the latter genus. Both have spicule tracts internally, as well as an isodictyal unispicular skeleton which produces a pulpy texture.

*Rhizochalina* has abundant spongin and a reticulate spicule-fibre skeleton (Topsent 1920, Bergquist 1965) which produces firm solid sponges. Recently de Weerdt & van Soest (1986) suggested that *Rhizochalina* should be retained as a subgenus for sponges with strongly developed spongin fibres and a subgenus *Oceanapia* should be reserved for pulpy sponges.

The two species examined on the Great Barrier Reef have different textures. *Oceanapia fistulosa* is very pulpy as the bulk of the skeleton is formed by a unispicular reticulation with few tracts of spicules and no fibre development. In contrast, *Oceanapia n.sp.* is a firm, incompressible sponge with a bark-like dermal region and tangential layers of spicules and fibre internally (cf. Figs. 3.7a, b, 3.8c, 3.9c). This species has the strong skeletal development described for the genus *Rhizochalina* and may need to be reallocated if further study suggests *Rhizochalina* should have generic status.

***Oceanapia fistulosa*** **(Bowerbank 1873)**

**Synonymy.**

*Desmacidon fistulosa* Bowerbank, 1873: p.19, pl.4, fig.7, 8; Carter, 1882: p.121.

*Rhizochalina fistulosa var. infradensata* Ridley, 1884: p.420.

*Rhizochalina fistulosa* Ridley and Dendy, 1887: p.32, pl.8, fig.2, 2a, pl.9, fig.4.

**Occurrence.** Little Pioneer Bay, Orpheus Island, 20 metres on a silty/sandy flat bottom (see Fig. 2.1).

**Description.** A massive sponge that is buried except for the fistules, and the whole sponge can be dug from the sediment by hand. The hollow fistules are up to 25 cm long and 1 cm in diameter, and the fistule walls are 1 mm thick (Fig. 3.7a).

**Colour.** The live sponge has long fawn turrets, inhabited by a pink zooanthid, and a cream-fawn interior. In alcohol the fistules are fawn or light brown and the internal colour is unchanged.

**Texture.** The sponge is compact but crumbly and forms a pulp around some supporting stringy fibre.

**Surface.** There is a surface crust or skin 2 mm thick which is microscopically hispid (Fig. 3.7a).

**Skeleton.** The choanosome is a unispicular isodictyal reticulation with rings of parallel spicules where the fistules extend upwards (Fig. 3.8c). Tracts of spicules are aligned within fibres and reinforce the isodictyal reticulation (Fig. 3.8c). The fistules have reticulate tracts of 20 spicules centrally, with a closer reticulation towards the surface of the fistule consisting of less densely packed tracts with fewer spicules (Figs. 3.2e, 3.8b).

**Spicules.** (Table 3.5). Hastate oxeas of 3 size categories (Fig. 3.8d).

**Discussion.** The Great Barrier Reef material was compared with Bowerbank's *Desmacidon fistulosa* from Fremantle, Australia (BMNH.1877.5.21.1353, 2 slides) and Ridley and Dendy's Challenger material of *Rhizochalina fistulosa* (BMNH.1887.5.2.246,264 & 1954.2.16.30). The skeletal characters agree well with the type material although the spicules of the Great Barrier Reef specimens are slightly thinner and there is an additional smaller size category. Ridley and Dendy (1887) mention small dermal spicules, 213 µm in length, in one specimen they examined. Small spicules are apparent in their material (BMNH.1954.2.16.30) and measure 83-114 x 5.2-7.3 µm. Thin forms of the large oxeas were also present measuring 208 x 3.1 µm. Small spicules were found in the slide (BMNH.1887.5.2.264) and measured 94-114 x 5.2-6.2 µm.

The Great Barrier Reef specimens have extremely long fistules, up to 25 cm. No previous descriptions of the species record fistules of this length but many report that fistules were broken prior to, or during, collection of the sponges.

This species has an extensive distribution and depth range, from 10 metres in Puerto Rico to 1800

metres in the Azores. It is likely that closely related species are being recorded as *O. fistulosa*, and the characters used to define it permit the incorporation of all these specimens.

**Distribution.** West coast, Australia; Arafura Sea, Azores, New Guinea, Puerto Rico.

*Oceanapia n.sp.*

**Synonymy.**

*Oceanapia fistulosa* Desqueyroux-Faundez, 1987a: p.203, fig.10a,b; figs.43-48.

**Occurrence.** Hawkesbury Island, 12 metres and Orpheus Island at 20 metres depth (see Fig. 2.1). This species occurs on fringing reefs with high sediment loadings. The Hawkesbury Island specimen was found encrusting around a coral branch, and the specimen from Orpheus Island was buried in sediment with fistules protruding.

**Description.** A massive spherical sponge with erect tapering fistules 3 to 5 cm tall (Fig. 3.7b). The fistules are hollow with a 1 mm thick wall, normally rounded at the apex, but a few oscular fistules are open apically.

**Colour.** Alive, the sponges have an orange surface and fistules with a pale orange interior, and in alcohol the whole sponge is medium to dark brown.

**Texture.** Firm and incompressible. The tissue is compact, fibrous, arranged radially and parallel to the surface in concentric bands (Fig. 3.7b).

**Surface.** The exterior is a smooth crust with the same appearance and texture as the fistule walls.

**Skeleton.** The ectosomal skeleton is a fibrous reticulation; each fibre centrally cored by 4-6 spicules and 160-200 µm wide (Fig. 3.9b). The mesh spaces between them are 330-670 µm in diameter. A thin dark coloured layer of tissue covers the fibre skeleton. The internal skeleton is a reticulation of fibre cored by spicules forming dense fibre bands parallel to the surface (Fig. 3.9c). The fibres are 320-530 µm across and enclose 10-20 spicules. These areas of fibre are interspersed with areas of pulpy mesohyl where the spicules are dispersed irregularly (Fig. 3.2f). The fistule walls are supported by a thick fasciculate spongin fibre tract up to 350 µm across packed with up to 50 spicules (Fig. 3.9d). Spicules extend at right angles to these tracts and irregular spicule fans support the surface (Fig. 3.9d).

**Spicules.** (Table 3.6). Smooth strongyles of four size categories although the two large thick sizes intergrade. Thin forms occur as do very thin toxa (Fig. 3.9e, f).

**Discussion.** *Oceanapia n.sp.* is readily recognised by its orange colour, fistular habit and very concentric skeletal development.

*Oceanapia n.sp.* looks like *O. fistulosa* Bowerbank (1873) described and figured by Desqueyroux-Faundez (1987a). Desqueyroux-Faundez commented on the variability in skeletal characters of specimens assigned to *O. fistulosa* and found differences between her specimens and the type material of *O. fistulosa*, but preferred to use that species name. Comparison of her material from New Caledonia (MHNG 978/534) with material of *Oceanapia n.sp.* from the Great Barrier Reef, leaves no doubt that these specimens belong to the same species. They are identical in external morphology and colour, skeletal architecture and spicule morphology. The absence of toxas and thin, probably developing, strongyles in the New Caledonian specimens is not considered sufficient reason to divide the specimens into two species.

Comparison of type material of *O. fistulosa* (BMNH 1877.5.21.1353, 1887.5.2.246, 264 & 1954.2.16.30) with *Oceanapia n.sp.* showed that these were not conspecific.

The only species of *Oceanapia* previously described with toxas, *O. arcifera* Dendy (1924), has oxeas as the principal megasclere and *Oceanapia n.sp.* does not conform to this species description.

**Distribution.** New Caledonia.

## DISCUSSION.

Both families examined in this order are easily recognised, and distinctive from each other, using arrangement of the skeleton and ratio of spicule to spongin fibre. However no conclusion could be reached, from examination of these characters, as to their use in distinguishing the Petrosida from the Haplosclerida. As will be discussed in Chapters Four & Five, chemical and reproductive characters provide conflicting information regarding the separation of these orders.

Problems at the generic level were found in the genus *Xestospongia*. *X. exigua* has quite different skeletal characters from *X. testudinaria* and *Xestospongia n.sp.1* and other differences have been found in chemical and reproductive characters (Chapters Four & Five). This supports the findings of Bergquist *et al.* (1980, 1986) who found, on chemical evidence, this genus may contain more than one generic group.

# CHAPTER FOUR.
# CHEMOTAXONOMY USING STEROL COMPLEMENTS.

## INTRODUCTION.

Sterols are membrane constituents and modulate the passive movement of solutes across the membrane bilayer. They occur in all eukaryotic organisms. Eukaryotic organisms not able to synthesize sterols require an exogenous source of these molecules. The greatest diversity of sterols is encountered among the most primitive animals (Bergmann 1949). As complexity increases from invertebrates to vertebrates there is a progressive reduction in the number of sterols occurring and an increasing occurrence of cholesterol and its 24-methyl and 24-ethyl derivatives. Nes and Nes (1980) relate this increasing dominance of cholesterol to the development of the nervous system. Sponges have a primitive non-tissue organisation of cells, lack a nervous system and yield the most diverse array of sterols found among the invertebrate phyla (De Rosa *et al.* 1973, Goad 1978).

Bergmann (1949), carried out the first chemotaxonomic study of sponges to compare the occurrence of sterols with taxonomy based on morphological characters. He found sterols suitable for comparative biochemical research because of their ubiquity and stability. Free sterols are particularly stable so auto-oxidation is less likely to occur than in other lipid classes (Popov *et al.* 1976). Bergmann's work was undertaken when methodological limitations meant that few minor sterols of an organism could be identified with any confidence. Many of his sterol entities proved to be mixtures of two or three compounds and it is only since the 1960's that greatly improved separation methods and technical advances in analytical equipment have made possible the identification of sterol components that constitute only 0.1% or less of a complex sterol mixture (Goad 1978).

In the 1970's and early 1980's a large number of new sterol structures were isolated from marine sources (Djerassi 1984) and the most thorough chemotaxonomic studies on sterols in sponges were undertaken by De Rosa *et al.* (1973) and Bergquist *et al.* (1980, 1986). In these studies, sterol structural parameters such as molecular size, ring structure, position of double bonds and configuration of side-chain double bonds were examined in species of the class Demospongiae and evaluated in relation to biological and morphological characters of the species. The range of sterols present and their quantitative relationships were found to be consistent in individuals of two species, collected at different times and from different localities (Bergquist *et al.* 1980).

Bergquist (1980) established a new order Nepheliospongida (in this study called Petrosida) for a group of sponges which had previously been included within the order Haplosclerida. Amongst the distinguishing characters of this order, discussed in Chapter One, was the incidence of cyclopropene rings or derivative structures in the side chains of principal sterols, in three species. *Calyx nicaensis* contained 52% of calysterol which had a cyclopropene ring in the side chain

(Fattorusso *et al.* 1975); *Petrosia ficiformis* contained 60% of petrosterol which had a cyclopropane ring in the side chain (Sica & Zolla 1978) and *Strongylophora durissima* contained 90% of strongylosterol (Bartolotto *et al.* 1978), which had a derivative structure associated with the cyclopropene ring structure (Bergquist 1980).

However, Bergquist *et al.* (1980, 1986) found other species of the order Petrosida did not have these novel side chain structures. They suggested that more generic and family groups occurred than were presently recognised in this order, and two distinct groups may exist within the genus *Xestospongia*.

The objective of this study was to investigate the division of the orders Haplosclerida and Petrosida using sterol complements as chemotaxonomic characters, and to examine relationships between species and genera within each of these groups. Advances in separation methods and analytical equipment have made it possible to identify the majority of the sterols and their quantities occurring in a sponge sample. With this capability the current study examined whether species retained the same sterol complements irrespective of locality collected, and if the quantities of these sterols in a species remained consistent. For this reason samples were collected from geographic localities, other than the Great Barrier Reef, to allow comparison of the same genera from different areas. Three analyses, (agglomerative hierarchical classifications, Williams 1971) were used to define relationships between species based on sterols present and their quantities. This pattern analysis technique had not previously been applied to sterol chemotaxonomic data sets. To allow for comparison with earlier studies, sterol structural parameters were examined. No relationships between species and genera in the two orders were assumed *apriori*.

## METHODS.

### Collection of material.

Species from the sponge orders Haplosclerida and Petrosida were collected from the Great Barrier Reef (29 samples), Darwin, Northern Territory, Australia (3 specimens), New Zealand (4 specimens) and Puerto Rico (3 specimens, Table 4.1). The samples were frozen at -10°C immediately after collection.

### Chemical analyses.

Sterol extraction methods, with slight modification, were based on those described in Itoh *et al.* (1983), and Kerr *et al.* (in press). Sponge samples were freeze-dried prior to extraction. Approximately 10 grams of sample was placed in ca. 500 ml. chloroform for about 24 hours at 5°C. The chloroform extract was treated with decolorizing charcoal, filtered and then concentrated in vacuo. The lipid fractions were obtained from the extract by column chromatography on silica gel, and eluted with 30% diethyl ether in *n*-hexane. Florisil was added to the silica columns for

additional colour removal.

Separation of the component sterols in each fraction was undertaken by preparative Thin Layer Chromatography (TLC) using silica coated TLC plates. A sample of each of the column fractions was added to the silica gel plate along with cholesterol which was used as a standard sterol indicator. Plates were developed using 10-20% ethyl acetate/hexane as eluent. The sterol zone was detected by spraying with sulphuric acid and ceric sulphate and the plates were heated. Fractions with sterols present travel a similar distance on the TLC plate to the standard cholesterol or slightly higher if methylated sterols are present. Those fractions with sterols were combined and concentrated in vacuo to give a total weight of sterol.

High Performance Liquid Chromatography (HPLC) was carried out on a Waters Associates HPLC system (M6000A and M45 pumps and R401 differential refractometers). Altex Ultrasil-Si normal phase columns (10 µm, 10 mm i.d. x 25 cm L., with 6% ethyl acetate in *n*-hexane as mobile phase) were used to separate various sterol nuclei, and further purification was achieved by using a reverse-phase system of two Altex Ultrasphere ODS columns (10 µm, 10 mm i.d. x 25 cm L., with methanol as mobile phase) connected in series. Each fraction obtained from the reverse phase HPLC was analysed by Analytical Gas Chromatography (GC) using a Carlo Erba model 4160 instrument with FID (HP fused silica capillary column, crosslinked 5% phenyl methyl silicone, 0.52 µm film thickness, 0.32 mm i.d. x 25 m L., 280°C to 290°C at 1°C/min., FID detector at 295°C, on-column injector). Low resolution mass spectra were recorded with a Gas Chromatogram-Mass Spectrometer (GC/MS). Varian 400-MHz $^{1}$H-NMR spectra (Nuclear Magnetic Resonance spectra) were obtained where necessary for structural elucidation.

Quantification of the sterol compositions in sponge samples was by integration of reverse phase HPLC and GC chromatograms. The percentage error (2 standard deviations) in integration of HPLC and GC chromatograms was calculated for a range of sterol quantities (%, Table 4.2), for example, if a sterol occurred in a quantity between 20-40%, the analytical error was 2.5-4% (GC).

**Statistical analyses.**

Variability of sterol quantities within individuals of the same species from different localities was determined by calculation of standard deviations (SD) about the mean (X) for *X. testudinaria* (Table 4.3). Variability within an individual was calculated using five samples taken from one specimen of *X. testudinaria* at Orpheus Island (SD1). Sterol variability between individuals from the same locality was calculated using one sample from each of three specimens of *X. testudinaria* from Orpheus Island (SD2, Table 4.3). To determine locality differences, one sample was collected from a sponge from Pandora Reef, a second from a sponge from Cape Tribulation and a third from a sponge from Low Isles (SD4).

The SD within an individual (SD1) were subtracted from the SD between individuals from the same locality (SD2), and gave an estimate of variability between individuals (~SD3) after within individual variability was removed (equation 1). Variability between individuals (~SD3) was subtracted from SD between individuals from different localities (SD4, Table 4.3). This provided an estimate of the variability due to locality differences (~SD5, equation 2).

$$\sim SD3 = \sqrt{(SD2^2 - SD1^2)} \quad (1)$$

$$\sim SD5 = \sqrt{(SD4^2 - \sim SD3^2)} \quad (2)$$

A more statistically accurate measure of variation would be to replicate within individuals and use a nested analysis of variance to give an estimate of variability between individuals when variability within individuals has been removed. This analysis was not possible with the replicate samples available in this study.

Cluster analyses.

The relationships between species, with sterols as attributes, were examined using agglomerative hierarchical classifications on the PATN package (Belbin 1987). Clustering techniques, such as hierarchical classifications, aggregate similar objects (species) into groups using a set of attributes (sterols). This method allows groups to be related to each other on the basis of similarities of the attributes they contain. The data consisted of a matrix of 38 sponge individuals (objects) representing 18 species, by 50 sterol types (attributes, Tables 4.1 & 4.4).

Presence/absence data were examined using the Jaccard association measure with Ward's Incremental Sum of Squares (ISS) fusion strategy (Fig. 4.1). Jaccard ignores the incidence of double zeroes and therefore does not group species on joint absences of sterols. Three species, *Xestospongia sp.4*, *X. muta (type 3)* and *Amphimedon n.sp.2*, had unique sterol complements and failed to group with any other sponges in the classification (Fig. 4.1, Table 4.5). A quantitative analysis would not indicate any relationship between them and the rest of the species in the data set. For this reason they were not included in quantitative analyses. This exclusion also avoided any masking of subtle differences between the remaining species in the data set (Williams, pers. comm.).

The reduced data set (35 sponges and 38 sterols) was examined quantitatively, i.e. using sterols as a percentage of the total amount of sterol in each sponge, with the Bray-Curtis similarity coefficient. Bray-Curtis, like Jaccard, ignores the incidence of double zeroes. Two different fusion strategies were used, one was space conserving (Flexible unweighted pair group average, UPGMA, B=0, Fig. 4.2) while the other was space dilating (Ward's incremental sum of squares, ISS, Fig. 4.3). Space dilating

strategies enlarge the object groups to include members with related attributes that in the conservative space conserving strategies, would not be grouped together (cf. Fig. 4.2 & 4.3). Consequently, both analyses were performed for comparison in this study.

Pseudocramer values (Abel *et al.* 1985) were used to identify those sterols (attributes) contributing most to the diagnoses of sponge groups (Table 4.6). This technique gives an average value of the attributes (sterols) for each sponge group, and thereby assesses each attribute's contribution to the definition of the groups.

The data was transposed and the three analyses performed on sterols, as objects, with species as attributes. The distribution of groups of sterols among groups of sponges was investigated using two way tables generated from the analyses. The two way table generated from the space dilating analyses (Bray Curtis/Ward's ISS) is presented in Figure 4.3.

$X^2$ values were calculated from contingency tables and converted to a Cramér value to investigate resemblance between the 3 analyses. This value allows a descriptive comparison between the three analyses (Cramér 1946, Table 4.7).

## RESULTS.

Fifty-four sterols were separated and identified from the thirty-eight sponges examined (Table 4.2). Twelve of the sterols were only found in three species, *Xestospongia sp.4, X. muta (type 3)* and *Amphimedon n.sp.2* (Tables 4.4, 4.5). These three species with major qualitative differences were not included in the subsequent quantitative analyses.

The principal differences between these species and the remainder of the sponges in the data set were as follows. *Xestospongia sp.4* and *X. muta (type 3)* contained unique sterols in large quantities (95.1%, 37%, Table 4.5). *Amphimedon n.sp.2* contained large amounts of three sterols (94.8%) that were in trace amounts (<1.40%) in the remainder of the data set.

*Orina sp.* also grouped separately in the presence/absence classification (Fig. 4.1). This species had a large percentage of 24-methyl cholestanol (sterol 15, 26.3%), a sterol which was also found in other species in the data set (Fig. 4.3), hence *Orina sp.* was included in the quantitative classifications.

### Sterol variability within a species.

Estimates of variability of sterol content within a species were low within individuals (SD1) (<4.47%), and between individuals (~SD3) (<3.06%) except for one exception (Table 4.3, sterol 17 = 15.40%). Variability of over half the sterols examined was less within individuals (SD1) than

between individuals (˜SD3 Table 4.3, sterol nos. 3,4,6,14,17,19,23,37). Of the remainder, four of the sterols varied more within individuals (Table 4.3, sterol nos. 2,13,18,20) and the remainder could not be compared because they did not occur in all sponge samples of *X. testudinaria* examined (Table 4.3).

Comparison of variability between individuals from the same locality (˜SD3) and from different localities (SD4) showed that variability as a result of locality differences was low (<1%). Variability of half the sterols was more between localities than between individuals (Table 4.3, sterol 2,13,15,18,19,20,54). In the remainder of the sterols the situation was reversed (Table 4.3).

These results indicate that variability in sterol content within the species *X. testudinaria* is low, irrespective of the individual sampled or its geographic location. This supports the findings of Bergquist *et al.* (1980) with other sponge species, e.g. *Phakellia arnensis*, and *Pseudaxinyssa sp.* The small variations in sterol quantities could be a result of the dynamics of the sponge at the time of sampling or the experimental error during analysis.

**Resemblance between analyses.**

$\chi^2$ expected values were too low for a test of resemblance, between the three analyses, to be statistically valid. However, the measure was used for descriptive comparison by converting the $\chi^2$ value to a Cramér value between 0 and 1 (equation 3).

$$C = \left[ \frac{\chi^2}{N \min (s-1, n-1)} \right] 1/2 \qquad (3)$$

(Cramér, 1946)

Values close to 0 indicate that agreement between the analyses is poor, values close to 1 indicate that agreement between the analyses is good (Williams pers. comm.).

The three analyses were in close agreement. There were few differences between sponge groups generated from the presence/absence analysis and sponge groups generated from the quantitative analyses (Cramér values 0.80 & 0.83, Table 4.7).

Principal differences between the presence/absence and the Bray Curtis/Ward's ISS (space dilating) analyses are as follows. Sponges in groups 3 & 4 in the latter analysis are in one group in the former (cf. Figs. 4.1 & 4.3). In addition, in the presence/absence analysis, *Niphates n.sp.* and *X. muta (type 1)* are associated with *Xestospongia sp.2, Amphimedon viridis, X. coralloides* and *Petrosia n.sp.* (Fig. 4.1). *Niphates n.sp.* and *X. muta (type 1)* are not associated with each other, or the species listed above, in the space dilating strategy (Fig. 4.3, cf. groups 2, 3 & 5). *Orina sp.* is most closely associated with *X. muta (type 3)* in the presence/absence classification but the latter species was excluded from the quantitative analyses, hence the two species were not

compared quantitatively.

The space conserving strategy (Bray Curtis/Flex.UPGMA) computed similar sponge groups to the space dilating strategy (Bray Curtis/Ward's ISS, Cramér value 1.00). The principal differences between the two quantitative analyses (Figs. 4.2, 4.3) were as follows. *Petrosia n.sp.* and *Xestospongia sp.2* were separated in the space conserving strategy, but were in the same group in the space dilating strategy. The group containing *Niphates n.sp., Gelliodes fibulata* and *Callyspongia aerizusa* was associated with the group containing *X. testudinaria* in the former analysis. In the latter analysis, this group was associated with the group containing *Petrosia australis, Haliclona symbiotica,* and *Callyspongia confoederata* (Figs. 4.2 & 4.3). As the two quantitative analyses produced similar sponge groupings, only the latter analysis is used for purposes of discussion.

**Pseudocramer values.**

Pseudocramer values (Abel *et al.* 1985) were generated from the Bray Curtis/Ward's ISS analysis. Average quantities of the sterols (%) are presented in Table 4.6. Those sterols contributing most to the diagnoses of sponge groups have the highest average values (Table 4.6). For example, the three sponges in sponge group one (*Xestospongia n.sp.1* and *X. muta (type 2)* were characterised by an exceptionally large amount of xestosterol (sterol no.20, 59.27%). They were the only sponges to contain xestostanol (no.21, 4%), and 28-dehydro-24-isopropyl cholesterol (no.56, 2.47%, Table 4.6).

Sponges in group 2, i.e. *Xestospongia sp.2, Petrosia n.sp., X. coralloides* and *Amphimedon viridis*, were characterised by a high quantity of cholestanol (sterol no.2, 22.58%) and also by the presence of 26-nor-22-dehydro cholestanol (no.13, 3.00%), and 22-dehydro-24-methyl cholesterol (no.11, 1.13%, Table 4.6).

Sponges in group 3 (*Niphates n.sp., Gelliodes fibulata* and *Callyspongia aerizusa*) contained a high amount of cholesterol (sterol no.1, 20.15%) and 22-dehydro-24-methyl cholesterol (no.55, 19.98%). They also contained 22-dehydro cholesterol (no.8, 6.70%) and 26-nor-22-dehydro cholesterol (no.23, 1.20%) in higher quantities than any other group (Table 4.6).

Group 4 (*Petrosia australis, Haliclona symbiotica,* and *Callyspongia confoederata*) contained 24-methylene cholesterol (sterol no.3) in particularly high amounts (42.67%, Table 4.6).

Group 5 (*Haliclona amboinensis, X. muta type 1, Xestospongia sp.3* and *X. testudinaria*) contained isofucosterol (sterol no.17 = 27.48%) in a significantly greater quantity than any other group (Table 4.6).

Group 6 (*Orina sp.* and *X. exigua*) was characterised by an exceptionally high amount of 24-ethyl cholesterol (no.6, 68.43%, Table 4.6).

Bray Curtis/Ward's incremental sum of squares (ISS) analysis.

This analysis identified six groups that were characterized as follows. The two most closely linked sponge groups, 2 & 3 (Fig. 4.3), with few exceptions contained all the sterols in sterol group 1. Sponge group 3 had greater quantities of these sterols than group 2. An additional complement of three sterols (sterol group 2, nos. 13,11,15), were found in group 2 but not in group 3 (Fig. 4.3).

Group 4 was linked to the previous two groups but differed from them in containing greater quantities of 24-methylene cholesterol (sterol group 1, no.3). Group 4 also lacked sterols belonging to sterol group 2 (Fig. 4.3).

Group 1, which linked next in the analysis (Fig. 4.3), contained low amounts of sterols in sterol groups 1 & 2 and had >45% of xestosterol (sterol group 3, no.20). This sterol was found in low quantities in other sponge groups.

Group 5, with two exceptions, contained all the sterols in sterol group 1 and six of the ten sterols in sterol group 2. This group was characterised by high levels of isofucosterol (sterol group 1, no.17, Fig. 4.3).

Group 6 was substantially different from groups one to five (Fig. 4.3). It contained very high levels of 24-ethyl cholesterol (sterol group 1, no.6). This group also lacked many of the sterols in sterol groups 1 & 2 (Fig. 4.3).

Aberrant species in the analysis were *Petrosia n.sp., Xestospongia sp.3,* and *Orina sp. Petrosia n.sp.* contained trace amounts of sterols in sterol groups 6 & 7. These sterols were rare, or absent, in other sponges examined. In addition, *Petrosia n.sp.* contained 13.4% of 24-ethyl lathosterol (Fig. 4.3, sterol group 6, no.22), a sterol present in trace amounts in only one other species in the data set.

*Xestospongia sp.3* contained most of the sterols common to group 5 but also contained 4.7% of 24,26-dimethyl cholesta-7,24(28)-dien-3B-ol, 6.7% of mutasterol and 11.2% of verongulasterol (Fig. 4.3, sterol group 5, nos. 41, 43 & 42 respectively).

*Orina sp.* did not fit well in group 6 except for the presence of 24-ethyl cholesterol and cholestanol (Fig. 4.3). It contained more sterols in sterol group 2 than other members of this group, and, in particular, contained 26.3% of 24-methyl cholesterol (Fig. 4.3, sterol no.15).

**Chemical Parameters: Carbon chain lengths.**

The distribution of sponges based on the length of their sterol carbon chains is presented in Figure 4.5. Group 1 had large amounts of C30 sterols. In Group 2 *Xestospongia sp.2* differed in having a high proportion of C28 sterols and *Petrosia n.sp.* had a high percentage of C29 sterols (Fig. 4.5). Groups 3, 4 and 5 were consistent except for *Xestospongia sp.3* which contained C30 sterols. Group 6, with the exception of *Orina sp* which had a high percentage of C28 sterols, also had consistent carbon chain length proportions.

The three aberrant species, excluded from the quantitative classifications, had substantially different proportions of carbon chain lengths from the rest of the sponges in the data set (Fig. 4.5). *Xestospongia sp.4* contained only C30 sterols, *X.muta (type 3)* was most similar to *Xestospongia sp.3* and *Amphimedon n.sp.2* was more similar to *Xestospongia sp.2* than any other species examined.

**Nucleus saturation.**

Grouping of sponges on the basis of nucleus saturation indicated that most had a similar quantity of sterols with a Δ5 nucleus, and the sponge groups were not distinguishable on the basis of this chemical parameter (Fig. 4.6).

Exceptions to this finding were *Petrosia n.sp.* and *X. muta (type 3)* which contained 7 sterols. The sample of *X. exigua* from Darwin was distinguished from the remaining individuals of this species in having a small Δ5,7 component that the others lacked.

The three aberrant species contained proportions of nucleus saturation in their sterol complements not found in other species of the data set. *Xestospongia sp.4* contained only Δ5 sterols, *X. muta (type 3)* had a small percentage of Δ0 and Δ7 sterols, and *Amphimedon n.sp.2* contained only Δ5,7 sterols.

**Nor sterols.**

In this study these sterols (Table 4.3, nos.13,23,33,34) were found in amounts less than 6% of the total sterol complement of the species, and therefore were not examined in detail.

**Unusual sterols.**

The unusual sterols in this data set (Fig. 4.4, Table 4.8) were the following. Verongulasterol and mutasterol (nos. 42 & 43) found in *Xestospongia sp.3* (11.2 & 6.7%) and *X. muta (type 3)* (8.7 & 6.2%), 24(28)-dehydroaplysterol (no.45) found in *X. muta (type 3)* (33.2%), and xestosterol and

xestostanol (nos.20 & 21) found in *Xestospongia n.sp.1* and *X. muta (type 2)* (av.59.27 & 4.0%), and in very low amounts in *X. testudinaria* (av.0.33%). Other unusual sterols that occurred in low amounts were 24,26-dimethylcholesta-7,24(28)-dien-3B-ol (no.41) in *Xestospongia sp.3* (4.7%), and 24,26,27-trimethyl-25(26)dehydro-lathosterol and 24,25,26-trimethyl-24(28)dehydro-lathosterol (nos.52 & 53) in *X. muta (type 3)* (0.40 & 0.20%) (Fig. 4.4, Table 4.8, see Appendix III for sterol structures). These sterols were all alkylated at C26.

## DISCUSSION.

The purpose of using clustering techniques was to provide a classification that would clarify and conceptualize information about sterol complements of species in the orders Haplosclerida and Petrosida. The use of three analyses differing in similarity indices or fusion strategies increased the reliability and robustness of the method. The close agreement of the three analyses indicated that the patterns found in the data were sound.

### Ordinal distinctions based on sterol complements.

The most striking result from the study was the absence of any well defined split of the species into the two orders Haplosclerida and Petrosida (Fig. 4.3). Bergquist's (1980) separation, on chemical parameters, of the two orders was based on the occurrence of novel sterols with cyclopropene ring structures in the side chain of some species of Petrosida. Chemical examination of this data set disclosed the absence of any such unusual sterol structures in the species examined (Fig. 4.4, Table 4.8, see Appendix III for sterol structures).

The unusual sterols in this data set were all alkylated at C26, a side chain modification previously reported by Bergquist *et al.* (1980) in some species of the order Petrosida as well as other species, for example in the order Verongida.

In this study all species examined and found to have alkylation at C26 belonged to the order Petrosida. These species belonged to the genus *Xestospongia* but not all species of this genus contained these sterols. Species lacking these sterols were *Xestospongia sp.2, X. coralloides, X. testudinaria* and *X. exigua*. Two species of the genus *Petrosia* (O. Petrosida) examined did not contain sterols alkylated at C26. In addition, no species of the order Haplosclerida contained sterols with this capability.

Bergquist *et al.* (1980, 1986) also found that not all species of the order Petrosida contained novel side chain structures. They suggested more generic and family groups occurred, in this order, than were presently recognised. This hypothesis is supported in this study.

**Sterol groupings at the family level.**

Very few groupings resulting from the analyses clustered species presently assigned to the same family. *Amphimedon viridis* (Fig. 4.3, group 2), *Niphates n.sp* and *Gelliodes fibulata* (Fig. 4.3, group 3) are presently classified, using morphological characters, in the family Niphatidae (O. Haplosclerida). The two species of *Callyspongia* are located in the family Callyspongiidae (O. Haplosclerida) and the two species of *Haliclona* are placed in the family Haliclonidae (O. Haplosclerida). None of these species, presently classified in the same families, were grouped together (Fig. 4.3). However all these species contained the majority of sterols from sterol group 1 with an occasional sterol from sterol groups 2 & 3 and are not markedly different. This result supports the location of these species in one order but the family allocations of these species were not supported in this analysis.

**Sterol patterns within genera.**

Nes & Nes (1980) suggested that organisms which on morphological grounds have sufficiently close affinities to be considered species within a genus, usually have close relationships in their lipids. Bergquist *et al.* (1980, 1986) found consistent sterol patterns within genera, e.g. *Iophon*, *Spongia*, and *Tethya*. In this study similar sterol complements within species of the same genus were not found. Two species in each of the genera *Callyspongia, Haliclona* and *Petrosia* were separated into different groups (Fig. 4.3). These species were more closely associated with species from other genera than intrageneric species. Species of *Xestospongia* were also separated, frequently widely, from each other, for example *Xestospongia n.sp.1, X. testudinaria* and *X. exigua* (Fig. 4.3).

The chemotaxonomic differences found between species of the same genus in this study, do not correlate with the present generic allocation of these species based on morphological characters. This result suggests that most of the genera examined in this study, are generic complexes, or sterol patterns are not valid at the generic level.

Bergquist *et al.* (1980, 1986) suggested that two distinct groups were found within the genus *Xestospongia*. In this study *X. exigua* is quite distinctive from the other species examined in this genus. Samples of *X. exigua* contained fewer sterols (8 in contrast to 19 in *X. testudinaria*, Fig. 4.3) than any other species examined. This species also lacked many sterols consistently found in other sponges, and was the only species to contain very high levels of 24-ethyl cholesterol (Fig. 4.3). These findings suggest different biosynthetic pathways are in operation in this species.

*Xestospongia n.sp.1* was also distinct from the remainder of the species in the genus *Xestospongia.* The principal difference was the large quantity of xestosterol (Fig. 4.3 no.20) in its sterol complement.

*Xestospongia sp.2, X. coralloides* and *Petrosia n.sp.* were grouped together (Fig. 4.3) and their generic diagnoses should be examined. They may be more correctly assigned to the same genus, and a new genus may need to be established to receive them.

*Petrosia australis* may be more correctly assigned to the order Haplosclerida, but more characters should be examined to support such a move (Fig. 4.3).

This study grouped species of the genus *Xestospongia* into four groups. These groupings should be examined further using additional character sets. The groups are as follows: Group 1: *Xestospongia n.sp.1* and *X. muta (type 1)*, Group 2: *Xestospongia sp.2, Petrosia n.sp.* and *X.coralloides*, Group 3: *X. muta (type 1), X. testudinaria* and *Xestospongia sp.3*, and Group 4: *X. exigua* (Fig. 4.3).

Sterol complements of species.

This study supported the results of Bergquist *et al.* (1980) that sponge species have a sterol fingerprint that includes a particular set of sterols as well as the same relative quantities of these sterols. Replicate samples of *Xestospongia testudinaria, Xestospongia n.sp.1, X. exigua, Callyspongia aerizusa* and *Callyspongia confoederata* contained the same sterol complements in similar quantities (Fig. 4.3).

This is all the more interesting when current research lists 4 sources of sterols for sponges: 1. denovo biosynthesis by the sponge, 2. from symbionts, 3. from diet without chemical modification, and 4. from diet with chemical modification. (Djerassi 1981, Kerr *et al.* in press). It appears therefore that regardless of the origin of the sterols, they are selectively retained in consistent amounts by a sponge species. These sterol complements remain constant over geographic locality and season. In addition, the quantities within a species do not fluctuate markedly.

In contrast, the three individuals of *Xestospongia muta* were always separated and contained sterol profiles that were distinct from each other. *X. muta (type 2)* grouped with *Xestospongia n.sp.1* (Fig. 4.3), *X. muta (type 1)*, in the quantitative analyses, grouped with *X. testudinaria* (Fig. 4.3), and *X. muta (type 3)* contained nine sterols that were not found in the remainder of the data set (Table 4.5).

The three samples of this species were collected from the same locality on the shelf edge at Puerto Rico and were indistinguishable, by the collector, in the field (Kerr pers. comm.) No differences in colour, consistency, size or shape of the sponges were reported (Kerr pers. comm.), however the distinctive sterol profiles of these samples suggest that they belong to more than one species. Other character sets, such as morphological and reproductive characters, should be examined, and calibrated with the samples examined in this study to determine additional differences at a species level. To resolve which of the samples is the species *X. muta*, the type material in the Paris

Museum must be examined.

**Unusual species in the analyses.**

The incidence of very high levels of unusual sterols separated three species, *Xestospongia sp.4, X. muta (type 3)* and *Amphimedon n.sp.2*, from the remainder of the species examined. *X. muta (type 3)* contained 33.2% of a sterol, 24(28) dehydroaplysterol, which was not found in the remainder of the data set (Table 4.5).

*Xestospongia sp.4* was totally different from other species in the data set as it contained only two sterols (Table 4.5, Δ22-24-isopropyl cholesterol and 24-isopropyl cholesterol) which did not occur in any other species examined. These two sterols have previously been reported from *Pseudaxinyssa sp.* (O. Axinellida) from the Great Barrier Reef, (Hofheinz & Oesterhelt 1979, Bergquist *et al.* 1980). *Xestospongia sp.4* may be a field misidentification and belong to the order Axinellida. Taxonomic allocation of this sponge awaits collection of additional samples.

*Amphimedon n.sp.2* contained a total of five sterols and was the only sponge to have 7,22-didehydro cholesterol (Table 4.5). This species is unusual in that the sterol fraction is not a major component by weight of the lipid extract (Garson *et al.* 1988), and a major terpene component has been reported (Garson pers. comm.). Large terpene components are not found in other species in this data set. The lack of common sterols in this species completely separates it from *Amphimedon viridis*, a species of the same genus, and all other Haplosclerida examined. However, this sponge has morphological characters that place it within this order.

**Common sterols.**

Some sterols were common and occurred in most of the species examined (Fig. 4.3). Twenty-eight of the sponge individuals examined contained 8 or more of the sterols in sterol group 1 (Fig. 4.3). Included in this group of sterols was cholesterol and nine sterols that were alkylated at C24 if at all, which is the normal site of alkylation for plant sterols. These sterols are common throughout the marine environment with cholesterol the major sterol component of the plankton and some diatoms (Goad 1978), and consequently may be diet derived.

**Sterol abundance.**

Bergquist *et al.* (1986) noted great variation in the number of sterols present in sponges. Most commonly there were seven to ten sterols present, in greater than trace quantities, in the species they examined. In this study, some species have a large number of sterols (*X. testudinaria* ~18), while others contain very few (*X. exigua* ~6 and *Petrosia australis, Haliclona symbiotica* and *Callyspongia confoederata* <11). These variations in sterol numbers may reflect the necessity of sponge species to contain particular sterols for unique membrane functions, or alternatively, reflect biosynthetic shifts occurring in different species. Marine filter feeders such as sponges have not developed the need for one specific sterol, such as cholesterol, which is required by higher animals

(Catalan *et al.* 1985).

**Chemical parameters.**

Previous taxonomic studies incorporating sterol analyses (De Rosa *et al.* 1973, Bergquist *et al.* 1980, 1986) have compared structural parameters of sterols such as percentage of carbon chain lengths C27-30, degree of nucleus saturation, and position of alkylation. In this study these chemical parameters were examined to allow for comparisons with previous taxonomic studies. In addition, it was possible to determine if the numerical analysis had grouped sponges that had similar chemical characters.

The analyses of general chemical parameters in this data set have supported the groupings determined by the cluster analyses. Consequently the cluster analyses displayed groupings with a sound chemical basis.

The proportions of carbon chain lengths were generally consistent within groups generated by the cluster analyses (Fig. 4.5).

The predominance of Δ5 sterols was expected as, in most organisms, the dominant sterols have a Δ5 nucleus (Nes & McKean 1977, Fig. 4.6). De Rosa *et al.* (1973) found Δ5 sterols were the most widely distributed sterols in the twenty-five species of sponges they examined. Catalan *et al.* (1985) noted that in sponges the predominant sterol types have only a few different nuclei, but many different side chains. This is consistent with the results of this study.

The lack of Δ5 sterols in *Amphimedon n.sp.2* suggests that, if de novo biosynthesis is involved in the sterol complement of this sponge, then the Δ7 reductase enzyme required to complete the Δ5 pathway (Nes & Nes 1980) has been lost. The Δ5,7 sterols found in this species could have a dietary source as they are known to occur in marine yeasts (Teshima & Kanazawa 1971). However, whatever the source of these sterols their presence, and absence of Δ5 sterols, shows this species is distinct from any other species examined.

Nor sterols were found in some sponges examined. These sterols do not have the usual generalized structure of a plant sterol. In addition, they are not peculiar to sponges, are relatively common in marine invertebrates and, in the small amounts found in this study, may have been of dietary origin (Kerr pers. comm.).

This study used a unique character set to examine sponges renowned for their lack of morphological characters. Such a data set cannot be treated in isolation from other character sets, but has approached the taxonomy of sponges in a novel manner and supplied a new set of groupings that can be thoroughly evaluated against, and in conjunction with, more readily available techniques.

# CHAPTER FIVE.
# REPRODUCTIVE BIOLOGY OF SPECIES OF THE HAPLOSCLERIDA AND PETROSIDA.

## INTRODUCTION.

Lévi (1953) proposed a division of the class Demospongiae into two subclasses, the Ceractinomorpha and Tetractinomorpha utilising as a major discriminator two types of reproductive mode, oviparity in Tetractinomorpha and viviparity in Ceractinomorpha. At that time there was relatively little information regarding demosponge reproductive patterns and information from a few known reproductive sequences was extrapolated to embrace genera and families with comparable morphology (Bergquist 1980).

Sponges belonging to the order Haplosclerida (subclass Ceractinomorpha) are now known to conform to the typical ceractinomorph reproductive mode and to incubate eggs and larvae. This pattern has been reported for the following marine species of the Haplosclerida, *Haliclona loosanoffi* Fell (1976), *H. permollis* Elvin (1976), *Adocia sp, Haliclona sp, Chalinula sp, Callyspongia sp.* and *Reniera sp.* Bergquist *et al.* (1979), *H. oculata, H. xena* and *H. rosea* Wapstra & van Soest (1987) and *Niphates sp.* and *Chalinula sp.* Ilan & Loya (1988, 1990).

In contrast, the only observation of reproduction in the Petrosida prior to this study was of release of eggs (oviparity) in *Xestospongia muta* in the Caribbean (Reiswig 1976), and the report of synchronously developing oocytes in *Petrosia ficiformis* (Liaci *et al.* 1973).

Recently authors have suggested that reproductive mode in sponges may be related to life history characteristics promoting survival of species in particular habitats (Bergquist *et al.* 1970, Reiswig 1973, Ayling 1980, Hoppe 1988). Consequently, studies on the reproductive biology of a species cannot examine reproduction in isolation from the distributions and habitats of the organisms as the habitat of an organism may, in part, influence its reproductive mode.

Comparative larval morphology has been used as a systematic character in defining higher order taxonomic relationships. The Ceractinomorpha incubate parenchymella larvae while the Tetractinomorpha have either blastula or parenchymella larvae (Bergquist 1980). Bergquist *et al.* (1979) found little uniformity in structure or behavioral patterns of larvae within the order Haplosclerida. Species of *Haliclona* and *Callyspongia* had parenchymella larvae with a posterior fringe of long cilia while *Reniera sp.* had small round parenchymella larvae with cilia of uniform length. *Niphates sp.* and *Chalinula sp.* Ilan & Loya (1990) conform to the former larval structure and ciliation pattern.

Bergquist *et al.* (1979) concluded that while most orders of the Ceractinomorpha have larvae which display distinct ordinal characteristics the Haplosclerida do not. The group either required major revision at the generic and family level, or larvae in this order seemed only to show marked similarities at the species level. More recently Wapstra and van Soest (1987) concluded that haplosclerid larvae have a ring of long cilia around the posterior pole, although not all species examined possessed one. These authors concluded that ciliation pattern and sometimes colour of larva were characteristics that could be used at ordinal level, while differences in larval morphology at family and generic level were unclear. Major specific features within the Haplosclerida were ciliation pattern, colour and size, and to a lesser degree, shape and behaviour differences (Wapstra & van Soest 1987).

No studies on larva of any species of Petrosida have been undertaken prior to this study. Liaci *et al.* (1973) studied the reproductive biology of *Petrosia ficiformis* but failed to find larvae in this species in four years of regular sampling in the Mediterranean. Obtaining larval material in oviparous species is difficult when the timing of the spawning event is unknown.

All regulatory aspects of reproduction in sponges are poorly known (Bergquist 1978). Information on timing of reproduction in sponges is now being accumulated and although ecological factors are unlikely to be relevant to discussions of higher order systematics, they are important in detecting differences between sympatric species. Temporal separation of spawning events in two closely related species has been cited as a reproductive isolating mechanism between species (Fromont 1988, Appendix IV).

Water temperature is assumed to be a major environmental factor which regulates reproduction of sponges in regions where there are large seasonal changes in this environmental variable (Fell 1983). Few studies have been conducted on reproduction in sponges in tropical regions where temperature differences are reduced. Three studies have examined reproductive timing of Caribbean species (Reiswig 1976, Hoppe & Reichert 1987, Hoppe 1988). Two studies have examined sponges occurring in coral reefs in the Red Sea, (Ilan & Loya 1988, 1990) and one study on Indo-pacific species has been published (Fromont 1988, Appendix IV).

Ilan and Loya (1988) found the appearance of reproductive products in the brooding sponge *Niphates sp.* to be related to an increase in water temperature and suggested this reproductive activity over the summer period also may be related to seasonal disappearance of benthic algae. Although increasing environmental temperature is correlated with the onset of gametogenesis in many species, in other reports gametogenesis has been associated with a decrease in temperature or no change (Simpson 1984).

Elvin (1976) found that initiation of oogenesis in the temperate intertidal brooding species *Haliclona permollis* was most closely related to an increase in incident light.

Synchronization of release of gametes in oviparous sponges has been reported in four genera in the Caribbean (Reiswig 1970, 1976, Hoppe & Reichert 1987, Hoppe 1988) and was strongly correlated to lunar phase in *Neofibularia nolitangere* (Order Poecilosclerida). No detailed reproductive studies, including timing and regulating factors of gametogenesis and the release process, have been undertaken on species of the Petrosida.

The prime objective of this study was to examine reproductive patterns in the two orders Haplosclerida and Petrosida. Primary importance was attached to establishing the reproductive mode of the species examined i.e. viviparity (brooding) or oviparity (broadcasting), and describing the habitats in which the species occur. Secondly, comparative larval morphology was examined for differences between species within and between the two orders. The onset of gametogenesis and timing of the release events were monitored in an effort to provide a complete picture of the reproductive patterns of the species examined.

## MATERIALS AND METHODS.

The sponges investigated were located in three subtidal reef communities in the central section of the Great Barrier Reef on the North-east coast of Queensland, Australia (Fig. 5.1). Two of the habitats were fringing reef communities; Geoffrey Bay, Magnetic Island and Pioneer Bay, Orpheus Island, and the third site, John Brewer Reef, is a mid-shelf reef off Townsville.

The sampling programme at Magnetic Island began in winter 1986 and continuous monthly monitoring ceased after summer 1988. Twenty individuals of each species were collected during the 1988 reproductive period (Table 5.1). The sampling programme at Orpheus Island began in winter 1986 and proceeded with discontinuities, when no reproductive products were present, until November 1989. The sampling programme at John Brewer Reef began in autumn 1986 and was discontinued after autumn 1987 (Table 5.1).

At least five individuals of each species were sampled at one month intervals (Table 5.1). On a few occasions, because of poor visibility, less than five individuals were found at the study sites. The number of individuals sampled was increased for at least one sampling during the reproductive period of each species, except those occurring at John Brewer Reef. Individuals of *X. testudinaria* and *Xestospongia n.sp.1* were sampled at two additional sites, Little Pioneer Bay, Orpheus Island and the adjacent Pelorus Island, during the 1987 reproductive period.

Sampling consisted of taking a plug of "tissue" from the central third of an individual with a 5 mm cork borer. Tissue samples were fixed in marine Bouin's solution for at least 24 hours, washed with successive changes of 70% alcohol and embedded in paraffin. The paraffin blocks were sectioned at 10 µm thickness, stained in Mayer's haematoxylin-eosin and mounted with DPX for examination by light microscopy. The sections were examined for presence and development of

eggs and sperm.

Physical data was obtained from various sources. Sea temperature at 2-5 metres depth in Geoffrey Bay, Magnetic Island, and 5 m depth in Pioneer Bay, Orpheus Island were recorded with data loggers maintained by the James Cook University Coral Group. Sea temperatures were not available from Orpheus Island in 1989 owing to a malfunction of the logger. Total monthly sunshine hours and rainfall (mm) were supplied by Townsville Meterological Office and moon phases and tides movements were taken from Australian National tide tables.

## RESULTS.

**Description of species and habitats of the Haplosclerida: *Haliclona amboinensis, H. symbiotica, Niphates n.sp.* and *Amphimedon n.sp.2.***

*Haliclona amboinensis*, *H. symbiotica* and *Niphates n.sp.* occurred on intertidal and subtidal reef flats to four metres depth in Geoffrey Bay, Magnetic Island (Fig. 5.1). Individual colonies of *H. amboinensis* and *Niphates n.sp.* formed small and thick encrusting mats or solid ramose branches (see Fig. 2.2a, d, e). The average size of colonies of *H. amboinensis* was 42 $cm^3$ +/- 22 $cm^3$ (n=20) and the average size of colonies of *Niphates n.sp.* was 42 $cm^3$ +/- 32 $cm^3$ (n=20). *H. amboinensis* and *Niphates n.sp.* occurred in cracks, crevices and beneath small boulders and coral heads covered in the macroalgae *Sargassum*, and additionally, *Niphates n.sp.* was found in greater illumination on the tops and sides of coral heads. Both species were common at the study site. *H. symbiotica* is a widespread species found on fringing reefs from Darwin to the Whitsunday Islands. Within these areas large spreading specimens consisting of interwoven branches can reach diameters of one metre (see Fig. 2.2b). This species occurs in symbiosis with the macroalga *Ceratodictyon spongiosum*.

*Amphimedon n.sp.2* is a massive erect species, with a patchy distribution, found on mid-shelf reefs in the Cairns and Central Sections of the Great Barrier Reef to depths of 30 metres (see Fig. 2.8d).

**Reproductive mode in species of the Haplosclerida.**

*Amphimedon n.sp.2* did not have reproductive products throughout the sampling period from September 1986 to September 1987. Sampling at the study site at John Brewer Reef ceased after this period because individuals of *Amphimedon n.sp.2* and *X. testudinaria* were losing their tissue integrity and were thought to be diseased. Later visits to the site failed to find either species, although both were found in a healthy condition further along the reef at sites that were inaccessible for monthly monitoring because of logistical problems.

The three species studied at Geoffrey Bay, *Haliclona amboinensis*, *H. symbiotica* and *Niphates n.sp.* were viviparous and brooded larvae. Size of the individuals did not show any obvious relationship to sexual maturity (Fig. 5.2). Small individuals (<20 $cm^{-3}$) of the three species contained female reproductive products. There is a trend in *H. amboinensis* for an increased number of brood chambers to be present in individuals of larger size, while in *Niphates n.sp.* no such trend was apparent. Small females (<20 $cm^{-3}$) of *H. symbiotica* were less likely to be gravid than large individuals.

Occurrence of female reproductive products.

Oocytes, embryos and larvae were present simultaneously and, in *H. amboinensis* and *Niphates n.sp.*, were found in brood chambers (Fig. 5.3a, b). These chambers were located toward the base of the sponges and were approximately 3 mm wide, 5 mm long and up to 3 mm deep. Densities of oocytes, embryos and larvae within an area of a brood chamber 0.5 $cm^2$ are plotted in Figures 5.4a & 5.5a. The maximum density of male and female reproductive products in each species is presented in Table 5.2.

During the later reproductive months many brood chambers had spaces where larvae had been released showing that the release of larvae is asynchronous over a number of months. Occasionally, immature oocytes were seen dispersed evenly throughout the interior of the sponge prior to the presence of mature oocytes, embryos and larvae. They were sometimes found within the brood chambers with mature products, implying that they may migrate from the mesohyl, where they arise, to the brood chambers (Fig. 5.3c).

*Haliclona symbiotica* did not contain brood chambers but oocytes, embryos and larvae were in patches aligned along the midline of the branches (Fig. 5.3d, e). Density of female reproductive products was less in samples of this species than in *H. amboinensis* and *Niphates n.sp.* (Table 5.2, Fig. 5.6a).

For two reasons, sampling of tagged individuals of the three brooding species ceased after the first reproductive season, and was replaced by random sampling of five individuals per species. As the female reproductive products were aggregated in brood chambers, sampling with a 5 mm tissue core had a large chance of missing the chamber and therefore underestimating the incidence of gravid females in the population. Two species, *Haliclona amboinensis* and *Niphates n.sp.*, had small individuals where more than one sampling could cause tissue loss sufficient to kill the sponges. Therefore, individuals of *H. amboinensis* and *Niphates n.sp.* were destructively sampled and all parts of the sponges examined for brood chambers.

**Occurrence of male reproductive products.**

In these three species sperm cysts were not in restricted areas but occurred in high densities throughout the tissues of adults that did not contain female reproductive products (Fig. 5.4b, 5.5b, 5.6b, Table 5.2). These species are contemporaneously gonochoric (separate sexes) and possibly successive hermaphrodites. Sequential sampling of the same individuals was not possible and sex changes through time could not be determined.

**Development and larval morphology in the Haplosclerida.**

Development proceeded from small immature oocytes (10-30 µm wide) with pronounced nuclei and nucleoli to large mature oocytes (240-340 µm wide) with a barely visible nucleus (Fig. 5.3c, f, 5.7a, b). Subsequent development resulted in an embryo made up of cells of equal size (Fig. 5.7c-f, 5.8a). The larvae were 240-350 µm wide and differentiated into superficial ciliated, elongated, epithelial cells with dark staining nuclei at the outer surface, and a solid mass of internal cells (Fig. 5.7d-f, 5.8a-d). Spicules were found in all larvae but were particularly abundant in larvae of *H. symbiotica* (Fig. 5.8d).

Larvae of the three species were creamy white with a dark coloured ring at the posterior pole (Fig. 5.3a, 5.7f, 5.8c, d). Cilia were visible around the edge of mature larvae but longer cilia were not apparent at the posterior pole (Fig. 5.8a-d). No free larvae, released naturally from the adult sponges, were observed.

Sperm cysts were small in the three species. *H. amboinensis* sperm cysts were 30 µm diameter (n=10), *Niphates n.sp.* cysts were 23 µm (n=10) and in *H. symbiotica* were 17 µm (n=10) (Fig. 5.8e, f, 5.9a).

**Timing of reproduction in the Haplosclerida.**

The development of reproductive products coincided with an increase in water temperature in 1986 and 1987 and cessation of activity occurred with a decrease in water temperature (Fig. 5.10). Temperatures were 27°C in September 1986, 31°C in February and 26.5°C in April 1987. In 1987 the temperatures were lower in September, 25°C. The temperature in April 1988 was 27°C (Fig. 5.10a). The timing of reproduction also coincided with increasing total sunshine hours. Sunshine hours may decrease, due to cloud cover, at the onset of the rains, a variable summer event occurring in January 1987, December 1987, February 1988, December 1988 and February to May 1989 (Fig. 5.10b).

*H. amboinensis* contained female reproductive products for 10 of the 20 months sampled. Female gametes were present in samples collected in September, and December to April (spring to

autumn) in 1986 to 1988 (Fig. 5.11a). Male gametes were present in colonies for eleven months and were found one month after female gametes in October 1986 and 1987 to April 1986 and 1987 (Fig. 5.11a). Sperm cysts were in two colonies in May 1987.

Gravid females of *Niphates n.sp.* were present in August and September 1986, 1987 and 1988, November 1986 and March 1987 i.e. spring to autumn (Fig. 5.11b), in 10 of the 22 months sampled. Sperm cysts were present in most months of the years examined (Fig. 5.11b). Sperm cysts were present prior to eggs being found in the samples and were found in 16 of the 22 months sampled.

*Haliclona symbiotica* had reproductive products in fewer months (5 out of 17 months sampled) than either *H. amboinensis* or *Niphates n.sp.* Gravid females were sampled in January 1987, December 1987 to March 1988 and April 1989. Sperm cysts were usually present in the months when female products were found; December 1986, January 1987, and December 1987 to March 1988 (Fig. 5.11c).

**Description of species and habitats of the Petrosida: *Xestospongia n.sp.1*, *X. testudinaria*, and *X. exigua*.**

*Xestospongia testudinaria* is a common Great Barrier Reef species found on inshore and midshelf reefs subtidally at depths of 5-15 m. *Xestospongia n.sp.1* has been found at similar depths but only on inshore reefs. Individuals of both species are large, erect, cup-shaped sponges (volcanoes) with vertical flukes or ridges on the outer surface (see Fig. 3.1d, e). Specimens can range in height from 5 cm to 1.5 m. The average number of individuals of *X. testudinaria* on the fringing reef in Pioneer Bay was 2.4 +/- 0.5 in a 10 $m^2$ quadrat (n=5); the average size of the individuals was 16483 $cm^3$ +/- 26300 $cm^3$ (n=9). The average number of individuals of *Xestospongia n.sp.1* was 2.2 +/- 0.8 in 10 $m^2$ quadrats (n=5) and average size of the individuals was 3405 $cm^3$ +/- 2000 $cm^3$ (n=11).

*Xestospongia exigua* is a widespread and common species occurring in Australia from Darwin to Townsville (see Fig. 2.1). It was found subtidally on fringing reef flats and reef slopes, and midshelf and outer barrier reefs to depths of 15 m. It is a thick encrusting to massive species which may extend finger-like projections vertically from a flat and spreading base (see Fig. 3.1b, c). In a study site in Blue Lagoon at Lizard Island (see Fig. 2.1) 17.3 +/- 9.0 individual colonies were recorded along a 50 metre transect (n=3).

**Reproductive mode in the Petrosida.**

**Occurrence of female reproductive products.**

*Xestospongia n.sp.1* and *X. testudinaria* are oviparous and develop and release eggs in a synchronous spawning event (see Fig. 3.7c, d, 5.12-5.14). Both species are gonochoric and individuals monitored over the four year period did not change sex. There was no size related

maturity or differentiation of sex on the basis of specimen size (Fig. 5.15a, b). A disproportionately large number of females, compared with the number of males, of *Xestospongia n.sp.1* were recorded at the study site (Fig. 5.15a).

Eggs are not aggregated in special areas but are present throughout the interior of females in densities, shortly before spawning, of 104 to 143 per 0.5 $cm^2$ (Table 5.3). A female of *Xestospongia n.sp.1* spawned an estimated 1.4 million eggs in aquaria in 1988. The fertilisation rate was 71.4%.

Individuals of *Xestospongia exigua* rarely had reproductive products in four years of sampling (Fig. 5.16). Eggs were found in females collected in January 1987 and 1988, February 1987 and 1990, and in September 1987. This species is apparently gonochoric; eggs and sperm were in separate individuals, but random sampling of specimens did not exclude successive hermaphroditism. Development of eggs was synchronous within individuals from the same population, and the eggs were evenly distributed throughout the adults in densities of 950 per 0.5 $cm^2$, prior to spawning, in February 1990 (Table 5.3, Fig. 5.9b).

Occurrence of male reproductive products.

Sperm cysts are evenly spread throughout males of *Xestospongia n.sp.1* and *X. testudinaria* in densities of 600 to 833 cysts per 1 $cm^2$ (Table 5.3). Reproductive products developed synchronously within individuals and between individuals of each species, from three localities near Orpheus Island in 1987 (Fig. 5.1, 5.12-5.14).

Sperm cysts were seen in males of *X. exigua* in February 1990 (Fig. 5.16b). Development of sperm was synchronous within individuals from the same population, and cysts were evenly distributed throughout the adults in densities of 889 cysts per $cm^2$ (Table 5.3, Fig. 5.9c).

Development and larval morphology in the Petrosida.

Early development of oocytes in *Xestospongia n.sp.1* and *X. testudinaria* was similar. Individuals with small oocytes of 15 µm diameter were observed in May and oocytes gradually increased in size (1-5 µm mean diameter per month) until September. In *Xestospongia n.sp.1* an increase in growth rate of the oocytes occurred in the month prior to spawning (Fig. 5.12a, 5.13a) while in *X. testudinaria*, except for 1989, the growth increment remained similar throughout the period of oocyte development (Fig. 5.14a, c). Early oocytes were small with a visible nucleus and nucleolus. The oocytes contained granular yolk near their outer edges which was not always clearly bounded by an epithelial layer.

No obvious morphological changes occurred in the oocytes until less than 4 weeks before spawning when the cytoplasm of the eggs differentiated. The peripheral region extended projections over

a clear area between the yolk and the bounding epithelium thus producing scalloped edges (Fig. 5.9d, e). Many oocytes, particularly in *Xestospongia n.sp.1* were atrophying (Fig. 5.17a, b). Immediately after spawning negatively buoyant eggs, with villi, were found in a mucus film adhering to the adults (Fig. 5.17c, d). The fertilised eggs were oval and cleavage was total and equal (Fig. 5.17e). Five hours after spawning the eggs had undergone numerous cell divisions and had formed morulae (Fig. 5.17f, 5.18a). Development of eggs of *X. testudinaria* did not proceed in aquaria and the process is unknown. In 1989, four days after the spawning event in *Xestospongia n.sp.1*, white larvae, 160 x 98 µm, were observed on the bottom of aquaria (Fig. 5.18b).

Sperm development was rapid in *Xestospongia n.sp.1* and *X. testudinaria* and was not detected in every year of sampling. In 1987 and 1989, immature sperm cysts were first observed in individuals of *Xestospongia n.sp.1* three days before spawning (Fig. 5.12b, 5.13b). The spermatogonia initially were barely defined as cysts and the nuclei were scarcely visible (Fig. 5.18c). Mature sperm with small dark staining nuclei, were present in well defined cysts 57 µm in diameter (n=5) in *Xestospongia n.sp.1* two days before spawning occurred in 1988 (Fig. 5.18d). Sperm with strong staining nuclei, were found in *X. testudinaria* 4 days before spawning in 1989 and mature sperm were found in cysts 33 µm in diameter (n=5), one day before spawning in 1987 (Fig. 5.18e, f).

Release of sperm from males was not observed and did not occur at the same time as spawning of eggs. Sperm were absent in males after release of eggs from females. It is assumed that sperm are released into seawater prior to the spawning of eggs and fertilisation is external.

Development of eggs in *Xestospongia exigua* is very rapid, being complete in about 58 days. Small eggs, 24 µm in diameter (n=20), with prominent nucleus and nucleolus, were apparent in some females in January 1987 (Fig. 5.19a). Mature oocytes, 58 µm in diameter (n=20), were found in February 1987 and 1990. Many of the mature oocytes contained bright staining symbionts peripherally (Fig. 5.19b). These symbionts are usually found in adult sponge tissues, near the exhalant canals, in most months of sampling throughout the year.

Sperm cysts were observed once, in samples taken in February 1990. They were 56 µm in diameter (n=10) and their development time is not known, but is less than the development time of the oocytes (Fig. 5.19d).

<u>Timing of reproduction in the Petrosida</u>.

Female gametes in *Xestospongia n.sp.1* and *X. testudinaria* were observed from May onwards when sea temperatures were decreasing (Fig. 5.20a). Male products are not found until four days prior to spawning, in the months of October and November, when sea temperatures have been increasing since September (Fig. 5.20). The spawning events of the two species were temporally isolated and separated by at least 15 days (Table 5.4). Spawning of *Xestospongia n.sp.1* occurred

above temperatures of 27°C and less than 28°C, and *X. testudinaria* spawned when temperatures were between 26 and 28°C (Fig. 5.20, Table 5.4).

No clear monthly lunar pattern was obvious (Fig. 5.21, 5.22, Table 5.4). *Xestospongia n.sp.1* spawned one day after new moon in 1986, four days after new moon in 1988, seven days after new moon in 1989 and six days after full moon in 1987. These spawning dates coincided with the end of neap tides and the beginning of spring tides in 1987 and 1989, with the end of spring tides in 1988 and with the middle to end of spring tides in 1986 (Fig. 5.21, 5.22, Table 5.4). One male in the field population was found with sperm cysts on 1st November 1988 and these were no longer present on 4/11/88, when two females in aquaria spawned a small amount of eggs. This apparent "false alarm" occurred 7 days after a full moon, and at the beginning of spring tides. These females spawned completely on 14 & 15th November with the remainder of the population. The male was not found with sperm again after the 4/11/88.

*X. testudinaria* spawned two days after full moon in 1986 and 1989, and one day before new moon in 1987. These dates coincided with the end of spring tides in 1986 and 1989 and the middle of spring tides in 1987 (Fig. 5.21, 5.22, Table 5.4). The spawning of *X. testudinaria* was not observed in 1988. Eggs were present on 20/9/88 and no eggs were observed at the next sampling on 21/10/88. During this period there was a full moon on 26/9/88 and a new moon on 11/10/88 (Fig. 5.22a). Temperatures above 26°C occurred during the week ending 8/10/88. Therefore, if minimum temperature is important, spawning most likely occurred around the new moon in October rather than the full moon at the end of September. One male was found with dark staining cells, thought to be individual mature sperm, four days before the spawning event on 13/10/89 (Fig. 5.18f). This is probably an incidence of premature maturation in this colony and suggests maturation of male products is not synchronised between all colonies.

Diel timing of spawning was consistent during the four years 1986 to 1989 and for both species was observed between 0700 and 1100 hours. Spawning of oocytes was observed in *Xestospongia n.sp.1*, over two consecutive mornings in 1987 and 1988. Sperm release was never observed in either species in the field or aquaria.

Females of *X. exigua* contained eggs in January 1987 and 1988, and mature eggs and sperm in February 1987 and 1990, suggesting spawning was about to occur. Sea temperatures during this period were 29.5 - 30.5°C at Orpheus Island in 1987, and 30.0 - 30.5°C at Magnetic Island in the same year. Eggs were found in some individuals from both localities at this time. Eggs were also found in two females from Orpheus Island in September 1989 when temperatures would not have been above 26°C (Fig. 5.19c, e).

# DISCUSSION.

## Reproductive mode.

Lévi (1957) put forward the hypothesis that reproductive modes in sponges were phylogenetically determined. Viviparity (brooding) occurs in the subclasses Ceractinomorpha and Homoscleromorpha, and ovipary (broadcasting) in the Tetractinomorpha.

An alternative observation to explain the reproductive modes occurring in sponges relates to life history characteristics. Species adopt reproductive strategies that optimise their ability to survive in particular habitats. Large, long-lived species will have short reproductive periods and ovipary, limiting the energy expended in production of larvae. They are specialists that have a narrow habitat and depth distribution (Reiswig 1973, Hoppe 1988). Small species are short-lived, have a rapid population turnover, and a wide habitat and depth range. They will produce large larvae of short planktonic life that are liberated over an extended reproductive period. These reproductive strategies span a range from K-selected stress tolerant species to r-selected opportunistic species (Hoppe 1988). Ayling (1980) related differences in reproductive strategy to the predictability and level of disturbances sponges normally encounter, with species living successfully in highly disturbed environments being invariably viviparous and producing large larvae with a high probability of success in settling.

This study supports Lévi's proposition that reproductive mode in sponges is phylogenetically determined. Three species of the Haplosclerida, *H. amboinensis*, *H. symbiotica* and *Niphates n.sp.*, are viviparous, and brood eggs and larvae. The fourth species of the Haplosclerida examined, *Amphimedon n.sp.2*, did not contain reproductive products during the period of sampling.

In addition, the viviparous reproductive mode of *H. amboinensis*, *H. symbiotica* and *Niphates n.sp.* conforms to the phylogenetic pattern previously attributed to the order Haplosclerida (Bergquist *et al.* 1979), as well as to the reproductive mode characteristic of the Ceractinomorpha.

Species of the order Petrosida, *Xestospongia n.sp.1* and *X. testudinaria*, are oviparous and broadcast eggs in a synchronous spawning event. A spawning event was not observed for *X. exigua*, but the synchronous development of eggs and absence of embryos and larvae implies oviparous development.

Synchronous spawning in *Xestospongia muta* was reported by Reiswig (1976), and subsequently Bergquist (1980) suggested that oviparity may be found to characterise the Petrosida. This requires that Lévi's original concept be modified. Ovipary is contrary to the expected reproductive mode of the Ceractinomorpha. The oviparous development of the three species of the genus *Xestospongia* examined in this study, supports her hypothesis that species of the Petrosida have

oviparous development.

However, in part, this study supports the alternative observation. The two large species with oviparous development, *Xestospongia n.sp.1* and *X. testudinaria*, occurred in environments disturbed during very bad storms and high winds, but which otherwise are stable. Few small specimens of these species were found and the adults appear to be long-lived, grow slowly and are replaced rarely. *Niphates n.sp.*, *Haliclona amboinensis*, and *H. symbiotica* are smaller encrusting or branching individuals, occurring in fringing reef environments prone to periodic, unpredictable disturbances such as wave generated turbulence and exposure to high ambient temperatures during summer spring tides. These three species have a viviparous reproductive mode.

*X. exigua* individuals, which are of a similar size to individuals of *H. symbiotica*, occurred in both the environments mentioned. *X. exigua* is common over a wide depth range, sediment loading conditions and reef aspects, and could be considered an opportunist with a large physiological tolerance to temperature, current and turbidity changes. This species is apparently oviparous with synchronous development of eggs. In addition, the brittle texture of the individuals and the growth form of projecting erect nodes and turrets suggest that asexual fragmentation may supplement sexual recruitment of this species. In this case, the local recruitment of populations may depend on fragmentation, while planktonic larvae provide gene flow between populations, and the potential to colonise distant habitats. The ability of adults of some species to fragment and therefore colonise nearby substrata, provides a life history trait supplementary to sexual reproduction, and decreases the limitations of a particular sexual reproductive mode. If asexual reproduction is possible, opportunistic species could be sexually oviparous, and incorporate asexual fragmentation for rapid local colonisation. Recent studies (Bergquist *et al.* 1970, Wulff 1985, Battershill & Bergquist, in press) have suggested a significant role for asexual reproduction in some sponge species. Hypotheses on reproductive mode relating to size of adults, environmental disturbances, and evolutionary implications, may all need to be modified following further study of asexual reproduction in sponge species.

Vivipary or ovipary may be determined phylogenetically and be coincidentally related to habitat or adult size. In addition, conclusions from short term studies, relating reproductive strategies to local environmental perturbations must be considered part of a larger, as yet incomplete picture.

If reproductive mode in sponges is phylogenetically determined, this contrasts with scleractinian corals where it is thought to be a variable life history trait (Harrison 1985). Sex determination, hermaphroditism or gonochorism, is phylogenetically determined in corals and characterises orders, families and genera (Harrison 1985). Simpson (1984) proposes that sex determination in sponges is physiologically determined, but little is known of the physiology in either group.

In this study, all of the species examined had separate sexes (gonochorism) and males and females

occurred contemporaneously. The inability to monitor the same individuals continuously, because of their small size, failed to eliminate the possibility of successive hermaphroditism in *H. amboinensis*, *H. symbiotica* and *Niphates n.sp.* The gonochoric condition in species of the Haplosclerida in this study is in contrast to the findings of Ilan & Loya (1988, 1990) who found that Red Sea species of *Niphates* and *Chalinula* were simultaneous hermaphrodites, but is similar to the findings of Fell (1970, 1976) who reported a gonochoric condition in *Haliclona ecbasis* and *H. loosanoffi*, Elvin (1976) who found gonochorism in *H. permollis* and Liaci *et al.* (1973) who reported it in *H. elegans*. The incidence of gonochorism in some species of the Haplosclerida, but hermaphroditism in others, supports the view of Simpson (1984) that sex determination in sponges is likely to be physiologically rather than phylogenetically determined.

None of the species examined had size-related maturity or showed differentiation of sex on the basis of size; small colonies were as likely to be gravid as large ones. Size-related maturity has been reported in some species of sponges. Egg production in *Suberites ficus* has been reported to be restricted to specimens smaller than 5 cm (Lévi 1956) and in *Mycale sp.*, small and large specimens produce sperm, but only specimens exceeding 200 ml. in net volume produce eggs and incubate larvae (Reiswig 1973).

Fecundity of all the sponges examined was very high. In brood chambers in viviparous sponges (0.5 $cm^{-2}$), up to 200 eggs, embryos and larvae were present at one time, and sperm cysts were present in males in numbers up to 360 per $cm^2$. Density of sperm was even greater in the broadcasting species with up to 900 sperm cysts per $cm^2$. Eggs in these sponges were in numbers up to 150 per 0.5 $cm^2$, and 950 per 0.5 $cm^2$ in *X. exigua*, and were more abundant than in the brooding species as they occurred throughout the sponge tissue. No other studies on sponges have reported the density of reproductive products in adults. Broadcasting reproductive mode seems to be associated with high fecundity to offset the mortality experienced during the larva's pelagic phase (Crisp 1977).

**Development and larval morphology.**

Eggs, embryos and larvae were found in brood chambers in *H. amboinensis* and *Niphates n.sp.* and development was asynchronous with eggs, embryos and larvae present contemporaneously. The brood chambers consistently occurred in basal regions of the sponges and may be a protective mechanism to prevent predation, damage from turbulence, or dehydration. *H. symbiotica* did not have brood chambers, instead eggs, embryos and larvae were aligned along the central axis of branches of the adult. This is most likely a spatial confinement related to the symbiotic association this sponge maintains with the macroalgae *Ceratodictyon spongiosum*. Spaces between algal fronds are narrow and too small for the location of a brood chamber.

Brood chambers, isolated pockets of female reproductive products, have been described in two

species of the Haplosclerida, *Niphates sp.* and *Chalinula sp.* (Ilan & Loya 1988, 1990) but female reproductive products were evenly dispersed in *Haliclona loosanoffi* (Fell 1976).

Males of the three species contained sperm cysts evenly distributed throughout the mesohyl.

Brooding sponges in this study contained female reproductive products for periods of five months (*H. symbiotica*) and up to 8 months (*Niphates n.sp.* and *H. amboinensis*), which is similar to the findings of Ilan & Loya (1988) who found *Niphates sp.* had a period of reproductive development spanning 8 to 9 months (May to January), including summer. Fell (1983) found the complete process of oogenesis, from immature oocytes to larvae, in *H. loosanoffi* takes 2-3 weeks but length of development from egg to larvae could not be determined in this study.

In species of the Petrosida, eggs and sperm were evenly distributed throughout individuals, sexes were separate, and in *Xestospongia n.sp.1* and *X. testudinaria*, individuals retained the same sex throughout the four year period of the study. Gonochorism has been reported for one other species, *Petrosia ficiformis*, in this order (Liaci *et al.* 1973).

Development of eggs and sperm could be determined for the broadcasting species. In *Xestospongia n.sp.1* and *X. testudinaria* eggs developed over a period of 7 months, larval differentiation from the fertilised egg took 4 days in *Xestospongia n.sp.1*, and sperm development was less than 5 days. In comparison, *X. exigua* had extremely rapid oocyte development, less than two months, and sperm developed in a shorter time span that was not determined. In *Petrosia ficiformis*, development of oocytes spanned 8 months, April to November, which is a similar period to that in *Xestospongia n.sp.1* and *X. testudinaria*, and sperm were present for 15-20 days at the end of October (Liaci *et al.* 1973) which is considerably longer than the 5 days or less for the oviparous species examined in this study. Spermatogenesis occurred within a 7 day period in males of *N. nolitangere* (Hoppe & Reichert 1987). In oviparous species such as *Axinella damicornis, A. verrucosa* (Siribelli 1962) and *Suberites massa* (Diaz 1973), development of the oocytes spans a period of 2 months or more (Fell 1983). The elapsed time between expulsion of eggs and development of free larvae was approximately 24 hours in *Raspailia pumila* (Lévi 1956), *Polymastia robusta* (Borojevic 1967) and *P. granulosa* (Bergquist *et al.* 1970).

Oocytes of *Xestospongia n.sp.1* and *X. testudinaria* increased in diameter at a similar rate until October when the growth increment increased in *Xestospongia n.sp.1.* Abortive oocytes, where the integrity of these cells was observed to be degenerating, were present in *Xestospongia n.sp.1* and *X. testudinaria* prior to spawning and subsequent fertilisation. Other studies have reported the presence of abortive oocytes, but few have determined the reason for their occurrence. Fell (1983) found that abortive oocytes frequently possessed two nuclei and that the degeneration of maturing oocytes may be related to failure of fertilization. In the coral *Diploria strigosa* Wyers (1985) suggested that degenerating oocytes may be a response to unspecified adverse

environmental conditions.

The reason for the presence of degenerating oocytes in *Xestospongia n.sp.1* and *X. testudinaria* is unknown, but it is not related to failure of fertilisation as oocytes were degenerating prior to the fertilisation event. The presence of degenerating oocytes in the four years of the study also suggests that adverse environmental conditions are not related to the presence of the abortive oocytes, and that resorption may be a metabolic alternative. Oocytes may degenerate because the individuals are limited in the number of oocytes they can spawn or the oocytes degenerate in areas away from exhalant canals. The abortive oocytes were more common in individuals of *Xestospongia n.sp.1* than in individuals of *X. testudinaria*.

Distinct layering of yolk material was noted in *Xestospongia n.sp.1* and *X. testudinaria* and eggs of both species showed a scalloping effect at the edges. This differentiation was previously observed only in *Xestospongia n.sp.1* (Fromont 1988, Appendix IV). The layering of cytoplasmic material conforms to the differentiation process reported to occur in mature oocytes of *Spongilla lacustris*, which prior to cleavage, have peripheral yolk containing large spherical granules while the central yolk around the nucleus has small granules (Saller & Weissenfels 1985).

*Xestospongia n.sp.1* and *X. testudinaria* spawned oval eggs that were negatively buoyant and which shrouded the sponge in a fine mucous sheath. In the oviparous sponge *Polymastia sp.* a thick mucus is exuded with the oocytes (Bergquist 1978), while in *Hemectyon ferox* and *Agelas sp.* eggs are released through the dermis and enveloped in sheets or cords of gelatinous matrix which cover the parent sponge (Reiswig 1976). In corals, negatively buoyant, sticky eggs have been suggested to be an adaptation to retaining larvae in the vicinity of the adult (Kojis & Quinn 1981), and this may also be the case in sponges.

Eggs of a number of oviparous sponges are surrounded by nurse cells when they are released. This has been reported to have a nutritive function in *Cliona celata* Warburton (1961), *Hemectyon ferox*, Reiswig (1976), and *Chondrosia reniformis* Lévi & Lévi (1976). Liaci *et al.* (1973) described very large mature oocytes (300 µm) in *Petrosia ficiformis* prior to spawning. This genus is closely related to *Xestospongia*. These oocytes were always surrounded by nutritive cells. Nutritive cells were not apparent around spawned eggs of *Xestospongia n.sp.1* or *X. testudinaria* and the eggs were considerably smaller (60-100 µm in diameter) than the mature oocytes of *P. ficiformis* and *Neofibularia nolitangere* (200 µm, Hoppe and Reichert 1987).

The three brooding species examined in this study contained larvae that conform in shape, colour and ciliation pattern to previous reports of larval morphology in the Haplosclerida (Bergquist *et al.* 1979, Wapstra & van Soest 1987, Ilan and Loya, 1990). The larvae were creamy white parenchymella, oval in shape, with a dark coloured ring at the posterior pole. Wapstra and van Soest (1987) found that most species of the Haplosclerida possess a ring of longer cilia around the

posterior pole. Free larvae, naturally released from the adult sponge, were not seen in this study. Therefore, the absence of long cilia may be because the larvae were not fully matured. In contrast, larvae of *Xestospongia n.sp.1* were white and lacking pigmentation. No larvae of species of the order Petrosida have previously been described.

**Timing of reproduction.**

In *H. amboinensis*, *H. symbiotica*, and *Niphates n.sp.* presence of reproductive products coincided with increasing water temperature. Individuals of *Niphates n.sp.* and *H. amboinensis* contained reproductive products in September when temperatures were 25 to 27°C, and continued to reproduce through February when temperatures reached 31°C. Reproduction ceased after April when temperatures fell below 27°C. Male gametes were found 1 month later than female gametes in *H. amboinensis*, and in *Niphates n.sp.*, sperm was present in most months of the years examined. *H. symbiotica* contained reproductive products in December when temperatures were increasing or at their maximum and reproduction ceased after April when temperatures fell below 27°C. Sperm cysts were found at the same time as female gametes.

Total sunshine hours and rainfall increased over the summer months when the sponges were reproductively active. During this period the macroalga *Sargassum* increases in biomass and forms large and dense stands (Price 1989), covering and shading the benthic organisms beneath it. Sediment is accumulated on the dense foliage rather than falling to the substrate beneath, where the sponges occur. Light is therefore unlikely to be significantly increased to the sponges over summer because of the shading effects of the algae. Rainfall increases during the summer period but onset and duration of the wet season is highly variable. Peak rainfall occurred in January 1987, December 1987, March 1988, December 1988 and April 1989 (refer Fig. 5.10b). It is unlikely that an environmental occurrence, so variable in this location, could trigger the onset of reproduction in these species.

The onset of reproduction in *H. symbiotica* coincided with the peak sexual and vegetative reproductive period of its algal symbiont, *Ceratodictyon spongiosum*. *C. spongiosum* lacks reproductive products in winter (June, July & August) but in other months is found with sexual products and tetrads at the tips of branches. This alga has separate male and female colonies (Price pers. comm.). It is assumed that one of the organisms comprising the symbiotic association must recolonise the juvenile of the other after release, although it is uncertain how they find each other.

Light has been implicated in the timing of reproduction in *Haliclona permollis* in temperate waters (Elvin 1979) and onset of reproduction in *Niphates sp.* is triggered by increase in water temperature, and depressed by a decrease in water temperature (Ilan & Loya 1988).

Oocyte development of *Xestospongia n.sp.1* and *X. testudinaria* was initiated in May when sea temperatures were decreasing. Oocytes continued to develop through the winter when temperatures, with a minimum of 23°C, were at their lowest. Temperatures undergo a rapid spring rise between late August and September and spawning occurs in October and November, prior to summer and before temperatures have reached their maximum. The opposite temperature regime for initiation of gametogenesis occurred in the brooding species *H. amboinensis*, *H. symbiotica* and *Niphates n.sp.*

*X. exigua* develops eggs and sperm in January, and spawns in February when temperatures are either at maximum or continuing to increase. It is possible that because of the rapid development time of reproductive products in this species, and the presence of eggs in September as well as January and February, more than one spawning per year can occur. This would preclude high sea temperatures in summer being an environmental cue. Alternatively, the presence of eggs in September may not be normal for this species. Also it could be indicative that more than one species occurs, and *X. exigua* is a species complex, with separate species having temporal reproductive isolation. Sufficient gross morphological diversity occurs within this species to support this concept.

Both brooding and broadcasting species in this study were reproductively active during a period of high temperatures over the period spring to autumn. However, different temperature regimes occurred at the onset of oocyte development. *H. amboinensis* and *Niphates n.sp.* began producing eggs in spring after temperatures had risen above their winter minimum and *H. symbiotica* produced eggs at the beginning of summer as temperatures were approaching their maximum (refer Fig. 5.10a). In contrast, *Xestospongia n.sp.1* and *X. testudinaria* initiated oocyte development at the end of autumn when temperatures were decreasing and approaching their winter minimum (refer Fig. 5.20a). These opposing temperature regimes may be related to differences in the duration of oocyte development. *H. amboinensis, Niphates n.sp.* and *H. symbiotica* produce mature larva in less than 7 months while *Xestospongia n.sp.1* and *X. testudinaria* develop mature oocytes in more than 7 months. If the latter species require a maximum temperature at spawning equivalent to early summer temperatures, this long development time necessitates development throughout winter.

The onset of spermatogenesis in *Xestospongia n.sp.1* and *X. testudinaria*, less than 5 days before spawning, supports the observation of Hoppe and Reichert (1987) that it is correlated with the completion of oogenesis.

Babcock (1984) found that three successive levels of environmental cues, in increasingly fine time scales, were responsible for the synchronous spawning event in broadcasting species of scleractinian corals. Annual sea temperature patterns initiated development, lunar or tidal cycles synchronised the month of spawning, and diel light cycles triggered simultaneous spawning.

Annual sea temperatures are important in synchronising egg development in the oviparous species examined in this study. Timing of the spawning event of the oviparous sponges could not be related to lunar periodicity. *Xestospongia n.sp.1* spawned between one to seven days after new moon in 3 of the years examined, but 6 days after full moon in 1987. Two spawning dates, 1987 and 1989, were associated with a period of decreased tidal movement, when the neap tides had ended and the spring tides were beginning. The apparent "false alarm" during which one male and two females spawned in 1988 conformed to this pattern, occurring at the onset of spring tides 7 days after a full moon. The other two spawnings were prior to neap tides, at the middle or end of the springs when tidal differences were still large (refer Fig. 5.21, 5.22, Table 5.4).

*X. testudinaria* may have a semilunar periodicity spawning 2 days after full moon in two years and one day before new moon in 1987, dates which coincided with spring tides and increased tidal movement.

The spawning dates of the two species were separated consistently by at least 15 days (refer Table 5.4). *X. testudinaria* spawned at least 15 days before *Xestospongia n.sp.1* in 2 years of observations and spawned an equivalent number of days after *Xestospongia n.sp.1* in a 3rd year (refer Table 5.4). The timing of these events suggests some periodicity is operative which prevents spawning of both species at the same time. The development and growth rates of oocytes is similar in both species, as are the temperature regimes observed at spawning (refer Table 5.4). The apparent irregularity of lunar periodicity, especially in *Xestospongia n.sp.1*, provides little information which points to a lunar cycle. Watanabe (1978) has evidence that a pheromone may synchronise spawning of males and females of two species of *Tetilla* and an endogenous mechanism such as this, which was not investigated in this study, may account for the regular separation of the two spawning events.

Synchronous spawning has been observed in oviparous sponge species, but few studies have determined the cues that trigger the synchronous release event. *Hemectyon ferox* spawned eggs simultaneously throughout local populations and over a range of several thousand miles a few days before a new moon (Reiswig 1976). *Neofibularia nolitangere* has a highly synchronized 3 day spawning event, beginning on the third day after full moon (Hoppe & Reichert 1987). Peak spawning of scleractinian corals on the Great Barrier Reef occurs on the third to sixth nights after a full moon (Babcock *et al.* 1986).

In contrast, *Xestospongia n.sp.1* does not show a clear lunar cycle and *X. testudinaria* may have a semilunar rhythm which will only be completely established with further study of the timing of the spawning event. The most surprising aspect of this synchronous spawning relates to the ability of the two species to separate their spawning events consistently when mechanisms suggested as cues in reproductive timing, such as lunar period, are apparently not implicated in one species.

Diel timing of the spawning event was consistent for both species during the 4 year study. For

each species the annual spawning event occurred between 0700 and 1100 hours, and in *Xestospongia n.sp.1*, was observed at this time on two consecutive mornings in 1987 and 1989. It is possible that this timing is related to light:dark regimes. Change from light to dark was thought to trigger gamete release in *Neofibularia nolitangere* which begins spawning at 1400 hours, has a maximum intensity at 1600 hours, and the event ceases around 1830 hours, after sunset (Hoppe & Reichert 1987). Spawning of corals tends to occur after a specific period of darkness, ranging from a few minutes to over four hours after sunset (Babcock *et al.* 1986). This diel spawning cycle in corals is associated with predator avoidance. Spawning at night minimizes predation by visual coral feeders such as planktivorous fishes (Babcock *et al.* 1986).

The tide regimes at time of spawning were not consistent in *Xestospongia n.sp.1*, but *X. testudinaria*, in three years, spawned on an incoming tide (refer Table 5.4). This may provide the means of retaining eggs near the parent population, and near optimal habitats, when they are most viable. Corals spawn during an extended period of slack water which has the advantage of increasing fertilisation opportunities during periods of low water motion and low water volume (Babcock *et al.* 1986).

The reproductive patterns in *Xestospongia n.sp.1* and *X. testudinaria* are clear examples of reproductive isolation between two sympatric species. These species were assumed to belong to the one species, *X. testudinaria*, prior to this study (Fromont 1988, Appendix IV).

Reproduction of the three species of *Xestospongia* examined in this study, the two species that form discrete, massive individuals and the smaller, spreading *X. exigua*, differed markedly in length of development of oocytes, 7 months in the former and less than 2 months in the latter, and timing of gametogenesis and spawning. *X. exigua* developed eggs when temperatures were at a maximum or increasing and spawned at the same time, while *Xestospongia n.sp.1* and *X. testudinaria* developed eggs as temperatures were decreasing and spawned when temperatures were increasing. These patterns may be physiologically determined or have systematic importance. Further study of oviparous species from this order, especially study of larval morphology, is necessary to expand the findings from this study.

# CHAPTER SIX.
# FINAL DISCUSSION.

Two of the major findings resulting from this multidisciplinary study are: firstly, all the character sets examined (morphological, chemical, reproductive, and electrophoretic) distinguished between sponges at the species level. Secondly, above the species level, three of the character sets (morphological, chemical, and reproductive) resulted in conflicting taxonomic conclusions. This suggests that discrepancies still occur in the generic, family and ordinal classifications of these groups.

The taxonomic framework developed in this study which is based on analysis of morphological characters is presented in Table 6.1. It most closely follows the work of Bergquist and Warne (1980) separating species into two orders, the Haplosclerida for sponges with spicule and/or fibre development, and the Petrosida (Nepheliospongida, Bergquist, 1980) for sponges with an enhanced mineral skeleton.

At family level, this system follows van Soest (1980) and, on the basis of morphological characters, the species examined in this study divided into five well defined families (Table 6.2). The morphological characters of primary importance for separating families were development of the internal skeleton, principally its components and their quantities, in conjunction with the design of the skeleton.

At the generic level, the development of the ectosomal skeleton was found to differentiate genera. For example, *Niphates* and *Amphimedon* were separated because of the presence of a well defined ectosomal skeleton in the former (Table 6.2). The presence or absence of microscleres was considered a species specific character (Table 6.2) although some variability occurred in the new species of *Oceanapia*. This species had toxas in Great Barrier Reef specimens but did not have them in specimens from New Caledonia.

Previous authors have placed primary emphasis on design of the internal skeleton (van Soest 1980, Bergquist & Warne 1980, de Weerdt 1985), but have differed on the importance of ectosomal skeletons and presence of microscleres. Van Soest (1980) considers them to be minor characters, but Bergquist & Warne (1980) place considerable importance on ectosomal skeletons. The latter reported distinguishing forms at the family level, and as a consequence use the family Haliclonidae for sponges without ectosomal skeletons and the Adociidae for sponges with them (Table 6.1). The presence of microscleres was used as a generic character in their work. De Weerdt (1985) also used ectosomal skeletal characters at the family level but microscleres at the species level. In this study these characters were used to distinguish genera (ectosomal characters) and species (microsclere component). Neither were used as descriptive characters at the family level.

Analyses of sterol chemistry of sponge species from both orders were undertaken to determine if they could be used to distinguish sponge taxa. The analyses undertaken were on quantities, and diversity of structure, of sterols in sponge individuals. Sponges with similar sterols in similar quantities were grouped more closely to each other than those which had different sterols or significantly different quantities of the same sterols (refer Fig. 4.3).

There was no clear separation of species into two groups corresponding to the orders Haplosclerida and Petrosida (Fig. 6.1). Species of the Haplosclerida, except for *C. aerizusa* and *C. confoederata*, contained all of the sterols in sterol group 1 and few of the other sterols in the data set (see Fig.4.3). With the exception of *A. viridis* and *H. amboinensis*, haplosclerid species occurred in closely related groups (Fig. 6.1). Species of the Petrosida grouped closely in some cases, (e.g. *Xestospongia sp.2*, *Petrosia n.sp.* and *X. coralloides*) but the greatest differences between groups, based on sterol chemistry, occurred within this order (e.g. *X. exigua*, *X. testudinaria*, and *Xestospongia n.sp.1*, Fig. 6.1).

Bergquist (1980) reported the occurrence of cyclopropene or cyclopropane ring structures or their derivatives in sterol side chains of some species of Petrosida. Subsequently, Bergquist *et al.* (1980, 1986) found species (in the Petrosida) with 26-methyl substitution, a C26 alkylation, in their sterol side chains. These authors suggest that 26-methyl substitution and cyclopropene or cyclopropane ring structures are indicative of similar biosynthetic pathways in these species. They also report that some petrosiid species lack these sterol modifications, and suggest, on the basis of this and morphological evidence, that more than one group of sponges occur in this order.

In this study, *Xestospongia n.sp.1*, *Xestospongia sp.3* and *X. muta (type 3)* were the only species containing sterols with C26 alkylation in significant quantities, and none of the species examined had cyclopropene or cyclopropane ring structures. Clearly, many species presently located in the Petrosida lack the biosynthetic pathway reported by Bergquist *et al.* (1980, 1986).

Few genera placed in the same family using morphological characters grouped together using sterol complements. For example, a species of the genus *Callysponiga* (family Callyspongiidae) grouped with *Gelliodes* and *Niphates* (family Niphatidae, Fig. 6.1). This grouping suggests two possibilities. Either species have been incorrectly assigned to genera, or family boundaries are poorly defined and overlap. Van Soest (1980) found species of the families Callyspongiidae and Niphatidae difficult to separate on skeletal characters and this chemical analysis supports his view.

Discrepancies between groupings based on sterol complements and those based on morphological characters also occurred at the generic level. Species of the same genus on morphological grounds invariably failed to group together on chemical grounds, for example, *C. aerizusa* and *C. pseudoreticulata*, *H. symbiotica* and *H. amboinensis*, and species of the genus *Xestospongia* (Fig. 6.1). These results suggest that generic and family boundaries based on morphological characters

are not supported by sterol chemistry, or sterol patterns are not valid at the generic level.

Bergquist *et al.*(1986) found consistent sterol patterns within some genera they examined (for example *Iophon*) but in others, such as *Xestospongia* and *Petrosia*, more than one sterol pattern was found. In the latter situation, they concluded that some species probably were wrongly assigned to the genera. In this study, some species of *Xestospongia* and species of *Petrosia* also failed to have similar sterol patterns (for example, *Petrosia australis* and *Petrosia n.sp.*, and *X. exigua*, *X. testudinaria*, and *Xestospongia n.sp.1*, Fig. 6.1). Consequently, this supports the findings of Bergquist *et al.* (1986) that generic complexes occur within these genera.

All species examined for sterol complements clearly separated from each other. This result supports the findings of Bergquist *et al.* (1980) that sponge species have a sterol fingerprint that includes a particular set of sterols as well as the same relative quantities of those sterols. The one exception in this study, *X. muta*, is thought to be a species complex (Fig. 6.1).

Reproductive processes are influenced by environment, physiological aspects of the sponge, and phylogeny, and caution should be exercised when drawing conclusions from these characters.

However, one clear conclusion was reached from examination of sponge reproductive characters (Chapter Five) in this study. On the basis of reproductive mode the species examined completely differentiated into two orders. Three of the four Haplosclerid species examined (*H. amboinensis*, *H. symbiotica* and *Niphates n.sp.*) brooded larvae; the characteristic mode of reproduction in orders in the subclass Ceractinomorpha. The fourth species, *Amphimedon n.sp.2.*, was not found with reproductive products.

In contrast, the three species of the Petrosida examined in this study, *X. testudinaria*, *Xestospongia n.sp.1* and *X. exigua*, were oviparous and broadcast eggs and sperm in synchronous spawning events. This mode of reproduction had previously been suggested by Bergquist (1980) to characterise this order.

In the Haplosclerida, the only comparison of reproductive characters that could be made at the family level was between *Niphates n.sp.* (F. Niphatidae), and *H. amboinensis* and *H. symbiotica* (F. Haliclonidae). No distinguishing reproductive characters at the family level were observed. Comparison of reproductive characters of two species in the same genus, *Haliclona amboinensis* and *H. symbiotica*, showed distinctive differences at this level. The two species had few characters in common. *H. symbiotica* lacks the brood chambers observed in *H. amboinensis*, has a shorter reproductive period, and is less fecund. As discussed in Chapter Five, these factors are closely related to the symbiotic association this species has with a red algae which does not occur in the other species.

In the Petrosida, *X. testudinaria* and *Xestospongia n.sp.1* had been considered to be one species prior to this study. These species were found to have very similar oocyte development, length of development (seven months), and timing of spawning (spring), but are distinguished by a temporal reproductive isolating mechanism (Fromont 1988, Appendix IV).

In contrast, there were marked differences between these two species and *X. exigua* which had oocytes different in morphology and size, rapid oocyte development (two months), and timing of spawning (summer). These differences support those apparent on the basis of chemical and morphological characters between *X. exigua* on the one hand and *X. testudinaria* and *Xestospongia n.sp.1.* on the other, *X. exigua* is clearly distinguished from the latter two species, is unlikely to be congeneric with them and, in future, should be relocated to another, possibly new, genus in the family.

The preliminary electrophoretic study (Appendix II), on *Niphates n.sp.* and *H. amboinensis*, supported the conclusion of Sole Cava & Thorpe (1986) that enzyme electrophoresis is useful for distinguishing between sponge species.

Three other studies, using character sets other than skeletal characters, have examined species of the Haplosclerida and/or the Petrosida. Pomponi (1976) examined cytological characters in six species of Haplosclerida and found differences in cell types in species from different families. This study showed some species were intermediate between the two family groups examined (Table 6.3).

Langenbruch (1988) examined the position of choanocytes in the mesohyl of demosponges and found they are invariably in contact with mesenchymal tissue. In marine Haplosclerida, the choanocytes may also be separated from the mesenchyme and either partly, or completely, surrounded by superficial cells (pinacocytes). He found species of the Haplosclerida, and one species of Petrosida, were divided into three categories and these did not correspond to their generic and family allocations based on morphological characters (Table 6.3).

Desqueyroux-Faundez (in press) examined silica content of species of the Petrosida and Haplosclerida and found that the families Petrosiidae and Oceanapiidae have higher mean silica contents (57.27% and 33.7%) than families in the Haplosclerida, where mean silica content was 29.59% or less (Table 6.3).

Only the latter study examined the division into two orders and supported the separation. The other two studies suggest discrepancies between cytological character sets and taxonomic decisions based on morphological characters.

In this study the two orders are retained as reproductive mode was found to clearly separate the species into two groups corresponding to the orders. The sterol chemistry was not useful in

distinguishing the ordinal division. Morphological characters defined a five family division of the species examined. More characters, preferably genetic, may be useful to further examine the two order separation.

The results of this study suggest that more work is required on the family Oceanapiidae and the genus *Siphonodictyon*. These two groups were found to be very similar in morphological characters but were not studied for chemical or reproductive characters. Rützler (1971) observed larvae in a species of *Siphonodictyon*, which suggests this genus may have viviparous development in keeping with its location in the order Haplosclerida. In this study, *Oceanapia n.sp.* was found with large oval bounded cell masses 190 x 280 µm in size, and similar masses were found in a species of *Oceanapia* from south-west Australia. These bounded masses do not have differentiated cells, normally found in sponge larvae, or a nucleus, normally present in sponge oocytes, and it is possible that they are asexually derived reproductive products. Bergquist (pers. comm.) has found similar products in other species of the subclass Ceractinomorpha and concludes that they are asexually derived. The reproductive mode of these species could provide new information on their relationships with other sponge species and should be examined.

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## APPENDIX I.

Glossary of terms used principally in Chapters Two and Three. (from Wiedenmayer, 1977a and Bergquist 1978).

Axial canal: Central growing region of spicule.
Centrangulate sigma: a sigma with a sharp bend in the centre of the curve.
Choanocyte: Flagellate cell crowned by a collar of cytoplasmic tentacles and responsible for generating the water current.
Choanosome: The area of the sponge where choanocyte chambers occur and in general terms refers to the interior of the sponge.
Conulose: Cone-shaped surface protrusions, generally over a fibre end.
Diactinal: A category of megasclere pointed at both ends.
Ectosome: The superficial region of a sponge.
Endosome: Internal skeleton, a term interchangeable with choanosome.
Fasciculate: Fibres collected together in groups.
Fibre: A discrete column of spongin and/or spicules and one of the chief structural elements of the skeleton of many demosponges.
Fistule: A hollow erect structure like a pipe or reed and used to describe erect columns extending vertically from the sponge surface.
Fusiform: Shaped like a spindle; tapering at both ends.
Haematoxylin-eosin: A histological stain that colours collagen light pink.
Hastate oxea: A spear-shaped oxea.
Hispid: Rough or bristly.
Interstitial: Spaces or openings between the skeletal tracts.
Isodictyal: A skeletal network where the meshes are commonly triangular in all directions.
Isotropic: disorganised, random.
Mammiform: A spicule ending in the shape of a breast.
Megascleres: The larger structural spicules.
Mesohyl: The intermediate or central area of the sponge body.
Microscleres: Smaller spicules which are frequently of ornate shape and used for reinforcing membranes or for packing the skeleton.
Oxeas: Megasclere spicules pointed at both ends.
Renierid: The type of reticulate structure in the family Haliclonidae (Renieridae) i.e. a simple, generally unispicular, isodictyal reticulation.
Reticulate skeleton: Skeletal architecture characterized by branching and anastomosing of fibres, tracts, or megascleres to form a ladder-like network.
Sigma: a c or s-shaped type of microsclere.
Spicule: a discrete skeletal element consisting of silica in the Class Demospongiae.
Spongin: Collagenous material deposited in the form of fibres.
Strongyle: A megasclere with both ends blunt and rounded.
Strongyloxea: An oxea with either one (anisostrongyloxea) or both ends blunt but not rounded - between an oxea and a strongyle.
Style: A megasclere with one end rounded and the other pointed.
Tangential skeleton: Orientated parallel to the surface.
Toxa: A bow-shaped type of microsclere.

## APPENDIX II.

**Results of a pilot study, using enzyme electrophoresis, to distinguish between two sponge species.**

**INTRODUCTION.** The aim of this study was to use enzyme electrophoresis to distinguish two sponge species, belonging to two genera. These results were compared with those from morphological, chemical and reproductive data. The two taxa *Niphates n.sp.* and *Haliclona amboinensis* occur in shallow waters, less than 5 metres deep, at Magnetic Island. Both species form small, thick encrustations on boulders and limestone substrata under the macrophyte *Sargassum*.

Horizontal starch gel enzyme electrophoresis has been used on sponges from other orders with promising results (Sole Cava & Thorpe 1986, 1987). Sponges of three morphotypes of *Suberites ficus* (order Hadromerida) that did not show any significant differences in spicule size or types could be easily differentiated on isozyme patterns (Sole Cava & Thorpe 1986). Significant differences in isozyme patterns, between samples, can indicate genetic differentiation implying reproductive isolation and hence identify separate species (Sole Cava & Thorpe 1986). In general, the more closely related species are, the greater the similarity in their electrophoretic patterns (Ferguson 1980).

**METHODS.** Five replicate colonies of *Niphates n.sp.* and *H. amboinensis* were collected alive from Magnetic Island, returned to the laboratory in aerated seawater and kept in aquaria overnight. The next morning a piece of each sample (approx. 1 $cm^{-2}$) was cleaned of foreign material and symbionts and homogenised with equal amounts of indicator-extractant solution (0.1 ml mercaptoethanol, 0.1 g bromophenol blue and 10 g sucrose per 100 ml distilled water) using a pestle and mortar. Small rectangles of chromatography paper (Whatman No.3) were soaked in the homogenate and were applied to horizontal starch gels (12% w.v. Electrostarch, Otto Hiller Co., Wisconsin). Two buffer systems were used, Tris-citrate pH8 (TC8) and Tris-EDTA-borate (TEC7) and the gels were run for 4 hours at 50 mA. Gels were sliced and stained for the following enzymes: leucyl tyrosine peptidase (Pep(Lt)), 6-phosphogluconate dehydrogenase (6pgd), glucosephosphate isomerase (Gpi), isocitrate dehydrogenase (Idh), mannosephosphate isomerase (Mpi), hexokinase (Hk), phosphoglucomutase (Pgm), and malate dehydrogenase (Mdh). The number of bands and their location were scored to provide an objective description of the zymograms (Figure A), but no further analysis was undertaken with this data.

**RESULTS.** Three of the enzyme systems showed banding patterns which were consistent within a species, (Pep(Lt)), 6pgd and Idh, and different between species. Three systems had non-specific bands at the top of the gel, Hk, Pgm and Mdh (Figure A). These were run on a Tris-EDTA-borate buffer, and consequently, the non-specific loci may be related to the presence of EDTA. Complex, multibanding patterns were found for Mpi, Mdh, Hk and Pgm. The Pgi banding pattern suggested a dimeric structure produced by a single locus (Figure A). Activity was intense for this enzyme system and (Pep(Lt)), 6Pgd and Idh.

**DISCUSSION.** This pilot study showed that two species of the Haplosclerida were amenable to enzyme electrophoretic techniques, suggesting that, if time had permitted, enzyme electrophoresis would be a useful technique for resolving species complexes. The species used in this experiment were morphologically distinguishable and the banding patterns of the two species were distinctive. It is important that a minimum of two diagnostic fixed differences are used for differentiation between sympatric species (Richardson *et al.* 1986). A fixed difference occurs when two species fail to share any alleles at a locus. These species had species specific alleles in three of the enzyme systems used: (Pep(Lt)), 6pgd and Idh.

FIGURE A. Buffer = TC8 Gels after 2.0 hours

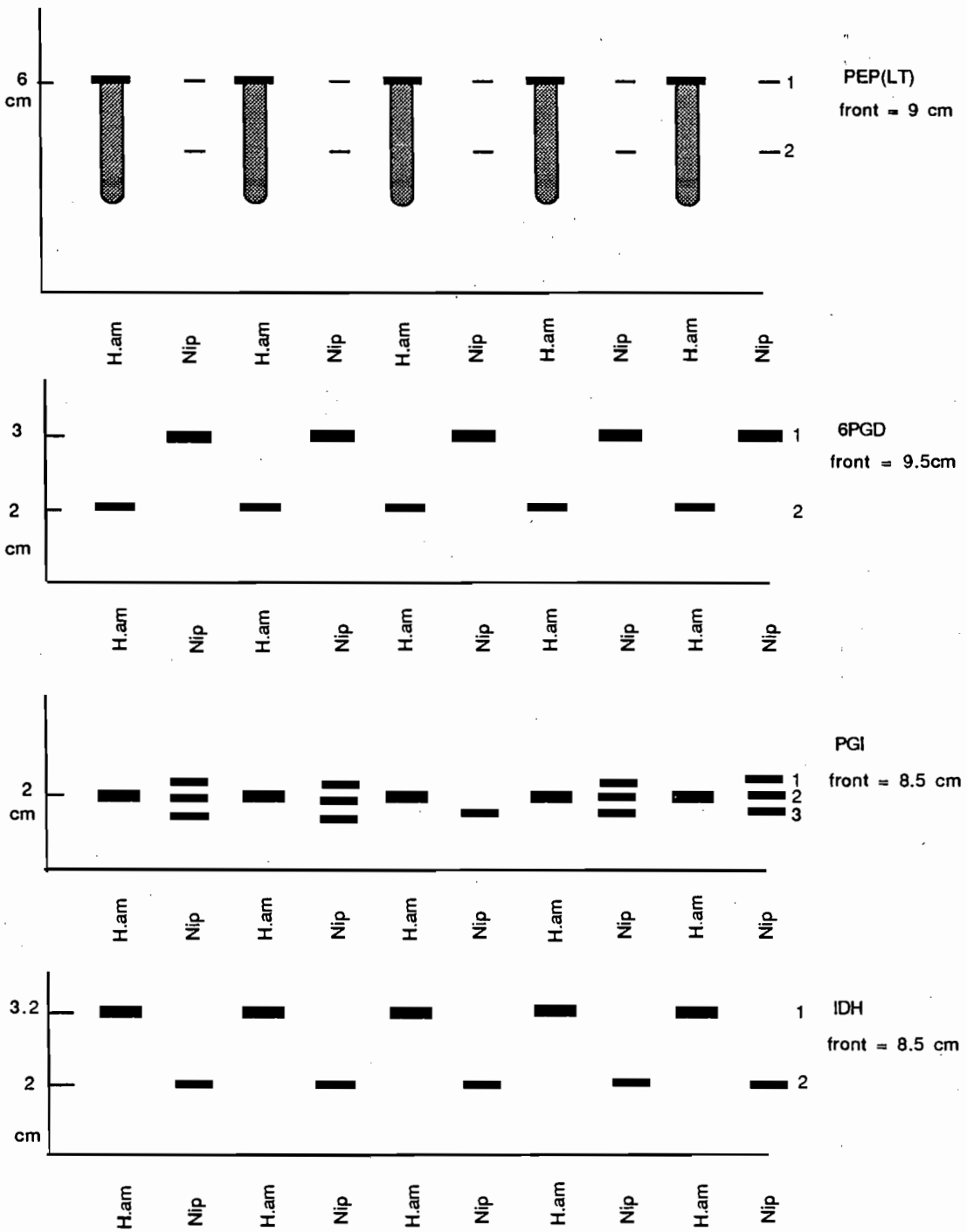
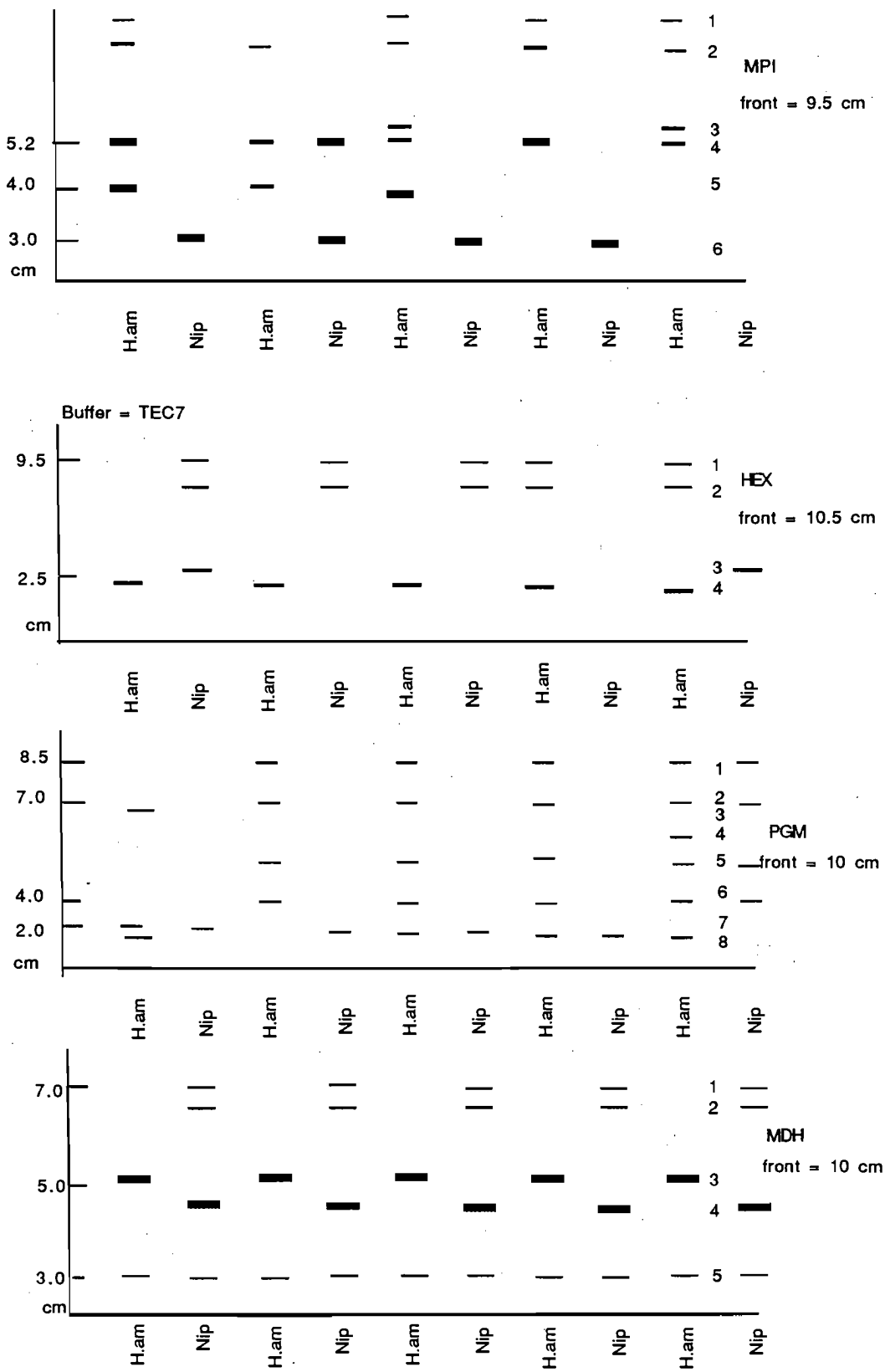
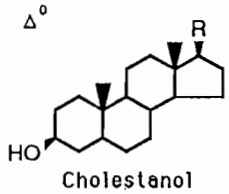


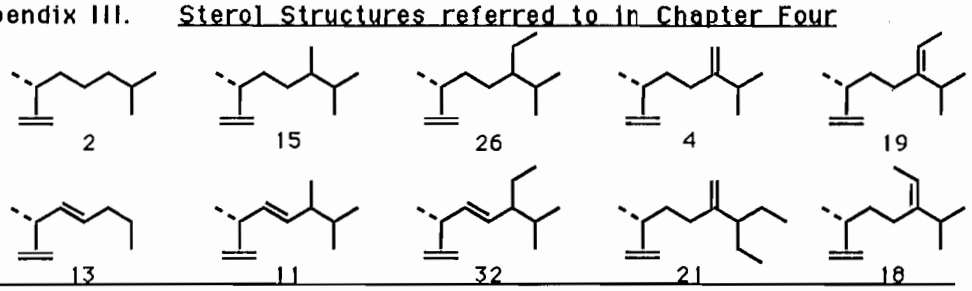
FIGURE A Cont. Buffer = TC8 Gels after 2.5 hours

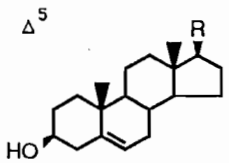


## Appendix III. Sterol Structures referred to in Chapter Four

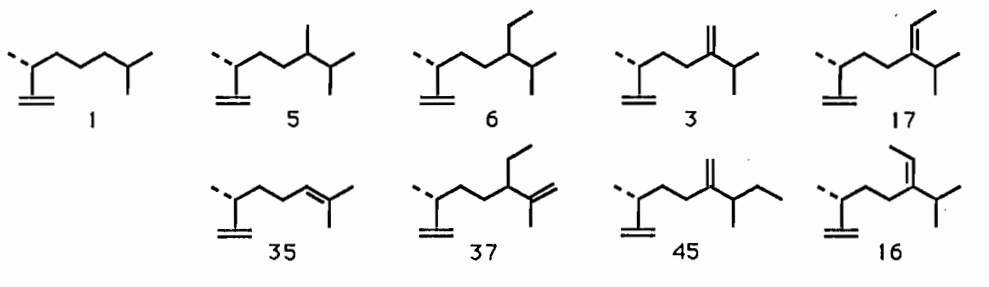


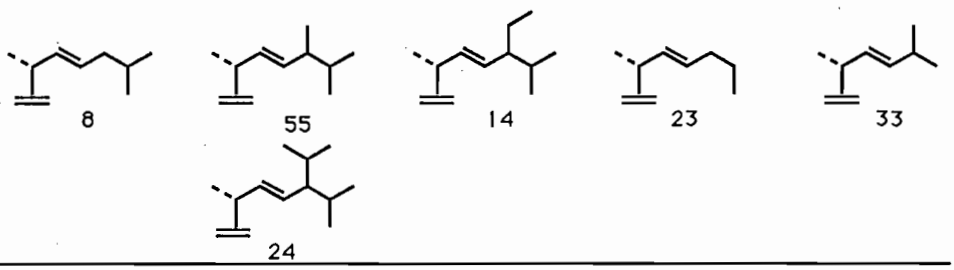

Cholestanol

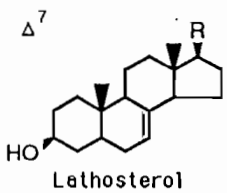


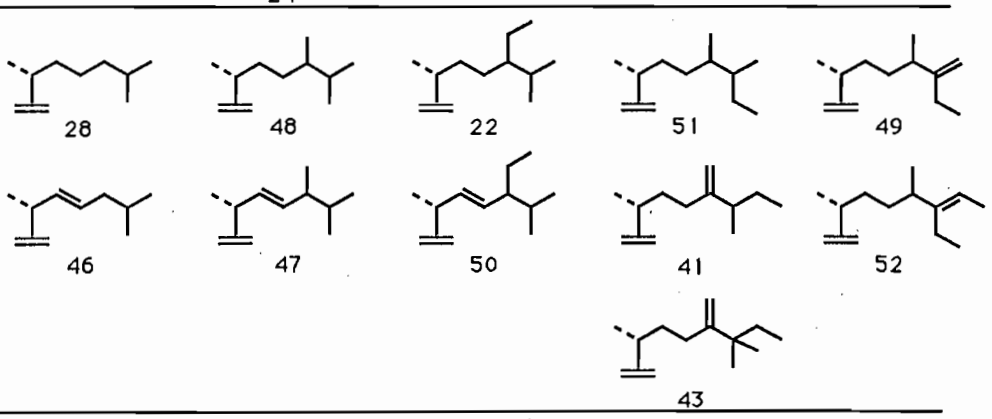

Cholesterol

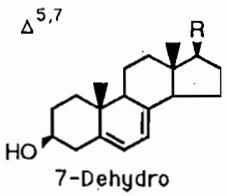


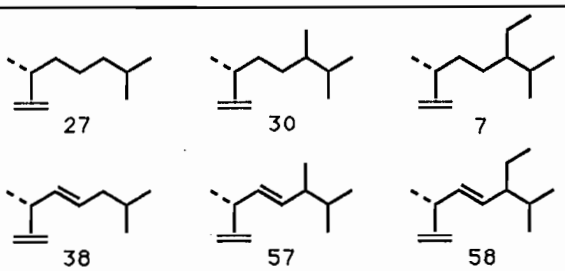




Lathosterol




7-Dehydro cholesterol



# APPENDIX IV.

Proceedings of the 6th International Coral Reef Symposium, Australia, 1988, Vol. 2

## ASPECTS OF THE REPRODUCTIVE BIOLOGY OF *XESTOSPONGIA TESTUDINARIA* (GREAT BARRIER REEF)

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### ABSTRACT

The reproductive biology of a species of marine sponge thought to have an oviparous (broadcasting) mode of reproduction, was investigated. In 1986 it was established that *Xestospongia testudinaria* broadcast eggs in a synchronous spawning event in November at the inshore site of Orpheus Island on the Great Barrier Reef. In addition it was established that two distinct forms of this species occurred in sympatry at this site. In 1987 each form was monitored to determine the sequence and timing of gametogenesis and environmental factors were assessed as possible regulatory mechanisms of the observed synchrony of their reproductive development and spawning. The results indicate that pronounced differences occur between the forms in the later stages of oocyte development and that the spawning of the forms occurs as two temporally separate events. These results are presented in detail and the reproductive biology of the forms discussed with reference to the existence of a reproductive isolating mechanism.

### INTRODUCTION

This study of the reproduction of *Xestospongia testudinaria* Lamarck was undertaken as part of a larger study on the reproductive biology of five species of sponges on the Great Barrier Reef. These investigations are one aspect of a multidisciplinary project addressing the taxonomy of two Orders of the Class Demospongiae (Phylum Porifera).

Prior to this study little information was available concerning reproduction in the genus *Xestospongia*. The Caribbean species, *Xestospongia muta* (Schmidt), has been reported to have gonochoric development and release sperm and eggs in a synchronous spawning event (H.M. Reiswig, pers. comm.). In November 1986 individuals of the Indo-Pacific species *Xestospongia testudinaria* from the Great Barrier Reef were observed with extruded eggs over the external surface of the sponge and the surrounding substrate. These observations suggest that both species employ an oviparous reproductive strategy, releasing unfertilised eggs directly into the water, and that oviparity may be characteristic for the genus. To further investigate oviparity in *Xestospongia*, a field programme was designed to monitor the reproductive development of populations of *X. testudinaria*.

Initial field monitoring revealed that two distinct morphologies of this species existed and occurred in sympatry within the study area (figure 1, a-f). These forms were termed 'soft' and 'hard' on the basis of the consistency of their skeletons. The hard form has spongin fibre encasing complete tracts of spicules while the soft form has spongin fibre development binding the junctions of the spicule tracts. The forms varied in colour with the hard form tending to be reddish-brown and the soft form maroon. The external growth form and spiculation of the two forms were identical and their geographic distributions overlapped.

As a consequence of the discovery of the two forms, the monitoring programme was expanded to provide detail of gametogenesis and the timing of gamete release in both forms.

A correlation was sought between the timing of gametogenesis and spawning and environmental factors that could act as triggering mechanisms for these events. Sea temperature, moon phase and tidal rhythms have been implicated in the timing of spawning in other sponges (Hoppe and Reichert 1987) and in coral spawning events on the Great Barrier Reef (Babcock *et al.* 1986). The November 1986 observations on spawning of each form of *Xestospongia testudinaria* on the Great Barrier Reef were discrete events, which occurred during the same month as the coral spawning. Finally, if reproduction and spawning events in the two forms proved to be different, and these differences were not symptoms of differences in distribution, depth, and other environmental factors between the two forms, argument for separation at species level would be provided.

### METHODS

The study reef was at Orpheus Island in the Palm Island group, a continental island off the east coast of Australia (figure 2). Site A was established in the Southwest corner of Pioneer Bay adjacent to the James Cook University marine research station at Orpheus Island. The population was initially assessed for the proportion of the hard and soft forms (table 1).

Table 1. Occurrence of morphological forms of *X. testudinaria* per site (n=20)

| SITE | SOFT FORM | HARD FORM |
|---|---|---|
| A Pioneer Bay | 11 | 9 |
| B Pioneer Bay | 15 | 5 |
| C Pelorus Island | 16 | 4 |

Samples were removed from each of three females of each form at monthly intervals from July 1986 until the predicted spawning period (November 1987). The predicted period of spawning between October and December was based on the observed spawning events in November 1986. Late in October 1987 two additional sampling sites were established, Site B at the Northern end of Pioneer Bay

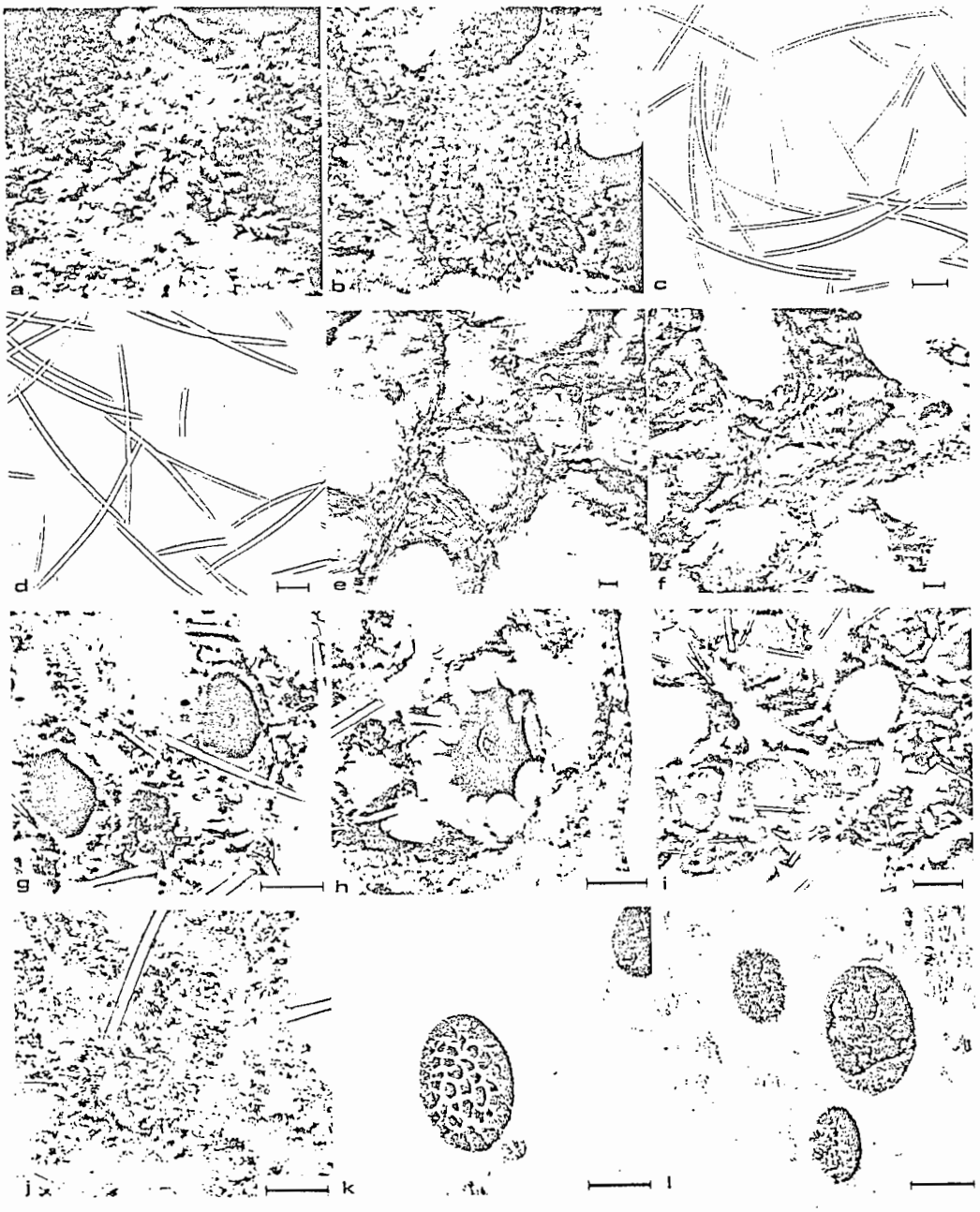


Figure 1. a) whole specimen of a soft form; b) a hard form; c) spicules of a soft form; d) spicules of a hard form; e) skeleton of a soft form; f) skeleton of a hard form; g-l) reproductive development in soft forms; g) developing oocytes (30/9/87); h) mature eggs with scalloped edges, differentiated yolk and a clear region around the nucleus (9/11/87); i) atrophying eggs (9/11/87); j) sperm (9/11/87); k) fertilised eggs after spawning (13/11/87); l) an early larvae (13/11/87).
——— = 50 micro-metres (um)

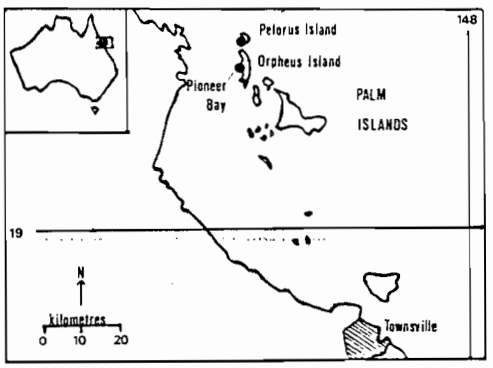


Figure 2. Location of Orpheus Island and Pelorus Island, Great Barrier Reef, Australia

and Site C at Pelorus Island located one kilometre north of Orpheus Island. The sample size was increased to a total of twenty individuals per site and the sampling frequency was increased from monthly to less than six day intervals.

Sampling consisted of taking a plug of "tissue" from the central third of each sponge with a 5.0 mm cork borer. Samples were fixed in Bouin's fixative, washed in 70% ethanol and embedded in paraffin. The paraffin blocks were sectioned at 10um thickness, stained in Mayer's hematoxylineosin and mounted with DPX for examination by light microscopy. The sections were examined for presence and development of eggs and sperm. In order to determine the mean oocyte diameter for each female during each sampling period ten oocytes from each female were measured each sampling period. To decrease the sampling variation, only oocytes sectioned through the nucleus were measured.

Sea temperatures were recorded hourly on a data logger situated at a depth of five metres in Pioneer Bay. Tidal range and moon phase were extracted from the Australian National Tide Tables.

## RESULTS

In the reef areas of Orpheus and Pelorus Islands where this study was undertaken, the soft form was more abundant then hard forms at all sites examined (table 1). However, observations of midshelf and outer reefs indicate the reverse is the case in these areas on the Great Barrier Reef (Fromont, unpublished data).

## GAMETOGENESIS AND SPAWNING

### Soft Form

Following the November spawning in 1986, sponges contained no visible sperm or oocytes until May 1987 when developing oocytes with a mean diameter of 16um were first observed. Oocytes increased slowly in size (1-5um mean diameter per month) until September when a marked increase in growth of the oocytes occurred (figure 3a). From September to spawning in November the monthly increase in mean oocyte diameter was from 6-14um.

The early oocytes were small with a visible nucleus ~7um in diameter (figure 1g). The oocytes contained granular yolk near their outer edges. The exterior edge of the oocyte was not always clearly bounded by an epithelial layer, and some cellular exchange appeared to be occurring between the oocyte and the parent tissue. Small cells were observed closely associated with the developing oocytes but could not be distinguished morphologically from cells occurring throughout the adult sponge.

No obvious morphological changes occurred in the oocytes until late October and early November when the yolk of the eggs clearly differentiated (figure 1h). At this time the peripheral yolk stained mauve and the central yolk stained red. The peripheral mauve staining yolk extended projections over a clear region between the yolk and external epithelial layer, hence the yolk had pronounced scalloped edges (figure 1h). Immediately adjacent to the nucleus was a clear region where yolk was absent. While differentiation of the yolk of some oocytes was occurring others (up to 50% of the total oocytes) were atrophying and being reabsorbed into the adult tissue (figure 1i).

Sperm development was not detected until 9/11/87, two days prior to spawning. Development was rapid as no sperm were found in males from the previous sampling on 7/11/87. The sperm present on 9/11/87 were at an early developmental stage, consisting of small purple staining cells with no aggregation into spermatocysts (figure 1j). No flagella were visible at this stage.

Spawning in the field occurred on 12 and 13/11/87, 175 and 176 days respectively after the first observation of developing oocytes. Spawning was not observed in the field populations but eggs were found coating the outer surface of the females and the surrounding substrate at 1200 hours on 13/11/87. The eggs were negatively buoyant, ovoid and white, and many of them were coated in a fine layer of silt. It is probable that these eggs were spawned on the previous day (12/11/87). Microscopic examination revealed that these were early embryos undergoing cell divisions and development was not synchronous (figure 1k, l). Some of the embryos contained multiple nuclei, and a few had columnar shaped cells localised at the periphery of the embryo. The remainder of the eggs were not coated in sediment and were obviously newly released. These were embryos undergoing cell divisions but none appeared to contain the developmentally advanced columnar cells. No sperm release was seen in the field nor were they observed to be associated with the eggs.

In an attempt to view the spawning event, two females of the soft form were put into aquaria at Orpheus Island on 23/10/87 and monitored in the hope they would act as indicators of the progress of the females in the field. However, these sponges spawned two and three days earlier than those in the field. This spawning occurred on consecutive mornings (9 & 10/11/87) between 7 and

FIG. 3A MEAN OOCYTE DIAM. (SOFT)

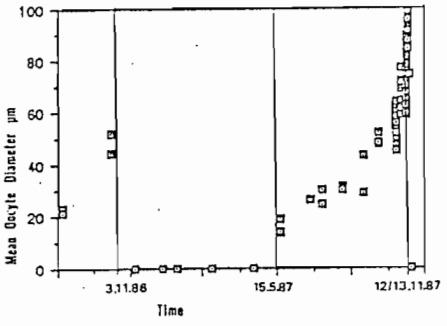


FIG. 3B MEAN OOCYTE DIAM. (HARD)

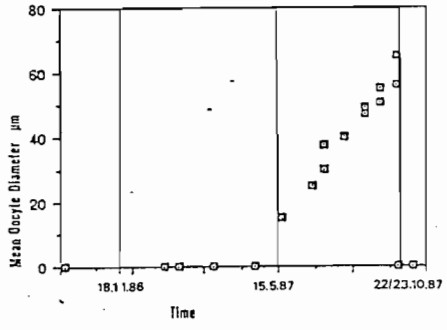


FIG. 3C AVERAGE WEEKLY SEA TEMP.

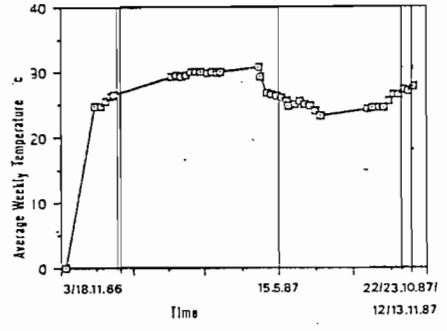


Figure 3. Monthly increase in oocyte diameters and average weekly seawater temperatures in Pioneer Bay in 1986 and 1987. In Figure 3A and 3B, ⊡ = mean oocyte diameter of one female

9 am. A third adult female was placed in aquaria on 10/11/87 and spawned on 12/11/87, at the same time as the field population. Eggs of the females in aquaria retained homogenous yolk with a central nucleus (figure 4a). No male colonies were observed in aquaria, and it was not determined whether sperm and ova were released synchronously, or whether sperm were released before ova and fertilisation occurred internally.

Hard Form

In females of the hard form, developing oocytes with a mean diameter of 15 um were first observed in May 1987. Oocyte mean diameter increased slowly and steadily (4-10um per month) until spawning occurred in late October. This form showed no late rapid increase in egg size (cf soft form) and at spawning had a mean oocyte diameter of 61um (figure 2b).

The early oocytes were indistinguishable from those of the same stage occurring in the soft form (Figure 4b). The centrally orientated nucleus (~6um mean diameter) and nucleolus (~2um mean diameter) were visible during the earliest stages of development. The eggs were irregularly ovoid in shape with parts of the peripheral yolk closely associated with the parental tissue such that some exchange of cytoplasm appeared to be occurring. Coarsely granular yolk was aggregated in the outer regions of the egg. In contrast to the soft form, no degeneration of egg tissue was observed and the majority of the eggs that developed to maturity retained an ovoid shape (figure 4c). In addition no scalloping of yolk was observed and histological samples from late October had mature eggs with an undifferentiated, dense homogeneous yolk and fine irregular cell processes extending into a clear area between the yolk and follicular epithelium. Unlike the centrally oriented nucleus of the soft form, the nucleus of the hard form was peripheral and lacked a visible nucleolus.

Sperm were not observed in the monthly sample on 30/9/87. Sperm development was detected one day (21/10/87) prior to spawning, but no information was available as to the rate at which sperm developed in this form. The sperm observed on 21/10/87 were well developed and clustered in large spermatocysts 60um in diameter (figure 4d). Individual sperm were too small to examine in detail, but tails were visible on the most mature sperm.

Spawning of hard forms was not observed in the field. Eggs and sperm were present in sponges on 21/10/87 but not on the 23/10/87 at Sites B and C or 24/10/87 at Site A. This suggests spawning may have occurred on 22/10/87 or 23/10/87. Storm conditions prevailed in the area at this time and eggs released on the morning of 23/10/87 would have been rapidly washed from the sponge surfaces. Spawning of the hard forms therefore occurred 154 and 155 days after oocyte development was first noted, and 21/22 days before the soft form.

## DISCUSSION

Results of this study indicated that true ovipary occurred in the soft form. Females of this form in aquaria, in the absence of males, released unfertilised eggs. Spawned eggs of the hard form were not obtained in the present study so this question will be reinvestigated in the 1988 breeding season. It was also possible to establish, at least for the soft form, that sexes remain unchanged between breeding seasons. Two females

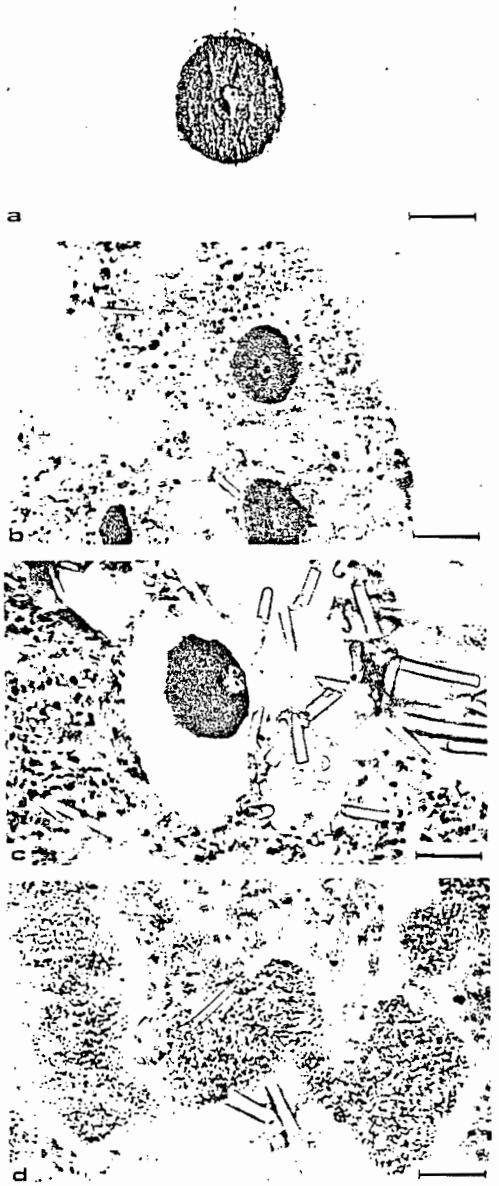


Figure 4. a) an unfertilised egg released from a soft form in aquaria (10/11/87); b) developing oocytes from a hard form (30/9/87); c) mature ovoid eggs with peripheral nucleus from a hard form (21/10/87); d) sperm from a hard form (21/10/87). ——— = 30 micro-metres (um).

of the soft form which were observed with eggs in 1986 also produced eggs in 1987.

Both forms have separate sexes (gonochorism) and both have egg development preceding sperm development (protogyny). The development of the eggs in both forms (5-6 months) is the longest period recorded for sponge oocytes while the sperm development of less than five days in the soft forms, is the shortest development yet recorded in the Porifera.

Little is known of the regulatory mechanisms associated with oocyte and sperm development in sponges (Fell 1983), and comparison between onset of gametogenesis and average seawater temperature failed to show any distinct correlation for either form in this study. The detection of oocyte development in May 1987, did not establish the precise timing for the onset of egg development. Previous samples examined for the presence of reproductive products were taken on April 14 1987 when no oocytes were detected. Consequently, it is possible that the onset of egg development may have occurred any time since the April sampling, and any correlation of development with environmental factors can only be inferred on a coarse scale. However, average weekly temperature recordings from Pioneer Bay showed seawater temperature was decreasing when oocyte development was first detected in both forms in May 1987 (figure 3c). Conversely, sea temperature was increasing when sperm development was first observed in both forms (figure 3c). However, it is quite likely that the trigger for sperm production is also related to oocyte development and other endogenous or exogenous factors, in addition to temperature.

The fact that regulatory mechanisms are in existence in both forms is evident from the observed synchrony of oocyte development which was almost perfect within individuals and within and between populations. Males within each form developed sperm during the same time period, and sperm development was synchronous within a spermatocyst but asynchronous between spermatocysts in any one individual. This phenomenon occurred in all the males examined, and as egg spawning occurred over two consecutive days, it is possible that asynchronous sperm development is a mechanism which enables males to release reproductive products on both days of egg release.

No correlation between measured environmental parameters and spawning was detected for the soft form. In 1986 the soft form spawned the day after a new moon at peak tidal range and when the average daily seawater temperature was 27.40C (figure 5a, b). In 1987, the soft forms, including the same individuals whose spawning was observed in 1986, spawned six and seven days after a full moon at minimum tidal amplitude and when the average seawater temperature was 280C (figure 5c,d).

A possible correlation between spawning and lunar cycle was detected for the hard forms. In 1986 the hard forms spawned one day after full moon and just after maximum tidal amplitude. No temperature data is available for this time but as it was the onset of summer, it is assumed that temperatures were increasing. In 1987 the hard forms spawned at new moon when tidal amplitude had peaked and the average daily seawater temperature

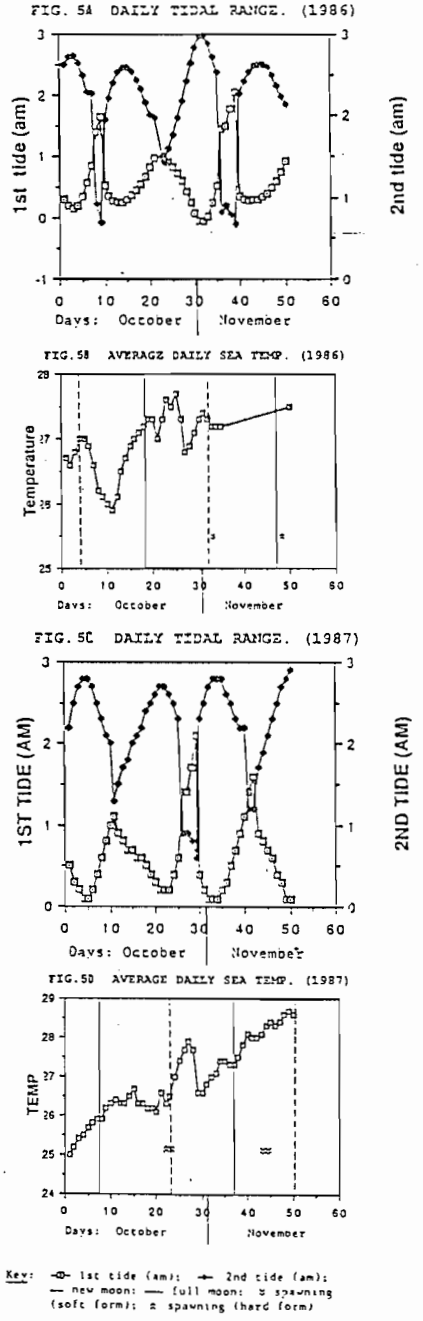


Figure 5. Tidal amplitudes, average daily seawater temperatures (Pioneer Bay), moon phase and spawning times of *X. testudinaria* in 1986 and 1987

was 26.1°C (figure 5c, d).

In contrast to the afternoon spawning of *Neofibularia nolitangere* in the Caribbean (Hoppe and Reichert 1987) and evening spawning of corals on the Great Barrier Reef (Babcock *et al.* 1986) these sponges spawned in the morning between 7 and 10 am both in 1986 and 1987. Their spawning pattern closely resembles the reported spawning of *X. muta* from the Caribbean (H. Reiswig pers. comm.), which occurred on two consecutive days in March 1976, at approximately 9.30 am. This consecutive two day period is the same strategy as described in the present study for *X. testudinaria*.

The diel timing of the spawning events of these forms is consistently a morning occurrence, and it is possibly linked to hours of daylight following sunrise. The diel light:dark regime is suggested to be the ultimate factor in triggering the release event in *N. nolitangere* (Hoppe and Reichert, 1987). The data suggests that the hard form has a semilunar rhythm providing a periodicity that acts at the time of full moon and at new moon, but these data are insufficient for detailed correlations to be made. It is intended that the sequence of development and time of spawning will be investigated in more detail in 1988, to determine more conclusively which environmental factors are used as spawning cues.

Although few environmental factors could be correlated with the onset of gametogenesis or the timing of spawning in these forms, it was obvious that the forms spawned at distinctly different times, separated in 1986 and 1987 by fifteen and twenty days respectively. The differential timing of these spawning events is indicative of a reproductive isolating mechanism between the forms.

Other differences were also observed in the reproductive biology of these forms. The late and rapid increase in size of the oocytes and yolk differentiation in soft forms did not occur in the hard forms. Eggs of the hard form differed in retaining an ovoid shape throughout development and the nucleus of the eggs altered position, moving to the periphery of the yolk prior to spawning. Scalloping of yolk edges in oocytes of the soft form and atrophying of eggs were not found in the hard form. Degeneration and resorption of a large number of oocytes prior to spawning has also been reported in populations of *Verongia cavernicola* and *V. aerophoba*. However, that phenomenon was only known to occur during certain years, and Fell (1983) suggests that it may be related to unfavourable environmental conditions or to a failure of fertilization to occur. It is unlikely that the latter explanation sufficiently explains the present data since the appearance of atrophying eggs was observed to occur prior to the fertilisation event. The possibility that the soft form was detrimentally affected by environmental factors in 1987 cannot be negated and this aspect will be addressed further in the 1988 breeding season.

The formal differentiation of two forms of *X. testudinaria* has not been reported previously in the literature, although both forms have been separately recorded as the same species in recent publications. Vacelet *et al.* (1976) and Bergquist (1980) include photographs of the hard form while

Desqueyroux-Faundez (1987) reports the occurrence of *X. testudinaria* in New Caledonia and includes a photograph of a soft form. Topsent (1933) noted that the holotype of *X. testudinaria* was missing from the Lamarck collection in the Paris Museum, and the species is used *sensu* Ridley and Dendy (1887). Consequently it is not possible to determine which of the soft or hard forms is typical for the species. The differences in colour, the consistency of their skeletons, and the degree of spongin fibre development in their skeletal structure are morphological characters distinguishing these forms. The results of this study on their reproductive biology provides conclusive evidence that the forms are distinct species. Obvious differences in oocyte development became apparent in the month prior to spawning; no temporal overlap in sperm development occurred between the two forms; and a 15-20 day temporal separation of the spawning events was observed between forms.

The biological species concept is based upon reproductive isolation between species. A temporal reproductive isolating mechanism decreases the likelihood of cross fertilization occurring and non-viable offspring being produced from closely related species with overlapping distributions. From the results of this study it is concluded that the hard and soft forms are closely related and reproductively isolated species. Additional evidence for this conclusion is supported by an analysis of their sterol chemistry (Fromont, unpublished data) which yielded two distinctive sets of sterols that coincide with the division of the species based on reproductive characters.

## ACKNOWLEDGEMENTS

I thank the following: Porferio Alino, Kerrie Licastro, Ian Goodwin, Chey Rayner, Michelle Kelly, Allan Horsup, Anne Jackson and David Sutton for voluntary field assistance; Paul Muir for assistance with computing; David Sutton and Russell Babcock for critical comments on the manuscript; Cathy Robinson for typing the manuscript; the Coral Reproduction Group, James Cook University for sea temperature data; and the Orpheus Island Research Station personnel for the use of the facilities.

The work was supported by a special Research Grant from James Cook University and a Commonwealth Postgraduate Research Award.

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