Marine Lakes of Indonesia

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Marine Lakes of Indonesia

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To my mother and sister

for their inspiration and support

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I discovered in nature the nonutilitarian delights that I sought in art. Both were a form of magic, both were a game of intricate enchantment and deception.

Vladimir Nabokov, 1951

“Speak, Memory”

Summary

The objective of this thesis is to obtain insight into the processes that play a role in biodiversity patterns of tropical marine species by using marine lakes as a model. It has long been hypothesized that marine species in general have large geographic ranges with large population sizes, and face weaker barriers to dispersal than terrestrial organisms. Recent population genetic and phylogenetic studies, however, show a different picture of population differentiation at small spatial scales. This suggests there may be many more barriers for dispersal and consequently more opportunities for allopatric speciation for marine organisms than initially assumed. The marine lake setting with clearly delineated contours provides an opportunity to study species assemblage patterns and early stages of evolution in coastal marine taxa in isolated environments. Marine lakes are little known anchialine habitats. The term anchialine refers to landlocked water bodies that maintain a marine character through narrow submarine connections to the sea. They display a tidal regime that is typically delayed in phase and dampened in amplitude compared to the adjacent sea. The lakes are usually situated in natural inland depressions in between hills; hence they are not visible from the coast. It is unknown exactly how many marine lakes there are worldwide, but the number is estimated at approximately 200 with clusters of ten or more lakes occurring in karstic limestone areas such as Croatia, Bermuda, Vietnam, Palau, and Indonesia. The lakes we see today were formed less than 1500 years ago. Each lake is thus ephemeral on a geological timescale, but the marine lakes ecosystem has probably been present through time. The few studies that focused on the biodiversity of marine lakes, conducted in Palau and Vietnam before the present thesis, portrayed a scenario of isolated populations and a high abundance of species rare or absent elsewhere. These results indicate that marine lakes are remarkable marine ecosystems with an untapped potential for studies on marine biodiversity and evolution, i.e. natural laboratories of evolution. In this thesis I have studied recently discovered marine lakes in two regions of Indonesia: the Berau region in East Kalimantan (Borneo island) and the Raja Ampat region in West Papua (New Guinea island). The aim was to unveil spatial biodiversity patterns in marine lakes in order to establish to what extent they represent isolated coastal environments. The following questions were addressed:

1. What are the different types of marine lakes in Indonesia?
2. Are the species assemblages in marine lakes distinct from those in the adjacent coastal environments?
3. To what extent are the populations in the lakes isolated?
4. Can marine lakes in Indonesia be considered natural laboratories of evolution?

When I began my PhD in 2007 little was known about marine lakes in Indonesia. As a result much descriptive groundwork (e.g. locating the lakes, describing their geographical and physical characteristics and unraveling the taxonomy of the species residing in these lakes) was a prerequisite before any further analytical studies could be performed. Sponges were chosen as a target group to measure biodiversity, because sponges are one of the most diverse taxa in the lakes and concomitantly constitute important players in reef and mangrove systems outside of the lakes in terms of diversity, biomass and filtering activities. This made them an ideal candidate to compare biodiversity inside and outside the lakes. Three different aspects of isolation were considered in order to establish whether the lakes in Indonesia are isolated environments:

(a) the physical degree of connection of the water between the lakes and the sea; the amount of exchange of water with the adjacent sea differs per lake and can function as a proxy for the degree of physical isolation of a lake.

(b) the patterns of species assemblages of sponges; variation in species assemblages between localities can provide information on marine area relationships or connectivity, reflecting the processes operating in those areas.

(c) the genetic patterns of populations of two typical species of marine lakes: the sponge *Suberites diversicolor* (Porifera: Demospongiae: Hadromerida: Suberitidae) and the mussel *Brachidontes* sp. (Mollusca: Bivalvia: Mytilidae); molecular markers are well suited to estimate levels of connectivity between natural populations and to estimate levels of diversity and divergence within populations.

If the lakes are in high connection to the adjacent sea and to each other we would expect to find similar species assemblages and little genetic differentiation between populations, particularly between geographically close localities.

1. *What are the different types of marine lakes in Indonesia?*

The results of this thesis show that there is a large diversity in types of marine lakes and many more remain to be documented in Indonesia (CHAPTER 1). There is a gradient in the degree of connection to the sea. The higher the connection the more the lake resembles a lagoon in both water chemistry and biota, while the more isolated lakes have brackish water and contain unique species that are rarely found in the adjacent sea.

2. *Are the species assemblages in marine lakes distinct from the adjacent coastal habitats?*

The spatial variation in sponge species composition of assemblages in marine lakes, coastal mangroves and coral reefs in Berau (East Kalimantan, Indonesia) was systematically and quantitatively measured. These comprehensive studies show that marine lakes are true sponge gardens containing strikingly different assemblage of sponge species with just a subset of the adjacent sea fauna (CHAPTERS 2, 3, 4 & 5). The lake assemblages consist of three groups of sponge species: (a) widespread species known from various coastal locations in Indo-Pacific reefs, (b)lake species that only occur in lake systems, (c) endemic species restricted to a single lake. These marine lakes significantly contribute to the regional diversity due to the presence of lake and endemic species. Over half of the species in these marine lakes do not have a scientific name and need to be described in a taxonomic framework (CHAPTERS 1, 3, 4 & 5).

3. *To what extent are the populations in the lakes isolated?*

In addition to a unique species diversity, lakes can harbor genetic variants not found elsewhere (CHAPTERS 6 & 7). In both *Suberites diversicolor* and *Brachidontes* populations, two highly diverged lineages were detected that may represent cryptic species(CHAPTERS 6 & 7). Furthermore, in both species we see a pattern emerging of possible recent local diversification in the largest and most isolated marine lake in Indonesia (Kakaban lake in East Kalimantan). The patterns of genetic variation found in the marine lake populations are generally consistent with populations in isolated environments. Isolation of marine lake species assemblages and populations may be the result of strong barriers to dispersal and/or different selective regimes within the lakes.

4. *Can marine lakes in Indonesia be considered natural laboratories of evolution?*

The lakes are no older than 15000 years old, yet much of the species and genetic diversity appears to be restricted to each lake (all CHAPTERS). Given the areal definition of an endemic as spatially restricted species, centers of endemism could be areas where species arise and remain (cradles), and/or the last stand of previously widespread species (refuge). The lakes appear to be cradles of diversity resulting from recent divergence of evolving populations within the lakes (Chapter 6 & 7). The lakes also may serve as refugia for ancient lineages, relicts of marine or older anchialine lake species and populations (Chapter 3, 6 & 7). The role of marine lakes in supporting endemism may thus reflect enhanced survival of endemics, with the possibility of population differentiation that in time may lead to speciation. This thesis only hints at some of the consequences of short term isolation on structuring marine assemblages and populations, but a wealth of information can be gained from studying the interplay of organisms and environments in the marine lakes of Indonesia. Further study of marine lakes will enhance our understanding of some of the physical and ecological processes responsible for diversification in tropical shallow marine environments.

The findings of this PhD research also have important implications for conservation. The marine lakes share characteristics with island systems: they are well-defined geographically (CHAPTER 1), harbor unique biota with a large proportion of endemics and/or an abundance of species rare elsewhere (CHAPTERS 2, 3, 4 & 5), and isolated populations (CHAPTERS 6 & 7). Like island systems marine lakes are vulnerable to anthropogenic threats such as exploitation and alien species introduction. All chapters of this thesis reveal that much species diversity remains to be described. As a result of their many special features, marine lakes should play a prominent role in the marine conservation planning of both Berau and Raja Ampat.

Thesis outline

This thesis contains four sections divided into seven chapters.

Section 1: General introduction

Chapter 1 provides a general introduction to anchialine systems and a description of the study sites in Indonesia. Extensive exploration in Indonesia, using local knowledge, a Drifter water plane, and Google Earth satellite images, resulted in the discovery of 23 anchialine systems new to science. This chapter gives a thorough description of these systems. Based on parameters such as bathymetry, size, coastline, salinity, water temperature, pH, degree of connection to the sea, and the presence-absence of selected key taxa, three types of (non-cave) anchialine systems are distinguished in the Indo-Pacific: (a) marine lakes with large and deep basins containing brackish to almost fully marine waters, (b) anchialine pools consisting of small and shallow basins containing brackish water and low diversity of macrofauna, (c) blue pools in chasms that contain water with a clear halocline which are are possibly connected to anchialine caves. Marine lakes show a range in the degree of connection to the sea with the result that the higher the connection the more the lake resembles a lagoon in both water chemistry and biota, while the more isolated lakes have brackish water and contain species that are rarely found in the adjacent sea.

Section 2: Species assemblages

For this section the spatial variation in sponge species composition of assemblages and abundance was systematically and quantitatively measured in the Berau region (East Kalimantan in Indonesia) in the marine lakes Kakaban and Haji Buang, and the adjacent mangroves and coral reefs. The aim was to assess if the assemblages varied between sites and relate the variation to environmental, habitat and spatial variables. In **Chapter 2** we recorded the sponge species in the reefs of Berau. A total of 168 species were identified in the reefs. Sponge composition varied in relation to distance from the Berau River and water visibility, in addition to sand cover and cover of encrusting corals. Sponges in the Berau reefs appeared to thrive in inshore reefs near the river outlet which is an area with species poor coral communities.

In **Chapter 3** we documented the sponge species diversity in marine lakes and mangroves in the Berau region. A total of 115 sponge species were identified, 33 of which were restricted to Kakaban lake, 18 to Haji Buang lake and 30 to coastal mangroves. Our results show that marine lakes may represent a distinct environment from marine coastal mangroves with significantly higher sponge cover and abundance as well as a markedly different species composition. In both lake and outer coastal mangrove environments there was a pronounced gradient in composition away from the shore with the primary difference being between solid (root or rock) and soft substrates (mud or sand).

Section 3: Taxonomy

Taxonomy is the science of classifying organisms, or put more simply the study of naming and describing species. The marine lakes studied in this thesis are situated in an area known as the Indo-Australian Archipelago which houses the world's largest concentration of marine biodiversity, a large portion of which is yet undescribed. The objective in this section was to produce the taxonomy of the target species that could be used for subsequent population genetic analysis.

In **Chapter 4** the sponge *Suberites diversicolor* sp. n. (Porifera: Demospongiae: Hadromerida: Suberitidae) is described from four marine lakes located in Indonesia and from a brackish inshore area in Singapore. *Suberites diversicolor* sp. n. differs from known shallow water species of the genus *Suberites* in the tropical Indo-Pacific due to its diverse display of color-morphs and the presence of larger tylostyles with a wide size range. This species is typical of marine lake environments in the Indo-Australian Archipelago and the study of its populations allows comparison of multiple lakes with varying degrees of connection to the sea. **Chapter 5** contains a revision of the genus *Placospongia* (Porifera: Hadromerida: Placospongiidae) from the Indo-Pacific was revised. Species of the genus *Placospongia* are common within the tropical Indo-Pacific, occurring in a wide variety of environments from marine lakes, coral reefs and mangroves. There are at least four species of *Placospongia* within the wider Indo-Pacific that can be distinguished by internal skeletal spicule features, but not by external habitus and coloration: *Placospongia anthosigma, Placospongia carinata, Placospongia mixta, Placospongia melobesioides, Placospongia santodomingoae* sp.n.. Two additional, possibly morphologically cryptic, species have been identified by molecular markers.

Section 4: Phylogeography

Phylogeographic studies of taxa inhabiting marine lakes provide excellent opportunities to study biogeographical relationships and population structures of marine species in isolated habitats. Phylogeography is a field of study concerned with the principles and processes governing the geographic distributions of genealogical lineages, especially those within and among closely related species. The discipline focuses on historical and phylogenetic components of population structure. The aim of this section was to estimate levels of diversity and divergence within marine lake populations of two target species and to assess if they are isolated. If marine lakes are isolated environments we would expect to find genetic and/or morphological differentiation between the lake populations.

In **Chapter 6** we studied the phylogeography of the sponge *Suberites diversicolor* (Porifera: Demospongiae: Hadromerida: Suberitidae) in seven marine lake populations and three coastal populations using two mitochondrial and two nuclear markers. We found two divergent lineages (A & B) in populations of *S. diversicolor* that may constitute morphologically cryptic species. There was strong spatial structuring of the genetic populations based on the molecular markers we used. Kakaban lake (Berau, East Kalimantan) housed the highest genetic diversity with genetic variants that were not found in any of the other populations. Kakaban may be an area where multiple putative refugia populations have come into secondary contact, resulting in the high genetic diversity.

In **Chapter 7** the results of chapter 6 are supplemented by studying the phylogeography of a co-distributed but unrelated taxon, the mussel *Brachidontes* sp. (Mollusca: Bivalvia: Mytilidae) in Indonesia and Palau. We sequenced three genes (one mitochondrial and two nuclear) of four populations of *Brachidontes* sp. from three marine lakes and one coastal mangrove in Indonesia. Subsequently we examined variation in shell shape using a geometric morphometric approach. By combining our data with sequences from *Brachidontes* populations from Palau we detected that the Indonesian populations of *Brachidontes* harbored deeply diverged lineages that were strongly supported by morphological characters. The Indonesian marine lake *Brachidontes* sp. populations are isolated from each other with possible local diversification within the lakes.

I GENERAL INTRODUCTION

We have concluded that all collecting trips to fairly unknown regions should be made twice; once to make mistakes and once to correct them.

John Steinbeck, 1951
“Log from the Sea of Cortez”

Recently discovered landlocked basins in Indonesia reveal great habitat diversity in anchialine systems

Leontine E. Becking, Willem Renema, Nadiezhda K. Santodomingo, Bert W. Hoeksema,
Yosephine Tuti, Nicole J. de Voogd

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Abstract

In this paper the variability of physical settings of anchialine systems in Indonesia is discussed together with the consequences these settings have for the environment and biota within the systems. Exploration in two karstic areas (Berau, East Kalimantan and Raja Ampat, West Papua) has resulted in the discovery of 20 previously unknown anchialine systems in Indonesia. Based on parameters such as bathymetry, size, coastline, salinity, water temperature, pH, degree of connection to the sea, and the presence-absence of selected key taxa we distinguish three types of (non-cave) anchialine systems in the Indo-Pacific: 1. Marine lakes with large and deep basins containing brackish to almost fully marine waters. Marine lakes show a range in the degree of connection to the sea with the result that the higher the connection the more the lake resembles a lagoon in both water chemistry and biota, while the more isolated lakes have brackish water and contain unique species that are rarely found in the adjacent sea. 2. Anchialine pools with small and shallow basins containing brackish water and low diversity of macrofauna. 3. Blue pools in chasms that contain water with a clear halocline and are possibly connected to anchialine caves. Study of the many unique features of anchialine systems will enhance our understanding of the physical and ecological processes responsible for diversification in tropical shallow marine environments.

Keywords: Anchialine pools • marine lakes • Raja Ampat • Berau • mangroves • karstic limestone

Introduction

Anchialine systems are small bodies of landlocked seawater that are isolated in varying degrees from the surrounding marine environment, containing water at sea level in natural depressions, craters, and caves, either in lava or limestone. The marine character of these systems is maintained by subterranean tunnels, fissures, cracks, or small dissolution channels in the surrounding rock, connecting the lakes to the adjacent sea. This environment has set the stage for small, isolated, rapidly evolving populations, and endemic (sub) species (Tomascik & Mah 1994, Dawson & Hamner 2005, Martinez et al. 2009). Many rare and novel genera and species across a large spectrum of taxa have been found in anchialine systems (Holthuis 1973, Maciolek 1983, Tomascik & Mah 1994, Kott 1995, Fransen & Tomascik 1996, Massin & Tomascik 1996). The anchialine systems that we observe today are thought to be a young phenomenon, having originated during the Holocene, somewhere between 7000-15000 years before present (Dawson et al. 2009). In their present location these systems may be ephemeral in a geological timescale, but anchialine systems have probably always been present through time (Iliffe 2000, Sathiamurthy & Voris 2006).

The term anchialine was originally defined by Holthuis (1973) as a system "with no surface connection to the sea, containing salt or brackish water, which fluctuates with the tides". Brock & Kam (1997) subsequently provided a working definition for anchialine pools as "pools isolated from other bodies of water at the highest tides." Since the 1970's there has been heightened interest in anchialine systems, particularly anchialine caves – systems mostly covered by land with restricted exposure to open air (e.g. Iliffe 1991, Iliffe 2000, Humphreys & Eberhard 2001, Jaume et al. 2009, Martinez et al. 2009). As a result the anchialine cave system has been comprehensively defined (Sket 1996, Iliffe 2000). In fact, the interest in caves was so great that Stock et al. (1986) proposed to amend the definition of 'anchialine' by adding the phrase "usually with restricted exposure to open air". Their rationale being that the majority of anchialine systems would be cave-like, open lakes being a rare phenomenon. Since then, however, numerous authors have located anchialine lakes, pools, and ponds (i.e. systems exposed to air) from a variety of geographic localities, for example, in the Mediterannean (e.g. Benivic et al. 2000, Katsanevakis 2005), Caribbean (Thomas 1992), Palau (e.g. Hamner & Hamner 1998, Dawson & Hamner 2005), Micronesia (Ng et al. 1996), Hawaii (e.g. Brock & Kam 1997), Vietnam (e.g. Cerrano et al. 2006), and Indonesia (e.g. Tomascik et al. 1997, Hoeksema 2004, Becking & Lim 2009, *CHAPTER 4*). It is evident from this bulk of literature, however, that a variety of terms have been used intermittently for these systems and with little demarcation between the different types of lakes and pools. Most noteworthy is the term 'marine lake' that has become common place in the scientific literature as well as in popular science for anchialine lakes (Hamner & Carleton 1979, Hamner & Hamner 1998, Dawson et al. 2001, Dawson et al. 2009). For the benefit of continuity we adopt this term, though we would like to stress that marine lakes are to be considered anchialine systems.

Anchialine pools can occur in high abundances in both karstic limestone as well as in irregular porous lava flows (Holthuis 1973, Iliffe 2000). Large numbers (over 100) of anchialine pools have been found in the lava-rock of Hawaii (e.g. Holthuis 1973, Brock & Kam 1997). The number of marine lakes worldwide is estimated at approximately 200 based on direct and indirect reports, as well as maps and satellite images (Dawson et al. 2009). Areas where clusters of ten or more lakes occur are located in Croatia, Bermuda, Vietnam, Palau, and Indonesia (Dawson et al. 2009). These areas have karstic settings in common, even though their geologic histories are widely different.

A large portion of anchialine taxa have geographically widespread distributions, even though they are adapted

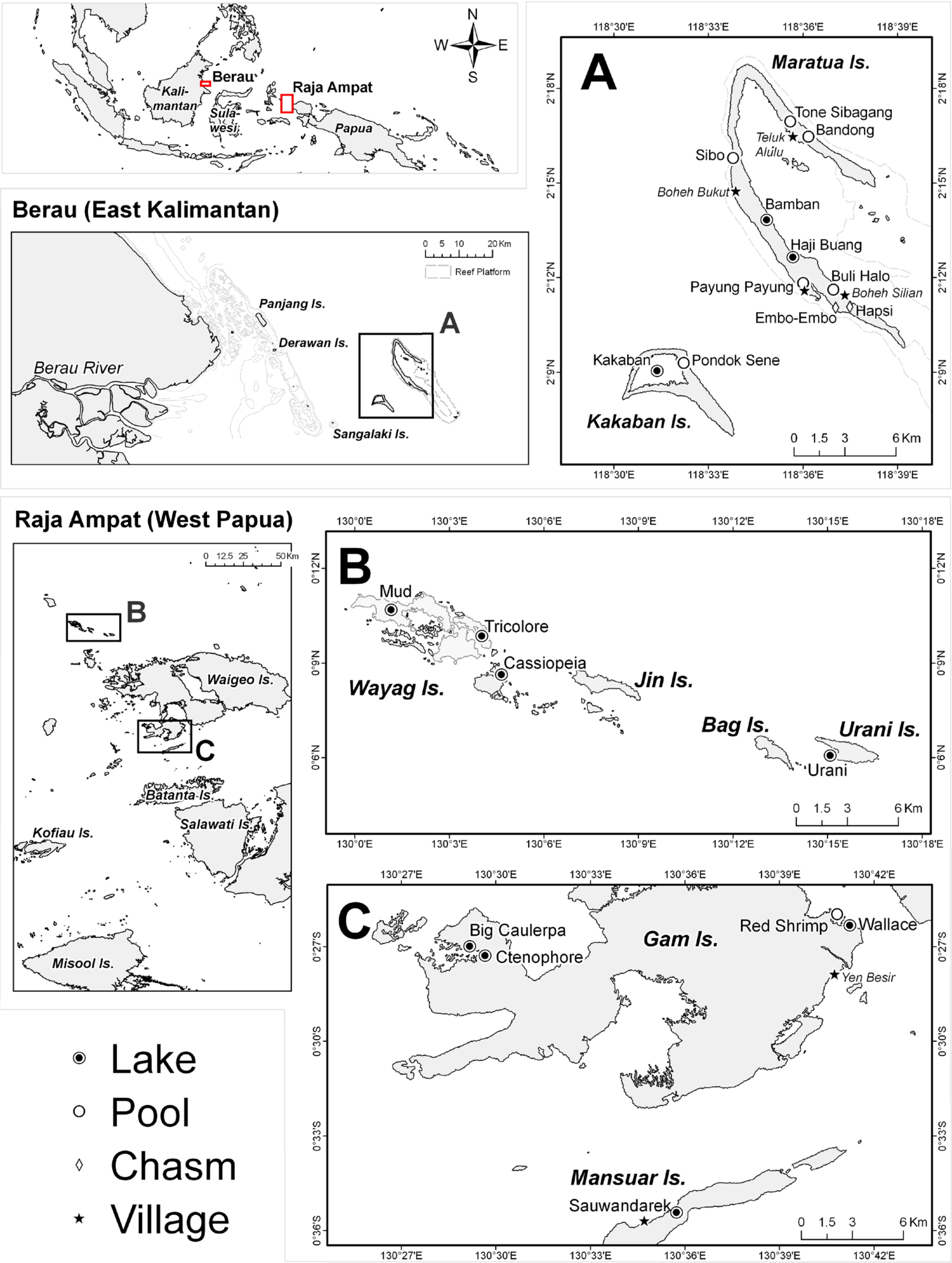

Fig. 1 Study areas in Indonesia. **A** Berau, East Kalimantan, **B** northern Raja Ampat, **C** central Raja Ampat, West Papua. Filled circles, empty circles, diamonds, and stars represent lakes, pools, chasms, and villages, respectively. Names of islands, anchialine systems, and villages indicated in the map

to unique niche environments. For example *Antecaridina lauensis* (Edmondson 1935) and *Parhippolyte uveae* Borradaile 1899 are shrimp species with red integumentary pigment and almost only occur in anchialine environments, yet have an extensive (disjunct) geographical distribution from the Red Sea to Hawaii (Holthuis 1973, Maciolek 1983, Fransen & Tomascik 1996). In the Hawaiian archipelago the small red shrimp *Halocaridina rubra* Holthuis, 1963 typifies the anchialine pools and the high evolutionary diversification between the various populations was probably driven by population fragmentation and isolation in the aquifers within the islands (Craft et al. 2008, Santos 2006). A remarkable feature of marine lakes is the vast populations of several subspecies of the jellyfish *Mastigias papua* (Lesson 1830) that occur enclosed in certain lakes in Palau and Indonesia and most likely radiated from the ubiquitous common ancestor in the sea (Dawson & Hamner 2005). These subspecies have an adapted morphology compared to the 'ancestral' *M. papua* morphotype from the sea*,* where a correlation was observed between the presumed age of the lake and the degree of adaptation to the environment (Dawson 2005).

The physical and chemical characteristics of the lakes and pools have ecological implications for the flora and fauna that reside in them. It is necessary to have a good baseline description of the systems in order to comprehend the distributions and adaptations of the unique anchialine taxa. From 2007 to 2009 we conducted an extensive search and survey of anchialine lakes and pools in Indonesia. In this paper, we discuss the variability of the setting in which these systems occur, and the implications of these settings for the environments and biota within the systems.

Study area

We surveyed anchialine lakes and pools on islands in two regions in Indonesia; the islands of Kakaban and Maratua in the Berau region, East Kalimantan Province (Fig. 1A) and the islands Wayag, Urani, Mansuar, and Gam in the Raja Ampat region, West Papua Province (Fig. 1BC). Monthly precipitation in Berau and Raja Ampat ranges from 200-275 mm with no clear seasonal pattern (Renema 2006*,* Prentice & Hope 2007).

Kakaban island is a trapezoidal shaped island with a maximal (diagonal) length of 7 km and a 40-60 m high Pliocene limestone ridge encircling a large marine lake (Figs. 1A & 2A). The southern coast of Kakaban island has a beach with *Avicennia* mangroves; the remainder of the coast surrounding the island is exposed rock in direct contact with the sea. Steep reef walls surround the island and the maximum depth is approximately 200 m. A general description of flora and fauna of Kakaban Lake was provided by Tomascik & Mah (1994) and Tomascik et al (1997). Maratua is a horse-shoe shaped island with a rim of raised Pliocene limestone that is 0.3-1.4 km wide and 10-120 m high (Figs. 1A & 2A). The island hugs a large lagoon of approximately 29.5x6.5 km with a depth of 0.5-5 m at low tide. Tomascik et al. (1997) mentioned the existence of 'anchialine lagoons' on the inner side of the raised rim of Maratua with the presence of *M. papua*, but they gave no further information on the location or characteristics of these lakes. The first records of species and localities of the Maratua lakes were published in a technical report resulting from a KNAW-Naturalis-LIPI expedition to the Berau Region (Hoeksema 2004). Two lakes, Haji Buang and Bamban, separated by a limestone cliff and a mangrove swamp, were reported on the western arm of Maratua Island.

Raja Ampat constitutes a group of islands at the northern tip of Bird's Head peninsula in West Papua and is an intricate and rugose karst system of late Miocene limestone. Lakes were found on the islands of Mansuar, Gam, Wayag and Urani (Figs. 1A & B). Each of these islands is characterized by a karstic scenery including a complex shaped coastline and frequent occurrence of inland depressions (Figs. 5L and S5E). The islands of Wayag and Urani in Northern Raja Ampat are characterized by the scarcity of freshwater sources and as such are practically uninhabited. The lakes and pool on Gam and Mansuar islands were located during the EWIN-LIPI-Naturalis expedition to Raja Ampat in 2007 (Becking et al. 2007). Previous descriptions of biota from lakes on Gam and Mansuar island only include ascidians Monniot (2009).

Methods

Locating lakes

In Berau the locations of three lakes had been reported in literature: Kakaban lake, Haji Buang lake and Bamban lake (Kuenen 1933 Tomascik et al. 1997, Hoeksema 2004) and the local people from Maratua island were knowledgeable of the anchialine pools present on the island. Many of the islands of Raja Ampat are only sparsely inhabited and we had to use other means than local knowledge to locate the lakes: Google Earth satellite images and a Drifter water-airplane (Fig. S5F).

Measurements

In Berau salinity, pH and temperature was measured with a handheld multimeter YSI63-50. At least three recordings were made per sample site, unless mentioned otherwise. In Raja Ampat, a STX-3 Salinity Refractometer (Vee Gee Instruments) was used to determine the salinity (in parts per thousand, ppt) and a Waterproof Multimeter Testr35 (Oakton) to determine the pH. Both instruments had been calibrated with the YSI63-50 in salinity and pH. Measurements were made in September 2008 and in May 2009. The minimal distance to the sea (over land) was measured from the rim of the lake to the nearest outer rim of the surrounding island. The outlines of the lakes were obtained by using the track-option in a handheld GPS device (Garmin GPS 60) walking, swimming or rowing along the perimeter. Satellite images (Berau, Landsat ETM 2001 Path 116/Row58; Raja Ampat, Landsat ETM 1999 Path 108/Row 60) and aerial photographs were used as a reference to adjust the coastline tracks. Depth measurements were made every 10-25 meters using a handheld sonar system PX Hawk Eye CE and these measurements were georeferenced along a straight axis from one end to the other of the lake, subsequently zig-zag tracks were made from opposite sides of the lakes crossing the initial straight axis. The obtained perimeter and georeferenced depth-measurements were analyzed in ESRI ArcGIS v9.3 software. Kriging interpolations were used to produce bathymetry maps, where separate models were tested by cross validation (spherical variogram model, small nugget component); the models shown here had mean prediction errors of less than 5%. The tidal fluctuations and temperature were measured with HOBO U20 Water Level Loggers (ONSET Computer Corporation, U.S.A.). The loggers were read out in the software Hoboware Pro version 2.5.0. The dataloggers were deployed for at least 24 hours in the lake and in the adjacent sea to obtain tidal measurements simultaneously in both locations with a logging frequency of 10 minutes. The degree of dampening of the tides was calculated as the relative amplitude of the lake compared to the sea. Relative tide amplitude was calculated as the percentage of tide variation inside the lake ($\Delta\text{Tide}_{\text{lake}}$) relative to the sea ($\Delta\text{Tide}_{\text{sea}}$)

The degree of tidal delay and dampening was used a proxy for the variation in the degree of connectivity between the lake and the sea (Hamner & Hamner 1998, Colin 2009). Tidal measurements were made in Kakaban lake and Haji Buang lake (East Kalimantan) and Cassiopeia lake and Tricolore lake (West Papua). In all other locations the tidal amplitude was estimated based on the intertidal zone determined at low tide. A Secchi disc was used to estimate vertical visibility around noon (from 11:00 to 14:00 hours) in Kakaban lake and Haji Buang lake.

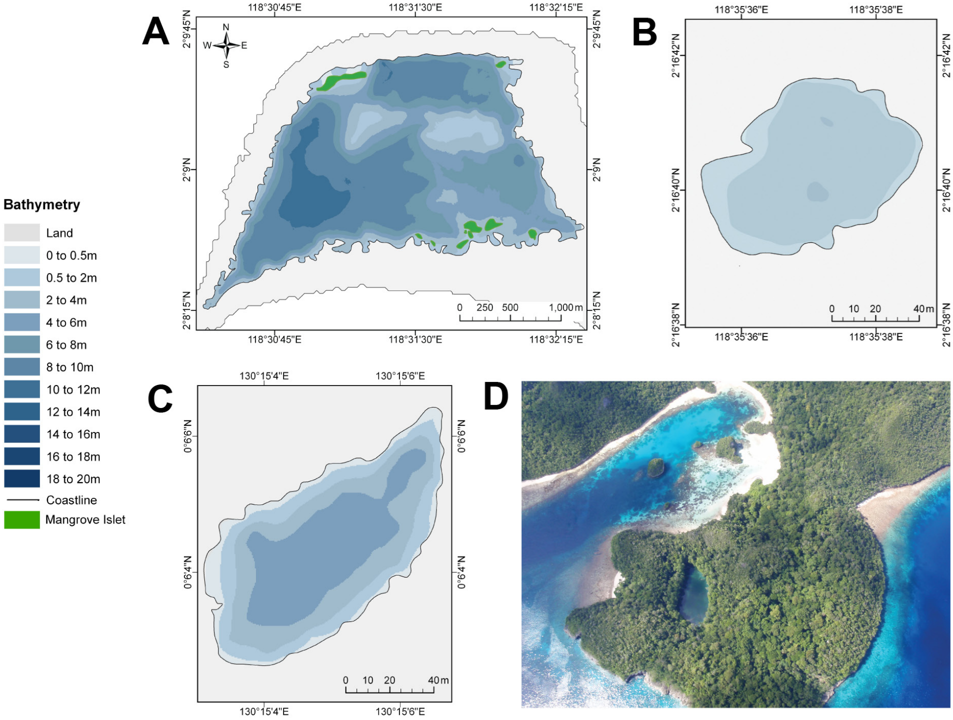

Fig. 2 Bathymetric maps of **A** Kakaban lake, **B** Tone Sibagang pool, **C** Urani lake, **D** aerial view of Urani lake. Note that scale differs per map

Recording target flora and fauna

Based on preliminary surveys of lakes in Indonesia, Vietnam and Palau (Hamner & Hamner 1997, Hoeksema 2004, Cerrano et al. 2006, de Voogd et al. 2006, Becking et al. 2008, Becking & de Voogd 2008) sponges, algae, molluscs, and mangroves were the most dominant macro-biota in terms of abundance and/or diversity. To provide biological indicators to help demark the different types of anchialine lake systems the presence of selected taxa was recorded, namely, two sponge species known from anchialine lakes *Suberites diversicolor* Becking & Lim, 2009 and *Darwinella* aff. *gardineri* Topsent, 1905 (de Voogd et al. 2006, Becking & Lim 2009, *CHAPTER 3&4*)(Fig. 3D), two sponge species common in reef flats and lagoons *Spheciospongia vagabunda* (Ridley, 1884) and *Clathria reinwardti* Vosmaer, 1880 (de Voogd et al. 2009)*;* two shrimp species known from anchialine systems *Antecaridina lauensis* (Fig. S2E) and *Parhippolyte uveae* (Fig. S4G) (Maciolek 1983); two jellyfish species *Mastigias papua* and *Cassiopeia ornata* Haeckel, 1880 (Fig. 3B) (Dawson 2005)*;* the algae genera *Caulerpa* spp. and *Halimeda* spp. (Hoeksema 2004); the mangrove genera *Bruguiera* spp. and *Rhizophora* spp.; in more general terms gobies, mussels, oysters, and stony corals. Representative voucher specimens have been deposited at the Naturalis Biodiversity Center. The size of the fish was estimated in categories: small (<10 cm), medium (10-15 cm), and large (> 15 cm). Sponge diversity was categorized as: low (<10 species), medium (10-20 species), and high (>20 species). A detailed detailed description of the sponge fauna of Indonesian marine lakes is in preparation.

Distinction between lakes

A Multidimensional Scaling (MDS) plot was used to produce a two-dimensional graphical representation of the similarity between the lakes and pools included in this study. IBM® SPSS® Statistics 18 was used to calculate the Euclidean distances and to make an MDS plot with S-stress diminishing by less than 0.0001 during successive iterations, in five trials. Classified abiotic attributes were: connection to sea (high, medium, low), maximum depth at low tide(m: >20/ 10-20/ 6-10/1-5/ <1), maximum length/diameter (m: <100/ 100-500/ >500), salinity (average ppt: 32-34/29-31/26-28/23-25/20-22/<20). Classified biotic attributes were: mangrove dominant (yes/no), mussel/oyster presence (mussel, oyster, none), sponge diversity (high/medium/ low/absent), fish presence and size (large/medium/small/ absent), and the presence or absence of: hard coral, large jellyfish populations, *Mastigias papua*, *Cassiopeia ornata*, *Suberites diversicolor*, *Darwinella* aff. *gardineri*, *Spheciospongia vagabunda*, *Clathria reinwardti*, *Antecaridina lauensis*, *Parhippolyte uveae*, and gobies. These attributes were recorded during timed interval surveys of two hours.

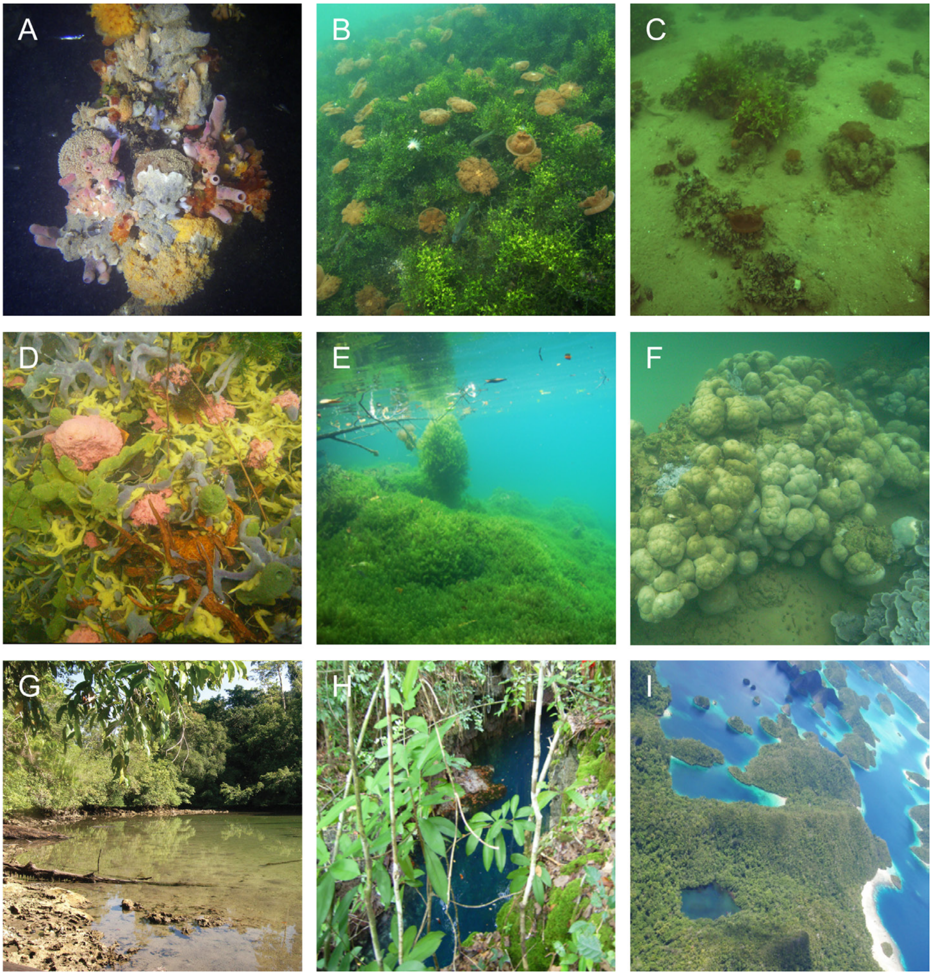

Fig. 3 A mangrove root studded with sponges in Kakaban lake (photograph: B. W. Hoeksema), **B** *Halimeda* algae buildup with *Cassiopeia* jellyfish, **C** Kakaban lake floor with patches of mussels, sponges, *Halimeda* algae, and *Cassiopeia* jellyfish, **D** full sponge cover in Haji Buang lake (green sponge *Suberites diversicolor*, pink sponge *Darwinella* aff. *gardineri*), **E** 'blanket' of *Caulerpa* algae in Haji Buang lake, **F** coral in Wallace lake, **G** Buli Halo pool, **H** Embo-Embo blue pool in chasm, **I** aerial view of Mud lake and the surroundings. (all photographs except A: L. E. Becking)

Results

A total of 24 anchialine lakes and pools were located of which 20 are new to science. 20 lakes and pools were surveyed for this study, 16 of which are newly catalogued (eight in East Kalimantan and eight in West Papua). None of the lakes and pools in West Papua have been formally named and only one lake had a local name (Sauwandarek). As such we use our fieldnames where appropriate. All lakes and pools were situated in depressions in karstic limestone, Pliocene reefal limestone in east Berau and late Miocene limestone in Raja Ampat. Geographical, physical, chemical, biological characters are summarized in Table 1A&B.

MDS analysis

The MDS resulted in two clusters representing pools and lakes (Fig. 4), which are primarily distinguished by the features: size, depth and presence of selected crustaceans. Within the lake cluster there is a gradient primarily determined by the degree of connection, salinity, and the presence or absence of the selected sponge species. Within the pool cluster two subgroups could be recognized: the first one composed by Embo Embo and Hapsi (two blue pools in chasms), and the second one grouping the remaining six pools. The distinction of the two groups is driven by differences in salinity, depth and presence/absence of fauna.

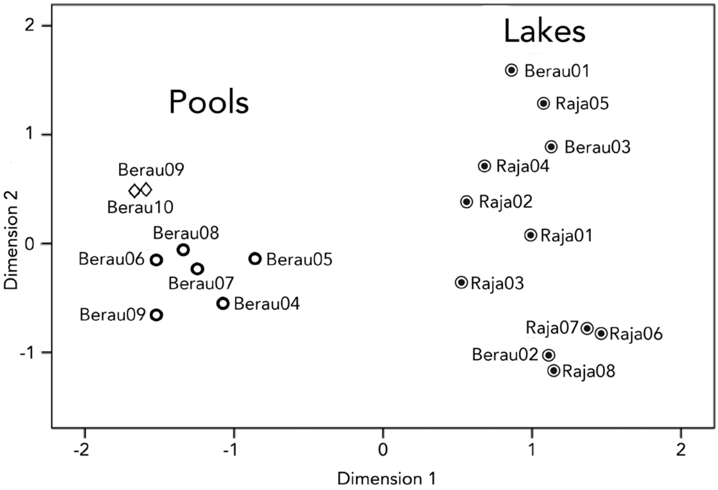

Fig. 4 Multidimensional scaling ordination based on Euclidian distances of characters of lakes and pools in Indonesia (Stress: 0.08 and R2: 0.97). See Table 1A&B for codes. Filled circles, empty circles, diamonds represent lakes, pools, chasms, respectively. Closer points indicate a higher similarity in the set of characters

Marine lakes

Twelve marine lakes were studied in East Kalimantan and West Papua (Fig. 1A, B & C): Kakaban (Fig. 2A), Pondok Sene, Haji Buang, Bamban, Sauwandarek, Ctenophore, Wallace, Big Caulerpa, Mud (Fig. 3J), Urani (Fig. 2C&D), Tricolore, Cassiopeia. A description of each lake and its biota is provided in the Supplementary Material and Table 1A&B. The majority of the lakes were separated from the sea by high (5-100 m) limestone cliffs or hills (Fig. 2C, 3J & S6A). The smaller lakes had uniform basins with the maximum depth in the central area, while the larger lakes had a heterogeneous bathymetry with multiple depressions (Fig. 2A, B

& C). The lakes have a maximum length of more than 80 m with basins deeper than 2 m at low tide, salinities ranging from 23-33 ppt, and tidal amplitudes ranging from 11% to almost 100% of the adjacent sea amplitudes (Table 1A & B). The shape of the lakes can be circular, elongated or irregular. All lakes had lower salinities and pH compared to the adjacent sea, while the temperatures were a few degrees higher (Table 1A & B). The connection to the sea is high (90-100% of adjacent sea tidal amplitude and < 1 hr delay), medium (60 - 90% relative tide amplitude and 1-2 hrs delay), or low (< 50% relative tide amplitude and > 2hr delay). Average tidal amplitude of the sea across all sites is 1.5-2 m (Table 1A&B)

The dominant biota, in terms of abundance, in marine lakes typically consisted of mangroves (e.g. *Bruguiera gymnorrhiza*), algae (*Caulerpa* spp. and *Halimeda* spp.), sponges, ascidians (e.g. *Styela complexa, Eudistoma* spp.), bivalves (e.g. *Brachydontes* spp.), gastropods (e.g. *Nerita* sp., *Terebralia* sp., *Cerithium* sp.), holothurians (e.g. *Synaptula* sp., *Holothuria* sp.), ophiuroids (*Ophiarachnella* sp.), asteroids (*Echinaster* spp.), fish (e.g. gobies, halfbeaks, soldierfish), shrimp (e.g. *Antecaridina lauensis, Parhippolyte uveae, Kemponia demani* (Kemp 1915)), crabs (e.g. *Orcovita saltatrix*), and in many cases also included annelids, and cnidarians (scyphozoans and anthozoans). The lakes with high connection to the sea contained more reef flat species, such as stony corals (e.g. *Porites* sp., Fig. 3F) and the sponges *Spheciospongia vagabunda* and *Clathria reinwardti*. The more isolated lakes contained *Suberites diversicolor, Darwinella* aff. *gardineri* (Fig. 3D), and few reef species. All marine lakes had a high cover of bivalves (Fig. 3C), either mussels or oysters were observed but never both in one lake except for in Ctenophore lake (West Papua). In all lakes *Caulerpa* was the dominant algae cover (Fig. 3E), except in Kakaban lake (East Kalimantan) where *Halimeda* was dominant (Fig. 3B) and Sauwandarek lake (West Papua) where both algae were rare. One lake (Haji Buang lake) contained seagrass (*Enhalus* sp.).

Anchialine pools

Five anchialine pools were studied on Maratua, Berau, East Kalimantan (Fig. 1A, B & C): Buli Halo (Fig. 3G), Sibo, Bandong, Payung Payung and Tone Sibabang (Fig. 2B); and one on Gam, Raja Ampat, West Papua. A description of each pool is provided in the Supplementary Material. This is the first description of these pools. The anchialine pools were separated from the sea only by sufficient elevation and distance (50 – 400 m) to prevent waves from entering. The pools are small, typically circular, basins of 20-100 m maximum width and with gently shelving basins where the maximum depth is located in the central area (Fig. 3G). The depths are less than 0.75 m (the majority less than 0.5 m) at low tide and with a range of salinities of 20-26 ppt. Two types of pools can be distinguished, those where the basin dries entirely at low tide and those where the basins remain minimally submerged depending on depth of the basin.

The dominant biota in anchialine pools consisted of algae (*Caulerpa* spp.), gastropods (e.g. *Nerita* sp, *Terebralia* sp., *Cerithium* sp.), ascidians (e.g. *Eudistoma* sp.*)*, shrimp(e.g. *Antecaridina lauensis*), and in some pools cnidarians (anthozoans). Sponges and crabs only occurred in some pools in low abundance and diversity, and jellyfish and corals were never observed.

Blue pool in chasm

Two blue pools (Embo-Embo and Hapsi) were studied on the southern end of the western arm of Maratua island (Fig. 1C) where the only source for fresh water is located (according to inhabitants of nearby villages). The blue pools are located 75-200 m inland, separated by limestone rock from the sea coast. The pools are present in chasms in the ground running parallel to the coastline, which with 1-20 m almost vertical walls (Fig. 3H). The depth of the pools is 5-6 m with deep blue color, and a visible halocline at 1-2 m depth. Due to logistical restraints, only samples of surface water (above the halocline) were collected to measure salinity (11 ppt) and temperature (25-28 °C). The bottom of the pool consisted of organic detritus, silt, and fallen tree trunks. The only biota observed were shrimp *(Antecaridina lauensis and Metabetaeus minutus* (Whitelegge, 1897)) and red encrusting algae.

Discussion

The discovery and subsequent survey of 20 lakes and pools in Indonesia has revealed great habitat diversity in anchialine systems. Here we will discuss the geomorphology, degree of connection to the sea, chemical water parameters, biota, and human influence. We will end with a synthesis of three types of anchialine systems that are present in the Indo-Pacific.

Connection to the sea and water chemistry

We observed different tidal regimes per location, most of them dampened and delayed compared to the outside sea. Comparing the tidal regime between the anchialine systems and the adjacent sea provided a proxy to estimate the degree of connection to the sea (Hamner & Hamner 1998). Counter to expectations, there did not appear to be a correlation between the distance of the anchialine system from the sea (i.e. the length of the land barrier) and the degree of connection. Differences in limestone rock porosity, presence of larger channels or tunnels will have a strong effect on the degree of connection to the adjacent sea as well as the residence time of water in anchialine systems (Mylroie & Carew 1995, Iliffe 2000). The residence time of the water will be a factor of actual exchange of seawater, the size and the depth of the anchialine system. It must be noted here that the tidal fluctuations are not necessarily only a result of active exchange of sea water, but could in part also be due to isostatic pressure from the surrounding sea. Particularly in the case of Kakaban lake, which is a large lake in a small island, the fluctuation of the damped tides is expected to be largely a result of isostatic pressure and not actual exchange of water with the adjacent sea. The degree of connection has an effect on the water chemistry of the lakes. For example, the salinity and pH were lower within lakes with restricted connection to the sea. However, the pools in contrast had little dampening of the tides, but the salinity was much lower than the sea and than most of the lakes. The shallow basins with a low volume to surface ratio likely allowed for more dilution by groundwater or rainwater.

In this study all lakes and pools consistently had lower salinities and pH compared to the adjacent sea, while the temperatures were a few degrees higher (see Table 1A&B). The whole spectrum of anchialine systems is typified by a wide range of water qualities. Anchialine cave systems are generally stratified with (meteoric) fresh water or brackish water overlying seawater and separated by a mixing zone. These waters typically have very low concentrations of oxygen at depth, containing hydrogen sulphide and supporting a complex aerobic and anaerobic microbial community (Humphreys 1999, Illife 2000). The deep marine lakes from Palau similarly show stratification with an increase of salinity and a decrease of oxygen towards the bottom. At the crossover to the anoxic layer a cyanobacterial mat is formed (Hamner et al. 1982, Hamner & Hamner, 1998, Dawson et al. 2009). Though we were not able to measure the oxygen concentration we were able to observe through indirect means (such as the presence of sponges and mussels at the bottom, and the absence of a cyanobacterial mat) that the majority of the presently investigated Indonesian lakes were not stratified. In Kakaban, Tomascik & Mah (1994) had measured lower oxygen levels at greater depth, but not anoxic levels (5.5-5.6 mg l^{-1}). Only Sauwandarek lake and Cassiopeia lake (West Papua) may have had anoxic

Table 1 List of all recorded characters of lakes and pools in (**A**) East Kalimantan, (**B**) West Papua

A. East Kalimantan	**Sea**	**Kakaban**	**Pondok Sene**	**Haji Buang**	**Bamban**	**Tone Sibagang**
code in Fig. 4		*Berau01*	*Berau02*	*Berau03*	-	*Berau04*
PHYSICAL CHARACTERS						
island		Kakaban	Kakaban	Maratua	Maratua	Maratua
latitude		N02° 08' 57.3"	N02° 09'18"	N02°12'31.2"	N02° 13' 50.0"	N02° 16' 39.6 "
longitude		E118° 31' 26.4"	E118° 32'18"	E118°35'46.8"	E118° 34 50.7	E118° 35 37.1
type	sea	lake	lake	lake	lake	pool
shape		trapezoidal	elongated	elongated	elongated	circular
connection to sea		low	high	low	low	high
tidal amplitude (m.)	1.5-2	0,2	1.5-2	0,9	0.5-1	1.5-2
tunnel visible		no	yes	no	no	no
max. depth at low tide (m.)		12	2	17	n.a.	0,75
max. length (m.)		3850	530	800	600	86
area (m2)		40*10^5	26500	14*10^4	12*10^4	4900
min dist to sea (m.)		120	20	325	115	50
salinity range (ppt)	33-34	23-24	33-34	26-28.5	26	20.8-22.6
pH range	8.2-8.5	7.0-7.8	8.0-8.2	7.3-7.8	n.a.	7.2-7.4
temperature range (°C)	28-30	29-31.5	28-30	29-30	29-30	28-29
BIOTA						
mangrove dominant		yes	no	no	yes	no
sponge diversity		high	high	high	medium	low
fish size		small	large	small	n.a.	large
hard coral		-	+	-	-	-
Suberites diversicolor		+	-	+	+	+
Darwinella aff. *gardineri*		+	-	+	+	-
Spheciospongia vagabunda		-	+	-	-	-
Clathria reinwardti		-	+	-	-	-
Antecaridina lauensis		-	-	-	n.a.	+
Parhippolyte uvaea		+	-	+	n.a.	-
gobies		+	-	+	+	-
HUMAN INFLUENCE		tourism, agriculture, sea turtle		tourism, logging	consumption mussels, logging	fishpond, sea turtle

B. West Papua	**Sea**	**Cassiopeia**	**Tricolore**	**Mud**	**Urani**	**Sauwandarek**
code in Fig.4		*Raja01*	*Raja02*	*Raja03*	*Raja04*	*Raja05*
PHYSICAL CHARACTERS						
island		Wayag	Wayag	Wayag	Urani	Mansuar
latitude		N0° 08' 36.9"	N0° 09' 47.9"	N0° 10' 40.3"	N0° 06' 03.8"	S0° 35' 19.6"
longitude		E130° 04' 39.7"	E130° 04' 05.4"	E130° 01' 09.3"	E130° 15' 04.3"	E130° 35' 48.8"
type	sea	lake	lake	lake	lake	lake
shape		circular	oval	circular	oval	oval
connection to sea		medium	medium	medium	medium	low
tidal amplitude (m.)	1-1.5	1,2	0,8	0.5-1	0.5-1	0.1-0.5
tunnel visible		no	no	yes	no	no
max. depth at low tide (m.)		4	2	2	6	19
max. length (m.)		125	250	170	140	500
area (m2)		13*10^3	18*10^3	19*10^3	6800	84*10^3
min. dist to sea (m.)		60	70	270	100	300
salinity range (ppt)	33-34	28-30	31-33	31-33	28-30	28-30
pH range	8.0-8.3	7.2-7.8	7.2-7.8	7.2-7.8	7.2-7.8	7.2-7.8
temperature range (°C)	28-29	30-31	29-30	29-30	30-31.5	31-34
BIOTA						
mangrove dominant		no	yes	yes	yes	yes
sponge diversity		medium	high	medium	high	medium
fish size		absent	medium	medium	small	small
hard coral		-	-	-	-	-
Suberites diversicolor		+	-	+	+	+
Darwinella aff. *gardineri*		-	+	-	+	+
Spheciospongia vagabunda		-	-	-	-	-
Clathria reinwardti		-	-	-	-	-
Antecaridina lauensis		-	-	-	-	-
Parhippolyte uvaea		-	+	-	+	+
gobies		-	+	+	+	+
HUMAN INFLUENCE		sea turtle?	absent	absent	absent	sea turtles, village

n.a., character not recorded; -, absent; ?, present. Areas, depths, and maximum lengths are all approximate values. Maximum depths are relative to low tide. See results section for definitions of categories

Buli Halo	**Payung Payung**	**Sibo**	**Bandong**	**Embo-Embo**	**Hapsi**
Berau05	*Berau06*	*Berau07*	*Berau08*	*Berau09*	*Berau10*
Maratua	Maratua	Maratua	Maratua	Maratua	Maratua
N02° 11' 16.4"	N02° 11' 45.7"	N02° 15' 47.3"	N02° 16' 22.3"	N02° 11' 03.0"	N02° 11' 04.2"
E118°37' 06.4"	E118°36' 09.9"	E118°33' 46.9"	E118°35' 55.0"	E118° 37 01.9	E118° 37' 28.7"
pool	pool	pool	pool	chasm	chasm
circular	circular	circular	circular	elongated	elongated
high	high	high	high	medium	medium
1.5-2	1.5-2	1.5-2	1.5-2	0.5-1	0.5-1
yes	no	yes	no	no	no
<0.5	0	0	<0.5	5	5
100	80	20	140	30	30
7295	4800	195	6785	165	105
400	300	60	80	75	200
26	26	20-23	20-23	11	11
n.a.	n.a.	n.a.	n.a.	n.a.	NA
n.a.	n.a.	n.a.	n.a.	26-28	26-28
no	no	**no**	no	no	no
low	absent	low	low	absent	absent
large	absent	absent	absent	absent	absent
-	-	-	-	-	-
-	-	-	+	-	-
-	-	-	-	-	-
-	-	-	-	-	-
-	-	-	-	-	-
+	-	-	+	+	+
-	-	-	-	-	-
-	-	-	-	-	-
fishpond, sea turtle	toilet	local recreation	toilet	local recreation	local recreation

Wallace	**Ctenophore**	**Big Caulerpa**	**Red shrimp**
Raja06	*Raja07*	*Raja08*	-
Gam	Gam	Gam	Gam
S0°26'31.08"	S0° 27' 17.3"	S0°26'58.85"	S0°25'58.23"
E 130°41'8.04"	E130° 29' 34.3"	E 130°29'10.17	E130°40'49.74"
lake	lake	lake	pool
circular	L-shaped	circular	circular
high	high	high	n.a.
1-1.5	1-1.5	1-1.5	1-1.5
yes	yes	yes	no
n.a.	9	6	0,5
200	230	85	20
8640	25*10^3	4100	n.a.
50	75	50	n.a.
31-33	31-33	31-33	n.a.
n.a.	7.7-8.0	7.7-8.0	n.a.
29-31	29-31	29-31	n.a.
no	no	no	no
high	high	high	absent
large	large	large	absent
+	-	+	-
-	-	-	-
-	-	-	-
+	+	+	-
+	+	+	-
-	-	-	+
-	-	-	-
-	-	-	-
fishpond	absent	absent	absent

layers at the bottom, revealed by changes in temperature (increased), water color (to dark brown-orange) with depth, and biota (absence). In any case the system was not stable as we observed that the depth of the warmer brown-orange colored water layer changed several meters on different visits, either days or months apart. This change in the position of the anoxic and H_2S zone has also been observed in Bundera Sinkhole in Australia (Humphreys 1999, Seymour et al. 2007)

Biota

The flora and fauna of the anchialine systems are of marine origin, but the lakes and pools harbor only a subset of the adjacent sea flora and fauna. The difference in species assemblages between different lakes and pools can for a large part be attributed to both the nature of the barrier between the lake and sea as well as the various environmental characters within the anchialine systems. First, the biota has to be able to colonize the lake. This colonization process will be different between lakes depending on the external setting which could involve direct connection to the lake or pool from lagoon through a tunnel (e.g. Ctenophore lake), from lagoon through dissolution channels in rock (e.g. Kakaban lake), or from lagoon through mangrove swamp and subsequently through dissolution channels in rock (e.g. Haji Buang lake). In the case of colonization the environmental barrier to the lake will act as a filter for the faunal composition of the lakes. An extreme illustration of the effect of the size of connection is that in more restricted lakes only small mangrove associated fish were observed, while in the lakes connected by tunnels to the sea large reef fishes were occasionally observed. Following colonization the taxa have to be able to tolerate the environmental conditions inside the lake. This often includes a broad range of and, in some cases, sudden modifications to environmental parameters. The lower salinities and higher temperatures in all lakes and pools compared to the sea will likely prove a limiting factor for many marine biota. There are taxa that are typically good at colonizing and surviving in anchialine environments, while some groups are only present in highly connected systems. Algae, sponges, molluscs, ascidians, and in many cases mangroves are the most dominant macroorganisms in terms of abundance and/or diversity in the lakes and pools of Indonesia, while corals were absent, except in the most connected marine lakes (Tomascik & Mah 1994, Hoeksema 2004a, Becking et al. 2007, this study). The sponge cover and abundance is generally high, but this does not necessarily correspond with high diversity, particularly compared with the numbers of species found in the adjacent reefs (de Voogd et al. 2009). In Vietnam there is a great variation in the sponge fauna between the different marine lakes, where the local assemblage can vary due to extreme variability of the environment (Cerrano et al. 2006, Azzini et al. 2007).

Types of anchialine systems

Different anchialine systems can be characterized based on environmental gradients, shape, depth, water parameters, and degree of connection to the sea (e.g. Illife 1991). To understand the biological processes within the anchialine systems it is necessary to make a distinction between the different types. The anchialine cave environment has been extensively described in the past (e.g. Sket 1996, Iliffe 2000) and is continued in this special issue of Hydrobiologia. There are numerous anchialine caves documented in the Indo-Pacific (e.g. Iliffe 1991, Iliffe 2000, Humphrey & Eberhard 2001, Jaume et al. 2009), yet in the present study we limit our focus to anchialine systems in the Indo-Pacific that are exposed to air, i.e. the lakes and pools. We identified three types of these anchialine environments: marine lakes, anchialine pools, and blue pools in chasms. The distinction and spectrum of these systems is evident in Fig. 4.

Marine lakes

Marine lakes are represented by large and deep basins that remain submerged during the whole tidal cycle. Within this category of marine lakes we include the 57 lakes in Palau (from Hamner & Hamner, 1997, Colin 2009), the eight lakes in Vietnam (from Cerrano et al. 2006, Azzini et al. 2007), as well as the 12 lakes from the present study. There is a range of features within marine lakes that is apparent in Fig. 4 where the variation is mostly influenced by the degree of connection to the adjacent sea, which in its turn influences the salinity and the species assemblages in the lakes. In Palau and Vietnam the marine lakes also display a wide variety in the degree of connection to the sea and subsequent environmental regimes within the lakes (Hamner & Hamner 1998, Cerrano et al. 2006, Azzinni et al. 2007, Colin 2009). In the marine lakes of Palau the range of tidal dampening (tidal amplitude 8-100% of the adjacent sea) and salinities (20-33.5 ppt) fit within the range that we have observed in Indonesia (Hamner & Hamner 1998). Hamner & Hamner (1998) and Colin (2009) make a distinction within the marine lakes of Palau between holomictic lakes where the water column is mixed and meromictic lakes where the water column is stratified due to little mixing and low connection to the adjacent sea. Essentially the marine lakes represent a geomorphological gradient starting by 1) lakes that are highly connected to the adjacent sea (through large and apparent tunnels or caves) with tides that are minimally delayed or damped and with well mixed waters with salinities comparable to the adjacent sea, continuing to 2) moderately isolated lakes with at least 1 hour delay in tidal amplitude and reduced salinities compared to the adjacent sea, and extending to 3) highly isolated lakes with tidal amplitudes damped by more than 50% and a delay in phase of more than 2 hours compared to the sea and with very low salinity, often meromictic. Beyond this spectrum are lakes that contain saline waters but without any connection to the sea at all, such as Motitoi lake on Satonda island near Sumbawa, Indonesia. Indeed, Motitoi lake is non-tidal and has a very high salinity range of 31.4-41.8 ppt (Tomascik et al. 1997, Pisera et al. 2010). In the present study, the greater the connection to the sea was, the more similar the lagoonal and lake assemblages were owing to the presence of, for example, stony corals and the sponges *Spheciospongia vagabunda* and *Clathria reinwardti*. The more connected lakes in Palau and Vietnam also contained coral and reef associated species (Azzini et al. 2007, Colin 2009). The more isolated lakes in Indonesia had unique assemblages that were typified by the presence of the sponges *Darwinella* aff. *gardineri* and *Suberites diversicolor,* the large red shrimp *Parhippolyte uveae,* and in many cases the jellyfish *Mastigias papua* and *Cassiopeia ornata.* It is remarkable that the sponge species *Suberites diversicolor* is not only present in more isolated lakes in East Kalimantan and West Papua (de Voogd et al. 2006, Becking & Lim, 2009, this study), but also in marine lakes with reduced salinities and high temperatures in Ha Long Bay in Vietnam (Cerrano et al. 2006, Azzini et al. 2007, Becking & Lim, 2009) and in Palau (pers. comm. L.J. Bell, Coral Reef Research Foundation, Palau). A related species of the genus *Suberites* has even been found in the completely isolated Motitoi lake near Sumbawa (Reitner et al. 1999, Pisera et al. 2010). It is noteworthy that the largest and most isolated lake of the present study, Kakaban lake, contained a unique fauna that we did not observe in any of the other lakes in Indonesia, such as two holothurians (*Holothuria (Lessonothuria) cavans* and *Synaptula spinifera*), a jellyfish eating actiniarian (*Entacmaea medusivora*), and an ascidian (*Styela complexa*) (Tomascik & Mah 1994). On a cautionary note with respect to inferring the degree of connection, Cassiopeia lake (West Papua) would be considered a moderately connected lake based on the degree of dampening of the tidal amplitudes, however faunistically this lake would represent a more restricted system with *Cassiopeia ornata, Mastigias papua,* and *Suberites diversicolor.* More detailed research on how the variation of the species assemblages relate to the lake characters is required to establish predictors.

Anchialine pools

Anchialine pools are represented by small, circular basins that have a shallow depth (<1 m.) or are dry at low tide. Within the category anchialine pool we include the pools and ponds as described in Hawaii (Holthuis 1973, Brock & Kam 1997), the Philippines (Wear & Holthuis 1976), Funafuti (Holthuis 1973), as well as the six pools in the present study. On the islands of Hawaii and Maui much smaller anchialine pools than in Indonesia have been found ranging in size from 0.5-12 m. in length and in salinities from 8-30 ppt (Holthuis 1973, Brock & Kam 1997). The Hawaiian pools are formed in lava rock, while those in Indonesia, the Philippines, and Funafuti are formed in karstic limestone.

In general the anchialine pools are expected to show great fluctuations in salinity and temperature within a short period within one pool, due to the small size, shallow depth, and the strong influence of the tides. Therefore, they constitute an extreme environment in which predominantly euryhaline species can tolerate for longer periods. As a consequence the diversity in these pools is much lower than the more stable systems of the marine lakes. In Hawaii the small red shrimp *Halocaridina rubra* typifies the anchialine pools (Maciolek 1983, Santos 2006). Experiments of red shrimp from pools in Hawaii showed that they can survive fresh to hypersaline (50 ppt) water (Holthuis 1973). In Indonesia and other locations in the Indo-Pacific the anchialine pools are dominated by another small red shrimp, *Antecaridina lauensis* (Holthuis 1973, Maciolek 1983, this study). In the Indonesian pools some individuals of sponge specimens were observed, but sponges are rarely recorded from the other geographic locations.

Blue pools in chasm

We observed two pools with striking blue colored water in chasms in the ground that ran parallel to the coast. Holthuis (1973) described a fissure north of Lohena Rock on Maui where he collected ‘red shrimp’ which match the chasms presently recorded from Indonesia. Ng et al. 1996 again described the same system at Vaikona chasm and Anatuku chasm in Niue, Polynesia. These blue pools may, in addition, be the same type of systems as the ‘grietas’ as described by Illife (1991) in the Galapagos. As our two observations do not seem to be singular to the island of Maratua in Indonesia, we have allocated a category to blue pools in chasms. This system is represented by a large brackish water lens which presumable lies above warmer saline water. We have only documented the surface layer of low salinity (11 ppt), whereas Ng et al. (1996) and Holthuis (1973) report salinities ranging from 1.5-8 ppt at the surface and 31 ppt at depth, the depths ranging from 6-32m. Possibly the blue pools are connected to anchialine caves, or may in fact be the exposed part of an anchialine cave systems. These pools only contained shrimp and algae and as such had the lowest biodiversity of all three anchialine systems described in this study.

Conservation

The isolated water bodies of anchialine lakes and pools are, like island systems, vulnerable to anthropogenic influence. Precedence of uncontrolled tourism development and alien species introduction in marine lakes in Palau and Vietnam has resulted in ecological disarray (Dawson et al. 2001, Cerrano et al., 2006, Azzini et al. 2007). While the lakes in Wayag and Urani (West Papua) appeared to be free of human influence, most of the other systems have already been exploited in various ways. For example, in East Kalimantan the lakes are beginning to face an increase in tourism and local exploitation. In Vietnam the lakes are no longer pristine, having been used for fisheries, mollusk harvesting, and aquaculture (Azzini et al. 2007). In addition, a large number of lakes and pools that we visited had an introduced green sea turtle for various reasons such as consumption and animistic rites. The presence of sea turtles may have a disruptive effect on the anchialine ecosystems, while the impact for the sea turtles may also be negative since there is little food available to ensure their long-term survival.

The two Indonesian regions that contain high abundances of anchialine systems, Berau in East Kalimantan and Raja Ampat in West Papua, are situated in the Coral Triangle, the centre of maximum marine species richness (Hoeksema 2007). Within this centre these regions are among the most diverse with regard to species and marine habitats (Hoeksema 2004a, Renema, 2006). This habitat diversity is partly related to the limestone underground, which is reflected in irregularly shaped coastlines that are fringed by coral reefs that may contain unique benthic species assemblages (e.g. Hoeksema 2004b, Renema 2006, de Voogd et al. 2009). Where the karstic limestone emerges above sea level, relatively isolated anchialine biotopes have developed into unique ecosystems, each with its own specific species assemblage. Many species here are rare and even endemic (Tomascik & Mah 1994, this study). As a result of their many special features, anchialine systems should play a prominent role in the marine conservation planning of both Berau and Raja Ampat.

Conclusions

This study provides the first overview of recently located anchialine systems in Indonesia. These systems vary from each other in terms of size, bathymetry, degree of connection to the sea, salinity, and species composition. Both the various characters within the lakes and pools as well as the nature of the barrier from the sea determine the species assemblages encountered in the systems. To understand the biological processes within the systems it is necessary to make a distinction between the different types. We propose to distinguish between three anchialine systems, though we caution that that these categories are transitional: 1. marine lakes, 2. anchialine pools, 3. blue pools in chasms. We adopt the term marine lakes (*sensu* Hamner & Hauri 1981) as a type of anchialine system. Within the marine lakes there is a clear gradient in characters which is related to the degree of connection to the sea which in its turn affects the salinity, pH, and species present. The discoveries of and continued research in anchialine systems will contribute significantly to a further understanding of the biogeography, connectivity, and genetic divergence of taxa in shallow tropical marine ecosystems.

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Supplementary description

Berau, East Kalimantan

Kakaban island

Kakaban is a trapezoidal shaped island with a maximal (diagonal) length of 7 km and a 40-60 m high ridge encircling a large marine lake (Figs. 1A & 2A, and see Tomascik et al. 1997). The southern coast of Kakaban island has a beach with *Avicennia* mangroves; the remainder of the coast surrounding the island is exposed rock in direct contact with the sea. Steep reef walls surround the island to a maximum depth of 200 m. Kakaban lake is one of the largest marine lakes presently known to science and was first scientifically described by Kuenen (1933) during the Dutch "Snellius" expedition to Indonesia from 1929-1930. The lake and its biota were described in more detail by Tomascik & Mah (1994) who called the lake "*Halimeda* lagoon". As a result of their fieldwork many rare and novel genera and species were found across a variety of taxa: a varunine crab (*Orcovita saltatrix* Ng & Tomascik 1994), two holothurians (*Holothuria (Lessonothuria) cavans* Massin & Tomascik, 1996 and *Synaptula spinifera* Massin & Tomascik 1996) (Fig. S1A), and an ascidian (*Styela complexa* Kott 1995). Since 2001 there is a jetty and walkway towards the lake built for easy access for tourists.

Kakaban lake

The average depth in the lake is 8m with two deeper areas of 10-12 m in the north and in the southwest separated by a shallow *Halimeda* bank of 0-2 m depth. A large portion of the lake in the center towards the east is very shallow (Fig. 2A). The west and south coasts are bordered by a flat nearshore zone and are fringed by a 1-5 m wide mangrove belt (predominantly *Bruguiera gymnorrhiza* Lam. and *Rhizophora mucronata* Lam.) which results in an irregular coastline with mangrove islets and bays (Fig. 2A, S1D). The eastern part of the south coast contains *Nypa* palm in addition to the other mangroves. The submerged roots of the mangroves are highly intertwined and meshed, providing a wall-like structure (Fig. S1B). In some locations the roots hang above the bottom while in others they are rooted in the bottom. The northern cliff coast is near vertical and mangroves are rare, resulting in a predominantly steep rocky shoreline (Fig. S1C). The east coast consists mostly of exposed rock interspersed by patches of mangroves. There are areas on the east coast with cavern formations 1-2m inland, these are all dead-ended. The bathymetry of the lake is indicated in Fig. 2A. The rocky coast and the submerged mangrove roots were covered with mussels, sponges, ascidians and algae (Fig. 3A), amidst which were holothurians, asteroids, and ophiuroids (Fig. S1A). Sponges had a high abundance and diversity (>40 species) in the lake. Along the upper part of the intertidal area there were high abundances of gastropods (predominantly *Nerita* sp., *Terebralia* sp., *Cerithium* sp.) and bivalves (predominantly *Brachydontes* sp.). The sediment below the roots was dark black-brown scattered with sponges fallen from the roots. The bottom in front of the roots was covered with *Halimeda* where *Cassiopeia ornata* jellyfish, and *Holothuria cavans* sea cucumbers were abundant, with few sponges (Fig. 3B). *Halimeda* spp. were the most dominant algae in terms of biomass. The shallow lake slope was covered by *Halimeda* algae down to a depth of ~5-6 m. The sediment at the lake floor was dominated by dead *Halimeda* thalli. The benthic habitat at >6 m depth was dominated by fine mud where locally patches mussels provided a solid substrate which was usually colonized by sponges (Fig. 3C). The lake housed large swarms of the jellyfish *Mastigias papua*. A detailed description of flora and fauna was provided by Tomascik & Mah (1994) and Tomascik et al (1997). A sea turtle had been released in the lake by humans before 2008 and was in poor health with decomposing flesh in 2009.

Kakaban lake had the smallest measured tidal amplitude (19 cm), which was damped to 11% of the adjacent sea amplitude (175 cm) and showed the largest delay (3 h 30 min) compared those in the surrounding sea (Table1A). The water had a vertical transparency of 6-8 m and had a green tinted color which transitioned to a light orange-brown color at depths greater than 5 m.

Pondok Sene

Outside Kakaban lake, along the eastern coast, separated by a steep cliff from the sea was a second lake. Ponok Sene is enclosed by land, except for a visible and large tunnel by which seawater gushes in and out with wave action and changing tides. At low tide the depth is 2 m in the north and 10-50 cm in the south. The sediment in this lake consisted of light colored carbonate sand. Pondok Sene most resembled a lagoon containing stony coral and the reef flat sponges *Spheciospongia vagabunda* and *Clathria reinwardti*. Despite its proximity (<100 m) the fauna in this lake was not similar to that of the larger and more isolated Kakaban lake.

Maratua island

Maratua is a horse-shoe shaped island with a rim of raised limestone that is 0.3-1.4 km wide and 10-120 m high. The island hugs a large lagoon of approximately 29.5x6.5 km with a depth of 0.5-5 m at low tide. Tomascik et al. (1997) mentioned the existence of 'anchialine lagoons' on the inner side of the raised rim of Maratua with the presence of *M. papua*, but they gave no further information on the location or characteristics of these lakes. The first records of species and localities of the Maratua lakes were published in a technical report resulting from a KNAW-Naturalis-LIPI expedition to the Berau Region (Hoeksema, 2004). Two lakes, Haji Buang and Bamban, separated by a mangrove swamp, are present on the western arm of Maratua Island (Fig. 1C). The mangrove swamp separating the two lakes is dominated by *Bruguiera gymnoriza* with the presence of gastropods (*Cerithium* sp*., Terebralia* sp*., Nerita* sp.) and algae (*Caulerpa* spp.), but no sponges and mussels. A steep limestone cliff separates Haji Buang lake from this mangrove swamp. We located five anchialine pools on Maratua: Buli Halo, Sibo, Bandong, Payung Payung and Tone Sibabang. This is the first description of these pools and we will describe Tone Sibagang in detail as an example. Additionally, we located two blue pools (Embo-Embo and Hapsi) at the southern end of the western arm of Maratua (Fig. 1C) where the only source for fresh water on Maratua is located (according to inhabitants of nearby villages).

Haji Buang lake

Haji Buang lake is a large, elongated lake separated from the sea by a steep limestone ridge of ~100 m height to the west and a lower limestone ridge of 20-40 m to the east. Between the western ridge and Maratua lagoon runs a mangrove swamp. The average depth of the lake is 8-10 m with two deeper areas in the north (14 m) and in the south (17 m) (Fig. S6AB). The majority of the coastline of Haji Buang lake is formed by limestone rocks covered in sponges (Fig. 3D). The coastline in the east is rimmed by a shallow plateau extending 1-2 m from the coast which is almost fully exposed to air at low tide (Fig. S1G). This plateau transitions into a steep wall-like slope that ends in the sediment at 3-4 m depth, from which the bottom sediment gently slopes down. The majority of the west coast is a vertical limestone wall which coves inwards at some places at approximately 1 m depth. Along the northern end of the west coast a shallow plateau extends 2-3 m from the coast with a 0.5-1 m depth, followed by a steep slope ending in the sediment at 2-3 m depth. Only the southern coast of Haji Buang lake is fringed by mangroves (predominantly *Bruguiera gymnorrhiza*) with a seagrass field (*Enhalus* sp.) in front of it (Fig. S1F). Along and below the intertidal area on the rock there were high abundances of gastropods (predominantly *Nerita* sp, *Terebralia* sp., *Cerithium* sp., *Chicoreus* sp.). Sponges occurred in high abundance and diversity (>30 species) along the coast (Fig. 3D). Along the east coast the sponges were covered by a thick 'blanket' of algae (*Caulerpa* spp.) (Fig. 3E). *Caulerpa* algae were generally abundant in the lake, and abundance decreased with increasing depth. We observed no *Caulerpa* algae at depths >6 m. *Cassiopeia ornata* was abundant on *Caulerpa* (densities of 5-15 ind. m^{-2}). Haji Buang lake contained a dense population of *M. papua.* In the south near the mangroves we observed, in September 2008 and May 2009, swarms of juvenile *M. papua* (0.5-1.5 cm width) (Fig. S1E). A green sea turtle had been introduced to the lake before 2008, but was not observed in 2008 or 2009.

The tidal amplitude in the lake was damped to 48% of that in the sea with a delay of 2 h 30 min (Table1A). We observed water flowing through the porous limestone rock at high tide. The water had a vertical transparency of 6-7 m and a general color of a milky green which transitioned to more brown-orange in water deeper than 5-6 m. In the southern end of the lake the visibility was lower than in other parts. There are two paths leading to the Haji Buang lake: one from the east, used by tourists from the nearby diveresorts, and one from the west, used by people from the nearby village Payung Payung.

Bamban lake

The second lake on Maratua, located north of Haji Buang lake, is a large, elongated lake (Fig. S1H). On the east coast the rock was covered in patches by mussels interspersed with sponges at a lower diversity and abundance than in Kakaban lake and Haji Buang lake. Small numbers of *M. papua* were present and high numbers of sea urchins. Due to the presence of a saltwater crocodile we were not able to survey this lake comprehensively. One surface water sample was taken for salinity (26 ppt). The lake was accessed from the east along a 150 m pass over a 10 m high ridge.

Tone Sibagang pool

Tone Sibagang is located in the village Teluk Alulu, approximately 20 m east from the main road and is separated from the sea by low limestone rock (Fig. 1C). The pool is small, circular with a uniform bowl-shaped basin and a maximum depth of 0.75 m at low tide (Fig. 1F, S2A). The pool was fringed by lowland tropical forest vegetation or mangrove associated flora, the *Bruguiera* and *Rhizophora* mangroves were absent. The bottom consisted of a mixture of areas of exposed rock and areas covered with a layer of dead leaves, *Caulerpa* algae, detritus and silt (Fig. S2B). Dense populations of small red shrimp *Antecaridina lauensis* (> 20 ind m^{-2}) and gastropods (*Nerita* sp, *Terebralia* sp., *Cerithium* sp.) were present. We observed in total only four individuals of the three sponge species *Suberites diversicolor, Spirastrella* aff. *decumbens* Ridley 1884*, Lissodendoryx* aff. *similis* Thiele, 1899. Humans had released large reef fish and one green sea turtle in the pool. Contrary to Haji Buang lake, there was little tidal dampening (pool amplitude 90-100% of adjacent sea amplitude) and the delay was less than one hour. The salinity, however, was significantly lower than in the adjacent sea (Table 1A). The water had a horizontal visibility of 10 m and a green-blue tinted color.

Other pools on Maratua island

All the other pools on Maratua island represented small, uniform bowl-shaped basins with maximum depths at low tide of <0.5 m. Buli Halo (Fig. 1E, 3G) near the village Boheh Silian contained the red shrimp *Antecaridina lauensis* and *Caulerpa* algae in the central pool. A tunnel connecting the pool to the adjacent sea had a high cover of an assemblage of seven sponge species (*Haliclona* sp., *Geodia* sp., *Placospongia melobesioides* Gray, 1867, *Placospongia mixta* Thiele, 1900*, Higginsia* sp., *Axinyssa* aff. *pitys* (de Laubenfels 1954), *Spirastrella* aff. *decumbens*), gastropods (*Nerita* sp., *Terebralia* sp.), cnidarians (anthozoans), and algae (*Caulerpa* spp.)*,* as well large reef fish. Sibo was covered in *Caulerpa* algae and its basin is dry at low tide (Fig. S2G), except for a small cavern area toward the east side. This cavern contained sponges (*Cliona* aff.

peleia (de Laubenfels 1954)*, Tethya* aff. *coccinae* Bergquist & Kelly-Borges 1991*, Spirastrella* aff. *decumbens*), ascidians (*Eudistoma* sp.), molluscs (*Nerita* sp, *Terebralia* sp., *Cerithium* sp.)*.* Bandong is located behind the gradeschool in Teluk Alulu and is used as a public toilet (Fig. S2D). This pool had a high cover of *Caulerpa* algae (Fig. S2G) and contained a few individuals of one sponge species (*Suberites diversicolor*), ascidians (*Eudistoma* sp.), and gastropods (*Nerita* sp, *Terebralia* sp., *Cerithium* sp.)*.* Payung Payung pool is located in the village Payung Payung and is heavily used as a public toilet (Fig. S2C) and the flora and fauna in the other pools were not present here.

Embo-Embo

This blue pool is located 75 m inland, separated by limestone rock from the sea coast that is fringed with *Avecinnia* and *Sonneratia* mangroves. The pool is present in a chasm in the ground running parallel to the coastline which was approximately 1-3 m deep to the water level with almost sheer vertical walls (Fig. 3H). The pool was accessed through a cave to the north of the pool, which opens exposing the pool to air. The depth of the pool is 5-6 m with very deep blue color, and a visible halocline at 1-2 m depth. Only one sample of surface water (above the halocline) was collected to measure salinity (11 ppt) and temperature (25-28 °C). The bottom of the pool consisted of organic detritus, silt, and a tree trunk.

Hapsi

This pool is located 200 m inland and approximately 500 m from the village Boheh Silian. Similar to Embo Embo, Hapsi is also situated in a chasm with vertical walls of up to 20 m. A portion of this pool is roofed over by rock. The water is deep blue with a visible halocline at 1m depth.

Raja Ampat, West Papua

Raja Ampat represents a group of islands at the northern tip of Bird's Head peninsula in West Papua and is an intricate and rugose karst system. Lakes were found on the islands of Mansuar, Gam, Wayag and Urani (Fig1A&B). Each of these islands is characterized by a karstic scenery including a complex shaped coastline and frequent occurrence of inland depressions (Figs. 5L and S5E). The islands of Wayag and Urani in Northern Raja Ampat are characterized by the scarcity of freshwater sources and as such are practically uninhabited. None of the lakes and pools have been formally named and only one lake had a local name (Sauwandarek). As such we use our fieldnames where appropriate. In total nine lakes and pools were surveyed and here we provide their first description. The lakes and pool on Gam and Mansuar islands were located during the EWIN-LIPI-Naturalis expedition to Raja Ampat in 2007 (Becking et al. 2007) in collaboration with researchers from the University of California, Merced and Coral Reef Research Foundation, Palau. Previous biota descriptions from lakes on Gam and Mansuar island are only of ascidians by Monniot (2009).

Sauwandarek lake, Mansuar island

This lake is located on Mansuar island and is the type locality for the species *Suberites diversicolor,* a sponge frequently found in marine lakes (Becking & Lim 2009). Sauwandarek is a medium sized, oblong shaped lake, separated from the sea to the north by a low pass and to the south by a mangrove swamp and limestone ridge (Fig. S6C). The average depth is 8 m with three deeper areas: one in the center (20 m), one in the southwest (19 m) and one in the south (17 m) (Fig. S6D). The majority of the coastline is fringed by mangroves (predominantly *Bruguiera* sp.) (Fig. S3A). Along the southwestern coast there is a 20-25 m area of exposed limestone rock with a plateau extending 1-3 m from the coast with a depth of 0.25-1 m. In the southern part there is a mangrove islet (Fig. S6CD). The mangrove created an intertwined wall of roots, as in Kakaban lake. The depth along the coastline ranges from 0.75-1 m and gently slopes down to greater depths. Mussels, sponges, ascidians and algae covered most the coast consisting of mangrove roots, fallen tree trunks, and rock (Fig. S3B). Cover decreased with increasing distance from the coast. There was a high abundance of sponges, but only of moderate diversity (18-20 species). In contrast to the larger lakes in East Kalimantan, neither *Caulerpa* nor *Halimeda* algae were very dominant in biomass. The mangrove roots and epibionts were largely covered by brown-purple filamentous algae. At 1-4 m depth there were patches of mussels, some partly covered by sponges. At least two green sea turtles had been introduced by humans before 2007 and these were still present in 2009. The skin of the turtles had turned a deep yellow color.

The delay in tidal phase was at least two hours and the amplitude appeared to be damped as judged by an intertidal zone in the lake of less than 0.5 m, between 20-50% of the amplitude in the surrounding sea (1-1.5 m). The water turned to a dark brown-orange color at 2-4 m depth in 2007 and at 0.5-1 m depth in 2009. The temperature was up to 34°C at this and greater depth. The lake was accessed from the north side of the island along a path of 500 m in length with little elevation. This path continues towards Sauwandarek village beyond the lake, hence the locals refer to the lake as Sauwandarek.

Ctenophore lake, Gam island

This lake is located in northern Gam (Fig. 1B) and is a small L-shaped lake that is separated from the sea by high limestone ridges from all wind directions (Fig. S6E). The lake is highly connected to the adjacent lagoon by means of a wide (1-2 m) and low (<0.5 m) tunnel on the western coast (Fig. S3C). Ctenophore lake has a uniform basin with a maximum depth of 8.5 m in the central part of the lake (Fig. S6F). The perimeter of the lake is mostly exposed or mud covered rock with sparsely distributed *Bruguiera* and *Rhizophora* mangroves. The periphery lake floor was covered with shell fragments, but further from the edge the bottom was covered in leaves and silt. Sponges, ascidians, oysters and mussels covered the mangrove roots and rocky coast, the abundance and biomass decreasing from the coasts. There was both a high cover and diversity of sponges (>30 species) that were predominantly reef flat species. We observed high densities of ctenophores and *Aurelia* sp. in the middle of the lake, and fewer than 10 individuals of *Cassiopeia* sp. along the rim. Large reef fish (e.g. *Acanthurus* spp.) rushed in and out of the lake. Judged by the intertidal zone, the tidal amplitude was 90-100% of that of the surrounding sea. After heavy rain a layer of fresh water of 5-25 cm thick remained visible for at least a day. Ctenophore lake was accessed from the west along a 80 m long and 10 m high pass.

Big Caulerpa Lake and Wallace Lake, Gam island

Two other lakes are present on Gam (Fig. 1B), which are similar to Ctenophore lake with high connection to the sea by means of tunnels, few mangroves, and a high diversity and cover of predominantly reef flat sponges (Fig. S3EF). In contrast to Ctenophore lake, these lakes contained living stony corals (Fig. 3F). Big Caulerpa lake was surrounded by steep clifs (Figs. S3D & S5C) and contained a high abundance of *Caulerpa* algae with large globular thalli. Wallace lake was designated this name as a reference to the six week stay of Alfred Russel Wallace, during his travels in the “Malay Archipelago”, in the village Bessir (now named Yen Bessir) just south of this lake (Wallace 1869).

Red Shrimp pool, Gam island

The Red Shrimp pool near Wallace Lake is similar to the small anchialine pools in East Kalimantan, with a uniform , bowl-shaped, shallow basin (<0.5 m at low tide). It is full of small red shrimp *Antecaridina lauensis* and some *Nypa* palms are present.

Cassiopeia lake, Wayag island group

This lake is located in the eastern part of the Wayag island group (Fig. 1A) and is a small, almost circular lake that is separated from the sea by a low limestone ridge (<10 m high) from all directions except to the east where there is little elevation (Fig. S5A). The lake has a uniform, bowl-shaped basin with a maximum depth of 4m in the center. The lake is rimmed by a wide (1-4 m) and shallow plateau with a depth of <0.5 m at low tide. In the east there is a shallow bay. The coastline is composed of predominantly exposed rock interspersed with single trees of *Rhizophora* mangrove. Patches of mussels and sponges covered the lake floor amidst which there were bristleworms, red worms and green zoanthids (Fig. 5E, S4B). Fields of *Caulerpa* algae covered the subtidal area until 3 m depth. The lake floor at >3 m depth was covered by light colored sand with occasional occurrence of sponges (predominantly the species *Suberites diversicolor)*. In the northern area we observed many dead crabs and bleached or dead sponges (Fig. S4A). There was a high abundance of sponges but only of a moderate diversity (15 species). In the central area there were a small number of *M. papua* jellyfish and along the shallow plateau there were high abundances of large *Cassiopeia ornata* jellyfish (5-15 ind m^{-2}). There was a green sea turtle, but it was unknown how it came here. We observed no fish in this lake. The tidal amplitude in the lake was damped to 89% of the sea amplitude , with a 1 h 20 min delay (Table 1B). During one visit the visibility in the lake was highly reduced towards the western coast (horizontally less than 1 m) where the water was a brown-yellow color. During our second visit after a rainstorm, however, the visibility increased in the whole lake (1- 5 m) and we observed the brown-yellow water layer below 2 m depth. The lake was accessed from the east along a 60 m pass with little elevation.

Tricolore lake, Wayag island group

This small, shallow, hourglass-shaped lake is located in the central part of the Wayag island group (Fig. 1A). Tricolore lake is separated from the sea by a high limestone ridge (>20 m) to the east and a low (<5 m) ridge to the west. The lake has three basins split by mangrove patches and has four deeper areas of 2m. depth, one each in the west, the northwest, the north and in the central area (Fig. S5B). The lake is fringed by *Rhizophora* and *Bruguiera* mangroves, except along the west side which consists of exposed rock and a 1-2m deep cavern with no visible connection to the sea. The lake bottom was covered with *Caulerpa* algae amidst which there were high abundance of bristleworms and white bullomorph opistibranch molluscs (Fig. S4C). The deeper part of the lake bottom consisted for a large part of mollusc fragments covered by a thin layer of detritus and of mangrove leaves, especially in the deeper area of the northwest part. The other deeper areas of the basins were covered with *Caulerpa* algae*.* Contrary to the other investigated lakes, oysters instead of mussels were abundantly attached to all available hard substrates. Sponges and ascidians were attached to mangrove roots in patches in the center of the lake (Fig. S4D), and in the cavern along the west side, but these were notably absent in the southeast part of the lake. In the whole lake the sponge abundance was low compared to other lakes but the diversity was relatively high (>20 species). The tidal amplitude in the lake was damped to 68% of the tidal amplitude of the sea, with a 2hr20 m delay in phase (Table1B). The color of the water was blue-green with a linear visibility 5-6m. The lake is named after its apparent three colors from the air: blue-green, dark brown, and orange-red (Fig. S5B). The lake was accessed from the west along a 70 m pass with little elevation of 2-4m.

Mud lake, Wayag island group

This lake is located in the western part of the Wayag island group (Fig. 1A). Mud lake is a small, shallow, oval-shaped lake with a uniform basin with a maximum depth of 2.3m in the central area. The lake is separated from the sea to the north by a high limestone ridge (>20 m high) and to the south by a low one (<5 m high) and a wide mangrove swamp. The lake coastline is fringed by mangroves (predominantly *Bruguiera* sp.). At the east side of the lake there is a cave which may have a direct connection to the sea. The south coast is muddy and is <0.25 m deep up to 5 m distance from the coast (Fig. S4E). The depth along the rest of the coast ranged between 0.5-1m. All sessile biota was covered with fine mud and filamentous brown algae (Fig. S4F). Translucent shrimp were abundantly present. Sponges and ascidians were sporadically attached to mangrove roots and tree trunks, and had the highest cover in the cave. The degree of connection to the sea is expected to be moderate judged by an intertidal zone of 0.5-1m (70-80% of the adjacent sea amplitude). The color of the water was blue-green and below 0.5-1m depth transitioned to brown-yellow with a high content of flocculent sedimentation. The vertical visibility was 1-2m. The lake was accessed from the south along a 500 m pass through a mangrove swamp and over an elevated limestone ridge of 3-5 m.

Urani lake, Urani island

This lake, located in the western side of Urani island (Fig. 1A), is a small, tear shaped lake that is separated from the sea in all wind directions by 20-40 m high limestone ridges (Fig. 2C). The lake has a uniform, bowl-shaped basin with a maximum depth of 6m in the central area of the lake (Fig. 2D). A dense mass of *Caulerpa* algae on the lake floor may have biased our handheld sonar measurements, obscuring the actual basin depth. A 1-5 m wide belt of mangroves (predominantly *Bruguiera*) fringes the perimeter of the lake. The west coast is represented by exposed limestone and a cavern with no visible connection to the sea. The depth along the coast ranges from 1-2m and slopes down steeply. Many trees had fallen into the lake (Fig. S5D). There was a mixture of *Caulerpa* and *Halimeda* algae in the lake, but *Caulerpa* was the most abundant. Large portions of the mangrove roots and the lake bottom are covered by *Caulerpa* algae. Sponges, ascidians and mussels were attached to all available substrate, amidst which were orange worms and ophiuroids (Fig. S4H). The large red shrimp *Parhippolyte uveae* appeared abundantly present (Fig. S4G). The degree of connection to the sea was expected to be moderate, judged by an intertidal zone of 0.5-1m (70-80% of the adjacent sea amplitude). The water was tinted a milky blue-green color and the vertical visibility was 4-5 m.

Lakes located by air

We located seven additional lakes by air, but these are not part of the present survey: WAY05 (Wayag island, N0° 10’ 35.9’ E130° 01’ 18.1”), WAY06 (Jin island, N0° 08’ 14.0” E130° 09’ 00.7”), WAY07 (Jin island, N0° 08’ 10.7” E130° 09’ 04.1”), WAY08 (Bag island, N0° 06’ 28.6” E130° 12’ 57.5”), GAM01 (Gam island, S0° 26’ 59.3” E130° 30’ 02.3”), GAM02 (Gam island, S0° 26’ 57.2” E130° 30’ 04.7”), FAM01 (Fam island, S0° 36’ 01” E130° 45’ 08”).

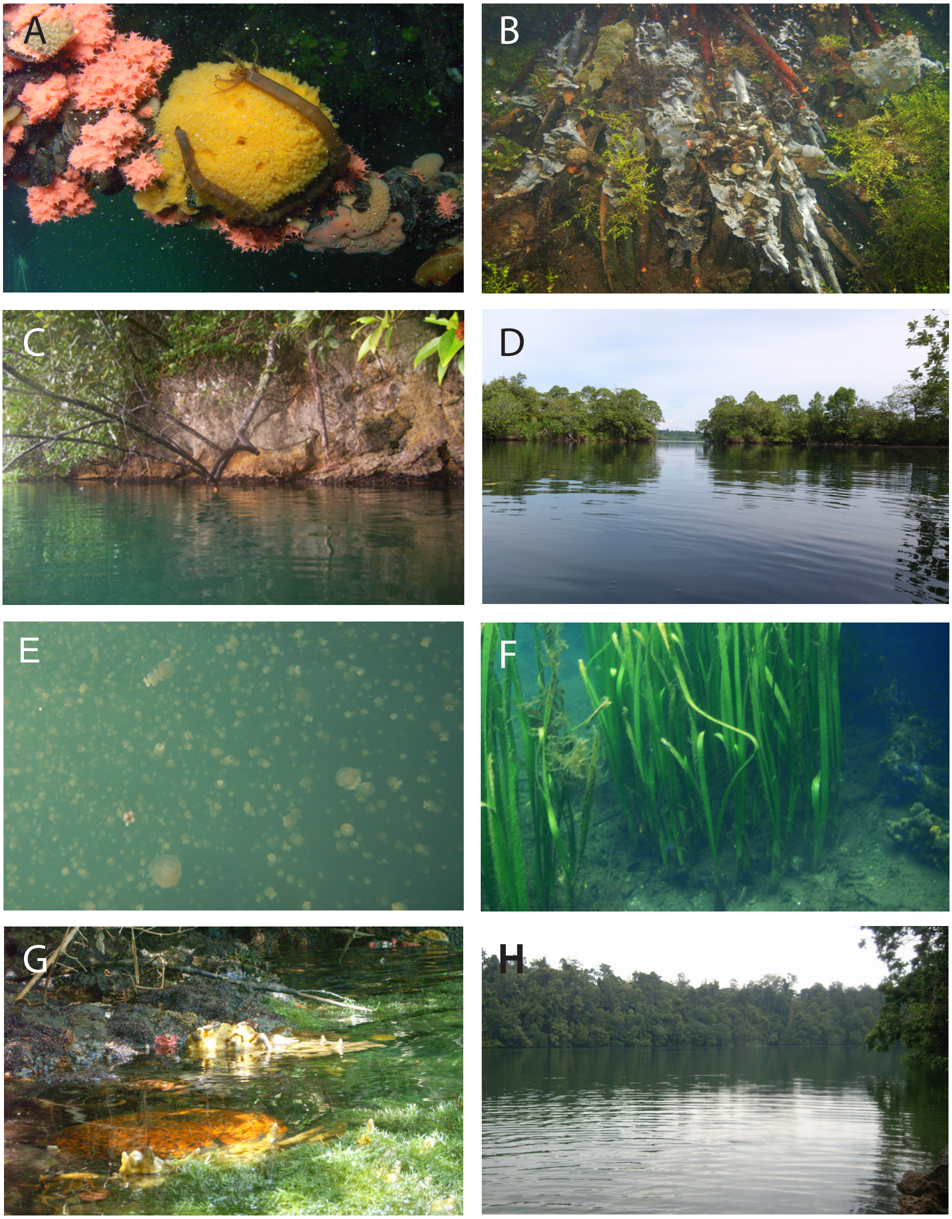


Figure S1. *In situ* photographs of marine lakes in Berau (East Kalimantan, Indonesia): Kakaban lake **A.** mangrove root covered in sponges with holothurian, **B.** mangrove roots intertwined as wall , **C.** north coast rocky shore, **D.** mangrove baylet; Haji Buang lake **E.** swarm of small juvenile *Mastigiaspapua* jellyfish, **F.** *Enhalus* sp. seagrass, **G.** sponges and algae along coast exposed to air at lowtide, **H**. overview of Bamban lake. All photographs by L.E. Becking, except **F.** by N.J. de Voogd.

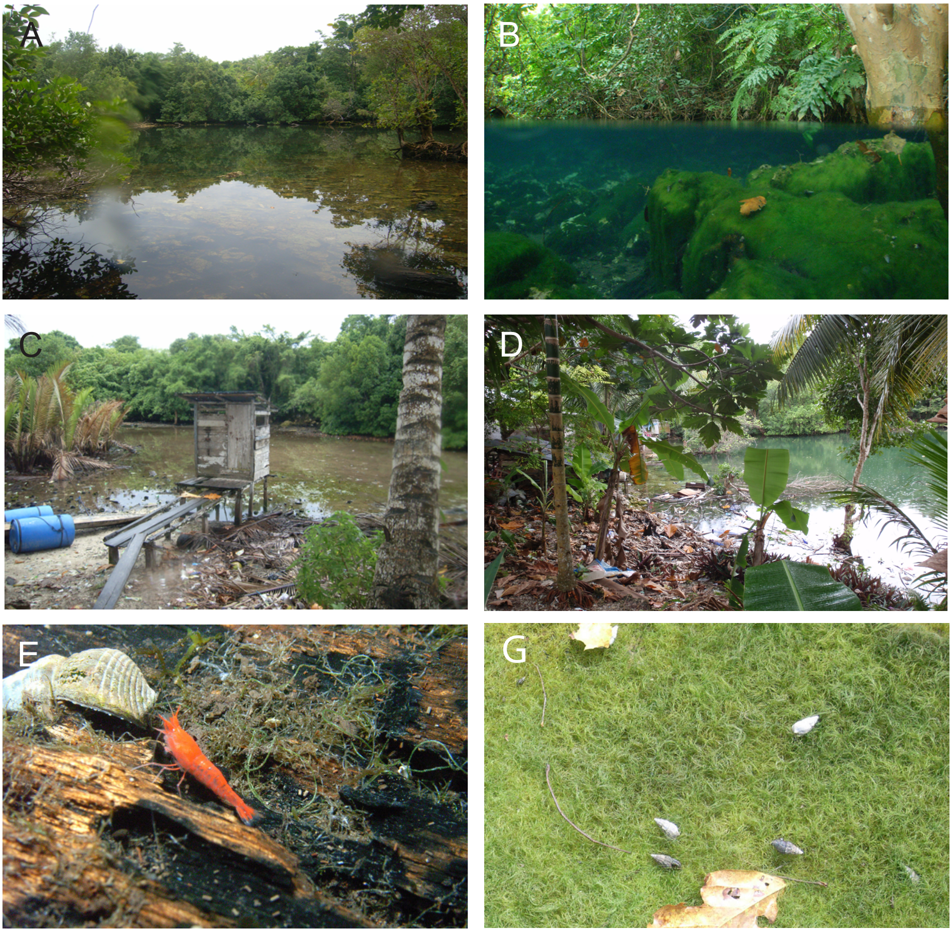


Figure S2. *In situ* photographs (by L.E. Becking) of anchialine pools in Berau (East Kalimantan, Indonesia): **A.** Tone Sibagang overview, **B.** Tone Sibagang at high tide, **C.** Payung Payung pool with outhouse, **D.** Bandong pool, **E.** *Antecaridina lauensis,* **F.** algae covering rocks in pools

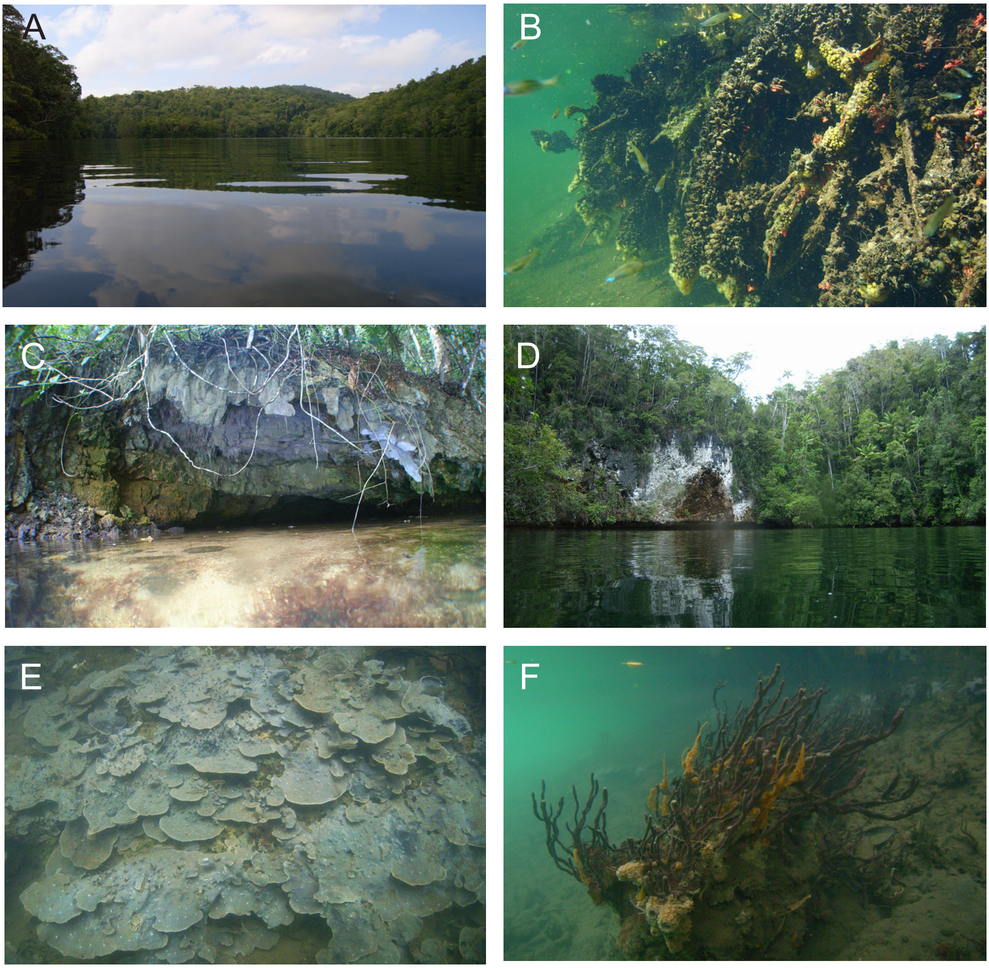
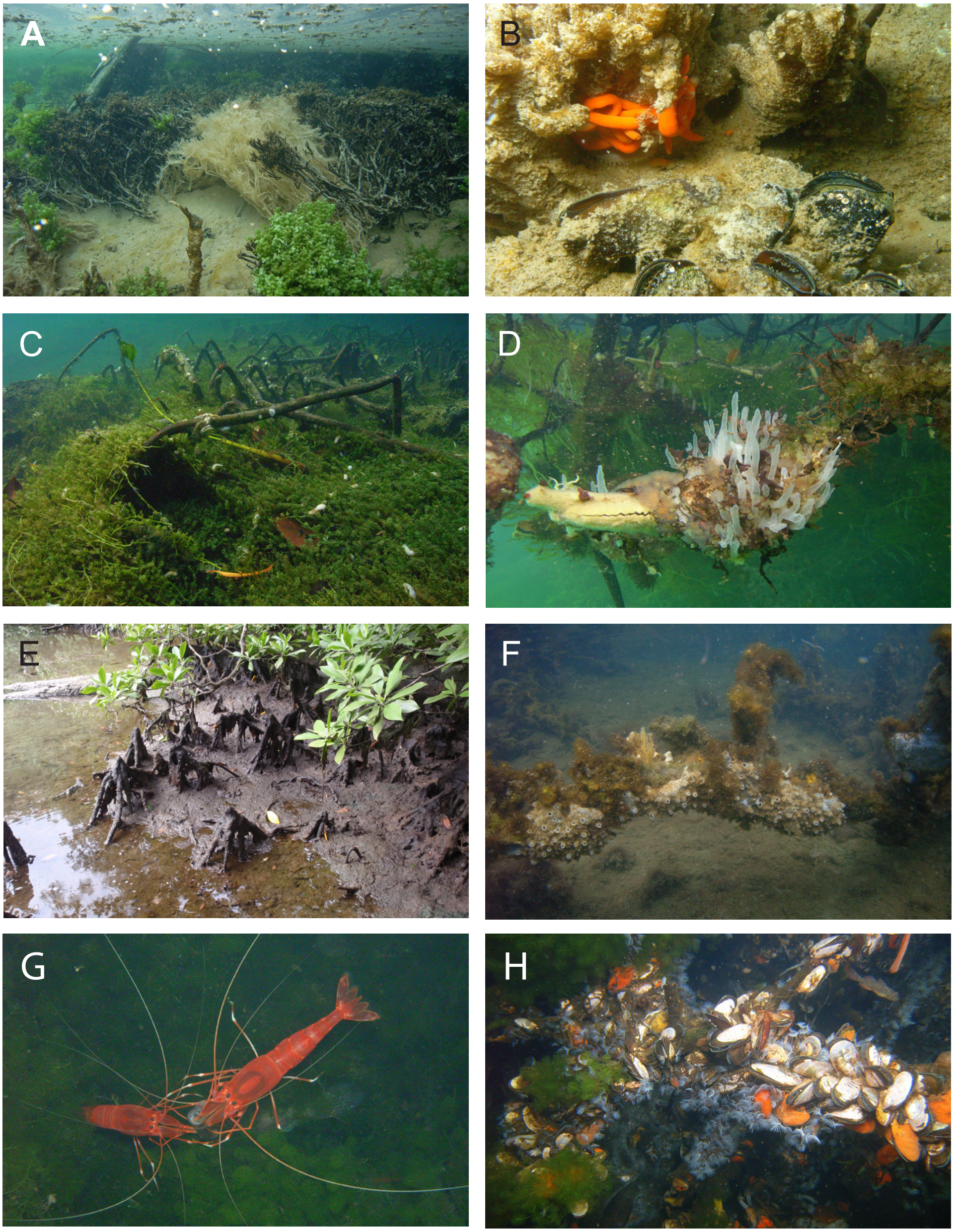
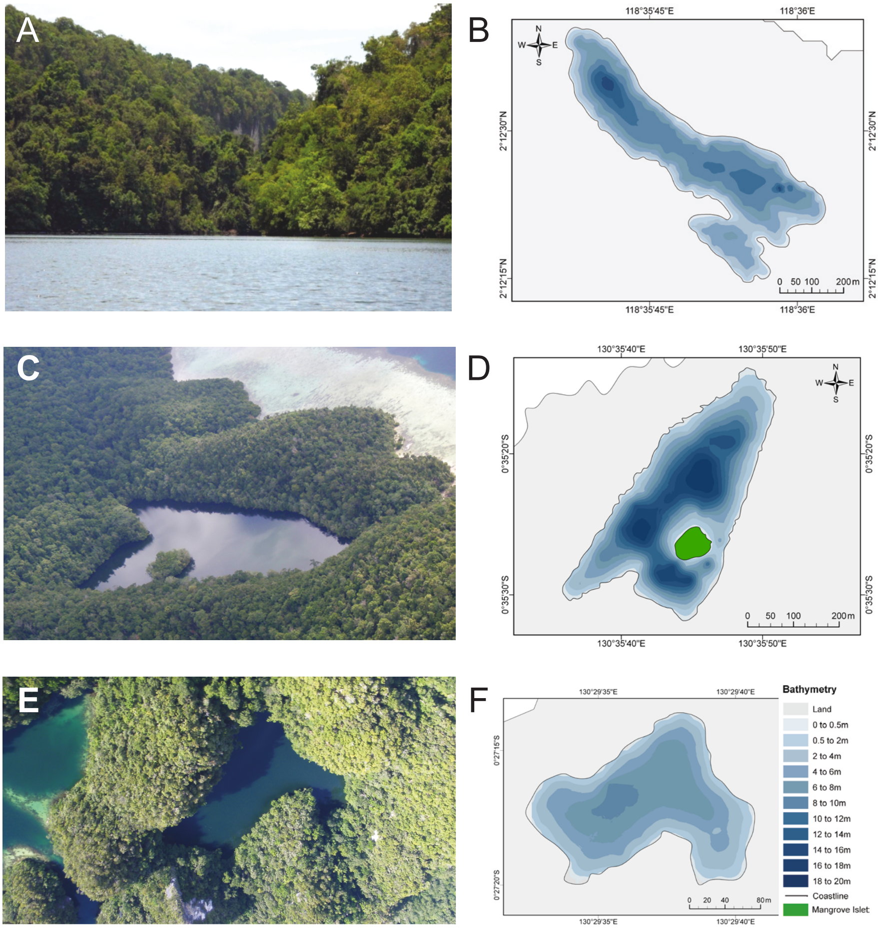


Figure S3. *In situ* photographs (by L.E. Becking) of marine lakes in central Raja Ampat (West Papua, Indonesia): **A.** overview of Sauwandarek lake, **B.** mussels, ascidians and sponges on mangroves in Sauwandarek lake, **C.** tunnel in Ctenophore lake, **D.** cliff in Big Caulerpa lake, **E**. spongereef in Wallace lake, **F.** elongated sponges in Wallace lake.



S4. In situ photographs (by L.E. Becking) of marine lakes in northern Raja Ampat (West Papua, Indonesia):
Cassiopeia lake **A.** branching brown sponge (*Haliclona* sp.) partially bleached, **B.** red worms and mussels; Tricolore lake **C.** mangrove roots with *Caulerpa* algae, oysters, and bullomorph opistibranch molluscs (white), **D.** mangrove root with sponges, mussels, ascidians, and algae; Mud lake **E.** muddy coast, **F.** sponge and lake floor covered in silt and algae; Urani lake **G.** *Parhippolyte uveae*, **H.** branch covered in mussels, sponges and tubeworms.

S5. Aerial photographs of Raja Ampat (West Papua, Indonesia): **A.** Cassiopeia lake, **B.** Tricolore lake, **C.** Big Caulerpa lake, **D.** close up of Urani lake (note fallen trees on surface), **E.** overview of Wayag island group, **F.** Drifter water aiplane. All photographs by L.E. Becking, except **F.** by E. Dondorp.



S6. In situ photograph (by L.E. Becking) and bathymetric map of **A.**,**B.**Haji Buang lake, aerial photographs (by L.E. Becking) and bathymetric maps of **C.**,**D.** Sauwandarek lake, **E.**,**F.** Ctenophore lake.

II SPECIES ASSEMBLAGES

Speak not of lakes […] to one who knows the splendor of the sea

Pu Songling
“Grace and Pine” short story #21 in “Strange tales from a Chinese Studio”

Sponge community composition in the Derawan Islands, NE Kalimantan, Indonesia

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Abstract

Coral reef ecosystems in Indonesia are among the most diverse in the world. Conservation, restoration and management of marine biodiversity hotspots such as Indonesia's coral reefs require accurate baseline knowledge of the constituent species and the environmental conditions under which these species thrive. Here we present a study on the habitat structure and diversity, composition and abundance of reef sponges in the Derawan Islands, East Kalimantan, Indonesia. Mean live coral cover across depths and sites was just under 30%, while the mean cover of rubble and dead coral exceeded 40%. The distribution of live coral cover was patchy; although lower inshore, some offshore sites had very low coral cover due to the effects of blast fishing. Rubble cover was highest inshore and beyond the barrier reef, whereas dead coral was most abundant in shallow-water and midshore reefs. A total of 168 sponge species or morphospecies were identified, of which *Stelletta clavosa*, *Lamellodysidea herbacea*, *Niphates* sp., *Ircinia ramosa* and *Petrosia nigricans* were the most common. Sponge composition varied in relation to distance from the Berau River and water visibility, in addition to sand cover and cover of encrusting corals. Importantly, sponges in the Derawan Islands appeared to thrive in inshore reefs that already had depauperate coral communities. This is in marked contrast to findings elsewhere in Indonesia (NW Java, SW Sulawesi) where inshore communities were depauperate for all taxa sampled.

Key words: Sponges • Coral reefs • Marine diversity • East Kalimantan • Berau

Introduction

Indonesian coral reefs are among the most diverse and threatened in the world. Proper conservation and management of Indonesia's coral reefs requires accurate baseline studies of the constituent taxa and environmental conditions (Mora et al. 2003). The acquisition of spatially explicit environmental data is essential to understand how spatial and environmental processes (including human-induced disturbance) interact to structure marine assemblages.Most reef surveys have tended to focus on charismatic groups such as corals or fishes and have generally taken place in areas which have already experienced massive losses in biodiversity and shifts in composition as a result of historical disturbances. In the Thousands Islands, NW Java, for example, historical coral collections were compared with recent reef surveys (van der Meij et al. 2009) and revealed that the diversity of corals has declined dramatically over a time span of only ca. 70 yr. Once diverse reefs close to the city of Jakarta had in fact virtually disappeared by 1995. Other studies, close to the city of Makassar (SW Sulawesi), reflected these findings in identifying a strong onshore–offshore gradient in composition with depauperate communities close to the city (Becking et al. 2006, Cleary et al. 2008). In addition to studying a wide array of coral reef taxa and using the limited historical data available to compare coral reef environments, it is also important to study the few remaining relatively undisturbed areas.

The Berau Delta and barrier reef system in East Kalimantan (Derawan Islands), Indonesia, is an intricate coastal system with a variety of coastal landforms and associated ecosystems. The Berau River basin and delta is composed of 2 major estuaries and is surrounded by mangrove forest. At the delta-front there is a barrier reef system that extends to the offshore islands of Kakaban and Maratua with oceanic reefs that border the Makassar Strait (Tomascik et al. 1997). Although the coastal region is reported to still have a number of relatively pristine characteristics, fish and shrimp ponds are gradually replacing the natural coastal vegetation and offshore reefs are becoming increasingly damaged by destructive, albeit illegal, fishing techniques such as blast fishing (Ismuranty 2003, Estradivari 2008). Furthermore, the Derawan Islands are unique and of global interest due to the presence of several marine lakes located within the islands of Kakaban and Maratua (Tomascik et al. 1997, Becking & Lim 2009, CHAPTERS 1, 3 & 4). In addition to the lakes, the Derawan chain contains Indonesia's largest nesting site of the endangered green sea turtle.

In the present study, we assessed the habitat structure (e.g. cover of branching coral, massive coral, sand or rubble), abiotic environmental variables (e.g. temperature, salinity, pH) and composition and abundance of larger reef sponges. Sponges have often been left out of biodiversity surveys because of difficulties in identifying taxa, even at higher taxonomic levels. They are, however, an important coral reef benthic group, and play a key role in nutrient cycling, water filtering, bioerosion, reef stabilization, spatial competition and as habitat for other reef invertebrates (Aerts & van Soest 1997, Skilleter et al. 2005, Wulff 2006, Bell 2008). The loss of sponge species could accelerate declines in coral reefs as they are fundamental in increasing water clarity, binding live corals to the reef frame and facilitating reef regeneration (Wulff 2006, Bell 2008). The aims of the present study were to: (1) assess to what extent the reefs of the Derawan Islands are undisturbed by quantifying the area of live coral cover and other structural components, including coral rubble and dead coral; (2) quantify spatial variation in sponge composition, abundance and species richness across a large spatial scale; and (3) quantify to what extent variation in composition can be explained by abiotic environmental variables, habitat structure variables or purely spatial variables.

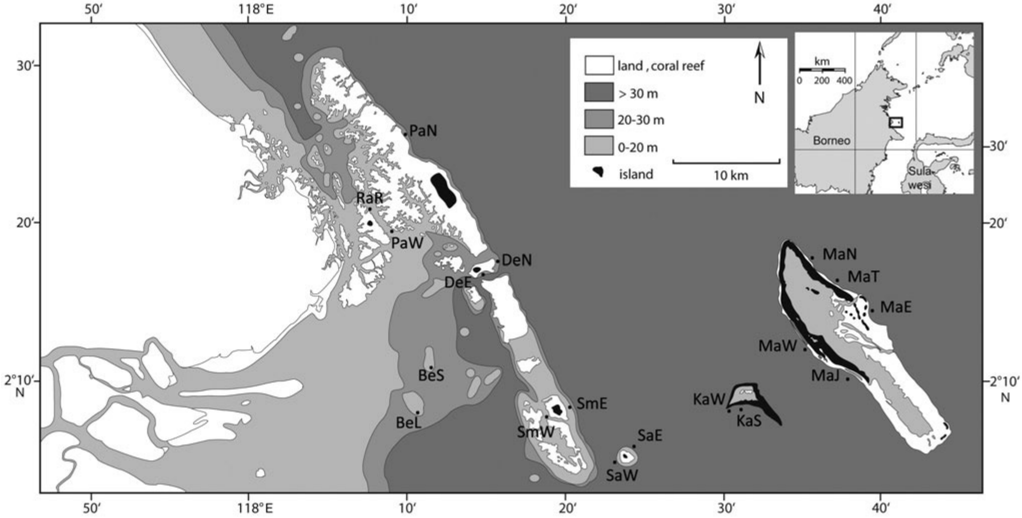

Figure 1. Derawan Islands, East Kalimantan, Indonesia. BeL: Berau 'Lighthouse'; BeS: Berau South; DeE: Derawan 'Jetty Point'; DeN: Derawan 'Coral Garden'; KaS: Kakaban Southwest; KaW: Kakaban West; MaN: Maratua Northwest; MaE: Maratua 'Midnight Snapper'; MaW: Maratua 'Traffic'; MaT: Maratua 'Parade'; MaJ: Maratua 'Johnny's Reef'; PaW: Panjang West; PaN: Panjang Northeast; RaR: Rabu Rabu; SmE: Samama East; SmW: Samama West; SaE: Sangalaki East; SaW: Sangalaki West. Site information provided in Table 1. Map adapted from Renema (2006a) with permission.

Materials & Methods

Study area. Research for the present study took place in the Derawan Islands, NE Kalimantan, Indonesia. Coral reefs in this area are found across a water gradient from fluvially influenced to fully oceanic, separated by a barrier reef. The reefs inside the barrier reef are under direct influence of the Berau River, and the river plume can extend 15 to 30 km from the mainland during the rainy season. The depth of the coral reefs inside the barrier reef varies from 10 m close to the river mouth to more than 150 m close to the barrier. Inshore reefs have a relatively low coral cover, with high densities of filter feeders such as sponges, soft corals and crinoids, and the rubble is covered by fine mud and silt (Renema 2006a). The outside barrier is comprised of diverse reef types, dominated by dense stands of corals and coarse sand. The annual rainfall in Berau is approximately 2000 mm with no particular rainy season, though there is less rainfall June-September (average 110-125 mm per month) compared to the rest of the year (average 150-200 mm per month) and an increase of strong winds from the North between December-March (Tomascik et al. 1997, Mantel 2001, Renema 2006).

Sponges. Sampling took place using SCUBA diving from 10 to 23 August 2008. Surveys were made at 2 depths (5 and 10 m) at 18 different sites (Fig. 1, Table 1). Sponge species and their abundance were noted in 1 m^2 quadrats laid every 1 m along a 30 m line-transect. Smaller (cryptic, boring and thinly encrusting) sponge specimens were excluded from the present study. Species were visually identified in the field, and fragments of all species were collected for closer examination and identification to species level by N. J. de Voogd. ethyl alcohol and deposited in the Porifera collection of Naturalis Biodiversity Center (RMNH POR.).

Environmental variables. Vertical water visibility, temperature, pH, salinity and depth were assessed as local abiotic environmental variables. Vertical water visibility was measured using a Secchi disc following English et al. (1994) at around 12:00 h near the surveyed sites. Depth was measured using a computerized depth meter (Suunto). Geographic coordinates were recorded at each transect with a handheld GPS device (Garmin GPS 60). Temperature, salinity and pH were measured with an YSI Model 63 handheld pH, conductivity, salinity and temperature system. In addition to these variables, we also include the distance of each site to the mouth of the Berau River. We assumed this was a proxy of processes including sedimentation and land-based contamination, as the Berau River is the main conduit of these factors into the research area.

Habitat structure. The habitat structure was assessed during the line intercept transect (LIT) method for surveys (English et al. 1994, Edinger & Risk 2000). In the present study, the cover of 28 life forms (see English et al. 1994) was assessed along two 30 m line transects located at 5 and 10 m depth in each site. The life forms (including non-living substrate) were hard dead coral (dead coral, dead coral with algae), *Acropora* corals (branching, encrusting, submassive, digitate, tabular), non-*Acropora* corals (branching, encrusting, foliose, massive, submassive, mushroom, *Heliopora*, *Millepora*,*Tubipora*); other fauna (soft corals, sponges, zoanthids and other invertebrate taxa); algae (algal assemblages, coralline algae, *Halimeda*, macroalgae, turf algae); and abiotic (sand, rubble, rock). The LIT method was used to estimate the cover of a life form and non-living substrate, in this case along a 30 m transect. The cover or percentage was calculated by the fraction of the length of the line that was intercepted by the life form in question.

Analytical framework. All analyses were performed and figures were made using R (www.r-project.org). For rarefaction and estimation of species richness using the Chao1 and Chao2 richness estimators, we used the vegan and fossil packages, respectively. Two ordination techniques were used to analyse the species: environmental and spatial data matrices. Principal components analysis (PCA) was used as an unconstrained ordination technique to explore the major axes of variation in the species × sites data matrix. Redundancy analysis (RDA) was used as a constrained ordination technique to relate sponge species to environmental variables (Legendre & Gallagher 2001). Input for the PCA and RDA consisted of loge(x + 1) species abundance data that were first transformed using the decostand function in the vegan package. Through this transformation, the species abundance data were adjusted so that the PCA and RDA preserved the chosen distance among objects (sample sites). In the present case, the Hellinger distance was used, as recommended by Legendre & Gallagher (2001).

Spatial variation in the study area was modelled using principal coordinates of neighbor matrices (PCNM). PCNM is a novel method for quantifying spatial trends across a range of scales and is based on eigenvalue decomposition of a truncated matrix of geographic distances among sampling sites (Borcard & Legendre 2002). For a detailed description of PCNM, see Borcard & Legendre (2002) and Dray et al. (2006). Significant PCNM eigenvectors were selected using the *quickPCNM* function with 999 permutations. Significant environmental and habitat structure variables were selected using the *forward.sel* function in the *packfor* package with 999 permutations. (Both *quickPCNM* in the PCNM library and *packfor* are available at the website of Pierre Legendre, www.bio.umontreal.ca/legendre/indexEn.html). The forward selection test used was based on a novel forward selection procedure that corrects for the inflated Type I error and overestimation of explained variance associated with classical forward selection (Blanchet et al. 2008). All significant PCNM, environmental and spatial variables were used in an RDA using the *rda-Test* function (www.bio.umontreal.ca/legendre/indexEn.html). Finally, we used variance partitioning (with the *varpart* function in vegan) to partition the variance explained by spatial, environmental and habitat structure variables.

Table 1. Characteristics of all transects sampled during the course of this study. Distance river: distance from the mouth of the River Berau, Lat: latitude in decimal degrees, Long: longitude in decimal degrees. Abundance: number of individual sponges sampled, Richness: rarefied number of species observed based on the minimum number of individuals sampled on a transect (n = 35). Life form data representing the percentage cover of coralline algae, turf algae, dead corals, rubble, sand, sponges and all live corals combined are also presented.

Site code	Location	Depth (m)	Visibility (m)	pH	T (°C)	Salinity (ppt)	Distance river (km)	Lat
BeL	Berau 'Lighthouse'	5	6.5	8.24	28.8	33	9.66	2.16
BeL	Berau 'Lighthouse'	10	6.5	8.21	28.9	33.55	9.66	2.16
RaR	Rabu-Rabu	5	7.5	8.25	28.85	33.8	11.02	2.35
RaR	Rabu-Rabu	10	7.5	8.215	28.9	33.8	11.02	2.35
BeS	Berau 'South'	5	8	8.24	28.95	32.95	12.43	2.20
BeS	Berau 'South'	10	8	8.195	28.85	33.35	12.43	2.20
PaW	West Panjang	5	11	8.235	28.75	33.6	13.86	2.33
PaW	West Panjang	10	11	8.18	28.6	33.6	13.86	2.33
PaN	Northeast Panjang	5	21	8.285	28.9	33.95	16.66	2.43
PaN	Northeast Panjang	10	21	8.255	28.95	33.9	16.66	2.43
DeE	Derawan 'Jetty Point'	5	21.5	8.29	28.6	34	19.40	2.28
DeE	Derawan 'Jetty Point'	10	21.5	8.25	28.6	34	19.40	2.28
DeN	Derawan 'Coral Garden'	5	26	8.295	29.4	34.1	21.00	2.30
DeN	Derawan, Coral Garden	10	26	8.26	28.9	34.05	21.00	2.30
SmW	West Samama	5	13	8.225	28.75	33.6	26.18	2.13
SmW	West Samama	10	13	8.185	28.65	33.8	26.18	2.13
SmE	East Samama	5	24.5	8.26	28.85	33.9	28.86	2.13
SmE	East Samama	10	24.5	8.21	28.8	33.75	28.86	2.13
SaW	West Sangalaki	5	25	8.23	28.65	34	34.49	2.09
SaW	West Sangalaki	10	25	8.195	28.7	34.05	34.49	2.09
SaE	East Sangalaki	5	28.5	8.26	28.7	33.15	35.89	2.09
SaE	East Sangalaki	10	28.5	8.21	28.75	34.1	35.89	2.09
KaS	Southwest Kakaban	5	41.5	8.25	28.45	34.15	47.11	2.14
KaS	Southwest Kakaban	10	41.5	8.205	28.25	34.05	47.11	2.14
KaW	West Kakaban	5	40	8.27	28.15	34.15	48.02	2.14
KaW	West Kakaban	10	40	8.24	28.05	34.1	48.02	2.14
MaW	West Maratua 'Turtle traffic'	5	45.5	8.265	28.8	33.95	56.69	2.20
MaW	West Maratua 'Turtle traffic'	10	45.5	8.24	28.8	33.75	56.69	2.20
MaJ	Southwest Maratua 'Johnny's Reef'	5	41	8.29	28.8	34	61.88	2.17
MaJ	Southwest Maratua 'Johnny's Reef'	10	41	8.245	28.2	34.1	61.88	2.17
MaN	Northeast Maratua 'Macronesia'	5	42.5	8.245	28.9	33.65	56.87	2.29
MaN	Northeast Maratua 'Macronesia'	10	42.5	8.225	28.9	33.45	56.87	2.29
MaT	East Maratua 'Turtle parade'	5	46.5	8.295	29	33.9	60.57	2.26
MaT	East Maratua 'Turtle parade'	10	46.5	8.255	29	33.9	60.57	2.26
MaE	Southeast Maratua 'Midnight snapper'	5	27.5	8.225	29.3	33.6	63.63	2.24
MaE	Southeast Maratua 'Midnight snapper'	10	27.5	8.22	29.05	33.65	63.63	2.24

Long	Abundance	Richness	Coraline algae (%)	Turf algae (%)	Dead coral (%)	Rubble (%)	Sand (%)	Sponge (%)	Total coral (%)
118.17	161.00	14.79	0.00	3.00	6.00	5.33	8.00	16.00	25.67
118.17	231.00	18.29	1.17	0.50	0.00	38.50	7.17	16.33	4.50
118.13	163.00	19.48	0.00	2.00	9.67	24.33	9.00	9.67	34.67
118.13	205.00	21.05	1.50	8.00	5.67	33.50	10.83	3.50	30.00
118.19	321.00	18.54	1.00	0.33	5.70	19.50	17.00	12.47	22.67
118.19	270.00	13.13	2.67	0.00	1.83	11.00	10.33	10.63	53.37
118.18	224.00	15.54	0.00	0.00	8.33	18.00	9.00	11.50	41.00
118.18	141.00	20.85	0.00	0.67	1.00	57.00	7.33	3.17	22.83
118.16	100.00	14.60	4.97	0.00	10.47	47.67	4.27	1.27	23.73
118.16	283.00	15.52	3.27	2.27	4.10	36.60	2.60	6.27	16.17
118.25	106.00	15.45	0.00	0.00	19.33	54.33	11.17	0.67	9.00
118.25	132.00	13.13	5.77	0.20	12.70	33.10	17.50	2.40	27.33
118.26	110.00	14.48	3.00	0.00	24.67	9.33	9.33	4.33	35.33
118.26	312.00	18.41	8.20	0.00	17.67	16.33	3.00	3.80	33.50
118.32	174.00	13.49	0.00	0.33	19.33	8.33	6.33	3.80	51.83
118.32	135.00	15.26	4.50	0.00	8.00	23.00	20.00	1.33	34.50
118.34	122.00	16.40	2.33	1.83	11.17	11.77	1.33	0.17	33.17
118.34	211.00	15.23	1.17	0.17	10.17	25.90	32.00	4.33	14.50
118.39	58.00	14.35	0.00	0.67	52.33	19.00	0.33	0.00	23.50
118.39	140.00	10.81	1.67	2.67	47.00	10.33	10.00	3.67	20.00
118.40	101.00	14.63	0.00	0.00	63.33	6.67	0.00	2.33	20.33
118.40	187.00	16.47	3.17	1.00	23.17	15.33	2.67	5.33	36.00
118.51	117.00	18.63	1.80	0.00	16.47	16.17	0.00	4.23	54.27
118.51	444.00	19.62	11.53	0.00	4.50	14.60	0.00	17.90	34.40
118.51	953.00	2.83	0.00	0.00	8.67	71.67	0.33	1.67	15.83
118.51	794.00	5.87	2.67	0.33	12.00	35.67	3.00	1.00	42.67
118.59	159.00	14.45	0.00	1.00	36.00	33.67	0.00	2.17	21.00
118.59	199.00	17.00	2.17	0.67	13.00	31.33	0.67	8.50	31.83
118.64	194.00	14.26	14.33	0.00	7.67	14.33	3.00	5.00	42.00
118.64	260.00	16.34	14.17	0.67	8.33	9.00	0.67	6.67	32.83
118.59	114.00	13.03	1.67	2.00	38.00	26.67	0.00	0.33	29.67
118.59	259.00	16.60	4.33	1.67	6.00	32.00	0.67	3.33	27.67
118.63	35.00	14.00	1.33	4.67	13.67	13.00	0.00	3.67	55.67
118.63	170.00	16.04	3.67	28.67	14.33	21.67	0.00	1.67	13.67
118.65	65.00	6.11	1.00	5.00	22.00	48.00	2.00	0.00	7.67
118.65	160.00	13.45	5.00	8.33	19.33	23.67	4.00	5.00	19.17

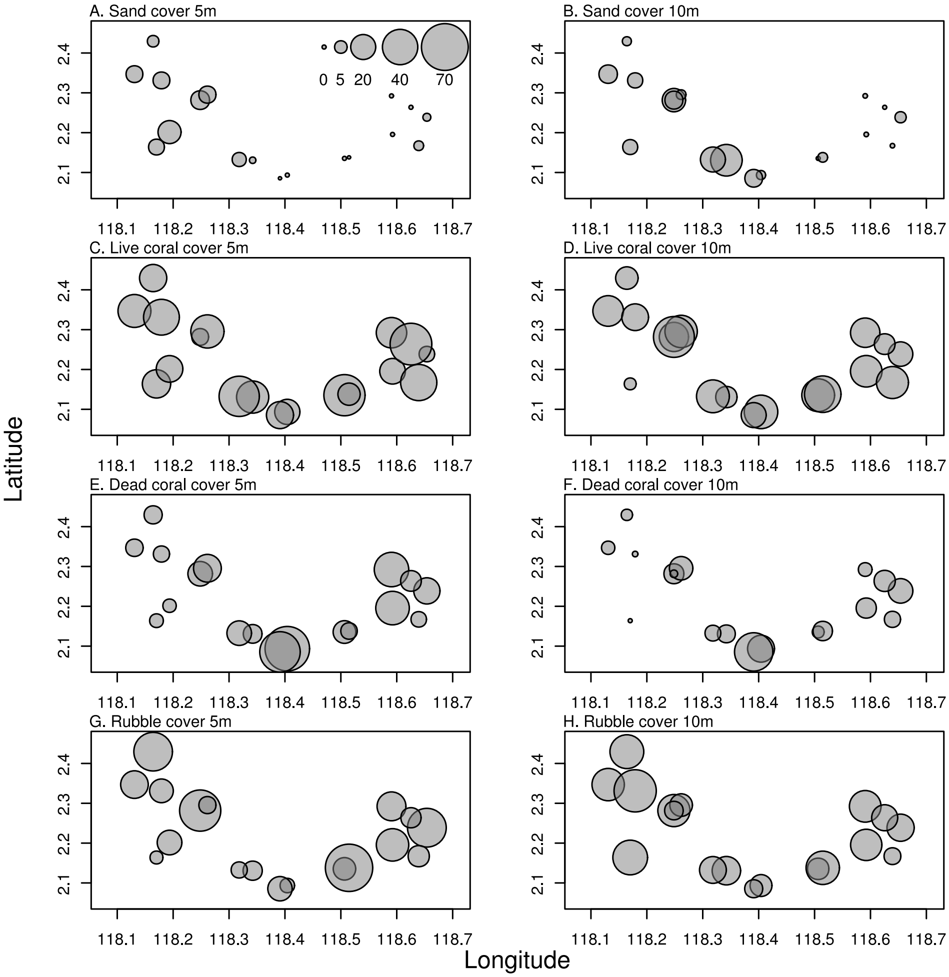

Figure 2. Cover of sand, live coral cover, dead coral and rubble across the Derawan Islands. (A) Sand at 5 m (range = 0 to 17.0%), (B) sand at 10 m (0 to 32.0%), (C) live scleractinian coral at 5 m (7.7 to 55.7%), (D) live scleractinian coral at 10 m (4.5 to 54.4%), (E) dead coral at 5 m (5.7 to 63.3%), (F) dead coral at 10 m (0 to 19.3%), (G) rubble at 5 m (5.3 to 71.7%) and (H) rubble at 10 m (9 to 57%)

Results

Live scleractinian coral cover ranged from 7.7 to 55.7% at 5 m and 5.7 to 63.3% at 10 m depth (Fig. 2, Table 1). Average live coral cover across sites and depths was 28.9%. The percentage cover of rubble was highest inshore and offshore and markedly less midshore, and across all sites ranged from 5.3 to 71.7% (at KaS; see Fig. 1 for site abbreviations) with a mean of 25.4%. Dead coral cover appeared to have quite a different pattern to rubble. Dead coral cover was higher midshore than inshore or offshore and was also somewhat higher at 5 than 10 m depth (Fig. 2). Overall, dead coral cover ranged from 0 to 63.3% with a mean of 16.1%. We sampled 7810 individual sponges and identified a total of 168 sponge species belonging to 62 genera and 37 families (see the complete species list in Table S1 in the electronic supplement at www.int-res.com/articles/suppl/m396p169_app.pfd). The median number of individuals recorded per transect was 119.5 (range = 35 to 953 individuals) at 5 m depth and 202 (range = 132 to 794 individuals) at 10 m depth (Fig. 3). Mean sponge cover varied from 4.40% (range = 0 to 16%) at 5 m depth to 5.37% (range = 1 to 17.9%) at 10 m depth. Rarefied species richness varied from 14.17 species (range = 2.83 to 19.48 species) at 5 m depth to 15.73 species (range = 5.87 to 21.05 species) at 10 m depth (Fig. 3). Only 8 species were common (as defined by Kaandorp 1986), i.e. co-occurred in >23 different transects of the total of 36 transects (66% level oftransects), namely *Carteriospongia foliascens*, *Cinachyrella* spp., *Clathria reinwardti*, *Hyrtios erectus*, *Ircinia ramosa*, *Lamellodysidea herbacea*, *Niphates* sp. 'blue'and *Petrosia nigricans*. The 10 most abundant species sampled during the present study were *Amphimedon paraviridis* (234), *Clathria reinwardti* (237), *Carteriospongia foliascens* (250), *Hyrtios erectus* (254), *Haliclona* aff. *amboinensis* (280), *Petrosia nigricans* (298), *Niphates* sp. 'blue' (304), *I. ramosa* (327), *L. herbacea* (603) and *Stelletta clavosa* (1540). The distribution of 4 of these species is shown in Fig. 4. *Haliclona* aff. *amboinensis* had a pronounced preference for inshore sites whereas *A. paraviridis* was more patchily distributed, although it attained high local abundance. The sponge *P. nigricans* was found in most sites in moderate densities. *Stelletta clavosa*, the most abundant species overall, was curiously enough only recorded in 3 transects. In one transect (DeE 5 m) only 4 individuals were recorded, whereas 645 to 891 individuals were recorded in the other 2 transects (KaW 5 m and KaW 10 m).

Species composition, based on the first 2 axes of a PCA, showed a largely inshore–offshore gradient in composition along the first (8.69% of total variation explained) and second axes (7.59% of total variation explained) (Fig. 5). The most distinct sponge assemblages were found at sites RaR 10 m (score on PC1 = 11.63) and KaS 10 m (score on PC1 = 13.28; PC2 = –15.44). Both sites had a number of species that were not recorded in any other transect (5 for RaR 10 m and 6 for KaS 10 m). One species, *Axinyssa* sp. '118', was represented by 9 individuals at KaS 10 m. In total, 38 species were only recorded at a single transect, indicating that actual diversity was higher than recorded. In addition, a total of 26 singletons and 11 duplicates were recorded at the study area. Estimates using the Chao1 and Chao2 richness estimators both yielded an expected lower bound richness of 198 species compared to the 168 species we observed. There was a significant relationship between space and community composition. Using a forward selection procedure, 5 PCNM variables were selected out of a total of 11. Significant PCNM variables are shown in Fig. S1 in the supplement. The same technique yielded 4 significant environmental variables and 4 significant habitat structure variables. Significant environmental variables included the distance from the river (F = 3.259, $p < 0.001$, R2 adj = 0.061), water transparency/visibility (F = 1.925, p = 0.002, R2 adj = 0.025), tempera ture (F = 1.855, p = 0.002, R2 adj = 0.023) and pH (F = 1.447, p = 0.028, R2 adj = 0.012). The variation in visibility is shown in Fig. S1 in the supplement, revealing an increase in visibility away from the river. Significant habitat structure variables included cover of sand (F = 2.135, $p < 0.001$, R2 adj = 0.031), encrusting coral (F = 1.643, p = 0.007, R2 adj = 0.018), turf algae (F = 1.549, p = 0.011, R2 adj = 0.016) and rubble (F = 1.372, p = 0.050, R2 adj = 0.011).

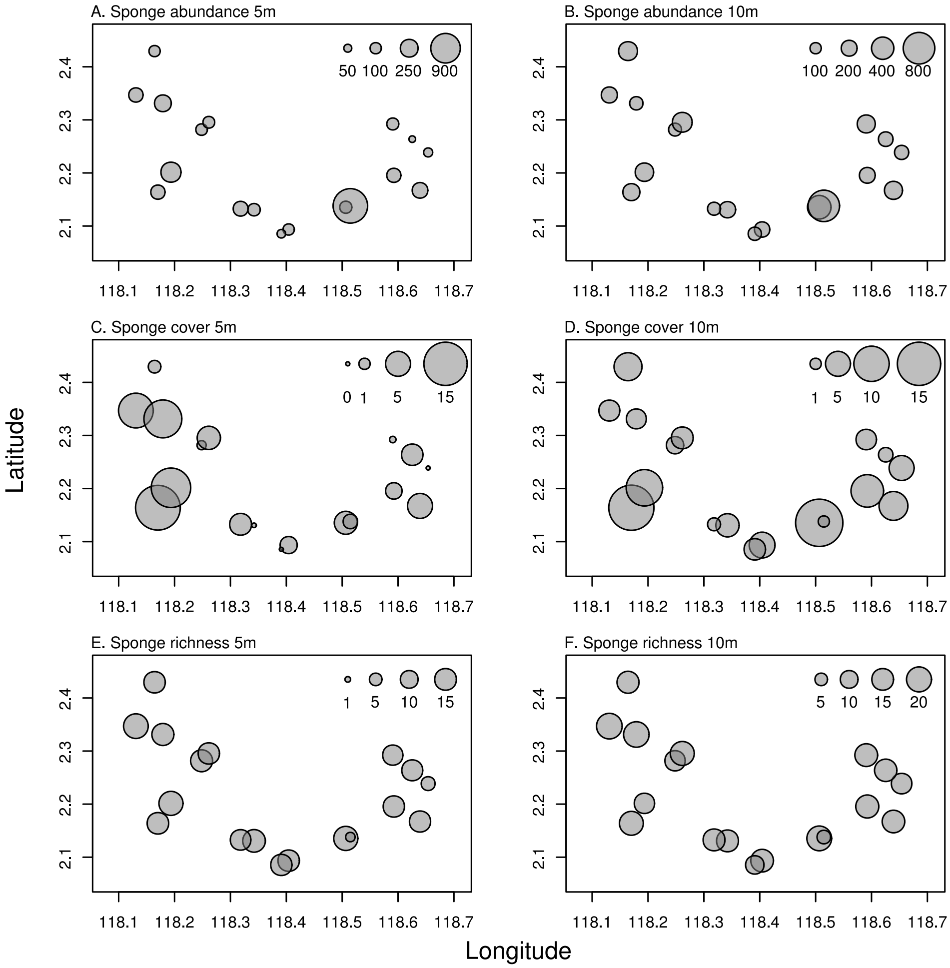

Figure 3. Abundance, cover and species richness of sponges in the Derawan Islands. (A) Sponge abundance at 5 m (range = 35 to 953 individuals per transect) and (B) 10 m across sampling sites (132 to 794 individuals per transect). (C) Sponge cover as percentage of total cover at 5 m (range = 0 to 16% per transect) and (D) 10 m across sampling sites (1 to 17.9% per transect). (E) Sponge rarefied (n = 35 individuals) species richness at 5 m (range = 2.83 to 19.48 species per transect) and (F) 10 m across sampling sites (5.87 to 21.05 species per transect)

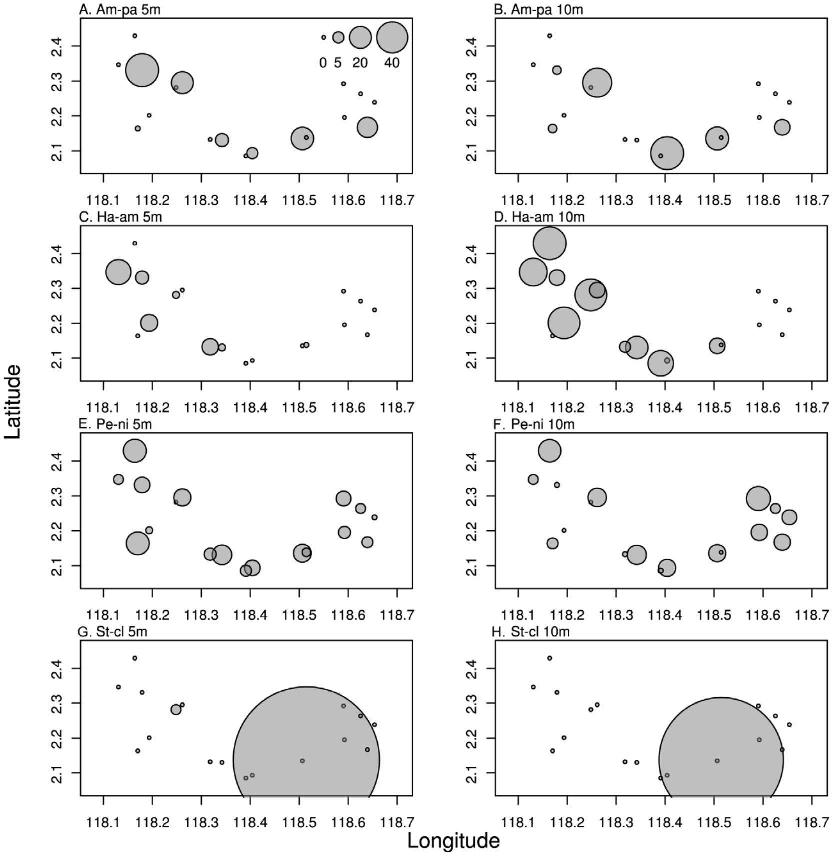

Figure 4. Variation in the abundance of selected species across the Derawan Islands. (A) *Amphimedon paraviridis* (Am-pa) at 5 m (range = 0 to 45 individuals per transect) and (B) 10 m across sampling sites (range: 0 to 26 individuals per transect). (C) *Haliclona* aff. *amboinensis* (Ha-am) at 5 m (0 to 26 individuals per transect) and (D) 10 m across sampling sites (0 to 45 individuals per transect). (E) *Petrosia nigricans* (Pe-Ni) at 5 m (0 to 22 individuals per transect) and (F) 10 m across sampling sites (0 to 24 individuals per transect). (G) *Stelletta clavosa* (St-cl) at 5 m (0 to 891 individuals per transect) and (H) 10 m across sampling sites (0 to 645 individuals per transect).

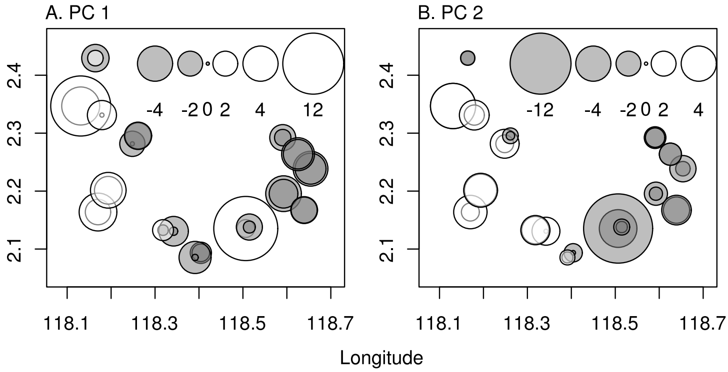

Figure 5. Variation in site scores of (A) PC1 (range: –4.12 to 13.26) and (B) PC2 (–15.44 to 6.60). Open symbols indicate positive values and shaded symbols negative values. The size of the symbol is proportional to the score

The spatial variation in community composition in relation to environmental variables is shown in Fig. 6. There was a highly significant relationship between the set of spatial, environmental and habitat structure variables and community composition ($F = 1.916$, $p < 0.001$, R2 = 0.531, R2 adj = 0.254). Space, environment and habitat structure together thus explained more than 25% of the variation in composition. Space, environment and habitat structure alone explained 9, 10 and 5% of total variation in composition, respectively. The major axis of variation was determined by sites with high sand cover versus sites that were distant from the river and had good water transparency/visibility. More or less perpendicular to this axis, there was a gradient from sites with a high cover of encrusting corals to sites with high turf algae cover. Species with high values along axis 1 (high sand cover) included *Haliclona* aff. *amboinensis*, *Echinodictyum mesenterinum*, *Paratetilla* aff. *bacca* and *Stylissa carteri*, whereas species with low values along axis 1 (good water visibility) included *Placospongia melobesioides* and *Petrosia corticata*. Species with low values along axis 2 included *Niphates* sp. 'blue', *Haliclona* (*Soestella*) 'brown' and *Amphimedon paraviridis*, whereas species with high values along axis 2, thus associated with areas of relatively high rubble and turf algae cover, included *Hyrtios erectus*, *Lamellodysidea herbacea* and *Agelas* aff. *nemoechinata*.

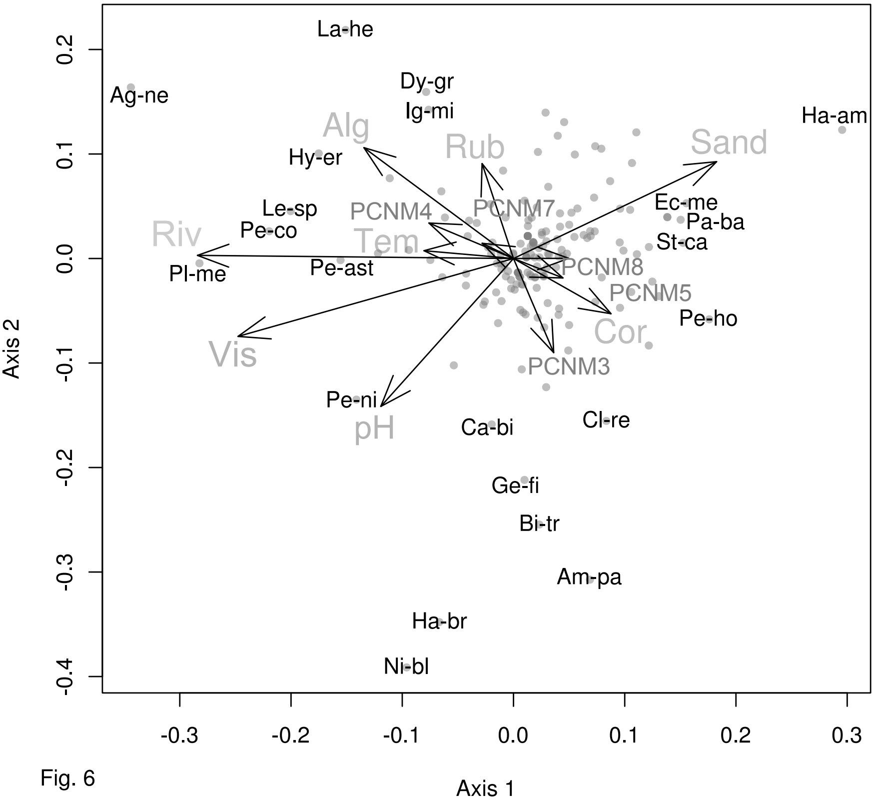

Fig. 6

Figure 6. Ordination of sponges based on redundancy analysis. Arrows represent significant spatial, habitat and environmental variables, and their direction and length indicates their contribution to variation along those axes. Significant variables include principal coordinates of neighbor matrices (PCNM) 3, 4, 5, 7 and 8, the cover of sand, encrusting corals (Cor), turf algae (Alg) and rubble (Rub), temperature (Tem), pH, water transparency/visibility (Vis) and the distance from the Berau River (Riv). Species are indicated by dots; selected species are indicated by codes: Ag-ne: *Agelas* aff. *nemoechinata*; Am-pa: *Amphimedon paraviridis*; Bi-tr: *Biemna triraphis*; Ca-bi: *Callyspongia biru*; Cl-re: *Clathria reinwardti*; Dy-gr: *Dysidea granulosa*; Ec-me: *Echinodictyum mesenterinum*; Ge-fi: *Gelliodes fibulata*; Ha-am: *Haliclona* aff. *amboinensis*; Ha-br: *Haliclona* (*Soestella*) sp.; Hy-er: *Hyrtios erectus*; Ig-mi: *Igernella mirabilis*; La-he: *Lamellodysidea herbacea*; Le-sp: *Lendenfeldia* sp.; Ni-bl: *Niphates* sp.; Pa-ba: *Paratetilla* aff. *bacca*; Pe-ast: *Petrosia* aff. *strongylata*; Pe-co: *Petrosia corticata*; Pe-ho: *Petrosia hoeksemai*; Pe-ni: *Petrosia nigricans*; Pl-me: *Placospongia melobesioides*; St-ca: *Stelletta clavosa*

Discussion

In general, coastal coral reefs are being increasingly exposed to elevated nutrient and sediment loads. Terrestrial runoff is therefore a growing concern for many coral reefs across the globe and can, if unabated, lead to serious degradation (Fabricius 2005). Although the coral reefs of the Derawan Islands have always been subjected to fluctuating sedimentation rates originating from the Berau River, particle influx may have gradually increased in recent years due to intensified terrestrial runoff into the river following large-scale deforestation as a result of logging and forest fires (Siegert et al. 2001, Cleary 2003, Cleary & Mooers 2004). The combination of terrestrial-based pollution and other sources of disturbance such as blast fishing appear to have adversely affected the coral reefs of the Derawan Islands. Average live coral cover across sites and depths was only 28.9%, hardly what one would consider pristine. The combined mean cover of rubble and dead coral (41.6%) was in fact well above the mean of live coral cover. Only 4 of the 36 transects, furthermore, had more than 50% live coral cover and would thus fall into the 'good' category of Gomez & Yap (1988), whereas 15 of the 36 transects had less than 25% live coral cover and would be classified as 'poor'. Various and possibly different scenarios may be responsible for the high cover of rubble. On the more offshore islands, the rubble is almost certainly the result of illegal blast fishing, a nefarious practice that has shifted to more remote sites following increased policing of the more accessible reefs (Erdmann 1998). Inshore, in contrast, the rubble may be the remnant vestiges of coral reefs that died in the more distant past; the exact sources of disturbance that led to this demise remain unknown. The large cover of dead coral midshore and in more shallow reefs suggests a different mechanism. Among other things, this may be the result of severe coral bleaching (Brown & Suharsono 1990), a crown-of-thorns starfish outbreak (DeVantier & Done 2007), pollution such as chronic oil spills, or a combination of these factors. In NW Java, Cleary et al. (2008) also observed high dead coral cover offshore. They also noted that offshore live coral cover had dropped dramatically between surveys conducted there in 1985 and 1995. As is probably the case in the Berau region, they attributed this loss to a number of documented sources of environmental stress including a marked increase in the number of crown-of-thorns starfish observed during that time period. In the present study, the highest number of sponge species (between 45 and 57) was found at the offshore sites of Kakaban (KaS) and some inshore reefs (BeL, BeS, RaR) at both depth intervals. These results are in concordance with the high sponge cover at those locations. The inshore sites of BeL, BeS and RaR are under the direct influence of the Berau River, and were typified by low visibility (less than 6 m); these reefs were also covered by a fine layer of sand, mud and silt. Large coral colonies were scarce in these sites with only small patches of encrusting and massive corals present; the dense fields of branching and tabular corals characteristic of many offshore sites were virtually absent. The marked absence of branching and tabular corals such as *Montipora* spp. and *Acropora* spp. from inshore sites is in line with findings that these species are less resilient to environmental stress than other corals such as the massive *Porites* (Edinger & Risk 2000).

Environmental conditions in the Derawan Islands appeared to have a positive effect on filter-feeding heterotrophic benthic taxa, in contrast to corals. Not only did we observe the highest number of sponge species, including numerous records of unique species, at the inshore reefs, the sponge individuals also tended to be larger at these sites. In particular, the species *Echinodictyum mesenterinum*, *Ianthella basta*, *Iotrochota purpurea* and *Xestospongia testudinaria* attained larger sizes close to the river. This pattern was in marked contrast to other studies of the inshore sponge fauna in NW Java and SW Sulawesi (de Voogd et al. 2006, de Voogd & Cleary 2008). In both of these areas, the inshore sponge fauna was markedly depauperate compared to the offshore fauna, indicating that urban-related disturbances have had an overwhelming impact on all taxa of inshore reefs adjacent to the large cities of Jakarta and Makassar. The inshore reefs of the Derawan Islands had very low live coral cover, but the lack of a major conurbation and thus severe environmental stress has enabled other taxa to flourish and to a large extent occupy space that presumably was previously occupied by coral. In inshore sites close to Jakarta, for example, the majority of the substrate consisted of sand and turf algae (Cleary et al. 2008). The lowest number of sponge species was found at several sites at the eastern side of the offshore Maratua atoll. The eastern side of Maratua borders the Makassar Strait, and has a narrow reef crest with a well developed spur-and-groove zone in contrast to the wide reef on the western side (up to 300 m) (Tomascik et al. 1997). The eastern reef crest abruptly drops to several hundred meters and has a maximum visibility of more than 45 m. The reefs of the southeastern sides of Maratua have, however, been heavily damaged by blast fishing, and long patches of unstable coral rubble probably prevent recolonization of benthic taxa (Fox & Caldwell 2006). Some sites had very high rubble cover including MaE (almost 50% rubble) due to blast fishing, but this did not appear to have a pronounced effect on sponge composition. However, the sponges that we observed in these rubble fields were, in general, small and had the tendency to glue loose pieces of rubble together. These species may therefore play a hitherto undescribed, but important, role in consolidating the coral rubble and thus facilitating reef regeneration.

A total of 38 (22%) unique species (only found in a single transect) and a high number of singletons (26species) were observed indicating that actual diversity is higher than recorded. Many of these unique species and singletons are new records for Indonesia or have not yet been described. Van Soest (1989) showed that different geographic regions within the Indo-West Pacific all have some endemic species but are, in the complement of their common species, very similar. Indeed, many of 59 mentioned common species observed by van Soest (1989) at various localities were found in the Berau region. Within a sponge community, some species can be self-seeding and are important for maintaining the local sponge population, whereas others may act as a source for downstream regions. For example, *Amphimedon paraviridis* showed a more patchy distribution across the Derawan Islands and was sometimes locally abundant. This species was also very common in the Spermonde Archipelago, whereas it has only been sporadically observed from other regions within Indonesia. *Haliclona* aff. *amboinensis* had a pronounced preference for the more inshore sites, whereas *Petrosia nigricans* was found in most sites in moderate densities. The species *Stelletta clavosa* accounted for a high proportion of the total sponge abundance; however, this was largely due to very high abundances on 2 transects, at 5 and 10 m depth, near the island of Kakaban (KaS), where more than 500 individuals were recorded at both depth intervals. The southeastern side of Kakaban consists of a steep carbonate wall, where to the east the coral reef is interrupted by valleys of fine coral rubble overgrown with macroalgae (Renema 2006a,b). The fine coral rubble is highly unstable, and the small globular *S. clavosa* seems good at attaching to this substrate and as such is able to dominate the local sponge assemblage. Quantitative studies on sponges in the Indo-Pacific region remain rare. However, in the Dampier Archipelago, Fromont et al. (2006) observed pronounced spatial heterogeneity in species composition. Composition varied with environmental factors such as substrate type, aspect, substrate configuration and depth. Likewise, in the central Torres Strait, Duckworth & Wolff (2007) found strong variation in the composition of dictyoceratid sponges across small spatial scales. They concluded that these patterns were largely species-specific and were explained by localised disturbance events, differences in food availability and patterns of water transport affecting larval dispersal.

Space, abiotic environmental conditions and habitat all contributed to structuring sponge assemblages across the Derawan Islands. Both spatial and abiotic environmental variables, however, explained more variation than local habitat structure. The most important habitat structure variables were sand cover and encrusting coral cover. There appeared to be a clear effect of the river on the cover of sand, with sites closer to the river having a higher sand cover. The cover of encrusting corals and turf algae was, in contrast, patchier, while the cover of coral rubble was higher at the most inshore and offshore sites. Generally, sand dominated sites are associated with a low density and diversity of constituent species (Nakamura & Sano 2005, Carballo 2006). Sand cover in the Derawan Islands, however, did not exceed 17% at 5 m depth and 32% at 10 m depth, compared to a high of >90% for inshore reefs in Jakarta Bay (Cleary et al. 2008). The most important abiotic environmental variables were distance from the Berau River and water visibility. Depth proved to be a poor predictor of variation in composition, in contrast to expectations: in previous studies, the diversity of coral reef sponges increased with depth (Adjeroud 1997, Hooper & Kennedy 2002, de Voogd et al. 2006, Fromont et al. 2006). Lesser (2006) suggested that food supply and, therefore, bottom- up processes significantly influenced the distribution and abundance of sponges with increasing depth in coral reefs located in Florida, Belize and the Bahamas. In the present study, rarefied species richness did not vary much between the 2 depth intervals. Remarkably, at a depth of 5 m, sponge cover was higher at the sites closer to the river than further away from the river. Our results may be explained by the pronounced onshore–offshore gradient in water visibility; much less light, for example, reached inshore reefs at 5 m than reached offshore reefs at 10 m. In marine environments, there are often pronounced depth-related gradients in a number of environmental parameters, including current velocity and temperature, but one of the most biologically important parameters is the amount of photic energy, which generally decreases with depth.

Although our set of spatial, environmental and habitat structure variables were able to explain a significant amount of spatial variation in sponge composition, a large amount of variation remained unexplained. In addition to previously mentioned factors that may be operating at different spatial scales, there are a number of unmeasured sources of variation. Biotic processes such as predation and competitive interaction play an important role in the population dynamics and size structure of sponges on coral reefs (Duffy & Paul 1992, Aerts & van Soest 1997). In addition to biotic processes, large-scale oceanographic processes or local physical differences that change with depth, such as flow velocities, might also structure sponge assemblages (Wilkinson & Evans 1989, Lesser 2006). In conclusion, we found a highly significant relationship between the variation in sponge species composition and a set of spatial, environmental and habitat structure variables in the research area. Sponge diversity and abundance is notably high when compared to other surveyed coral reefs within the Indonesian Archipelago (van Soest 1989, Bell & Smith 2004, de Voogd et al. 2006, Cleary & de Voogd 2007).

Although disturbances, including riverine transport of sediments and nutrients inshore and blast fishing offshore, have adversely affected coral cover and composition, these disturbances do not appear to have had a seriously adverse effect on sponge diversity and composition. The distinct difference in the impact of disturbance on corals and other benthic taxa differs from that found in areas close to major conurbations and merits further study.

Acknowledgements

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Supplementary material

Sponge community composition in the Derawan Islands, NE Kalimantan, Indonesia
Systematic list of sponge species observed at the sampled locations in the the Derawan Islands.

Demospongiae
Homosclerophorida
Plakinidae
    Corticium niger Pulitzer-Finali, 1996
    Plakortis lita (de Laubenfels, 1954)
    Plakortis nigra Lévi, 1953
Spirophorida
Tetillidae
    Cinachyrella australiensis (Carter, 1886)
    Cinachyrella sp.
    Paratetilla merguiensis (Carter, 1883)
Astrophorida
Ancorinidae
    Dercitus (Halinastra) berau van Soest, Beglinger, de Voogd, 2010
    Jaspis splendens (de Laubenfels, 1954)
    Melophlus sarassinorum Thiele, 1899
    Penares sollasi *Thiele, 1900*
    Rhabdastrella globostellata (Carter, 1883)
    Stelletta clavosa *Ridley, 1884*
    Stelletta sp.
Geodiidae
    Erylus aff. *lendenfeldi* Sollas, 1888
Hadromerida
Clionaidae
    *Spheciospongia vagabunda (*Ridley, 1884)
Placospongiidae
    Placospongia melobesioides Gray, 1867
    Placospongia aff. *carinata (*Bowerbank, 1858)
Suberitidae
    Aaptos suberitioides Brønsted, 1934
    Suberites aff. *diversicolor* Becking & Lim, 2009
Tethyidae
    Tethya cf. *seychellensis* (Wright, 1881)
'Lithistid' Demospongiae
Theonellidae
    Theonella swinhoei Gray, 1868
    Theonella mirabilis (de Laubenfels, 1954)
Scleritodermidae
    Aciculites aff. *ciliate* (Wilson, 1925)
Poecilosclerida
Microcionina
Microcionidae

Clathria (Clathria) basilana Lévi, 1961
Clathria (Isociella) eccentrica (Burton, 1934)
Clathria (Thalysias) cervicornis (Thiele, 1903)
Clathria (Thalysias) reinwardti Vosmaer, 1880
*Clathria (Thalysias) vulpina (*Lamarck, 1813)
Clathria (Wilsonella) mixta Hentschel, 1912
Echinochalina (Protophlitaspongia) mucronata (Topsent, 1897)
Raspailiidae
Echinodictyum mesenterinum (Lamarck, 1814)
Endectyon (Endectyon) fruticosum aruense Hentschel, 1912
Thrinacophora cervicornis Ridley & Dendy, 1886
Rhabderemiidae
Rhabderemia forcipula (Lévi & Lévi, 1989)
Rhabderemia aff. *indica* Dendy, 1905
Myxillina
Chondropsidae
Chondropsis sp.
Coelosphaeridae
Lissodendoryx (Acanthodoryx) fibrosa (Lévi, 1961)
Crambeidae
Monanchora dianchora de Laubenfels, 1935
Iotrochotidae
Iotrochota baculifera Ridley, 1884
*Iotrochota purpurea (*Bowerbank, 1875)
Mycalina
Desmacellidae
Biemna aff. *humilis* Thiele, 1903
Biemna trirhaphis (Topsent, 1897)
Biemna / *Stelletta* sp.
Esperiopsidae
Ulosa aff. *ada* (de Laubenfels, 1954)
Mycalidae
Mycale (Naviculina) flagellifera Vacelet & Vasseur, 1971
Mycale (Arenochalina) aff. *euplectelloides* (Row, 1911)
Mycale (Mycale) vansoesti Calcinai, Cerrano, Totti, Romagnoli & Bavestrello, 2006
Podospongiidae
Diacarnus megaspinorhabdosa Kelly-Borges & Vacelet, 1995
Isodictydidae
Coelocarteria agglomerans Azzini, Calcinai & Pansini, 2007
Halichondrida
Axinellidae
Ptilocaulis aff. *fusiformis* Lévi, 1967
Dictyonellidae
Acanthella cavernosa Dendy, 1922
Acanthella aff. *cavernosa* Dendy, 1922
Dictyonella sp.
Liosina paradoxa Thiele, 1899

Stylissa carteri Dendy, 1889
Stylissa sp.
Halichondriidae
Axinyssa aff. *aplysinoides* Dendy, 1922
Axinyssa spp.
Halichondria cartilaginea Esper, 1794
Halichondria (Halichondria) sp.
Heteroxyidae
Didiscus sp.
Higginsia mixta Hentschel, 1912
*Myrmekioderma granulata (*Esper, 1794)
Agelasida
Agelasidae
Agelas ceylonica Dendy, 1905
Agelas aff. *nemoechinata* Hoshino, 1985
Agelas nakamurai Hoshino, 1985
Haplosclerida
Haplosclerina
Callyspongiidae
Callyspongia (Cladochalina) aerizusa Desqueyroux-Faundez, 1984
Callyspongia (Cladochalina) aff. *confoederata* Ridley, 1884
Callyspongia (Cladochalina) joubini (Topsent, 1897)
Callyspongia (Cladochalina) samarensis (Wilson, 1925)
Callyspongia (Cladochalina) aff. *subarmigera* (Ridley, 1884)
Callyspongia (Toxohalina) aff. *ramosa (*Gray, 1843)
Callyspongia (Euplacella) biru de Voogd, 2004
Callyspongia spp.
Chalinidae
Chalinula confusa (Dendy, 1922)
*Chalinula hooperi (*Bakus & Nishiyama 2000)
Cladocroce acuelata Pulitzer-Finali, 1982
Cladocroce sp.
Haliclona (Gellius) aff. *amboinensis* Lévi, 1959
Haliclona (Gellius) cymaeformis (Esper, 1794)
Haliclona (Gellius) sp.
Haliclona (Halichoclona) vanderlandi de Weerdt & van Soest, 2001
Haliclona (Reniera) fascigera Hentschel, 1912
Haliclona (Reniera) poseidon (de Laubenfels, 1954)
Haliclona (Soestella) sp.
Haliclona spp.
Niphatidae van Soest, 1980
Amphimedon paraviridis Fromont, 1988
Cribochalina sp.
Dasychalina fragilis Ridley & Dendy, 1886
Gelliodes fibulata (Carter, 1881)
Gelliodes aff. *hamata (*Thiele, 1903)
Gelliodes spp.

Niphates olemda (de Laubenfels, 1954)
Niphates spp.
Pachychalina sp. Schmidt, 1868

Petrosina

Phloeodictyidae
Aka sp.
Oceanapia sagittaria Sollas, 1902

Petrosiidae
*Acanthostrongylophora ingens (*Thiele, 1899)
*Neopetrosia exigua (*Kirkpatrick, 1900)
Neopetrosia aff. *carbonaria (*Lamarck, 1814)
Neopetrosia spp.
Petrosia (Petrosia) alfiani de Voogd & van Soest, 2002
Petrosia (Petrosia) hoeksemai de Voogd & van Soest, 2002
*Petrosia (Petrosia)*aff. *microxea* (Vacelet, Vasseur & Lévi, 1976)
Petrosia (Petrosia) nigricans Lindgren, 1897
Petrosia (Strongylophora) corticata (Wilson, 1925)
Petrosia (Strongylophora) aff. *strongylata* Thiele, 1903
Xestospongia aff. *mammillata* Pullitzer-Finali, 1982
Xestospongia vansoesti Bakus & Nishiyama, 2000
Xestospongia testudinaria (Lamarck, 1813)
Xestospongia spp.

Dictyoceratida

Irciniidae Gray, 1867
Ircinia ramosa Keller, 1889
Ircinia spp.

Thorectidae Bergquist, 1978
Thorectinae
Cacospongia mycofijiensis (Kakou,Crews & Bakus, 1987)
Dactylospongia elegans (Thiele, 1899)
Fascaplysinopsis reticulata (Hentschel, 1912)
Hyrtios erectus Keller, 1889
Hyrtios reticulatus Thiele, 1899
Hyrtios sp.
Luffariella variabilis (Polejaeff, 1884)
Semitaspongia sp.

Phyllospongiinae
Carteriospongia foliascens (Pallas, 1766)
Lendenfeldia sp.
Phyllospongia papyracea (Esper, 1806)
Phyllospongia aff. *lamellose (Esper, 1794)*
Strepsichordaia aliena Wilson, 1925

Spongiidae
Hippospongia sp.
Leiosella ramosa Bergquist, 1995
Spongia (Spongia) ceylonensis Dendy, 1905

Dysideidae

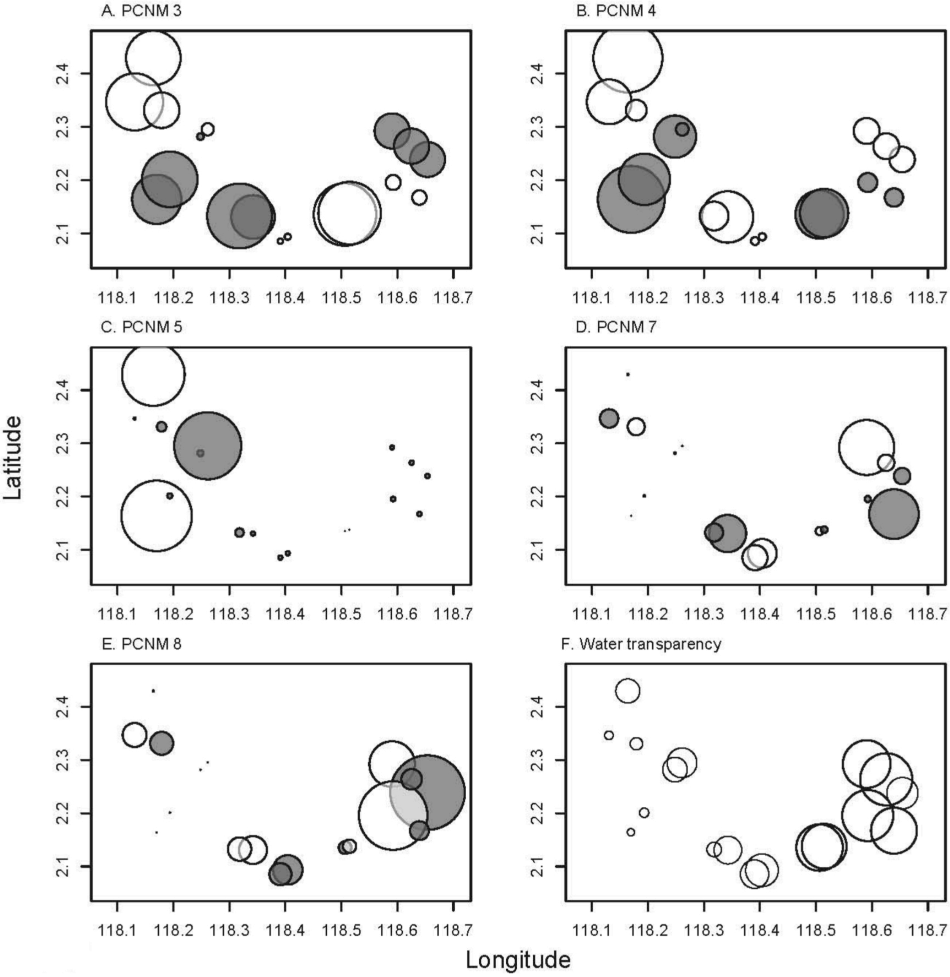

Fig. S1 Scores for (A) PCNM 3 (range in scores: -0.23 - 0.22, F = 1.587, P = 0.022, R^2_{adj} = 0.017), (B) PCNM 4 (range in scores: -0.24 – 0.25, F = 1.753, P = 0.005, R^2_{adj} = 0.021), (C) PCNM 5 (range in scores: -0.24 – 0.25, F = 1.617, P = 0.012, R^2_{adj} = 0.017), (D) PCNM 7 (range in scores: -0.04 – 0.05, F = 1.480, P = 0.038, R^2_{adj} = 0.014)), (E) PCNM 8 (range in scores: -0.03 – 0.03, F = 1.537, P = 0.013, R^2_{adj} = 0.016) and (F) water transparency/visibility (range in visibility: 6.5 – 46.5 m). For (A) to (E), clear symbols indicate positive values and shaded symbols negative values. The size of the symbol is proportional to the score. The size of the symbol is proportional to the score. Large clear symbols, therefore, have similar PCNM scores and very different scores from large shaded symbols. Note that the significant PCNM's represent intermediate scale variation.

Sponge species composition, abundance, and cover in marine lakes and coastal mangroves in Berau, Indonesia

Leontine E. Becking, Daniel F.R. Cleary, Nicole J. de Voogd

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Abstract

This is the first study to show that mangroves in the Indo-Pacific harbor a diverse array of sponge species. Our aim was to quantify variation in the species composition, abundance and cover of sponges in two marine lakes (Kakaban lake and Haji Buang lake) and adjacent coastal mangroves in the Berau region, East Kalimantan, Indonesia. We recorded a total of 115 sponge species, 33 of which were restricted to Kakaban lake, 18 to Haji Buang lake and 30 to coastal mangroves. Only 13 species were shared among all three locations. Assemblages of the two marine lakes at 10 km distance were more similar to each other than to the coastal mangrove systems at just 200-500 m distance. Our results show that marine lakes may represent a distinct habitat from coastal mangroves with significantly higher sponge cover and abundance as well as a markedly different species composition. In both lake and outer coastal mangrove habitats there was a pronounced gradient in composition away from the shore with the primary difference between hard (root or rock) and soft substrates (mud or sand). Each substrate type harbored different sets of species in both lake and outer mangrove habitats. Time was not a significant predictor of variation in sponge species composition, abundance and cover between semi-permanent transects sampled in 2008 and 2009. The isolated habitat of marine lakes with many new species to science warrants further study and conservation.

Keywords: mangrove roots • species assemblage • Principal Coordinates Analysis • Borneo • East Kalimantan • Kakaban • anchialine systems

Introduction

Mangrove forests grow pantropically on protected coasts and can host diverse colorful assemblages of epibionts (Farnsworth & Ellison 1996, Diaz et al. 2004). The vast majority of mangrove-epibiont studies have been conducted in the Caribbean where sponges together with algae, cnidarians and ascidians are the most dominant taxa covering mangrove roots (e.g. Ellison et al. 1996, Farnsworth & Ellison 1996, Diaz 2005). There is a facultative mutualism between sponges and the mangroves: mangroves provide the only hard substrate for sponges in the mangrove systems, and passively leak carbon from their roots that is assimilated by sponges, while sponges directly protect roots from isopod attack and enhance cable root growth by inducing adventitious rootlet formation (Ellison et al. 1996**.** Sponge communities in Caribbean mangrove systems are spatially heterogeneous, whereby a species can dominate one site, and be absent from another (Farnsworth & Ellison 1996, Diaz et al. 2004, Hunting et al. 2008, Guerra-Castro et al. 2011). Sponge communities in mangroves are, furthermore, distinct from those in reefs and contain species that appear to be specifically adapted to survive extremes in salinity, temperature and sedimentation (Wulff , Rützler 1995, Wulff 2000, Engel & Pawlik 2005, Wulff 2005b, Pawlik et al. 2007, Nagelkerken et al. 2008). Biotic factors such as predation and competition for space with other marine invertebrates likely limit their distribution in coral reefs (Nagelkerken et al. 2008, Pawlik 1997, Dunlap & Pawlik 1997, Aerts & van Soest 1997, de Voogd et al. 2004). Competition for space in the mangroves is, however, also intense as there is limited hard substrate which most sponges require to survive, favoring species with high growth rates and the production of possible allelochemicals to ward off spatial competitors (Engel & Pawlik, 2005; Wulff 2006, Nagelkerken et al. 2008). The composition of these epibiont communities can either be seasonally dynamic (Florida, Jamaica) or be stable over longer time periods (Belize, Venezuela) (Sutherland 1980, Bingham & Young 1995, Farnsworth & Ellison 1996, Wulff 2004, Pawlik et al. 2007).

Indonesia contains the most diverse mangrove ecosystems in the world covering an area of almost 50000 km^2 (Spalding et al. 1997, Alongi 2002), but to date there have been no comprehensive studies of mangrove epibionts. A recent review of the habitat function of mangroves for terrestrial and marine fauna suggested that the mangrove systems in the Indo-Pacific may not house the high diversity of sponges found in the Caribbean (Nagelkerken et al. 2008). Indeed, East African mangroves have a low diversity of sponges with just three species (Barnes 1999). Furthermore, two surveys of the faunal diversity in mangroves in the Indian Ocean and Malaysia did not record any sponge species associated with mangrove roots (Rützler 1964, Sasekumar & Chong 1998). There is, however, a relative paucity of information on sponges and mangroves in the Indo-Pacific, e.g. only three published papers in the Indo-Pacific referred to sponges and mangroves compared to over 40 for the Caribbean. In Indonesia, mangroves do not only occur along coasts, but also within marine lakes (Becking et al. 2011, *CHAPTER 1*). Marine lakes are anchialine systems, which are small bodies of landlocked seawater isolated to varying degrees from the surrounding marine environment (Holthuis 1973, Hamner & Hamner 1998, Colin 2009, Becking et al. 2011, *CHAPTER 1*). The marine character of these systems is maintained by subterranean tunnels, fissures, or small dissolution channels in the surrounding rock, connecting the lakes to the adjacent sea, and as such display a wide variety in the degree of connection to the sea and environmental regimes within the lakes (Hamner & Hamner 1998, Cerrano et al. 2006, Azzini et al. 2007, Becking et al. 2011, *CHAPTER 1*). The total number of marine lakes worldwide is estimated at only ~ 200 with clusters of ten or more lakes occurring in areas with a karstic limestone landscape such as Croatia, Bermuda, Vietnam, Palau, and Indonesia (Dawson et al. 2009). Preliminary surveys of macro-biota in marine lakes in Indonesia, Vietnam and Palau have shown that sponges, algae, ascidians and mollusks are the most dominant taxa in terms of abundance and diversity (Hoeksema 2004, Cerrano et al. 2006, Colin 2009, Becking et al. 2011, *CHAPTER 1*). This enclosed environment has set the stage for small, isolated, rapidly evolving populations, and possibly endemic (sub)species (Holthuis 1973, Maciolek 1983, Tomascik & Mah 1994, Fransen & Tomascik 1996, Massin & Tomascik 1996, Dawson & Hamner 2005). The present study is the first comprehensive assessment of sponges in marine lakes and mangroves in Indonesia. Our aim is to quantify spatial and temporal variation in the abundance and species composition of sponges in marine lakes and coastal mangroves in the Berau region, East Kalimantan, Indonesia. We relate variation in composition to the habitat (marine lake or coastal mangrove), substrate and sampling event. Our aims are to 1) assess if sponge composition in the marine lakes is distinct from that in the outer coastal mangroves, 2) identify species that are unique to each major habitat, 3) relate variation in sponge abundance, cover and species composition to substrate type and cover of other spatial competitors, and 4) determine if these sponge communities are stable over longer time periods (> 6 months).

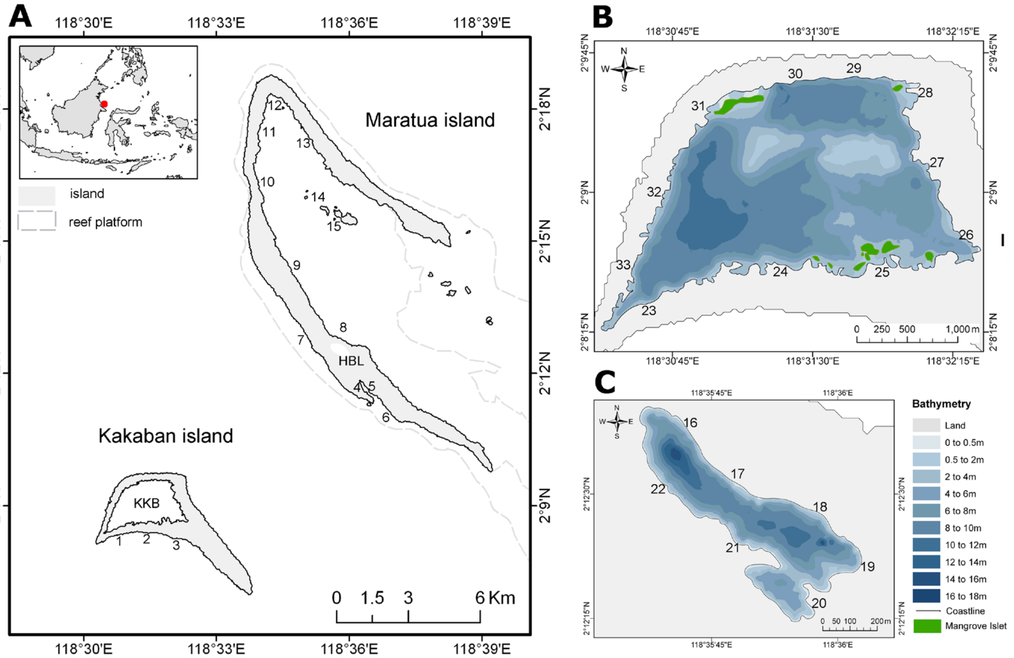

Figure 1 A Map of Kakaban and Maratua islands in Berau region, East Kalimantan Province, Indonesia, **B** bathymetry map of Kakaban lake, **C** bathymetry map of Haji Buang lake (note that scale differs between B and C). Sample site locations indicated with numbers 1-33. Sites 1, 2, 3, 6, 7, 10, 11, 12, and 14 in coastal mangroves contained no sponge epibionts.

Material & Methods

Study area

The comparison of sponge fauna was between mangroves within the marine lakes, and mangroves along the coast (and other substrata that co-occur in both habitats). We surveyed marine lakes and coastal mangroves of the islands Kakaban and Maratua in the Berau region, East Kalimantan Province (Fig. 1). These islands fall within the Berau Marine Protected Area and provide a setting to survey sponge assemblages at varying scales (metres to kilometres) in marine lakes and coastal mangroves (Tomascik et al. 1997, Becking et al. 2011, *CHAPTER 1*). The annual rainfall in Berau is approximately 2000 mm with no particular rainy season, though there is less rainfall June-September (average 110-125 mm per month) compared to the rest of the year (average 150-200 mm per month) and an increase of strong winds from the North between December-March (Tomascik et al. 1997, Mantel 2001, Renema 2006).

A general description of the lakes of Kakaban and Maratua islands is provided by Tomascik & Mah (1994), Tomascik et al (1997) and Becking et al. (2011). Kakaban island is a trapezoidal shaped island with a protected southern coast that is fringed by mangroves dominated by *Avicennia marina* with aerial roots exposed to air at low tide. In the centre of Kakaban island is a large, approximately 4 km^2, marine lake with the southern, western and eastern coasts fringed by mangroves dominated by *Bruguiera gymnorrhiza* with roots submerged in the water (Fig 2D); the northern coast is a predominantly rocky shore. The dominant algae in this lake are species of the genus *Halimeda.* Bathymetric surveys revealed that Kakaban lake averages 8 m depth, with a 12 meter deep depression at the western portion of the lake (Figure 1B). The tidal amplitude in Kakaban lake (19 cm) is dampened to 11% of adjacent sea amplitude (200 cm) and the tidal phase has a delay of 3 h 30 min compared that to the surrounding sea, indicating that there is little connection between the lake and the sea (Becking et al. 2011, *CHAPTER 1*).

Maratua is a horse-shoe shaped island that partly encloses a large lagoon of approximately 29.5 x 6.5 km with a depth of 0.5-5 m at low tide. Parts of the lagoon are rimmed by patches of *Avicennia marina, Sonneratia alba,* and *Rhizophora mucronata* mangroves with aerial- or proproots exposed to air at low tide (Fig. 2B). The mangrove transitions to a sandy reef flat away from the coast. On Maratua island there are at least nine anchialine systems, of which Haji Buang lake is the largest marine lake (Fig 2A). Haji Buang lake is an elongated lake of 0.14 km^2 surface area located on the western arm of Maratua. Haji Buang lake averages 10 m depth, with two deep depressions at the northern (19 m) and southern (15 m) end of the lake (Figure 1C). The largest part of the lake's coastline is formed by limestone rock with only a small patch of the southern coast fringed by mangroves (predominantly *B. gymnorrhiza*) with a seagrass field (*Enhalus* sp.) in front. The dominant algae in this lake are species of the genus *Caulerpa.* The tidal amplitude in the lake (90 cm) is dampened to 48% of the adjacent sea amplitude (200 cm) and the tidal phase had a delay of 2 h 30 min (Becking et al. 2011, *CHAPTER 1*), indicating that the connection to the sea is limited, but higher than in Kakaban lake. Coastal mangrove were patchy along the coasts of Maratua and Kakaban islands. In addition to this, sponge abundance varied greatly within the mangroves; a number of locations along the coast of Maratua and Kakaban did not contain a single sponge. Transects were laid down randomly in the coastal mangroves, but in 9 sites out of 15 we recorded no sponges (Fig. 1).

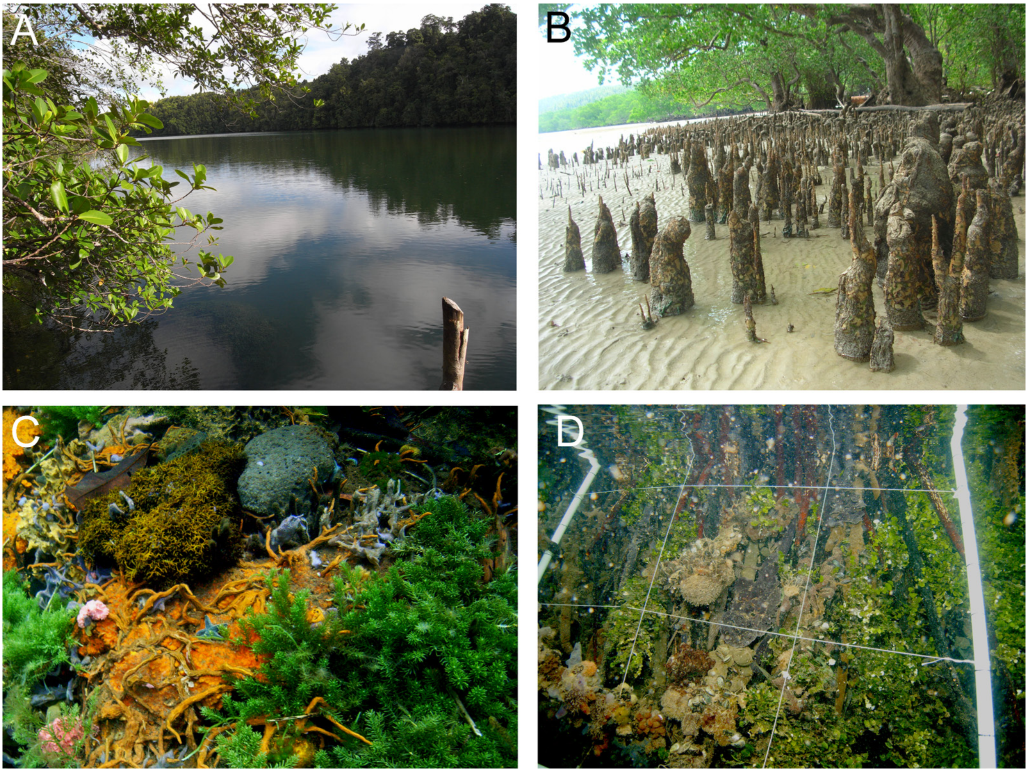

Figure 2 Images of the two habitats (marine lakes and outer coastal mangroves) in Berau, East Kalimantan, Indonesia. **A.** Haji Buang marine lake, **B.** coastal mangrove in Maratua bay with roots fully exposed to air and sun at low tide, **C.** Sponge and algae cover in in Haji Buang Lake, **D.** 'root-wall' and quadrat in mangroves of Kakaban lake.

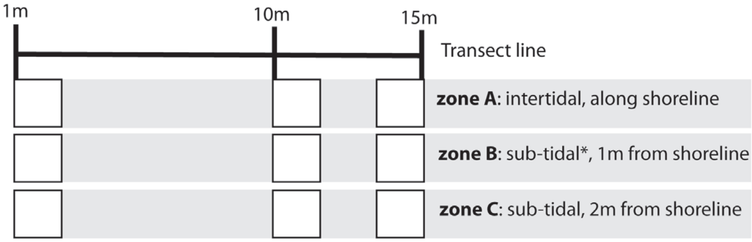

Figure 3 Sketch of experimental set-up. A 15 m transect line was placed along the shore, squares represent 1x1m quadrats that were placed along the transect and away from shore. Not to scale. *zone B was intertidal in coastal mangrove habitat.

Data collection

Transects

Study sites were randomly distributed within Kakaban lake, Haji Buang lake, and Maratua Bay (Fig. 1 & 2), within each site three transects were surveyed (Fig. 3). Some sites were eventually omitted from analysis because they contained no sponges. Likewise some transects were omitted because they contained less than 5 sponge individuals. We recorded data in semi-permanent transects in two periods: between 30 August – 20 September 2008 and 10 - 31 May 2009. The location of the transect was marked with biodegradable red tape and the coordinates were located with a handheld GPS (Garmin GPS60) with 5m variation. The roots of the mangroves in the lakes were so intertwined/meshed, that they created a 'wall-like' structure (Fig. 2D), which did not allow us to use the more common method of quantifying mangrove epibiont species richness and diversity by haphazardly selecting roots along a transect to be surveyed (e.g. Ellison & Farnsworth 1992, Farnsworth & Ellison 1996, Hunting et al. 2008). An alternative method was thus devised to quantify epibiont species abundance and composition on these 'root-walls' that enabled a consistent comparison with the variety of substrates that occur in the lakes and the coastal mangroves (e.g. rock-wall, sand, reef flat): a 15m transect was placed along the shore and three 1 m^2 quadrats were surveyed at the 1m, 10m and 15m mark, this was repeated three times away from the shore in order to determine if there was a zonation in species composition with distance from shore (Fig. 3). Zone A: quadrat on the shore within the tidal zone; zone B: 1m offshore, following the contour of the bottom or directly in front of the mangrove roots; Zone C: 2m offshore, following the contour of the bottom. The quadrats in Zone A were fully exposed to air at low tides in the coastal mangrove systems, were mostly (75-90%) exposed to air in Haji Buang lake, and were only partially (5-10%) exposed to air in Kakaban lake. The maximum depth of the quadrat was recorded, as well as the general substrate within the quadrat (root, rock, root & rock, sand, mud).The percentage cover within the quadrat was measured visually with the aid of 33x33 cm subdivisions of the quadrat. The following categories of cover were recorded: algae, ascidians, coral, mud, mussels, rock , root, sand, seagrass and sponges. These classes of higher taxonomic groups of associated organisms were selected based on preliminary data that showed that these groups were abundant in marine lakes (Hoeksema 2004, Cerrano et al. 2006, Colin 2009, Becking et al. 2011, *CHAPTER 1*) and potentially may affect the species composition, abundance and cover of sponges. Subsequently, sponge species and their abundance (number of individuals per species) were recorded within the 1 m^2 quadrats. Only sponge individuals larger than 1 cm^2 were included in the present study. We also recorded what species were exposed to air at low tide.

Sponge species were visually identified in the field. Of all species two or more vouchers were collected for closer examination of skeletal structure for taxonomic identification. Sponges were identified based on spicule characters and skeletal structure by L.E. Becking and N. J. de Voogd, preserved in 70% ethanol and deposited in the Porifera collection of the Naturalis Biodiversity Center (catalogued as RMNH POR). Ideally, the specimens were identified to species level, however many of the surveyed sponges belonged to novel or little known species and rare genera, so these were preliminary assigned to morphospecies awaiting formal description. Some sponges consisted of a species complex and could not be separated visually in the field; these were grouped together such as *Placospongia* spp (consisted of *P. carinata*, *P.melobesioides* and *P. mixta* (CHAPTER 5) and *Cinachyrella* spp (consisted of *C. porosa*, *C.australiensis*, *C.paterifera* (Santodomingo & Becking *in prep*).

The salinity, temperature and pH was measured twice in each location at each transect in both sampling periods with a handheld multimeter YSI63-50. Where possible, measurements were made at the surface, 1m and 2m depth.

Data analysis

Abbreviations used for locations: KKB - Kakaban lake, HBL - Haji Buang lake, BAY - coastal mangrove habitat on Maratua island.

Sponge composition

Data matrices of species abundance per transect were $\log_{10}(x + 1)$ transformed and distance matrices constructed using the Bray-Curtis index with the *vegdist* function in the *vegan* package in R (Oksanen et al. 2009). The Bray-Curtis index is one of the most frequently applied (dis)similarity indices used in ecology (Legendre & Gallagher 2001, Cleary 2003, Cleary et al. 2005, de Voogd et al. 2006). Matrices were constructed for 1. all transects and 2. all transects within each location (KKB, HBL and BAY) separately. Transects with five or fewer individuals (24 out of 153 transects) were removed prior to analysis. Of the 15 transects dropped, 12 were in Kakaban and 3 in Maratua; 14 of the transects were, furthermore, on a mud substrate and one on a rock substrate. After removal, 60, 33 and 36 transects from KKB, HBL and BAY (respectively) remained for the analyses. Variation in sponge species composition among location class groups (KKB-mud, KKB-root, KKB-rock, HBL-mud, HBL-root, HBL-rock, BAY-reef, BAY-root, BAY-sand) and sample year was assessed with Principal Coordinates Analysis (PCO) using the *cmdscale* function in R with the Bray-Curtis distance matrix as input. Variation among location and year was tested for significance using the *adonis* function in vegan. In the *adonis* analysis, the Bray-Curtis distance matrix of species composition was the response variable with location and sample year as independent variables. We also included the interaction term between location and year. The number of permutations was set at 999; all other arguments used the default values set in the function. For the analyses within each habitat, we followed the above analytical approach but used the *adonis* analysis to test for significant variation in substrate (mud, rock and root for KKB and HBL; reef, rock and root for BAY) and sample year. We also included the interaction term between substrate and year. The *adonis* function is an analysis of variance with distance matrices using permutations that partitions distance matrices among sources of variation; in this case location class and sampling year. In addition to the above, we also tested for associations between species composition and sample depth and measured substrate class variables, namely the percentage cover of mussels, algae, soft substrate (mud and sand), rock, mangrove roots, seagrass, ascidians, corals and sponges. These were fit onto PCO ordinations of each habitat with the *envfit* function in vegan. Using the *envfit* function, we also tested for significant relationships between these variables and PCO ordinations of sponge composition using 999 permutations; all other arguments in the function were left as default. The contribution of sponge species to the ordinations was assessed using weighted averages scores for the first two axes of each ordination with the *wascores* function in the vegan package. Detailed descriptions of the functions used here can be found in R (e.g., *?cmdscale*) and online in the reference manuals (e.g. http://cran.r-project.org/web/packages/vegan/index.html; checked 2011 04 08).

In addition to environment and time, spatial processes may also play an important role structuring assemblages. We tested for an effect of space on species composition using principal coordinates of neighbor matrices (PCNM), a novel method for quantifying spatial trends across a range of scales. PCNM is based on eigenvalue decomposition of a truncated matrix of geographic distances among sampling sites (Borcard & Legendre 2002, Dray et al. 2006). Significant PCNM eigenvectors were selected using the PCNM function with 999 permutations in the PCNM package in R. Additional arguments for the PCNM function included Hellinger transformed matrices of species abundance and distance matrices (longitude, latitude); analyses were run for each location (KKB, HBL and BAY) separately. The forward selection test used was based on a novel forward selection procedure that corrects for the inflated Type I error and overestimation of explained variance associated with classical forward selection (Blanchet et al. 2008). The PCNM package is freely available at the website of Pierre Legendre (http://www.bio.umontreal.ca/legendre/indexEn.html, last checked: 20-09-2011)

Sponge abundance and cover

After testing for normality with the *shapiro.test* function in R (all tests deviated significantly ($P < 0.001$) from a normal distribution), we tested for significant differences in sponge abundance and cover at the transect level among substrate type (mud, rock and root for KKB and HBL and reef, rock and root for BAY) and sampling year within locations (KKB, HBL and BAY) with 'distribution free' *Adonis* tests using the *adonis* function in vegan (Oksanen et al. 2009). In the *adonis* analysis, the Euclidean distance matrix of sponge abundance or sponge cover was the response variable with substrate type and sample year (2008 or 2009) as independent variables. We also included the interaction term between substrate type and depth. The number of permutations was set at 999; all other arguments used the default values set in the function.

Table 1 Recorded species in marine lakes and mangroves in East Kalimantan, Indonesia. Majority of marine lake species are undescribed, therefore many species are denoted with ‘sp.’ or ‘aff.’. Given are the species codes that are used in the methods and results section, whether a species was recorded exposed to air (exp.), the occurrence on substrate type (root, rock, sand, mud, reef), and the occurrence in the three locations of this study Kakaban lake (KKB), Haji Buang lake (HBL), and the costal mangroves of Maratua island (BAY). See appendix 1 for images of marine lakes species.

code	species	exp	substrate	KKB	HBL	BAY
	ASTROPHORIDA					
	Ancorinidae					
St-sp	*Stelletta* sp. "icing"	no	root/rock	•	•	
	Chondrillidae					
Ch-ch	*Chondrilla* aff. *chucala*	yes	rock	•	•	
Ch-bl	*Chondrilla australiensis*	yes	root/rock	•	•	•
	Geodiidae					
Ge-bl	*Geodia* sp. "black"	yes	root/rock	•		
Ge-ch	*Geodia* sp. "chimney"	yes	rock		•	
Ge-ye	*Geodia* sp. "yellow"	yes	root/rock	•		
	CLATHRINIDA					
	Clathrinidae					
Cl-wh	*Clathrina* sp. "white"	no	root/rock	•	•	
Cl-ye	*Clathrina* sp. "yellow"	no	rock	•		
	DENDROCERATIDA					
	Darwinellidae					
Da-sp	*Darwinella* aff. *gardineri*	yes	root/rock	•	•	
De-bl	*Dendrilla* sp. "black"	no	root	•	•	
De-pa	*Dendrilla* sp. "pale"	no	root	•		
De-pu	*Dendrilla* sp. "purple"	no	root	•		
	Dictyodendrillidae					
Ac-bu	*Acanthodendrilla*sp. "burgandy"	no	root	•		
Ch-go	*Chelonaplysilla*sp. "gold"	no	root	•		
Sa-gr	*Spongionella* sp. "green"	no	root	•	•	
Sa-pu	*Spongionella*sp. "purple"	no	root	•	•	
	DICTYOCERATIDA					
	Dysideidae					
Dy-bl	*Dysidea* sp. "blue"	yes	root/rock	•	•	
Dy-ta	*Dysidea*sp. "tan"	no	root/rock	•		
Dy-wh	*Dysidea*sp. "white"	no	root/rock	•	•	
La-bl	*Lamellodysidea*	yes	root/rock	•		
La-he	*Lamellodysidea herbacea*	yes	rock/sand			•
	Irciniidae					
Ir-sp	*Ircinia ramosa*	yes	rock/sand			•
	Spongiidae					
Sp-br	*Spongia* sp. "black"	no	rock	•		

code	species	exp	substrate	KKB	HBL	BAY
Sp-sh	*Spongia* sp. "shiny"	no	rock		•	
Sp-fs	*Spongia* sp. "fistules"	yes	rock	•		
Sp-do	*Spongia ceylonensis*	yes	sand/reef			•
	Thorectidae					
Ca-bl	*Cacospongia* sp. "shiny"	yes	root/rock	•		
Se-ho	*Cacospongia*sp. "honeycomb"	no	rock		•	
Se-sp	*Semitaspongia* sp. "black"	no	root	•		
Sm-sp	*Smenospongia* sp. "beige"	yes	root/rock		•	
	HADROMERIDA					
	Clionaidae					
Sp-pe	*Cliona peleia*	yes	rock	•	•	•
Cl-or	*Cliona* sp. orange"	no	rock	•	•	
Sc-va	*Spheciospongia vagabunda*	yes	sand/reef			•
	Placospongiidae					
Pl-cx	*Placospongia* spp.	yes	root/rock/mud/reef	•	•	•
	Spirastrellidae					
Si-de	*Spirastrella* aff. *decumbens*	yes	root/rock	•	•	•
Si-gr	*Spirastrella* aff. *solida*	yes	root	•		
Si-or	*Spirastrella* sp. "orange"	yes	root/rock	•		
Si-tu	*Spirastrella* sp. "tubes"	yes	rock		•	
	Suberitidae					
Ps-sp	*Pseudosuberites andrewsi*	yes	rock		•	
Su-di	*Suberites diversicolor*	yes	root/rock	•	•	•
	Tethyidae					
He-sp	*Laxotethya* sp. "spaghetti"	no	rock/mud	•		
Te-as	*Tethytimea*aff. *tylota*	yes	root/rock	•	•	
Te-bu	*Tethya* aff. *bulae*	yes	root/rock	•		
Te-co	*Tethya* aff. *coccinae*	yes	root/rock	•	•	•
Te-se	*Tethya* aff. *sechellensis*	yes	root/rock	•		•
Ti-ye	*Timea* sp. "yellow"	no	root	•	•	
	HALICHONDRIDA					
	Dictyonellidae					
St-ca	*Stylissa carteri*	yes	root/rock/sand	•	•	•
St-ma	*Stylissa massa*	yes	root/rock/sand			•
	Halichondriidae					
Am-gc	*Amorphinopsis*sp.	yes	root			•
Ax-pi	*Axinyssa mertoni*	yes	root/rock/mud	•	•	•

code	species	exp	substrate	KKB	HBL	BAY
Ax-or	*Axinyssa* sp. "orange"	no	rock	•		
Ha-tg	*Halichondia*sp. "green"	yes	sand/reef			•
Ha-cr	*Halichondria* sp. "crown"	no	mud	•		
Ha-or	*Halichondria* sp. "orange"	no	root			•
Sp-ye	*Hymeniacidon* sp. "yellow"	yes	root/rock	•	•	
	Heteroxyidae					
Hi-mi	*Higginsia* mixta	yes	root/rock	•	•	•
Hi-en	*Higginsia* sp. "encrust"	yes	root/rock	•	•	
My-gr	*Myrmekioderma granulata*	yes	root/rock	•	•	
	HAPLOSCLERIDA					
	Callyspongiidae					
Ha-pp	*Callyspongia diffusa*	no	root/rock/reef			•
	Chalinidae					
Ch-co	*Chalinula confusa*	yes	sand/reef			•
Cl-bu	*Cladocroce burapha*	yes	reef/rock			•
Ha-pi	*Haliclona* aff. *baeri*	yes	root/rock	•	•	•
Ha-am	*Haliclona amboinensis*	yes	sand/reef			•
Ha-bc	*Haliclona* sp. "blue crumble"	yes	root			•
Ha-sl	*Haliclona* sp. "blue slimy"	yes	reef			•
Ha-bk	*Haliclona* sp. "blue soft branch"	no	root/rock	•		
Ha-bs	*Haliclona* sp. "blue sticky"	yes	root	•		•
Ha-wk	*Haliclona* sp. "blue-white cloud"	yes	root/rock	•		
Ha-bm	*Haliclona*sp. "blue branch"	yes	root/rock		•	
Ha-xs	*Haliclona* sp. "bordeaux soft"	yes	root			•
Ha-bo	*Haliclona* sp. "bordeaux"	yes	root	•		
Ha-bb	*Haliclona* sp. "brown branch"	no	mud	•		
Ha-pa	*Haliclona* sp. "pale beige"	yes	rock		•	
Ha-pc	*Haliclona* sp. "pink cloud"	no	root	•		
Ha-pr	*Haliclona* sp. "purple branch"	no	root/rock		•	
Ha-pb	*Haliclona* sp. "purple soft branch"	yes	rock		•	
Ha-pt	*Haliclona* sp. "purple tough"	yes	root			•
Ha-wo	*Haliclona* sp. "purple-white"	yes	rock		•	
Ha-py	*Haliclona* sp. "purple-yellow"	no	root/rock	•		
Ha-sp	*Haliclona* sp. "soft spikes"	no	root/rock	•	•	
Ha-k2	*Haliclona* sp. "spicky"	no	rock	•		
Ha-vb	*Haliclona* sp. "violet branch"	no	root/rock	•		
Ha-vc	*Haliclona* sp. "violet cloud"	no	root/rock	•	•	
Ha-vs	*Haliclona* sp. "violet soft"	yes	root/rock		•	
Ha-vt	*Haliclona* sp. "violet tube"	yes	root	•		
Ha-vv	*Haliclona* sp. "violet vase"	yes	root		•	
Ha-wm	*Haliclona* sp. "white cloud"	no	root/rock		•	
Ha-we	*Haliclona* sp. "white extentions"	yes	root/rock	•		

code	species	exp	substrate	KKB	HBL	BAY
Ha-rh	*Haliclona* sp. "yellow branch"	yes	root/rock	•	•	
Ha-ym	*Haliclona* sp. "yellow cloud"	yes	root/rock		•	
Ha-yk	*Haliclona* sp. "yellow"	yes	root/rock	•		
Ha-lo	*Haliclona* sp." white"	no	root/sand			•
	Niphatidae					
Am-br	*Amphimedon queenslandica*	yes	root/rock/sand			•
Am-pa	*Amphimedon paraviridis*	no	reef			•
Am-gr	*Amphimedon* sp. "blue-green"	yes	root			•
	Petrosiidae					
Ha-ca	*Neopetrosia* aff. *carbonaria*	yes	reef/rock			•
Ne-ex	*Neopetrosia exigua*	yes	reef/rock			•
Ha-bt	*Neopetrosia* sp. *blue*	yes	root			•
	Phloeodictyidae					
Ak-mu	*Aka mucosa*	yes	sand			•
Oc-wh	*Oceanapia*sp. "white"	yes	sand			•
	POECILOSCLERIDA					
	Acarnidae					
Ac-sp	*Acarnus* sp.	no	root/rock		•	
	Coelosphaeridae					
Bi-fo	*Biemna fortis*	yes	root/mud/sand	•	•	•
Li-sp	*Lissodendoryx* aff. *similis*	yes	root/rock		•	
	Iotrochotidae					
Io-ba	*Iotrochota baculifera*	yes	reef			•
	Isodictyidae					
Co-si	*Coelocarteria singaporensis*	yes	sand			•
	Microcionidae					
An-th	*Antho ridleyi*	no	root/rock	•		
Cl-re	*Clathria reinwardti*	yes	rock/reef/sand			•
	Raspailiidae					
Ah-sp	*Acanthostylotella cornuta*	yes	rock		•	
Ec-sl	*Echinodictyum conulosum*	yes	rock/reef/sand			•
Ra-bl	*Raspailia* aff. *bifurcata*	yes	rock/sand		•	•
Ra-ye	*Raspailia* aff. *clathrata*	no	mud	•		
	Tedaniidae					
Te-re	*Tedania diraphis*	yes	root/rock			•
	SPIROPHORIDA					
	Tetillidae					
Ci-au	*Cinachyrella* spp.	yes	root/rock/mud/sand/reef	•	•	•
Pa-cx	*Paratetilla bacca*	yes	root/rock/mud/sand/reef	•	•	•
	VERONGIDA					
	Ianthellidae					
He-in	*Hexadella*aff. *indica*	no	root/rock	•		

Results

Abbreviations used for locations: KKB - Kakaban lake, HBL - Haji Buang lake, BAY - coastal mangrove habitat on Maratua island. Abbreviations used for species are indicated in Table 1. The salinity, temperature and pH did not differ between sampling periods or between transects within a location. The range of environmental variables per location is indicated in Table 2. A total of nine sites in the coastal mangrove system did not contain epibionts: Sites 1-3 (on Kakaban island) 6, 7, 10, 11, 12, and 14 (on Maratua island) (Figure 1A). These were not included in the analyses.

Table 2 Dominant mangrove genera and range in salinity, temperature and pH in Kakaban lake (KKB), Haji Buang lake (HBL) and the coastal mangroves of Maratua island (BAY). Measurements were taken in September 2008 and May 2009.

Lake	Salinity (ppt)	Temp. (°C)	pH	Mangroves
KKB	23-24	29-32	7.0-7.8	*Bruguiera*
HBL	26-28	29-30	7.3-7.8	*Bruguiera*
BAY	33-34	28-32	7.8-8.3	*Avicennia, Rhizophora, Sonneratia*

Sponge composition

10167 sponge individuals were recorded, belonging to 115 species, 10 orders, 32 families and 52 genera (Table 1). A total of 67 species were recorded in KKB, 53 species in HBL, and 47 in BAY. A total of 33 species were restricted to KKB, 18 to HBL and 30 to BAY. The two lakes (KKB & HBL) shared 18 species; only 13 species were recorded across all three locations. The lake habitat (KKB & HBL combined) harbored 85 species, 16 of which were shared with the coastal mangrove habitat (Table 1).

Sponge species composition differed significantly among locations (*adonis* $F_{2, 123} = 34.74$, $P < 0.001$, $R^2 = 0.359$) but not between years (*adonis* $F_{1, 123} = 0.40$, $P = 0.96$). There was no significant interaction between location and year (*adonis* $F_{2, 123} = 0.20$, $P = 1.000$). Each location formed a distinct cluster (Fig. 4A), but the two lakes were clearly more similar to one other than to the mangroves (Fig. 4B). The lake transects on a mud substrate resembled the coastal transects more closely than the other transects (Fig. 4A). There was also some temporal variation in species composition as indicated by the vectors, particularly in lake habitats, although this differed among transects (Fig. 4A).

In all three locations, there was a significant difference in species composition among substrate types (adonis; KKB: $F_{2, 54} = 8.58$, $P < 0.001$, R^2 2= 0.236; HBL: $F_{2, 27} = 15.31$, $P < 0.001$, $R^2$2 = 0.514; BAY: $F_{2, 30} = 13.96$, $P < 0.001$, R^2 2 = 0.481), but no significant difference between years (adonis; KKB: $F_{1, 54} = 0.62$, $P = 0.776$; HBL: $F_{1, 27} = 0.63$, $P = 0.690$; BAY: $F_{1, 30} = 0.05$, $P = 0.999$). There was also no significant interaction (adonis; KKB: $F_{2, 54} = 0.49$, $P = 0.955$; HBL: $F_{2, 27} = 0.63$, $P = 0.791$; BAY: $F_{2, 30} = 0.03$, $P = 1.000$). There was also no significant effect of spatial processes on species composition based on results of the PCNM analyses (all $P > 0.10$).

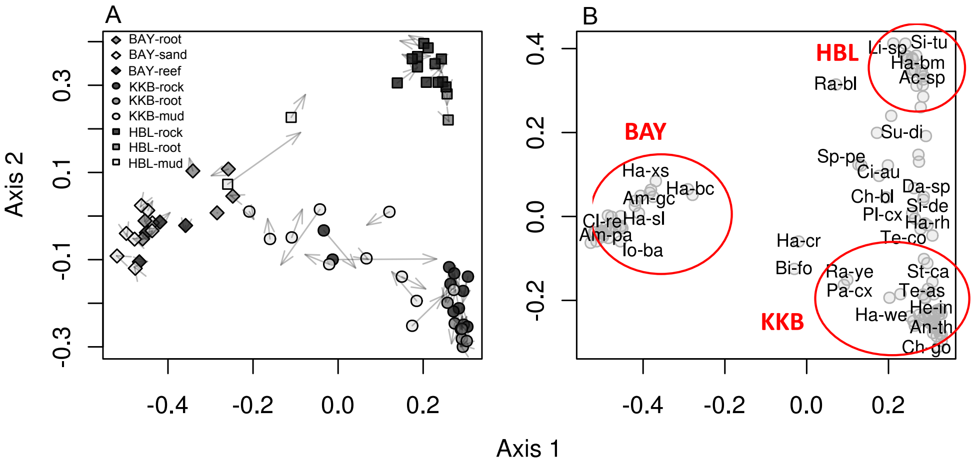

Figure 4 Ordination scores of transects (**A** and Weighted Averages scores of sponge species (**B**) along the first and second Principal Coordinates Analysis (PCO) axes representing variation in sponge community composition among locations (KKB, HBL and BAY) and substrate types (mud, rock, root, reef and sand). Arrows in **A** represent temporal variation in composition from transects sampled in 2008 (symbols) to the same transects sampled in 2009 (arrow heads). In **B** Species are indicated by dots; selected species are indicated by codes following Table 1. The principal axis of variation represents variation from transects in marine lake habitats (KKB and HBL) to transects in coastal mangroves. The secondary axis of variation (axis 2) is primarily related to variation between lake habitats. Note that mud transects from both lakes tend to exhibit the greatest similarity in composition with mangrove transects. Weighted Averages scores of sponge species was estimated using the *wascores* function of the vegan library in R and based on the first two PCO ordination axes.

In KKB, the main gradient in composition was related to variation between transects on a mud substrate at greater depth and transects on a hard substrate with high mussel and ascidian cover (Fig. 5A and B). Species associated with a mud substrate include *Raspaillia* aff. *clathrata*, *Halichondria* sp. “crown”, and *Higginsia mixta*. Species mainly associated with rock and root substrates include *Placospongia* spp., *Cinachyrella* spp., *Cliona peleia, Tethytimea* aff. *tylota*. Variation in HBL was primarily related to differences between transects on mud versus hard (root and rock) substrates and secondarily between rock substrate with higher ascidian cover and root substrate with higher mussel cover (Fig. 5C and D). *Biemna fortis* was the most prevalent species on a mud substrate. Species strongly associated with a rock substrate included *Semitaspongia* sp., *Cliona peleia* and *Antho ridleyi*, *Tethytimea* aff, *tylota*. Species strongly associated with a root substrate included *Spongionella* sp. “green”, and *Haliclona* spp.. In BAY, the main gradient in composition was related to variation from transects on a root substrate in shallow water to transects in deeper water on a sand or reef substrate (Fig. 5E and F). Species mainly associated with a root substrate included *Tethya* aff. *coccinae, Haliclona* spp. and *Amorphinopsis* sp.. There was a secondary gradient related to transects with higher coral cover versus transects with higher sand and rock cover. Species more strongly associated with higher coral cover included *Placospongia* spp., *Iotrochota baculifera and Amphimedon paraviridis,* whereas species more strongly associated with higher sand and rock cover included *Axinyssa mertoni, Biemna fortis, Cliona peleia, Cinachyrella* spp..

Sponge abundance and cover

Sponge abundance differed significantly among substrate types in the marine lake habitat but not in the coastal mangrove habitat (adonis; KKB abundance: $F_{2,54} = 28.70$, $P < 0.001$, $R^2 = 0.502$; HBL abundance $F_{2,26} = 21.64$, $P < 0.001$, $R^2 = 0.590$; BAY abundance: $F_{2,30} = 2.33$, $P = 0.129$, $R^2 = 0.134$;). Sponge cover also differed significantly among substrate types in the marine lake habitat but again not in the coastal mangrove habitat (adonis; KKB cover: $F_{2, 54} = 43.89$, $P < 0.001$, $R^2 = 0.617$; HBL cover $F_{2, 26} = 10.28$, $P < 0.001$, $R^2 = 0.429$; BAY cover: $F_{2, 30} = 1.65$, $P = 0.214$, $R^2 = 0.098$). Sponge abundance and cover was highest on root and rock substrates in the marine lake habitat and was lowest on mud substrate (Fig. 6). There was no significant difference in abundance between years in any habitat (adonis; KKB abundance: $F_{1, 54} = 1.18$, $P = 0.252$, $R^2 = 0.010$; HBL abundance $F_{1, 26} = 2.48$, $P = 0.132$, $R^2 = 0.034$; BAY abundance: $F_{1, 30} = 0.00$, $P = 1.000$, $R^2 = 0.000$; BAY cover: $F_{1, 30} = 0.18$, $P = 0.660$, $R^2 = 0.010$). There were also no significant interactions (adonis; KKB abundance: $F_{2, 54} = 0.84$, $P = 0.419$, $R^2 = 0.015$; HBL abundance $F_{2, 26} = 0.30$, $P = 0.738$, $R^2 = 0.008$; BAY abundance: $F_{2, 30} = 0.04$, $P = 0.960$, $R^2 = 0.002$). There was also no significant difference in cover between years in any habitat (adonis; KKB cover: $F_{1, 54} = 0.46$, $P = 0.511$, $R^2 = 0.003$; HBL cover $F_{1, 26} = 0.36$, $P = 0.557$, $R^2 = 0.008$; BAY cover: $F_{2, 30} = 0.18$, $P = 0.845$, $R^2 = 0.010$).

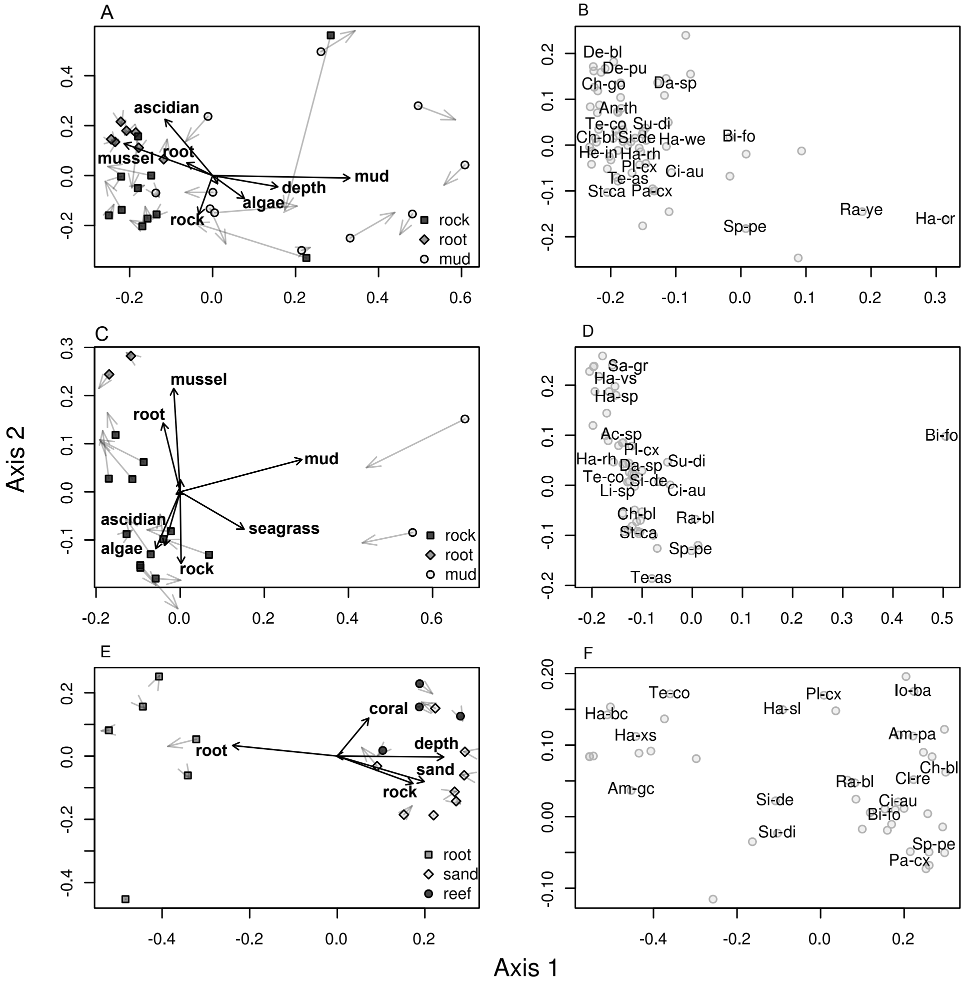

Figure 5 Ordination scores of transects and Weighted Averages scores of sponge species along the first and second Principal Coordinates Analysis (PCO) axes for Kakaban lake (**A, B**) Haji Buang lake (**C, D**) and coastal mangroves (**E, F**). Transects on different substrate types (mud, rock, root, reef and sand) are indicated by symbols. Codes in B, D and F represent sponge species following Table 1. Grey arrows represent temporal variation in composition from transects sampled in 2008 (symbols) to the same transects sampled in 2009 (arrow heads of grey arrows). Black arrows are vectors of environmental variables (cover of ascidians, mussels, mud, seagrass, bare rock, bare root, and coral in transect, depth of transect) representing maximal correlations with the ordination configuration obtained using the *envfit* function in the vegan library in R. The principal axis of variation (axis 1) represents variation among solid and soft substrate.

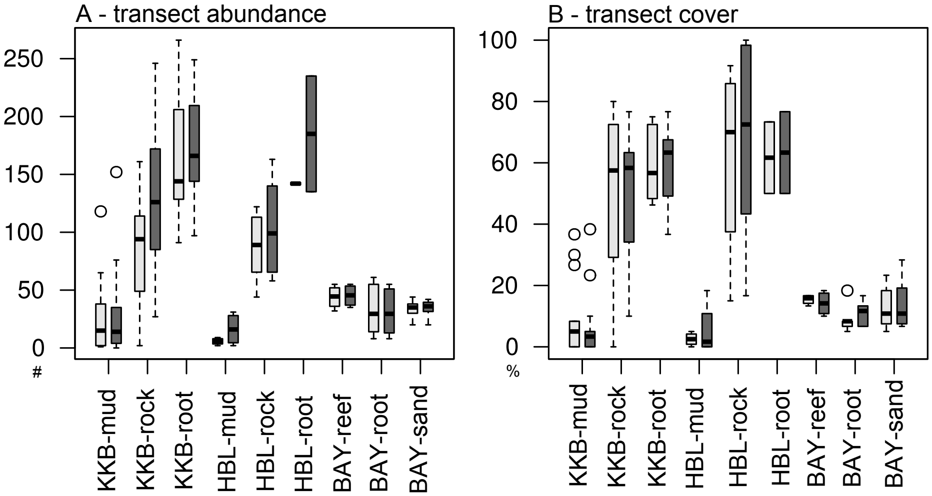

Figure 6 Box-and-whisker plots of **A** the abundance and **B** the cover of sponges across locations (KKB, HBL and BAY) and substrate types (mud, rock, root, reef and sand). Light grey represents data from 2008, dark grey from 2009. The thick black line intersecting each box-plot represents the median values. The boxplot hinges are versions of the first and third quartiles. Open circles represent outliers and the upper and lower whiskers represent the most extreme data point given that it is no more than 1.5 times the interquartile range from the box (graph created using the default values for the *boxplot* function in R)

Discussion

Differences between habitats

The marine lakes are true sponge gardens containing distinct assemblages of sponge species. The lakes displayed a markedly higher abundance and cover of sponges compared to the outer coastal mangrove habitat surrounding the islands containing the marine lakes (Figs. 2 & 5). Transects in the lakes could harbor great densities of sponges more than 200 individuals in an area of only 3 m^2, occupying at times up to 100% of available substrate. These abundances are comparable to the amounts recorded in areas 10 times as large (30 m^2) in the adjacent reefs in Berau (de Voogd et al. 2009). The marine lake habitat provides a continuously sheltered and submerged environment with large sponges, while in contrast the coastal mangrove habitat is more exposed to waves and air at low tide, harboring smaller and fewer sponges (Figs. 2 & 5). Sponges were patchily distributed in the coastal mangrove system where some locations along the coast of Maratua and Kakaban did not contain a single sponge. This patchiness has frequently been noted in Caribbean mangroves and has been attributed in part to biotic factors, but mostly to abiotic environmental factors (e.g. Ellison & Farnsworth 1992, Farnsworth & Ellison 1996, Diaz et al. 2004, Wulff 2005a, Hunting et al. 2008, Guerra-Castro et al. 2011). Exposure to currents was, for example, the most reliable predictor of percentage cover and diversity of species in Belize mangroves (Farnsworth & Ellison 1996). Roots on protected coasts are typically dominated by massive sponges, ascidians, sea anemones, and fleshy algae, while the high energy windward coasts are generally dominated by hydroids and filamentous algae with no sponges (Farnsworth & Ellison 1996).

The three locations of this study (Kakaban lake, Haji Buang lake and the coastal mangroves of Maratua island) each had distinct assemblages (Fig. 4). The two marine lakes at 10 km distance, were, however, more similar to each other than to the coastal mangrove systems at just 200-500 m distance. The marine lakes harbored only a subset of the adjacent sea flora and fauna. The difference in species composition between different lakes and mangroves can for a large part be attributed to both the nature of the barrier between the lake and sea (the putative source of the species pool) as well as the various environmental charactistics within the lake systems (Becking et al. 2011, *CHAPTER 1*). Depending on the degree of connection to the sea, the lakes can more or less resemble a coastal lagoon in geomorphology and species assemblage. Kakaban lake and Haji Buang lake are highly isolated lakes following the classification of Becking et al. (2011). The substantially different environmental variables (salinity, pH, degree of exposure to waves and air) as well as the different species of mangroves between both habitats are possibly forces that structure sponge composition (Table 2, Fig. 5); salinity is lower in the lakes than in the adjacent coastal mangroves and coral reefs, which can be a limiting factor for sponges. Likewise the coastal mangrove habitat has a higher chance of extended periods of exposure to air, which only few sponge species can tolerate (Rützler 1995). In the Caribbean, mangroves and coral reefs have been shown to differ substantially in composition (Ellison & Farnsworth 1992, Farnsworth & Ellison 1996, Wulff 2000, Diaz et al. 2004, Hunting et al. 2008, Guerra-Castro et al. 2011). Species within the typical mangrove sponge community are specifically adapted to survive extremes in abiotic factors such as salinity, temperature and sedimentation, either through tolerance or through rapid recovery after storms (Rützler 1995, Engel & Pawlik 2005, Wulff 2005b, Pawlik et al. 2007, Nagelkerken et al. 2008). Pawlik et al. (2007) observed that reef species declined in health and died within 60 days of being transplanted to mangrove sites. This result was attributed to the inability of reef species to tolerate the abiotic conditions of high temperature, rainfall events and sedimentation in mangroves.

The lake and coastal mangrove habitats share only a few species and most of these shared species (e.g. *Cinachyrella* spp*., Paratetilla bacca, Biemna fortis, Suberites diversicolor*) are also known to occur in perturbed areas or extreme environments in Indonesia, Singapore, and Australia (e.g. Hooper et al. 2002, McDonald et al. 2002, Cleary & de Voogd 2007, de Voogd & Cleary 2007, Becking & Lim 2009, de Voogd & Cleary 2009, *CHAPTER 4*). Though the lakes are minimally perturbed by human influence, they do share some characteristics with perturbed bay environments such as high turbidity and low salinity. All sponge species in the coastal mangrove habitat are common, widely distributed and have been recorded in tropical sheltered bays, sandy reef flats or coral reefs across the Indonesia (e.g. Becking et al. 2006, de Voogd & Cleary 2008, de Voogd et al. 2009, de Voogd & Cleary 2009, *CHAPTER 3*). In contrast, a large portion of the species in the lakes is not known from any published records of the reefs; many are undescribed species new to science. Both Kakaban and Haji Buang lake housed species that were only recorded in one lake and possibly represent local endemics. In Kakaban lake, half the species recorded were restricted to this lake. Many rare and novel genera and species among a variety of taxa have been described from this highly isolated lake, such as a crab (*Orcovita saltatrix*), two holothurians (*Holothuria cavans* and *Synaptula spinifera*), and an ascidian (*Styela complexa*) (Ng & Tomascik 1994, Tomascik & Mah 1994, Massin & Tomascik 1996). It is nevertheless possible that these 'endemic' lake-dwelling species with large growth forms may occur elsewhere in cryptic locations such as crusts in reefs or marine caves and may thus have been overlooked during reef surveys (Pawlik 1998). We did, however, thoroughly scrutinize the coastal coral reefs, mangroves and sandy reef flats in search of these lake species.

To summarize, the lake assemblages contained three groups of species: 1) species that are widely distributed (e.g. *Cinachyrella* spp and *Placospongia* spp.*),* 2) species that are shared by both lakes and that have been observed in other Indo-Pacific lakes (e.g. *Darwinella* aff. *gardineri* and *Tethytimea* aff. *tylota),* and 3) species that have (thus far) only been observed in a single lake (e.g. several species of the genus *Haliclona*). The only representative study on sponge fauna in marine lakes in the Indo-Pacific so far was conducted in Ha Long Bay in Vietnam (Cerrano et al. 2006, Azzini et al. 2007). The Indonesian and Vietnamese marine lakes share few species, though similar patterns in assemblage structures are seen in both. A qualitative survey of species presence and absence on the rocky shore within lakes and adjacent coastal habitats in Vietnam revealed that the more isolated lakes harbored unique species where 23 species (of a total of 63 species) were only present in the lakes, 18 of which were uniques (i.e., species that were only observed in a single lake). In the lakes of Vietnam, there is a wide variation in the degree of connection to the sea. The well connected lakes harbored coral and sponge assemblages that did not differ from those in the adjacent bays, while the more isolated lakes had much less compositional overlap (Azzini et al. 2007). The mangrove fringed ponds of the Pelican Cays in Belize, though not anchialine, show some similarities to Indo-Pacific marine lakes. Comparison of the sponge fauna in these ponds with more common open mangrove systems indicated that the ponds contained a distinct species assemblage with a high number of rare and undescribed taxa (Rützler et al. 2000).

Differences between substrates and cover

In all habitats there was a clear zonation away from the shoreline. In the marine lakes, the hard substrates such as rock and root along the coast harbored multitudes of sponges, while the abundance, cover and number of species declined markedly with just 1-2 m distance from the shore in the soft flocculent mud substrate (Fig. 6). Mud and sediments can clog the oscula and pores of sponges, which can adversely affect their pumping (Gerrodette & Flechsig 1979) Certain species have adapted their morphology to circumvent the negative effects, for example by means of psammobiontic growthforms (e.g. *Biemna fortis)* or a hairy-bristly surface which can capture fine sediments (*Paratetilla bacca, Cinachyrella* spp, and *Raspaillia* spp.). In our study root, mussel, ascidian and algae cover was strongly related to variation in sponge composition in both Kakaban lake and Haji Buang lake. Roots almost always had a high cover of mussels, while the cover of mussels on a rock substrate was much lower (or absent as was the case in in Haji Buang lake). The relationship between mussel cover and sponge composition may therefore be simply correlative and not due to spatial competition or other inter-taxon interactions. Algae occurred on roots in Kakaban lake, but increased dramatically to almost 100% cover with depth in the more muddy substrates. In Haji Buang lake the subtidal quadrats could be 100% covered by algae that formed a blanket over an equally high sponge cover underneath. Remarkably this covering did not seem to adversely affect the sponges. More detailed research, however, is needed to understand how the various benthic taxa within marine lakes interact.

Within both habitats (marine lakes and coastal mangroves) sponge species composition differed markedly between mangrove roots and other substrates with certain species only occurring on mangrove roots. In Haji Buang lake there were only two transects in the mangrove roots (due to limited mangrove cover in this lake), yet these assemblages contained species that were not present in the remainder of the lake. Differential response of sponge larvae to concentration of tannins in mangrove roots may play a role in structuring the assemblages associated with mangroves (Hunting et al. 2008, Hunting et al. 2010). The ability to resist tidal exposure may, furthermore, be a strong selective trait for species survival in mangrove systems. The most speciose order associated with roots in the lakes and coastal mangroves is composed of the Haplosclerida, particularly the family Chalinidae and within that the genus *Haliclona.* The majority of *Haliclona* spp. were recorded as exposed to air during low tide. In Caribbean mangroves, the Chalinidae is generally the most speciose family (e.g. De Weerdt 2000, Diaz et al. 2004, Wulff 2004, Guerra-Castro et al. 2011). Many Caribbean *Haliclona* spp. can tolerate strong water movement, sudden oscillations in temperature and salinity, as well as exposure to air (de Weerdt et al. 1991, De Weerdt 2000). Certain *Haliclona* spp. are able to resist desiccation, including 4 hrs of exposure to sun, by being able to retain more water during exposure to sun than deeper water species (Rützler 1995).

Temporal variation
Sponge species composition, abundance and cover in marine lakes and coastal mangroves in Indonesia did not vary significantly between sampling dates. There is little evidence of a strong and consistent change in composition across plots based on the ordinations. Individual transects do exhibit, sometimes pronounced, differences in composition between sampling events, yet here are marked differences in the degree (and direction) of compositional change among plots as indicated in Figs 3 & 4. Our lack of directional change differs somewhat from results obtained in Vietnamese marine lakes where degeneration of species and subsequent variation in local assemblages was observed after half a year due to heightened temperature and extreme rainfall during the monsoon season (Cerrano et al. 2006, Azzini et al. 2007). Our contrasting results with Vietnam may be due to the fact that seasonal differences are much less pronounced in the Berau region than in Vietnam. That being said, longer term monitoring is necessary in order to gain a fuller understanding of sponge community dynamics in marine lake environments.

Sponges in Indo-Pacific mangroves
This is the first study to illustrate that mangroves in the Indo-Pacific can harbor a diverse array of sponge species. The number of sponge species associated with mangroves recorded for the Caribbean (scale of 1000’s of kms) is just under 150 based on papers from the 1960s up until now (e.g., Rützler 1969, Diaz et al. 2004, Guerra-Castro et al. 2011). In only a single study at a much smaller scale (10 km), we have recorded a total of 115 sponge species in the marine lakes and coastal mangroves of Berau, of which 74 species were attached to mangrove roots. A large portion of the Indonesian coastline is fringed by a great diversity of mangrove species (Spalding et al. 1997, Alongi 2002) that have yet to be explored for epibionts. In recent years, over 45 marine lakes new to science have been discovered in Raja Ampat, Indonesia (Becking et al. 2011, Becking pers. obs., *CHAPTER 1*). As such, a vast amount of diversity remains to be unveiled in Indonesia, and likely beyond in the wider Indo-Pacific. The unique habitat and species composition of marine lakes warrants further study and conservation.

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Appendix 1. *In situ* images of sponges from Kakaban lake and Haji Buang lake in Berau, East Kalimantan, Indonesia

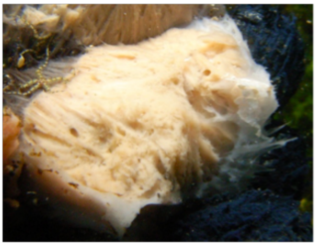
CALCAREA Bowerbank, 1864
Clathrinida Hartman, 1958
Clathrinidae Minchin, 1900

Clathrina sp. "white"

Clathrina sp. " yellow"

DEMOSPONGIAE Sollas, 1885
Astrophorida Sollas, 1887
Ancorinidae Schmidt, 1870

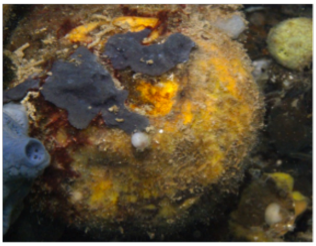
Stelletta sp. "icing"



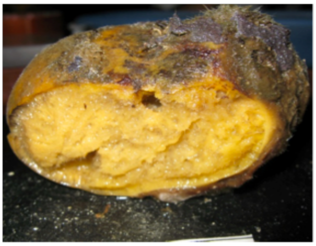
Stelletta sp. "icing"

Geodiidae Gray, 1867

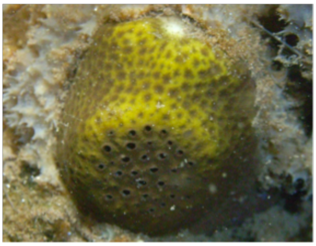
Geodia sp. "black"



Geodia sp. "yellow"



Geodia sp. "yellow"



Geodia sp. "chimney"

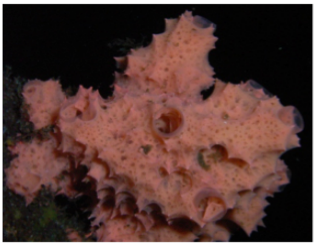
Chondrosida Boury-Esnault & Lopès, 1985
Chondrillidae Gray, 1872

Chondrilla aff. *australiensis*

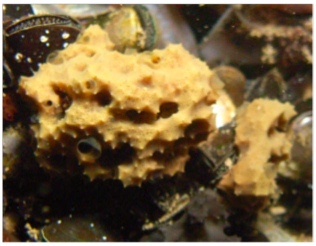
Chondrilla aff. *australiensis*

Chondrosia aff. *chucalla*

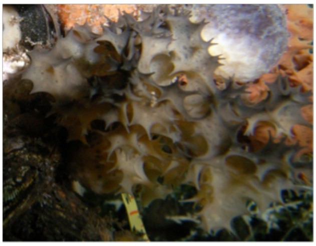
Dendroceratida Minchin, 1900
Darwinellidae Merejkowsky, 1879



Darwinella aff. *gardineri*



Chelonaplysilla sp. "gold"



Chelonaplysilla sp. "black"

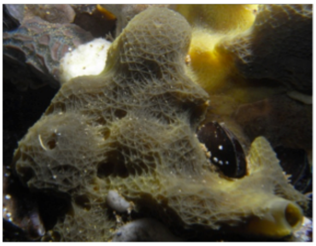
Dendrilla sp. "black"

Dendrilla sp. "black"

Dendrilla sp. "purple"

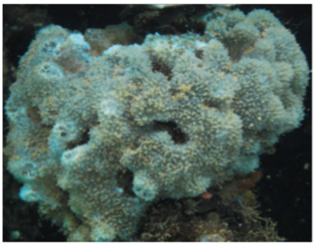
Dictyodendrillidae Bergquist, 1980

Spongionella sp. "purple"

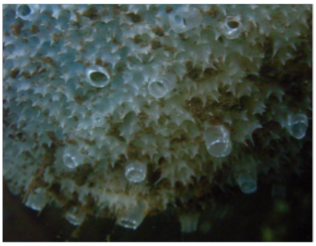


Spongionella sp. "green"

Dictyoceratida Minchin, 1900
Dysideidae Gray, 1867



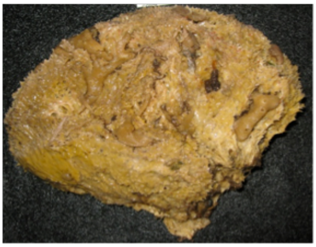
Dysidea sp. “blue”



Dysidea sp. “blue”

Dysidea sp. “beige”

Dysidea sp. “white”

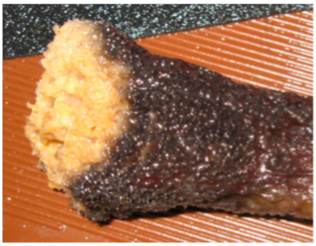


Dysidea sp. “white”

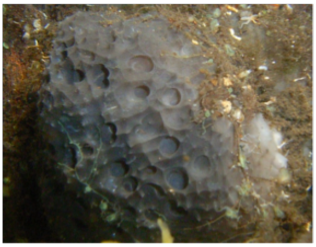
Lamellodysidea “blue”

Thorectidae Bergquist, 1978

Cacospongia sp. *“shiny”*

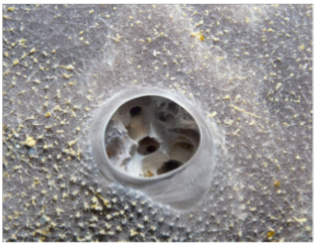


Cacospongia sp. *“shiny”*



Cacospongia sp. “honeycomb”

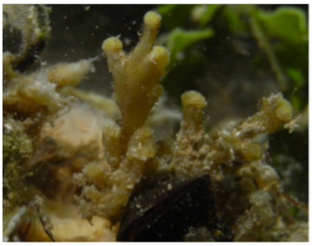
Semitaspongia sp.



Semitaspongia sp.

Smenospongia sp.

Spongiidae Gray, 1867

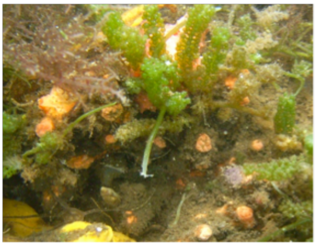


Spongia sp. "fistules"

Spongia sp. "black"

Hadromerida Topsent, 1894

Clionaidae d'Orbigny, 1851



Cliona aff. *peleia*

Cliona aff. *peleia*

Placospongiidae Gray, 1867

Placospongia carinata

Placospongia mixta

Placospongia melobesioides

Spirastrellidae Ridley & Dendy 1886

Spirastrella aff. *decumbens*

Spirastrella sp. "tubes"

Spirastrella sp. "orange"

Spirastrella sp.”solida”

Suberitidae Schmidt, 1870

Pseudosuberites andrewsi

Suberites diversicolor

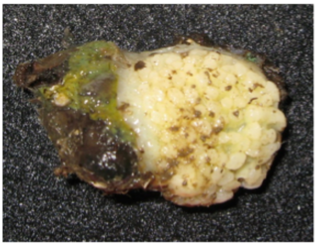
Suberites diversicolor

Tethyidae Gray 1848

Laxotethya sp. “spaghetti”

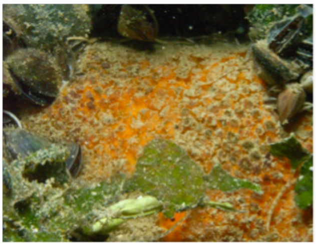
Tethya aff. *bullae*

Tethya aff. *seychellensis*

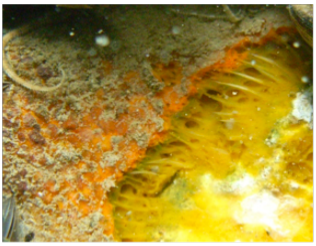


Tethya aff. *coccinae*

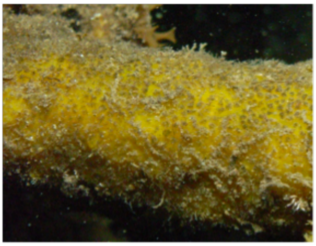
Tethya aff. *coccinae*



Tethytimea aff. *tylota*



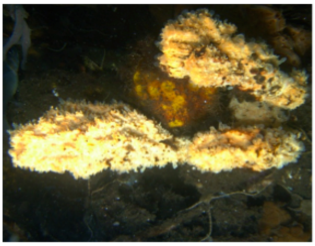
Tethytimea aff. *tylota*



Timea sp. “yellow” ‘

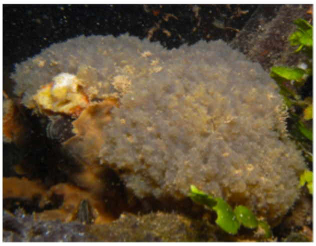
Halichondrida Gray, 1867
Dictyonellidae Van Soest, Diaz & Pomponi, 1990

Stylissa carteri

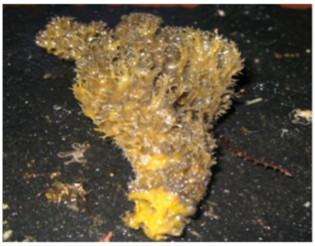


Stylissa carteri

Halichondriidae Gray, 1867

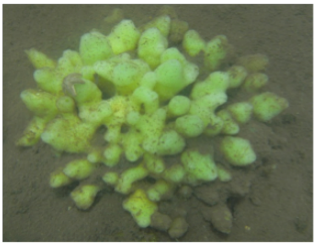


Axinyssa mertoni



Axinyssa mertoni

Axinyssa sp. "orange"

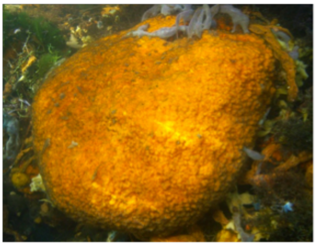


Halichondria sp. "crown"

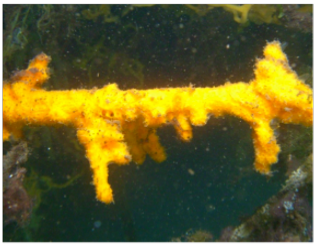
Halichondria sp. "crown"

Hymeniacidon sp. "yellow"

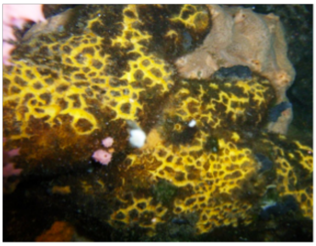
Heteroxyidae Dendy, 1905



Higginsia mixta



Higginsia sp. "encrust"



Myrmekioderma granulatum

Haplosclerida Topsent 1928
Chalinidae Gray 1867


Haliclona sp. “blue sticky”

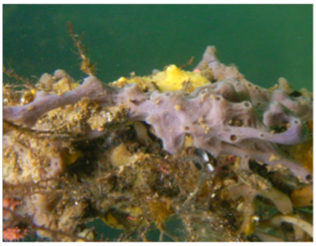

Haliclona sp. “blue sticky”


Haliclona sp. “blue soft branch”


Haliclona sp. “blue branch”


Haliclona sp. “violet branch”


Haliclona sp. “purple branch”

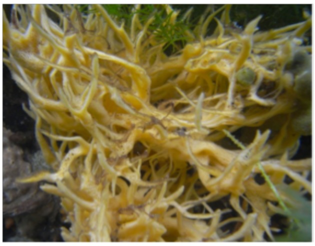

Haliclona sp. “purple soft branch”


Haliclona sp. “brown branch”


Haliclona sp. “brown branch”

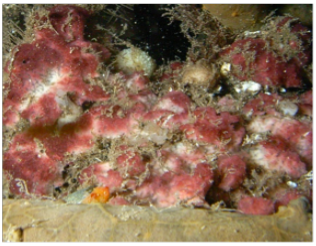

Haliclona sp. “yellow branch”

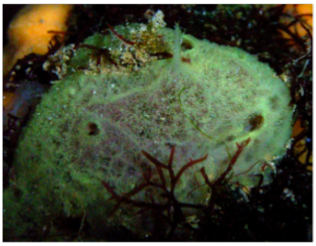

Haliclona sp. “yellow branch”


Haliclona sp. “yellow branch”


Haliclona sp. “translucent”


Haliclona sp. “violet tube”

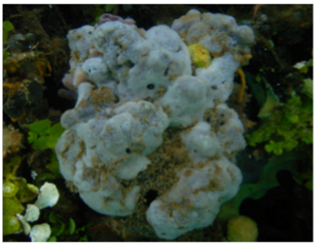

Haliclona sp. “bordeaux”



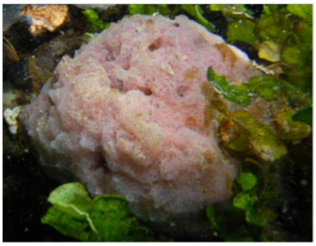
Haliclona sp. “purple-yellow”

Haliclona sp. “white oscules”

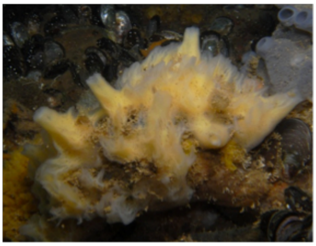
Haliclona aff. *baeri*



Haliclona sp. “violet cloud”



Haliclona sp. “pink cloud”

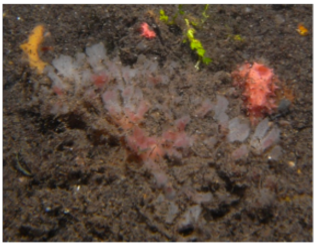


Haliclona sp. “yellow cloud”

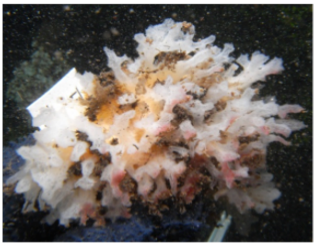
Haliclona sp. “soft spikes”

Haliclona sp. “blue-white cloud”

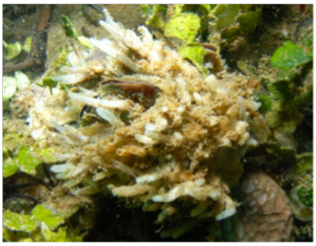
Haliclona sp. “purple vase”



Haliclona sp. “crunchy”

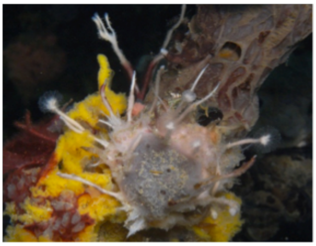


Haliclona sp. “crunchy”



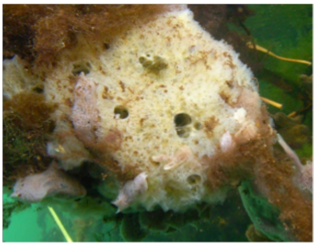
Haliclona sp. “crunchy extentions”

Haliclona sp. “white extention”



Haliclona sp. “white extention”

Haliclona sp. “white”



Haliclona sp. “yellow”

Haliclona sp. “beige”

Poecilosclerida Topsent, 1928

Acarnidae Dendy, 1922

Acarnus sp.

Acarnus sp.

Coelosphaeridae Dendy, 1922

Lissodendoryx aff. *similis*

Desmacellidae Ridley & Dendy, 1886

Biemna fortis

Biemna fortis

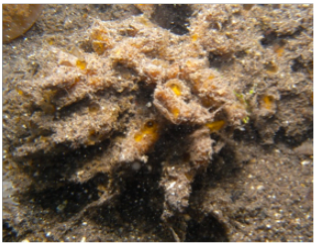
Biemna fortis

Microcionidae Carter 1875

Antho ridleyi

Antho ridleyi

Raspailiidae Nardo, 1833

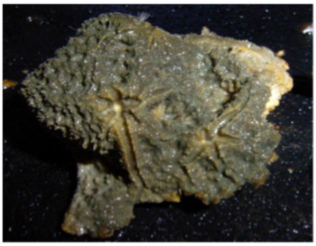


Raspailia aff. *clathrata*

Raspailia aff. *clathrata*

Raspailia aff. *bifurcata*

Acanthostylotella cornuta

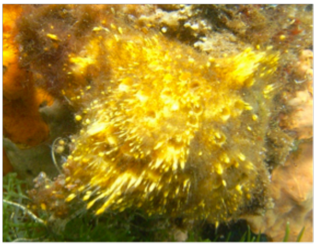


Acanthostylotella cornuta

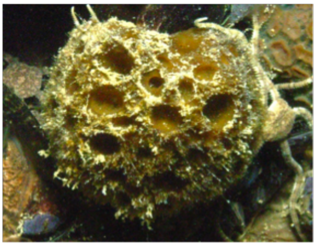
Spirophorida Bergquist & Hogg 1969

Tetillidae Sollas, 1886

Cinachyrella sp.



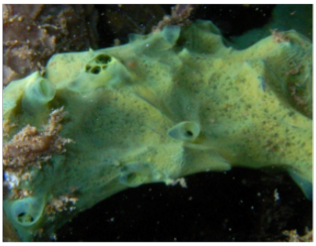
Cinachyrella sp.



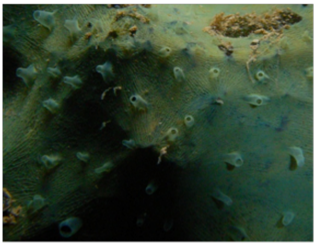
Paratetilla bacca

Verongida Bergquist, 1978

Ianthellidae Hyatt, 1875



Hexadella aff. *indica*



Hexadella aff. *indica*

III TAXONOMY

The work on hand is largely data processing and, as with any scientific data processing, complex matters of assorting, assigning, codifying and evaluating, and of establishing rules of classification and units and symbols, form the major part of the work. Once these are done the mathematical manipulation of figures is a rapid, easy and pleasant 'play'.

D. Carleton Gajdusek, 1959

Gajdusek to Adels, April 17,1959, box 1, Gajdusek Correspondence, National Library of Medicine (NLM)

Chapter 4

A new *Suberites* (Porifera: Demospongiae: Hadromerida: Suberitidae) from the tropical Indo-West Pacific

Leontine E. Becking , Swee Cheng Lim

Zoologische Mededelingen Leiden (2009) 83: 853-862

Abstract

In this paper we describe *Suberites diversicolor* sp. n. (Porifera: Demospongiae: Hadromerida: Suberitidae) from four enclosed anchialine lakes located in Indonesia and from a sheltered system in Singapore. Initially this species was thought to be specific to anchialine lakes, but further comparison to coastal areas indicated that it is more widespread in inshore brackish systems. We have used morphological characters to distinguish this species and a molecular marker to confirm that all types are the same species. *Suberites diversicolor* sp. n. is encrusting or massive with small protrusions or larger globular branches. The external color can be olive-green, blue, purple, red-orange, or orange-yellow. *Suberites diversicolor* sp. n. differs from known shallow water species of the genus *Suberites* in the tropical Indo-Pacific due to its diverse display of color-morphs and the presence of larger tylostyles without size classes.

Key words: Porifera • Hadromerida• *Suberites* • new species • Indonesia • Singapore • anchialine lakes • brackish water.

Introduction

Anchialine lakes, *sensu* Holthuis (1973), are small bodies of seawater that are entirely surrounded by land (Fig. 2A). These lakes are variably connected to the open sea through porous rock or through small subterranean channels. The landlocked pools of water are subjected to a tidal regime which is typically delayed (ranging from 20 minutes to 4 hours) and damped (ranging from 20 cm to 1.5 m) compared to the adjacent sea. The flora and fauna of anchialine lakes are sparsely documented, with a notable exception of a study by Azzini et al. (2007) on eight lakes in Vietnam. Recently, three campaigns were held in Indonesia in 2003 (NWO-KNAW East Kalimantan Program), 2007 (E-WIN Naturalis Raja Ampat Expedition) and 2008 (fieldtrip by first author to anchialine lakes in East Kalimantan) at which time 15 lakes were located and theirsponge fauna was documented. Sponges are one of the most dominant taxa in anchialine lakes in terms of species diversity and biomass (de Voogd et al. 2006, Becking & de Voogd 2008). During the surveys of the lakes one species of the genus *Suberites* was frequently observed and collected. Not all anchialine lakes that were visited contained this species, but when present it was typically rather abundant (often > 1 individual per m2). Individuals were generally large (> 8 cm), growing in the mud, on mangrove roots or on limestone rock. As this *Suberites* species had not been recorded in the coral reefs, it was initially suspected to be a species restricted to isolated anchialine lake systems (de Voogd et al. 2006, Azzini et al. 2007). This thought was further enforced by records of endemic subspecies and new species of other taxa from these isolated systems, such as the ascidean *Styela complexa* Kott 1995 and the decapod crab *Orcovita saltatrix* Ng & Tomascik, 1994. However, in the same period of the lake surveys a similar species of *Suberites* was observed in Singapore (by the second author). Though the external color and growth forms can vary greatly between specimens depending on locality and even within localities, we have concluded that these are indeed the same species based on a closer inspection of the morphology and a molecular marker. We have subsequently encountered this species in coastal mangrove systems in Indonesia and received material from similar lake systems in Vietnam and a man-made marine pool in Darwin, northern Australia. This species is therefore not necessarily restricted to anchialine lakes, yet all localities seem to have in common that the salinity is on average low (29 promille or less) and in all these localities the sponges have the potential to be exposed to air and the sun for up to four hours a day. This species appears to be able to tolerate and thrive in such an extreme, intertidal, estuarine environment. We conclude that this is an inshore species with a wide distribution in the Indo-West Pacific in areas with lower salinity than fully marine. Comparison with available literature on *Suberites* species and examination of type material show that this species is new to science.

Material & Methods

Specimens from Indonesia, Vietnam and Australia were collected while snorkelling and the specimens from Singapore were collected from intertidal reef flats during low tides. Where possible the material was preserved in 96% ethanol for DNA analysis and the voucher specimens were preserved in 70% ethanol and deposited in the collections of the Naturalis Biodiversity Center, Leiden (RMNH Porifera) and the Zoological Reference Collection, Raffles Museum of Biodiversity Research, National University of Singapore (ZRC). The external morphology, skeletal architecture and shape and size of spicules were examined for all material. Spicule dimensions are based on 25 measurements and given as minimum-average-maximum length × minimum-average-maximum width in the text. To examine the skeletal architecture, hand-cut tangential sections of the ectosome and perpendicular sections of the choanosome were made. The sections were air-dried, mounted in Durcupan® ACM on a microscope slide, and studied under Leica light microscope. Spicule preparations were made by dissolving the organic tissue of a small fragment of the specimen in commercial bleach, after which the spicules were washed 10 times with distilled water and once with 96% ethanol. The spicules were air-dried on microscope slides and mounted with Durcupan® ACM. The spicules were also mounted on aluminium stubs, coated with gold-palladium and studied with a Jeol Scanning Electron Microscope. For RMNH. POR. 4670, 4672, 4673, 4675, 2433, 2434, 2263, 1716. A fragment of the mitochondrial cytochrome oxidase subunit I (COI) was amplified using the universal barcoding primers by Folmer et al. (1994) LCO1490 (GGT CAA CAA ATC ATA AAG ATA TTG G) and HCO2198 (TAA ACT TCA GGG TGA CCA AAA AAT CA) with an annealing temperature of 49°C. PCR products were purified and sequenced by Macrogen Inc (Korea and The Netherlands). We compared the obtained sequences to those of other species of *Suberites* available on GenBank and computed a basic similarity matrix in BioEdit version 5.0.9 (Hall, 1999). Further abbreviations used in this paper: Zoologisches Museum für Naturkunde an der Universität Humboldt zu Berlin, Berlin, Germany (ZMB), British Museum of Natural History (BMNH).

Systematics

Phylum Porifera Grant, 1835
Class Demospongiae Sollas, 1885
Order Hadromerida Topsent, 1894
Family Suberitidae Schmidt, 1870
Genus *Suberites* Nardo, 1833
Suberites diversicolor sp. nov.
(figs 1A-B, 2B-D, 3A-C; table 1)
Suberites flabellatus; *sensu* Dendy, 1916: 135 (not Carter, 1886)

Material.— **Holotype**: RMNH POR. 4672, Indonesia, W Papua, Raja Ampat, Mansuar Island, anchialine lake; 0°35’19.6”S 130°35’48.8”E; 1 m. depth, 20.xi.2007, coll. L.E. Becking, #RAJ04/MOL037. **Paratypes**: RMNH POR. 4673 , Indonesia, W Papua, Raja Ampat, Mansuar island, anchialine lake; 0°35’19.6”S 130°35’48.8”E; 1 m. depth, 20.xi.2007, coll. L.E. Becking, #RAJ04/MOL035; RMNH POR. 2263, Indonesia, NE Kalimantan, Berau, Maratua Island, Danau Haji Buang, anchialine lake; 02°12’31.2”N 118°35’46.8”; 0.5-2 m. depth, 17.x.2003, coll. N.J. de Voogd, #BER18/171003/NV187; RMNH POR. 4670, Indonesia, NE Kalimantan, Berau, Maratua Island, Danau Tanah Bamban, anchialine lake; 02°13’48.8”N 118°34’48.0”E, 0.5-2 m. depth, 26.x.2003, coll. R. Moolenbeek, #BER44/RM15; RMNH POR. 4675 Singapore; Johor Strait, 0 m. depth, 01°26’02.34”N, 104°02’54.31”E; 20.viii.2008, coll. S.C. Lim; ZRC. Por. 0005 Singapore; Johor Strait, 0 m. depth, 01°26’02.34”N 104°02’54.31E”; 20.viii.2008, coll. S.C. Lim. Additional material examined: BMNH 1925.11.1.350, labelled ‘*Suberites glabellatus*’ (spelling mistake of *Suberites flabellatus*), Okhamandal, H.I.S.II.1, Dendy Coll.. RMNH POR. 4674, Vietnam, Ha Long Bay, Dau Be Island, small anchialine lake; 20°45’01”N 107°08’53”E, 1-2 m. depth, 27.iv.2004, coll. M. Pansini, F. Azzini & B. Calcinai, #HL182; RMNH POR. 4677, Australia, Northern Territory, Darwin, Lake Alexander, man-made marine lake; 12°25’S, 130°50’E, 0.5-1 m. depth, 15.ii.2008, coll. B. Alvarez, # BAG080215-07. RMNH POR. 4680, Indonesia, W Papua, Raja Ampat, Mansuar island, anchialine lake; 0°35’19.6”S 130°35’48.8”E; 1 m. depth, 20.xi.2007, coll. L.E. Becking, #RAJ04/MOL010; RMNH POR. 4681, Indonesia, W Papua, Raja Ampat, Mansuar island, anchialine lake; 0°35’19.6”S 130°35’48.8”E; 1 m. depth, 20.xi.2007, coll. L.E. Becking, #RAJ04/MOL043; RMNH POR. 4682, Indonesia, W Papua, Raja Ampat, Mansuar island, anchialine lake; 0°35’19.6”S 130°35’48.8”E; 1 m. depth, 20.xi.2007, coll. L.E. Becking, #RAJ04/MOL471; RMNH POR.1716, Indonesia, NE Kalimantan, Berau, Maratua Island, Danau Haji Buang, anchialine lake; 02°12’31.2”N 118°35’46.8”E; 0.5-2 m. depth, 17.x.2003, coll. N.J. de Voogd, #BER18/171003/NV189; RMNH POR. 2433, Indonesia, NE Kalimantan, Berau, Maratua Island, Danau Tanah Bamban, anchialine lake; 02°13’48.8”N 118°34’48.0”E, 0.5-2 m. depth, 26.x.2003, coll. R. Moolenbeek, # BER44/RM16; RMNH POR. 2434, Indonesia, NE Kalimantan, Berau, Maratua Island, Danau Tanah Bamban, anchialine lake; 02°13’48.8”N 118°34’48.0”E, 0.5-2 m. depth, 26.x.2003, coll. R. Moolenbeek, # BER44/RM07; ZRC. Por. 0012 Singapore; Johor Strait, 0 m. depth, 01°26’02.34”N 104°02’54.31”E; 25.vii.2003, coll. S.C. Lim; ZRC. Por. 0014 Singapore; Johor Strait, 0 m. depth, 01°26’02.34”N 104°02’54.31”E; 11.x.2006, coll. S.C. Lim; ZRC. Por. 0015 Singapore; Johor Strait, 0m. depth, 01°26’02.34”N 104°02’54.31”E; 25.vi.2006, coll. S.C. Lim.

Shape and size. — The holotype is irregularly massive with short, rounded, fingershaped processes of up to 4 cm in length (fig. 1A&B), approximately 10 × 6 × 9 cm (length × width × height). Larger specimens can occur up to 40 cm in height. Shape is to some degree dependant on the type of habitat. In exposed areas such as coastal tidal mangrove systems and buoys in the sea, this species is encrusting up to 2 cm thick. In areas with little exposure the sponge is massive, irregularly shaped with at times erect, globular and slightly flattened branches (fig. 2B-D). Paratype RMNH POR. 4670 has globular branches projecting from an ill-defined stalk. The terminal ends of the processes are rounded and without oscules. Compound oscules with a wide diameter range (0.2- 2.0 cm) occur sparingly on the upper part of the sponge. When encrusting, oscules are not visible to the naked eye and when in protected areas with high sedimentation, the oscules are greatly enlarged.

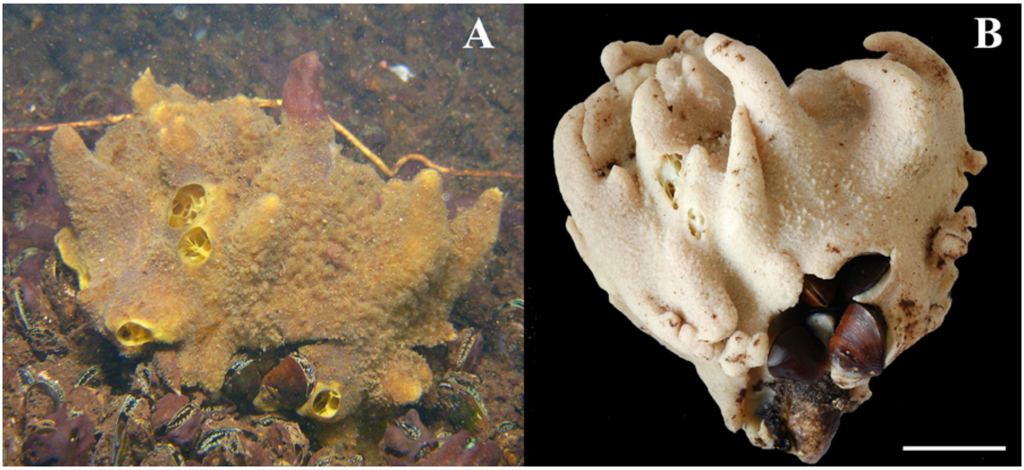


Figure 1. *Suberites diversicolor* sp. n. holotype (RMNH POR. 4672) **A** habit *in situ*, **B** after preservation in ethanol (scale bar = 2 cm).

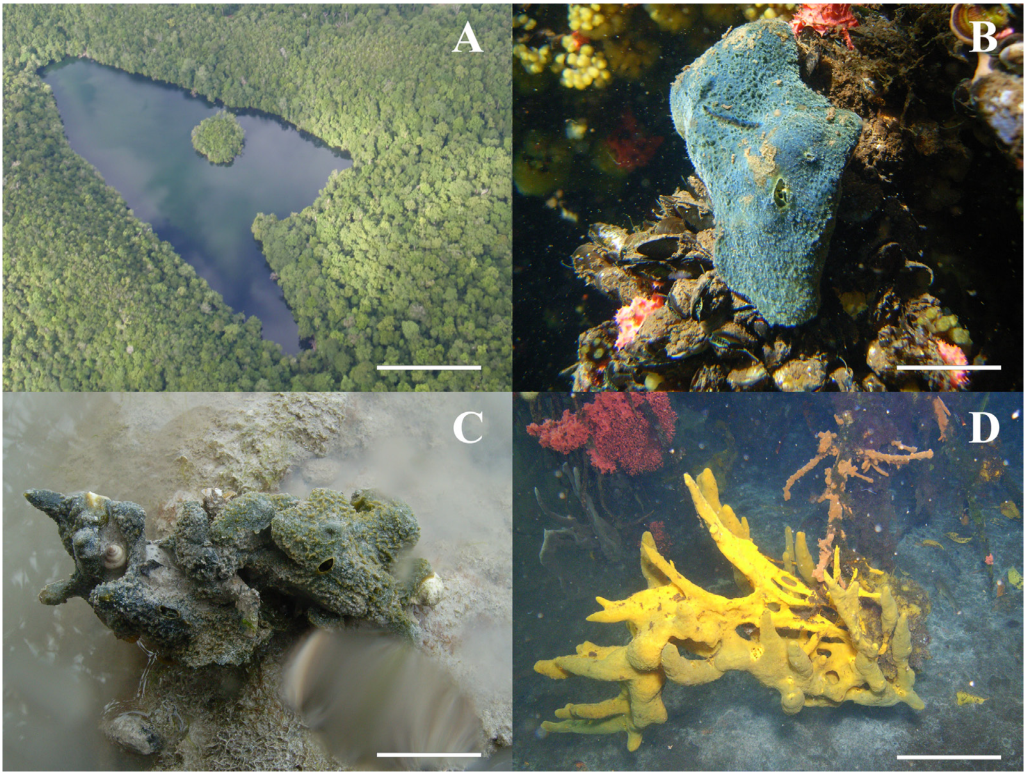


Figure2. A aerial photograph of anchialine lake on Mansuar Island in Raja Ampat, West Papua, Indonesia (scale bar = 100 m.), **B** blue morphotype (scale bar = 5 cm.), **C** green morphotype exposed to air (scale bar = 5 cm.), **D** green-yellow morphotype with globular branches (scale bar = 10 cm.).

Color.— The holotype is purple-brown externally. Living individuals have a wide range of external colors: olive-green, blue-green, blue-purple, purple, or red-orange (fig. 2B-D); most of the Singapore encrusting material exhibits the same external and internal color, yet all other material is bright to dark yellow internally. The variable external coloration may be due to the presence and type of photosynthesizing symbionts. All specimens turned light beige after preservation in ethanol.

Surface.— Regularly microhispid to velvety, but can be papillate with irregular rounded protrusions of approximately 30 mm length and 10 mm diameter.

Consistency.— Firm, slightly compressible and elastic with a meat-like consistency.

Skeleton.— Ectosomal skeleton consists of smaller tylostyles (up to 500 µm) at the periphery directed outwards in palisade, carried by tracts of larger tylostyles. Tangential spicules absent. There is no recognizable cortex. The interior skeleton comprises of densely packed tylostyles in vague tracts and/or in confusion. Peripheral choanosomal skeleton consists of closely packed diverging tracts of 60-100 µm in diameter (fig. 3A). The tylostyles have a wide size range and the typical two size categories tylostyles found in *Suberites* species (see definition in Van Soest, 2002) are overlapping in this species.

Spicules.— The megascleres are tylostyles (fig. 3B, C). These are straight, smooth, and sharply pointed at the end; for holotype tylostyle dimensions are 165-499-810 µm length × 2.5-8.9-17.5 µm width (please refer to Table 1 for details of tylostyle dimensions per specimen). Modifications of shape and position of the tylostyles were observed;

some tylostyles were slightly curved and variation in form and position of the tylostyleheads (fig. 3C) was observed.

Ecology.— Found at 0-7 m. depth in anchialine lakes, mangrove systems, and tidal reef flats; growing in mud, on rock, on mangrove roots, on submerged treetrunks/ branches, on buoys, and among beds of mussels. Salinity range from 23 to 29 promille. Both solitary and associated with other invertebrates.

DNA Barcode.— The holotype, four paratypes and three specimens of the additional material from three regions described in this paper have one identical genotype for COI and this representative nucleotide sequence of 534 bp in length has been deposited in GenBank with Accession Number: FJ968448. Comparison of this nucleotide sequence

to the three available of the same fragment of other *Suberites* species in GenBank showed that *S. diversicolor* sp. n.differs by 1% to *Suberites aurantiacus* from the Caribbean (Accession number EF519681), 12% to *Suberites ficus* from the northern Atlantic (AJ843891), and 3% from an unidentified *Suberites* sp. from Australia (AY561966).

Distribution.— India, Singapore, Vietnam, Indonesia, and Northern Australia.

Etymology.— Name refers to wide variety of colors that this species can display.

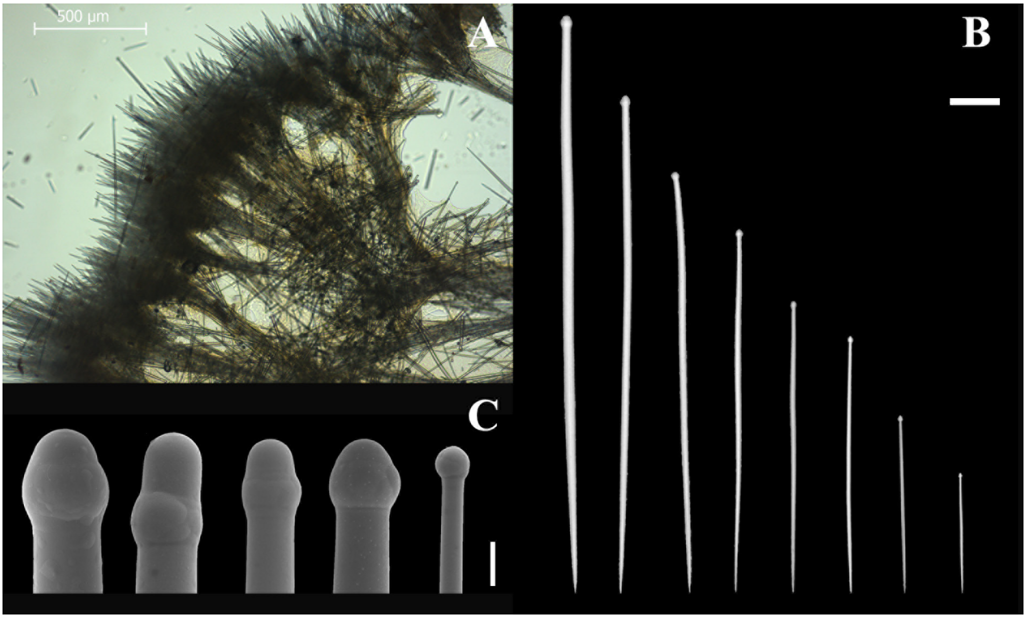


Figure 3. *Suberites diversicolor* sp. n. **A** Cross section of choanosomal skeleton of holotype, **B** Sequence of size ranges of tylostyles from paratype ZRC. Por. 0005 (scale bar = 100 µm), **C** Tylostyle head variations of paratype RMNH POR. 2263 (scale bar = 10 µm).

Table 1. Region of collection, color, habit, and tylostyle dimensions (in µm) of types of *Suberites diversicolor* sp. n.and additional material.

				Tylostyle length			Tylostyle width		
	Region	Colour	Habit	Min.	Average	Max.	Min.	Average	Max.
RMNH POR.4672	Papua	purple	massive with processes	165	499	810	2.5	8.85	17.5
RMNH POR.4673	Papua	olive green	massive with processes	183	470	830	2.5	8.4	15
RMNH POR.4680	Papua	blue	massive	127	486	850	5	10	20
RMNH POR.4681	Papua	olive green	massive	185	570	860	2.5	9.7	20
RMNH POR.4682	Papua	blue	massive	210	572	830	2.5	10.1	17.5
RMNH POR.2263	Kalimantan	orange	massive with globular branches	160	472	790	2.5	8.4	15
RMNH POR.1716	Kalimantan	olive green	massive with globular branches	165	476	760	2.5	8.1	12.5
RMNH POR.4670	Kalimantan	orange	massive with globular branches	138	537	930	2.5	7.2	15
RMNH POR.2433	Kalimantan	orange	massive with globular branches	155	481	770	2.5	6.5	10
RMNH POR.2434	Kalimantan	orange	massive with globular branches	133	491	890	2.5	6.7	12.5
RMNH POR.4675	Singapore	olive green	encrusting	143	486	840	2.5	8.1	15
ZRC.POR.0005	Singapore	olive green	encrusting	160	522	830	2.5	8.8	17.5
ZRC.POR.0012	Singapore	olive green	encrusting	180	556	930	2.5	8.7	17.5
ZRC.POR.0013	Singapore	olive green	encrusting	110	453	790	2.5	7.7	17.5
ZRC.POR.0014	Singapore	olive green	encrusting	140	466	780	2.5	8.1	17.5
ZRC.POR.0015	Singapore	olive green	encrusting	150	521	960	2.5	8.2	17.5
RMNH POR.4674	Vietnam	olive green	massive with globular branches	220	541	960	2.5	6.4	15
RMNH POR.4677	Australia	olive green	thick encrusting	117	493	890	2.5	7.9	15

Discussion

The genus *Suberites* is highly speciose, consisting of more than 70 species (van Soest et al. 2008). However, most of them are described from temperate seas and only six species are described from the tropical Indo-Pacific of which one is from Indonesia (van Soest et al. 2008). We will discuss and compare by region these six species as well as three additional species collected from the tropical Indo-Pacific to *Suberites diversicolor* spec. nov.*,* concluding with the additional material examined for this paper and some reports of unidentified *Suberites* sp. from brackish water systems.

The singular description from Ternate, Indonesia, of *Suberites radiatus* Kieschnick (1896) is extremely brief and vague. No mention is made of the dimensions of spicules. Thiele (1900) re-examined and described a part of Kieschnick's material from Ternate, but this species was not included. We could not locate Kieschnick's material at the ZMB (Zoologisches Museum für Naturkunde an der Universität Humboldt zu Berlin, Berlin, Germany). According to Thiele (1900), a part of his collection was lost and it is likely that *S. radiatus* might be among it. Annandale (1914, 1915) reported two *Suberites* species from the brackish water Chilka Lake in Orissa, India. One is *Suberites aquaedulcioris* (Annandale, 1914) which can occur in a variety of colors (deep orange yellow to bright green). The largest tylostyles in *S. aquaedulcioris* are 330 µm in length (Annandale 1914). In a later description Annandale (1915) designated this specimen as *Laxosuberites aquaedulcioris,* which in the present accepted classification would be *Protosuberites aquadulcioris*. *Suberites sericeus* Thiele 1898, originally described from Enoshima in Japan, was also reported by Annandale (1915) from Chilka Lake as both encrusting and irregularly massive with spicule dimensions of maximum 440 × 11.7 µm. We have made measurements in specimens of *S. diversicolor* sp. n.with a variety of size classes (from 5-40 cm in maximum length) and a variety of growth forms (thinly encrusting to massive with globular branches) from three different environmental situations (brackish water protected anchialine lakes, exposed reef flats, more saline inshore systems). All of these *S. diversicolor* specimens examined have a maximum tylostyle length of between 760-960 µm, which is at least two times as long as the maximum lengths reported for the Chilka Lake specimens. In fact, the maximum lengths reported for the Chilka lake specimens are lower than the average lengths of all the specimens of *S. diversicolor* spec. nov (see Table 1). We consider the spicule length of twice the size in *S. diversicolor* sp. n. as a strong distinguishing character. It is unfortunate that we could not examine Annandale's type materials, but we are confident that he was a trustworthy and reliable taxonomist, as there are no records of significant erroneous spicule measurements in his species descriptions. He described close to one hundred sponges species and the majority of them remain valid.

Two deep sea species *Suberites bengalensis* Lévi, 1964 and *Suberites pisiformis* Lévi, 1993 have been described from India (1190 m. depth) and New Caledonia (400-700 m. depth) respectively. *Suberites bengalensis* differs from the shallow water *S. diversicolor* sp. n. not only in its distinct deep sea habitat, but also in containing substantially larger tylostyles in two size categories measuring 280-1000 µm × 7-20 µm and 1200-1600 µm × 30-32 µm. *Suberites pisiformis* Lévi, 1993 also differs from the *S. diversicolor* sp. n. in having either an egg-shaped or spherical growth form and containing tylostyles in three size classes: principal tylostyles 500-700 µm × 8-10 µm, base tylostyles 300-450 µm × 5-8 µm, and peripheral tylostyles, 150-275 µm × 6-10 µm.

Examination of the type material of *Suberites clavatus* Keller, 1891 from the Eritrean Red Sea (ZMB 2696), revealed tylostyles measuring 300-449-530 µm × 5-9.8-15 µm. These are shorter than those of *S. diversicolor* sp. n. Similar spicule dimensions for *S. clavatus* were reported by Lévi (1965) from the island of Abulat, close to Jeddah in the Red Sea. Another Red Sea species *Suberites tylobtusa* Lévi, 1958 contains tylostrongyles which are not present in *S. diversicolor* sp. n. Both tylostyles and tylostrongyles in *S. tylobtusa* furthermore have much thicker spicule widths of up to 25 µm.

Based on examination of the type material (slide) of *Suberites laxosuberites* Sollas, 1902 (BMNH 1938.8.17.4) from peninsular Malaysia, we conclude that this species belongs to a different Suberitid genus, namely *Aaptos*. This species contains abundant strongyloxeas in the skeleton instead of tylostyles.

Dendy (1916) reported *Suberites flabellatus* (Carter, 1886), BMNH 1925.11.1.350, from Okhamandal, western India. The specimen described by Dendy has similar spiculation, skeletal structure and habit to *S. diversicolor* spec. nov., but unfortunately the live color was not recorded. The tylostyles have a similar wide size range, 110-475.5-755 µm × 4-8.4-15 µm, and there are no apparent size categories, comparable with *S. diversicolor* sp. n. However, *S. flabellatus sensu* Dendy is probably not conspecific with *S. flabellatus* (Carter, 1886). The latter was originally described from South Australia as flabellate and stipitate with a thick stem. In contrast, Dendy's material is encrusting, massive, irregularly shaped, with short digits. The average length of tylostyles of Carter's *S. flabellatus* is much shorter, about 241.3 µm in length, thus only about half the length of Dendy's (size range was provided by Carter). There are no subsequent reports of *S. flabellatus* which extend its distribution from South Australia to other areas besides India. It is also notably absent in recent studies conducted in the Dampier Archipelago (Fromont, 2004) and Great Barrier Reef (Hooper et al., 1999), suggesting that *S. flabellatus* may be confined to South Australia where it was first described.

Finally, we examined two specimens from an anchialine lake in Vietnam (RMNH POR. 4674) and from a man-made marine to brackish water lake in Australia (RMNH POR. 4677). This material has the same habit as *S. diversicolor* sp. n. and has a similarly wide tylostyle size range (see Table 1) without discrete size categories. As we have not been able to obtain the COI sequences, we have chosen not to include this material as paratypes in the present description. Though we strongly suspect that they are conspecific with *S. diversicolor* spec. nov., we prefer to prevent possible confusion in the future in the event that cryptic speciation is demonstrated.

Interestingly, some undetermined *Suberites* species were reported from Lake Motitoi on the island of Satonda in Indonesia which is a brackish water lake in an old volcano crater. Reitner et al. (1999) recorded two morphotypes belonging to a *Suberites* sp. and "*Laxosuberites* sp. (= *Protosuberites*)". They recorded green, brown, yellow-brown and yellow specimens with encrusting or somewhat erect growth forms and ectosomal plumose bundles of short tylostyles (150-200 µm) and larger tylostyles in the choanosome (300-500 µm). There have been additional reports of an olive-green colored *Suberites* sp. from from Ongeim'l Tketau (Jellyfish Lake) in Palau by L.J. Bell (Coral Reef Research Foundation). These reports may represent *S. diversicolor* sp. n. or possibly a closely related species.

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Diversity of the genus *Placospongia* (Porifera: Demospongiae: Hadromerida: Placospongiidae) in the West Pacific

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Zookeys (accepted)

Abstract

Species of the genus *Placospongia* are common within the tropical Indo-West Pacific, demonstrating a wide variety of colors and either branching or encrusting growth forms. A revision of Indo-West Pacific *Placospongia* is undertaken based on a redescription of the holotypes of species of *Placospongia* from the Indian Ocean and western Pacific and an examination of an additional collection of over 100 specimens of *Placospongia* spp. collected from Indonesia (including the Vosmaer & Vernhout 1902 material), Seychelles, India, Singapore and Micronesia. One mitochondrial (COI) and one nuclear (ITS) marker were subsequently used to differentiate species. All *Placospongia* species are characterized by selenasters and tylostyles in two size classes. The combination of microsclere diversity, morphology and megasclere size were shown to be informative morphometric characters, supported by molecular evidence. Live coloration and growth form is shown to be unreliable for diagnoses. The study of holotypes found that *P. mixta* is a valid species and that two genus transfers are necessary: *Geodinella anthosigma* is a *Placospongia* and *P. labyrinthica* is a *Geodia.* A new species is also described from an anchialine pool in Indonesia, *Placospongia santodomingoae* sp.n.; bringing the total fauna of *Placospongia* species in the Indo-West Pacific to five: *Placospongia anthosigma, Placospongia carinata, Placospongia mixta, Placospongia melobesioides,* and *Placospongia santodomingoae* sp.n. An identification key is given. Two additional species, possibly morphologically cryptic, have been identified by molecular markers.

Keywords: sponge • Indonesia • marine lake • coral reef • anchialine pool • ITS • COI

Introduction

Species of the genus *Placospongia* in the tropical Indo-West Pacific occurr in a wide variety of environments such as marine lakes, coral reefs and mangroves. They may display a variety of colors and growth forms, from encrusting to branching (Figs 1 & 2). Generally only two species have been recorded in species checklists within the Indo-West Pacific (e.g. Burton 1959, Hooper and Wiedenmeyer 1994, Hooper et al. 2000, de Voogd et al. 2006, de Voogd et al. 2008, de Voogd et al. 2009): *Placospongia melobesioides* Gray 1867, and *Placospongia carinata* (Bowerbank 1858). A recent collection of over 100 *Placospongia* specimens during fieldtrips to Indonesia in 2006 (Sulawesi), 2007 (Papua), 2008 (Berau), 2009 (Berau), and to Micronesia in 2010 (Yap) revealed, however, that there were more than two species present in these faunas.

The taxonomic literature records six valid species of *Placospongia* worldwide, of which there are three from the Indian Ocean and western Pacific: *P. carinata* (type locality "South Sea", presumably in the Pacific), *Placospongia labyrinthica* Kirkpatrick 1903 (type locality East London, South Africa, Indian Ocean), *P. melobesioides* (type locality Borneo, Pacific). In 1900 Thiele described the species *Placospongia mixta* from Ternate (Indonesia), which was later synonymized with *P. carinata* by Vosmaer and Vernhout in 1902. Vosmaer and Vernhout (1902) based their conclusions on a review of 26 specimens collected during the Siboga expedition to Indonesia which is presently housed at the Naturalis Biodiversity Center, The Netherlands. Subsequently, according to the World Porifera Database (van Soest et al. 2011) *Geodinella anthosigma* Tanita and Hoshino 1989 (type locality Sagami Bay, Japan) should be transferred to the genus *Placospongia,* and *P. labyrinthica* should in fact be transferred to the genus *Geodia.* These suggested genus transfers have, however, not yet been published in the peer-reviewed literature. A molecular phylogeny constructed using the internal transcribed spacer region (ITS) indicated that there are nine evolutionary lineages worldwide within the genus *Placospongia*, of which there are five distinct clades in the Indo-Pacific (clades C3, C4, C5, C6 & C9) that may represent five species (Nichols & Barnes 2005). The authors did not investigate the spicule morphology of the specimens in their study, therefore it is unclear which species name can be assigned to the different clades.

The objectives of the present study were to revise the genus *Placospongia* in the Indo-West Pacific by examining the holotypes of *P. melobesioides, P. carinata, P. mixta*, as well as over 100 specimens of *Placospongia* spp. that were collected from Indonesia (including Vosmaer & Vernhout's material), Singapore, Seychelles, Madagascar, and Micronesia. In order to obtain a full view of the species from the western Pacific and Indian Ocean the holotypes of the temperate species *G. anthosigma*, and *P. labyrinthica* were also examined. Subsequently it was the aim to determine if growth form and color can be used as diagnostic characteristics to identify different species of *Placospongia* in the field. Finally, an attempt was made to provide species names to the five clades of Indo-Pacific *Placospongia* as published by Nichols & Barnes (2005) by combing their published ITS sequences from GenBank with ITS sequences from identified species of Indo-Pacific *Placospongia.*

Figure 1. *In situ* underwater images of *Placospongia* spp. in Indonesia, displaying natural variation in color and growth form of live specimens. **A** *Placospongia mixta*, **B** *Placospongia carinata,* **C** *Placospongia carinata,* **D** *Placospongia melobesioides.*

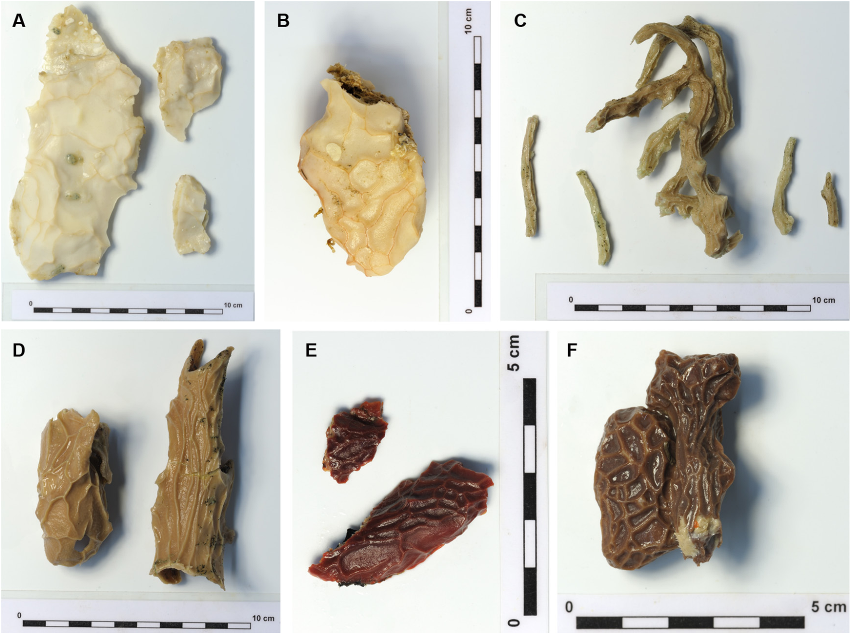

Figure 2. Gradation of external coloration in preserved specimens. A. *Placospongia mixta* RMNH POR. 4492, B. *Placospongia mixta* RMNH POR. 4113, C. *Placospongia carinata* RMNH POR. 4483*,* D. *Placospongia carinata* RMNH POR. 4483, E. *Placospongia mixta* RMNH POR. 3979, F. *Placospongia melobesioides* RMNH POR. 4114

Material & Methods

Specimens from Indonesia were collected via snorkeling in marine lakes and scuba diving on reefs. Where possible material was preserved in 96% ethanol for DNA analysis, and voucher specimens were preserved in 70% ethanol and deposited in the Porifera collections of the Naturalis Biodiversity Center, Leiden (RMNH POR.). Records were made on the external morphology, skeletal architecture and spicules of all material. Spicule dimensions were measured of a subset of specimens indicated in Table 1, based on 25 measurements (unless noted otherwise) and given in the text as minimum-**average**-maximum. The following dimensions were measured: tylostyles length x shaft width X head width; selenasters length x width; spirasters total length x ray length; spherasters diameter; rhabds length x width. Only fully developed spicules were measured. To study the skeletal architecture hand-cut perpendicular sections of the choanosome were made. The sections were air-dried, mounted in Durcupan® ACM on a microscope slide, and studied under a Leica high power microscope. Spicule preparations were made by dissolving the organic tissue of a small fragment of the specimen in commercial bleach, after which the spicules were washed >10 times with distilled water and once with 96% ethanol. The spicules were air-dried on microscope slides and mounted with Durcupan® ACM. The spicules were also mounted on aluminium stubs, coated with gold-palladium and studied with a Jeol Scanning Electron Microscope.

The following 25 specimens were selected for further molecular analyses:
Placospongia melobesioides: RMNH POR. 2464, 3166, 4497, 3942, 3976, 4495, 4496; ZMA Por. 10496
Placospongia mixta: RMNH POR. 3158, 3936, 4113, 4489, 4490, 4492, 4494, 4493
Placospongia carinata: RMNH POR. 4482, 4483, 4484, 4485, 4486, 4487, 4488; ZMA Por. 10727, ZMA Por. 11367

DNA extractions were made with Qiagen DNEasy animal blood and tissue extraction kit following the manufacturer's protocol. The polymerase chain reaction (PCR) reaction volume was 25 μl and contained 5 μl Phire ® Hot Start reaction buffer, 1 unit Hotstart Phire® Hot Start DNA polymerase (Finnzymes) , 2 μl 1 mM dNTPs (Gibco), 1 μl DNA template (5-20 ng) and 0.625 μl of 10mM each primer. The standard DNA-barcoding fragment of the mitochondrial cytochrome oxidase subunit I (COI) fragment was amplified by using a specific forward primer designed by the author for *Placospongia* P-COI-F: GCA GG ATG ATA GGA ACA GGW TTT AG and the degenerated reverse primer from Folmer et al. (1994) designed by Meyer et al. (2005): dgHCO2198:TAA ACT TCA GGG TGA CCA AAR AAY CA. Temperature regime: 94°C for 30s; followed by 35 cycles of 94°C for 5s; 50°C for 5s; 72°C for 12 s; followed by 71°C for 1 min). ITS was amplified with primers from Wörheide (1998) RA2: GTCCCTGCCCTTTGTACACA and ITS2.2: CCT GGT TAG TTT CTT TTC CTC CGC). PCR products were purified and sequenced by Macrogen Inc (Korea and The Netherlands). The poriferan origin of the obtained sequences was verified through BLAST searches (http://blast.ncbi.nlm.nih.gov/blast.cgi). Sequences were handled in SEQUENCHER 4.10.1 (Gene Codes Corporation) and aligned with CLUSTALW and MUSCLE implemented in DAMBE (Xia & Xie 2001). Species of the families Spirastrellidae and Clionaidae were selected as outgroup for the phylogenetic analyses. For the COI genetree fou specimens of *Spirastrella* aff. *decumbens* (RMNH POR. 4505, 4589, 4614) was taken. For the ITS genetree sequences of species from Spirastrellidae were taken from GenBank, as well as ITS sequences of Indo-Pacific *Placospongia* spp. from the study by Nichols & Barnes (2005), for GenBank accession numbers see Figure 11. The best-fit DNA substitution model was selected as by the Akaike Information Criterion deployed in jMODELTEST v. 0.1.1 (Posada 2008) and this model (HKY for COI and GTR+G+I for ITS) was used for subsequent Bayesian and maximum likelihood phylogeny inferences. Phylogenetic reconstructions were performed under Bayesian inference criteria implemented in MrBayes v. 3.1.2. (Huelsenbeck & Ronquist 2001). Each analysis comprised of two independent runs of four Metropolis-coupled Markov-chains, sampled at every 1000th generation at the default temperature (0.2). Analyses were terminated after the chains converged significantly as indicated by an average standard deviation of split frequencies <0.001. Convergence was also checked in Tracer v. 1.5.0 (Rambaut & Drummond 2007). For comparison, maximum likelihood bootstrap analyses were conducted using MEGA v. 5.01 (Tamura et al. 2011) using a heuristic search with 1000 bootstrap replicates. Within-group and between-group uncorrected *p*-distances were calculated in MEGA.

Abbreviations used in this manuscript: Naturalis Biodiversity Center, Leiden (RMNH POR.), the Zoological Museum of the University of Amsterdam (ZMA POR.), Zoologisches Museum für Naturkunde an der Universität Humboldt zu Berlin, Berlin, Germany (ZMB), The Natural History Museum (BMNH).

Note on presentation of examined material:
In this study multiple specimens from the same location were examined. As a result, the information of examined material is provided in the following order: country, province, region, island, location, coordinates, habitat type, depth, collector, date: registration number (#fieldnumber in brackets) for all specimens from the specified location. Where certain information is unavailable this is omitted. First the information of the holotype is given, then the material from the Siboga Expedition that was reviewed by Vosmaer & Vernhout (1902), followed by other museum material (first RMNH POR., then ZMA Por.).

Phylum	Porifera Grant, 1836
Class	Demospongiae Sollas, 1885
Order	Hadromerida Topsent, 1894
Family	Placospongiidae Gray, 1867
Genus	*Placospongia* Gray, 1867

Placospongia Gray, 1867

Type species: *Placospongia melobesioides* Gray, 1867 by monotypy

Description, amended from Systema Porifera (Hooper and van Soest 2002): Encrusting to branching growth forms. Small encrustations of 3 cm^2 to large surfaces of >2m^2 to branching individual with total size of up to 45cm in length and branch diameter between 0.25-1.5cm. Total size of specimens is hard to establish as parts of the body may be encrusting within cracks. Dried material is hard, alcohol preserved and live specimens remain compressible as the choanosome is of more pliant material than the cortex. The surface is made up of smooth cortical plates separated by contractible grooves which form a kind of network on the surface while these are firmly closed in preserved specimens. See Vosmaer & Vernhout (1902) and Rützler (2002) for an extensive description of the genus. In live specimens grooves are open and oscules are visible inside contractile ridges, running between plates. Live color white, cream, orange, reddish brown to dark black-brown (Fig. 1 & 2) and this color is usually retained after alcohol preservation. The contact lines between the plates ridge up slightly and are generally a darker shade than the color of the plates.

Skeleton: the cortical plates consist of densely packed selenasters and can also contain auxiliary microscleres. Developmental stages of selenasters occur throughout the choanosome. Tylostyle tracts support the margins of the cortical plates. In branching specimens radial tylostyle tracts run from the centre core (consisting of densely packed selenaster) to the cortical plates, in encrusting specimens tracts run in direction from substrate to cortex. The sharp ends of the smaller tylostyles are projected beyond the cortex surface. Microscleres occur in the cortex and scattered in choanosomal skeleton. For a detailed description of external morphology and anatomy see Vosmaer & Vernhout (1902).

Spicules: Megascleres are tylostyles two size classes, microscleres are selenasters, and can include choanosomal and ectosomal spirasters, spherasters, spherules, or acanthose microrhabds. Selenasters often remain pigmented after treatment with bleach or nitric acid.

Table 1. Measurements of spicules of *Placospongia carinata* , *Placospongia melobesioides, Placospongia mixta,* and *Placospongia santodomingoae* sp.n.
Sample location, growth form, color and spicule measurements provided per specimen. Spicule dimensions are based on 25 measurements and given in the text as minimum-**average**-maximum. Spheraster measurements in *P.melobesioides* based on less than ten measurements, due to low of abundance of these spicules in specimens.

	region	growthform	color live	Tylostyle blunt end length	max width	head width	Tylostyle sharp end length
P.melobesioides							
BMNH52.4.1.14 (holotype)	Borneo	branching	dark brown	670-**879.6**-1010	10-**13.2**-18	10-**16.3**-20	205-**293.4**-420
RMNH4495	N Kalimantan (Kakaban lake)	encrusting	dark brown	480-**717.6**-1040	5-**9.5**-15	8-**10.3**-15	190-**297.6**-370
RMNH4496	N Kalimantan (Kakaban lake)	branching	dark brown	580-**778.4**-900	8-**11.7**-15	10-**14.1**-18	230-**272.8**-400
RMNH4497	N Kalimantan	branching	dark brown	620-**745.2**-860	10-**12.2**-15	13-**14.8**-18	250-**320.8**-450
RMNH3935	N Kalimantan	encrusting	dark brown	460-**660.9**760	10-**11.6**-15	10-**13.7**-18	210-**325.8**-450
RMNH3166	N Sulawesi	encrusting	dark brown	460-**704.8**-810	8-**11.4**-13	10-**13.2**-15	200-**288**-470
RMNH3976	Moluccas	branching	dark brown	600-**793.6**-910	10-**12**-15	13-**14**-18	190-**321.2**-450
RMNH3977	Moluccas	branching	brown	510-**683.6**-780	10-**11.5**-13	13-**13.9**-15	200-**326**-450
RMNH758	W Papua	branching		630-**853.2**-1020	10-**13.3**-15	13-**15.8**-18	210-**253.2**-310
RMNH757	W Papua	branching		550-**829.2**-960	10-**13.3**-16	13-**15.8**-18	260-**302.1**-370
RMNH2464	Singapore	branching		710-**933.4**-1080	12.5-**15**-17.5	13-**15.7**-20	240-**326.7**-330
ZMA10459	Seychelles	branching	brown	520-**670.8**-820	7.5-**11.4**-12.5	10-**13.4**-17.5	310-**362.5**-430
P. carinata							
R122b-86g-BK1390 (holotype)	"South Sea"			500-**710.4**-800	10-**13.4**-15	10-**15.3**-18	140-**317.4**-450
RMNH4482	N Kalimantan (Haji Buang lake)	branching	orange	660-**726**-800	10-**12.3**-15	10-**14.5**-18	180-**263**-410
RMNH4483	N Kalimantan (Haji Buang lake)	encrusting	crème	610-**703.8**-800	10-**13.1**-15	13-**14.9**-18	190-**286.7**-470
RMNH4484	N Kalimantan (Kakaban lake)	encrusting	crème	560-**709.16**-920	8-**11.7**-18	10-**13.9**-18	175-**267.1**-550
RMNH4485	N Kalimantan (Kakaban lake)	branching	dark	550-**761.2**-930	10-**14**-18	13-**15.5**-18	210-**295.2**-450
RMNH744	Moluccas	encrusting	light purple	450-**748.6**-980	8-**11.1**-13	10-**13.2**-15	195-**256.8**-550
RMNH754	Philippines	branching	white	540-**705.8**-830	10-**12.8**-15	13-**15.2**-18	280-**355.5**-500
RMNH755	W Papua	branching	crème	560-**764.7**-910	8-**12.2**-15	10-**14.7**-18	250-**311.8**-360
ZMA10727	Seychelles	encrusting		620-**738.7**-840	8-**11**-13	13-**15.5**-18	240-**258.3**-270
ZMA9189	India	branching		550-**703.3**-820	10-**12.8**-15	13-**15**-18	210-**318.8**-410
P. mixta							
ZMB3204 (holotype)	Moluccas	encrusting		355-**672.4**-940	8-**12.1**-18	8-**15.6**-20	165-**226.4**-275
RMNH4112	Moluccas	encrusting	red	480-**870**-1040	10-**12.7**-15	13-**15.8**-28	210-**288**-410
RMNH4113	Moluccas	encrusting	crème	550-**817.6**-1030	10-**13.1**-15	13-**15.6**-18	160-**260**-350
RMNH742	Moluccas	branching	red	550-**759.2**-850	10-**11.9**-15	10-**14.9**-20	120-**230**-380
RMNH4489	N Kalimantan (Kakaban lake)	encrusting	crème	630-**886.6**-1010	10-**12.9**-15	13-**15.4**-19	175-**221.5**-320
RMNH4490	N Kalimantan (Kakaban lake)	encrusting	crème	510-**727.6**-970	8-**13.1**20	13-**16.3**-23	150-**240**-310
RMNH4491	N Kalimantan	encrusting	brown	780-**1001.4**-1200	10-**14.8**-18	15-**17.5**-20	240-**284**-350
RMNH4492	N Kalimantan	encrusting	white	610-**995.8**-1250	10-**16**-20	13-**19**-25	260-**274**-290
RMNH3158	N Sulawesi	encrusting	crème	550-**990**-1210	13-**16.9**-20	13-**17.5**-20	130-**267.8**-400
RMNH745	S Sulawesi	encrusting	brown	760-**914.1**-1030	13-**17**-23	10-**18**-25	250-**366.6**-480
RMNH4493	W Papua	encrusting	brown	460-**761.6**-1070	10-**14.6**-23	13-**17.38**-25	220-**323.6**-430
RMNH4494	W Papua	encrusting	brown	540-**758**-900	10-**12.2**-18	10-**13.8**-20	180-**216.9**-350
P. santodomingoae sp.n.							
RMNH4486 (holotype)	N Kalimantan (Hapsi Bulo pool)	branching	brown	430-**605.6**-660	13-**15.5**-20	13-**18.1**-23	240-**261.3**-290
RMNH4487	N Kalimantan (Hapsi Bulo pool)	branching	orange	530-**652.4**-740	13-**16**-20	15-**18.0**-23	220-**274.7**-310
RMNH4488	N Kalimantan (Hapsi Bulo pool)	branching	orange	480-**633.2**-760	15-**17.2**-20	18-**19.6**-23	190-**273.2**-380

		Selenaster		Spheraster	Spiraster		Microrhabd	
max width	head width	length	width	diameter	total length	length ray	length	width
5-**9.9**-13	5-**9.9**-13	58-**63.1**-68	45-**51.7**-68	15-**16.8**-18				
3-**5.8**-8	3-**6.1**-8	45-**56.6**-70	30-**41.6**-50					
5-**7.4**-10	8-**9.1**-10	45-**60**-75	35-**45**-63					
5-**8.8**-10	5-**9.4**-13	63-**70.8**-83	45-**59.6**-65					
3-**7.4**-13	3-**8.3**-13	45-**63.9**-70	38-**51.3**-60	**15-20**				
3-**9.5**-13	5-**10.8**-15	60-**63.6**-70	50-**50.2**-55					
5-**8.5**-13	5-**9.6**-13	48-**66.8**-75	48-**55.2**-65					
5-**7.5**-10	8-**9.5**-13	58-**63.3**-68	40-**46**-53					
5-**9.5**-13	8-**11.8**-15	50-**55.2**-62.5	35-**42.3**-50	**15**				
8-**9.6**-13	10-**11.2**-15	55-**60.4**-65	43-**48.0**-53					
5-**9.2**-13	5-**10.8**-15	67.5-**81**-87.5	60-**72.5**-85					
5-**8.8**-10	5-**10.1**-13	62.5-**68.9**-72.5	50-**55.5**-65					
5-**8.4**-12.5	8-**9.3**-13	80-**90**-98	60-**71.3**-85		23-**33.8**-43	8-**11.6**-15	8-**12.0**-18	2,5
3-**5**-7.5	8-**7.5**-8	65-**71.5**-75	50-**58.5**-65		15-**34**-48	10-**13.0**-15	8-**11.7**-15	2,5
5-**6.4**-10	5-**8.6**-13	60-**80**-85	60-**62.9**-70		20-**33.7**-40	10-**13.2**-15	8-**11.9**-18	2,5
3-**4.4**-10	5-**6.4**-13	50-**61.8**-70	35-**47.4**-55		25-**29.7**-35	8-**11.0**-15	10-**13.3**-18	2,5
3-**5.6**-8	5-**7.6**-10	28-**63**-73	38-**50**-58		20-**27.6**-38	5-**9.0**-13	5-**9.4**-13	<2.5
5-**6.2**-10	5-**6.7**-8	60-**66.3**-70	50-**55.6**-65		25-**29.9**-38	10-**12.9**18	8-**10.8**-13	<2.5
5-**7.0**-10	5-**8.6**-13	55-**67.7**-75	45-**51.8**-55		25-**30.9**-38	8-**9.5**-13	8-**12.3**-18	2,5
5-**7.3**-8	5-**8.2**-10	55-**61.1**-65	38-**47.5**-55		30-**32.9**-38	8-**9.8**-13	8-**10.2**-13	2,5
3-**3.3**-5	3-**4.6**-8	50-**58.8**-78	35-**42.5**-63		25-**27.6**-38	8-**11.1**-15	8-**8.1**-10	<2.5
5-**7.5**-10	5-**9.7**-13	63-**72.2**-78	50-**56.8**-65		30-**35**-48	8-**10.7**-15	8-**9.2**-13	2,5
3-**6.1**-8	3-**7.8**-10	55-**69.8**-75	43-**55.4**-73	20-**25**-30	15-**23.9**-33	3-**7.6**-13	5-**7.1**-10	<2.5
5-**6.2**-10	5-**7.2**-10	50-**66.6**-75	38-**50.7**-58	18-**20.2**-25	18-**23.7**-35	5-**6.4**-10	5-**6.4**-10	<2.5
5-**7.3**-10	5-**8.2**-12.5	62.5-**66**-70	45-**53**-57.5	20-**22.1**-25	20-**24.8**-30	5-**5.7**-8	5-**7.5**-10	2,5
3-**5.9**-10	3-**7.6**-10	50-**65.4**-73	33-**46.5**-56	22-**23.4**-25	15-**22.2**-35	2-**5.7**-8	5-**7.4**-10	<2.5
3-**3.9**-8	2-**7.2**-10	60-**68**-75	43-**50.8**-58	18-**20.6**-25	20-**26.1**-35	8-**10.8**-15	8-**8.5**-10	<2.5
3-**5.3**-8	2-**6.4**-8	55-**70.4**-83	40-**53.3**-65	13-**20.5**-25	15-**21.7**-30	5-**6.4**-13	8-**9.2**-13	<2.5
5-**6.3**-8	5-**8.3**-10	60-**71**-75	48-**57.5**-63	18-**23**-25	20-**27.3**-35	5-**7**-10	5-**6.3**-8	2,5
8-**9**-10	8-**9**-10	58-**71**-78	45-**54.6**-70	15-**20.2**-25	18-**24.8**-33	10-**11.2**-15	5-**8.6**-18	<2.5
5-**8.8**-15	8-**9**-10	65-**71**-75	50-**56.5**-63	23-**23.8**-25	23-**28.4**-35	5-**8.7**-13	5-**6.6**-8	<2.5
3-**8**-13	3-**9**-13	45-**73.6**-80	45-**60**-70	20-**23.9**-25	20-**23.7**-30	3-**6.4-9**	5-**7.5**-10	<2.5
8-**9.1**-13	10-**11.3**-15	73-**80.3**-85	53-**65.3**-73	20-**26.5**-30	18-**23.4**-30	15-**8.1**-10	8-**8.7**-13	<2.5
3-**3.3**-5	4-**4.4**-8	50-**59.1**-68	35-**42.3**-58	15-**20.9**-28	23-**26.9**-30	8-**10.4**-13	8-**8.5**-10	<2.5
5-**7.2**-8	5-**8.8**-10	80-**84.8**-90	60-**67.3**-75				8-**12.3**-18	2.5-**2.7**-3.5
5-**8.2**-13	8-**9.5**-15	*63*-**82.9**-93	60-**66.3**-73				5-**10.5**-20	2.5-**2.6**-3.5
5-**7.9**-10	8-**10.3**-13	80-**87**-93	58-**69**-75				8-**13.5**-18	2.5-**2.9**-3.5

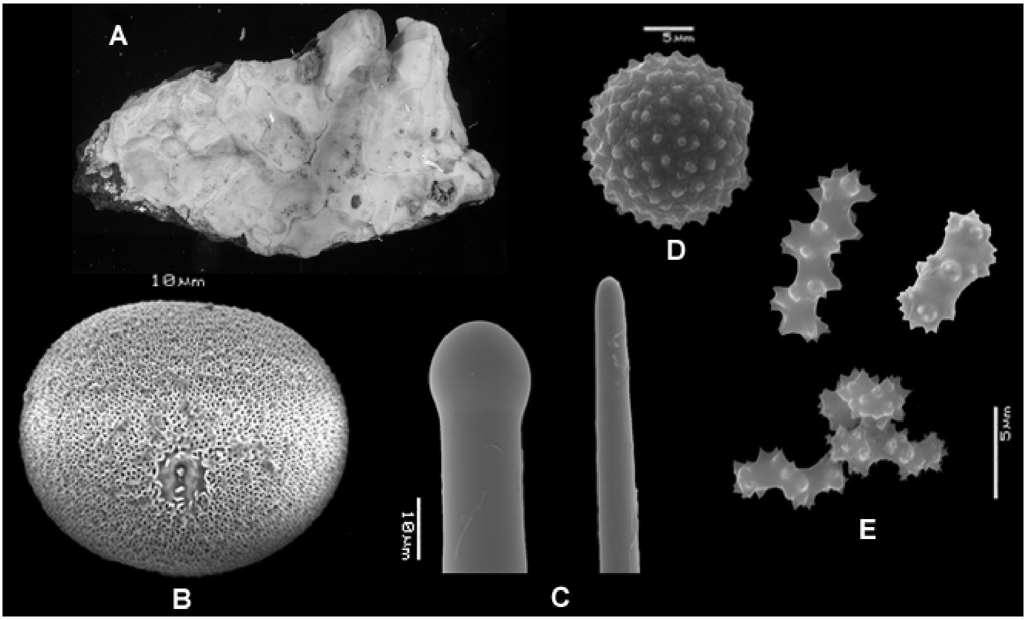

Figure 3. *Placospongia anthosigma* holotype (NSMT-Po R288) A. type specimen (image taken from website of Museum of Nature and Science, Tokyo, Japan), B. selenaster, C large tylostyle (head and blunt end), D. spheraster, E. spirasters referred to as 'anthosigma' by Tanita & Hoshino (1989)

Placospongia anthosigma **(Tanita & Hoshino, 1989)**
Figure 3

Geodinella anthosigma Tanita & Hoshino, 1989: Fig. 16, Plate III Fig. 1

Material examined:
Holotype. Japan, Kannonzuka-dashi, Amadaiba, Sagami Bay, depth 62-67m: NSMT-Po R288 (National Museum of Nature and Science, Tokyo, Japan).

Description: Holotype NSMT-Po R288 encrusting specimen in three pieces of 1-2cm^2 and 5mm thick, beige to pink in alcohol (Figure 3A).

Spicules: Megascleres large tylostyles with blunt point 520-797-930 x 15-18-20 x 18-20-23 μm**,** small tylostyles with blunt point 250-320-410 x 10-12-18 x 13x14-18 μm**;** microscleres selenasters 85-90-98 x 70-73-80 μm, spherasters 15-19-25 μm, stout spirasters with two or three contortions and acanthose spines spirally placed on shaft 8-11-18 x 3-4.5-5 μm (Fig. 3)

Skeleton: as genus description with addition that anthosigma form a layer over and amidst the selenaster cortex and are also prevalent in choanosomal tissue. Spherasters amidst selenaster cortex and dispersed in choanosome.

Distribution: type locality Sagami Bay, Eastern Japan, presently not recorded from any other locality.

Ecology: on rock substrate in deep temperate waters**.**

Remarks: Originally described by Tanita and Hoshino (1989) as *Geodinella anthosigma*. *Geodinella* is not a no longer a valid genus. *Geodinella anthosigma* should be transferred to the genus *Placospongia* based on the external morphology with the characteristic cortical plates and the presence of selenasters, tylostyles and spherasters. *Placospongia anthosigma* is distinguished from the other *Placospongia* spp. by the absence of small acanthose microrhabds, presence of stout elongated spirasters referred to by Tanita and Hoshino (1989) as 'anthosigma' and the small class of tylostyles with blunt points. The spirasters of *P. anthosigma* are larger than the acanthose microrhabds of *P. carinata* and *P. mixta* and are furthermore spirally decorated.

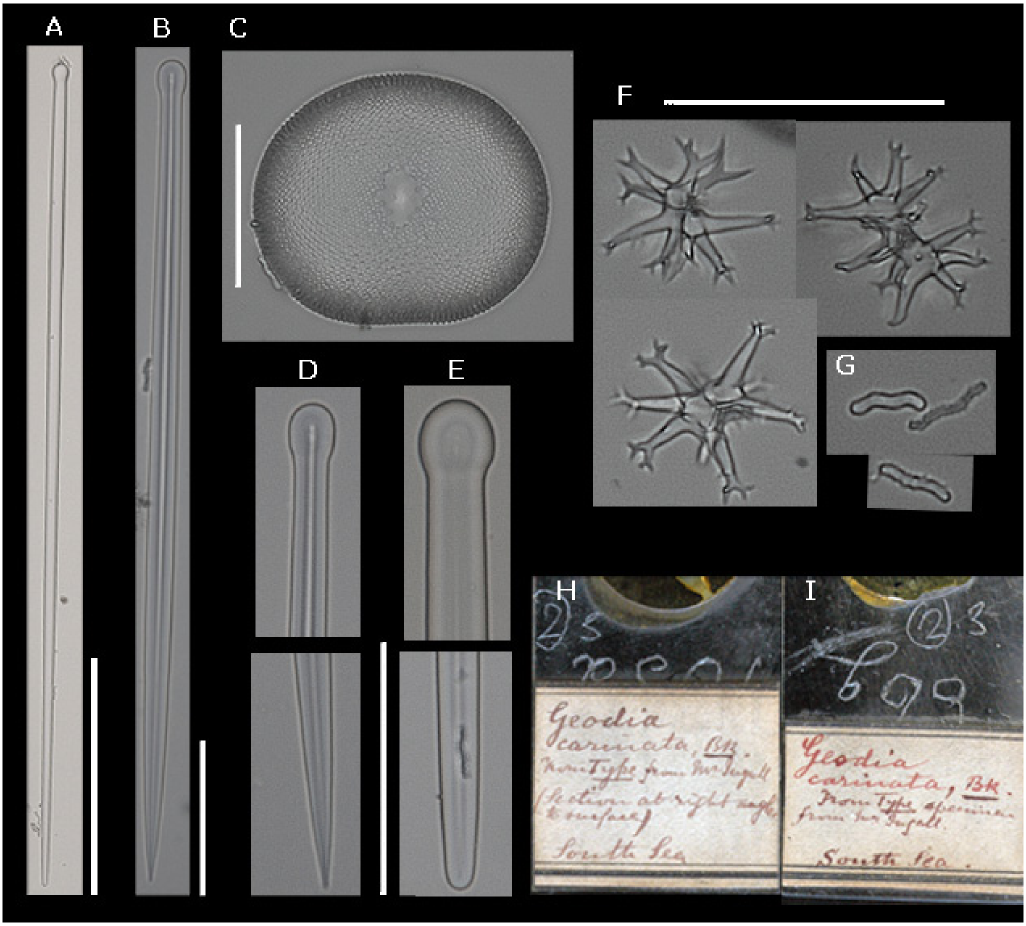

Figure 4. *Placospongia carinata* slide of holotype (BMNH, R1228, 86g, Bk.1390; R1275, PE01, Bk1390). A. large tylostyle (scale=200 µm), B. small tylostyle (scale=50 µm), C. selenaster (scale=50 µm), D. close up of large tylostyle (scale=50 µm), E. close up of small tylostyle, F. spirasters (scale=50 µm), G. microrhabds, H. original slide of thick section of holotype, I. original slide of spicules of holotype.

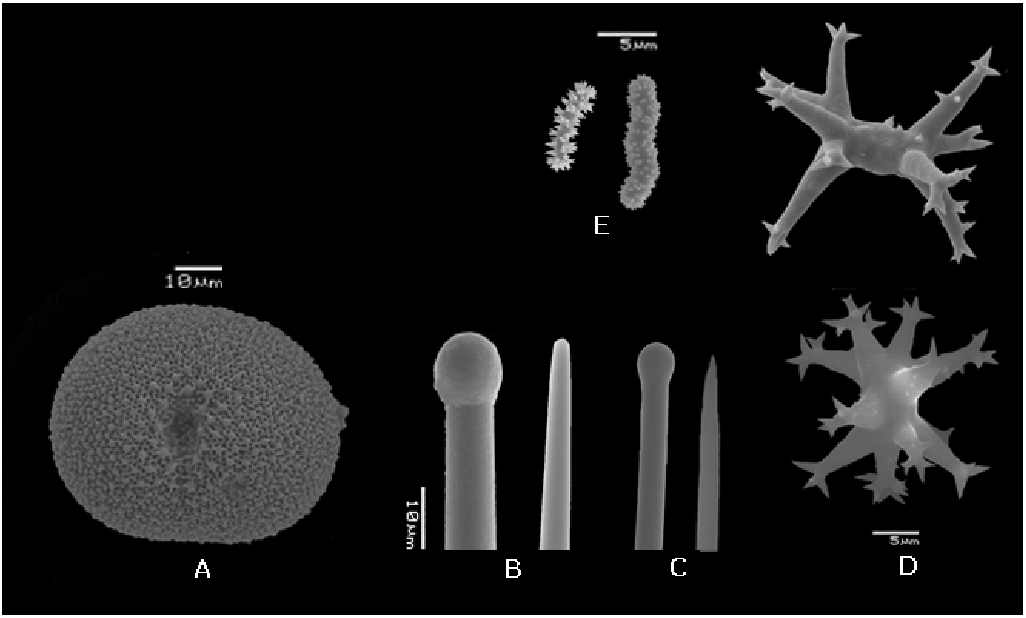

Figure 5. *Placospongia carinata* (RMNH POR. 4483). A. selenaster, B large tylostyle (head and blunt end), C. small tylostyle (head and hastate end), D. spirasters, E. acanthose microrhabds

Placospongia carinata **(Bowerbank, 1858)**

Figures 4 & 5

Geodia carinata Bowerbank, 1858: plate XXV Fig. 19
Geodia carinata Bowerbank, 1874: plate XLVI Figs 1-5

Material examined:

Lectotype. “South Sea”: BMNH R1228 - 86g - Bk.1390 (slide), R1275 - PE01 - Bk1390 (slide).

Vosmaer & Vernhout (1902), Siboga expedition. Indonesia, West Papua province, Raja Ampat region, SE Misool, Siboga stat. 164, S02°28’.5 E131°3’.3 E, 32m. depth: RMNH POR. 755 (#1848); Philippines, Sulu Sea region, Ubian Islands, Siboga stat. 99, 6°7.5’N 120°26’E, 16-23m. depth: RMNH POR. 754 (#1458); Indonesia, Moluccas province, W of Aru, Kur Island, Siboga stat. 250, 20-40m. depth: RMNH POR. 744 (#1500). **Other material.** Indonesia, East Kalimantan province, Berau region, Kakaban island, Kakaban lake, N02° 08’ 57.3” E118° 31’ 26.4”, marine lake, 0-2m. depth, coll. L.E. Becking, ix.2008: RMNH POR. 4484 (#KKB/mol1107), RMNH POR. 3943 (#KKB/mol716), RMNH POR. 3944 (#KKB/mol754), RMNH POR. 4485 (#KKB/mol763), RMNH POR. 3945 (#KKB/mol780), RMNH POR. 3946 (#KKB/mol810), RMNH POR. 3947 (#KKB/mol814), RMNH POR. 3948 (#KKB/mol825), RMNH POR. 3949 (#KKB/mol713), RMNH POR. 3950 (#KKB/mol1068); Indonesia, East Kalimantan province, Berau region, Maratua island, Haji Buang lake, N02° 12’31.2” E118° 35’46.8”, marine lake, 0-2m. depth, coll. L.E. Becking, ix.2008: RMNH POR. 3951 (#MA/mol700), RMNH POR. 3952 (#MA/mol975), RMNH POR. 3953 (#MA/mol947), RMNH POR. 3954 (#MA/mol1055), RMNH POR. 3955 (#MA/mol1012), RMNH POR. 4482 (#MA/mol1061), RMNH POR. 3956 (#MA/mol1001), RMNH POR. 3957 (#MA/mol1009), RMNH POR. 4483 (#MA/LE172), RMNH POR. 3958 (#MA/mol1500); Indonesia, Nusa Tenggara province, Komodo, NE cape, Snellius II Expedition, coll. R.W.M. van Soest, 1984: ZMA Por. 8813; Singapore, Pulau Salu: ZMA Por. 09578; Seychelles, Mahé, coll. R.W.M. van Soest, 1992: ZMA Por. 11367, ZMA Por. 16584, ZMA Por. 10727, ZMA Por. 1818, ZMA Por. 10481, ZMA Por. 20735; India, Laccadive Islands, Agatti Island, depth 20-25m, coll. National Institute of Oceanography, 1987: ZMA POR.9189.

Description: Reviewed material is encrusting and/or branching. External morphology follows the description of the genus. Color of live specimens can be purple brown, chocolate brown, milk coffee brown, orange brown, orange, or cream (Fig. 1 & 2). Color of choanosome is pale beige. After preservation in ethanol colors are similar to live specimens.

Spicules: Holotype slide with spicules R1228-86g-Bk.1390 (BMNH) and slide with thick section R1275-PE01-Bk1390 (BMNH) (Fig. 4): megascleres large straight tylostyles with blunt ends 500-**710**-820 x 10-**13**-15 X 10-15-18 µm, small straight tylostyles with sharp ends 140-**317**-450 x 5-**8**-25 X 8-**9**-13 µm; microscleres selenasters 80-**90**-98 µm, spirasters with varying number of rays (5-10) with bifurcating endings or tufts 23-**34**-43 x 8-15 µm, acantho microrhabds 8-**12**-18 x 1-2.5 µm, spherasters absent. The range within the examined material (Table 1 & Fig. 5): megascleres large tylostyles 540-990 x 8-18 X 10-18 µm, small tylostyles 175-550 x 3-10 X 3-13 µm; microscleres selenasters 50-85 x 35-70 µm, spirasters 15-48 x 5-18 µm, acantho microrhabds rhabds 5-18 x 1-2.5 µm, spherasters absent.

Skeleton: as genus description with addition that microrhabds form a layer over and amidst the selenaster cortex and are also prevalent in choanosomal tissue. Spirasters scattered in choanosome.

Distribution: Originally described from the 'South Sea', presumably the South Pacific Ocean. This has been interpreted by some (Rützler 2002, van Soest et al. 2011) to be Palau or Vanuatu, but this remains speculative. Based on the reviewed material and literature the minimal distribution is from Madagascar (Lévi 1956), to the Seychelles, and across Indonesia to the Aru Islands (Fig. 9). Distribution may extend further the East.

Ecology: In Indonesia rarely found in reef environment, but in high abundances in marine lakes. Possibly higher prevalence in reef in Eastern Africa, based on the ZMA Por. collection from the reefs in the Seychelles and the publication from Madagascar by Levi (1956).

Remarks: The Bowerbank description from 1858 should be considered as the original description of '*Geodia carinata*' , now accepted as *P. carinata,* with plates XXV Fig. 19 and XXVI Fig. 10 representing the spirasters ("arborescent elongo-subsphero-stella"). Subsequently in 1874 Bowerbank published a more extensive description of "*Geodia carinata"* including a drawing of the spirasters (Fig. 3, p.299) and spined microrhabds ("minute multiangulated cylindrical retentive spicula", fig.2, p.299) that he described as characteristic of the species. In neither publication registration numbers are given, however. The habitus drawing in Fig 5, p299 of Bowerbank publication in 1874 is identical to the specimen BMNH95.6.7.1 that I received from the BMNH after requesting the holotype for *P. carinata*. In addition, I received the slides of spicules (codes: R1228, 86g, Bk.1390) and of the thick cut (codes: R1275, PE01, Bk1390) that were labeled to belong to the holotype (Fig. 5). Upon inspection I discovered that the specimen BMNH 95.6.7.1 is in fact a *P. melobesioides*, while the two slides do indeed represent *P. carinata* containing the characteristic spirasters with bifurcating endings and the micro rhabds as indicated in the Bowerbank images and in the images taken from these slides in Fig. 5. The slides clearly do not represent specimen BMNH 95.6.7.1. In the 16 years between Bowerbank's 1858 and 1874 publications, I fear that there has been some exchange or misinterpretation of the labels of the specimens resulting in the incorrect assignment of specimen BMNH 95.6.7.1 to the slides and as the holotype of *P. carinata*. Furthermore, specimen BMNH 95.6.7.1 has two labels attached to it: one with "*Geodia carinata*", and one with "*Placospongia melobesioides*". According to Bowerbank (1874) three specimens had been reviewed for his manuscript: one received from his friend Mr. Thos. Ingall in 1854, one placed by Dr. Baird from the coral to the sponge collection in the BMNH, and one specimen purchased by the author in 1864. The first mentioned specimen is presumably the holotype, but as this specimen has not been located, I propose to designate the slides R1228- 86g-Bk.1390 and R1275-PE01-Bk1390 as representing the lectotype of *P. carinata*.

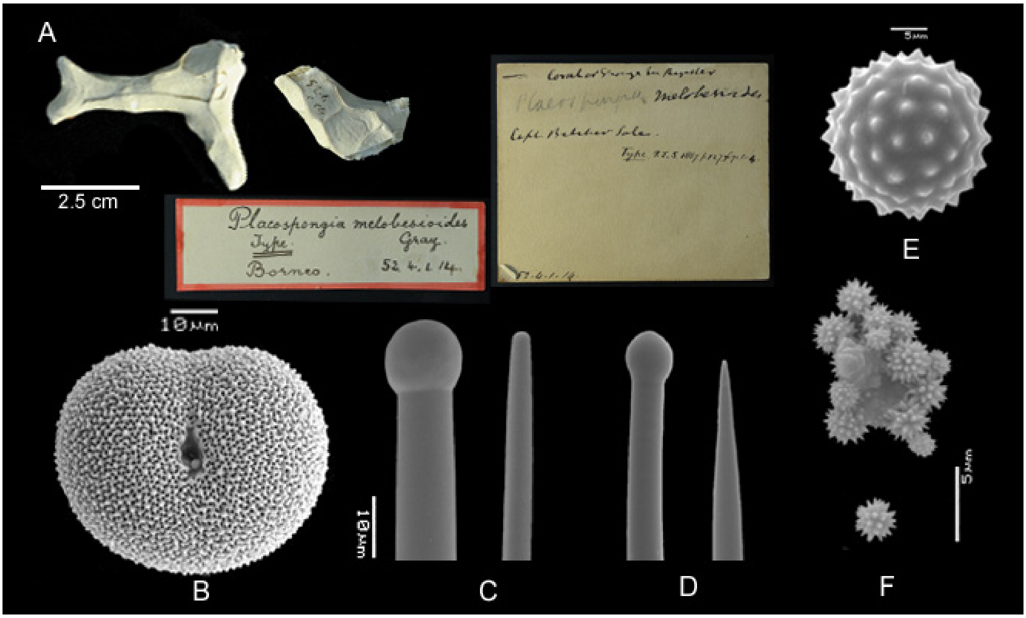

Figure 6. *Placospongia melobesioides* holotype (BMNH 52.4.1.14). A. Holotype with two labels, B. selenaster, C. large tylostyle (head and blunt end), D. small tylostyle (head and hastate end), E. spheraster, F. spherules

Placospongia melobesioides Gray, 1867

Figure 6

Placospongia melobesioides Gray (1867): Figs 1-4

Material examined:

Holotype. Indonesia, Borneo: BMNH 52.4.1.14

Vosmaer & Vernhout 1902. Indonesia, Nusa Tenggara province, N of Sumbawa, S07° 12.6' S E118° 7.7', 36m. depth: RMNH POR. 756 (#660); Indonesia, S of Moluccas, E04° 12' S129° 20.4', 45m. depth: RMNH POR. 761 (#1033); Indonesia, West Papua province, SE of Misool, Banda Islands, 32m. depth: RMNH POR. 758 (#1847), RMNH POR. 757 (#1849), RMNH POR. 760 (#1851), RMNH POR. 759 (#1853). **Other material.** Indonesia, East Kalimantan province, Berau region, Kakaban island, Kakaban lake, N02° 08' 57.3" E118° 31' 26.4", marine lake, 0-2m. depth, coll. L.E. Becking, ix.2008: RMNH POR. 3933 (#KKB/mol766), RMNH POR. 3934 (#KKB/mol767), RMNH POR. 4495 (#KKB/mol1075), RMNH POR. 3932 (#KKB/mol866), RMNH POR. 4496 (#KKB/mol776), RMNH POR. 4114 (#KKB/mol795); Indonesia, East Kalimantan province, Berau region, NE Maratua island, N02° 17' 32.3" E118° 35' 26.1", reef, 5-10m depth, coll. N.J. de Voogd, viii.2008: RMNH POR. 3935 (#BER113/mol689), RMNH POR. 3936 (#BER113/mol687); Indonesia, East Kalimantan province, Berau region, Sangalaki Island, N02° 05' 36.6" E118° 24' 15.2", reef, 5-10m. depth, coll. L.E. Becking, viii.2008: RMNH POR. 4497 (#BER107/mol603), RMNH POR. 3937 (#BER107/mol604), RMNH POR. 3938 (#BER107/mol608), RMNH POR. 3939 (#BER108/mol601); Indonesia, Moluccas province, Ternate, reef, 5-10m. depth, coll. N.J. de Voogd, xi.2009: RMNH POR. 3976, RMNH POR. 3977, RMNH POR. 3978 (#PM-TER02, #PM-TER08, #PM-TER12); Indonesia, North Sulawesi province, Bunaken, reef, 5-30m. depth, coll. L.E. Becking, ix.2006: RMNH POR. POR3166 (#LEMD13/69), RMNH POR. 3177

(#LEMD22/87), RMNH POR. 3154 (#LEMD05/30); Singapore, Pulau Semakau northwest Side, N01° 13’70”, E103° 45’61’”, reef, 10-12m. depth, coll. N.J. de Voogd, III.2006: RMNH POR. 2463 (# Sin05/270306/025), RMNH POR. 2464 (# Sin05/270306/026); Micronesia, Yap island, N09° 31’ 36.7” E138° 07’ 48.7”, reefflat in front of mangrove, 1-3m. depth, coll. L.E. Becking, viii.2010: RMNH POR. 3940 (#P-YAP1), RMNH POR. 3941 (#P-YAP2), RMNH POR. 3942 (#P-YAP3); Indonesia, South Sulawesi province, Spermonde archipelago, reef, 5-30m. depth, coll. N.J. de Voogd: ZMA Por. 13097; Seychelles, Mahé, coll. R.W.M. van Soest, xii.1992: ZMA Por. 10459, ZMA Por. 10496.

Description: Holotype BMNH 52.4.1.14 dry, chalky white angular branches, hard. Other examined material encrusting to branching, hard, thicker specimens slightly compressible. External morphology follows the description of the genus. Size ranging between 5-50 cm, though encrusting specimens may cover larger areas hidden within crevices. Ectosome color in living specimens ranges from purplish brown, dark black brown, chocolate brown, orange brown to light beige (Fig. 1 & 2). Choanosome pale beige. After preservation color of ectosome is similar to live color.

Spicules: Holotype BMNH 52.4.1.14 (Fig. 6): Megascleres large straight tylostyles with blunt ends 670-**880**-1010 x 10-**13**-18 x 10-16-20 μm, small concave to straight tylostyles with sharp ends 205-293-420 x 5-**10**-13 x 5-**10**-13 μm. Microscleres selenasters 58-**63**-68 x 45-**52**-68 μm, spherasters 15-17-18 μm (five measurements, not abundant), spherules 1-2-3 μm. The range within the examined material (Table 1): large tylostyles 460-1040 x 5-16 X 8-18 μm, small tylostyles 190-470 x 3-13 X 3-15 μm, selenasters 45-83 x 30-65 μm, spherules 1-3 μm, spherasters only found in singles in some individuals 15-20 μm.

Skeleton: as in genus description with addition of sporadic spherasters lodged amidst selenasters in cortex and high abundance of spherules in choanosome and cortex.

Distribution: Type locality: Borneo**.** Distribution from Seychelles to Micronesia (Fig. 9). Possibly further east to Central Pacific.

Ecology: Depth: 0-45m. Reefs, rocky shores, reefflats, mangroves, and marine lakes.

Remarks: In the original description by Gray (1867) there is no mention of two size classes of tylostyles. Reexamination of the original slide revealed that the holotype contains two size classes of tylostyles; the larger tylostyles with blunt endings and the smaller tylostyles with hastate endings. The Systema Porifera indicates that the holotype has two size classes, the large 720-**963**-1200 x 13-**14.1**-19 μm and the small 350-**438.8**-560 x **8-9.1**-10.5 μm, based on 10 measurements per spicule type (Rützler 2002). These measurements deviate from the holotype measurements in the present study that were based on 25 measurements per spicule type (670-**880**-1010 x 10-**13**-18 μm and 205-**293**-420 x 5-**10**-13 μm respectively), and also deviate from the range of sizes within the examined material of this study (Table 1). There is great variation in tylostyle length and spherasters are only sporadically present, often absent.

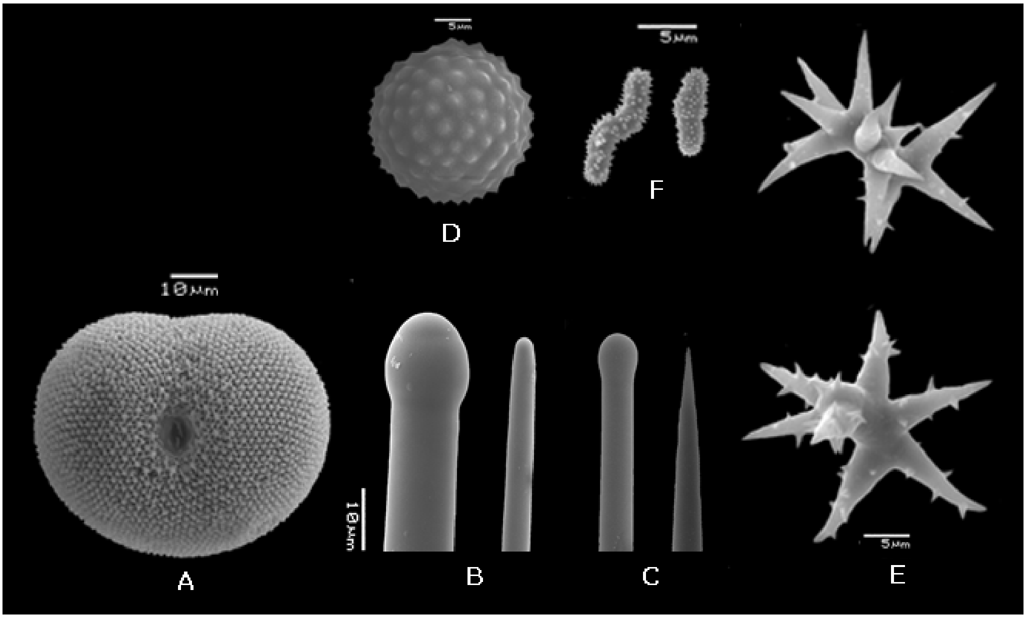

Figure 7. *Placospongia mixta* holotype (ZMB 3204) A. selenaster, B large tylostyle (head and blunt end), C. small tylostyle (head and hastate end), D. spheraster, E. spirasters, F. microrhabds

Placospongia mixta Thiele 1900

Figure 7

Placospongia mixta Thiele, 1900: Plate III, Fig. 25.

Material examined:

Holotype. Indonesia, Moluccas, Ternate: ZMB 3204

Vosmaer & Vernhout 1902. Indonesia, Moluccas province, Aru Islands, Siboga stat.273, 13m. depth: RMNH POR. 742 (#163a); Indonesia, South Sulawesi province, N. of Kabia Island, Siboga stat.213, 36m. depth: RMNH POR. 745 (#577); Indonesia, West Papua province, Raja Ampat region, E. of Misool, S01° 42.5’ E130° 47.5’, 32m. depth: RMNH POR. 753 (#311), RMNH POR. 763 (#1004), RMNH POR. 764 (#1850), RMNH POR. 765 (#1854), RMNH POR. 766 (#1856), RMNH POR. 751 (#1857). **Other material.** Indonesia, East Kalimantan province, Berau region, Kakaban island, Kakaban lake, N02° 08’ 57.3” E118° 31’ 26.4”, marine lake, 0-2m. depth, coll. L.E. Becking, ix.2008: RMNH POR. 4489 (#KKB/mol721), RMNH POR. 4490 (#KKB/mol830), RMNH POR. 3959 (#KKB/mol827), RMNH POR. 3960 (#KKB/mol829), RMNH POR. 3961 (#KKB/mol851), RMNH POR. 3979 (#KKB/mol 779); Indonesia, East Kalimantan province, Berau region, lighthouse near Berau river, N 02° 09’ 49.9” E 118° 10’ 12.8”, reef, 10m. depth, coll. L.E. Becking, viiI.2008: RMNH POR. 4491 (#BER109/mol629); Indonesia, East Kalimantan province, Berau region, Kakaban island, N02° 08’ 07.5” E118° 30’ 23.3”, reef, 10m. depth, coll. N.J. de Voogd, viii.2008: RMNH POR. 4492 (#BER111/mol666), RMNH POR. 3962 (#BER111/mol1203), RMNH POR. 3963 (#BER111/1209), RMNH POR. 3964 (#BER111/1213), RMNH POR. 3965 (#BER111/mol1219); Indonesia, North Sulawesi province, Bunaken, reef, 19m. depth, coll. L.E. Becking, ix.2006: RMNH POR. 3158 (#LEMD08/42), RMNH POR. 3148 (#LEMD40/21), RMNH POR. 3163(#LEMD11/52), RMNH POR. 3155 (#LEMD06/32), RMNH POR. 3157 (#LEMD08/39); Indonesia, Moluccas province, Ternate, reef, coll. N.J. de Voogd, xi.2009: RMNH POR. 4112, RMNH POR. 4113 (#P-TER11, #P-TER22); Indonesia, West Papua province, Raja Ampat region, Gam island, Ctenophore lake, S0°27’17.46” E130°29’33.77”, marine lake, 0-2m. depth, coll. L.E. Becking, xi.2007: RMNH POR. 4494 (#RAJ23/mol199), RMNH POR. 3966 (#RAJ23/mol195), RMNH POR. 3967 (#RAJ23/mol187); Indonesia, West Papua province, Raja Ampat region, Waigeo Island, Teluk Mayabilit, S00°18’17.04” E130°54’15.60”, reef, 10m. depth, coll. L.E. Becking, xii.2007: RMNH POR. 4493 (#RAJ64/mol428), RMNH POR. 3968 (#RAJ64/mol429), RMNH POR. 3969 (#RAJ64/mol430), RMNH POR. 3970 (#RAJ64/mol431), RMNH POR. 3971 (#RAJ64/mol432), RMNH POR. 3972 (#RAJ64/mol433); Indonesia, West Papua province, Raja Ampat region, Fam Island, S00° 36’ 01.5” E130° 45’ 08”, rocky shore, 0-1m. depth, coll. L.E. Becking, xi.2007: RMNH POR. 3973 (#RAJ39/mol249), RMNH POR. 3974 (#RAJ39/mol250), RMNH POR. 3975 (#RAJ39/mol254); Seychelles, Mahé, southeast coast, near Pointe Cocos, IOP-E stat.738/08, coll. R.W.M. van Soest, 1992: ZMA Por. 10495**;** Indonesia, South Sulawesi province, SW Salayer, reef N of Pulau Bahuluang, Snellius Expedition II stat.079/1, coll. R.W.M. van Soest, 1984: ZMA Por. 0896.

Description: Holotype ZMB3204 encrusting, size 5 x 2.5 cm and thickness 1–5 mm (as described by Thiele, now very small fragment), white after preservation in alcohol. The majority of the reviewed material is encrusting with a thickness of 4-10mm, but branching specimens also occur. External morphology follows the description of the genus. Color of the ectosome can be red, orange, brown orange, dark brown, chocolate brown, milk coffee brown, cream, or white (Fig. 1 & 2). Color of choanosome is pale beige. After preservation in ethanol color is similar to live specimens.

Spicules: Holotype ZMB 3204 (Fig. 6) Megascleres large straight tylostyles with blunt/rounded point 355-**672**-940 x 7.5-**12**-17.5 x 7.5-**16**-20 μm, small straight tylostyles with sharp point 165-**226**-275 x 2.5-**6**-7.5 x 2.5-**8**-10 μm*;* microscleres selenasters 55-**70**-75 x 42.5-**55**-72.5 μm, spherasters (abundant) 20-**25**-30 μm, spirasters typically with well developed axis and with 4-9 rays with hastate endings, rays can be spined, but do have no bifurcations of the tips 15-**24**-32.5 x 2.5-**8**-12.5 μm; acanthose microrhabs with straight or zig-zag axis 5-7-10 x <2.5 μm. The range within the examined material (Table 1): large tylostyles 460-1250 x 8-23 X 10-25 μm, small tylostyles 120-430 x 3-15 X 2-15 μm, selenasters 50-85 x 22-73 μm, spherasters 13-30 μm, spirasters 15-35 x 2-15 μm, rays 5-18 x 1-2.5 μm .

Skeleton: as description of genus with addition that microrhabds form a layer over and amidst the selenaster cortex and are also prevalent in choanosomal tissue. Spirasters scattered in choanosome. Spherasters amidst selenasters in cortex and scattered in choanosome.

Distribution: East African coast to eastern Indonesia. Possibly further east to Central Pacific. Pulitzer-Finali (1993) identified a '*P. carinata'* from East Africa (Mombasa) that fits the description of *P. mixta* based on the length of the tylostyles (up to 1200 μm) and the presence of spherasters. No *P. mixta* specimens were observed in the Seychelles material deposited at ZMA.

Ecology: Common reef species, also occurs in marine lakes.

Remarks:

In 1900 Thiele described a new species named *P. mixta*, of which the holotype was originally identified as *P. melobesioides* by Kieschnick (1896). The specific epithet *mixta* was given because the specimen contained a mixture of spicules: both spirasters like *P. carinata* as well as large spherasters like *P. intermedia* and *P. melobesioides*, which are absent in *P. carinata*. In 1902 Vosmaer & Vernhout decided that *P. mixta* was a junior synonym of *P. carinata*, because they saw no distinction between the different shapes of spirasters and stated that spherasters are never very abundant – in some 'exceedingly rare and in some we failed to find them at all' – and could therefore not be seen as a distinguishing character. In the present study the specimens of Vosmaer and Vernhout (1902) were reexamined (see material and Methods). After inspection, the specimens labeled '*P. carinata*' could be clearly and consistently divided into two species: *P. carinata* without spherasters, with spirasters displaying bifurcating endings, and tylostyles up to 980 μm, and *P. mixta* with abundant spherasters, with spirasters displaying hastate endings, and tylostyles up to 1250 μm. In none of the specimens of Vosmaer & Vernhout (1902), nor in the other specimens reviewed for this study was there a mixture of the two types of spirasters. These species also show molecular distinction in both mitochondrial and nuclear markers (Fig. 9, Table 2 & 3).

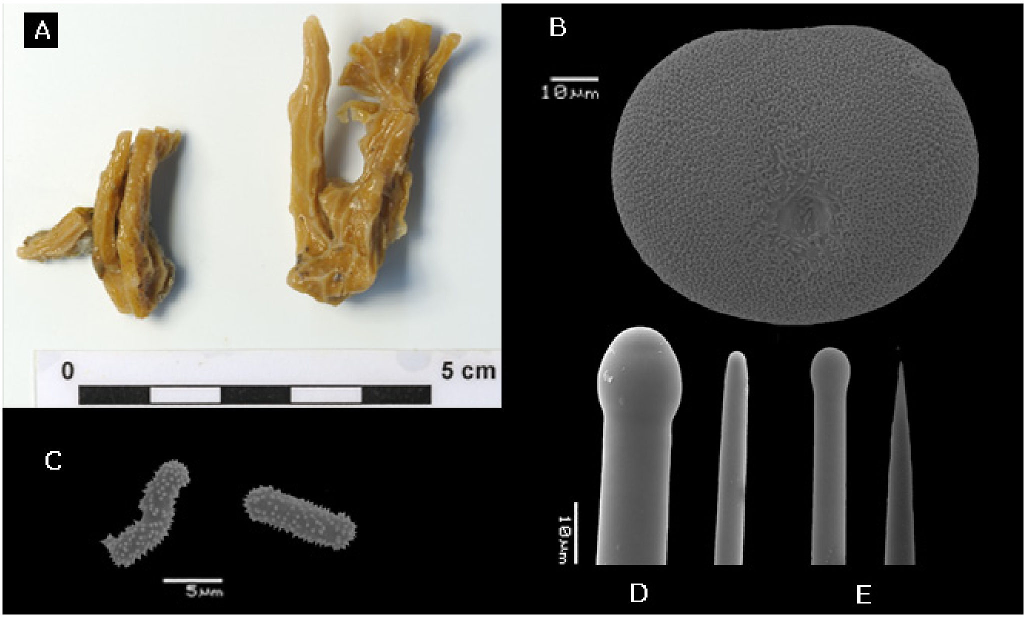

Figure 8. *Placospongia santodomingoae* sp.n. (RMNH POR. 4486) A. ethanol preserved specimen, B. selenaster, C large tylostyle (head and blunt end), D. small tylostyle (head and hastate end), E. microrhabds

Placospongia santodomingoae **sp.n.**

Figure 8

Holotype. Indonesia, East Kalimantan province, Maratua island, Buli Halo anchialine pool, N02° 11' 16.4" E118° 37' 06.4", anchialine pool, 0-1m. depth, xi.2008, coll. N.K. Santodomingo & Estradivari: RMNH POR. 4486 (#BER128/mol1147). **Paratypes.** Indonesia, East Kalimantan province, Maratua island, Buli Halo anchialine pool, N02° 11' 16.4" E118° 37' 06.4", anchialine pool, 0-1m. depth, xi.2008, coll. N. K. Santodomingo & Estradivari: RMNH POR. 4487 (#BER128/1125), RMNH POR. 4488 (#BER128/1156).

Description: Holotype and paratypes are branching and encrusting, size 8cm in length.Total size of specimens *in situ* is hard to establish as parts of the body may be encrusting within cracks. Alcohol-preserved and live specimens are hard but slightly compressible. The surface is made up of typical *Placospongia* cortical plates separated by contractible grooves which form a network on the surface. Oscules are present in the grooves. Color of the live holotype was orange-brown, the paratypes were orange, and these colors become slightly lighter after alcohol preservation (Fig. 8A).

Spicules: Holotype (Fig. 8) megascleres large straight tylostyles with blunt point 430-**605**.5-660 x 13-**15**.5-20 x 13-**18.1**-23 μm, small straight tylostyles with sharp point 240-**261.3**-290 x 5-**7.2**-8 x 5-**8.8**-10 μm; microscleres selenasters 80-**84.8**-90 x 60-**67.3**-75 μm, acanthose microrhabds 8-**12.3**-18 x 2.5-**2.7**-3.5 μm. Range of the paratypes (Table 1) large straight tylostyles with blunt point 430-**480**-760 x 13-**20** x 15-23 μm, small straight tylostyles with sharp point 190-380 x 5-13 x 8-15 μm, microscleres selenasters 63-93 x 58-75 μm, acanthose microrhabds with straight axis 5-20 x 2.5-3.5 μm.

Skeleton: the cortical plates consist of densely packed selenasters, microrhabds form a layer over and amidst this selenaster cortex and are also prevalent in choanosomal tissue. Developmental stages of selenasters occur throughout the choanosome. Tylostyle tracts support the margins of the cortical plates in radial tracts from the centre core (consisting of densely packed selenaster) to the cortical plates. The sharp ends of the smaller tylostyles can be projected beyond the cortex surface.

Distribution: presently only recorded from Buli Halo anchialine pool on Maratua island, Berau, East Kalimantan, Indonesia (Fig. 9).

Ecology: occurs in anchialine pool, can be exposed to air during low tide and can tolerate great fluctuations in salinity (from 24 to 33 ‰)

Etymology: named in honor of Nadiezhda K. Santodomingo, the collector of the types, for her years of tireless work in marine science including research on anchialine systems.

Remarks: *Placospongia santodomingoae* sp. n. is similar to *P. carinata,* yet lacks spirasters and has shorter tylostyles. *Placospongia santodomingoae* sp. n. likewise differs from *P. mixta* by the absence of spirasters as well as the absence of spherasters. *Placospongia santodomingoae* sp. n. differs from *P. anthosigma* by the absence of anthosigma, and by having hastate endings of the smaller tylostyles.

Geodia labyrinthica (Kirkpatrick, 1903)

Placospongia labyrinthica Kirkpatrick 1903: Plate V Fig. 1a-b, Plate VI Fig. 1a-f

Reviewed material:

Holotype. South Africa, East London Coast, S33° 06’ 30” E028° 11’: BMNH 02.11.16.1

Spicules: megascleres styles, oxea; microscleres sterrasters, chiasters

Remarks:

This species was originally described as ‘*Placospongia labyrinthica’,* but does not have the characteristic cortical plates of *Placospongia* and has sieve pores, sterrasters with star-like plates, euasters, styles and oxea characteristic of the Geodiidae. In the original description by Kirkpatrick (1903) stated “the presence of chiasters is so exceptional that I thought at first that I had to deal with a geodine sponges, but there were no triaenes to be found” and as a result placed this species in the *Placospongia* rather than *Geodia*. Genus transfer to *Geodia* is required as suggested on the World Porifera Database (van Soest et al. 2011).

Identification key for Indo-Pacific species of *Placospongia*

1. Spirasters absent..........2

Spirasters present..........3

2. Spherules present..........*P. melobesioides*

Spherules absent, acanthose microrhabds present..........*P. santodomingoae* sp.n.

3. Spirasters with elongated rays of 4-18 μm, acanthose microrhabds present..........4

Spirasters stout with short acanthose spines spirally placed on shaft..........*P. anthosigma*

4. Spirasters have rays with ends bifurcating or with tufts..........*P. carinata*

Spirasters have rays with hastate ends, spherasters present..........*P. mixta*

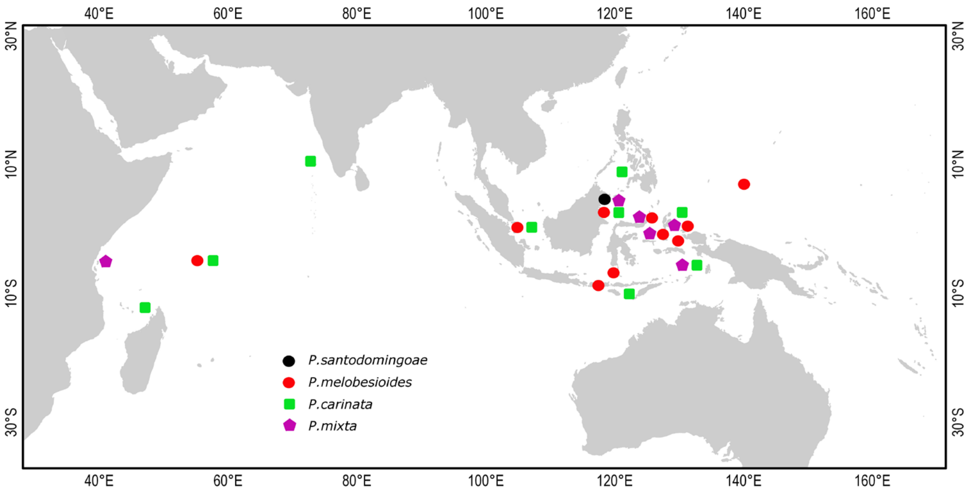

Figure 9. Distribution of *Placospongia* spp. in the Indo-West Pacific. Location of symbols is approximate.

Genetic data analysis

Final alignments (excluding primers) were obtained for the sponge *Placospongia* of 581 bp for COI with three genetic variants (25 individuals) and 13 polymorphic sites. The three genetic variants correspond to the three species *P. melobesioides, P. mixta,* and *P. carinata* that represent monophyletic groups which are strongly supported by both Bayesian and maximum likelihood inference methods (Fig. 10). There was no intra-specific variation within each species, regardless of geographic locality. The inter-specific *p*-distances ranged between 0.5-2.1% (Table 2). There were 11 substitutions between *P. melobesioides* and *P. carinata,* 12 substitutions between *P. melobesioides* and *P. mixta,* and three substitutions between *P. mixta* and *P. carinata*. The specimens of *P. carinata* and of *P. santodomingoae* sp.n. had identical genotypes for COI. No molecular work could be done on the dried holotype of *Placospongia anthosigma* and fresh material was not available. Final alignments (excluding primers) of 788bp were obtained for ITS with 18 genetic variants from the present study (21 individuals) and 27 genetic variant from GenBank (for GenBank accession numbers see figure 11). The ITS sequences represented five clades that were strongly supported by both Bayesian and maximum likelihood inference methods (Fig. 11). These five divergent clades (see Table 3 for uncorrected inter- and intaspecific *p*-distances) correspond to the clades C3, C4, C5, C6, and C9 as presented by the study of Nichols & Barnes (2005). Clade C9 represents specimens of the species *P. melobesioides,* clade C5 *P. mixta,* and clade C4 *P. carinata*. Clades C6 is represented by one specimen from the Solomon Islands (QM317896) and clade C3 by one specimen from Bynoe Harbour, Northern Territory, Australia (QM303439); none of the samples sequenced in the present study fell into either C3 or C6 clade. The specimens of *P. carinata* without spirasters represented a separate lineage within the *P. carinata* clade (C4) which was supported by Bayesian inference, but not by maximujm likelihood. The *p*-distance between *P. carinata* specimens and the specimens of *P. santodomingoae* sp.n. was 0.6%.

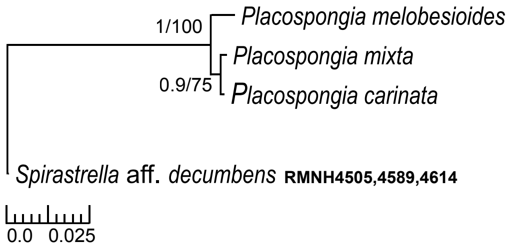

Figure 10 Bayesian/maximum Likelihood phylograms Cytochrome Oxidase I (COI) sequences from Indo-Pacific *Placospongia* spp. *Placospongia melobesioides* was represented by RMNH POR. 2464, 3166, 4497, 3942, 3976, 4495, 4496, ZMA POR.10496; *Placospongia mixta* by RMNH POR. 3158, 3936, 4113, 4489, 4490, 4492, 4494, 4493; *Placospongia carinata* by RMNH POR. 4482, 4483, 4484, 4485, 4486, 4487, 4488, ZMA Por. 10727, ZMA Por. 11367. Only posterior probabilities of >90 and Maximum Likelihood values of >70 indicated. Scale bars indicate substitutions/site.

Table 2 The number of base differences per site from averaging over all COI sequence pairs between *Placospongia* spp. groups are shown (uncorrected *p*-distances). Standard error estimate(s) are shown above the diagonal. The analysis involved 30 nucleotide sequences. There was no within-group difference. *Spirastrella* aff. *decumbens* was used as outgroup in the phylogenetic inference of Fig. 10.

% *p*-distance COI	*P. melobesioides*	*P. mixta*	*P. carinata*	*Spirastrella* aff. *decumbens*
P. melobesioides	*	*0.6*	*0.6*	*1.3*
P. mixta	2.1	*	*0.3*	*1.2*
P. carinata	1.9	0.5	*	*1.3*
Spirastrella aff. *decumbens*	12.2	11.5	11.7	*

Table 3 The number of base differences per site from averaging over all ITS sequence pairs between *Placospongia* spp. groups are shown (uncorrected *p*-distances). Standard error estimate(s) are shown above the diagonal. The analysis involved 73 nucleotide sequences. All positions with less than 5% site coverage were eliminated. Black cursive along the diagonal indicates within-group uncorrected *p*-distance. C9, C5, C6, C4, C3 refer to five clades with the Indo-West Pacific *Placospongia* as presented in Fig. 11.

% *p*-distance ITS	*P.melobesioides*	*P. mixta*	*P. carinata*	*P. santodomingoae* sp.n.	C9	C5	C6	C4	C3
P.melobesioides	***0.1***	*1.3*	*1.4*	*1.4*	*0.3*	*1.3*	*1.3*	*1.3*	*1.3*
P. mixta	13.8	***0.7***	*0.9*	*0.9*	*1.2*	*0.2*	*0.5*	*0.9*	*0.9*
P. carinata	14.7	6.3	***0.4***	*0.2*	*1.3*	*0.9*	*0.9*	*0.2*	*0.9*
P. santodomingoae sp.n.	13.2	5.8	0.6	***1.6***	*1.3*	*0.9*	*0.9*	*0.3*	*0.9*
C9	0.9	13.5	14.6	13.6	***0.1***	*1.2*	*1.2*	*1.2*	*1.2*
C5	13.5	0.9	6.6	6.1	12.9	***0.7***	*0.5*	*0.8*	*0.9*
C6	14.0	2.2	6.4	6.1	13.2	2.2	***0.1***	*0.8*	*0.8*
C4	14.8	6.3	0.5	0.9	14.3	6.3	6.0	***0.4***	*0.8*
C3	15.2	7.1	6.1	5.9	14.5	6.9	6.3	5.6	***0.9***

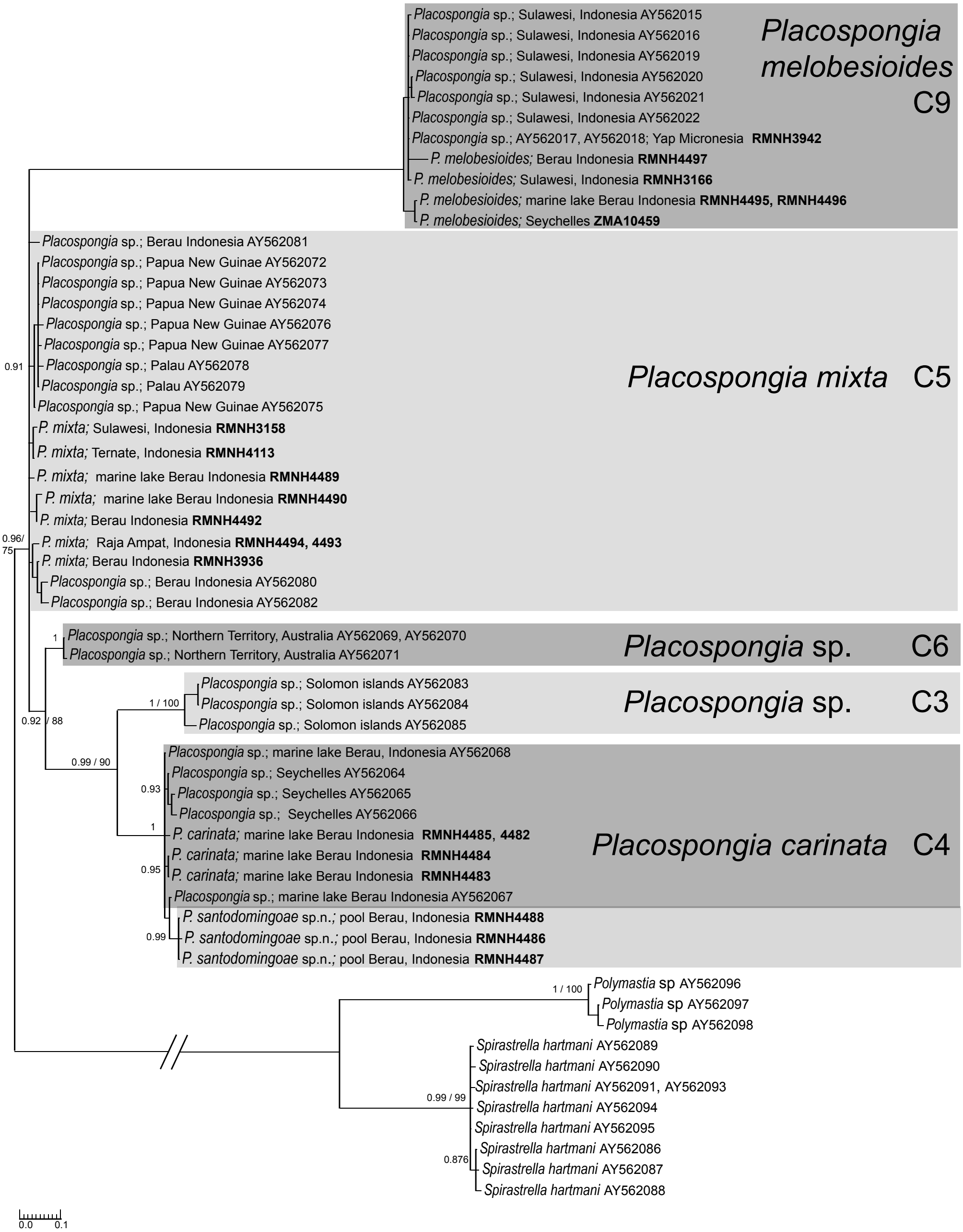

Figure 11 Bayesian/maximum likelihood phylograms of genotypes of the internal transcribed spacer region of nuclear ribosomal operons (ITS) of Indo-Pacific *Placospongia* spp. found in this study and related species from the same genus collected from GenBank. Clades C3,C4,C5,C6 & C9 refer to the clades presented in the study by Nichols & Barnes (2005). Taxon labels are organized as follows: Specimen - Locality - Genbank code or RMNH POR. Number. Only posterior probabilities of >90 and Maximum Likelihood values of >70 indicated. Scale bars indicate substitutions/site.

Discussion

Different species

In the Indo-West Pacific at least five species of the genus *Placospongia* can be identified based on spicule morphology. *Placospongia melobesioides, P. carinata,* and *P. mixta* can be distinguished with the DNA barcode marker (COI) and a nuclear marker (ITS). The species *P. santodomingoae* sp.n. and *P. carinata* have the same sequence of COI. The sequence variation for mitochondrial DNA and particularly COI in sponges is known to be low (Wörheide 2006, Xavier et al. 2010, Pöppe et al. 2011) and this is also the case in species of *Placospongia*, e.g. only 0.5% nucleotide distance between the species *P. mixta* and *P. carinata.* There is furthermore no intraspecific variation in COI within each of the *Placospongia* species, not even between populations at 1000s of km distance from each other (e.g. specimens ZMA Por. 11367 and ZMA Por. 10727 from the Seychelles are identical with specimens RMNH POR. 4483, RMNH POR. 4482, RMNH POR. 4484, RMNH POR. 4485 from Indonesia). The phylogenetic inference based on the ITS sequences does show a supported clade of *P. santodomingoae* sp.n. within the clade of *P. carinata* (Fig. 11)*,* though the degree of divergence between the two species is low (0.6%) (Table 3). *Placospongia santodomingoae* sp.n. should, however, be designated as a new species based on the spicule morphology: the absence of a distinguishing spicule type (spirasters) and consistently shorter and thicker tylostyles (maximum 760 x 20 µm) compared to *P. carinata* (maximum 980 x 17.5 µm) are valid arguments to distinguish a separate species within this genus. The specimens of *P. santodomingoae* sp. n. were collected from an anchialine pool. This kind of isolated environment has previously been shown to contain small, rapidly evolving populations, and many rare novel species across a large spectrum of taxa (e.g. Holthuis 1973, Tomascik & Mah 1994, Dawson & Hamner 2005, Becking et al. 2011, CHAPTERS 3, 4, 6 & 7). The divergence of *P. santodomingoae* sp.n. from *P. carinata* is likely too recent to be expressed in the molecular markers I used. Other, faster evolving, molecular markers might show a more distinct separation between species, but for the present significant morphometric differences in spicules are reliable characters in separating these sister species.

A molecular phylogeny using the internal transcribed spacer region (ITS) showed that there were five distinct clades within the genus *Placospongia* in the Indo-West Pacific (clades C3, C4, C5, C6 & C9) (Nichols & Barnes, 2005). Nichols & Barnes (2005) indicated that their results presented a conundrum that “specimens collected from Indonesian marine lakes that have been isolated from the surrounding marine environment since the Pleistocene are undifferentiated from individuals collected from the Seychelles indicating that populations from these geographically disparate regions are, or have recently been, connected by gene flow despite the lack of evidence of connectivity between these lakes and nearby reefs.” It is important to note here that the authors did not investigate the spicule morphology of the specimens in their study, while it is in fact the spicules that can largely explain the presented conundrum. In the present study over 30 specimens from the marine lakes Kakaban and Maratua and the adjacent reefs have been reviewed as well as the specimens from the ZMA Por. collection that were used in the Nichols & Barnes study. Clade C4 represents the material from the Seychelles (ZMA Por.11367) together with the marine lakes and can all be morphologically identified as *P. carinata sensu stricto*. The samples from the lakes and the Seychelles are thus conspecific, but the populations of the two locations are necessarily connected by gene flow. Subsequently clade C9 is *P. melobesioides* (specimens from Indonesia, Miscronesia and the Seychelles) and clade C5 is of *P. mixta* (specimens from Indonesia, Palau and Papua New Guinae). This explains three of the five clades from the Indo-West Pacific and leaves two undetermined: clade C3 represented by one specimen from Bynoe Harbour, Northern Territory, Australia (QM303439), and clade C6 represented by one specimen from the Solomon Islands (QM317896). The 'Mudmaps' of these specimens in the Queensland Museum portray images of spicules that fit the definition of *P. mixta* (in particular the spirasters with hastate endings). The morphology of these specimens should be further studied in order to determine if they may represent morphologically cryptic species.

Natural variation

Each of the five species of the genus *Placospongia* in the Indo-West Pacific can be distinguished based on spicule composition. The external morphology, however, does not allow species distinction. The most common species from the tropical Indo-West Pacific (*P. melobesioides, P. mixta, and P. carinata*) can have both encrusting and branching growth forms displaying a variety of colors from white to dark brown. All the red specimens appeared to belong to *P. mixta,* while all the dark black-brown specimens belonged to *P. melobesioides.* These two colors may be useful for field identifications, yet both species can also display the range of other colors (white, cream, beige, light brown) as well. The density of canals/ridges (or size of cortical plates) appears to be related to environment as this is higher in specimens from high sediment locations such as the marine lakes than in specimens from the reefs (Fig. 1). Within each species there is also some natural variation in the range of tylostyle length and spicule morphology. The spiraster morphology varies within species and even within individuals. Within one individual the number of rays can vary from 4-10 (Figs 3, 4) and between individuals the ornamentation and size of spines can be diverse. For example the spirasters of *P. carinata* specimens from Haji Buang marine lake are micro-acanthose while the specimens from other locations are not. Spherasters are always present and abundant in *P. mixta* and *P. anthosigma,* but are in low abundances or absent in *P. melobesioides*, as has been indicated previously by Vosmaer & Vernhout (1902). In *P. carinata* and *P. santodomingoae* sp.n. spherasters are always absent.

Ecology & Distribution

P. melobesioides and *P. mixta* are common in the reef environment. Most of the collected material from the reefs in Indonesia were one of these two species. *P. carinata* appears to be rare in the reefs, in Indonesia at least, while it is highly abundant in the marine lakes Haji Buang and Kakaban in East Kalimantan, Indonesia. *Placospongia santodomingoae* n.sp. is restricted to an anchialine pool. *Placospongia anthosigma* was not found in any of the examined collections from the tropical western Pacific, this species is restricted to more temperate and deeper waters. *Placospongia melobesioides* is indicated in the Systema Porifera to have a distribution from the Indo-West Pacific to the Tropical Atlantic (Rützler, 2002). Both *P. melobesioides* and *P. carinata* have been recorded from the Atlantic (e.g. de Laubenfels 1936, Hechtel 1976, Coelho & Mello-Leitão 1978, Pulitzer-Finali 1986, González-Farías 1989), which would imply that these are pantropical species. Recent molecular and more detailed morphological studies have, however, shown that many cosmopolitan sponge species are in fact species complexes either delineated by morphology or molecules (e.g. Reveilleud et al. 2010, Xavier et al. 2010). Van Soest (2009) has indicated that there are at least five species of *Placospongia* in the Caribbean that are morphologically different from the holotypes of *P. melobesoides* and *P. carinata.* Rua et al. (2006) and Nichols & Barnes (2005), furthermore, show that there are distinct lineages in the Caribbean and western Pacific, that are not shared between the two regions and that most likely represent undescribed species in the Caribbean. Considering these results as well as the large geographic distance between the Caribbean and the type localities of *P. melobesioides* and *P. carinata* (both Indo-West Pacific), it is highly unlikely that these species occur in the Tropical Atlantic. Further revision of the Atlantic and eastern Pacific material will shed more light on this issue.

Future biodiversity surveys and species checklists both in the Atlantic as well as in the Pacific are advised to check the spicule morphology of *Placospongia* specimens in order to identify species, as the external morphology and color will not give an indication to the number of species. The different *Placospongia* spp. can occupy the same type of habitats in the tropics. The epitome of this sympatry is represented in Kakaban lake where in the 4 km^2 area of the marine lake three common tropical species co-exist. Without review of the spicule morphology the true diversity of species in the study area would perhaps remain concealed.

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IV PHYLOGEOGRAPHY

Sponges - they say - they DO it!?

Cole Porter , 1928

“Let’s do it”, as sung by Billie Holiday

Chapter 6

Phylogeography of the sponge *Suberites diversicolor* in Indonesia: insights into the evolution of marine lake populations

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Abstract

Phylogeographic studies of taxa inhabiting marine lakes provide excellent opportunities to study biogeographical relationships and population structures of marine species in isolated habitats. Marine lakes are landlocked water bodies that maintain a marine character through narrow submarine connections to the sea and could be regarded as the marine equivalents of terrestrial islands. The sponge *Suberites diversicolor* (Demospongiae: Suberitidae) is typical of marine lake habitats in the Indo-Australian Archipelago. We employed four molecular markers (two mitochondrial and two nuclear) to study its genetic structure within and between marine lakes in Indonesia and three coastal locations in Indonesia, Singapore and Australia. Within the populations of *S. diversicolor* there were two strongly divergent lineages (A & B) (COI: p=0.4% and ITS: p=7.3%), that may constitute cryptic species. Lineage A only occurred in Kakaban lake (East Kalimantan), while lineage B was present in all sampled populations. Within lineage B, we found low levels of genetic diversity in lakes and strong spatial genetic population structuring. The Australian population is genetically differentiated from the Indonesian populations. Within Indonesia with we did not record an East-West barrier which has frequently been reported for other marine invertebrates. Kakaban lake is the largest and most isolated marine lake in Indonesia and contains the highest genetic diversity with genetic variants not observed elsewhere. Kakaban may be an area where multiple putative refugia populations have come into secondary contact, resulting in the high genetic diversity and the high number of endemics.

Keywords
anchialine • cryptic species • Indo-Australian Archipelago • marine lakes • COI • ITS

Introduction

It has long been hypothesized that marine species have large geographic ranges with large population sizes, and are faced with weaker barriers to dispersal than terrestrial organisms, thus resulting in relatively slow rates of speciation (Palumbi 1994). The assumed presence of circum-tropical species has for long supported this view. However, recent phylogeographic and population genetic studies on marine taxa portray a situation of ecologically heterogeneous environments on small spatial scales with several morphologically cryptic species instead of cosmopolitan species (e.g. Knowlton 2000, Barber et al. 2002, Peijnenburg et al. 2004, Nuryanto & Kochzius 2009, Malay & Paulay 2010, Reveillaud et al. 2010, Xavier et al. 2010a, Gaither et al. 2011). These results suggest that there may be many more barriers to dispersal at small spatial scales than we are able to observe (Palumbi 1994, Carpenter et al. 2011, Goetze & Peijnenburg *submitted*). The existence of multiple independently derived populations in landlocked marine lakes provides an opportunity for fundamental research into the role of isolation in population divergence and speciation in marine taxa (Dawson & Hamner 2005). Marine lakes are anchialine systems, which are landlocked water bodies that maintain a marine character through narrow submarine connections to the sea (Holthuis 1973, Hamner & Hamner 1998). The lakes are formed in natural inland depressions and display a tidal regime which is typically delayed and damped compared to the adjacent sea (Holthuis 1973, Hamner & Hamner 1998, Becking et al. 2011, *CHAPTER 1*). The level of obstruction of water echange, i.e. the degree of isolation, differs per lake as does the salinity and environmental regimes within the lakes (Hamner & Hamner 1998, Becking et al. 2011, *CHAPTER 1*). The number of marine lakes worldwide is estimated at approximately 200 with clusters of ten or more lakes occurring in areas with a karstic limestone landscape such as Croatia, Bermuda, Vietnam, Palau, and Indonesia (Dawson 2009).

The marine lakes share many characteristics with island systems (Dawson 2006): they are well-defined geographically (Hamner & Hamner 1998, Colin 2009, Becking et al. 2011, *CHAPTER 1*), harbor unique biota with high endemism and/or an abundance of species rare that are elsewhere (Tomascik & Mah 1994, Tomascik et al. 1997, Azzini et al. 2007, Dawson 2009, Becking et al. 2011, *CHAPTER 1, 3, 4 & 5*), and isolated populations (Dawson & Hamner 2005, Gotoh et al. 2009, Goto et al. 2011). The marine lakes in the Indo-Pacific were formed less than 15000 years ago (Dawson 2006, Sathiamurthy & Voris 2006), yet their biodiversity is unique. Recent comprehensive studies of sponge assemblages of marine lakes, coastal mangroves and coral reefs in Berau (East Kalimantan, Indonesia) indicated that these lakes harbor a significantly different assemblage consisting of a subset of the fauna of the adjacent (de Voogd et al. 2009, Becking et al. *accepted & unpublished data, CHAPTERS 2 & 3*). Extensive surveys have shown that the lake assemblages represent three groups of sponge species: 1. widespread species known from various coastal locations in Indo-Pacific reefs, 2. lake species that only occur in lake systems, 3. endemic species restricted to only one single lake (de Voogd et al. 2009, Becking et al. *accepted & unpublished data, CHAPTERS 2 & 3*). Marine lakes can significantly contribute to the regional diversity because they harbor predominantly lake and endemic species (Becking et al. *accepted & unpublished data, CHAPTER 3*). Consistent with the island biogeography theory (MacArthur & Wilson 1967, Whittaker & Fernandez-Palacios 2007, Rosindell et al. 2011) larger lakes have more species than smaller ones and the most isolated lakes contain few reef species and the highest number of putative endemics, while the more connected lakes are dominated by reef species (Becking et al. 2011, Becking et al. *accepted& unpublished data, CHAPTERS 1, 2 & 3*). In the present study our overall aim was to obtain insight into the role of isolation in genetic diversity of marine lake populations by studying population structures of a selected sponge species among Indonesian lakes and between the lakes and the sea.

Phylogeographic studies of anchialine systems across the world typically show high levels of genetic differentiation between marine lake populations, suggesting little to no gene flow at small spatial scales ranging from 10 to 100 km (see Table 1). Furthermore, molecular markers revealed the presence of highly divergent, but morphologically cryptic species in a number of taxa such as cnidarians, crustaceans, fish and mollusks (Dawson & Hamner 2005, Santos 2006, Craft et al. 2008, Page et al. 2008, Gotoh et al. 2009, Botello & Alvarez 2010, Bauzà-Ribot et al. 2011, Goto et al. 2011). There are, however, exceptions to this general pattern which have been interpreted as resulting from life history strategies involving greater dispersal capabilities (Russ et al. 2009). Too limited sampling may also have prevented any observation of patterns (Kano & Kase 2004). Here we have conducted the first phylogeographic study of Indonesian marine lake populations and this is also the first phylogeographic study on sponges in Indonesia. Sponges are one of the most dominant taxa in marine lakes in terms of biomass and species diversity (Tomascik & Mah 1994, Becking et al. 2011, *CHAPTER* 1). The sponge species *Suberites diversicolor* (Demospongiae: Suberitidae) is an ideal taxon to pursue this study as it allows comparison of multiple lakes at various scales and with varying degrees of connection to the sea. There are few other species that are prevalent in marine lakes (Becking et al. 2011, *CHAPTER* 1). *Suberites diversicolor* occurs in most moderately to highly isolated marine lakes in Indonesia (Becking et al. 2011), as well as in limited numbers of small populations in sheltered bays in Singapore, Indonesia and Australia (Becking & Lim 2009, *CHAPTER 4*). *Suberites diversicolor* shows great plasticity in adapting to harsh environments (low salinity and exposure to air) yet is absent in coral reefs (Azzini et al. 2007, de Voogd et al. 2009, Lim et al. 2009, Becking et al. 2011, *CHAPTER 1, 2 & 4*).

Our specific aims were: 1) to estimate levels of diversity and divergence of seven marine lake populations and three coastal populations using two mitochondrial and two nuclear markers, 2) to study the phylogeography of *S. diversicolor* populations in marine lakes across Indonesia, 3) to investigate the relationship between the genetic diversity and the level of isolation of the lakes.

Table 1. Overview of intra-specific genetic structure of populations in anchialine systems from literature.

Anchialine system	Location	Taxon	Marker(s)	Structure	Scale of differentiation	Reference
lake	Palau	*Mastigias papua*	mtDNA COI & nDNA ITS	yes, each lake private haplotypes	1-50km	Dawson & Hamner 2005, Dawson 2005
lake	Palau	*Brachidontes* sp.	mtDNA COI	yes, divergent species; each lake private haplotypes	1-50km	Goto et al. 2011
lake	Palau	*Sphaeramia orbicularis*	mtDNA control region	yes, lakes reduced diversity, private haplotypes	1-50 km	Gotoh et al. 2009
pool	Hawaii island	*Holocaridina rubra*	mtDNA COI	yes, each pool private haplotypes	30-50 km	Santos 2006
pool	Hawaii Archipelago	*Halocaridina rubra*	mtDNA COI	yes, each pool private haplotypes	10-50 km	Craft et al. 2008
pool	Maui &Hawaii	*Halocaridina rubra*	mtDNA COI	yes, each pool private haplotypes	1-100 km	Santos & Weese 2011
pool	Hawaii Archipelago	*Metabenaeus lohena*	mtDNA COI	panmixia	25-300 km	Russ et al. 2009
cave	Philippines	*Neritilia cavernicola*	mtDNA COI	panmixia	200 km	Kano & Kase 2003
cave	Australia	*Stygiocaris lancifera*	mtDNA COI, 16S	yes, divergent species	10-100 km	Page et al. 2008
cave	Spain	*Metacrangonyx longipes*	mtDNA COI 16S, histone	yes, divergent species	20-100 km	Bauza-Ribot et al. 2011
cave	Mexico	*Creaseria morleyi*	mtDNA COI, 16S	yes, divergent populations	10-100 km	Botello & Alvarez 2010

Sampling

Twenty four marine lakes and adjacent coastal habitats in Indonesia were thoroughly surveyed by snorkeling for the presence of the sponge *Suberites diversicolor.* Populations of *Suberites diversicolor* were located in seven marine lakes (29% of all surveyed lakes) in the region of Berau, East Kalimantan province (Kakaban lake, Haji Buang lake, Tanah Bamban lake) and the regions of Northern Raja Ampat (Cassiopeia lake, Urani lake, Sauwandarek lake) and Southern Raja Ampat (Misool Jellyfish lake) in West Papua province, and in mangroves along the coast of the island of Maratua in the region of Berau, East Kalimantan province. Additional coastal populations were sampled from Johor Straight in Singapore (collected by S.C. Lim) and the man-made Lake Alexander in Darwin, Australia (collected by B. Alvarez), resulting in a total of seven marine lake populations and three coastal populations sampled for this study (Figure 1A). The lakes Kakaban, Tanah Bamban, Haji Buang and Misool house immense perennial populations of the jellyfish *Mastigias papua* such as those that have been extensively documented in five marine lakes in Palau. For a full description of the sampled marine lakes, see Becking et al. (2011, *CHAPTER 1*). These landlocked pools of water are subjected to a tidal regime which is typically delayed in phase (ranging from 20 minutes to 4 hours) and dampened in amplitude (tide ranging from 20 cm to 1.5 m) compared to the adjacent sea (Tomascik & Mah 1994, Becking et al. 2011, *CHAPTER 1*). We used the amount of tidal delay and dampening as a proxy for the degree of connection between the lake and the adjacent sea (Hamner & Hamner 1998, Becking et al. 2011, *CHAPTER 1*). The relative degree of isolation of each marine lake is provided in Table 2.

Collections were made randomly along the entire coastline of each of the lakes and specimens were collected at least 25m distance from each other to avoid collecting clone siblings. Our aim was to collect 20 individuals per location, but in most locations the resident population size was too small to attain this target (see Table 2). Hence, sample sizes are small for some locations. The color and substrate of each specimen was recorded, and a photograph was taken either *in situ* or within 2 hours after collection. After collection, portions of the choanosome were cut into approximately 125 mm^3 cubes, avoiding the surface to minimize potential contamination with protists or other sponge associates, and preserved in 96% ethanol, which was refreshed after 24 hours. The remainder of the samples were preserved in 70% ethanol and deposited in the Porifera collection of the Naturalis Biodiversity Center, The Netherlands (RMNH POR.) as voucher specimens. The investigated specimens are listed in the Appendix 1 (page 167).

Table 2. Sample localities of ten *Suberites diversicolor* populations from marine lakes and coastal locations in Berau (East Kalimantan) and Raja Ampat (West Papua) in Indonesia, Darwin in Australia, and Singapore. Per locality relative connection to the adjacent sea is provided, and for the marine lake size, number of species and proportion of putative endemics. In addition, the density of the target sponge species *Suberites diversicolor,* color morphs, and number of samples per genetic marker (COI, COII, 28S, ITS) is provided per location.

Code	Location	Region	Latitude	Longitude	Connection	Size lake (1000 m^3)	# sponge species	% putative endemic sponge species	density *S.diversicolor* (ind. 50m^2)	color morphs *S.diversicolor*	nCOI	nITS	nCOII	n28S
Lake														
KKB	Kakaban lake	Berau	N02° 08' 23.5"	E118° 30' 31.9"	most isolated	4000	67	49%	1-15	green, red	22	21	21	5
HBL	Haji Buang lake	Berau	N02° 12' 30.4"	E118° 35' 40.8"	isolated	140	53	34%	15-50	green, red, blue, purple, yellow	20	20	20	2
TBB	Tanah Bamban lake	Berau	N02° 13' 50.0"	E118° 34' 50.7"	least isolated	120	17	0	0-2	green, red	4	4	4	2
RAJ	Sauwandarek lake	Raja Ampat	S0° 35’ 19.6”	E130° 35’ 48.8”	very isolated	84	20	20%	0-10	purple, blue, green	21	21	21	2
CAS	Cassiopeia lake	Raja Ampat	N0° 08' 36.6"	E130° 04' 39.8"	least isolated	13	15	0	0-10	green	10	10	10	2
URA	Urani lake	Raja Ampat	N0° 06' 05.1"	E130° 15' 05.5"	isolated	68	25	12%	0-2	green	8	8	8	1
MIS	Misool Jellfish Lake	Raja Ampat	S01° 55'	E130° 20'	isolated	12	8	25%	0-2	green	7	7	7	1
Coastal														
BER	Maratua mangrove	Berau	N02° 12' 52.3"	E118° 35' 34.1"	open				0-1	green, yellow	3	3	3	1
DAR	Lake Alexander, Darwin	Australia	S12° 25'	E130° 50'	open				0-1	green	6	6	6	2
SIN	Johor Strait	Singapore	N 01° 26’02.34”	E104°02’54.31”	open				0-1	purple, blue, green	4	4	4	2

DNA extraction, amplification and sequencing

Total DNA was extracted from 105 specimens using DNeasy tissue kit (Qiagen), following the instructions of the manufacturer. Partitions of four markers were amplified: two mitochondrial genes, cytochrome oxidase subunit 1 (COI) and subunit 2 (COII), and two nuclear markers, the nuclear ribosomal operons consisting of partial 18S rDNA, full-length internal transcribed spacer 1 and 2, 5.8S, and partial 28S rDNA fragments (ITS) and the D3-D5 region of the nuclear ribosomal 28S gene (28S). The nuclear markers are independent from the mitochondrial markers and therefore provide extra support in case of congruent results.

The standard DNA-barcoding fragment of COI was amplified by using a specific forward primer designed for *Suberites* SUB-COI-F: GGAATGATCGGGACAGCTTTTAGCATG and the degenerated reverse primer from Folmer et al. (1994) designed by Meyer et al. (2005): dgHCO2198:TAA ACT TCA GGG TGA CCA AAR AAY CA. COII was amplified with the primers from Rua et al. (2011): CO2F: TTTTTCACGATCAGATTATGTTTA and CO2R: ATACTCGCACTGAGTTTGAATAGG. ITS amplified with primers from Wörheide (1998) RA2: GTCCCTGCCCTTTGTACACA and ITS2.2: CCT GGT TAG TTT CTT TTC CTC CGC). 28S was amplified in a subset of samples with primers from McCormack and Kelly (2002) RD3A: GACCCGTCTTGAAACACGA and RD5B2: ACACACTCCTTAGCGGA . Amplifications were carried out in 25μl reaction volumes containing 5μl Phire® Reaction Buffer,3 μl dNTPs (1mM), 0.625 μl of each primer (10μM), 0.25 μl Phire® Hotstart-*Taq* polymerase DNA (Thermo Scientific, Finnzymes), and 1 μl of DNA (10-20 *ng*/μl). The temperature regime for amplification: 94°C for 30s; followed by 35 cycles of 94°C for 5 s; 50°C for 5 s; 72°C for 12 s; followed by 72°C for 1 min. PCR products were purified and sequenced by Macrogen Inc (Korea and The Netherlands).

Data analysis

Genetic diversity (COI, COII, ITS, 28S)

The poriferan origin of the obtained sequences was verified through BLAST searches (http://blast.ncbi.nlm.nih.gov/blast.cgi). Sequences were handled in SEQUENCHER 4.10.1 (Gene Codes Corporation) and aligned with CLUSTALW and MUSCLE implemented in DAMBE (Xia & Xie 2001) and SEAview v 4.3.0 (Gouy et al. 2010). Alignment was conducted under default settings and optimized by eye. Alignments were collapsed to contain only unique sequence types in DAMBE. Haplo/genotypes and nucleotide diversity as well as Tajima's D neutrality test was calculated per population with Arlequin v. 3.11 (Excoffier et al. 2005).

Phylogeography (COI, ITS, color morphs, substrate preference)

We used ITS outgroup sequences obtained from Genbank from the family Halichondriidae (Figure 1), as the available sequences for ITS of other Suberitidae were more distant than those from Halichondriidae. Several studies have shown that the families Suberitidae and Halichondriidae are sistergroups (Chombard et al. 1998, Chombard & Boury-Esnault 1999, Morrow et al. 2012). To be consistent we also used species of the family Halichondriidae for the outgroup of the COI phylogram. The best-fit DNA substitution model was selected by the Akaike Information Criterion deployed in jMODELTEST v. 0.1.1 (Posada 2008) and this model (COI: HKY and ITS:GTR+G+I for both markers) was used for subsequent Bayesian and maximum likelihood phylogeny inferences. Phylogenetic reconstructions were performed under Bayesian inference criteria implemented in MrBayes v. 3.1.2. (Huelsenbeck & Ronquist 2001). Each analysis consisted of two independent runs of four Metropolis-coupled Markov-chains, sampled at every 1000th generation at the default temperature (0.2). Analyses were terminated after the chains converged significantly as indicated by an average standard deviation of split frequencies <0.001. Convergence was also checked in Tracer v. 1.5.0 (Rambaut & Drummond 2007). For comparison, maximum likelihood bootstrap analyses were conducted using MEGA v. 5.01 (Tamura et al. 2011) using a heuristic search with 1000 bootstrap replicates. The Bayesian and maximum likelihood phylograms were combined and visualized using Treegraph 2 (Stöver & Müller 2010). Within group *p*-distance (uncorrected), as well as net nucleotide divergence between groups were calculated in MEGA. A Kruskall-Wallace test was performed to test whether color or substrate preference significantly differed between lineages. To test for spatial structuring of samples we performed an analysis of molecular variance (AMOVA) and calculated pairwise *Φst* values between separate populations using Arlequin 3.5.1.2 (Excoffier & Lischer 2010). Significance of pairwise *Φst* values (based on *p*-distances) was determined by 10000 permutations and exact tests of population differentiation in Arlequin.

Table 3. Genetic diversity indices based on ITS sequences per population of *Suberites diversicolor* of lineage A and B (location codes indicated in Table 2); gene diversity (*h*), nucleotide diversity (π), Tajima's D neutrality test. The majority of populations had only one haplotype resulting in 0 values for all indices calculated. All Tajima D values are not significant.

Code	Lineage	n ITS	*h* ITS	π ITS	Tajima's D
KKB	A	13	0.8242 +/- 0.0567	0.005656 +/- 0.003392	1.3927
KKB	B	8	0.5357 +/- 0.1232	0.001578 +/- 0.001318	1.4488
HBL	B	20	0	0	0
TBB	B	4	0	0	0
RAJ	B	21	0	0	0
CAS	B	10	0	0	0
URA	B	8	0	0	0
MIS	B	7	0.2857 +/- 0.1964	0.000842 +/- 0.000879	-1.23716
BER	B	3	0	0	0
SIN	B	4	0	0	0
DAR	B	6	0.3333 +/- 0.2152	0.000980 +/- 0.000997	-1.13197

Table 4. Pairwise *Φst* values between all populations of lineage B based ITS sequences (location codes indicated in Table 2). Values in bold and with asterisk indicate significant values (p<0.05).

	KKB	HBL	TBB	RAJ	CAS	URA	MIS	BER	SIN
HBL	**0.60591***								
TBB	0.30435	0							
RAJ	**0.60591***	**1***	**1***						
CAS	**0.4702***	**1***	**1***	0					
URA	0.42857	**1***	**1***	0	0				
MIS	0.13514	0.16749	-0.09804	**0.91393***	**0.86315***	**0.84466***			
BER	0.25	**1***	**1***	0	0	0	**0.76136***		
SIN	0.30435	**1***	**1***	0	0	0	**0.78544***	0	
DAR	**0.53451***	**0.9512***	**0.86348***	**0.83584***	**0.74359***	**0.71049***	**0.77327***	0.55882	0.60396

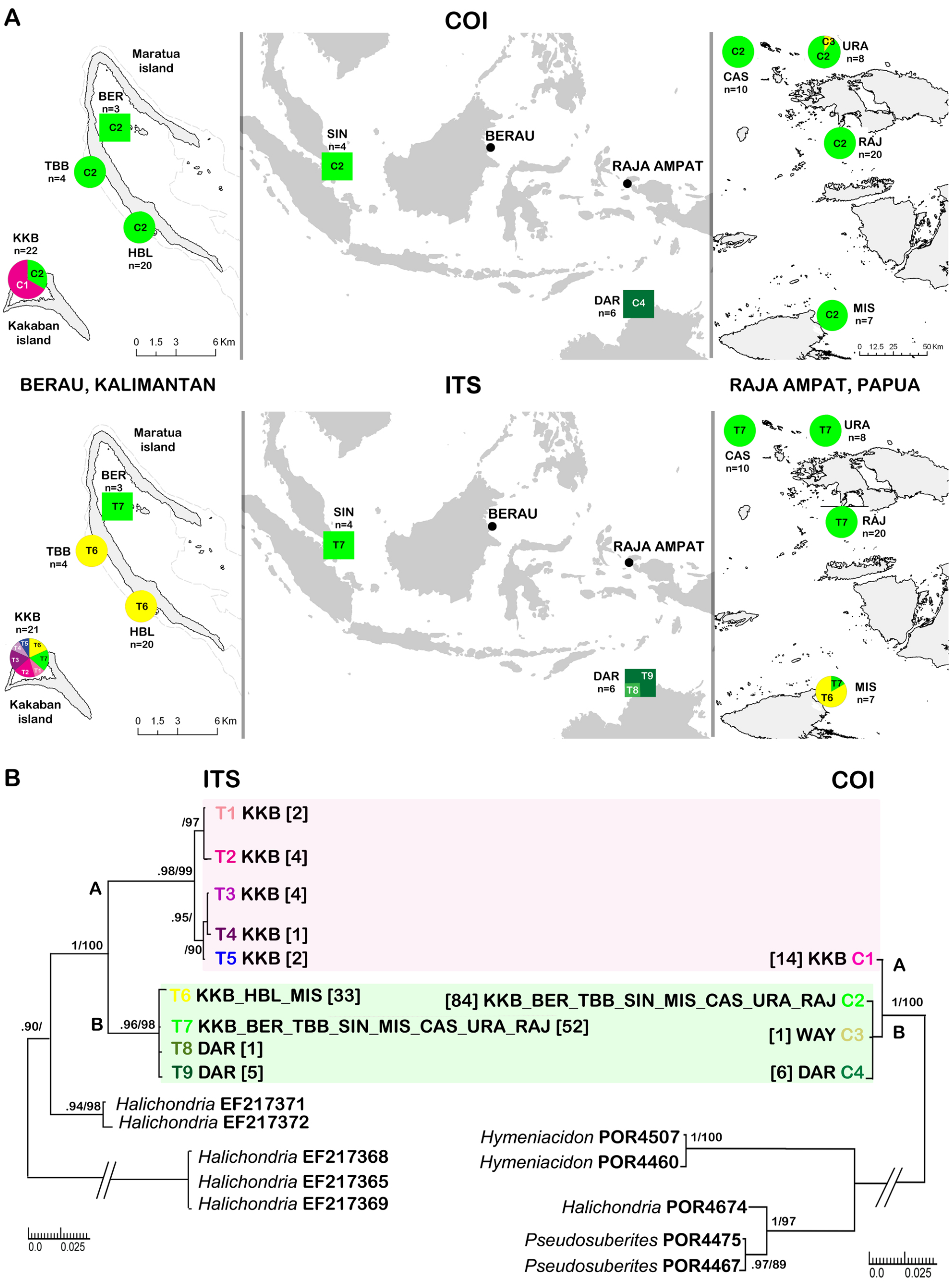

Figure 1. A Sample locations of the sponge *Suberites diversicolor*: top map represents distribution and frequencies of haplotypes for partial Cytochrome Oxidase I (COI) and below of genotypes of internal transcribed spacer region of nuclear ribosomal operons (ITS) in Indonesia, Singapore and Australia with insets of Berau, East Kalimantan (left) and Raja Ampat, West Papua (right) in Indonesia; location codes are explained in Table 2; circles represent marine lakes and squares are outer coastal populations; haplo/genotypes are indicated by number code (COI: C1-4 and ITS: T1-9) and color codes as provided in B. Note that scale differs per map. **B** Bayesian/ maximum likelihood phylogram of 105 COI sequences (right) and 104 ITS sequences (left); each haplo/genotype indicated by specific color, followed by location code and total number of samples in squared brackets. Only posterior probabilities of >90 and maximum likelihood values of >70 are indicated. Color blocks represent the same individuals for both molecular markers (i.e. lineage A (pink) and B (green) represented by the same individuals with both COI and ITS markers). Scale bars indicate substitutions/site.

Results

Sequence variation (COI, COII, ITS, 28S)

We obtained final alignments (excluding primers) for the sponge *S. diversicolor* of 519 bp for COI with four haplotypes (C1-4, 105 individuals), 331 bp for COII with one haplotype (105 individuals), 689 bp of ITS with nine genetic variants (T1-9, 104 individuals) (Table 2, Appendix 1). For a subset of 20 specimens we obtained 574 bp for 28S resulting in one genetic variant.

Divergent lineages in S. diversicolor

All COI and ITS sequences were obtained from the same specimens and fall apart into two major lineages, termed A and B. These lineages represent reciprocally monophyletic groups for both markers and were strongly supported by both Bayesian and maximum likelihood inference methods (Fig. 1B). Lineage A was represented by haplotype C1 for COI and genotypes T1-5 for ITS. Lineage B is represented by COI haplotypes C2-4 and ITS genotypes T6-9 (Figure 1B). Within lineage A there was no sequence variation in COI (n=14), while the average *p*-distance within lineage B was 0.25% (n= 91). The net nucleotide divergence between lineage A & B for COI was 0.38%. Haplotype C1 (lineage A) differed by two basepairs from C2 (the dominant haplotype from lineage B) of which one resulted in a non-synonymous substitution between two unpolar amino acids, from isoleucine to valine, when translated into proteins. For ITS the average *p*-distance within lineage A was 0.44% (n=13), while the average *p*-distance within lineage B was 0.29% (n=91). The net nucleotide divergence between lineages A & B for ITS was 7.26%. Several indels of 1-3 bp length were observed and were consistent within lineages and differed between lineages. There were insertions in lineage A with respect to lineage B from bp102-103 (either CT or TT), 380-381 (CA), 470-473 (GGA or GAA). There were gaps in lineage A with respect to lineage B from bp139, 178-180, 549-55. No double peaks were observed, and it is assumed that no intragenomic polymorphisms occur within this species. The level of intragenomic polymorphisms differ per species (Wörheide et al. 2004). We consider the risk of analyzing paralogous rDNA sequence types to be minimal as we see genealogical concordance across two unlinked loci. We did not, however, detect a significant difference between lineage A & B in color (p=0.249) or substrate preference (p=0.100) using the independent samples Kruskal-Wallis Test.

Diversity and spatial population structuring (COI & ITS)

Lineage A was only present in Kakaban lake while lineage B was present in all populations. The geographical distribution of COI haplotypes is shown in Fig. 1A. Of the four detected haplotypes in COI, haplotype C1 was restricted to Kakaban lake (East Kalimantan). Haplotype C3 only occurred in one individual in Urani lake (West Papua). The Darwin population was represented by haplotype C4 which was shared with no other population. Haplotype C2 was the most abundant haplotype, occurring in all populations except Darwin and was the dominant haplotype in the populations of Berau mangroves, Singapore, Sauwandarek lake, Cassopeia lake, Urani Lake, Misool Jellyfish lake. Of the nine detected genotypes of ITS, five were restricted to Kakaban lake (genotypes T1-5), all representatives of lineage A. Genotype T7 (lineage B) was the most abundant and was shared by all sampled populations except Haji Buang Lake and Tanah Bamban lake (Kalimantan) and Darwin (Australia). Darwin was represented by the private haplotypes T8-9. Haji Buang lake and Tanah Bamban lake were represented fully by a single genotype (T6) that was shared by Kakaban lake (Kalimantan) and Misool Jellyfish lake (Papua).

Within lineage A in Kakaban lake there was only a single haplotype of COI while the ITS gene diversity was 0.8242 +/- 0.0567, and ITS nucleotide diversity was 0.005656 +/- 0.003392. Within lineage B all populations contained a single COI haplotype except Urani lake, which had two haplotypes with a haplotype diversity of 0.3333 +/- 0.2152 and nucleotide diversity of 0.000624 +/- 0.000822. For ITS, the majority of the populations contained only a single genotype, except for Kakaban lake, Darwin and Misool Jellyfish lake. The population in Kakaban lake had the highest gene and nucleotide diversity in lineage B, followed by Darwin and Misool Jellyfish lake (Table 3). Tajima's D tests of neutrality were carried out per population. The majority of the populations had a zero value due to the presence of only one genetic variant. Values of Tajima's D for ITS were negative, but not significant ($p>0.1$) in Misool lake and Darwin (Table 3).

Spatial analysis of genetic structure of lineage B haplo/genotypes of COI and ITS showed that the Darwin population was strongly (Φst between 0.53-1) and significantly different from all marine lakes populations (Table 4 & Appendix 2). Besides Darwin there was no significant variation in COI between the different populations (Appendix 2). ITS showed more structure among the populations than COI (Figure 1, Table 3). The Berau lakes (East Kalimantan) Kakaban and Haji Buang lakes were significantly differentiated. The Raja Ampat lakes (West Papua) were not genetically differentiated from each other except Misool which was differentiated from all Raja Ampat lakes, yet not from the populations of the lakes Kakaban, Haji Buang and Tanah Bamban (East Kalimantan). The AMOVA analyses revealed that significant portions of the total variance within lineage B can be attributed to differences among the following three groups 1. Berau coast, Singapore coast, Northern Raja Ampat lakes (Sauwandarek, Cassopeia, Urani), 2. Berau lakes (Kakaban, Tanah Bamban, Haji Buang), Southern Raja Ampat (Misool), 3. Darwin. The among group variation was 84.6% ($p<0.001$) and the within population variation was 10% ($p<0.001$).

Discussion

Two divergent lineages

Two major lineages were uncovered in the populations of the sponge *S. diversicolor*. The congruent patterns of COI and ITS genetic markers and the degree of divergence between the two lineages (COI: 0.4% and ITS: 7.3%) is indicative of reproductive isolation, and thus we suggest that the two lineages (A and B) constitute sibling species. We searched for morphological and ecological characters to distinguish the two lineages, but genetic divergence can preclude morphological distinction. The skeletal structure and spicule lengths do not differ between lineages and fall within the natural variation of this species (Becking & Lim 2009, *CHAPTER 4*). The color and substrate preference are variable, but not consistent within a particular lineage. A related *Suberites* from Satonda lake (Sumbawa, Indonesia) displays different colors at different depths as a result of a symbiosis with the unicellular green algae *Chlorella* and a symbiotic bacteria (Arp et al. 1996). Phylogeographic studies in the Indo-Australian-Archipelago have uncovered numerous lineages in diverse marine taxa that may represent undescribed cryptic species (e.g. Barber et al. 2002, Crandall et al. 2008, Malay & Paulay 2010). Within sponges, molecular studies have revealed a high prevalence of morphologically cryptic sponge species (see review in Xavier et al. 2010a, Reveillaud et al. 2011).

The divergence between lineage A and B, points to a long standing isolation in spite the fact that they are sympatric in Kakaban lake (East Kalimantan). Within sponges there are several reports of sympatric cryptic species: *Tedania* spp. in mangroves (Wulff 2006), *Scopalina lophyropoda* (Blanquer et al. 2008), *Cliona* spp. (Xavier et al. 2010a), *Hexadella* spp. (Reveillaud et al. 2010). Differential reproductive traits and output can promote the co-existence of sibling species (Blanquer et al. 2008, Pérez-Porro et al. 2012). This observation of divergent lineages sympatric within one lake is, however, not common in the phylogeographic studies conducted thus far on populations in the marine lakes in Palau (Dawson 2005, Dawson & Hamner 2005, Gotoh 2009, Goto 2011). The Palauan studies on three distinct taxa (jellyfish, fish and bivalves) mostly show a pattern of one lineage occupying one lake (Dawson 2005, Dawson & Hamner 2005, Gotoh 2009, Goto 2011). One reason why Kakaban lake may contain two lineages is the sheer size of the lake – at almost 4 km^2 it is tenfold larger than any of the other lakes in Indonesia and the majority of Palau (Table 2, Colin 2009, Becking et al. 2011). Alternatively lineage B could be an introduction to the lake. Sponge fragments are known to be transported by waterfowl (Pronzato & Manconi 1994) and workers from the neighboring island Maratua who stay on Kakaban for short periods to attend small crops also may act as possible vectors of *Suberites diversicolor* from the Maratua lakes or the mangroves near their village.

Phylogeography

Lineage A is only present in Kakaban lake, while lineage B is present in all populations. Within lineage B the spatial genetic population structure shows three groups: 1. the three Berau lakes and southern Raja Ampat lake, 2. Berau coast, Singapore coast and the three northern Raja Ampat lakes, 3. Darwin. At present there is no comprehensive phylogeographic study of sponges spanning across the Indonesian archipelago, yet pronounced genetic differences in populations of other marine invertebrates and vertebrates are present between the Java Sea, the Indonesian Through Flow, and the seas of East Sulawesi (e.g. Barber et al. 2000, Barber et al. 2006, Timm & Kochzius 2008, Nuryanto & Kochzius 2009, Carpenter et al. 2011). The marine phylogeographic patterns of these studies strongly support the existence of a barrier in the area between the Sunda and Sahul shelves, where populations from Kalimantan are genetically isolated from those in Papua. Our data does not show a clear East to West geographic break, but the Darwin population, though small in sample size, is clearly genetically differentiated from the other populations. Dispersal potential and habitat specialization may determine how lineages are distributed and how fauna of different geographic regions are connected (e.g. Carpenter et al. 2011). The majority of sponge population genetic and phylogeographic studies based on less conserved fragments of mitochondrial DNA and nuclear markers reveal structured populations with in some cases evidence of (occasional) long distance dispersal events (Wörheide et al. 2002, 2005, 2008, Lopez-Legentil & Pawlik 2009, DeBiasse et al. 2010, Xavier et al. 2010b). This pattern is congruent with philopatric, shortlived larvae that recruit at short distances from the parental locations (Mariani et al. 2005, Mariani et al. 2006) together with the ability of sponges to disperse as viable fragments in the currents or rafting on various floating material (Wulff 1991, 1995, Maldonado & Uriz 1999). The reproductive cycle and larvae of *S. diversicolor* are unknown, but this species does produce asexual buds (Becking pers. obs.) which may survive a considerable amount of time in the plankton or by rafting before colonizing distant locations as proposed by Wörheide et al. (2008) for *Leucetta chagosensis*.

Within lineage B none of the Indonesian marine lakes contained private genotypes (or unique genetic diversity), and many lakes were identical in composition. The only studies on marine lake phylogeography have been in the islands of Palau on the jellyfish *Mastigias papua* (Dawson & Hamner 2005, Dawson 2005), the fish *Sphaeramia orbicularis* (Gotoh et al. 2009), and the mussel *Brachidontes* sp. (Goto et al. 2011). These studies show extreme genetic isolation, low genetic diversity, and in the cases of *Mastigias papua* and *Brachidontes* sp. rapid morphological evolution in the marine lakes (Dawson & Hamner 2005, Dawson 2005, Gotoh et al. 2009, Goto et al. 2011). The lack of a strong population structure between many of the Indonesian lakes of the present study may be caused by recurrent (recent and historic) geneflow among lakes. We suggest, however, that the lack of structure is a result of small sample sizes of some populations and that the genetic markers we used do not evolve fast enough for mutations to have accumulated to show the differentiation. It is still possible that all these lakes are completely isolated, i.e. do not exchange any migrants. Intraspecific variation in demosponge COI is generally low (e.g. Erpenbeck et al. 2006, Wörheide 2006) and interspecific variation of COI in sponges can be as low as 0-0.4% (*p*-distances) (e.g. Pöppe et al. 2011). However, COI can provide low but sufficient genetic variation in populations of some sponge taxa over relatively short geographic distances (Duran & Rützler 2006, DeBiasse et al. 2010). Of the four molecular markers used in the present study, ITS evolves the fastest and therefore gave the highest resolution of spatial genetic structure. There was no sequence variation in 28S and COII between any of the populations or between the two lineages. The D3-D5 of the 28S fragment has been used to distinguish genera and species of a wide range of demosponge taxa including halichondrids (e.g. McCormack & Kelly 2002, Erpenbeck et al. 2005), but also can be too conserved to discriminate closely related species in others (Reveillaud et al. 2010). COII was proposed as a polymorphic mitochondrial marker for sponge phylogeography by Rua et al. (2011). Rua et al. (2011) indicated that the variation of this marker could be low in halichondrid species *Hymeniacidon heliophila* but attributed their results to the collection of clone-mates. In the present study COII showed no variation between any of the samples spanning a wide geographic range. We conclude that COII is not a suitable marker for intraspecific variation or distinction between closely related species of the genus *Suberites* in particular, and probably more generally for the families Suberitidae and Halichondriidae (Morrow et al. 2012).

Isolation & genetic diversity

Kakaban lake is the largest and most isolated lake in Indonesia, and houses the highest proportion of endemic sponge species (Table 2). Within this lake the population of *Suberites diversicolor* displayed the highest genetic diversity with unique genetic variants that were not shared with the two lakes at just 6 km distance (Figure 1). These results are concordant with results from Palau where the degree of genetic distance between marine lake and adjacent sea populations was strongly correlated to the degree of connection from the lake to the sea and not the actual geographic distance between the populations (Dawson & Hamner 2005, Goto et al. 2011). Isolation acts to decrease the rate of immigration and thus to decrease the genetic diversity and the number of species expected at equilibrium in an island system (MacArthur & Wilson 1967, Whittaker & Fernandez-Palacios 2007, Chen & He 2009, Rosindell et al. 2011) Yet isolation can also enhance species formation, with the diminished gene flow allowing populations to diverge and ultimately form new species if they remain isolated (Emerson & Gillespie 2008, Chen & He 2009, Rosindell et al. 2011). With the molecular markers we used there was, however, not a direct relationship between moderate levels of isolation and the genetic diversity of the lakes. For example, the populations in northern Raja Ampat lakes (West Papua) are not genetically differentiated, despite the limited physical connections to each other and the adjacent sea.

Biogeographic scenario

Kakaban lake was probably filled with sea water less than 15000 years ago (Dawson 2006, Sathiamurthy & Voris 2006). Considering the deep divergence between lineages A & B, this divergence likely occured well before the formation of Kakaban. Wörheide et al. (2004) estimated an evolutionary rate of 1% per million years for ITS in a suberitid sponge *Prosuberites 'laughlini'* based on the formation of Isthmus of Panama. Implementing the 1 % mutational rate would mean that the two lineages diverged approximately 7 million years ago. Though this is a rough estimation with great error bars and rates of evolution may be higher for recently diverged lineages (Ho et al. 2011), the age is consistent with recent phylogeographic studies that suggest that many endemics from the Indo-Australian-Archipelago have origins in the early Pliocene-Miocene (3-20 million years ago) (e.g. Renema et al. 2008, Bellwood & Meyer 2009, Cowman & Bellwood 2011).
Kakaban lake houses a genetic and species diversity of sponges, that appears to be absent from the surrounding sea (Becking et al. *accepted & unpublished data, CHAPTERS 2, 3, 4, 5 & 6*). Each lake is ephemeral, but the marine lakes ecosystem probably has occurred in various locations during the past glacial-cycles (Sathiamurthy & Voris 2006). The Sunda shelf, which includes Borneo (Kalimantan), was exposed during the Last Glacial Maximum (LGM) when sealevels are estimated to have been approximately 110-140m lower than modern sea levels (Geyh et al. 1979, Voris 2000, Hoeksema 2007). Multiple larger and smaller depressions in the shelf have been recorded which presumably represented palaeo-lakes during the LGM (Sathiamurthy & Voris 2006) that could have become brackish marine lakes with the increase in sea level. What is more, during the LGM the Sunda Land region was dominated by mangroves (Morley 2000), and the water around the Sunda area would have been brackish due to the multiple river outlets (Hoeksema 2007), which are both environments amenable for *S. diversicolor.* Ancient lineages/endemics may have 'hopped' from lake to lake or from mangrove to lake, as the lakes formed and subsequently disappeared with the rise and fall in sealevel during the Plio-Pleistocene glacial cycles. Genetic signatures for glacial refugia are expected to be characterized by high genetic diversity and mixture of ancestral and private haplotypes (Hewitt 2000, Maggs et al. 2008). Kakaban matches this pattern. While Kakaban itself could not have been a refugium during LGM (it was dry), there may have been palaeo-lakes in the vicinity that served as such. Kakaban may be an area where multiple putative refugia populations have come into secondary contact, resulting in the high genetic diversity and the high number of endemics. Molecular studies on co-distributed taxa at larger scales including lakes from adjacent regions in Palau and Vietnam will enhance our understanding of the processes behind the unique marine lake diversity.

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Appendix 1.

Lineage	COI	ITS	Location	Color	Substrate	Fieldcode	RMNH POR
A	C1	*	KKB	Green	root	KKB905	6400
A	C1	H1	KKB	Green	root	SKKB03_120	6485
A	C1	H1	KKB	Green	root	SKKB1067	6401
A	C1	H2	KKB	Green	rock	SKKB1617	6402
A	C1	H2	KKB	Red	rock	SKKB790	6403
A	C1	H2	KKB	Green	rock	SKKB791	6404
A	C1	H2	KKB	Green	mud/sand	SKKB880	6405
A	C1	H3	KKB	Green	root	SKKB05	1397
A	C1	H3	KKB	Green	rock	SKKB1605	6406
A	C1	H3	KKB	Green	rock	SKKB826	6407
A	C1	H3	KKB	Green	rock	SKKB831	6408
A	C1	H4	KKB	Green	rock	SKKB1616	6409
A	C1	H5	KKB	Green	rock	SKKB797	6410
A	C1	H5	KKB	Green	rock	SKKB807	6411
B	C2	H6	HBL	Green	rock	MA1529	6430
B	C2	H6	HBL	Green	rock	SMA02	1438
B	C2	H6	HBL	Green	root	SMA1003	6431
B	C2	H6	HBL	Purple	rock	SMA1014	6432
B	C2	H6	HBL	Green	rock	SMA1018	6433
B	C2	H6	HBL	Green	rock	SMA1022	6434
B	C2	H6	HBL	green	root	SMA1028	6435
B	C2	H6	HBL	Green	rock	SMA1031	6436
B	C2	H6	HBL	Green	root	SMA1037	6437
B	C2	H6	HBL	Yellow	rock	SMA1043	6438
B	C2	H6	HBL	Green	rock	SMA1056	6439
B	C2	H6	HBL	Green	rock	SMA1510	6440
B	C2	H6	HBL	Green	rock	SMA1511	6441
B	C2	H6	HBL	Green	rock	SMA1514	6442
B	C2	H6	HBL	Green	rock	SMA1528	6443
B	C2	H6	HBL	Blue	rock	SMA709	6444
B	C2	H6	HBL	Green	rock	SMA948	6445
B	C2	H6	HBL	Green	rock	SMA951	6446
B	C2	H6	HBL	Green	rock	SMA959	6447
B	C2	H6	HBL	Green	mud/sand	SMA973	6448
B	C2	H6	KKB	Red	root	SKKB1559	6449
B	C2	H6	KKB	green	rock	SKKB1615	6450
B	C2	H6	KKB	Green	root	skkb747	6451
B	C2	H6	KKB	Green	root	skkb781	6452
B	C2	H6	MIS	Green	rock	SMIS1760	6457
B	C2	H6	MIS	Green	rock	SMIS1761	6458
B	C2	H6	MIS	Green	rock	SMIS1762	6459
B	C2	H6	MIS	Green	rock	SMIS1764	6460
B	C2	H6	MIS	Green	rock	SMIS1765	6461
B	C2	H6	MIS	Green	rock	SMIS1766	6462
B	C2	H6	TBB	Green	mud/sand	SMB01	2438
B	C2	H6	TBB	Green	mud/sand	SMB02	4670
B	C2	H6	TBB	Red	mud/sand	SMB03	2433
B	C2	H6	TBB	Red	mud/sand	SMB04	2434
B	C2	H7	BER	Green	mud/sand	SBER1128	6412
B	C2	H7	BER	Green	mud/sand	SBAY1637	6413
B	C2	H7	BER	Green	mud/sand	SBAY1645	6414

Lineage	COI	ITS	Location	Color	Substrate	Fieldcode	RMNH POR
B	C2	H7	CAS	Green	mud/sand	SWAY1720	6415
B	C2	H7	CAS	Green	mud/sand	SWAY1721	6416
B	C2	H7	CAS	Green	mud/sand	SWAY1722	6417
B	C2	H7	CAS	Green	mud/sand	SWAY1723	6418
B	C2	H7	CAS	Green	mud/sand	SWAY1724	6419
B	C2	H7	CAS	Green	mud/sand	SWAY1725	6420
B	C2	H7	CAS	Green	mud/sand	SWAY1726	6421
B	C2	H7	CAS	Green	mud/sand	SWAY1727	6422
B	C2	H7	CAS	Green	mud/sand	SWAY1728	6423
B	C2	H7	CAS	Green	mud/sand	SWAY1746	6424
B	C2	H7	KKB	Green	root	SKKB1066	6453
B	C2	H7	KKB	Red	mud/sand	SKKB1560	6454
B	C2	H7	KKB	Red	mud/sand	SKKB1561	6455
B	C2	H7	KKB	Green	rock	SKKB820	6456
B	C2	H7	MIS	Green	mud/sand	SMIS1763	6463
B	C2	H7	RAJ	Green	root	SRAJ07	6464
B	C2	H7	RAJ	Purple	root	SRAJ08	6465
B	C2	H7	RAJ	Blue	mud/sand	SRAJ10	4680
B	C2	H7	RAJ	Blue	root	SRAJ11	6466
B	C2	H7	RAJ	Blue	root	SRAJ13	6467
B	C2	H7	RAJ	Green	root	SRAJ1667	6468
B	C2	H7	RAJ	Green	mud/sand	SRAJ1668	6469
B	C2	H7	RAJ	Green	mud/sand	SRAJ1670	6470
B	C2	H7	RAJ	Green	mud/sand	SRAJ17	6471
B	C2	H7	RAJ	Green	root	SRAJ19	6472
B	C2	H7	RAJ	Purple	root	SRAJ1923	6473
B	C2	H7	RAJ	Green	root	SRAJ20	6474
B	C2	H7	RAJ	Green	mud/sand	SRAJ21	6475
B	C2	H7	RAJ	Purple	root	SRAJ26	6476
B	C2	H7	RAJ	Blue	root	SRAJ32	6477
B	C2	H7	RAJ	Green	root	SRAJ43	4681
B	C2	H7	RAJ	Purple	mud/sand	SRAJ37	4672
B	C2	H7	RAJ	Blue	root	SRAJ40	6478
B	C2	H7	RAJ	Green	mud/sand	SRAJ47	6479
B	C2	H7	RAJ	Green	root	SRAJ471	4682
B	C2	H7	RAJ	Blue	root	SRAJ35	4673
B	C2	H7	SIN	Green	mud/sand	SIN01	4675
B	C2	H7	SIN	Green	mud/sand	SIN02	6480
B	C2	H7	SIN	Green	mud/sand	SIN03	6481
B	C2	H7	SIN	Green	mud/sand	SIN04	6482
B	C2	H7	URA	Green	root	SW1740	6483
B	C2	H7	URA	Green	rock	SW1742	6425
B	C2	H7	URA	Green	rock	SW1743	6426
B	C2	H7	URA	Green	rock	SW1744	6427
B	C2	H7	URA	Green	root	SW1745	6428
B	C2	H7	URA	Green	root	SW1746	4677
B	C2	H7	URA	Green	root	SW1747	6429
B	C3	H7	URA	Green	rock	SW1741	6484
B	C4	H8	DAR	Green	mud/sand	SDAR09	6486
B	C4	H9	DAR	Green	mud/sand	SDAR01	6487
B	C4	H9	DAR	Green	mud/sand	SDAR02	6488
B	C4	H9	DAR	Green	mud/sand	SDAR03	6489
B	C4	H9	DAR	Green	mud/sand	SDAR07	6490
B	C4	H9	DAR	Green	mud/sand	SDAR05	6491

Appendix 2.

Code	KKB	HBL	TBB	RAJ	CAS	URA	MIS	BER	SIN
HBL	0								
TBB	0	0							
RAJ	0	0	0						
CAS	0	0	0	0					
URA	0,05138	0,22581	-0,08108	0,22581	0,09091				
MIS	0	0	0	0	0	0,02778			
BER	0	0	0	0	0	-0,15385	0		
SIN	0	0	0	0	0	-0,08108	0	0	
DAR	**1***	**1***	**1***	**1***	**1***	**0.85714***	**1***	**1***	**1***

Chapter 7

Are marine lakes cradles or refuges of diversity? A mussel's (*Brachidontes* sp.) perspective

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manuscript in preparation

Abstract

The marine lake setting, landlocked seawater with clearly delineated contours, provides a unique model to study early stages of evolution in coastal marine taxa. The phylogeography of the mussel *Brachidontes* sp. from marine lakes in Indonesia was studied by collecting and analyzing sequence data of the mitochondrial Cytochrome Oxidase I (COI) gene. In addition, we examined shell shape using a geometric morphometric approach. We found strong genetic and morphological differentiation between *Brachidontes* sp. populations in Indonesian marine lakes. The Indonesian populations of *Brachidontes* sp. harbored deeply diverged lineages (14.8% sequence divergence) that correspond to 'lineage A & B' which was previously recorded from marine lakes in Palau. The mussel populations in the three Indonesian marine lakes differed significantly in shell outline shape, concordant with three genetic lineages and these lineages may constitute a species complex of at least two undescribed species. Analysis of two nuclear genes (28S and 18S) confirmed the designation of the marine lake samples to the genus *Brachidontes* but did not allow species distinction within the genus. Each lake contained within lineage diversity that was unique to each lake and we suggest that this resulted from *in situ* divergence. Combined effects of stochastic processes (e.g. founder effects), local adaptation and increased evolutionary rates could produce high levels of differentiation in small populations such as in marine lake environments. The lakes appear to be cradles of diversity resulting from recent divergence of evolving populations within the lakes. The lakes also may serve as refuges for ancient lineages from the sea or older anchialine populations. The role of marine lakes in supporting endemism in the Indo-Australian Archipelago region may reflect enhanced survival of endemics, with the possibility of population differentiation that in time may lead to speciation.

Keywords

Phylogeography • Indonesia • Indo-Australian Archipelago • Raja Ampat • Berau • anchialine

Introduction

Marine lake populations provide an opportunity to study the early stages of evolution coastal marine taxa. Marine lakes are anchialine systems: small bodies of landlocked seawater isolated to varying degrees from the surrounding marine environment (Holthuis 1973, Hamner & Hamner 1998). A large number of marine lakes are located in the countries Indonesia and Palau (Dawson et al. 2009, Becking et al. 2011). Indonesia and Palau are situated within the world's largest concentration of marine biodiversity, an area known as the Indo-Australian Archipelago (IAA) (Roberts et al. 2002, Hoeksema 2007). Numerous factors have been proposed that may account for the high biodiversity within the IAA, including geological history of the area (Renema et al. 2008), its position downstream of the Pacific (Connolly et al. 2003, Kool et al. 2011), the large area of shallow water habitat during the Pleistocene low sea level stands (Voris 2000, Hoeksema 2007), great habitat heterogeneity (Hoeksema 2007), and large reef area (Bellwood et al 2005). The IAA is also remarkable for the high number of endemics. Given the areal definition of an endemic as spatially restricted species (Begon et al. 1996), centers of endemism could be areas where species arise and remain, and/or the last stand of previously widespread species (Briggs 2000, Roberts et al. 2002, Meyer et al. 2005, Bellwood & Meyer 2009). Interestingly, for many taxa in the IAA the majority of endemics lie in the peripheral areas which are characterized by a high degree of isolation (Bellwood & Meyer 2009, Malay & Paulay 2010).

Small peripatric populations such as those in marine lakes could provide an opportunity to study marine taxa in isolated environments (Dawson & Hamner 2005). The majority of marine lakes are shallower than 50 m., which means that during the Last Glacial Maximum when sea levels are estimated to have been approximately 110-140 m. lower than modern sea levels (Geyh et al. 1979, Voris 2000) the lakes would have been dry or contained fresh water (Dawson 2006). Based on the estimated sea level rise, the presumed dates of filling of the lakes with seawater are estimated at 5000-15000 years before present (Dawson 2006, Sathiamurthy & Voris 2006). Hence, marine lakes are young environments, yet their biodiversity is distinct from the adjacent sea (Dawson & Hamner 2005, *CHAPTER 1 & 3*). Several descriptive studies of marine lake fauna suggest high endemism or an abundance of species rare elsewhere (Tomascik & Mah 1994, Dawson 2005, Dawson & Hamner 2005, Azzini et al. 2007, Colin 2009, Becking et al. 2011, *CHAPTER 1*). The unique diversity within the lakes could have two origins: (1) it has resulted from recent divergence of rapidly evolving populations isolated from their ancestral population in the sea or (2) or it is composed of ancient lineages, which are relicts of the sea or of earlier anchialine populations. We refer to these hypotheses as the 'cradle' and 'refuge' hypothesis as adapted from Briggs (2000), Bellwood et al. (2005), and Bellwood & Meyer (2009) and do not consider them mutually exclusive.

Phylogeographic studies of the jellyfish *Mastigias papua* (Dawson 2005, Dawson & Hamner 2005), the fish *Sphaeramia orbicularis* (Gotoh et al. 2009), and the mussel *Brachidontes* sp. (Goto et al. 2011) from marine lakes of the islands of Palau show extreme genetic isolation, low genetic diversity, and in the cases of *Mastigias papua* and *Brachidontes* sp. rapid morphological evolution. In Indonesia the only phylogeographic study of marine lakes fauna conducted to date was on the sponge species *Suberites diversicolor* (*CHAPTER 6*). This study revealed two deeply diverged lineages and suggests that within one lineage there may have been local diversification in the largest and most isolated marine lake in Indonesia (Kakaban lake in East Kalimantan). Here we expand on these results by studying the phylogeography of a co-distributed but unrelated taxon, the mussel *Brachidontes* sp.

Species of the genus *Brachidontes* Swainson, 1840 (Mollusca; Bivalvia; Mytilidae) are marine mussels, which attach themselves to substrate in and below intertidal areas and can form large mytilid beds (Terranova et al. 2007). *Brachidontes* are broadcast spawners with external fertilization and only disperse during their planktonic larval stage for a duration of up to four weeks (Reunov et al. 1999, Monteiro-Ribas et al. 2006, Terranova et al. 2006). *Brachidontes* larvae can live in the plankton for up to four weeks (Monteiro-Ribas et al. 2006), yet it is unknown whether they are able to survive in the subterranean channels connecting marine lakes to the surrounding sea. Worldwide 31 species of *Brachidontes* have been described, but the phylogenetic position of the different species within this genus remains unclear with several reports of the occurrence of cryptic species (Lee & Foighil 2004, 2005, Aguirre et al. 2006). There is an undescribed species of *Brachidontes* that inhabits many marine lakes and when present is generally dominant in terms of space occupation and biomass in the lakes (Tomascik & Mah 1994, Colin et al. 2009, Becking et al. 2011, *CHAPTER 1*). In contrast, this species is extremely rare in coastal habitats (non-marine lake) (Colin 2009, Goto et al. 2011). A previous study of the *Brachidontes* sp. from marine lakes in Palau reported two genetically distinct and morphologically differentiated lineages that probably represent different species (Goto et al 2011). Moreover, the spatial genetic structure of *Brachidontes* sp. from Palau indicated that the majority of the marine lake populations were highly differentiated from each other, each containing private haplotypes (Goto et al 2011).

To study the phylogeography of *Brachidontes* sp. and to compare with data from Palau we collected sequence data of the mitochondrial Cytochrome Oxidase I (COI) gene from four populations of mussels. This is an informative marker at the within as well as between species level (Lee & Foighil 2004, 2005, Aguirre et al. 2006). We collected additional sequences of two nuclear ribosomal genes (28S and 18S) to determine species level relationships within Mytilidae (Lee & Foighil 2004, Aguirre et al. 2006). In addition we examined shell shape using a geometric morphometric approach to determine morphological differentiation. If marine lakes are isolated environments we would expect to find genetic and/or morphological differentiation between the lakes. With this data we addressed the following questions:

1. Are the same lineages present in *Brachidontes* sp. populations in the marine lakes of both Indonesia and Palau?

2. Are the Indonesian marine lake *Brachidontes* sp. populations isolated?

3. Can marine lakes be considered cradles and/or refuges of diversity?

Table 1 Sample localities of six *Brachidontes* sp. populations from Berau (East Kalimantan) and Raja Ampat (West Papua) in Indonesia. Per locality is provided: the salinity, size, relative degree of connection to the adjacent sea, number of individuals analyzed for morphometric analyses (morph.), number of individuals sequenced for 28S, 18S, and COI (number of unique sequences are given in brackets). For the Female-type (F-type) COI sequences the haplotype diversity (h) +/- standard deviations, nucleotide diversity (π) +/- standard deviations, and Tajima's D neutrality index was calculated per population, significant values in bold with asterisk.

										F-type	F-type	F-type	F-type
code	location	region	coordinates	Salinity (ppt)	size lake (x10^3 m^3)	connection	morph	28S	18S	COI	h	π (10-3)	Tajima's D
lake													
KKB	Kakaban lake	Berau	N 02° 08' 23.5" E 118° 30' 31.9"	23-24	4000	most isolated	67	2 (2)	2 (1)	13 (4)	0.4231 +/- 0.1645	1.858 +/- 1.490	-1.29575
TBB	Tanah Bamban lake	Berau	N 02° 13' 50.0" E 118° 34' 50.7"	26	120	least isolated	18			5 (1)	0	0	0
RAJ	Sauwandarek lake	Raja Ampat	S 0° 35' 19.6" E 130° 35' 48.8"	28-30	84	isolated	33	4 (3)	3 (1)	32 (8)	0.3952 +/- 0.1100	0.898 +/- 0.877	**-2.0796***
coast													
GAM	Gam mangrove	Raja Ampat	S 00° 30' 23.8" E 130° 38' 58.9"	32-33	-	open	22	4 (2)	3 (1)	15 (4)	0.3714 +/- 0.1532	1.208 +/- 1.099	**-1.91084***
MGR1	Maratua mangrove	Berau	N 02° 12' 16.3" E 118° 35' 18.9"	33-34	-	open	10	-	-	-	-	-	-
MGR2	Samama mangrove	Berau	N 02° 09' 03.6" E 118° 19' 31.6"	33-34	-	open	3	-	-	-	-	-	-

Material & Methods

Sample collection

All mussels collected in this study belong to the genus *Brachidontes* (Rob Moolenbeek, personal communication), and their morphology corresponds to morphological characters of *Brachidontes* provided by Goto et al. (2011). Mussels were collected from three marine lakes in Indonesia (Figure 1, Table 1): Kakaban lake (KKB, collected in 2003 & 2008), Tanah Bamban lake (TBB, in 2003, collected by R. Moolenbeek) in the Berau region of East Kalimantan province, and Sauwandarek lake (RAJ, in 2009) in Raja Ampat region of West Papua province. Mussels were abundant in the lakes displaying dense mussel beds along the shorelines or covering mangrove roots. *Brachidontes* sp. is rarely found outside of lakes (Colin 2009, Goto et al. 2011, Becking pers. obs.), but after extensive searching we were able to find small populations in Papua in a mangrove swamp by Gam island (GAM, in 2007) approximately 6km distance from RAJ on Mansuar island, and in Kalimantan in the large lagoon of Maratua island (MGR1, in 2003, collected by R. Moolenbeek) approximately 500m North of TBB, and in a tidal creek of Samama island (MGR2, in 2003, collected by R. Moolenbeek) approximately 20 km West from Kakaban island. We assume that these samples are well connected with populations in the surrounding sea. For a full description of the lakes see Becking et al. (2011). These landlocked pools of water have a tidal regime which is typically delayed (ranging from 20 minutes to 4 hours) and dampened (ranging from 20 cm to 1.5 m) compared to the adjacent sea (Hamner & Hamner 1998, Becking et al. 2011, *CHAPTER 1*). Based on the level of tidal dampening and delay, marine lakes can be ranked by their degree of connectivity to the surrounding sea. KKB was the most isolated, followed by RAJ, and TBB had the highest connection to the sea of all three lakes.

Additional sequences of *Brachidontes* sp. from Palau were obtained from GenBank (accession numbers given in Figure 2). Palau is located at approximately 1000 km north from Raja Ampat in Papua. The sample locations are indicated in Table 1. For a full description of the Palauan marine lakes see Hamner & Hamner (1998) and Colin (2009). All specimens were conserved intact in 96% ethanol, except for the samples collected in 2003, which were initially stored in 70% ethanol and transferred to 96% ethanol in March 2011.

DNA extraction, gene amplification and sequencing

Mantle and gill tissue samples were taken from *Brachidontes* sp. and incubated at room temperature for 24 hours in a lysis buffer consisting of 250mM EDTA, 5% SDS, 50mM Tris (pH = 8) (Holland 1993). Total DNA was purified using DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's protocol. Cytochrome Oxidase I (COI) is an informative marker at the within as well as between species level (e.g. Lee & Foighil 2004, Aguirre et al. 2006). Partial COI was amplified using the universal COI primers from Folmer (1994) LCO1490-F: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198-R: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'. PCR reactions were performed in 25µL volumes containing 10.75µL ddH$_2$O, 2µL 25mM MgCl$_2$, 5µL dNTP's (1mM each), 2.5µL 10x PCR Buffer (SpheroQ), 1µL BSA (10mg/mL), 0.3µL of both primers (10µM), 0.15µL Taq polymerase (5units/µL, home made following Pluthero (1993)) and 3µL template DNA. The PCR program consisted of an initial denaturation step of 94°C for 3m followed by 35 PCR cycles of 94°C for 45s, 45°C for 60s and 72°C for 120s, with a final extension step of 72°C for 10m. Quality of PCR products was assessed using gel electrophoresis on 1% agarose gels. If PCR product quality was insufficient, a new PCR reaction was performed in a 25µL volume containing PCR Beads (Illustra, GE Healthcare) using 21.6µL ddH$_2$O, 0.2µL of both primers (10µM) and 3µL template DNA, and an identical PCR cycling program.

To test whether highly divergent mitochondrial lineages were also differentiated at nuclear loci, a subset of 10 samples were amplified for two nuclear genes, 28S and 18S ribosomal DNA. The C1-D3 region of the nuclear ribosomal 28S was amplified using primers C1-F: 5'-ACCCGCTGAATTTAAGCAT-3' (Dayrat et al. 2001) and D3-R (5'-GACGATCGATTTGCACGTCA-3' (Vonneman et al. 2005). The partial nuclear ribosomal 18S was amplified using the primers A1-F: 5'-CTGGTTGATCCTGCCAGTCATATGC-3' and 1800-R: 5'-GATCCTTCCGCAGGTTCACCTACG-3' (Vonneman et al. 2005), and sequenced with the internal primers KP-F: 5'-TGGAGGGCAAGTCTGGTG-3' and KP-R :5'-TTCCCGTGTTGAGTCAAATTAAG-3' (Peijnenburg pers. comm.). PCR amplification of both 28S and 18S was performed in 20µL volumes containing 8.8µL ddH_2O, 4µL 5x Phire buffer (Phire), 2µL dNTP's (1mM each), 1.4µL 100% DMSO, 0.2µL BSA (10mg/ml), 0.5µL of each primer, 0.2µL Hot Start Taq (Phire) and 2.4µL template DNA. PCR cycling steps consisted of an initial denaturation step of 98°C for 30s, followed by 35 cycles of 98°C for 5s, 48°C for 5s and 72°C for 20s, a final extension step of 72°C for 60s and a cooling step of 4°C for 180s. PCR products were purified and sequenced by Macrogen Inc (Korea and The Netherlands).

Doubly uniparental inheritance (DUI)

Some species of the family Mytilidae display a special type of mitochondrial inheritance called 'doubly uniparental inheritance' (DUI) (Zouros et al. 1994), in which two different mitochondrial lineages, a male (M-type) and female (F-type) lineage, are present in male individuals (Terranova et al. 2007, Goto et al. 2011). Male and female mitochondrial lineages can be highly diverged (Saavedra et al. 1997, Kenchington et al. 2002). F-type mtDNA is commonly used as a genetic marker to study the phylogeny of mussels, because it exists in both sexes, and reference sequences for *Brachidontes* mussels are available in GenBank. The distinction between F and M-type sequences in our data was made by constructing a phylogeny and subsequent intraspecific analyses were focused on F-type sequences only.

Genetic data analysis

The bivalve origin of the obtained sequences was verified through BLAST searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were aligned and handled in Geneious Pro 4.8.5 (Drummond et al., 2010) and DAMBE 5.2.15 (Xia & Xie 2001). Outgroups of other species from the genus *Brachidontes* were collected from GenBank. Alignments were collapsed to contain only unique sequence types in DAMBE. The best-fit DNA substitution model was selected by the Akaike Information Criterion deployed in jMODELTEST v. 0.1.1 (Posada 2008) and this model (COI: GTR+G+I, 28S:GTR+G+I, 18S: K2+G) was used for subsequent Bayesian and maximum likelihood phylogeny inferences. Phylogenetic reconstructions were performed under Bayesian inference criteria implemented in MrBayes v. 3.1.2. (Huelsenbeck & Ronquist 2001). Each analysis comprised two independent runs of four Metropolis-coupled Markov chains, sampled at every 1000th generation at the default temperature (0.2). Analyses were terminated after the chains converged significantly as indicated by an average standard deviation of split frequencies of <0.001. Convergence of chains was subsequently checked in Tracer v. 1.5.0 (Rambaut & Drummond 2007). For comparison, maximum likelihood bootstrap analyses were conducted using MEGA v. 5.01 (Tamura et al. 2011) using a heuristic search with 1000 bootstrap replicates. The Bayesian and maximum likelihood phylograms were combined and visualized using Treegraph 2 (Stöver & Müller 2010). Intra- and interlineage uncorrected *p*-distances were calculated in MEGA v. 5.01. To assess genetic diversity within populations and to detect deviations of neutrality, haplotype diversity (h) and nucleotide diversity (π) were calculated and Tajima's D neutrality test (Tajima 1989) was performed using Arlequin v. 3.5.1.2 (Excoffier & Lischer 2010). Haplotype networks were computed to obtain graphical representations of evolutionary relationships between haplotypes using the minimum spanning network algorithm of Rohlf (1973) as and visualized using FigTree v1.3.1 and Adobe©

Illustrator© CS5. To test for spatial structuring of samples we performed an analysis of molecular variance (AMOVA) and calculated pairwise *Φst* values (based on uncorrected *p*-distances) between populations using Arlequin v. 3.5.1.2. Significance of pairwise *Φst* values was determined by 10000 permutations and exact tests of population differentiation in Arlequin.

Morphometric analysis

Brachidontes sp. mussels were photographed in a standardized orientation (Fig. 4) for geometric morphometric analyses. In total 153 digital images were stored as Nikon RAW format (.nef) and converted to 3008 x 2000 pixel JPEG images using Photoshop 5.0 (Adobe). JPEG images were sampled into TPS files using *tpsUtil* (Rohlf 2010a). Shell outlines were used to capture variation in shell shape of *Brachidontes* sp. We used a sliding semi-landmark analysis, in which semi-landmarks are allowed to slide along the outline of a shell in order to find the position that optimally matches the positions of corresponding semi-landmarks in a reference specimen, usually a consensus specimen (Bookstein 1997, Adams et al. 2004). Shell outlines were drawn as curves and digitized as 39 semi-landmarks at equal distance using *tpsDig* (Rohlf 2010b), using the beak of the mussel (umbo, see Fig. 4) as a standardized starting point for drawing an outline. A "sliders file" indicating sliding semi-landmarks was made using *tpsUtil* (Rohlf 2010a). To standardize for size and orientation we used *tpsRelw* (Rohlf 2010c) with Generalized Procrustes Superimposition (Rohlf 1999). Residuals from the superimposition were analysed with the thin-plate spline interpolating function, producing principal warps, followed by relative warp (RW) analysis. *TpsRelw* was used to obtain centroid size (Bookstein 1991) and RW scores for each individual. RW axes are analogous to the eigenvectors of principal component analysis, which combine the major patterns of shell shape variation in the data. Repeatability of RW axes was tested using regression analysis and a non-parametric analysis of similarity in PAST 2.11 (Hammer et al. 2001) of RW scores extracted from 16 specimens of *Brachidontes* sp., which were independently photographed by the same observer (A. Knegt). RW axes were considered repeatable when they showed a non-significant and close to zero R-value in the analysis of similarity and a strong (r > 0.7) and significant correlation. Only repeatable relative warp axes were included in further analyses of shell shape variation. Correlations of RW scores were tested with centroid size and if significant and strong (r > 0.7), we used residuals of the regression to correct for size in all further analyses of shell shape. Significant differentiation between populations was tested using a non-parametric analysis of similarity (ANOSIM, 10000 randomizations; Clarke 1993) based on Euclidian distance as implemented in PAST 2.11.

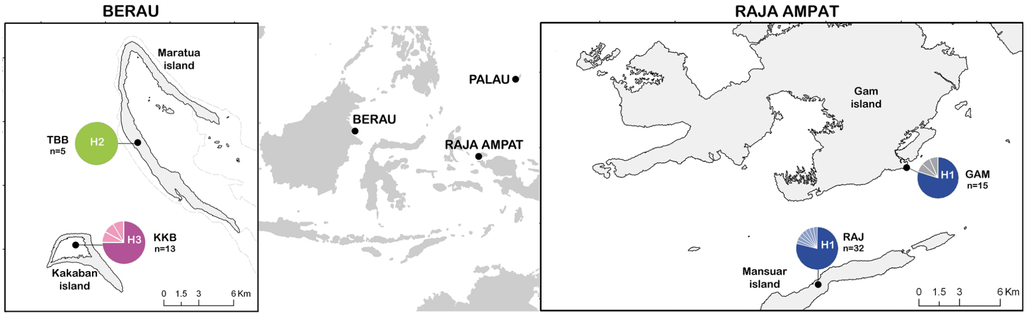

Figure 1 Sample localities of *Brachidontes* sp. populations. Left inset shows map of Berau, East Kalimantan province and right inset a map of Raja Ampat, Papua province (Indonesia). Haplotype frequencies of mitochondrial Cytochrome Oxidase I (COI) Female-type sequences given as pie charts per population of the mussel *Brachidontes* sp. in Kakaban lake (KKB), Tanah Bamban lake (TBB), Sauwandarek lake (RAJ) and Gam mangrove (GAM). Colors represent different haplotypes as shown in Figure 2. Haplotype H1, H2 & H3 refer to the three abundant haplotypes from the three lineage A, A2 and B respectively, as indicated in Figure 2.

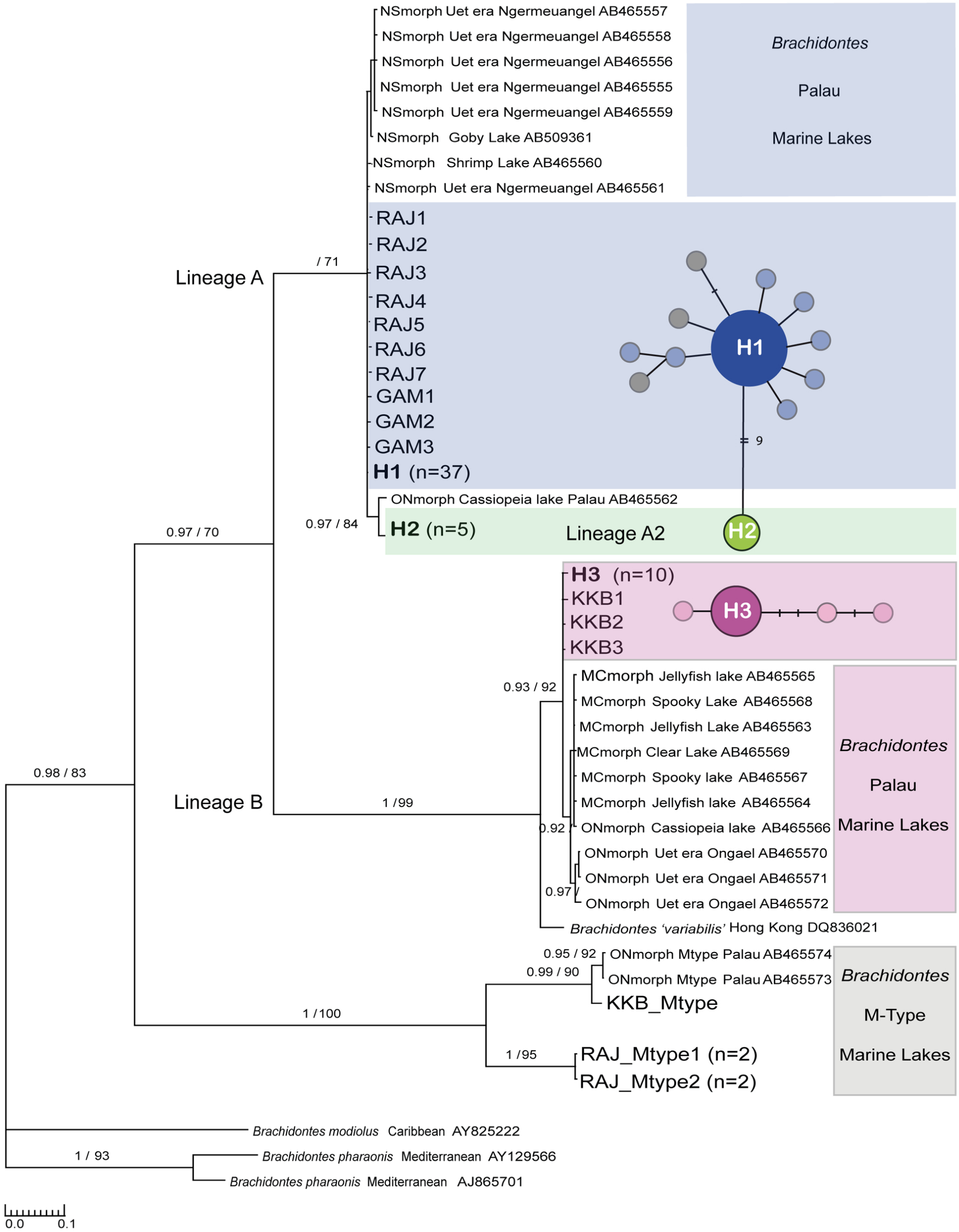

Figure 2 Bayesian/ maximum likelihood phylogram of 91 partial Cytochrome Oxidase I sequences of *Brachidontes* sp. sampled from Indonesia and Palau (see Fig. 1). The sequences from the present study from Indonesia are indicated by location codes (see Table 1), number of shared haplotypes indicated in brackets, no number in brackets indicates a unique haplotype. Sequences of *Brachidontes* from Palau marine lakes are from the study Goto et al. (2011) and are represented with the morphological type, lake name and Genbank accession number. The outgroup is presented with species name, sample location, and GenBank accession numbers. Only posterior probabilities of >90% and maximum likelihood support of >70% are indicated (see Material & Methods for more details). Scale bars indicate substitutions/site. To the right of the phylogram, minimum spanning networks are shown of haplotypes found in the Indonesian marine lakes of the present study. Each circle represents a single haplotype and its diameter is approximately proportional to the number of individuals carrying that haplotype, with the smallest circle representing a single individual. Lines connecting haplotypes represent one base substitution between two haplotypes with additional mutational steps indicated by hash marks and a number if more than thee substitutions.

Results

Sequence diversity

We were able to amplify the genetic markers in the populations from Kakaban lake (KKB), Tanah Bamban lake (TBB), Sauwandarek lake (RAJ) and Gam mangroves (GAM) to amplify the genetic markers, but in the other populations (MGR 1 & 2) the DNA-yield was too low to allow amplifications (Table 1). A total of 70 COI sequences of 552 bp were collected, resulting in 65 F-type sequences (16 haplotypes) and five M-type sequences (three haplotypes). Three abundant F-type haplotypes were collected: H1 in RAJ and GAM (n=37), H2 in TBB (n=5) and H3 in KKB (n=10). The remainder of the F-type haplotypes (n=13) were unique. No stop codons, indels or double peaks were observed in the F-type sequences. M-type sequences were designated based on a close similarity to the ON-morph M-type sequences from Palau (Figure 2). All M-type sequences revealed heteroplasmic “double peaks” at a small number of nucleotide positions in their sequence chromatograms. These double peaks are unlikely the result of amplification of both F- and M-type sequences in male individuals (resulting from DUI, explained in M&M) because both sequences were very distant from all sampled F-type sequences and were closely related to published M-type sequences. The heteroplasmic sequences did not contain any stopcodons, refuting the possibility of co-amplification of a pseudogene (Bensasson *et al.* 2001). All further phylogeographic analyses were focused on F type sequences only.

These double peaks were not a result of DUI (see Material andd Methods) as the sequences were very distant from all sampled F-type sequences and M-type sequences could not be assembled to reference F-type sequences. The heteroplasmic sequences did not contain any stopcodons, refuting the possibility of a pseudogene (Bensasson *et al.* 2001). All further phylogeographic analyses was focused on F type sequences only. A subset of ten individuals from the four Indonesian populations were sequenced for 28S resulting in 636 bp length fragments and three gene variants (Figure 3A). For 18S the same subset of samples as 28S was amplified, but resulted in only eight successful sequences of 326 bp and two genetic variants (Figure 3B). Amplifications of 28S and 18S of samples from TBB samples were unsuccessful.

Phylogeography (COI)

The COI sequences represented two major lineages that were strongly supported by both Bayesian and maximum likelihood analyses: lineage A was represented by the two abundant haplotypes H1 and H2, and the single haplotypes GAM1-3 and RAJ1-7, while lineage B comprised the abundant haplotype H3 and the single haplotypes KKB1-3 (Figure 1 and 2). The two highly divergent lineages (*p*-distance is 14.8%, Table 3) correspond to ‘lineage A’ and ‘lineage B’ from the marine lakes in Palau (Goto et al. 2011) which naming we adopt. In the present study lineage A was present in TBB, RAJ and GAM, while lineage B was present in KKB only. No haplotypes were shared between Indonesia and Palau, but comparison of GAM and RAJ populations with lineage A from Palau shows that the two regions were closely related with a between group *p*-distance of 0.84% (Table 3). Similarly, COI haplotypes from the KKB population in Indonesia were closely related but not identical to lineage B haplotypes from Palau with a between group *p*-distance of 1.43% (Table 3). In addition, mussels from TBB constituted a highly supported lineage A2 within lineage A with a between-group *p*-distance of 1.8 % between TBB and RAJ & GAM (Figure 2 and Table 3). The samples from TBB were closely related to the Palauan ON-morph *Brachidontes* AB465562 from Northern Cassiopea Lake (Fig. 2; Goto et al. 2011).

Lineage A & B were not distinguished by the 28S and 18S sequences (Figure 3A&B). The phylogram of 28S

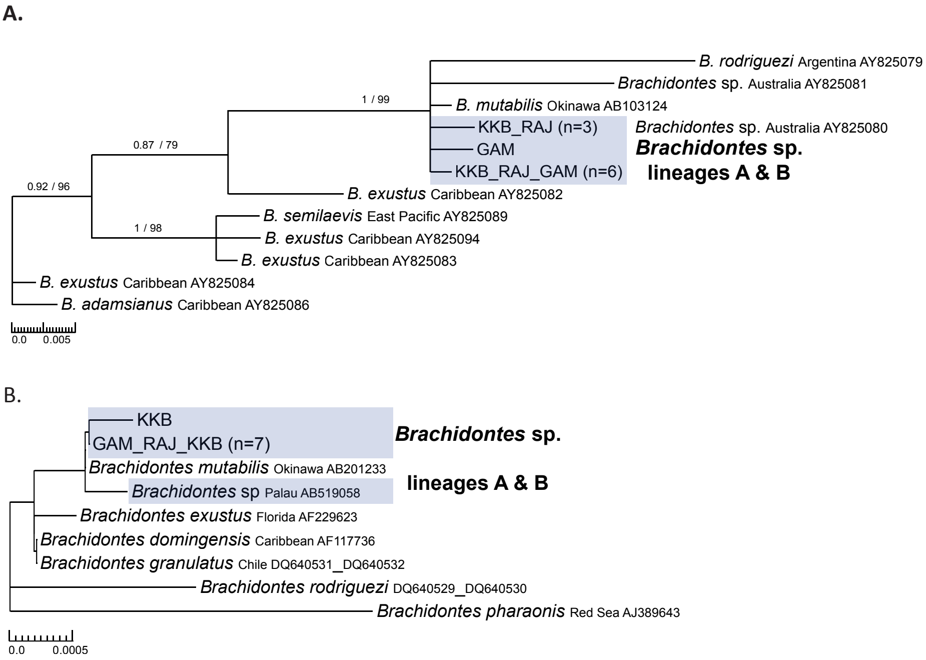

Figure 3 Bayesian/maximum likelihood phylograms of nuclear markers of *Brachidontes* sp. found in this study and related species from the same genus. **A.** partial 28S rDNA genes. **B.** partial 18S rDNA genes. Samples of the present study indicated by location codes (see Table 1) and separated by underscores followed by the number of individuals in brackets. For samples downloaded from GenBank species names are provided, followed by the sample location and GenBank accession number. *Brachidontes* sp. lineage A and B indicated by blue boxes. Only posterior probabilities of >90 and maximum likelihood values of >70 indicated. The 18S resulted in an unresolved tree. Scale bars indicate substitutions/site.

gene sequences (Figure 3A) shows a strongly supported polytomy including the Indonesian *Brachidontes* as well as the species *B. mutabilis* (collected in Okinawa), *Brachidontes* sp. (Darwin, Australia), and *B. rodriguezi* (Argentina). One 28S sequence representing both lineages A and B from the present study was identical with a *Brachidontes* sp. 28S sequence from Darwin, Australia (Genbank accession AY825080). The 18S sequences resulted in an unsupported phylogeny (both Bayesian and Maximum Likelihood), Fig. 3B. Our results of 28S and 18S confirmed the designation of the marine lake samples to the genus *Brachidontes* but did not allow species distinction within the genus (Lee & Foighil 2004, 2005, Aguirre et al. 2006, Santaclara et al. 2006). Genetic diversity indices based on F-type COI sequences are shown in Table 1. Haplotype diversity was highest in KKB (0.4231 +/- 0.1645) and lowest in TBB (0). Nucleotide diversity was highest in KKB (1.858 +/- 1.490) and lowest in TBB (0). Neutrality index Tajima's D was negative and significant for the populations of GAM and RAJ, suggesting deviations from neutrality in these samples (Table 1). All COI haplotypes found in KKB and TBB were unique for that location, whereas RAJ and GAM shared the COI haplotype H1 (Figure 1). The analysis of molecular variance (AMOVA) showed that there was a significant spatial genetic structure within the samples. All populations were strongly and significantly differentiated (*Φst* ranged from 0.95 to 0.99), except RAJ and GAM that were not significantly differentiated (Table 2).

Table 2 Pairwise *Φst* based on partial Cytochrome Oxidase I Female-type sequences between populations of *Brachidontes* sp. in Kakaban lake (KKB), Tanah Bamban lake (TBB), Sauwandarek lake (RAJ) and Gam mangrove (GAM). Significant *Φst* indicated in bold with asterisk (p<0.05).

Φst	KKB	TBB	RAJ
TBB	**0.99118***		
RAJ	**0.99214***	**0.95454***	
GAM	**0.98985***	**0.94805***	-0.00591

Table 3 Between- and within-group uncorrected *p*-distances based on partial Cytochrome Oxidase I sequences of *Brachidontes* sp. from Indonesia and Palau provided below the diagonal, standard deviations provided in italics above the diagonal. Black cursive along the diagonal indicates within-group uncorrected *p*-distance. For location codes refer to Table 1. Lineage A and B refer to the two major lineages obtained in Palau by Goto (2011), 'Outgroup' to *B.pharaonis* (GenBank accession numbers AY129566 and AJ865701) and *B.modiolus* (AY825222).

%	lineage A	lineage B	KKB	TBB	RAJ_GAM	OUTGROUP
lineage A	**0.80**	*1.43*	*1.42*	*0.45*	*0.25*	*1.29*
lineage B	14.93	**0.59**	*0.40*	*1.44*	*1.43*	*1.40*
KKB	15.11	1.43	**0.51**	*1.43*	*1.42*	*1.39*
TBB	1.75	14.80	14.81	**0.00**	*0.49*	*1.29*
RAJ_GAM	0.84	14.85	14.86	1.83	**0.38**	*1.29*
OUTGROUP	17.29	18.79	18.66	17.10	17.10	**14.27**

Morphometric analysis

The relative warp axes 1-4 were significantly repeatable, explaining 92.80% of total observed variation in shell shape. The population sampled at MGR2 was excluded from statistical analyses because its sample size was too small (n < 5). Variation in shell outline was found in shell length to width ratio and the position of the umbo relative to the longitudinal axis of the shell. A scatter plot was made for relative warp axes 1 and 2, showing how 85.11% of total observed variation in shell outline is distributed between sample locations and along these axes of shell outline variation (Fig. 5). RW1 represents variation in length to width ratio, whereas relative warp axis 2 incorporates the position of the umbo relative to the longitudinal axis of the shell. This suggests that individuals from RAJ and GAM have a larger length to width ratio than individuals from KKB, and individuals from MGR1 have an umbo that is positioned farther away under the longitudinal axis of the shell than individuals from other populations. Overall shell shape variation among populations was highly significant (ANOSIM, global R = 0.3704, P = 0.0001) and all pair wise comparisons were significant except between RAJ and GAM (Table 4).

Table 4 Analysis of similarity for *Brachidontes sp.* based on relative warp axes 1-4. R-values are shown below the diagonal, sequential Bonferroni corrected *P*-values are shown above the diagonal. Bold R-values with asterisk indicate significant values (p<0.05). The population sampled at MGR2 was excluded from statistical analyses because its sample size was too small (n < 5).

	KKB	TBB	RAJ	GAM	MGR1
Sample size	67	18	33	22	10
KKB		0.0121	0.0001	0.0001	0.0001
TBB	**0.1646***		0.0001	0.0001	0.0003
RAJ	**0.392***	**0.5065***		-0.03051	0.0001
GAM	**0.2774***	**0.5002***	0.7815		0.0001
MGR1	**0.5144***	**0.3608***	**0.5865***	**0.6728***	

Figure 4 Standardized orientation the mussel *Brachidontes* sp. for the morphometric analyses. Morphology was characterized by a curve capturing shell outline, consisting of 39 semi-landmarks at equal distance starting from the umbo, indicated by red dot. Representatives are shown of the three dominant haplotypes (see Figure 2) from the localities Gam mangrove (GAM), Tanah Bamban lake (TBB), and Kakaban lake (KKB).

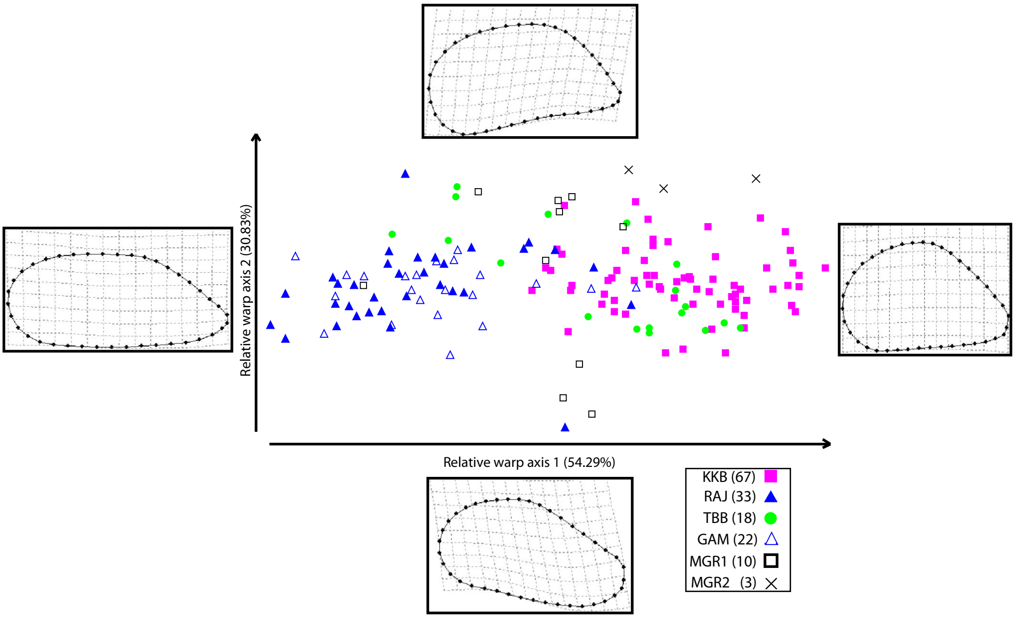

Figure 5 Shell outline shape variation in six populations of *Brachidontes* sp. mussels from Indonesia (sample codes are detailed in Table 1, sample size in brackets). Ordination of data in the plane identified by relative warp (RW) axis 1 and 2, together explaining 85.11% of the variance. Corresponding thin-plate splines of the most positve and negative deformations along the axes are shown. Both axes have been corrected for their correlation with centroid size.

Discussion

Divergent Brachidontes *lineages in Indonesia and Palau*

We detected three mitochondrial Female-type (F-type) lineages based on COI sequences, viz. lineage A, A2, and B, in *Brachidontes* sp. mussels sampled in marine lakes and adjacent sea habitats in Indonesia. The two major lineages are closely related to the lineages A and B reported by Goto et al. (2011) for *Brachidontes* sp. from marine lakes in Palau. Considering the degree of genetic divergence (almost 15%), lineages A and B of *Brachidontes* sp. in the marine lakes of Indonesia and Palau may constitute a species complex of at least two undescribed species. Furthermore, it is possible that the lineage A2 represents an incipient species that will continue to diverge if the populations remain isolated. The genetic distances between lineage A, A2, and B were comparable to those reported between (cryptic) species of other mussels (Lee and Foighil 2004, 2005, Terranova et al. 2007). Unfortunately, the two nuclear markers we used (18S and 28S) could not resolve species level relationships, probably because these markers were too conserved (Lee & Foighil 2004, 2005, Santaclara et al. 2006, Goto et al. 2011).

Patterns of COI genetic differentiation of the *Brachidontes* sp. populations were strikingly congruent with morphological differentiation. The mussel populations in the three Indonesian marine lakes differed significantly in shell outline shape, concordant with the three genetic lineages. Our results are supported by Wesselingh et al (*in review)* who concluded that the *Brachidontes* sp*.* in Kakaban lake probably represents an as yet undescribed species as it could not be attributed to any known species from the region. Morphology can provide a proxy for underlying genetic variation and adaptive evolution (Dawson 2005, Dawson & Hamner 2005, Mariani et al. 2011). The morphometric differentiation we found is probably partly genetic but also may represent ecophenotypic plasticity related to different environments which is common in mussels (Aguirre et al. 2006, Groenenberg et al. 2011, Mariani et al. 2011). Each of the three lakes of the present study have a different environmental regime (Table 1; Becking et al. 2011, *CHAPTER* 1) which may also cause the differences in shell shape that we observe (Groenenberg et al. 2011).

In a study of the population structure of a co-distributed sponge species in marine lakes in Indonesia, Becking et al. (*CHAPTER 6*) found a highly divergent lineage in Kakaban lake that was not observed in any of the other populations. By combining our data with Goto et al. (2011), we find that unique *Brachidontes* sp. lineages in specific marine lakes in Indonesia are in fact, also found in other geographically very distant regions in the IAA. This finding stresses the importance of extensive sampling throughout the Indo-Pacific in order to establish true endemism.

Differentiation of populations in Indonesian marine lakes

We found strong genetic differentiation between *Brachidontes* sp. populations in Indonesian marine lakes that was congruent with differences in shell outline shape. Not one haplotype was shared between the different marine lakes populations of *Brachidontes* sp. from Indonesia and Palau, suggesting that dispersal between marine lakes is highly limited. If gene flow would be common between marine lakes and adjacent sea populations, we would have expected to see haplotype sharing between populations, particularly between geographically close lakes. Dawson & Hamner (2005) found that the genetic distance between marine lake and lagoon populations of the jellyfish *Mastigias papua* was highly correlated with the degree of physical isolation of the lake to the adjacent sea, and not the actual geographic proximity of populations to each other.

Our data shows that the largest and most isolated lake, Kakaban lake in Berau, harbors the highest nucleotide diversity of all sampled *Brachidontes* populations in Indonesia and Palau (Goto et al. 2011). Kakaban lake also harbors the highest genetic diversity of all populations of the co-distributed sponge *Suberites diversicolor* sampled from seven marine lakes and three coastal locations in Indonesia, Singapore and Australia (Becking et al *CHAPTER 6*). Isolation acts to decrease the rate of immigration (MacArthur & Wilson 1967, Whittaker & Fernandez-Palacios 2007, Chen & He 2009, Chen et al. 2011, Rosindell et al. 2011), but isolation can also enhance diversification as diminished gene flow allows populations to diverge and ultimately form new species (Emerson & Gillespie 2008, Chen & He 2009, Rosindell et al. 2011). The degree of physical connection of a lake to the adjacent sea may, however, not fully dictate the genetic structure and diversity within a lake. Sauwandarek lake (RAJ) in Raja Ampat has a low physical connection with the surrounding sea, yet is not differentiated from the adjacent mangrove population (GAM). The lack of strong population differentiation between RAJ lake and the coastal mangrove may be caused by recurrent (recent and historic) gene flow. However, they do not share any of the unique haplotypes, which is suggestive that these populations are in fact isolated. The two populations may be too recently diverged to show strong differentiation in the molecular markers that we used.

Isolation of marine lake populations may be the result of strong isolating barriers and/or different selective regimes in the lakes. The subterranean channels that connect each lake with the surrounding sea may provide a formidable dispersal barrier for propagules. Alternatively, a propagule may be able to enter but may not be able to survive due to the environmental regime within the lake or competition with resident founder lineages/species. The pattern that is almost consistently seen in a variety of taxa (jellyfish, fish, and bivalves) is that each lake harbors a single lineage per taxon (Dawson 2005, Dawson & Hamner 2005, Gotoh et al. 2009, Goto et al. 2011). An explanation for this pattern may be that the first colonizers enter, proliferate and out-compete any subsequent migrating newcomers. This hypothesis is supported by the observation that lakes either contain mussels or oyster as the dominant bivalves, rarely both (Becking et al. 2011). The strong morphological differentiation in shell shape suggests that different lake habitats provide different selective regimes, as is also illustrated by differentiated species assemblages (Becking et al. 2011, Becking et al. *unpublished data , CHAPTERS 1, 2, 3, 4 & 5)*.

Marine lakes: cradles or refuges of diversity?

The marine lakes of Indonesia and Palau are situated in the Indo-Australian Archipelago (IAA) of highest marine biodiversity, including a high number of endemics (Hoeksema 2007). The high biodiversity in this hotspot probably resulted from current ecological and environmental conditions as well as the biogeographical history of the area (e.g. Bellwood et al. 2005, Hoeksema 2007). The processes that have lead to this pattern of biodiversity remain much debated. In the 'cradle hypothesis' allopatric speciation in isolated marine environments during the Pleistocene low sea level stands is believed to have contributed to the current marine biodiversity in the IAA (e.g. Briggs 2000, 2005), while the 'refuge hypothesis' contends that isolated marine environments during Pleistocene may have provided refuge for marine shallow-water species that went extinct elsewhere (e.g. Bellwood et al. 2005, Renema et al. 2008, Bellwood & Meyer 2009). Recent phylogenetic studies strongly suggest that endemics in the IAA are not exceptionally young – most endemics having origins in the early Pliocene-Miocene age (Renema et al. 2008, Cowman & Bellwood 2011). Though there are still few examples in favor of the 'cradle hypothesis' (e.g. Barber et al. 2006), most studies have concluded that different and possibly combined processes will be relevant for different taxa (e.g. Barber & Bellwood 2005, Carpenter et al. 2011, Cowman & Bellwood 2011).

Both hypotheses are supported when taking the marine lakes as small natural laboratories for the scenario of the Pleistocene low sea level stands. We assume that the floodwaters that filled the lakes during the Holocene sea level rise (<15000 years before present) allowed for independent, chance colonization of lakes by propagules from the surrounding sea and that these propagules were the progenitors of the present day populations (Dawson & Hamner 2005, Dawson 2006). The deep divergences that are observed in the mussel populations are probably ancient lineages that have taken refuge there. It is unlikely that these have diverged within the timeframe of the lake because if this were the case we would have unrealistically high evolutionary rates of more than 100% COI sequence divergence per million years. Our results show that no marine lake shares any of the COI haplotypes with any other lake. With similar patterns of genetic variation in COI for the jellyfish *Mastigias papua* populations in marine lakes in Palau, Dawson & Hamner (2005) conclude that the pattern resulted from random redistribution of haplotypes that existed in the sea during the formation of each lake. It is, however, remarkable that of the 35 haplotypes of *Brachidontes* sp. that have been uncovered in Palau and Indonesia, almost 90% of the haplotypes are not shared among the 12 lakes whereas there is extensive haplotype sharing within each of the lakes. Therefore we suggest an alternative hypothesis, namely that the within lineage diversity that is unique to each lake resulted from *in situ* divergence. This scenario would also result in relatively rapid evolutionary rates, but such rates are not uncommon for recently diverged taxa (e.g. Genner et al. 2007, Ho et al. 2011). For example, if we take the within *p*-distances of the Kakaban lake population (0.51%) and the maximum age that the lake could be (15000 years), this would result in a mutation rate of 35% per million years for *Brachidontes* sp. This is higher than the evolutionary rate that has previously been estimated at 18.3-24.4% per million years for the F-type COI third codon positions in *Brachidontes* spp.(Lee & Ó Foighil 2004). Combined effects of stochastic processes (e.g. founder effects), local adaptation and increased evolutionary rates could produce high levels of differentiation in small populations such as in marine lake environments (Dawson & Hamner 2005, Ho et al. 2011). The patterns of genetic variation found so far in marine lake populations of *Mastigias papua* (Dawson & Hamner 2005)*, Brachidontes* sp. (Goto et al. 2011, this study)*, Suberites diversicolor* (Becking et al. *CHAPTER 6*)*, Sphaeramia orbicularis* (Gotoh et al 2009) are generally consistent with taxa evolving in isolation in peripatric environments, such as islands or satellite lakes of ancient rift lakes in Africa (e.g. Genner et al. 2007, Emerson & Gillespie 2008, Chen & He 2009, Rosindell et al. 2011). The role of marine lakes in supporting endemism in the IAA region may reflect enhanced survival of endemics, with the possibility of population differentiation that in time may lead to speciation.

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Samenvatting

Dit proefschrift beoogt inzicht te geven in de processen die een rol spelen in biodiversiteit van tropische kustgebieden. Daarbij worden mariene meren, dat wil zeggen door land omsloten delen van de zee, als model gebruikt. Lange tijd werd aangenomen dat in zee levende soorten grote verspreidingsgebieden en grote populaties hebben, waardoor ze minder sterke barrières bij hun dispersie zouden ondervinden dan organismen op het land. Recent populatie genetisch en fylogenetisch onderzoek laat echter zien dat er ook op kleine schaal al grote genetische differentiatie kan voorkomen. Dit betekent dat er aanzienlijk meer barrières voor dispersie, en daaraan gerelateerde mogelijkheden voor allopatrische soortsvorming, zijn voor mariene organismes dan aanvankelijk vermoed werd. Onderzoek naar mariene meren, die gekenmerkt zijn door duidelijk omschreven barrières, kan nieuwe inzichten opleveren in de ecologische en evolutionaire processen die van invloed zijn op de verspreidingspatronen van tropische mariene soorten.

In dit proefschrift komen uiteenlopende aspecten van deze relatief onbekende habitats aan de orde. Het gaat om zogenaamde anchialine systemen, kleine zeewaterlichamen, die alleen door ondergrondse verbindingen via kanalen of door poreus gesteente met de open zee in verbinding staan. Daardoor bevatten ze zout of brak water, in tegenstelling tot “normale” meren die uit zoet water bestaan. De mariene meren bevinden zich vaak in glooiend landschap, waardoor ze vanaf de kust niet zichtbaar zijn. Het is dan ook onbekend hoeveel van dergelijke meren er precies op de wereld bestaan; naar schatting zijn het er ongeveer 200. In gebieden met uitgebreide karstlandschappen, zoals Kroatië, Bermuda, Vietnam, Palau en Indonesië, bevinden zich concentraties van 10 of meer meren. De weinige studies naar de biodiversiteit van mariene meren, uitgevoerd in Palau en Vietnam voordat mijn promotieonderzoek begon, laten zien dat die meren gekenmerkt worden door sterk geïsoleerde populaties en een groot aantal soorten die buiten de meren niet aanwezig zijn of zeer zeldzaam zijn. Deze bevindingen geven aan dat het om opmerkelijke ecosystemen gaat, die nog veel mogelijkheid bieden voor onderzoek aan mariene biodiversiteit en evolutie. Mariene meren kunnen als natuurlijke laboratoria worden beschouwd, waarmee veel ecologisch-evolutionaire vraagstukken bestudeerd kunnen worden.

Voor dit onderzoek zijn mariene meren onderzocht die onlangs ontdekt zijn in twee regio’s in Indonesië: Berau in Oost Kalimantan (Borneo) en Raja Ampat in West Papua (Nieuw Guinea). De volgende vragen zijn daarbij aan de orde gekomen:

1. Welke verschillende soorten mariene meren zijn er te onderscheiden in Indonesië?
2. In welke mate verschillen de soortengemeenschappen die we in mariene meren aantreffen van die in de nabijgelegen habitats in zee en aan de kust?
3. In welke mate zijn de populaties in de mariene meren geïsoleerd?
4. Kunnen mariene meren beschouwd worden als natuurlijke laboratoria van evolutie?

Bij aanvang van mijn promotieonderzoek in 2007 was er zeer weinig bekend over de mariene meren in Indonesië. Daarom was beschrijvend veldonderzoek een eerste vereiste voordat verder analytisch werk mogelijk was. Om de biodiversiteit van de meren te onderzoeken, was gekozen voor sponzen als indicatorsoorten. Ze zijn dominant aanwezig in de meren en spelen een belangrijke rol in de nabijgelegen koraalriffen en mangroves door hun soortenrijkdom, biomassa, en filteractiviteiten.

Om vast te stellen in hoeverre mariene meren geïsoleerde habitats zijn, is gekeken naar drie aspecten:

(a) De omvang van de uitwisseling van water tussen het meer en de zee. De hoeveelheid wateruitwisseling kan dienen als een benadering voor de mate van fysieke isolatie.

(b) De patronen van soortenassemblages van sponzen. Variatie in de compositie van soortenassemblages tussen de uiteenlopende locaties kan informatie verschaffen over de mogelijkheden tot verspreiding in het mariene milieu.

(c) De genetische patronen van populaties van twee model-soorten uit mariene meren: de spons *Suberites diversicolor* (Porifera: Demospongiae: Hadromerida: Suberitidae) en de mossel *Brachidontes* sp. (Mollusca: Bivalvia: Mytilidae). Met behulp van moleculaire methoden kan de mate van diversiteit binnen populaties, alsook de mate van genetische verwantschap en samenhang (‘gene flow‘) tussen natuurlijke populaties worden bepaald.

Als de meren in open verbinding zouden staan, met elkaar en met de zee, dan is de verwachting dat er dezelfde soortensamenstellingen gevonden zullen worden en dat er weinig genetische differentiatie zal zijn tussen de populaties.

1. *Welke verschillende soorten mariene meren zijn er te onderscheiden in Indonesië?*

We zien een grote diversiteit aan mariene meren in Indonesië (HOOFDSTUK 1). Ook moeten veel meren nog nader gedocumenteerd worden. Er is een gradiënt in de mate van verbinding tussen meer en zee. Een meer met een relatief grote zeeverbinding lijkt op een lagune, zowel in waterchemie als in biota, terwijl de meer geïsoleerde meren vooral brak water bevatten en unieke soorten die zelden of nooit in zee gevonden worden.

2. *In welke mate verschillen de soorten die we in mariene meren aantreffen van die in de nabijgele gen habitats in zee en aan de kust?*

De samenstelling van de sponzenfauna is systematisch en kwantitatief gemeten in mariene meren, kustmangroves en koraalriffen in Berau (Oost-Kalimantan, Indonesië). Deze studies laten zien dat mariene meren een significant andere sponzenfauna bevatten dan daarbuiten voorkomt, met slechts een gedeelte van de nabijgelegen zeefauna (HOOFDSTUKKEN 2, 3, 4 & 5). De sponssoorten in de meren kunnen in drie groepen worden onderverdeeld: (a) endemische soorten, die slechts in één meer voorkomen; (b) soorten die in verschillende meren voorkomen, maar niet daarbuiten (‘meersoorten’); en (c) wijdverbreide soorten, die behalve in de meren ook in zee voorkomen. Mariene meren dragen significant bij aan de lokale soortendiversiteit door de aanwezigheid van endemen en meersoorten. Meer dan de helft van de sponssoorten in de meren hebben nog geen wetenschappelijke naam en moeten dus nog beschreven worden. (HOOFDSTUKKEN 1, 2, 3, 4 & 5).

3. *In welke mate zijn de populaties in de mariene meren geïsoleerd?*

De meren bevatten een unieke diversiteit aan soorten en bovendien endemische genetische varianten (HOOFDSTUKKEN 6 & 7). In de populaties van zowel de spons *Suberites diversicolor* als de mossel *Brachidontes* sp. werden twee genetisch sterk verschillende varianten gevonden, die mogelijk als morfologisch cryptische soorten geïnterpreteerd moeten worden. De patronen van genetische variatie die in de meren gevonden zijn, lijken sterk op wat bekend is van populaties in geïsoleerde omgevingen (HOOFDSTUKKEN 6 & 7). In zowel de spons- als de mosselsoort zien we een patroon van lokale diversificatie in het grootste en meest geïsoleerde mariene meer in Indonesië (het Kakaban-meer in Oost-Kalimantan). Isolatie van populaties en soortengroepen in mariene meren kan ontstaan als gevolg van sterk isolerende barrières en/of door verschillende selectieregimes in de meren. Het gesteente tussen de meren en de zee, hoewel poreus, kan een belangrijke barrière vormen voor dispersie van een soort. Indien een immigrant een meer wel kan bereiken, bestaat de kans dat deze het niet overleeft door de milieufactoren binnen het meer of door competitie met de daar reeds aanwezige organismes.

4. *Zijn mariene meren natuurlijke laboratoria van evolutie?*

De onderzochte mariene meren kunnen niet ouder zijn dan 15.000 jaar, maar toch lijkt veel van de diversiteit in soorten en genetische samenstelling beperkt te zijn tot elk meer afzonderlijk (alle HOOFDSTUKKEN). Een gebied met een hoog endemisme kan een centrum zijn waar soorten ontstaan en blijven bestaan (‘kraamkamers’), maar kan juist ook het laatste gebied zijn waar een vroeger wijdverbreide soort nog overgebleven is (‘refugium’). Beide scenario’s kunnen van toepassing zijn op mariene meren. Het lijken inderdaad kraamkamers voor diversiteit te zijn, gezien de recent gedivergeerde en snel evoluerende populaties die er worden aangetroffen (HOOFDSTUKKEN 6 & 7). De meren zouden tevens ook als refugia kunnen dienen voor elders uitgestorven organismen, relicten uit de vroegere zee-fauna of uit oudere anchialine systemen (HOOFDSTUKKEN 3, 6 & 7). Gezien de hoge mate van endemisme die er wordt aangetroffen is het aannemelijk dat de meren habitats zijn waarin allopatrische differentiatie uiteindelijk tot soortvorming kan leiden. Dit proefschrift geeft slechts een voorzichtig beeld van de gevolgen van kortstondige isolatie op de mariene biodiversiteit. Verdere studie aan mariene meren kan een beter begrip bewerkstelligen van de snelheid en de aard van relatief snelle evolutionaire processen in tropische mariene ecosytemen.

De resultaten van dit promotieonderzoek hebben belangrijke implicaties voor natuurbescherming. Mariene meren lijken op eilandsystemen: ze zijn geografisch goed gedefinieerd (HOOFDSTUK 1), ze bevatten unieke, geïsoleerde biota (HOOFDSTUKKEN 6 & 7), waarbij een groot deel van de soorten endemisch is of in ieder geval elders zeldzaam is (HOOFDSTUKKEN 2, 3, 4 & 5). Net als eilanden zijn deze meren erg gevoelig voor veranderingen door menselijk toedoen, bijvoorbeeld de introductie van invasieve soorten. Dit proefschrift laat zien dat veel van de soortendiversiteit nog niet beschreven is. Juist om die reden en vanwege hun verschillende uitzonderlijke eigenschappen verdienen mariene meren een belangrijke rol in de natuurbescherming, zowel in Berau als Raja Ampat.

Ringkansan

Tujuan dari tesis ini adalah untuk mendapatkan wawasan tentang peran proses distribusi spesies laut tropis dengan menggunakan danau laut sebagai model. Selama ini banyak pendapat mengatakan, bahwa spesies laut pada umumnya memiliki rentang geografis yang luas dengan ukuran populasi yang besar, karenanya penyebaran organismenya agak lambat dibandingkan penyebaran organisme di darat. Studi terbaru terkait populasi molekul menunjukkan gambaran yang berbeda pada diferensiasi populasi dalam skala spasial kecil. Hal ini berarti, pada organisme laut tampaknya ditemukan lebih banyak hambatan dan kurangnya kesempatan, sehingga organisme laut lebih memiliki kemungkinan *allo-patric*. Danau-danau laut dengan penggambaran kontur jelas menyediakan kesempatan untuk mempelajari pola kumpulan spesies dan tahap awal evolusi pada laut dan pesisir di lingkungan yang terisolasi.

Penelitian ini mengangkat berbagai aspek dari berbagai habitat yang relatif kurang dikenal. Selama ini diketahui danau laut memiliki sedikit sistem *anchialine*. Istilah *anchialine* merujuk pada badan air yang terkurung daratan dan mempertahankan karakter kelautan melalui koneksi bawah laut yang menyempit. Oleh karena itu kawasan ini selain berair asin juga berair tawar, dan sangat berbeda dengan danau lain yang umumnya berair tawar. Danau laut pada umumnya ditemukan pada kawasan yang tersembunyi, pada cekungan pedalaman alami di antara bukit-bukit dan umumnya tidak terlihat dari pantai.

Berapa jumlah danau laut seperti ini di dunia tidak diketahui persis. Namun diperkirakan terdapat sekitar 200 danau yang tersebar di berbagai tempat, 10 diantaranya terdapat di daerah berkapur seperti Kroasia, Bermuda, Vietnam, Palau, dan Indonesia. Selama ini sangat sedikit dilakukan penelitian tentang keragaman danau laut di Palau dan Vietnam, tempat dimana penelitian saya dimulai. Di danau-danau laut ini, terlihat bahwa isolasi populasi telah mengakibatkan munculnya beragam jenis spesies yang langka dan unik. Kondisi ini disebabkan oleh sebuah sistem lingkungan tertentu, dan menawarkan potensi besar untuk penelitian lebih lanjut dan mendalam tentang keragaman laut dan evolusi. Danau laut merupakan sebuah laboratorium alam dimana banyak pertanyaan tentang evolusi lingkungan dapat dipelajari.

Dalam tesis ini saya meneliti danau laut yang baru-baru ini ditemukan di dua wilayah Indonesia: perairan Berau di Kalimantan Timur dan perairan Raja Ampat di Papua Barat. Tujuan tesis ini adalah untuk mengungkap pola spasial keanekaragaman hayati di danau laut dan mengetahui sejauh mana danau laut dapat mewakili isolasi lingkungan pesisir.

Penelitian ini akan membahas sejumlah pertanyaan, yaitu:

1. Apa saja jenis danau laut di Indonesia?

2. Jenis spesies apa saja yang ada di danau laut yang berbeda dengan jenis spesies yang ada dilingkungan pesisir yang ada di dekatnya?

3. Sejauh mana populasi di danau terisolasi?

4. Dapatkah danau laut di Indonesia dianggap sebagai laboratorium alam evolusi ?

Ketika saya memulai PhD saya pada tahun 2007, hanya sedikit informasi yang saya dapatkan tentang danau laut di Indonesia. Di awal penelitian, saya mengamati kondisi danau laut secara deskriptif - misalnya lokasi danau, penggambaran karakteristik geografis dan fisik, serta taksonomi dari spesies yang berada di danau ini. Landasan deskriptif ini merupakan prasyarat sebelum dilakukannya studi analitis lanjutan. Sebagai titik tolak penelitian, saya memilih *spons* sebagai kelompok sasaran dalam mengukur keanekaragaman hayati. Spons adalah salah satu taksa paling beragam di danau dan memiliki peran penting dalam pertumbuhan karang dan mangrove, baik oleh keragaman, biomassa maupun kegiatan penyaringannya. *Spons* adalah biota ideal untuk membandingkan keanekaragaman hayati di dalam dan di luar danau laut.

Untuk menentukan sampai sejauh mana danau laut di Indonesia termasuk dalam lingkungan yang terisolasi, ada tiga aspek yang dipertimbangkan:

(a) Tingkat koneksi fisik air antara danau dan laut. Jumlah pertukaran air dapat berfungsi sebagai pendekatan untuk tingkat isolasi fisik.

(b) Pola kumpulan spesies spons. Variasi dalam kumpulan spesies di berbagai tempat dapat memberikan informasi tentang hubungannya dengan lingkungan laut sekaligus mencerminkan proses penyebarannya.

(c) Pola genetik dari populasi dua spesies khas danau laut: spons *Suberites diversico lor* (*Porifera: Demospongiae: Hadromerida: Suberitidae*) dan remis *Brachidontes sp. (Mollusca: Bivalvia: Mytilidae*). Dengan menggunakan metode molekuler dapat diper kirakan kecocokannya baik secara polulasi maupun genetisnya, serta ketergantungannya pada populasi alamiah.

Jika sebuah danau laut berada pada sebuah daerah terbuka dengan kerangka ketinggian yang sama dengan laut; dapat diperkirakan akan ditemukan jenis-jenis organisme yang sama dan kurang terjadi perbedaan, baik secara genetis maupun populasi.

1. *Apa saja jenis danau laut di Indonesia?*

Penelitian ini menunjukan bahwa ada keragaman besar dalam jenis danau laut dan masih begitu banyak danau laut yang masih harus didokumentasikan di Indonesia (BAB 1). Di dalamnya terdapat berbagai gradasi hubungan antara danau dan laut. Semakin banyak hubungan antara keduanya, danau yang terbentuk semakin menyerupai sebuah laguna baik secara kimia air dan biota. Sementara danau-danau yang terisolasi, lebih memiliki air payau, dan mempunyai keunikan spesies yang tidak atau jarang ditemukan pada laut yang letaknya paling dekat.

2. *Sampai sejauh mana jenis spesies di danau laut berbeda dengan spesies di habitat pesisir yang berdekatan?*

Variasi spasial dalam komposisi spesies spons di danau laut, hutan bakau pesisir dan terumbu karang di perairan Berau (Kalimantan Timur, Indonesia) diukur secara sistematis dan kuantitatif. Studi yang komprehensif menunjukkan bahwa danau laut adalah "kebun spons" yang benar-benar mengandung kumpulan yang sangat berbeda dengan jenis spesies spons dari fauna laut yang berdekatan (BAB 2, 3, 4 & 5). Jenis spons yang ditemukan di danau dapat dibedakan dalam tiga kelompok jenis spons, yakni : (a) jenis endemik terbatas pada sebuah danau tunggal; b) jenis beragam yang ditemukan di beberapa danau, namun tidak ada di luar lingkungannya; dan c) jenis-jenis lain yang ditemukan baik di danau maupun di laut. Keberadaan danau-danau laut di tempat-tempat tertentu menyumbangkan arti besar bagi keragaman endemenis. Lebih dari setengah jenis-jenis spons di dalam danau laut belum memiliki nama ilmiah dan perlu dijelaskan dalam kerangka taksonomi (BAB 1, 3, 4 & 5).

3. *Sejauh mana populasi di danau terisolasi?*

Sebuah danau memiliki keragaman yang unik, lebih lagi mempunyai keragaman variasi genetik. (BAB 6 & 7). Dalam sebuah populasi seperti halnya spons *Suberites diversicolor* maupun populasi *Brachidontes* ditemukan dua garis keturunan yang sangat menyimpang dan sangat mungkin merupakan spesies samar. Pola variasi genetis yang ditemukan di danau laut, tampak sangat kuat terlihat sebagai jenis populasi yang berasal dari daerah yang terisolasi (BAB 6 & 7). Hal yang sama juga ditemukan pada jenis spons, juga jenis kerangnya, dimana kita dapat melihat pola keragaman lokal yang sama baik di danau laut yang terbesar maupun yang paling terpencil di Indonesia (Danau Kakaban di Kalimantan Timur). Pola variasi genetik yang ditemukan dalam populasi danau laut umumnya konsisten dengan populasi dalam lingkungan terisolasi yang lain.

4. *Dapatkah danau laut di Indonesia dianggap laboratorium alam evolusi?*

Usia danau laut kemungkinan besar tidak lebih dari 15 ribu tahun, kendati demikian, terdapat banyak keragaman spesies dan keragaman genetik yang saling berbaur, hingga memunculkan kekhasan tertentu di setiap danau (semua BAB). Mengingat definisi areal yang endemik adalahspesies dengan skala spasial yang dibatasi, pusat endemisme biasanya adalah daerah dimana sebuah spesies bertahan (berkembangbiak), tetapi bisa juga terdiri dari daerah terakhir dimana sebuah spesies tersebar (mengungsi). Kedua skenario ini dapat terjadi dalam danau laut. Dan tampaknya, jenis keragaman spesies yang mampu bertahan membuat keragaman baru pada populasi yang berkembang dalam danau (BAB 6 & 7). Danau laut juga dapat berfungsi sebagai tempat perlindungan untuk garis keturunan kuno, peninggalan dari laut atau lebih lagi spesies danau dan populasi *anchialine* (BAB 3, 6 & 7). Dengan kata lain peran danau laut dalam mendukung endemisme adalah menjaga kelangsungan dan menyempurnakan karakteristik endemis, dan pada saatnya memunculkan kemungkinan diferensiasi populasi dan spesiasi. Tesis ini hanya menunjukan beberapa konsekuensi isolasi jangka pendek pada penataan kumpulan jenis danau laut dan populasi struktur. Studi lebih lanjut tentang danau laut akan meningkatkan pemahaman kita tentang berbagai proses fisik dan ekologis yang bertanggungjawab dalam diversifikasi lingkungan tropis laut dangkal.

Penemuan dari penelitian PhD ini juga memiliki implikasi penting bagi pelestarian alam. Danau laut seperti halnya sistem kepulauan, secara geografis dapat didefiniskan dengan baik (BAB 1). Danau laut memiliki keunikan dan merupakan populasi terisolasi (BAB 6&7). Organisme yang hidup di danau laut sebagian besar adalah jenis endemis dan jenis sangat langka (BAB 2, 3, 4 & 5). Sama halnya dengan daerah kepulauan, danau laut pun sangat rentan terhadap ancaman perilaku manusia, seperti eksploitasi dan invasi. Semua BAB dari tesis ini menunjukkan bahwa masih dibutuhkan banyak penjelasan dan penelitian tentang keanekaragaman spesies. Karena keragaman dan keunikan yang dimilikinya sangat beralasan jika danau laut harus memainkan peran penting dalam perencanaan konservasi laut, baik di perairan Berau maupun perairan Raja Ampat.

Leontine Elisabeth Becking, also known as Lisa, was born on the 29th of November 1978 in Amsterdam. Lisa graduated from the Barlaeus Gymnasium Amsterdam in 1997 and in 1998 she commenced the study of Biology at the University of Amsterdam (UvA). Since she was a small child she has been mesmerized by the sea – in fact she could swim before she could speak. As a result, she decided to specialize in marine biology during her studies by taking all possible marine biology courses and conducted four research projects focused on marine biology topics in The Netherlands, Indonesia and the U.S.A. After receiving an MSc in Biology from the UvA in 2004, Lisa was selected for the Japan Prizewinners Programme, a postgraduate fellowship set up by the Dutch Ministry of Education, Culture and Science to study Japanese and conduct a seven month internship in academia and business in Tokyo. Upon returning to the Netherlands in 2005, she became a research associate in the Marine Zoology department at Naturalis (Leiden, The Netherlands) and a member of the Naturalis Zeeteam. During this time at Naturalis, she wrote a PhD proposal together with her supervisor/co-promotor Nicole de Voogd for the Netherlands Organisation of Science (NWO-ALW) in order to study marine lakes in Indonesia. In the months before the referees and committee members passed their wise judgments on the proposal, Lisa worked as a junior researcher in a project at the University College Dublin (Ireland) on the evolutionary history of plankton populations in open sea and isolated in marine lakes. In the Fall of 2007 she commenced her PhD at Naturalis and Leiden University (UL) with the funding of NWO-ALW. Once Lisa receives her PhD degree, she will start a two-year postdoc that is funded by an NWO-RUBICON grant to continue research on marine lakes at the University of California Berkeley. She is now also working part-time as a tropical marine ecologist in the Tropenteam of the Dutch Institute for Marine Resources and Ecosystem Studies Marine (IMARES).

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