

# Staying well connected – Lithistid sponges on seamounts

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*Three species of lithistid sponges, Neoaulaxinia zingiberadix, Isabella mirabilis and Neoschrammeniella fulvodesmus were collected from deep seamounts off New Caledonia to address questions about their population structure, gene flow and the relative contribution of sexual and asexual reproductive strategies to their populations. The sponges were tested by sequencing the ITS (internal transcribed spacer) and CO1 regions of their genomes. These rare and presumably ancient sponges have a distribution restricted to seamounts in the south-western Pacific. Deep seamounts represent geographically separated islands. Although the sponges could be expected to have sexual reproduction restricted to near neighbours due to low sexual dispersal opportunities via larvae, this study found surprisingly high levels of gene flow between the seamounts. Amongst the specimens of N. zingiberadix taken from two seamounts there was no population structure; CO1 resulted in identical genotypes. For the population structure within N. fulvodesmus, as revealed by ITS, most of the variation was within each individual from the six seamounts on which it occurred and CO1 revealed no difference between individuals or seamounts. The third species I. mirabilis showed four genotypes based on CO1, which were distributed across all the seamounts. Indirect measures of different species showed a range of reproductive strategies from asexual to sexual, but with much higher connection between seamounts than previously thought. Individual seamounts did not show a separate population structure as one might expect from ‘islands’. The conclusion must be that these sponges have mechanisms to attain greater dispersal than previously thought.*

**Keywords:** Lithistid, population genetics, seamounts, gene flow, dispersal, reproduction, *Neoaulaxinia zingiberadix*, *Isabella mirabilis*, *Neoschrammeniella fulvodesmus*

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## INTRODUCTION

Sponges occupy widely divergent biogeographic regions. This may be a testament to their ability to colonize over long distances, or their ability to adapt and evolve to suit changing environments. Yet the mechanisms whereby they do this, in particular the relative importance of both sexual and asexual reproduction strategies, is largely unknown for many sponges. Sponges found on seamounts are isolated, submerged and independent offering an opportunity to test various possibilities.

Sponges are known to use both sexual and asexual reproductive strategies to survive and prosper (Kaye, 1990) and both sexual and asexual components to enable long-range dispersal and colonization of new habitats (see overview in Maldonado & Riesgo, 2008). Active locomotion of entire individual sponges has been observed in response to environmental factors (Bond & Harris, 1988; Maldonado & Uriz, 1999b), but such an ability, used to readjust the sponge position at the microhabitat scale (displacements from millimetres to centimetres) cannot account for population dispersal. Dispersal of sexual stages is generally assumed to involve the

transmission of a sexual form, such as detached tissue still undergoing post fertilization development (Maldonado & Uriz, 1999b), sperm and eggs, or a swimming or crawling larval stage that lasts for only a few days (Maldonado & Riesgo, 2008). These are potentially transmitted via currents, waves, storms or using vectors such as fish or echinoderms. Asexual reproduction has also been observed and includes abiotic factors such as physical fragmentation (Wulff, 1991) and biotic factors such as fission, budding (e.g. Teixido *et al.*, 2006; Ereskovsky & Tokina, 2007) and gemmule formation (Sarà *et al.*, 2002). Both strategies have been shown to occur in individual sponges concurrently (Leong & Pawlik, 2010) or successively (Ereskovsky & Tokina, 2007). It is the sponges' ability to use both of these strategies synergistically, that improves their dispersal ability (Maldonado & Uriz, 1999a). The formation of external buds of hexactinellids in the stable environment of Antarctic waters (Teixido *et al.*, 2006) may enable the formation of a stable clonal population in the immediate vicinity. The formation of gemmules generally associated with freshwater sponges also occur in some marine sponges (Sarà *et al.*, 2002). However, these gemmules are expected to have dispersal abilities on par with sexual propagules. The formation of bubble-like buds in *Oscarella* spp. (Ereskovsky & Tokina, 2007) and *Leucetta changosensis* Dendy, 1913 (Wörheide *et al.*, 2008) shows a previously unnoticed far-reaching asexual dispersal mechanism. Merging of conspecific sponges (Wulff, 1990) also provides

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another possible asexual reproduction mechanism which is not usually accompanied by meiotic recombination but carried out by totipotent somatic cells in sponges.

Colonization of new locations by asexual stages may, alternatively, be more commonly due to the dispersal of fragments or entire sponges, via waves, currents, storms and cyclones, or as a passenger on substrates such as debris. There are several characteristics that may indicate an asexual or clonal population including frequent recovery of genotypes, correlation between different independent markers, linkage disequilibrium and the absence of sexual reproductive structures. Direct observation of deep sea sponge dispersal is not currently feasible, although observations indicate it can be done at great expense (Teixido *et al.*, 2006). In other sessile invertebrates such as corals, asexual reproduction is common and is an adaptation that succeeds both in moderate to high levels of disturbance (Le Goff-Vitry *et al.*, 2004; Miller & Ayre, 2004) and stable environments (Foster *et al.*, 2007) as it enables well-adapted individuals (Ayre & Willis, 1988) to rapidly colonize environments or maintain dominance in environments unfavourable for sexual reproduction (Wulff, 1991; Hughes *et al.*, 1992). Branching corals, which have relatively fragile morphology, are more likely to have high asexual components (Le Goff-Vitry *et al.*, 2004) than more stable massive corals (Foster *et al.*, 2007). By contrast, the coral excavating sponge *Cliona delitrix* Pang, 1973 was shown to not have clonal propagation, as was expected, resulting from coral breakage (Zilberberg *et al.*, 2006b).

Research has been carried out exploring the contributions of sexual and asexual recruitment on sponges from physical observations (Battershill & Bergquist, 1990; Wulff, 1991). However, other indirect methods to assess the relative input of sexual and asexual reproductive forms in the dispersal process have been used including histocompatibility (Neigel & Avise, 1983), allozymes (Zilberberg *et al.*, 2006a; Whalan *et al.*, 2008), mitochondrial DNA such as CO1 sequence data (Duran *et al.*, 2004a; Whalan *et al.*, 2008; Dailianis *et al.*, 2011), microsatellites (Duran *et al.*, 2002, 2004c; Blanquer *et al.*, 2005), introns (Bentlage & Worheide, 2007; Wörheide *et al.*, 2008) and nuclear ribosomal internal transcribed spacer (ITS) sequence data (Lopez *et al.*, 2002; Wörheide *et al.*, 2002a, b; Duran *et al.*, 2004a; Nichols & Barnes, 2005; Duran & Rützler, 2006; Hoshino *et al.*, 2008). Within an individual sponge, the multiple copies of the ITS have been suggested to be not yet homogenized by concerted evolution (Duran *et al.*, 2004a). However, intra-genomic polymorphisms (IGPs) within individual sponges have been detected in about half of sponge species tested (Duran *et al.*, 2004a; Wörheide *et al.*, 2004). This indicates that the variation occurring within individuals may be responsible for much of the variation attributed to different species, genera and even families. Indeed in phylogenetic studies the populations should always be screened before phylogenetic comparisons are carried out between different taxa. Other researchers have also detected ITS polymorphisms ranging from single base changes (Lopez *et al.*, 2002), several nucleotides (Duran *et al.*, 2004a) and variable sites (Schmitt *et al.*, 2005) through to examples of up to 13 sequence types per individual (Wörheide *et al.*, 2004). Intra-genomic variation is the presence of multiple genotypes within an individual sponge. However, it may also be an example of multiple sponges forming of chimeras (Blanquer & Uriz, 2011).

Population research in sponges has largely concentrated on biogeography (Nichols & Barnes, 2005), the movement of species into or out of the Mediterranean Sea (Duran *et al.*, 2004a), survival in the South Pacific (Wörheide *et al.*, 2002a) and ecological specialization (Duran & Rützler, 2006). Much of the work on sponge population genetics has shown genetic differentiation at geographic scales (Klautau *et al.*, 1999; Duran *et al.*, 2004a, b; Bentlage & Worheide, 2007; Hoshino *et al.*, 2008; Wörheide *et al.*, 2008; Xavier *et al.*, 2010); for a review see Uriz & Turon (2012). By comparison, only a small amount of research has been carried out on small scales as would occur on a seamount. Two Mediterranean studies on *Crambe crambe* (Schmidt, 1862) (Calderon *et al.*, 2007) and *Scopalina lophyropoda* Schmidt, 1862 (Blanquer *et al.*, 2009) showed genetic structure at this small scale consistent with what one would expect from a poorly dispersing sexual stage, although this result could be confused by the presence of chimeric individuals (Blanquer & Uriz, 2011). Seamounts have been predicted to have large amounts of population structure because of spatial separation and limited dispersal mechanisms coupled with high levels of asexual reproduction (Richer de Forges *et al.*, 2000; Samadi *et al.*, 2006). Seamounts are believed to be particularly susceptible to genetic drift due to their small size (Le Goff-Vitry *et al.*, 2004). Seamounts are not ephemeral and, coupled with low gene flow due to distance and hydraulics, structure within populations would be expected (Samadi *et al.*, 2006). However, research on crustaceans collected from New Caledonian seamounts showed that previous perceived high endemism (Richer de Forges *et al.*, 2000) was not the case for seamounts. Instead the crustaceans had high levels of gene flow between the seamounts. The only exception in fact was a gastropod that had limited larval dispersal (Samadi *et al.*, 2006).

This study focuses on three rare, deep and isolated species of lithistid ('rock') sponges from deep seamounts in New Caledonia that are several million years old (Richer de Forges *et al.*, 2000), *Neoaualaxinia zingiberadix* (Kelly, 2007), *Isabella mirabilis* (Schlacher-Hoenlinger *et al.*, 2005) and *Neoschrammeniella fulvodesmus* (Lévi & Lévi, 1983). Lithistids on these seamounts may be relict fauna from the Mesozoic Era (Lévi, 1991), surviving on refuge habitat (Samadi *et al.*, 2007). Lithistids are found in most parts of the world at great depths. They are believed to be long lived and slow growing and, as such, display K reproductive strategies such as ovipary (Reiswig, 1973; Fromont & Bergquist, 1994). Lithistid sponges constitute a polyphyletic collection of disparate families and genera grouped together by the common presence of desmas. However, the latter remains significantly unresolved based on morphology, and still awaits independent datasets to support or refute morphometric hypotheses (e.g. Pisera & Lévi, 2002). Indeed because lithistids represent an artificial group split into several unrelated orders (Schuster *et al.*, 2015), it is likely there will be varying reproductive strategies within the lithistids. Only one study on reproduction in lithistids has been reported (Maldonado & Bergquist, 2002; Maldonado & Riesgo, 2008). and this is based purely on studies from *Theonella*, which has been recently revised to *Astrophorida* (Hall *et al.*, 2014). The one study indicated the *Theonella* is gonochoric and oviparous which means they release zygotes or early embryos that develop externally (Maldonado & Bergquist, 2002; Maldonado & Riesgo, 2008). However, these reproductive

modes are more likely to be strategies to suit the current environmental conditions (Ereskovsky, 2010). Lithistids have been reported to have either parenchymella or clavablastula larval types, with free swimming planktonic or crawling demersal larval stages (Hooper & Van Soest, 2002). Some of the larval stages are thought to survive for only a few hours and up to 3 weeks (Maldonado & Bergquist, 2002). However, other astrophorid sponge genera *Thoosa* and *Alectona* produce an unciliated hoplitomella larvae, which shows great dispersal characteristics, using its protruding spicules to float for long periods of time (Maldonado & Bergquist, 2002; Borchiellini *et al.*, 2004; Maldonado, 2004; Bautista-Guerrero *et al.*, 2010). The sexual structures and larval stages have not been observed for the three species used in this research. However, because of the limited dispersal characteristics of sponge larvae in general, sponge populations have been thought to have local low genetic variability, high genetic structure and incipient species (Jablonski, 1986; Jackson, 1986; Wörheide *et al.*, 2005). Nonetheless, due to the large dispersal characteristics of some deep sea astrophorids, these lithistids may indeed have greater dispersal characteristics but require specific environmental conditions to survive that may occur only on deep seamounts.

The depths from which the lithistid sponges used in this study are recovered (270–1032 m depth range) would have enabled survival during the glacial ice age without having to move down the slope to colder deeper water. In addition, these populations are unlikely to have suffered disturbance from human activities, few surface climatic effects, little predation, and since they are on seamounts they are unlikely to have been affected by continental erosion. The main aim of this research is to evaluate if there are discrete populations of these three lithistid species on different seamount ‘islands’ up to almost 200 km apart, with the abyssal floor between these seamounts at 4000 m depth. Previous research on fauna from seamounts has found highly localized species distributions and apparent speciation between island groups or ridge systems in the south-western Pacific (Richer de Forges *et al.*, 2000). This research is the first attempt to investigate if specimens of an individual sponge species from different seamounts belong to genetically distinct populations (genotypes), or are truly widespread as their present taxonomy suggests. Further we will use direct (i.e. genotypes) and indirect (i.e. linkage disequilibrium) tests to measure the relative components of sexual and asexual reproduction within this species of sponge.

## MATERIALS AND METHODS

### Sampling, DNA extraction and sequencing

The lithistid specimens sponges were collected by dredge from deep seamounts off New Caledonia across 10 sites at depths ranging from 270 to 1032 m. The only specimens collected by trawling of up to 14 km transect length were the specimens from Eponge North. The sponges were frozen immediately following collection. All specimens were registered at the Queensland Museum, registration numbers between QM G329756 to QM G331837 (Table 1). The species were identified morphologically by soaking thin tissue section in Histo-Clear II (National Diagnostics, USA) for 24 h before mounting in Histomount (National Diagnostics, USA), and examined under an Olympus BH-1 light microscope.

Spicules were dissociated by dissolving sponge tissue in boiling nitric acid, rinsed in water and resuspended in absolute ethanol. They were then either mounted in Durcupan ACM (Sigma-Aldrich, Australia) and examined under an Olympus BH-1 light microscope, or ignited on an SEM stub and examined using a Hitachi H1000 SEM.

For molecular work, samples from 131 specimens were taken by cutting a 5 mm<sup>3</sup> section of sponge tissue from the middle of the specimen. DNA was extracted using a DNAeasy<sup>®</sup> Tissue Kit (Qiagen, Australia) according to the manufacturer’s instructions. The ribosomal internal transcribed spacer (ITS) region, including the entire ITS<sub>1</sub>, 5.8S rRNA and ITS<sub>2</sub> regions, was amplified using primers RA2: GTCCCTGCCCTTTGTACACA and ITS2.2: CCTGGTTAGTTTCTTTTCCTCCGC. PCR amplifications were carried out in a 25 µL volume reaction, with 1 unit of HotmasterTaq<sup>®</sup> (Eppendorf), 200 µM of mixed dNTPs and 10 µM of each primer. Reaction conditions consisted of a denaturing step of 2 min at 95°C, 35 cycles of 20 s at 95°C, 10 s at 58°C, and 1 min at 65°C, followed by a final extension of 10 min at 65°C. The PCR product was electrophoresed on a 0.5% agarose gel for 1 h at 90 volts, the single band excised, and the 1 kb product purified using Perfectprep Gel Cleanup kit (Eppendorf, Germany). The PCR product was then cloned using pDrive Vector (Qiagen) following the manufacturer’s instructions, modified to half concentration, i.e. ½X reaction and the addition of 800 µL of 37°C SOC during transformations. Successful recombinants were selected and grown in liquid culture and plasmid DNA isolated via alkaline lysis minipreps (Sambrook *et al.*, 1989). Between two and seven plasmids per specimen were sequenced directly in 12 µL reactions containing 3 µL of 5× BigDye Terminator Buffer (Applied Biosystems, Australia), 0.8 µM of either M13F or T7 universal primer, 200 ng of plasmid DNA, and 1 µL Big Dye Terminator Mix v3.1 (Applied Biosystems, Australia). Reactions were denatured for 5 min at 94°C, followed by 30 cycles of 10 s at 96°C, 5 s at 50°C, and 4 min at 60°C. The sequencing reactions were precipitated using 75 µL of a 70% ethanol solution with 0.0002 M MgSO<sub>4</sub> and centrifuged at 3600 rpm for 15 min before the supernatant was discarded. Sequencing was carried out on an ABI 3730 × 196 capillary automated DNA sequencer. Sequences were aligned using Sequencher<sup>™</sup> 4.5 (Genecodes) and BIOEDIT 7.0.4.1 (Hall, 1999).

CO1 data has been obtained during the barcoding of the Queensland Museum sponge collection in the course of the Sponge Barcoding Project ([www.spongebarcoding.org](http://www.spongebarcoding.org)) (Wörheide & Erpenbeck, 2007). The DNA extraction followed a plate-based extraction method (Vargas *et al.*, 2010). Fragments of CO1 standard barcoding fragment were amplified using the degenerated barcoding primers dgLCO1490 and dgHCO2198 (Meyer *et al.*, 2005) with annealing temperature of 43°C. The PCR products were purified with ExoSAP-IT (Affymetrix) or standard Ammonium Acetate-Ethanol precipitation before cycle sequencing both strands with the Big Dye Terminator Mix v3.1 (Applied Biosystems, Australia) following the manufacturer’s protocol and sequencing on an ABI 3730 automated sequencer. The poriferan origin of the sequences was checked by a BLAST search against the NCBI GenBank nr database (<http://www.ncbi.nlm.nih.gov/>). Sequences were base called and assembled to a 563 bp alignment in CodonCode Aligner v3.7.1.1 and subsequently aligned in Sea-View 4 (Galtier *et al.*, 1996) using Muscle 3.6

**Table 1.** List of the species and the collection data of all of the specimens used in this study.

Queensland Museum Registration	Species	Location	Depth Deploy (m)	Depth Retrieve (m)	Latitude (Degrees)	Longitude (Degrees)	ITS	CO1
G329825	<i>N. clavata</i>	Jumeau East	470	621	23.715 S	168.257 E	ITS	CO1
G329756	<i>N. fulvodesmus</i>	Jumeau East	470	621	23.715 S	168.257 E	ITS	CO1
G329757	<i>N. fulvodesmus</i>	Jumeau East	470	621	23.715 S	168.257 E	ITS	CO1
G329758	<i>N. fulvodesmus</i>	Jumeau East	470	621	23.715 S	168.257 E	ITS	
G329759	<i>N. fulvodesmus</i>	Jumeau East	470	621	23.715 S	168.257 E	ITS	
G329760	<i>N. fulvodesmus</i>	Jumeau East	470	621	23.715 S	168.257 E	ITS	
G329761	<i>N. fulvodesmus</i>	Jumeau East	470	621	23.715 S	168.257 E	ITS	CO1
G329762	<i>N. fulvodesmus</i>	Jumeau East	470	621	23.715 S	168.257 E	ITS	CO1
G329763	<i>N. fulvodesmus</i>	Jumeau East	470	621	23.715 S	168.257 E	ITS	
G329764	<i>N. fulvodesmus</i>	Jumeau East	470	621	23.715 S	168.257 E	ITS	
G329765	<i>N. fulvodesmus</i>	Jumeau East	470	621	23.715 S	168.257 E	ITS	
G329766	<i>N. fulvodesmus</i>	Zorro South	623	691	25.400 S	168.333 E	ITS	
G329767	<i>N. fulvodesmus</i>	Zorro South	623	691	25.400 S	168.333 E	ITS	
G329768	<i>N. fulvodesmus</i>	Zorro South	623	691	25.400 S	168.333 E	ITS	
G329769	<i>N. fulvodesmus</i>	Zorro South	623	691	25.400 S	168.333 E	ITS	CO1
G329770	<i>N. fulvodesmus</i>	Zorro South	623	691	25.400 S	168.333 E	ITS	
G329771	<i>N. fulvodesmus</i>	Zorro South	623	691	25.400 S	168.333 E	ITS	
G329772	<i>N. fulvodesmus</i>	Zorro South	623	691	25.400 S	168.333 E	ITS	
G329773	<i>N. fulvodesmus</i>	Zorro South	623	691	25.400 S	168.333 E	ITS	
G329774	<i>N. fulvodesmus</i>	Zorro South	623	691	25.400 S	168.333 E	ITS	
G329775	<i>N. fulvodesmus</i>	Zorro South	623	691	25.400 S	168.333 E	ITS	
G329776	<i>N. fulvodesmus</i>	Zorro North	666	1000	25.344 S	168.309 E	ITS	
G329777	<i>N. fulvodesmus</i>	Zorro North	666	1000	25.344 S	168.309 E	ITS	
G329778	<i>N. fulvodesmus</i>	Zorro North	666	1000	25.344 S	168.309 E	ITS	
G329779	<i>N. fulvodesmus</i>	Zorro North	666	1000	25.344 S	168.309 E	ITS	
G329780	<i>N. fulvodesmus</i>	Zorro North	666	1000	25.344 S	168.309 E	ITS	
G329781	<i>N. fulvodesmus</i>	Zorro North	666	1000	25.344 S	168.309 E	ITS	
G329782	<i>N. fulvodesmus</i>	Zorro North	666	1000	25.344 S	168.309 E	ITS	
G329783	<i>N. fulvodesmus</i>	Zorro North	666	1000	25.344 S	168.309 E	ITS	
G329784	<i>N. fulvodesmus</i>	Zorro North	666	1000	25.344 S	168.309 E	ITS	
G329785	<i>N. fulvodesmus</i>	Zorro North	666	1000	25.344 S	168.309 E	ITS	
G329786	<i>N. fulvodesmus</i>	Eponge North	530	540	24.887 S	168.364 E	ITS	
G329787	<i>N. fulvodesmus</i>	Eponge North	530	540	24.887 S	168.364 E	ITS	
G329788	<i>N. fulvodesmus</i>	Eponge North	530	540	24.887 S	168.364 E	ITS	
G329789	<i>N. fulvodesmus</i>	Eponge North	530	540	24.887 S	168.364 E	ITS	
G329790	<i>N. fulvodesmus</i>	Eponge North	530	540	24.887 S	168.364 E	ITS	
G329791	<i>N. fulvodesmus</i>	Eponge North	530	540	24.887 S	168.364 E	ITS	
G329792	<i>N. fulvodesmus</i>	Eponge North	530	540	24.887 S	168.364 E	ITS	
G329793	<i>N. fulvodesmus</i>	Eponge North	530	540	24.887 S	168.364 E	ITS	
G329794	<i>N. fulvodesmus</i>	Eponge North	530	540	24.887 S	168.364 E	ITS	
G329795	<i>N. fulvodesmus</i>	Eponge North	530	540	24.887 S	168.364 E	ITS	
G329796	<i>N. fulvodesmus</i>	Eponge South	518	586	24.937 S	168.361 E	ITS	
G329797	<i>N. fulvodesmus</i>	Eponge South	518	586	24.937 S	168.361 E	ITS	
G329798	<i>N. fulvodesmus</i>	Eponge South	518	586	24.937 S	168.361 E	ITS	
G329799	<i>N. fulvodesmus</i>	Eponge South	518	586	24.937 S	168.361 E	ITS	
G329800	<i>N. fulvodesmus</i>	Eponge South	518	586	24.937 S	168.361 E	ITS	
G329801	<i>N. fulvodesmus</i>	Eponge South	518	586	24.937 S	168.361 E	ITS	
G329802	<i>N. fulvodesmus</i>	Eponge South	518	586	24.937 S	168.361 E	ITS	
G329803	<i>N. fulvodesmus</i>	Eponge South	518	586	24.937 S	168.361 E	ITS	
G329804	<i>N. fulvodesmus</i>	Eponge South	518	586	24.937 S	168.361 E	ITS	
G329805	<i>N. fulvodesmus</i>	Eponge South	518	586	24.937 S	168.361 E	ITS	
G329806	<i>N. fulvodesmus</i>	Kaimon Maru	600	896	24.756 S	168.104 E	ITS	CO1
G329807	<i>N. fulvodesmus</i>	Kaimon Maru	600	896	24.756 S	168.104 E	ITS	
G329808	<i>N. fulvodesmus</i>	Kaimon Maru	600	896	24.756 S	168.104 E	ITS	
G329809	<i>N. fulvodesmus</i>	Kaimon Maru	600	896	24.756 S	168.104 E	ITS	
G329810	<i>N. fulvodesmus</i>	Kaimon Maru	600	896	24.756 S	168.104 E	ITS	
G329811	<i>N. fulvodesmus</i>	Kaimon Maru	600	896	24.756 S	168.104 E	ITS	
G329812	<i>N. fulvodesmus</i>	Kaimon Maru	600	896	24.756 S	168.104 E	ITS	
G329813	<i>N. fulvodesmus</i>	Kaimon Maru	600	896	24.756 S	168.104 E	ITS	CO1
G329814	<i>N. fulvodesmus</i>	Kaimon Maru	600	896	24.756 S	168.104 E	ITS	
G329815	<i>N. fulvodesmus</i>	Kaimon Maru	600	896	24.756 S	168.104 E	ITS	CO1
G329816	<i>N. zingiberadix</i>	Jumeau East	470	621	23.715 S	168.257 E		CO1
G329817	<i>N. zingiberadix</i>	Jumeau East	470	621	23.715 S	168.257 E		CO1

Continued

Table 1. Continued

Queensland Museum Registration	Species	Location	Depth Deploy (m)	Depth Retrieve (m)	Latitude (Degrees)	Longitude (Degrees)	ITS	CO1
G329818	<i>N. zingiberadix</i>	Jumeau East	470	621	23.715 S	168.257 E		CO1
G329819	<i>N. zingiberadix</i>	Jumeau East	470	621	23.715 S	168.257 E		CO1
G329821	<i>N. zingiberadix</i>	Jumeau East	470	621	23.715 S	168.257 E		CO1
G329822	<i>N. zingiberadix</i>	Jumeau East	470	621	23.715 S	168.257 E		CO1
G329823	<i>N. zingiberadix</i>	Jumeau East	470	621	23.715 S	168.257 E		CO1
G329824	<i>N. zingiberadix</i>	Jumeau East	470	621	23.715 S	168.257 E		CO1
G329826	<i>N. zingiberadix</i>	Introuvable	591	1032	24.663 S	168.674 E		CO1
G329827	<i>N. zingiberadix</i>	Introuvable	591	1032	24.663 S	168.674 E		CO1
G329828	<i>N. zingiberadix</i>	Introuvable	591	1032	24.663 S	168.674 E		CO1
G329829	<i>N. zingiberadix</i>	Introuvable	591	1032	24.663 S	168.674 E		CO1
G329830	<i>N. zingiberadix</i>	Introuvable	591	1032	24.663 S	168.674 E		CO1
G329831	<i>N. zingiberadix</i>	Introuvable	591	1032	24.663 S	168.674 E		CO1
G329832	<i>N. zingiberadix</i>	Introuvable	591	1032	24.663 S	168.674 E		CO1
G329833	<i>N. zingiberadix</i>	Introuvable	591	1032	24.663 S	168.674 E		CO1
G329834	<i>N. zingiberadix</i>	Introuvable	591	1032	24.663 S	168.674 E		CO1
G329835	<i>N. zingiberadix</i>	Introuvable	591	1032	24.663 S	168.674 E		CO1
G318751	<i>I. mirabilis</i>	Jumeau West	285	285	23.685 S	168.017 E		CO1
G329906	<i>I. mirabilis</i>	Jumeau West	285	285	23.685 S	168.017 E		CO1
G329908	<i>I. mirabilis</i>	Jumeau West	285	285	23.685 S	168.017 E		CO1
G329909	<i>I. mirabilis</i>	Jumeau West	285	285	23.685 S	168.017 E		CO1
G329910	<i>I. mirabilis</i>	Jumeau West	285	285	23.685 S	168.017 E		CO1
G329911	<i>I. mirabilis</i>	Jumeau West	285	285	23.685 S	168.017 E		CO1
G329912	<i>I. mirabilis</i>	Jumeau West	285	285	23.685 S	168.017 E		CO1
G329913	<i>I. mirabilis</i>	Jumeau West	285	285	23.685 S	168.017 E		CO1
G329914	<i>I. mirabilis</i>	Jumeau West	285	285	23.685 S	168.017 E		CO1
G329915	<i>I. mirabilis</i>	Jumeau West	285	285	23.685 S	168.017 E		CO1
G329916	<i>I. mirabilis</i>	Kaimon Maru	320	345	24.753 S	168.121 E		CO1
G329917	<i>I. mirabilis</i>	Kaimon Maru	320	345	24.753 S	168.121 E		CO1
G329918	<i>I. mirabilis</i>	Kaimon Maru	320	345	24.753 S	168.121 E		CO1
G329919	<i>I. mirabilis</i>	Kaimon Maru	320	345	24.753 S	168.121 E		CO1
G329920	<i>I. mirabilis</i>	Kaimon Maru	320	345	24.753 S	168.121 E		CO1
G329921	<i>I. mirabilis</i>	Kaimon Maru	320	345	24.753 S	168.121 E		CO1
G329922	<i>I. mirabilis</i>	Kaimon Maru	320	345	24.753 S	168.121 E		CO1
G329923	<i>I. mirabilis</i>	Kaimon Maru	320	345	24.753 S	168.121 E		CO1
G329924	<i>I. mirabilis</i>	Kaimon Maru	320	345	24.753 S	168.121 E		CO1
G329925	<i>I. mirabilis</i>	Kaimon Maru	320	345	24.753 S	168.121 E		CO1
G329926	<i>I. mirabilis</i>	Blanc Nouveau 2	275	348	23.277 S	168.233 E		CO1
G329927	<i>I. mirabilis</i>	Blanc Nouveau 2	275	348	23.277 S	168.233 E		CO1
G329929	<i>I. mirabilis</i>	Blanc Nouveau 2	275	348	23.277 S	168.233 E		CO1
G329930	<i>I. mirabilis</i>	Blanc Nouveau 2	275	348	23.277 S	168.233 E		CO1
G329931	<i>I. mirabilis</i>	Blanc Nouveau 2	275	348	23.277 S	168.233 E		CO1
G329932	<i>I. mirabilis</i>	Blanc Nouveau 2	275	348	23.277 S	168.233 E		CO1
G329933	<i>I. mirabilis</i>	Blanc Nouveau 2	275	348	23.277 S	168.233 E		CO1
G329934	<i>I. mirabilis</i>	Blanc Nouveau 2	275	348	23.277 S	168.233 E		CO1
G329935	<i>I. mirabilis</i>	Blanc Nouveau 2	275	348	23.277 S	168.233 E		CO1
G329936	<i>I. mirabilis</i>	Munida	270	350	22.998 S	168.363 E		CO1
G329937	<i>I. mirabilis</i>	Munida	270	350	22.998 S	168.363 E		CO1
G329938	<i>I. mirabilis</i>	Munida	270	350	22.998 S	168.363 E		CO1
G329939	<i>I. mirabilis</i>	Munida	270	350	22.998 S	168.363 E		CO1
G329940	<i>I. mirabilis</i>	Munida	270	350	22.998 S	168.363 E		CO1
G329941	<i>I. mirabilis</i>	Munida	270	350	22.998 S	168.363 E		CO1
G329942	<i>I. mirabilis</i>	Munida	270	350	22.998 S	168.363 E		CO1
G329943	<i>I. mirabilis</i>	Munida	270	350	22.998 S	168.363 E		CO1
G329944	<i>I. mirabilis</i>	Munida	270	350	22.998 S	168.363 E		CO1
G329945	<i>I. mirabilis</i>	Munida	270	350	22.998 S	168.363 E		CO1
G329952	<i>N. zingiberadix</i>	Jumeau East	470	621	23.715 S	168.257 E		CO1
G329953	<i>N. zingiberadix</i>	Introuvable	591	1032	24.663 S	168.674 E		CO1
G331823	<i>N. zingiberadix</i>	Introuvable	591	1032	24.663 S	168.674 E		CO1
G331825	<i>N. zingiberadix</i>	Introuvable	591	1032	24.663 S	168.674 E		CO1
G331827	<i>N. zingiberadix</i>	Introuvable	591	1032	24.663 S	168.674 E		CO1
G331828	<i>N. zingiberadix</i>	Introuvable	591	1032	24.663 S	168.674 E		CO1
G331829	<i>N. zingiberadix</i>	Introuvable	591	1032	24.663 S	168.674 E		CO1
G331830	<i>N. zingiberadix</i>	Introuvable	591	1032	24.663 S	168.674 E		CO1

Continued

Table 1. Continued

Queensland Museum Registration	Species	Location	Depth Deploy (m)	Depth Retrieve (m)	Latitude (Degrees)	Longitude (Degrees)	ITS	CO1
G331831	<i>N. zingiberadix</i>	Introuvable	591	1032	24.663 S	168.674 E		CO1
G331832	<i>N. zingiberadix</i>	Jumeau East	470	621	23.715 S	168.257 E		CO1
G331833	<i>N. zingiberadix</i>	Jumeau East	470	621	23.715 S	168.257 E		CO1
G331834	<i>N. zingiberadix</i>	Jumeau East	470	621	23.715 S	168.257 E		CO1
G331836	<i>N. zingiberadix</i>	Jumeau East	470	621	23.715 S	168.257 E		CO1
G331837	<i>N. zingiberadix</i>	Jumeau East	470	621	23.715 S	168.257 E		CO1

(Edgar, 2004). Sequences are published in NCBI GenBank (Acc. No. KR270646-KR270725) and in the Sponge Barcoding Database ([www.spongebarcoding.org](http://www.spongebarcoding.org)).

### Analyses of genetic variation

Genetic diversity was expressed as nucleotide diversity ( $\pi$ ; the probability that two randomly chosen homologous nucleotides differ) and gene diversity ( $h$ ; the probability that two randomly chosen genotypes differ), and calculated in Arlequin 3.1 (Excoffier *et al.*, 2005). The hierarchical distribution of genetic diversity was analysed in the AMOVA framework (Analysis of Molecular Variance) also using Arlequin 3.1, where genetic variation was partitioned into within-individual, among-individual within-population, and among-population components. Significance of variance components was assessed using 10 000 randomizations. Within-individual variation was included as a variance component due to the presence of IGPs. Pairwise  $F_{ST}$ s between sampled populations were calculated in Arlequin 3.1. To indirectly assess the contribution of sexual vs asexual modes of reproduction, we examined levels of linkage disequilibrium, where high levels indicate either non-random sexual reproduction (inbreeding) or asexual reproduction. Linkage disequilibrium was calculated in Arlequin 3.1 (Excoffier *et al.*, 2005). Analyses of the three lithistid species were carried out independently. Sequencing of the ITS was only carried out for *N. fulvodesmus*. The CO1 fragment of *N. fulvodesmus* was only sequenced on eight individuals to detect if there was any variation within this species, while all collected *I. mirabilis* and *N. zingiberadix* were screened.

### Phylogenetic analyses

Maximum likelihood reconstructions were performed with RAxML-7.2.5 (Stamatakis, 2006) under the GTR + GAMMA model of substitution. Branch support was inferred with 1000 rapid bootstrap replicates.

## RESULTS

### *Neoschrammeniella fulvodesmus*

A total of 114 ITS genotypes were recovered from the 57 specimens (Table 2). A higher number of genotypes, and higher gene and nucleotide diversities were found for ITS1 than for ITS2 and 5.8S. There were 24 nucleotide differences in the 5.8S rRNA section, with only six single base changes found in the 5.8S region. One sequence had two of these changes and only two sequences shared the same change and they were from different seamounts. Overall gene diversity was high ( $h = 0.9843$ ) when compared with nucleotide diversity ( $\pi = 0.127324$ ).

Six of the 114 genotypes were found in a single specimen. In two of the 57 specimens, only one plasmid was successfully sequenced. Of the remaining 55 individuals for which multiple sequences were obtained, 52 (94.5%) exhibited intra-genomic polymorphism, with between two and six genotypes present within an individual specimen.

An AMOVA looking at the individuals and the variation within each individual shows 84.57% of the variation is within the individuals, compared with 15.66% between the individuals within each seamount (Table 3). The most common genotype occurred 14 times and 63 genotypes occurred only once.

The AMOVA for all of the populations and for the entire sequence including ITS1 and ITS2 indicates that virtually all of the variation (97.38%) is contained within each seamount (Table 4).

Most of the genotypes (71/114) were found on a single seamount, with the number of unique genotypes ranging from six at Zorro North to 19 at Eponge South (Figure 1). Most shared genotypes were limited to two seamounts (40 genotypes), however, one genotype occurred at three seamounts (Jumeau East, Eponge North and Zorro North). The most common genotype (14 specimens) occurred at four seamounts (Jumeau East, Kaimon Maru, Eponge South and Zorro North)

Table 2. Comparison of diversities and  $F_{ST}$ s of *N. fulvodesmus* calculated from ITS on the seamounts in order of increasing latitude.

Seamount	Sample size (N)	Number of sequences	Number of genotypes ( $h$ )	Nucleotide diversity ( $\pi$ )	Gene diversity ( $h$ )	$F_{ST}$
Jumeau East	9	31	20	0.101965	0.9600	0.04672
Kaimon-Mar	9	39	35	0.248202	0.9638	0.01040
Eponge North	10	23	21	0.311829	1.0000	-0.00279
Eponge South	10	35	29	0.046155	0.8758	0.06069
Zorro North	9	31	27	0.315330	0.9815	-0.00678
Zorro South	10	38	29	0.100148	0.9644	0.04725
All	57	197	152	0.127324	0.9843	

**Table 3.** AMOVA results of the entire ITS sequence of *N. fulvodesmus* between seamounts, between individuals within seamounts and within individuals.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	$F_{ST}$
Between seamounts	5	625.243	-0.22734	-0.24	0.15426
Between individual genotypes within seamounts	43	5052.946	14.94180	15.66	
Within individuals	77	6211.692	80.67132	84.57	
Total	125	11,889.881	95.38578		

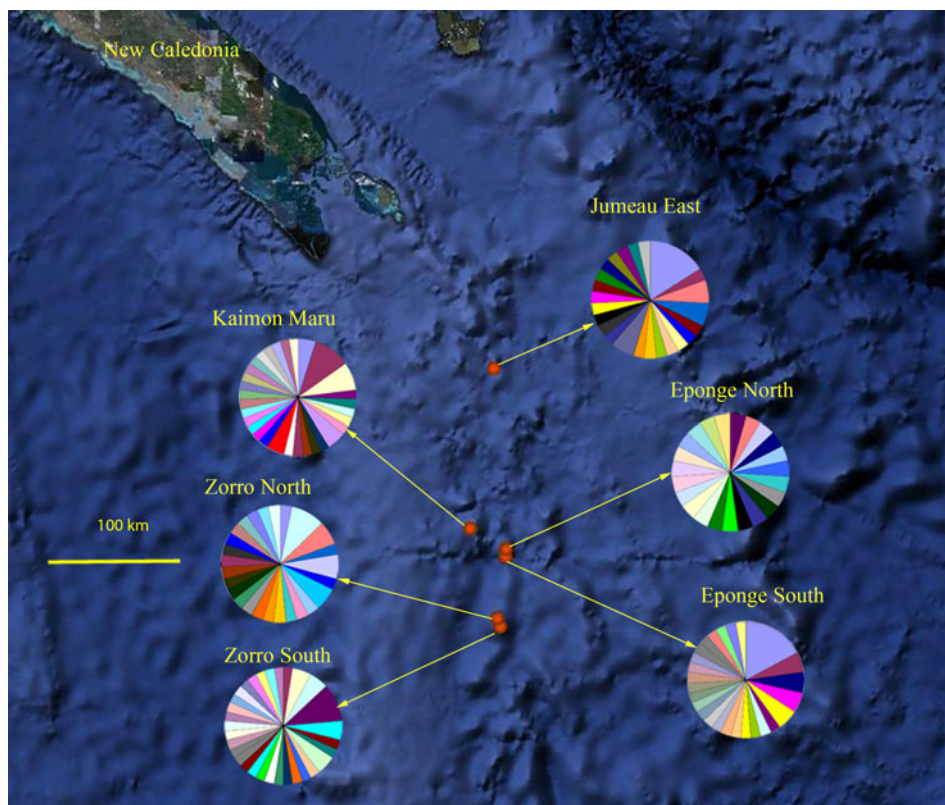
and the second most common genotype (8 specimens) also occurred at four seamounts (Jumeau East, Kaimon Maru, Eponge South and Zorro South). No genotype was shared across all six seamounts.

When each seamount is regarded as a distinct population, the pairwise genetic differences (Table 5) showed that the two pairs of populations that are physically closest together, i.e. Zorro North and Zorro South, and Eponge North and Eponge South, show significant pairwise  $F_{ST}$ . The only other significant pairwise differences are between Zorro South and Jumeau East, and between Zorro South and Eponge South.

When the seamounts are clustered geographically, the variation is distributed within each population rather than between populations or groups of populations even when adjacent seamounts are compared with other seamounts. There is no latitudinal gradient showing genetic flow from the north to south nor is there flow from the south to the north. There was no latitudinal gradient between the seamounts. Neither was there any longitudinal gradient for gene diversity, nucleotide diversity and haplotypic diversity between the seamounts. There were no significant bathymetric gradients, or bathymetric ranges amongst the genotypes or the

**Table 4.** AMOVA results of the entire ITS sequence of *N. fulvodesmus* between seamounts and within seamounts.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	$F_{ST}$
Between seamounts	5	592.141	2.02720	2.62	0.02624
Within seamounts	123	9254.269	75.23796	97.38	
Total	128	9846.411	77.26516		

**Fig. 1.** Distribution of the *N. fulvodesmus* ITS genotypes on the seamounts (ALA, 2014).

**Table 5.** Pairwise differences in  $F_{ST}$  of *N. fulvodesmus* calculated from ITS between the seamounts using the distance method.

Seamount	Jumeau East	Kaimon-Mar	Eponge North	Eponge South	Zorro North	Zorro South
Jumeau East	0.00000					
Kaimon-Mar	0.01597	0.00000				
Eponge North	0.06331	-0.02924	0.00000			
Eponge South	-0.01924	0.05118	0.11365*	0.00000		
Zorro North	-0.02223	0.01823	0.06110	0.00663	0.00000	
Zorro South	0.06132*	-0.02529	-0.03920	0.09611*	0.06125*	0.00000

\*Indicates significant difference from 0 at  $P = 0.05$ .

diversities. Most of the depths of the dredges on the seamounts from which the specimens of *N. fulvodesmus* were recovered were from around 500 m depth (ranging from 470 to 691 m) (Table 1), with the exception of Kaimon Maru and Zorro North which reached depths of 896 and 1000 m respectively. Interestingly the highest diversities were found from the Eponge North seamount with the smallest depth range of dredges.

All of the individual populations showed significant levels of linkage disequilibrium amongst the polymorphic loci as shown in Table 6.

Eight individuals were screened using CO1 from three seamounts to detect if there was any variation within this species. Table 7 compares the diversity of the seamounts, which is zero, and there were no polymorphic loci found in the CO1 fragment. All eight individuals were found to belong to the same mtDNA genotype and were found at all three seamounts (Figure 3). These eight individuals when analysed with ITS found 23 of the 114 genotypes. This ranged from two individuals with only one genotype detected to another individual with eight genotypes. These are likely to be related to the number of clones sequenced.

Pairwise differences in  $F_{ST}$  using the distance method between the seamounts for CO1 was not calculated for *N. fulvodesmus* because of the low numbers of individuals sampled from the population, likewise linkage disequilibrium could not be calculated because there were no polymorphic loci. All the loci at the CO1 fragment were monomorphic. No morphological differences were detected between the specimens.

### *Neoaualaxinia zingiberadix*

All 32 specimens of *N. zingiberadix* were identical for CO1 sequenced region (Figure 3). There was no latitudinal,

**Table 6.** Summary of significant polymorphic loci of *N. fulvodesmus* with linkage disequilibrium.

Seamount	Number of combinations	Loci significant disequilibrium ( $P = 0.05$ )	% of loci with significant linkage disequilibrium
Jumeau East	171	28	16.4%
Zorro South	435	50	11.5%
Zorro North	105	20	19.0%
Eponge North	231	13	5.6%
Eponge South	276	35	12.7%
Kaimon-Mar	435	63	14.5%
Combined populations	45	26	57.8%

longitudinal or depth separation. All specimens were from two seamounts Jumeau East and Introvable and there was no separation between the seamounts (Table 8).

Linkage disequilibrium was not calculated because of the monomorphism at all the loci of the CO1 fragment. No morphological differences were detected between the specimens.

### *Isabella mirabilis*

Sequencing of the CO1 fragment of all specimens of the species *I. mirabilis*, divided the specimens into four distinct genotypes with one very large genotype comprising the majority of specimens (23/38) (Figure 2). These sponges were from four seamount sites, each seamount with three or four genotypes (Table 9). There was one genotype present only in two specimens. There were other genotypes comprised of five and eight individuals (Figure 3). All four seamounts had three of the four genotypes in different combinations, so there was no specialization of genotypes for a specific seamount. All of the specimens were collected from between 270 and 348 m depth (Table 1) and the genotypes were not specific for any particular depth. Morphological analysis of the genotypes of *I. mirabilis* only revealed variation in the relative proportions of microscleres.

Significant differences were found in  $F_{ST}$  values in seamounts of the species *I. mirabilis* between Jumeau West and Munida, and Jumeau West and Blanc Nouveau 2 only (Table 10, see also Figure 2). In order to test for population subdivision the seamounts were clustered into two groups as identified by  $F_{ST}$  analysis. One group contained the two seamounts of Munida and Blanc Nouveau 2, and the other group contained the seamounts Jumeau West and Kaimon Maru. This revealed that 97.79% of the variation was found within the seamount clusters, so there was no population structure here. Clustering of the seamounts using a distance-related latitudinal gradient provided a higher signal of the variation spread between the populations, with clustering of Jumeau West, Munida and Blanc Nouveau 2 compared with Kaimon Maru. On all accounts most of the variation is included within the populations (92.66%).

Linkage disequilibrium was calculated for the polymorphic loci at the seamounts. For two sites, Munida and Blanc Nouveau 2, there were no polymorphic loci with significant linkage disequilibrium. This contrasts with the other two seamounts of Jumeau West and Kaimon Maru, which had both of the polymorphic loci showing significant ( $P = 0.05$ ) linkage disequilibrium. This is all overshadowed by the fact that for the CO1 region from the 597 usable nucleotide sites there were polymorphisms only at a maximum of three sites for Munida and all other seamounts had two polymorphic

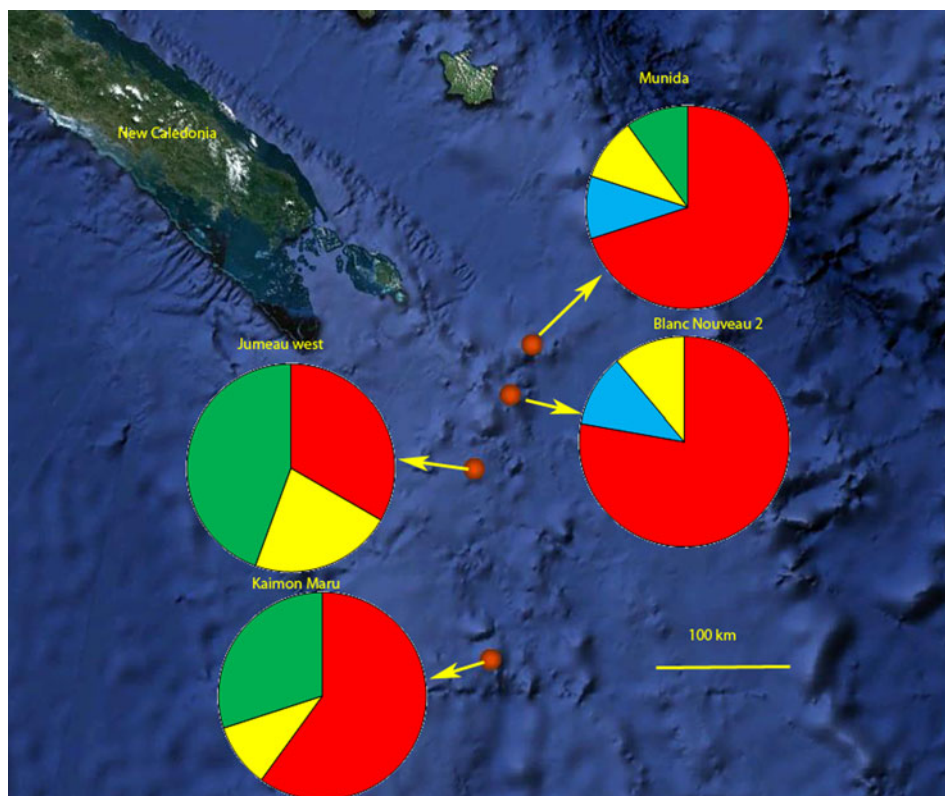


**Table 7.** Comparison of diversities of the seamounts of *N. fulvodesmus* from CO<sub>1</sub> in order of increasing latitude.

Seamount	Sample size (N)	Number of sequences	Number of genotypes ( <i>h</i> )	Nucleotide diversity ( $\pi$ )	Gene diversity ( <i>h</i> )
Jumeau East	4	4	1	0	1
Kaimon Maru	3	3	1	0	1
Zorro South	1	1	1	0	1
Total	8	8	1	0	1

**Table 8.** Comparison of diversities of *N. zingiberadix* calculated from CO<sub>1</sub> of the seamounts in order of increasing latitude.

Seamount	Sample size (N)	Number of sequences	Number of genotypes ( <i>h</i> )	Nucleotide diversity ( $\pi$ )	Gene diversity ( <i>h</i> )
Jumeau East	14	14	1	0	1
Introuvable	18	18	1	0	1
Total	32	32	1	0	1

**Fig. 2.** Distribution of the *I. mirabilis* CO<sub>1</sub> genotypes on the seamounts (ALA, 2014).**Table 9.** Comparison of diversities of *I. mirabilis* calculated from CO<sub>1</sub> of the seamounts in order of increasing latitude.

Seamount	Sample size (N)	Number of sequences	Number of genotypes ( <i>h</i> )	Nucleotide diversity ( $\pi$ )	Gene diversity ( <i>h</i> )
Munida	10	10	4	0.001266	1
Blanc Nouveau 2	9	9	3	0.000723	1
Jumeau WestWest	9	9	3	0.001719	1
Kaimon Maru	10	10	3	0.002049	1
Total	38	38	4	0.010	1



**Fig. 3.** CO1 maximum likelihood reconstruction of the samples analysed in this study. Numbers following the taxon names are Queensland Museum collection numbers (QM G) or GenBank accession numbers. Numbers on the branches indicate rapid bootstrap support values (>70). The scale bar depicts substitutions per site.

sites. This contrasts strongly to the two other species, which showed no polymorphism at all for the CO1.

## DISCUSSION

This study demonstrates that the three lithistid sponge populations have varying levels of population connectivity amongst seamounts, ranging from very well connected subpopulations on individual seamounts to subpopulations with moderate

gene flows between the seamounts. Seamounts are topographically small and isolated, suggesting an island model, which has led to an expectation of high endemism and species richness on seamounts (Richer de Forges *et al.*, 2000). From a purely geomorphological sense one would expect each separate seamount to behave at least as a discrete population. These seamounts would then have reduced gene flow and be particularly susceptible to genetic drift because of their small size (Le Goff-Vitry *et al.*, 2004). However, the results in the present study did not support this. Contrary to the island

**Table 10.** Pairwise differences in  $F_{ST}$  of *I. mirabilis* calculated from CO1 between the seamounts using the distance method.

Seamount	Munida	Blanc Nouveau 2	Jumeau West	Kaimon Maru
Munida	0.00000			
Blanc Nouveau 2	-0.08221	0.00000		
Jumeau West	0.21921*	0.36719*	0.00000	
Kaimon Maru	-0.00076	-0.00130	-0.01170	0.00000

\*Indicates significant difference from 0 at  $P = 0.05$ .

model, seamounts are not surrounded by hydrological barriers and populations might have high gene flow between seamounts, a finding also concluded by other researchers (Samadi *et al.*, 2007; Clark *et al.*, 2012). The lack of significant difference between the sites studied here indicates all the seamounts form one meta-population. The alleged high rates of gene flow of the species studied here amongst the seamounts are presumably caused largely by currents, e.g. migration of larvae, sponge tissue drifting or transmission on ghost nets. With potentially year-round reproduction sponges would be able to take advantage of differing seasonal currents to migrate (Longo *et al.*, 2012). The concept of a short-lived crawling stage as suggested for some sponge species (Hooper & Van Soest, 2002) is likely to be untenable here. Indeed, the presence of the large trough between these seamounts likely represents a serious barrier to crawling larvae. It is most likely that the sponges studied here have a highly mobile longer-lived egg and sperm stage or egg/zygote phase than previously thought which enables them to survive the distance between the seamounts. Other studies have found that species of molluscs with high genetic structure have larvae with low dispersal ability and species with low genetic structure have larvae with high dispersal ability (Todd *et al.*, 1998; Boisselier-Dubayle, 1999; Kyle & Boulding, 2000; Collin, 2001). Bohonak (1999) indicated there is a correspondence between dispersal and gene flow.

All specimens of *Neoaualaxinia zingiberadix* and *Neoschrammeniella fulvodesmus*, when sequenced for CO1, showed only one single genotype. CO1 sequencing of *Isabella mirabilis* revealed four genotypes and each seamount had three of the four genotypes. There was no specialization for genotypes on each seamount. Therefore this species is an example of a lithistid species that mixes well between seamounts. This also confirms that CO1 provides resolution at population level for this species. Other researchers using CO1 have also reported low numbers of genotypes, although in different sponge species (Wörheide, 2006; Hoshino *et al.*, 2008; Whalan *et al.*, 2008; Dailianis *et al.*, 2011). The presence of only one genotype in *N. zingiberadix* and *N. fulvodesmus* hints that genetic drift has occurred. The formation of external buds of hexactinellids in the stable environment of the Antarctic waters (Teixido *et al.*, 2006) may enable the formation of a stable clonal population in the immediate vicinity. Indeed the formation of buds was observed on one of the specimens of *I. mirabilis* in this study. If the ancestral population of one of these lithistid sponges was separated a long time ago but reproduced clonally we would expect two discrete populations. All the seamounts are most likely functioning as a single population suggested by the presence of one single CO1 genotype of *N. zingiberadix* and *N. fulvodesmus*. An alternative explanation to the lack of genotypic diversity in CO1 is because the rock

sponges are long lived and slow growing coupled with slow mitochondrial substitutions (Shearer *et al.*, 2002). The overwhelming proportion of diversity detected by ITS within the same specimens of *N. fulvodesmus* is contained within intragenomic diversity, and in comparison there are comparatively small differences within seamounts and virtually no differences between seamounts. With the ITS there were no examples of the same genotype occurring at all six sites, but there were many unique genotypes at each seamount. Because these species appear to be very slow growing and long lived there does not need to be much gene flow between the seamounts to maintain the genetic uniformity and the vagility of the larval stage may still be quite low. In theory, the exchange of a single individual between large populations is enough to counter any effects from genetic drift (Silva & Russo, 2000) and with a sponge with a generation time of potentially several hundred years this is not impossible.

Clusters of ITS genotypes in *N. fulvodesmus* on seamounts are likely to be the result of either: adjacent settling of larvae, clonality via asexual reproduction, colony fragmentation into discrete individuals, inbreeding populations or even self-fertilizing populations. However, it could also be a function of sampling as deep sea lithistids have been observed to adhere to the rock in distinct clusters (Ekins' personal observations from ROV research Deep Down Under Expedition; [www.deepdownunder.de](http://www.deepdownunder.de)). Much of the diversity in sponge populations has previously been attributed to sex and not asexuality (Dailianis *et al.*, 2011; Uriz & Turon, 2012). However, asexual components may also add to the diversity by colony formation of multiple genotypes such as chimeras. Chimeras have been reported in other sessile marine invertebrates, e.g. corals (Puill-Stephan *et al.*, 2009), ascidians (Sommerfeldt *et al.*, 2003) as well as sponges (Wulff, 1990; Maldonado, 1998; Blanquer & Uriz, 2011). Somatic mutations have also been suggested as a potential mean for increasing genotypic diversity within an individual (Buss, 1982), but these options appear less likely to explain the diversity observed here.

A sexual population is evidenced by the variation in the ITS region in *N. fulvodesmus*, high levels of gene and genotypic diversity coupled with low levels of nucleotide diversity. However, while within this species there are also indirect indicators of asexual components as evidenced by the occurrence of the same common genotypes on the different seamounts and significant linkage disequilibrium, however asexual reproduction in rock sponges has yet to be described. For *N. fulvodesmus* and *N. zingiberadix* the dominance of one genotype in CO1 may be seen as an indication of asexuality, however this has to be tempered by the conservation of the CO1 gene (Shearer *et al.*, 2002; Erpenbeck *et al.*, 2006). The multiple genotypes present in *I. mirabilis* indicate there is some balance between asexual and sexual reproduction at least in some part of the species history that has been maintained on different seamounts. This phenomenon has also been found in sponges (Zilberberg *et al.*, 2006a), corals (Foster *et al.*, 2007; Morrison *et al.*, 2011) and ascidians (Perez-Portela & Turon, 2008). For *I. mirabilis* it just means that it had recombination events at some point even prior to asexual transmission between the seamounts, which has been maintained in the population by asexual reproduction. The lack of subdivision indicates that either they all arose together and evolved very slowly with no continued divergence or they are mixing and interbreeding, with substantial gene flow between the seamounts.

Molecular studies of other sponges have detected strong spatial structure and restricted gene flow, reproductively isolating the populations and thus increasing the chance for speciation (e.g. Duran *et al.*, 2004a; Blanquer *et al.*, 2009). Cryptic speciation amongst what was thought to be cosmopolitan species correlates with potential speciation, presumably due to the low dispersal abilities of many sponges (Wörheide *et al.*, 2002b; Duran & Rützler, 2006; Blanquer & Uriz, 2007).

The lack of any genetic differences along latitudinal or longitudinal gradients indicates that currents around the seamounts potentially have eddies, caused by the unidirectional East Australian Current heading south-easterly, along the Western edge of the Norfolk Ridge (Ridgway & Dunn, 2003). The two pairs of seamounts that have physically close pairs of seamounts, i.e. Zorro North and Zorro South, and Eponge North and Eponge South are not genetically close. This result is in contrast to Duran & Rützler (2006) who only found virtually all of the populations of *Chondrilla* cf. *nucula* Schmidt, 1862, to be significantly different.

This study demonstrates that sponge populations on the deep seamounts studied here appear to be well connected. Despite being topographically small and isolated, the individual seamounts do not show high levels of population structure based on the molecular loci studied here. Not only do seamounts share the same sponge species they also share the same genotypes indicating a mixture of sexual and asexual reproductive strategies with better dispersal properties than previously thought.

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