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The Dictyotales (Phaeophyceae) in New Caledonia : DNA and morphological approaches and spatial analyses based on alpha, beta and gamma diversity

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The Dictyotales (Phaeophyceae) in New Caledonia : DNA and morphological approaches and spatial analyses based on alpha, beta and gamma diversity

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Abstract

The marine flora of New Caledonia (NC), is one of the major group contributing significantly to the high diversity of the coral reefs. The remarkable marine biodiversity is resulting from the combination of the diversity of marine habitats and climates as well as the stability of the seasonal temperature. However, the current figures probably underestimate the actual diversity as many groups have been under sampled and insufficiently studied. The present study aims to analyze, from local to global scale, the diversity of the Dictyotales, which represents the most species-rich order of the Phaeophyceae (brown algae). This study has relied on the abundant material housed at the Herbarium at IRD-Nouméa and the results accumulated in previous studies.

The first step was to supplement the previous data by documenting the diversity of three understudied genera namely *Distromium*, *Homoeostrichus* and *Lobophora* based on detailed morphological and molecular analyses using *rbcL* and psbA (both plastid genes) gene sequences. The second step aimed the analysis of the global diversity of the Dictyotales using alpha, beta and gamma diversity approaches. Finally, we have tested the reliability of the sampling effort and estimated the maximum of the species richness using accumulation curves. DNA analysis brought 32 new sequences some of which are related to species currently unidentified and belonging to *Distromium* (6 sequences with 2 clades), *Homoeostrichus* (12 sequences, 3 clades) and *Lobophora* (14 sequences, 5 clades).The new discoveries represent 2 species of *Distromium*, 5 species of *Lobophora* and 3 species of *Homoeostrichus* and bring the total number of Dictyotales in New Caledonia to 59 species. These species came from 188 sites sampled over the last ten years and grouped in six large geographical areas: Chesterfield Islands, Loyalty Islands, East Lagoon, West Lagoon, North Lagoon and South Lagoon.

Our study reveals that the long term sampling effort approaches adequately the real species richness. Of the various projections from the species accumulation curve tested, the extrapolation stands at 71 species i.e. 12 species (16%) more than we actually collected. For *Padina*, which is the most common and diverse genus, the projection met with the actual species number.

Alpha diversity evaluated at the sampling site scale ranges from 1 species of Dictyotales collected in a station to a maximum of 8 species. The mean species richness value for one site was less than 3 species per station over the six areas, suggesting that local alpha diversity of Dictyotales is low in each area. Gamma diversity was computed by pooling samples over large areas. Chesterfields appears to be the less diversified area with 11 species found in the whole area, whereas 36 species were collected in the South Lagoon.

Rare species appear in a large portion of the Dictyotales with 28.9% of the species restricted to one or two sampling sites which corroborate results from previous studies on various biological groups such molluscs or fish and puts forward the question of the significance of the rarity in the ecosystem functioning.

The Beta diversity, calculated by the Whittaker's $\beta_{W.}$ distinguished the Chertesfield area from the other areas on species composition. Because of the high proportion of rare species, multivariate analyses based on the dissimilarity between areas could not delimitate separate zone based on species composition. Only Chertesfield area show a high difference in species composition with all the other area, based on β_W .

Key words: *rbcL*, psbA, Alpha beta gamma diversity, rare species, spatial distribution, Dictyotales

Contents

Introduction

The marine ecosystems of New Caledonia (NC) have a high diversity of species (Payri and Richer de Forges, 2007), which can be linked to its diverse climates from North to South (tropical, subtropical, temperate), to the fast speciation under these conditions and the stability of the seasonal temperature (Mittelbach et al., 2007). Moreover, NC is close to the coral triangle which is one of the most bio-diverse region. In addition, the marine ecosystems in NC, display a wide diversity of habitats, including lagoonal coral reefs complexes, seagrass and macroalgal beds, reef slopes, or oceanic platform and atolls (Andréfouët et al. 2007). Several studies dedicated to the marine flora have shown an important species diversity for numerous groups at the New Caledonia scale (Payri, 2007, Bittner et al. 2008; Dijoux et al. 2012, Dalleau et al., 2009). However, there was no study focusing on the analysis of the spatial variation of macroalgal diversity from local (i.e. sites) to global scale (i.e. New Caledonia), based on Whittaker's (1960, 1972) concepts of α-β- and γ-diversity. Whittaker's idea was that the total species diversity in a landscape (γ-diversity) is determined by the mean species diversity in sites or habitats at a more local scale (α-diversity) and the differentiation among those habitats (β- diversity). The species richness along with the distribution of species and community differences should be also evaluated in order to better measure and understand the biodiversity patterns (Ellingsen, 2001).. This approach requires accurate inventories of species at the appropriate spatial scale. This condition forced to focus on the ecologically important biological groups of well known taxonomy. Among the very abundant material collected in NC and housed at the IRD center, the Phaeophyceae, also known as brown algae and particularly the order Dictyotales representing the third most diverse species-rich order have 10 of the 19 genera present in NC: *Dictyota, Dictyopteris, Distromium, Homoeostrichus, Lobophora, Padina, Spathoglossum, Stypopodium, Taonia* and *Zonaria*. Among those, the three genera, *Distromium, Lobophora* and *Homoeostrichus* required a deeper taxonomical investigation to get a more comprehensive picture of these groups and consider an in-depth diversity analysis. Before analyzing the diversity (alpha) and measuring its variations between communities and sites along environmental gradients at the NC scale, species identification for the three neglected genera were undertaken with a combination of DNA and morphological analyses.

The study aimed to clarify the classification of Dictyotales in NC for three genera (*Distromium, Homoeostrichus* and *Lobophora*) before the assessment of marine algae diversity in New Caledonia, by analyzing the diversity of the Dictyotales.

Materials and methods:

1. Study areas

New Caledonia is located in the Southwest Pacific Ocean between 15° and 25°S and 160° and 170°E, at 2.500 km east from Australia. This region was divided into six large areas : Chesterfield Islands Loyalty Islands (including Astrolabe and Beautemps-Beaupré in the north and Durand bank in the south); South lagoon (comprising "Ile des Pins"); North lagoon; East lagoon and West lagoon (including Nouméa), based on the geographical position of the regions (Fig. 1). The barrier reefs, which are 1.600 km long, enclose a wide lagoon around Grande Terre, providing several different habitats for marine species (Andréfouët et al., 2007).

2. Biological material

Algal collections considered in this study came from 188 sampling stations distributed within the lagoon and reef complexes (Fig 1.). For each specimen, GPS coordinates; description of the typology and morphology of the sampling site, according Andréfouët et al. (2006), are described. Typology 1 corresponds to the description of six categories of habitats : reef, coast, lagoon, open sea, coastal slope, and inner slope, while the typology 2 described the habitat at a more precise level with 12 different categories : fringing reef, submerged reef, barrier reef, intermediate reef, cay reef, seagrass, bay, pinnacle, lagoon floor, channel, outer slope, pass. Sites were also associated to depth categories: [0-5] meters, [6-20] meters, [21-40] meters, [41-60] meters and > 60 meters.

Raw data came from the collection of macroalgae housed at IRD (Institut de Recherche pour le Développement) in Noumea (New Caledonia).

3. Species identification

3.1 Morpho-anatomy approaches

About 700 specimens of Phaeophyceae, acquired over the ten last years in New Caledonia were considered in this work. Part of them has been previously identified at the species level using morpho-anatomy and DNA analysis. For the specimens unidentified, we used a rapid biodiversity assessment, based on the "parataxonomy method" described by Abadie et al.(2008) in order to assign specimens to morphotypes before histological and molecular analysis evaluation.

For anatomical purpose, small fragments removed from the dried specimen (herbarium voucher) were rehydrated before being sectioned in transversal and longitudinal sections using a freezing microtome. Slides were observed with an Olympus BH2 microscope. The taxonomical descriptions were based on the available literature such Abbott and Huisman (2004), Kraft (2009) and Womersley (1987).

The three genera were first separated based on the general organization of the thallus:

(i) *Lobophora* has erect or prostrate thallus. They are characterized by 3 tiers of layers of cells: ventral and dorsal layers with a variable number of cortical cells and a medullar layer. For *Lobophora*'s anatomical analyses, the length, width and height of cells were measured in cross and longitudinal sections.

(ii) *Distromium* has lobed, fan-shaped or dissected thallus, of brown color when living. In cross section, the genus is two-cells thick (excluding apical margin), cells are nearly uniform in shape and size throughout the thallus,

Those two genera have been often confused due to the strong external morphological resemblance. The number of cells in cross section strips away the ambiguity between the two genera.

(iii) *Homoeostrichus* has fan-shape blades multilayered, most often with a three layers of cells. The blade is thin with a conspicious holdfast and a high density of hairs throughout the frond. Old blades are golden brown and deeply lacerated while recent fronds are greenish and hairless.

For each genus, unidentified specimens were separated between the different morphotypes based on more accurate morphological and anatomical characters, as the size and the shape of the thallus, the number of lobes, the position of hairs and the number of cells in cross and longitudinal sections. The morphotypes were validated with the molecular results.

3.2 Molecular analysis

New sequences were provided from collection of tissues preserved in silicagel and from fragments carefully removed from the specimens in herbarium, following DNA analyses according to Bittner et al. (2008) and modified for the extraction step, following the protocol of the Molecular lab in Ghent University. The fragments of specimens were ground with liquid nitrogen and CTAB buffer before the DNA was extracted using chloroform and phenol. DNA was purified using the Wizard DNA Clean-up System Resin, following the manufacturer's instructions.

Based on previous works, two plastid genes: *rbcL* and psbA were chosen for DNA analysis. The gene (*rbcL*) has been extensively used in molecular phylogenetic studies of brown algae and has been demonstrated to be useful molecular marker by many authors (Siemer et al. 1998, Draisma et al. 2011, Lee and Bae 2002, Cho et al. 2004, Hoshina et al. 2004, De Clerck et al. 2006, Lane et al. 2006, Cho et al. 2007, Bittner et al. 2008, Ni-Ni-Win et al. 2008, 2010, Philipps et al. 2008), whereas *psbA* gene was less studied

Specimens considered in this study to generate sequences are listed in Table 1.

The *rbcL* gene (approximatively 1350 base pairs (bp)) was amplified and sequenced only for the first overlapping fragments (700bp), using the primers F68 and R708. The psbA gene (approximatively 1000 bp) was amplified using the primers psbA-F and psbA-R1.

The primers sequences used for the polymerase chain reaction (PCR) amplification and sequencing are described in the Table 2.

The PCR conditions for *rbcL* were as follows: an initial denaturation step at 94°C for 3 minutes, followed by 94°C for 45 seconds, annealing at 52°C for 45 seconds, extension at 72°C for 2 minutes for 40 cycles, and final extension at 72°C for 6 minutes.

The PCR conditions for psbA consisted in 40 cycles comprising an initial denaturation at 94°C for 3 minutes, followed by 94°C for 1 minute, annealing a 46°C for 1 minute, extension at 72°C for 2 minutes, and then, a final extension of 10 minutes at 72°C.

For both genes, the PCR-amplified DNA of some herbarium specimens could not be sequenced due to the bad quality of the extracted DNA, which can be very degraded, or because the PCR failed.

The unpurified products were sent for sequencing to MACROGEN (http://www.macrogen.com/eng/). Sequences were analysed using Sequencher ™ 4.1 (Gene Codes Corporation, Michigan) and were aligned with MUSCLE and then manually and adjusted with MEGA 5.1. Phylogenetic trees were inferred using Neighbor-Joining (NJ) algorithm that uses a matrix of pairwise distances estimated under the model for nucleotide sequences (Koichiro Tamura et al.,2011). All positions containing gaps and missing data were eliminated. In order to check for clustering of specimens assigned to a single species in a DNA sequence-based phylogeny, as well as for congruence of tree topologies, three alignments were created for the phylogenetic analyses: *rbcL*, psbA and combined *rbcL* + psbA. We also obtained phylogenetic trees using Maximum-Likehood (ML) (not shown) in order to compare the results from the two different methods..

The robustness of the results was tested by bootstrap analysis (Felsenstein, 1985) using 1000 replications in NJ analysis. The number on the node (between 0 and 100) means the number of times that this branch appears during the repetition. The cut-off limit to define if the node is surrogate or note was chosen at 55 %.

Sequences from GenBank, assigned to other Dictyotales (*Dictyota* , *Padina*,, and *Zonaria*) and one species belonging to the Sargassaecae (Phaeophyceae) : *Sargassum*, were chosen as out-groups in order to root the tree.

4. Assessment of diversity

Diversity analyses were done on the absence or presence of a species in a sampling station and on the number of occurrences (number of stations where we found the species) per large area. A total of 188 stations distributed in the 6 large areas were considered in the study.

Alpha diversity was considered as sample species richness (SR_s), measured in a sampling site and taken from a community. Gamma diversity was the species richness in large areas (SR_l).All the regions together constituted the largest scale studied, called the total area species richness (SR_T).

Following the terminology of Colwell & Coddington (1994). species restricted to only one site were considered as “unique” or “rare” and species occurring at two sites exactly were called “duplicate”. Based on the unique and duplicate species, the non parametric Chao 2 index, independent on the distribution of species (Colwell & Coddington, 1994), was used to estimate the theoretical number of species expected for the whole New Caledonia, using the PRIMER 6 software. Chao 2 is calculated from the number of species observed in all samples (Sobs) and the frequency of unique species ($Q1$) and duplicate species ($Q2$): Chao2 = Sobs + ($Q1^2/2Q2$). Species cumulative curves were calculated with the PRIMER 6 software, which

computes randomized species accumulation curves; we ran 1000 random permutations drawing of the 188 stations.

Beta diversity was estimated by using two indices.

First, Jaccard binary index measures the distance between two samples based on presence/absence of the species in samples and compares the specific composition between pairs of samples. Jaccard index does not consider the double absence of one species within pair of stations as a criterion of similarity between the two stations: $Sjk = 100\alpha / (a+b+c)$ with *a* the number of common species in 2 samples, *b* the number of species in sample j not present in the sample k and *c* the number of species in sample k not present in the sample j. From the resulting distance matrix, clusters are generated and correspond to assemblages of stations based on their similarities in their species composition. Jaccard's coefficients range from 0 (samples completely dissimilar) to 1 (identical samples). Multivariate statistical analyses, based on the resulting matrix were then computed. Hierarchical classification (CLUSTER), based on group-average linking (Clifford & Stephenson, 1975) and ordination by non-metric multidimensional scaling (MDS) (Kruskal & Wish, 1978) were computed to give a graphical presentation of the similarities between samples or areas. The typology 1 & 2, and the depth assigned for each sampling stations) were added to the multivariate analysesin order to identifiy potential clusters of stations of similar species composition.

Second, β Whittaker diversity is computed with the equation $\beta_W = (\gamma/\bar{\alpha}) - 1$, where γ is the total number of species resulting from merging a number of individual samples and $\bar{\alpha}$ is the average number of species per individual sample (Whittaker 1960, 1972). This calculates the proportion by which a given area is richer than the average of samples within it. β_W was measured over the large area scale corresponding to the six areas. Among taxa, β diversity is highest in those with the most restricted ranges and specialized habitats, whereas within taxa, β diversity may increase with the environmental dissimilarity between sites (Harrison et al., 1992).

Results

1. Morphological and genetic analyses

From the morphological and genetic analyses, the list of species considered in this study has been completed. (Table 3).

In the following text, "fully supported" relationships refers to a bootstrap support (BP) equal to 100, while "strong support" corresponds to a bootstrap support >88%. Other arbitrarily bootstrap values used are "moderately supported" (for 75-88% BP) and "weakly supported" (55-74%BP).

The combined *rbcL* and psbA alignment consisted of 44 sequences representing 21 *Distromium,* 11 *Lobophora*, 8 *Homoeostrichus* and 4 outgroups. (Fig 2).

The *rbcL* alignment consisted of 72 sequences representing 35 *Distromium*, 17 *Lobophora*, 13 *Homoeostrichus,* including 6 sequences of New Caledonia species that were published by Bittner et al., 2008. The psbA alignment consisted of 47 sequences, representing 21 *Distromium*, 11 *Lobophora*, 9 *Homoeostrichus*, including sequences from GenBank. (Appendix 1).

The phylogenetic trees inferred from separate and combined data were highly congruent, differing only in the position of some nodes that received little or not support, as the clade A of *Distromium* (=*Distromium decumbens*), that is monophyletic for the psbA tree and for the *rbcL* tree, but appear polyphyletic in the combined psbA + *rbcL* tree.

Trees inferred from ML and NJ analyses gave similar results. Only trees from NJ method (Saitou and Nei, 1987) that is a simplified version of the minimum evolution (ME) method (Rzhetsky and Nei, 1992), are shown in this paper.

NJ trees from *rbcL*, psbA and concatened *rbcL* + psbA shown similar topology, with slightly differences in the psbA tree due to some lack of sequences. The highest number of fully or strongly supported nodes was obtained with *rbcL*, whereas, the lowest number was observed with psbA. As a consequence, in the following text, only tree from *rbcL* sequences will be presented, to analyze the *Distromium*, *Homoeostrichus* and *Lobophora* diversity.

1.1 *Distromium* diversity

From the *rbcL* NJ tree (35 sequences) (Fig 3), the psbA NJ tree (21 sequences) (Table 1) and the combined *rbcL*+psbA NJ tree (20 sequences) (Fig 2), five well supported genetic groups or clades (clade A to clade E) have been found, corresponding to five different morphotypes. Representative specimens of each clade shared the same morphological characters and were significantly different from each other, except for the large clade A which displays a high morphological diversity.

Finally, the clade A, including the sequence EU579946 assigned to *Distromium decumbens* by Bittner et al (2008), which was also collected in New Caledonia, has a very high phenotypic plasticity. Individuals are present in all New Caledonia regions, except in the Chesterfield Islands, with a high morphological variability. Some morphological characters

can be associated to the area of collect, such as the dark color of the thallus, for the specimens from the eastern area.

This clade, strongly supported, is the largest clade of *Distromium* clustering specimens collected either in the lagoon or more commonly on the outer reef slopes from various areas in New Caledonia. It is not associated to any specific morphological characters due to the high phenotypic plasticity. .

The clade B well-supported belongs to the clade A (=*Distromium decumbens*) and corresponds to samples from Chesterfield area which is remote and geographically isolated from the others regions. Morphological results leaded to three different morphotypes clearly and easily distinguishable from each other. However, they appeared in the same genetic clade. This might be the result of a morphological divergence but stay closed genetically.

The clade C, well supported consists of specimens from Beautemps-Beaupré (Loyalty Islands) and from the North East coast of the Grande Terre.

Clade D, with IRD321 and IRD320 (GenBank reference, Bittner et al., 2008) sequences, corresponds to *Distromium didymothrix* only found in Ile des Pins (South of New Caledonia).

Finally, clade E is strongly supported in genetic analyses and morphologically homogeneous. Specimens came from the outer slope of the "Passe de Uitoé, ST254", they are browner than the other specimens from the others localities, and which are more greenish. This species differs significantly in morphological characters from the others species with a large and tall thallus, very thin and light brown.

1.2 *Homoeostrichus* diversity

From the *rbcL* NJ tree (12 sequences) (Fig 4), the psbA NJ tree (9 sequences) (Table 1) and the combined *rbcL*+psbA NJ tree (8 sequences) (Fig 2) *Homoeostrichus* specimens are split in three clades (A, B and C), Fig 4).

Clade A strongly supported in our genetic analyses. represents only species from Loyalty Islands. The particularity of the specimens clustered in this clade, is the gradient of color from the brown base to the light golden brown to submarginal region.

Clade B has entire or deeply incised blades, from dark brown to green, with hairs throughout the blade and on both sides. This clade, including EU579951, assigned to *Homoeostrichus* sp. in Bittner et al., (2008) is present in all the areas, except in the Chesterfield, either because this genus is not present in this region, or because it was not sampled.

Clade C including the sequence EU579952, also assigned to *Homoestrichus* sp. (Bittner et al., 2008) is strongly supported in all analyses. The very thin green to brown thallus is unilobed and has scarce hairs at the base. The specimens belonging to this clade came from, Loyalty Islands (Lifou) and the Eastern and Western areas of "Grande Terre" (Poindimié and Passe de Uitoé).

1.3 *Lobophora* diversity

From the *rbcL* NJ tree (17 sequences) (Fig 5), the psbA NJ tree (11 sequences) (Table 1) and the combined *rbcL*+psbA NJ tree (11 sequences) (Fig 2), 5 clades have been identified.

The clade A is constituted by specimens which have 5 or 6 cell layers, a very thin green brown thallus and no hair on the blade surfaces.

The clade B, including the specimen ST 276 identified as *Lobophora variegata* by Bittner (2008) has morphological and anatomical characters closed to the clade A.

Specimen of the clade C (IRD275) is a crustose and dark algae, found on the coral.

Clades D and E have a similar morphological pattern, but differ by their anatomical structures. IRD 7669 and specimens from the clade E have 9 cell layers, whereas IRD 7640 has only 5 cell layers in cross section. Actually, clade D comprises three different species, closed in genetic analyses with our markers, but separated with the cox 3 marker gene (ongoing study).

A lot of specimens are unidentified or undescribed species and need more description based on specific criteria that are not studied in this paper (ongoing study). Morphological studies (not shown in this paper) showed the high variability in morphological characters. Only morphological data is inadequate basis for identification of species and knowledge of species boundaries.

2. Species Richness

Alpha diversity (sample species richness SR_s) at 188 sampling sites was very low with a value of the mean of *SRs* inferior to three species per station for all areas (Table.4), with a maximum of height species found in only two stations of the West Lagoon : ST771 (Koumac), and ST254 (Passe de Uitoé). Mean SR_s was very low for all the stations in all the areas, but was highest for the South lagoon area (2.5) where the SR_l was also the most important (36 species) and the sampling effort was the highest (48 stations).

The highest number of rare species (unique species) was found in Chesterfield, which has also the lowest number of sampling sites (15 stations) and of the lowest species richness (mean SR_s = 1.5± 0.7 species per site and SR_l = 11 species).

Gamma diversity (SR_l) was variable, ranging from 36 species in South Lagoon, to 11 species in Chesterfield. A total of 59 species (SR_T) were collected at 188 sites in New Caledonia. (Table 3).

Significance of the sampling effort

The species accumulation curve, performed by PRIMER 6, plots the cumulative species count against sample number. On the Fig.6 (a), Sobs (the species accumulation curve observed for our data) reach asymptotic values, but the asymptote, indicating that the sampling is not

totally saturated. Projections from all the species accumulation curves extrapolate the total richness at the study region to over 65 species. The estimate of current species richness computed by the non parametric indice Chao 2 is 71 species, whereas our species richness observed was 59 species. Sobs underestimates the true richness based on Chao2 (Fig.6, a).

From the species accumulation curves (Fig.6) obtained for the dominant taxonomic groups *Padina* (16 species corresponding to 27.1% of the total number of species),(c) *Lobophora* and (d) *Dictyota* (respectively 11 and 10 species comprising 18.6% and 16.9 % of the total number of species) showed that the curves did not reach an asymptotic value for *Lobophora* and *Dictyota*, whereas *Padina* showed a bigger sign of stabilizing towards asymptotic values.

3. Distributions of species, ecological rarity

Padina is the most common genus in New Caledonia with 14 species present in 102 stations, following by *Lobophora* with 11 species and 36 occurrences, and *Dictyota* with 9 species in 53 stations. The most common genera are also the most diverse , except *Distromium* that occurs in 53 stations with only 5 different species (Table 5 and Fig 7). *Taonia* is the only genus which was found only one time in one region and represented by only one species.

No species was present in more than 50 % of the total sampling areas and only two species (*Padina australis* and *Padina melemele2*) were spanned the entire region (the six large areas). However, 8 species occurred on five over the six areas (*Distromium decumbens*, *Homoeostrichus* clade B, *Lobophora* clade D, *Padina minor*, *Padina okinawaensis*, *Padina stipitata*, *Stypopodium flabelliforme* and *Stypopodium* group 3). Those species except *Lobophora* clade D, are completely absent from the Chesterfield.

The distribution of species range size (Fig.8, a) showed a high number of rare or unique species, occurring in only one sampling site. 20% of the species (12 species) were restricted at single stations, and only 23% (14 species) were collected in more than ten sites. (Fig.9). Diversity statistics also showed the dominance of only some species (*Distromium decumbens*, *Padina melemele2*, *Padina australis*, *Homoeostrichus* clade B, *Padina stipitata*, *Spathglossum asperum*, *Dictyota friabilis*, *Padina macrophylla*, *Padina minor*, *Dictyota bartayresiana*, *Padina melemele1*, *Padina okinawaensis*, *Stypopodium* group3) that occur on more than 10 sampling sites over the 188 sites in New Caledonia.(Fig. 8,b).

According to Gaston's (1994) definition, less restrictive definition of the rarity, species are considered as "rare" if they belong to the less abundance quartile of species. For our data, these 25%, evaluated by the number of occurrences of the species instead of the abundance, correspond to the 15 species that occur in only one site (for the last 12species) and in two sites (for the 3 other species).

4. Dictyotales assemblages

Based on the Jaccard distance between pairwise of stations, hierarchical cluster were computed, using R and PRIMER softwares. The Jaccard matrix and the hierarchical cluster

were first computed for all the species of Dictyotales from New Caledonia. The clustering result showed a very high dissimilarity between stations and was highly divided into many groups that did not give any information about species assemblages. We computed then Jaccard distance matrix and hierarchical clustering with only rare species and species that occurs in less than 10 stations, and we deleted ubiquitous species (common species found in more than 10 sampling sites over the 188 samples sites) for the analyses, in order have a better representation of the assemblages if any. The results (Fig. 10) was almost the same than with all the species.

Clusters occurred over a wide range of similarities (0-66%, Fig. 10). Only two sites shared 66% of similarities, whereas the other groups of stations do not share more than 50 %. The cluster were also divided into many groups that did not give neither a clear geographical repartition of the sampling sites in large areas, nor a role of the typology or the depth on the grouping of stations. However, adding the geographical information (area belonging), three groups of few stations from three different areas are revealed in the Chesterfield area, in the Loyalty Islandsand finallyin the South lagoon.

Therefore, the Multidimensional scaling ordination did not give any relevant or clear results that it is worthwhile to be presented and discussed in this paper. Dissimilarity between the area is not strong enough to delimitate separate zone based on the species composition.

5. Beta diversity

Table 6 shows the matrix results of the Whittaker's β_W computed on R software for the six areas. The minimum of β diversity is the value of 1 when all sites share the same species thus there is no change in species composition between the two samples. The maximum value (=0) of the β_W is obtained when no one of the species is present in several sites and, as a consequence, the turnover of species composition is important and the β_W is the most important.

The β_W is highly variable from a minimum of 0.35 between South and East Lagoons that suggest a high number of common species shared by these two areas.

At the contrary, the maximum value between Loyalty and Chesterfield Islands shows that those two areas are the most different in New Caledonia and the change in species composition in very important; they do not share a lot of species in common.

Regarding to the all matrix, Chesterfield has the highest values of β_W that reflects a species composition different than the rest of the sampling area.

Discussion

1. New insight on species diversity

This study of the abundant material from the Herbarium collection has helped to gain more insight into the diversity of the Dictyotales. DNA analyses based on *rbcL* and psbA gene sequences brought a great number of new sequences (32 sequences) and revealed potential new species for the three targeted genera: *Distromium* (2), *Homoeostrichus* (1) and *Lobophora* (5). This richness was unexpected as specimens were currently assigned to the single *Lobophora variegata*.

DNA analysis also revealed cryptic diversity related to the geographical origin of the specimens. *Distromium decumbens,* from Chesterfield region forms a separate cluster like *Homoeostrichus* Clade A which was only found in Loyalty Islands.

The presence of new genetic clades, which might be considered as new species with further studies, shows that the species richness in New Caledonia is greater than previously estimated and might be improved with supplementary sampling and further study on the huge collection housed in the Herbarium.

The more effort are dedicated to the study of the collection the more new species are found. Our discoveries follow the previous studies dedicated to the Dictyotales which had already revealed 4 new species and one "New genus" (Bittner. et al, 2008). The present study increases the diversity of the Dictyotales by 13.5% of the total species richness.

2. Rarity

In New Caledonia, rare species are everywhere with a minimum of 38% of unique species in the Loyalty Islands and a maximum of 66% for the Chesterfield area. Genera, can be also rare with the extreme case of the genus *Taonia*, which is represented by one species found only one time at one station (ST246, South lagoon), or *Zonaria* and the "New genus" (Bittner et al., 2008) found only one or two times and restricted to one or two areas. The high number of rare species can be explained partly by the sampling itself, as Dictyotales were collected as the same time with all the other macroalgal groups, leading to some missing species. The sampling result is also dependent on the major goal of the collect (searching for particular species or genus) and on the time spent into the water. Moreover, some of brown blades can be confused underwater due to their similar gross morphology.

Nevertheless, even if the rarity of the species might be explained partly by the collect effort, rarity has an ecological meaning, and a number of studies have shown that a high diversity of species in an ecosystem, such as marine ecosystems, includes a high number of rare species. Bouchet et al.(2002) shown that mollusc fauna is represented by a considerable portion of rare species (32% species present at a single sites, and 20% of the species represented by a single specimen).. According to Mouillot et al., (2013) rare species are irreplaceable in an ecosystem and have an important role in ecological functions.

3. Significance of the effort sampling

The Species Accumulation Curve (SAC) shown (Fig6.) that neither the SAC (Sobs) nor the estimators (Chao 1 and 2, Jackniffe 1 and 2) reached perfect asymptote values and the

cumulative number of species is still slowly increasing (with a low slope). Our study reveals that the long term sampling effort approaches adequately the real species richness. Of the various projections from the species accumulation curve tested, the extrapolation stands at 71 species i.e. 12 species (16%) more than we actually collected.

SAC for the common taxa (*Dictyota* and *Lobophora*) did not reach the asymptotic values (10 species vs 14.5 projected by Chao 2 and 10 species vs 21 projected, respectively). The projected value for *Lobophora* is totally supported by the ongoing study (Christophe Vieira thesis study). For *Padina*, which is the most common and diverse genus, the projection met with the actual species number. Consequently, this suggests that an increase of the sampling effort is needed to get the stabilization of the curve at an asymptotic value. However, the sampling effort on the remote Chesterfield Islands (12 days and 39 stations in 2008), provided adequately results, with five genera (*Dictyota*, *Distromium*, *Lobophora*, *Padina* and *Stypopodium*) over the eight common Dictyotales found in NC (we do not consider *Zonaria*, *Taonia* and the "New genus" which are restricted to the South and West lagoon areas). For the Loyalty Islands, the sampling effort was comparable with the Chesterfield (35 stations and 39 stations respectively), and the two genera *Homoeostrichus* and *Spatoglossum* were found in the Loyalty Islands (3 and 1 species, respectively) in addition to the five also present in the Chesterfield. Regarding the sampling conditions, dependent on the difficulties to sample the Chesterfield area, results in terms of alpha diversity can be considered as satisfactory.

Bouchet et al (2002) suggested that the underestimation of actual richness might also be the result of insufficient coverage of spatial heterogeneity. In such diversified ecosystem, species can be confined to one kind of habitat or typology. If the sampling does not enough take into account this heterogeneity, species can be missed.

4. Spatial analysis

According to the SR_S values obtained for each station, Dictyotales are present everywhere in New Caledonia in similar and homogeneous proportion in each area.

From the diversity analyses, no real pattern based on geographical distribution was found. However, the cluster based on the Jaccard index showed small groups of sites corresponding to Chesterfield, Loyalty and South Lagoon (included "Ile des Pins") areas (Fig 10). These observations showed that the island systems separated from the "Grande Terre" can be discriminated by their species composition and could be considered as ecoregions. Moreover, the Whittaker's β showed a high variability in species composition at the large area scale, with lower values for the areas in the "Grande Terre" (from 0.35 to 0.47). The Chesterfield Islands appear as the most different area with high numbers of β_W that reflect a more singular species composition. This reflects the relative isolation of the Chesterfield Island and the less diverse habitats which are mainly exposed reefs and deeply open lagoons. These considerations corroborate observations based on the alpha diversity analyses.

Results obtained for Chesterfield, Loyalty and Iles des Pins Islands cannot be extent to the "Grande Terre" as multivariate approach using Jaccard matrix (using presence or absence data) could not discriminate the communities because of the high number of rare species which generate too many different groups of stations with low species in common. Probably, the station level is not the appropriate scale and a clustering of stations based on typology and bathymetric characters would be more adequate. Moreover, the low number of species involved in the study resulting in small numbers for alpha diversity could explain the low reliability of the approach for grouping stations according to their species composition. No

single mechanism can explain Dictyotales patterns and communities observed in New Caledonia. A combination of more environmental factors, such as the distance from the coast and the substratum type might provide information of importance to establish accurate comparison between sites.

Conclusion

Genetic and morphological analyses revealed eight potential new species bringing the total species richness of Dictyotales in New Caledonia at 59 species. These two complementary approaches provided new sequences of Dictyotales, and new morphotypes description, which will lead to new species descriptions.

This total species richness differs to the estimation of the true species richness. Higher number of species richness could be provided by improving the sampling effort and undertaking deep morphological and genetic analyses on specimens in the Herbarium that might contains new species, as the ongoing study on *Lobophora* showed by the discover of new and cryptic species among specimens traditionally attributed to a single species.

Multivariate analyses did not identify factors for the species distribution because of the high number of rare species and the low alpha diversity that make too many differences between two sampling sites. No clear spatial distribution was explained, except for Chesterfield, Loyalty and Ile des Pins Islands which were discriminated by their species composition and could be considered as ecoregions.

Local and gamma species richness varied between areas. Whittaker's (1960,1972) beta diversity, clusters and comparison of SR_l and SR_s between all areas considered Chesterfield area as the most divergent area compared to the five others, with a lowest alpha and gamma species richness. This difference is probably partly explained by the remoteness of the Chesterfield and the less diverse habitats and the distance with the "Grande Terre" which make difficult to go there to collect data.

This study was a first approach and the results are encouraging. Thus, it has to be extended to other groups of algae for which the ongoing studies are bringing the same kind of information (taxonomy and distribution), in order to enlarge the data set and avoid problems linked to the low values in the analyses of the diversity. Spatial approaches need more investment in order to find the appropriate level to conduct the analyses.

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Figure caption

Fig.1. Geographical position of the 188 sampling sites (represented by the small circle) in the six large areas in New Caledonia, based on the Millenium classification. (Andrefouët, et al. 2005)

Fig.2. NJ tree based on the combined *rbcL*+psbA gene sequences. Evolutionary relationships of taxa *Distromium*, *Lobophora* and *Homoeostrichus*, inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. The analysis involved 72 nucleotide sequences. Evolutionary analyses were conducted in MEGA5.

Fig.3. Neighbor-Joining (NJ) tree based on *rbcL* gene sequences for *Distromium*. Numbers at each nodes indicate bootstrap values (>55%) for NJ.

Fig.4. NJ tree based on *rbcL* gene sequences for *Homoeostrichus*. Numbers at each node indicate bootstrap value (>55%).

Fig.5. NJ tree based on *rbcL* gene sequences for *Lobophora* specimens. Numbers at each node indicate bootstrap values (>55%).

Fig.6 .Species accumulation curves , 1000 permutations of samples were performed for all the sampling sites in New Caledonia.(a) Species accumulation curves based on PRIMER 6 ; Jackniffe 1, Chao 1 and 2 richness estimators. Estimators of species richness are the total number of all species (Sobs) and the Chao 2 estimator of the true richness. (b)(c)(d) Species accumulation curve of the dominant groups of Dictyotales. (b) *Padina*,(c) *Lobophora* and (d) *Dictyota*.

Fig.7. Histogram of the number of occurrences of each genus of Dictyotales in New Caledonia.

Fig.8. (a). Distribution of species range sizes, with range size considered as the number of sites occupied by a species out of 188 sites.

Fig.8. (b) Histogram of the species range from the most abundant (occurred 43 times in the samples) to the less abundant (occurred only one time in the samples) in New Caledonia.

Fig 9. Ecological rarity of the Dictyotales from New Caledonia. Proportion of species in number of stations of occurrence.

Fig.10. Hierarchical, agglomerative clustering based on the Jaccard distance matrix, using data of presence/absence of the species on the 188 sites.

Table caption

Table.1.: List of taxa and specimens for which sequences were obtained. Sequences from GenBank are listed with authorship and accession number.

Table.2. List of primers used in the PCR amplification and sequencing.

Table.3. List of species considered in this study.

Table.4. Species richness (mean SR_s = mean of alpha diversity at each station of the whole area ± Standard deviation, SR_l = large area, SRT= total area (NC), and the proportion of rare species (unique species and duplicates species).

Table.5. Genera characterized by their number of species, the percentage of the species compared to the total number of species (59 species), for the six areas and the number of occurrences of the genus in New Caledonia.

Table.6. Whittaker's beta diversity (β_W) for the six large areas, computed on the R software.

Table.1.

Voucher specimen	Taxa	Genes	GenBank reference	Date of collection	Collection Site	Collector
IRD243	*Homoeostrichus*	psbA / rbcL	-	30th November 2005	ST460 Ile des Pins	C. Payri
IRD244	*Distromium*	psbA / rbcL	-	26th May 2004	ST254 Passe Uitoé	J-L Menou
IRD249	*Distromium*	psbA / rbcL	-	20th April 2004	ST720 Récif Tomboo	C. Payri
IRD253	*Lobophora*	psbA / rbcL	-	22nd June 2005	Baie de Saint Vincent	C. Payri
IRD255	*Distromium*	psbA / rbcL	-	7th December 2004	ST770 Koumac	C. Payri
IRD257	*Distromium*	psbA / rbcL	-	17th May 2004	ST254 Passe Uitoé	J-L Menou
IRD275	*Lobophora*	psbA / rbcL	-	2nd December 2004	ST609 Touho	C. Payri
IRD277	*Lobophora*	psbA / rbcL	-	7th April 2005	ST653 Beautemps-Beaupré	C. Payri
IRD280	*Distromium*	rbcL	-	5th April 2005	ST649 Astrolabe	C. Payri
IRD282	*Lobophora*	rbcL	-	4th May 2004	ST771 Baie de Ste Marie	C. Payri
IRD321	*Distromium*	psbA / rbcL	-	6th December 2005	ST996 Ile des Pins	C. Payri
IRD7383	*Distromium*	rbcL	-	10th July 2008	ST1156 Chesterfields	C. Payri
IRD7397	*Homoeostrichus*	rbcL	-	7th December 2004	ST770 Koumac	J-L Menou
IRD7400	*Homoeostrichus*	psbA / rbcL	-	8th February 2005	ST046 Goro	C. Payri
IRD7404	*Homoeostrichus*	psbA / rbcL	-	22th May 2006	ST254 Passe Uitoé	J-L Menou C. Payri
IRD7412	*Homoeostrichus*	rbcL	-	28th February 2005	ST254 Passe Uitoé	C. Payri
IRD7455	*Homoeostrichus*	psbA / rbcL	-	26th March 2005	ST632 Lifou	C. Payri
IRD7460	*Homoeostrichus*	psbA / rbcL	-	30th March 2005	ST640 Ouvéa	C. Payri
IRD7474	*Distromium*	psbA / rbcL	-	18th March 2007	ST1067 N'Goë toupeti	C. Payri
IRD7476	*Homoeostrichus*	rbcL	-	19th March 2007	ST1069 Port Bouquet	J-L Menou/ C.Payri
IRD7571	*Distromium*	psbA / rbcL	-	6th December 2005	ST996 Ile des Pins	C. Payri
IRD7576	*Distromium*	psbA / rbcL	-	16th March 2007	ST1062 Côte Oubliée	C. Payri
IRD7578	*Distromium*	rbcL	-	18th March 2007	ST1067 N'Goë toupeti	C. Payri
IRD7583	*Distromium*	rbcL	-	18th March 2007	ST1068 N'Goë toupeti	C. Payri
IRD7585	*Distromium*	rbcL	-	20th March 2007	ST1072 Port Bouquet	C. Payri
IRD7588	*Distromium*	psbA / rbcL	-	13th July 2008	ST1161 Chesterfields	C. Payri

Voucher specimen	Taxa	Genes	GenBank reference	Date of collection	Collection Site	Collector
IRD7592	*Distromium*	psbA / rbcL	-	19th July 2008	ST1172 Chesterfields	C. Payri
IRD7597	*Distromium*	psbA / rbcL	-	28th February 2005	ST254 Passe Uitoé	C. Payri
IRD7604	*Distromium*	psbA / rbcL	-	22nd May 2006	ST254 Passe Uitoé	J-L Menou
IRD7605	*Distromium*	rbcL	-	28th February 2005	ST254 Passe Uitoé	C. Payri
IRD7607	*Distromium*	rbcL	-	10th October 2007	ST756 Dumbéa	C. Payri / J-L Menou
IRD7612	*Distromium*	psbA / rbcL	-	17th March 2009	ST1197 Beautemps Beaupré	C. Payri
IRD7621	*Lobophora*	rbcL	-	14th May 2009	ST1190 Belep	
IRD7622	*Distromium*	psbA / rbcL	-	25th November 2005	ST914 Ile des Pins	C. Payri
IRD7626	*Lobophora*	rbcL	-	26th March 2007	ST1084 Baie Cap Tonnedu	C. Payri
IRD7627	*Distromium*	rbcL	-	26th March 2007	ST1083 Ouinné	C. Payri
IRD7628	*Distromium*	psbA	-	25th March 2007	ST1081 Récif du plaisir solitaire	C. Payri
IRD7629	*Distromium*	psbA / rbcL	-	25th March 2007	ST1081 Récif du plaisir solitaire	C. Payri
IRD7635	*Distromium*	psbA / rbcL	-	18th March 2007	ST1067 N'Goë toupeti	C. Payri
IRD7638	*Lobophora*	psbA / rbcL	-	5th April 2005	ST649 Astrolabe	C. Payri
IRD7639	*Distromium*	psbA / rbcL	-	4th April 2005	ST648 Astrolabe	C. Payri
IRD7640	*Lobophora*	rbcL	-	4th April 2005	ST647 Astrolabe	C. Payri
IRD7643	*Distromium*	psbA / rbcL	-	29th March 2005	ST638 Ouvéa	C. Payri
IRD7645	*Distromium*	rbcL	-	25th March 2005	ST630 Lifou	C. Payri
IRD7648	*Distromium*	rbcL	-	22nd March 2005	ST623 Maré	C. Payri
IRD7649	*Homoeostrichus*	psbA / rbcL	-	22nd March 2005	ST624 Maré	C. Payri
IRD7651	*Lobophora*	psbA / rbcL	-	21st March 2005	ST622 Maré	C. Payri
IRD7653	*Distromium*	psbA / rbcL	-	22nd June 2006	ST1039 Canal Woodin	C. Payri
IRD7663	*Distromium*	rbcL	-	13rd September 2002	ST657 Ile aux Canards	C. Payri
IRD7666	*Distromium*	rbcL	-	16th September 2002	ST196 Dumbéa	J-L Menou
IRD7667	*Distromium*	rbcL	-	29th April 2004	ST759 Ilot Signal	J-L Menou/ C. Payri
IRD7668	*Distromium*	psbA	-	13th October 2002	ST657 Ile aux Canards	C. Payri
IRD7669	*Lobophora*	psbA / rbcL	-	3rd October 2005	ST963 Port Boisé	C. Payri
IRD7670	*Lobophora*	rbcL	-	22nd May 2006	ST254 Fausse passé Uitoé	C. Payri
IRD7676	*Lobophora*	rbcL	-	4th July 2008	ST1140 Chesterfields	C. Payri
IRD7876	*Lobophora*	psbA / rbcL	-	24th April 2012	ST1482 Port boisé	C. Payri
IRD7878	*Lobophora*	psbA / rbcL	-	21st April 2012	ST1476 Canala	C. Payri
IRD7888	*Lobophora*	psbA / rbcL	-	21st April 2012	ST1476 Canala	C. Payri
IRD7897	*Distromium*	psbA / rbcL	-	25th April 2012	ST 1483 Port boisé	C. Payri
IRD7900	*Distromium*	psbA / rbcL	-	16th April 2012	ST1468 Poindimié	C. Payri

Voucher specimen	Taxa	Genes	GenBank reference	Date of collection	Collection Site	Collector
IRD7905	*Distromium*	psbA / rbcL	-	6th April 2005	ST651 Beautemps-Beaupré	C. Payri
IRD7909	*Homoeostrichus*	psbA / rbcL	-	6th May 2004	ST765 Poidimié	J-L Menou
IRD7910	*Distromium*	rbcL	-	29th April 2004	ST759 Ilot Signal	J-L Menou/C. Payri
IRD247	*Distromium decumbens*	rbcL	EU579946	7th September 2004	ST791 Tomboo Mato	J-L Menou/C. Payri
IRD274	*Distromium sp.*	rbcL	EU579950	7th April 2005	ST653 Beautemps-Beaupré	C. Payri
IRD320	*Distromium didymothrix*	rbcL	EU579948	6th December 2005	ST996 Ile des Pins	C. Payri
IRD1	*Homoeostrichus sp.*	rbcL	EU579951	12th February 2004	ST750 Mbere	J-L Menou/C. Payri
IRD4	*Homoeostrichus sp.*	rbcL	EU579952	6th May 204	ST765 Poindimié	C. Payri
IRD259	*Lobophora*	rbcL	EU579956	30th November 2005	ST460 Ile des Pins	C. Payri
IRD276	*Lobophora variegata*	rbcL	EU579957	25th March 2005	ST631 Lifou	C. Payri
-	*Lobophora sp.*	rbcL	AB665281	-	-	Sun, Z. et al., 2011
-	*Dictyota crenulata*	rbcL	JQ061121	-	-	Tronholm, A. et al., 2012
-	*Homoeostrichus sinclarii*	rbcL	DQ866935	-	-	Lee,W.J., et al., 2006
-	*Padina crassa*	rbcL	AB358909	-	-	Ni-Ni-Win et al., 2008
-	*Zonaria crenata*	rbcL	DQ866933	-	-	Lee,W.J., et al., 2006
-	*Zonaria sp.*	rbcL	AB665282	-	-	Sun, Z. et al., 2011
-	*Sargassum agarhianum*	rbcL	AY256964	-	-	Phillips, N.E., et al., 2005
-	*Dictyota bartayresiana*	psbA	GQ466071	-	-	Tronholm,A., et al., 2009
-	*Dictyota crenulata*	psbA	GU265782	-	-	Tronholm,A. et al., 2010
-	*Distromium decumbens*	psbA	AY422645	-	-	Lee.W.J., et al., 2003
-	*Homoeostrichus sinclarii*	psbA	DQ866953	-	-	Lee,W.J., et al., 2006
-	*Homoeostrichus sp*	psbA	DQ866951	-	-	Lee,W.J., et al., 2006
-	*Lobophora sp.*	psbA	DQ866942	-	-	Lee,W.J., et al., 2006
-	*Lobophora variegata*	psbA	DQ866944	-	-	Lee,W.J., et al., 2006
-	*Padina crassa*	psbA	AY422643	-	-	Lee.W.J., et al. , 2003
-	*Sargassum elegans*	psbA	FM958300	-	-	Draisma,S.G. A., 2010
-	*Zonaria crenata*	psbA	DQ866955	-	-	Lee,W.J., et al., 2006
-	*Zonaria dieseingiana*	psbA	AY528441	-	-	Lee,W.J., et al., 2004

Table.2.

Primer names	Gene	Primer sequence (5'-3')	Primer direction
F	psbA	ATGACTGCTACTTTAGAAAGACG	Forward
R1	psbA	GCTAAATCTARWGGGAAGTTGTG	Reverse
F68	*rbcL*	TGCCWAAATGGGRWAYTGGGATGC	Forward
R708	*rbcL*	TTAAGNTAWGAACCYTTAACTTC	Reverse

Table.3.

Taxa (Phaeophyceae_ Dictyotales)	***Genus and species***	**Authority**
Dictyopteris	*Dictyopteris australis*	(Sonder) Askenasy
	Dictyopteris delicatula	J.V Lamouroux
	Dictyopteris sp2	(in need of description)
	Dictyopteris sp3	(in need of description)
	Dictyopteris sp4	(in need of description)
Dictyota	*Dictyota bartayresiana*	J.V Lamouroux
	Dictyota canaliculata	O. De Clerck & E.Coppejans
	Dictyota ceylanica	Kützing
	Dictyota ciliolata	Sonder ex Kützing
	Dictyota dichotoma	(Hudson) J.V Lamouroux
	Dictyota dichotoma var. intricata	(C. Agardh) Schmidt
	Dictyota friabilis	Setchell
	Dictyota hamifera	Setchell
	Dictyota sp	(in need of description)
	Dictyota stolonifera	E.Y. Dawson
Distromium	*Distromium decumbens*	(Okamura) Levring
	Distromium didymothrix	Allender & Kraft
	Distromium Clade C	(in need of description)
	Distromium Clade D	(in need of description)
	Distromium Clade E	(in need of description)
Homoeostrichus	*Homoeostrichus Clade A*	(in need of description)
	Homoeostrichus Clade B	(in need of description)
	Homoeostrichus Clade C	(in need of description)
Lobophora	*Lobophora cf sp 5*	(in need of description)
	Lobophora cf sp8	(in need of description)
	Lobophora cf sp10	(in need of description)
	Lobophora sp 4	(in need of description)
	Lobophora sp 11	(in need of description)
	Lobophora Clade A	(in need of description)
	Lobophora Clade B	(in need of description)
	Lobophora Clade C	(in need of description)
	Lobophora Clade D	(in need of description)
	Lobophora Clade E	(in need of description)
	Lobophora nigrescens	J. Agardh
New genus	*New genus crassinervia*	(in need of description)
	New genus sp4	(in need of description)
Padina	*Padina australis*	Hauck
	Padina boryana	Thivy
	Padina gymnospera	(Kützing) Sonder
	Padina macrophylla	Ni-Ni-Win, M.Uchimura & H. Kawai
	Padina melemele1	I.A.Abbott & Magruder
	Padina melemele2	I.A.Abbott & Magruder
	Padina minor	Yamada
	Padina moffitiana	Abbott & Huisman
	Padina okinawaensis	Ni-Ni-Win, S.Arai & H.Kawai
	Padina santae-crucis	Borgensen
	Padina sp1	(in need of description)
	Padina sp2	(in need of description)
	Padina sp3	(in need of description)
	Padina sp11	(in need of description)
	Padina stipitata	Tanaka & Nozawa

Taxa (Phaeophyceae_ Dictyotales)	***Genus and species***	**Authority**
	Padina undulata	Ni-Ni-Win, S.Arai & H.Kawai
Spathoglossum	*Spathoglossum asperum*	J. Agardh
Stypopodium	*Stypopodium australasicum*	(Zanardini) Allender & Kraft
	Stypopodium flabelliforme	Weber-van Bosse
	Stypopodium group 3	(in need of description)
	Stypopodium group 4	(in need of description)
Taonia	*Taonia australasica*	J. Agardh
Zonaria	*Zonaria stipitata*	Tanaka & K. Nozawa

Table.4.

Area	Number of sampling sites	SR_s ± SD	SR_l	Unique (%)	Duplicates (%)	Unique + Duplicates (%)
Chesterfield	15	1.5 ± 0.7	11	63.6	9.1	72.7
Loyalty Islands	32	1.8 ± 1.2	21	38.1	19.0	57.1
East Lagoon	44	2.0 ± 1.3	33	39.4	15.2	54.6
North Lagoon	22	2.4 ± 2.0	27	55.6	22.2	77.8
West Lagoon	27	2.0 ± 1.6	26	46.2	34.6	80.8
South Lagoon	48	2.5 ± 1.8	36	41.7	19.4	61.1
Total SR_T	188	2.1 ± 1.5	59	20.3	8.5	28.9

Table.5.

Genera	Number of species	Percentage (%) of the total number of species	Number of occurrences
Dictyopteris	5	8.5	16
Dictyota	10	16.9	64
Distromium	5	8.5	60
Homoeostrichus	3	5.1	23
Lobophora	11	18.6	43
New genus	2	3.4	7
Padina	16	27.1	131
Spathoglossum	1	1.7	16
Stypopodium	4	6.8	35
Taonia	1	1.7	1
Zonaria	1	1.7	6

Table.6.

	Chesterfield	**Loyalty Islands**	**East Lagoon**	**North Lagoon**	**West Lagoon**
Loyalty Islands	0.81				
East Lagoon	0.77	0.47			
North Lagoon	0.74	0.46	0.42		
West Lagoon	0.79	0.54	0.39	0.44	
South Lagoon	0.66	0.51	0.35	0.37	0.43

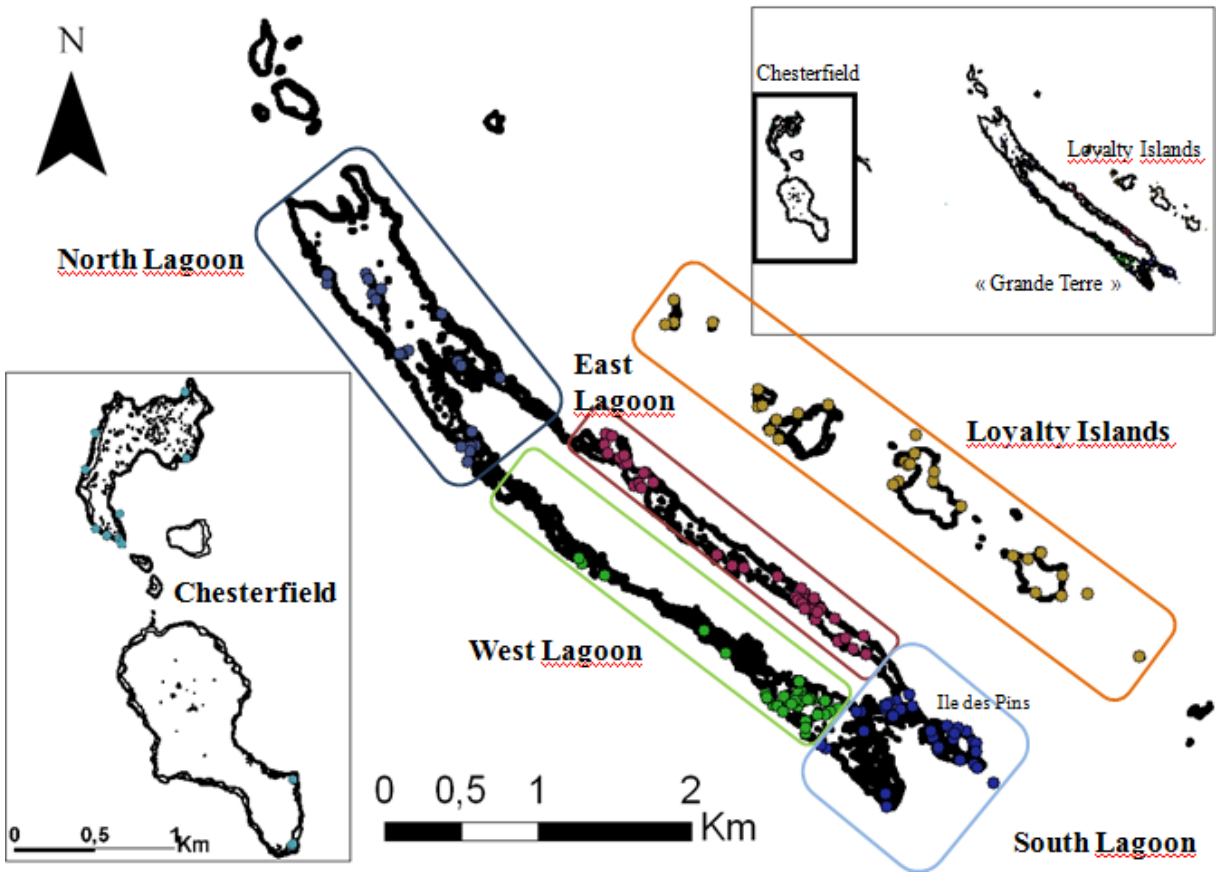

N
North Lagoon
East
Lagoon
West Lagoon
Loyalty Islands
Ile des Pins
South Lagoon
Chesterfield
0 0,5 1 2
Km
Chesterfield
Loyalty Islands
« Grande Terre »
0 0,5 1
Km

Fig.1.

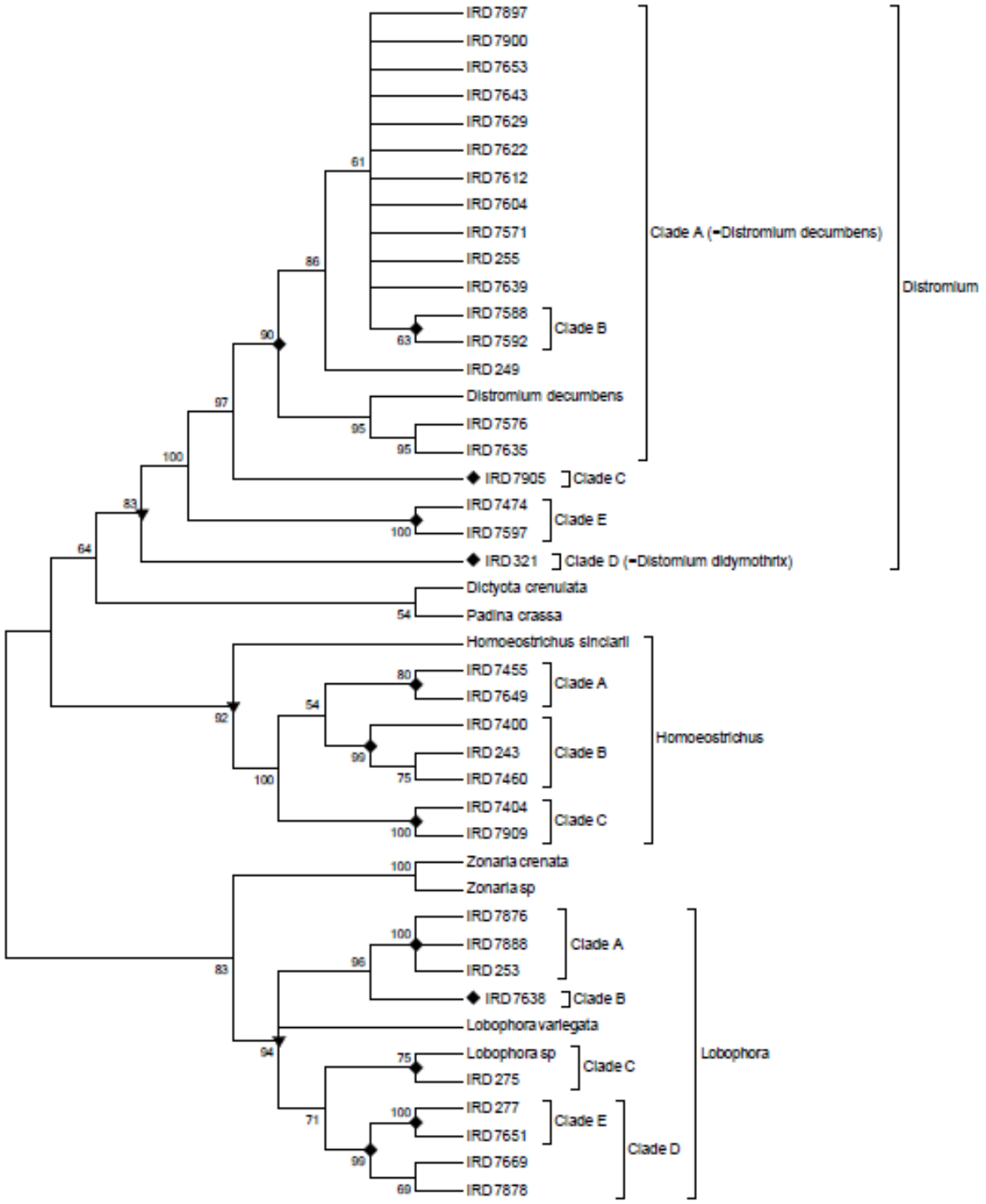

IRD 7897
IRD 7900
IRD 7653
IRD 7643
IRD 7629
IRD 7622
IRD 7612
IRD 7604
IRD 7571
IRD 255
IRD 7639
IRD 7588
IRD 7592
IRD 249
Distromium decumbens
IRD 7576
IRD 7635
IRD 7905
IRD 7474
IRD 7597
IRD 321
Dictyota crenulata
Padina crassa
Homoeostrichus sinclarii
IRD 7455
IRD 7649
IRD 7400
IRD 243
IRD 7460
IRD 7404
IRD 7909
Zonaria crenata
Zonaria sp
IRD 7876
IRD 7888
IRD 253
IRD 7638
Lobophora variegata
Lobophora sp
IRD 275
IRD 277
IRD 7651
IRD 7669
IRD 7878
Clade A (=Distromium decumbens)
Clade B
Clade C
Clade E
Clade D (=Distomium didymothrix)
Distromium
Clade A
Clade B
Clade C
Homoeostrichus
Clade A
Clade B
Clade C
Clade E
Clade D
Lobophora
61
86
90
63
97
95
95
100
83
100
64
54
92
80
54
99
75
100
100
100
100
96
83
94
75
71
100
99
69

Fig 2.

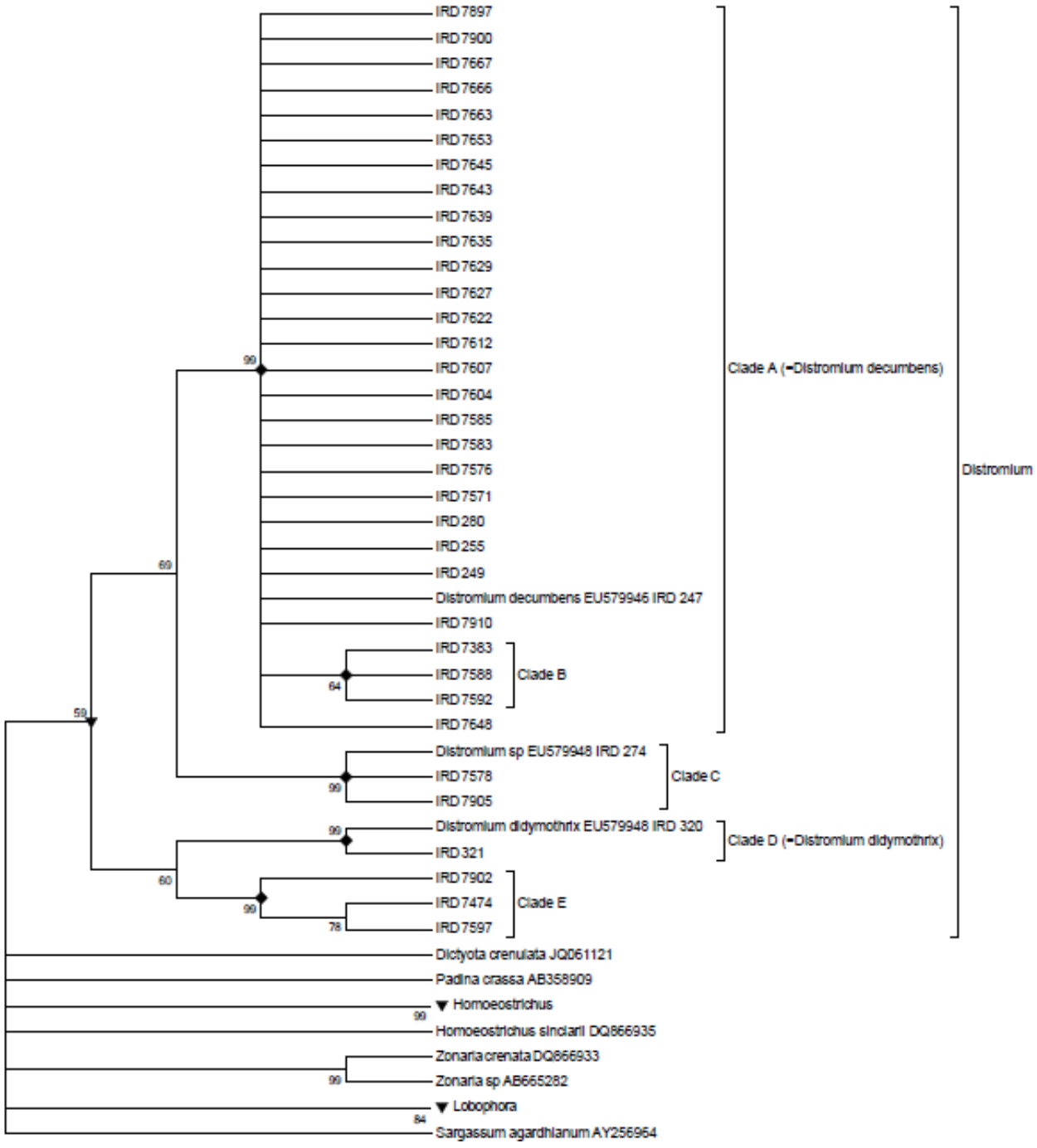

IRD 7897
IRD 7900
IRD 7667
IRD 7666
IRD 7663
IRD 7653
IRD 7645
IRD 7643
IRD 7639
IRD 7635
IRD 7629
IRD 7627
IRD 7622
IRD 7612
99
IRD 7607
IRD 7604
IRD 7585
IRD 7583
IRD 7576
IRD 7571
IRD 280
IRD 255
60
IRD 249
Distromium decumbens EU579946 IRD 247
IRD 7910
IRD 7383
IRD 7588
64
IRD 7592
Clade B
59
IRD 7648
Clade A (=Distromium decumbens)
Distromium sp EU579948 IRD 274
IRD 7578
99
IRD 7905
Clade C
99
Distromium didymothrix EU579948 IRD 320
IRD 321
Clade D (=Distromium didymothrix)
60
IRD 7902
99
IRD 7474
78
IRD 7597
Clade E
Distromium
Dictyota crenulata JQ061121
Padina crassa AB358909
99
▼ Homoeostrichus
Homoeostrichus sinclarii DQ866935
Zonaria crenata DQ866933
99
Zonaria sp AB665282
▼ Lobophora
84
Sargassum agardhianum AY256964

Fig 3.

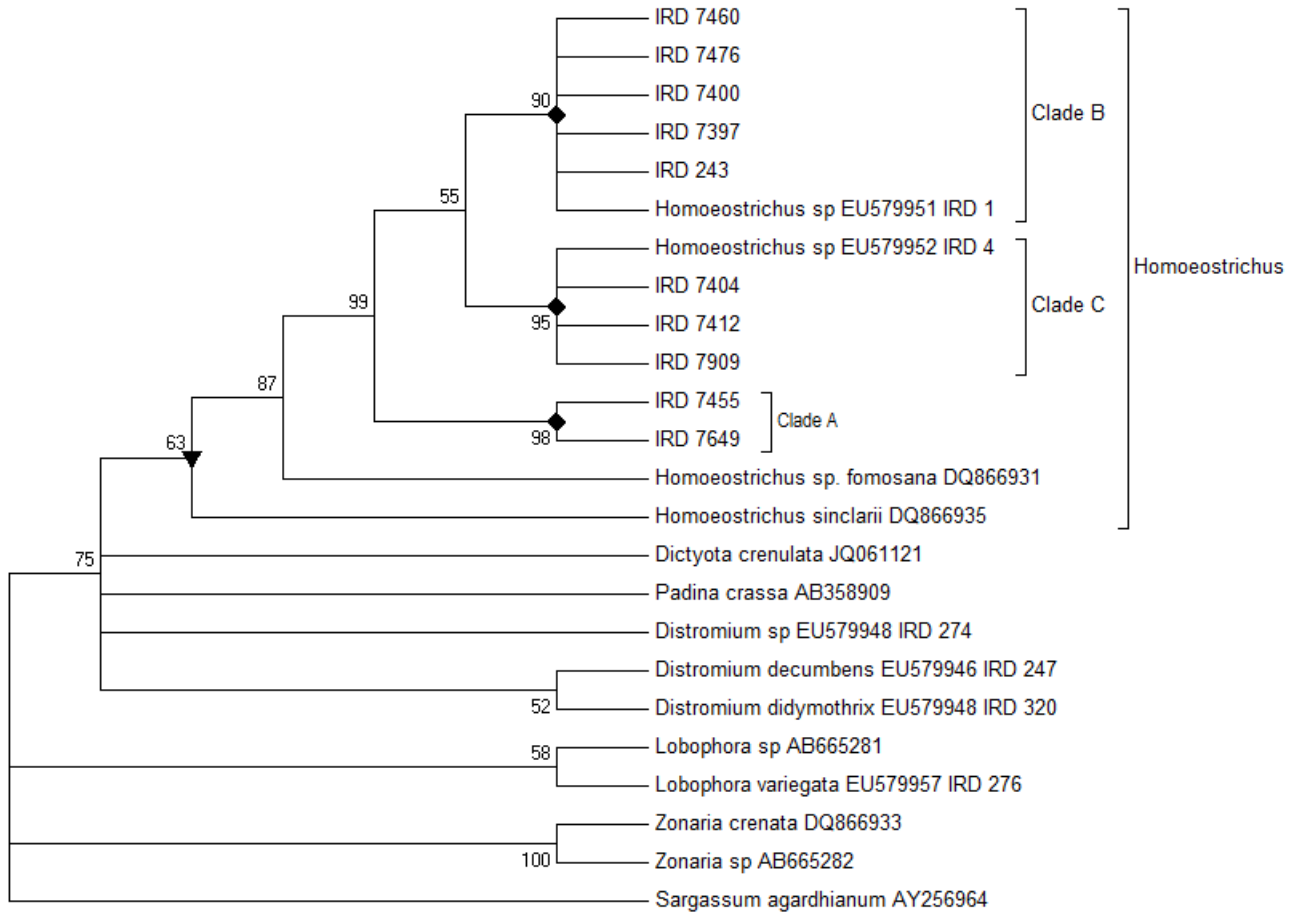

IRD 7460
IRD 7476
IRD 7400
IRD 7397
IRD 243
Homoeostrichus sp EU579951 IRD 1
Homoeostrichus sp EU579952 IRD 4
IRD 7404
IRD 7412
IRD 7909
IRD 7455
IRD 7649
Homoeostrichus sp. fomosana DQ866931
Homoeostrichus sinclarii DQ866935
Dictyota crenulata JQ061121
Padina crassa AB358909
Distromium sp EU579948 IRD 274
Distromium decumbens EU579946 IRD 247
Distromium didymothrix EU579948 IRD 320
Lobophora sp AB665281
Lobophora variegata EU579957 IRD 276
Zonaria crenata DQ866933
Zonaria sp AB665282
Sargassum agardhianum AY256964
Clade B
Clade C
Clade A
Homoeostrichus
90
55
99
95
87
98
63
75
52
58
100

Fig.4.

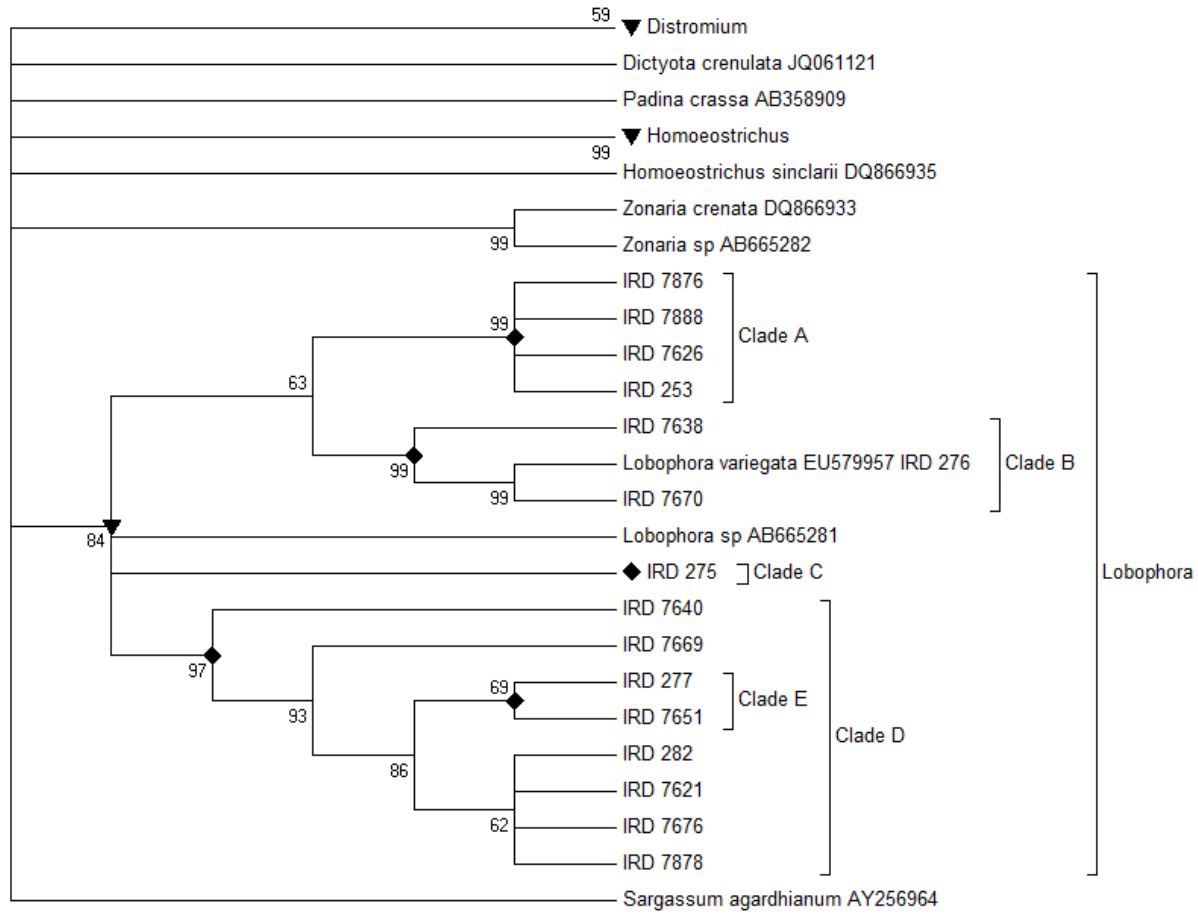
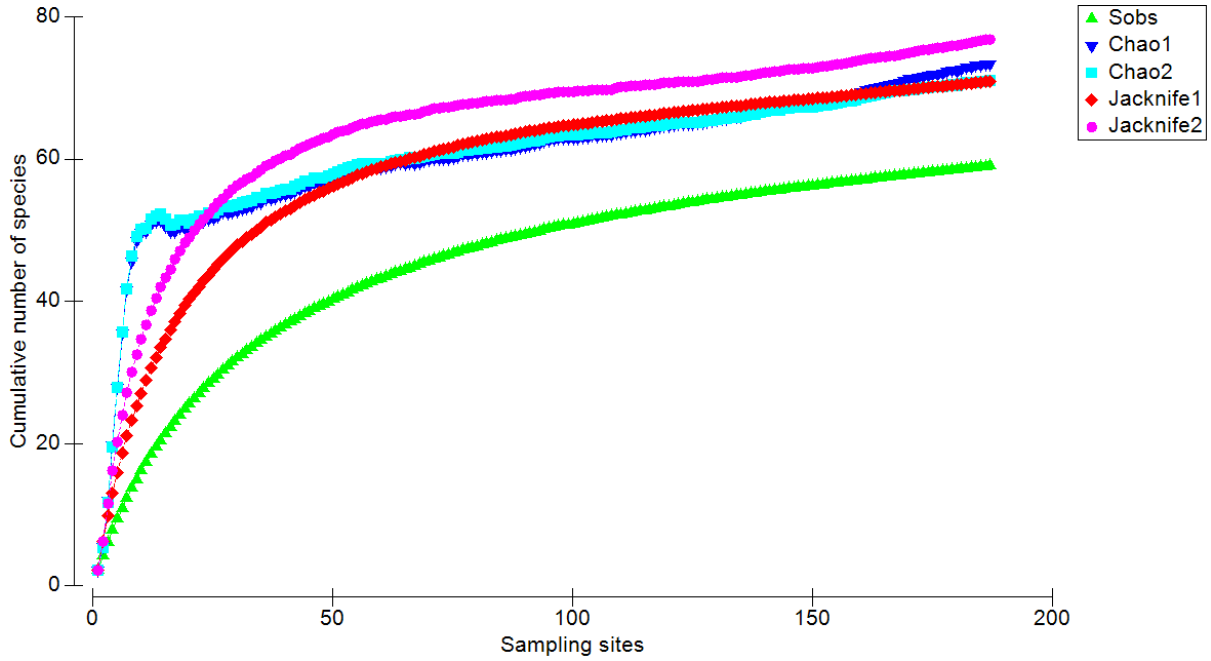
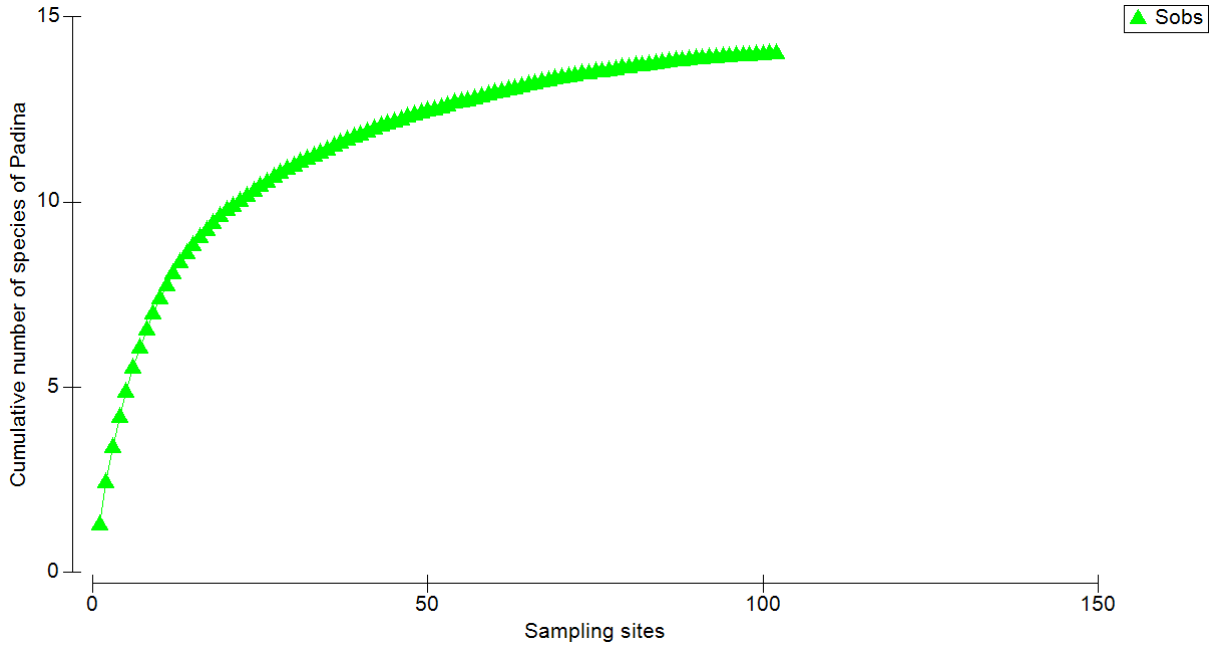

59
Distromium
Dictyota crenulata JQ061121
Padina crassa AB358909
Homoeostrichus
99
Homoeostrichus sinclarii DQ866935
Zonaria crenata DQ866933
99
Zonaria sp AB665282
IRD 7876
99
IRD 7888
Clade A
IRD 7626
63
IRD 253
IRD 7638
99
Lobophora variegata EU579957 IRD 276
Clade B
99
IRD 7670
84
Lobophora sp AB665281
IRD 275
Clade C
Lobophora
IRD 7640
IRD 7669
97
IRD 277
69
Clade E
93
IRD 7651
Clade D
IRD 282
86
IRD 7621
62
IRD 7676
IRD 7878
Sargassum agardhianum AY256964

Fig.5.

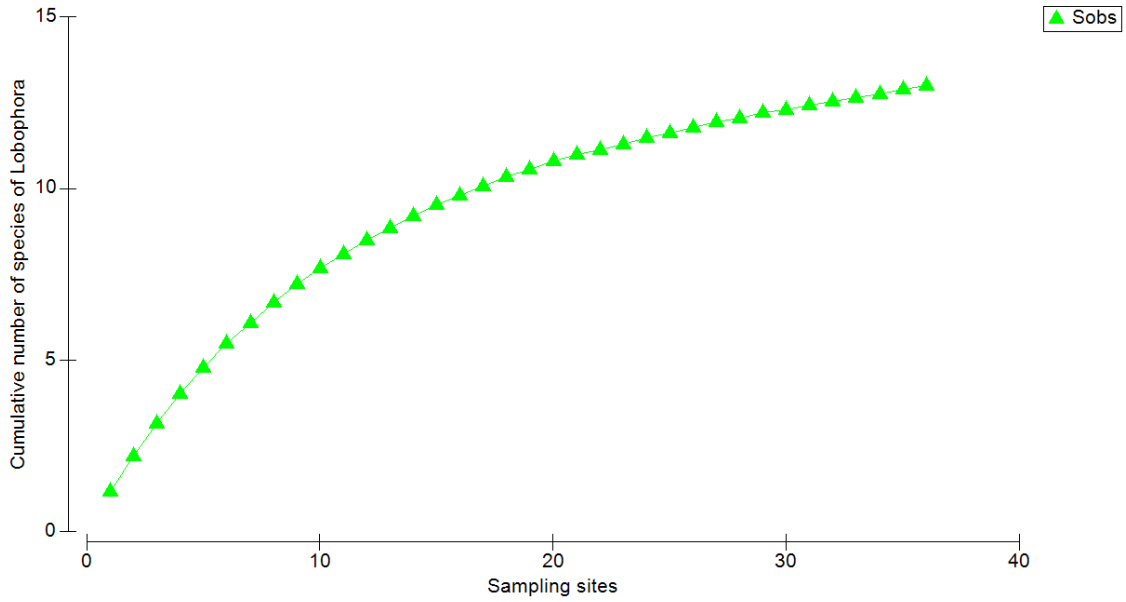
(a)


Sobs
Chao1
Chao2
Jacknife1
Jacknife2
Cumulative number of species
Sampling sites
0
20
40
60
80
0
50
100
150
200

(b)


Sobs
Cumulative number of species of Padina
Sampling sites
0
5
10
15
0
50
100
150

(c)


Sobs
Cumulative number of species of Lobophora
15
10
5
0
0
10
20
30
40
Sampling sites

(d)

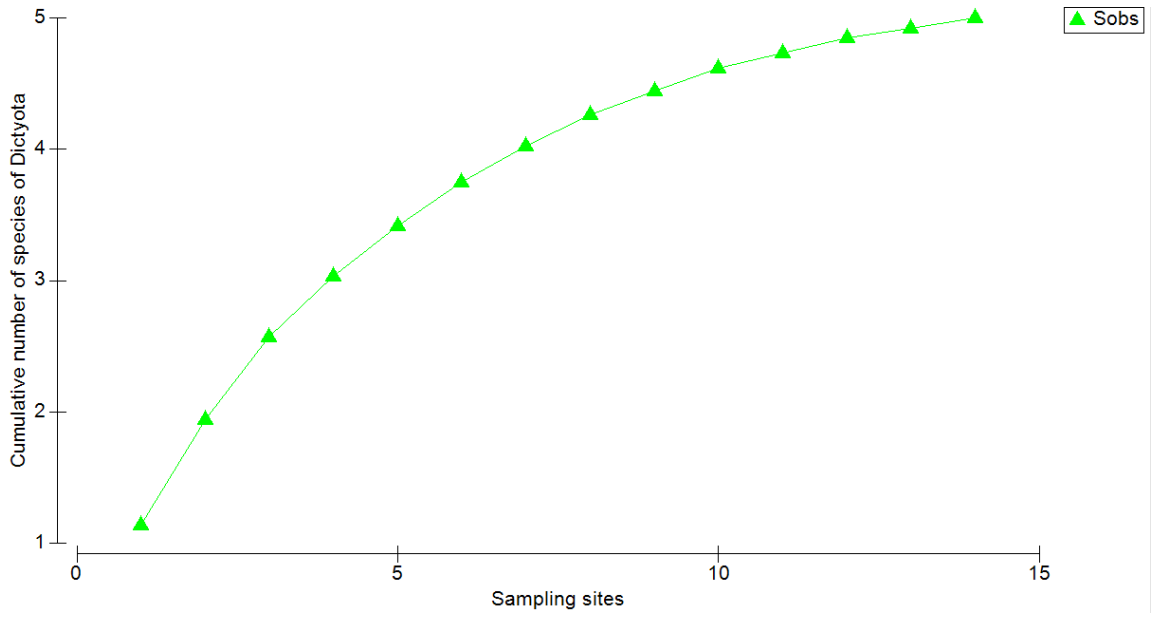

Sobs
Cumulative number of species of Dictyota
5
4
3
2
1
0
5
10
15
Sampling sites

Fig.6 (a) (b) (c) (d)

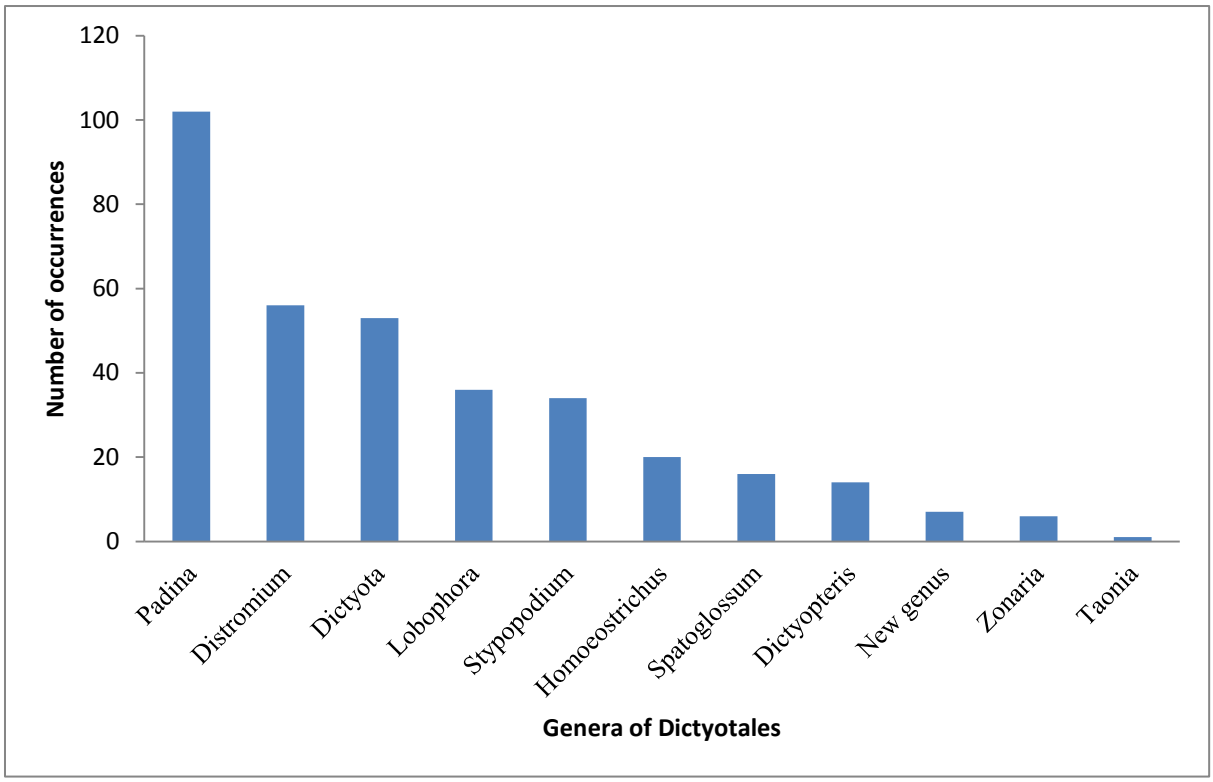

120
100
80
60
40
20
0
Number of occurrences
Padina
Distromium
Dictyota
Lobophora
Stypopodium
Homoeostrichus
Spatoglossum
Dictyopteris
New genus
Zonaria
Taonia
Genera of Dictyotales

Fig.7

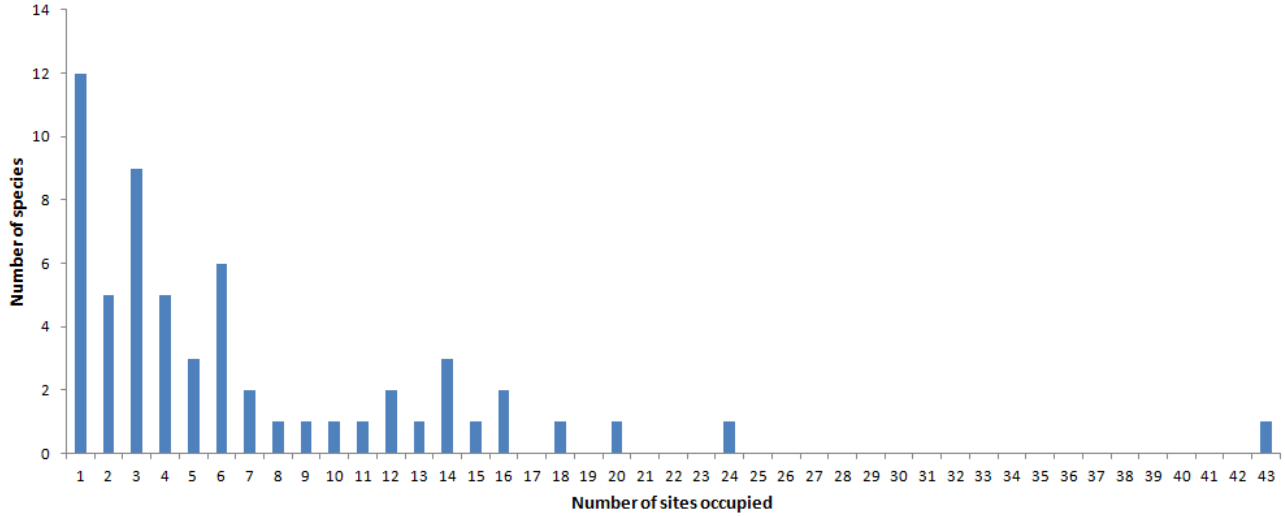

Number of species
Number of sites occupied
0
2
4
6
8
10
12
14
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43

Fig. 8 (a)

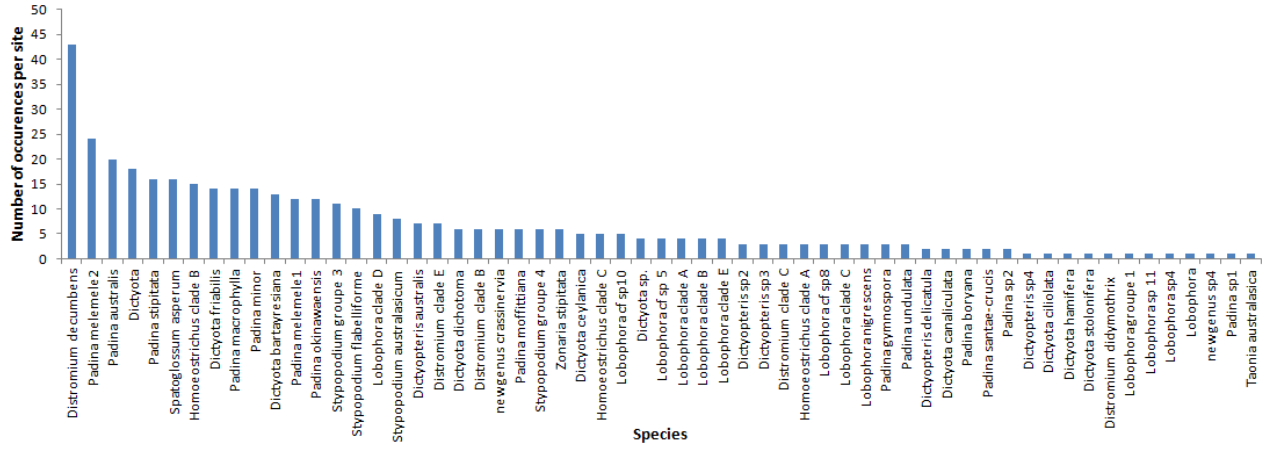

Number of occurences per site
Species
0
5
10
15
20
25
30
35
40
45
50
Distromium decumbens
Padina melemele2
Padina australis
Dictyota
Padina stipitata
Spatoglossum asperum
Homoeostrichus clade B
Dictyota friabilis
Padina macrophylla
Padina minor
Dictyota bartayresiana
Padina melemele1
Padina okinawaensis
Stypopodium groupe 3
Stypopodium flabelliforme
Lobophora clade D
Stypopodium australasicum
Dictyopteris australis
Distromium clade E
Dictyota dichotoma
Distromium clade B
new genus crassinervia
Padina moffittiana
Stypopodium groupe 4
Zonaria stipitata
Dictyota ceylanica
Homoeostrichus clade C
Lobophora cf sp10
Dictyota sp.
Lobophora cf sp 5
Lobophora clade A
Lobophora clade B
Lobophora clade E
Dictyopteris sp2
Dictyopteris sp3
Distromium clade C
Homoeostrichus clade A
Lobophora cf sp8
Lobophora clade C
Lobophora nigrescens
Padina gymnospora
Padina undulata
Dictyopteris delicatula
Dictyota canaliculata
Padina boryana
Padina santae-crucis
Padina sp2
Dictyopteris sp4
Dictyota ciliolata
Dictyota hamifera
Dictyota stolonifera
Distromium didymothrix
Lobophora groupe 1
Lobophora sp 11
Lobophora sp4
Lobophora
new genus sp4
Padina sp1
Taonia australasica

Fig. 8 (b)

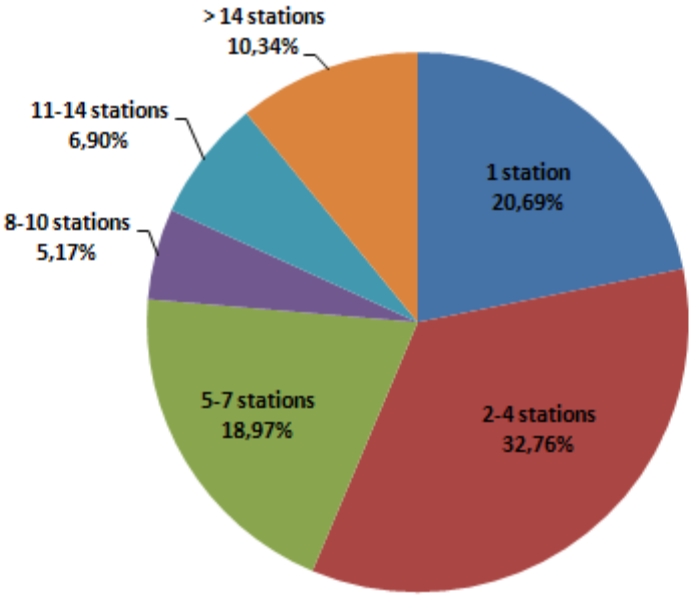

> 14 stations
10,34%
11-14 stations
6,90%
8-10 stations
5,17%
1 station
20,69%
5-7 stations
18,97%
2-4 stations
32,76%

Fig. 9

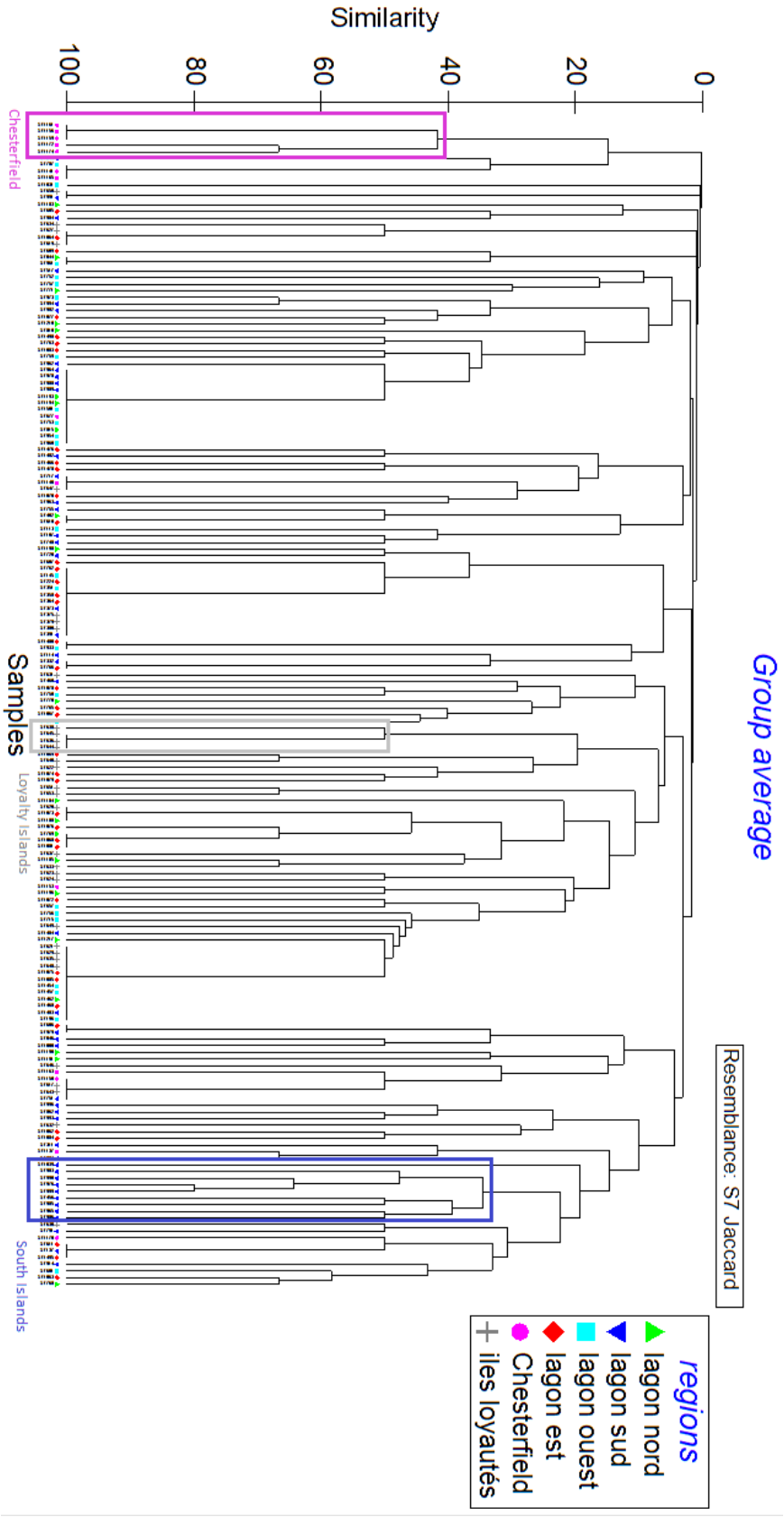

Similarity
100
80
60
40
20
0
Chesterfield
Samples
Loyalty Islands
South Islands
Group average
Resemblance: S7 Jaccard
regions
lagon nord
lagon sud
lagon ouest
lagon est
Chesterfield
iles loyautés

Fig.10

Appendix

Supplementary material.
The following supplementary material is available for this article
Annex 1 : Figures 11 ; 12 ; 13

<u>Annex 1 : Neighbor-Joining (NJ) trees based on psbA gene sequences</u>

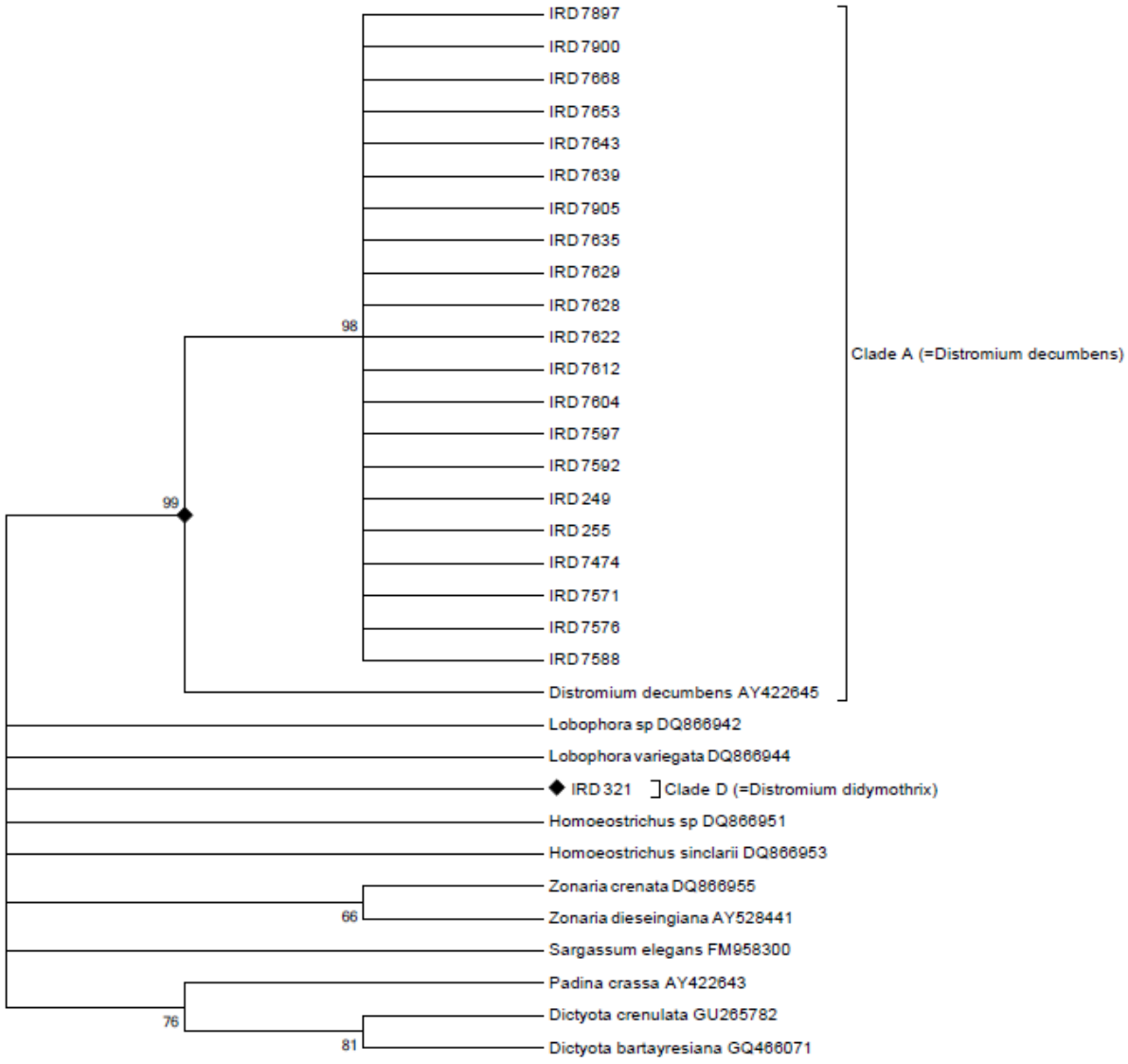


Fig. 11. Neighbor-Joining tree based on psbA gene sequences for *Distromium*.

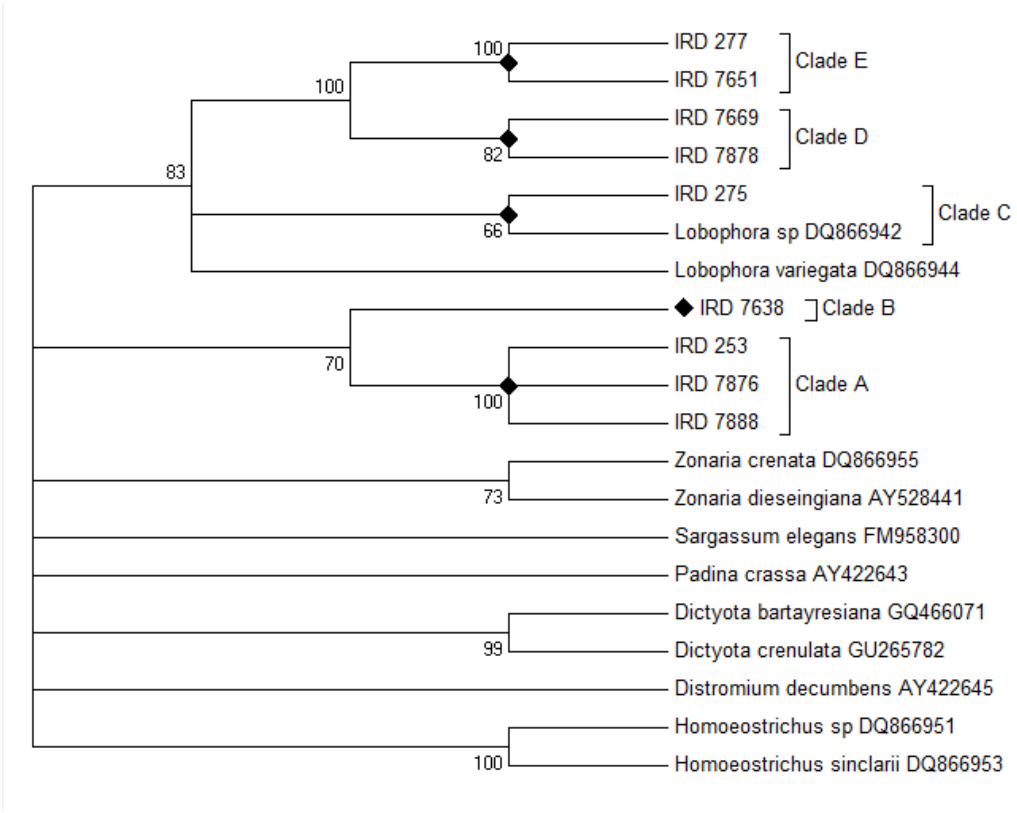


Fig 12. Neighbor-Joining (NJ) tree based on *psbA* gene sequences for *Lobophora.*

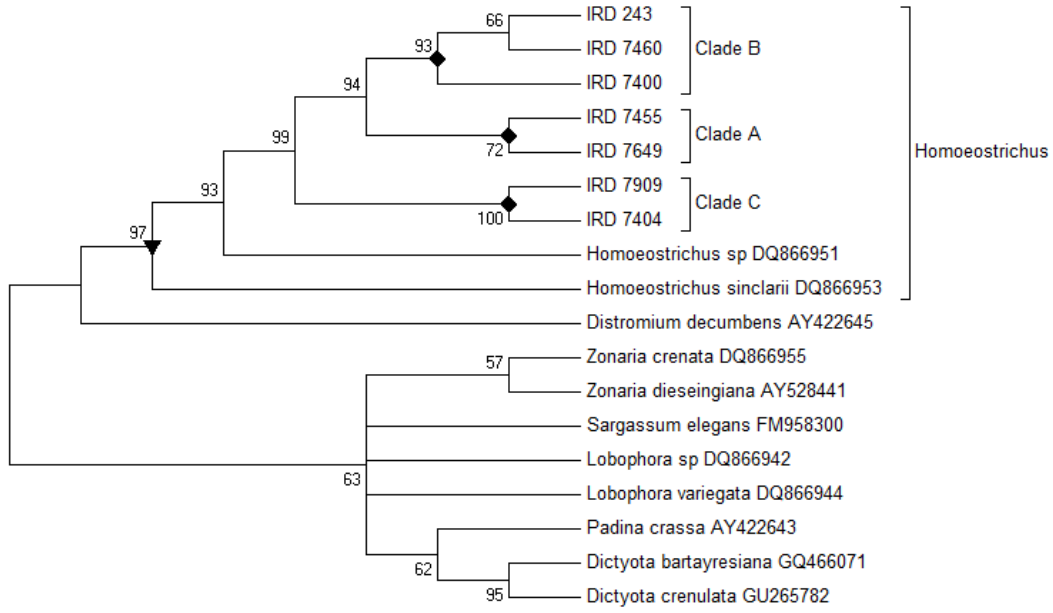


Fig 13. Neighbor-Joining (NJ) tree based on *psbA* for *Homoeostrichus.*