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Diversity of the *Bostrychia radicans / Bostrychia moritziana* species complex (Rhodomelaceae, Rhodophyta) in the mangroves of New Caledonia

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Abstract – Red algae of the *Bostrychia radicans / B. moritziana* species complex are common in mangrove habitats. This group consists of seven highly divergent evolutionary lineages based on a plastid-encoded marker (RuBisCo spacer). We sampled this species complex around the island of New Caledonia. On the west coast of the island most samples were of Lineage 1 (four haplotypes), the most common lineage in the western Pacific Ocean. On the east coast of the island Lineage 1 samples were less common, with samples from lineages 2, 6, and 7, which have a world-wide distribution, predominating. Lineage 1 samples from the west coast had mostly an asexual reproductive life cycle (i.e. recycling tetrasporophytes) while the ones on the east coast were mostly sexual. A set of samples collected at a nickel ore port (Kouaoua, east coast), and found in Lineage 7 had a haplotype identical to a Florida USA sample. This study shows that biodiversity of algae can not solely be determined from morphological data. This study also highlights the differences in diversity between the east and west coasts of New Caledonia and suggests that historical, ecological and recent factors may have contributed to this difference.

***Bostrychia radicans* / *Bostrychia moritziana* / Espaceur RuBisCo / genetic diversity / mangrove algae / New Caledonia / Rhodomelaceae / Rhodophyta**

Résumé – **Diversité du complexe d'espèces *Bostrychia radicans/Bostrychia moritziana* (Rhodomelaceae, Rhodophyta) dans les mangroves de Nouvelle-Calédonie.** Des algues rouges appartenant au complexe *Bostrychia radicans /B. moritziana* sont fréquemment observées dans les mangroves. Ce complexe d'espèces comprend 7 lignées évolutives bien distinctes basées sur l'analyse de la zone espaceur de la RuBisCo (marqueur plastidial). Nous avons échantillonné ces algues le long des côtes de la Nouvelle-Calédonie. La plupart des échantillons récoltés sur la côte ouest appartiennent à la lignée 1 (4 haplotypes), lignée la plus commune de l'Océan Pacifique ouest. Au contraire, cette lignée est peu fréquente sur la côte est, les lignées 2, 6 et 7, qui ont une distribution mondiale, étant majoritaires. La plupart des échantillons de la lignée 1 récoltés sur la côte ouest ne se reproduisent

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qu'asexuellement (par tétraspores) tandis que ceux de la côte est se reproduisent sexuellement. Les spécimens de la lignée 7, tous trouvés dans la région du port minéralier (nickel, côte est) de Kouaoua, ont le même haplotype qu'un spécimen provenant de Floride, USA. Ce travail montre que l'on ne peut pas se fier à la seule étude morphologique pour déterminer la biodiversité de ces algues et que l'analyse moléculaire est aussi nécessaire. Cette étude met aussi en lumière la différence de biodiversité entre les deux côtes, différence dont les causes pourraient être leur histoire évolutive, des conditions écologiques différentes ou des facteurs humains plus récents.

algues des mangroves / *Bostrychia radicans/ Bostrychia moritziana* / diversité génétique / Nouvelle-Calédonie / Rhodomelaceae / Rhodophyta / RuBisCo spacer

INTRODUCTION

The tropical mangroves of the world contain a unique complement of algae frequently consisting of the red algal genera *Bostrychia* and *Caloglossa* (Post, 1968; West & Zuccarello, 1999a; Kamiya *et al.*, 2003). These algae have been studied extensively over the last two decades and their taxonomy and molecular systematics have been thoroughly revised (King & Puttock, 1989, 1994; Kamiya *et al.*, 2003; Zuccarello & West, 2006). Although the taxonomy has been researched using many methods, certain taxonomic problems still remain. One of these is the evolutionary relationships within the genus *Bostrychia* and algae that are identified as either *B. radicans* (Montagne) Montagne or *B. moritziana* (Sonders *ex* Kützing) J. Agardh (following King & Puttock, 1989). The algae are ecorticated, have irregular branching and have specialized attachment structures called cladohaptera. King & Puttock (1989) distinguished *B. radicans* from *B. moritziana* by the latter possessing terminal lateral monosiphonous branches.

Since 1989 research based on several molecular markers (Zuccarello *et al.*, 1999a; Zuccarello & West, 2003, 2006) has shown that the character of monosiphonous laterals does not separate monophyletic evolutionary lineages nor is this character stable in culture in some isolates (Zuccarello & West, 1995). Molecular evidence based on organellar markers (plastid and mitochondrial) and ribosomal RNA genes (Zuccarello & West, 2003, 2006) shows that samples identified as either *B. radicans* or *B. moritziana* are found in seven distinct lineages (lineages arbitrarily named 1-7), with samples identified as either one or the other species found in most evolutionary lineages. These lineages appear to be reproductively isolated (Zuccarello & West, 1997, 2003) and therefore could be considered cryptic species. Interestingly, reproductive isolation was also noted within lineages (Zuccarello & West, 2003), potentially increasing the number of reproductively isolated cryptic species within this species complex.

Another feature of this species complex is that many isolates do not reproduce sexually in laboratory culture (West & Zuccarello, 1999a). These asexual isolates are especially prevalent within samples of Lineage 1 found in Australia (Zuccarello *et al.*, 1999a) but are also found in many samples from western New Caledonia (West & Zuccarello, 1999; Zuccarello *et al.*, 1999a).

Molecular studies have shown that morphologically indistinguishable isolates from different lineages can be sympatric (Zuccarello *et al.*, 1999a; Zuccarello & West, 2003) making an assessment of biodiversity based on morphological species characteristics problematic in this group.

New Caledonia is a tropical island situated in the south-western Pacific Ocean, lying south of the South Pacific Convergence Zone, between about 20-22°S and 164-167°E, in an approximately northwest-southeast direction (Hénin & Cresswell, 2005). Even though it has about 2,250 km of coastline and barrier coral reefs, the marine algal flora is poorly documented and only recently has work been initiated to document the seaweed diversity (Millar & Payri, 2006). Some species are shared between New Caledonia and tropical Australia (e.g. Kraft & Millar, 2005) and this includes algae associated with mangroves (West & Zuccarello, 1999).

This study was undertaken to determine the diversity in the *Bostrychia radicans / B. moritziana* species complex, the spatial distribution of this diversity, and the patterns of reproduction in New Caledonia. We were especially interested in seeing if there were different genotypes in the "west" and "east" side of the island.

MATERIALS AND METHODS

Conditions for collection, isolation and culture have been reported elsewhere (West & Zuccarello, 1999; West, 2005). Additional information on cultured isolates is listed in the URL: http://www.botany.unimelb.edu.au/West. Samples were also collected for molecular analysis by placing individual field collected plants directly into silica gel (Tab. 1, Fig. 1).

DNA extraction, PCR amplification, and sequencing of the RuBisCo spacer (ribulose-1-5-bisphosphate carboxylase/oxygenase large and small subunit intergenic spacer) have been presented elsewhere (Zuccarello *et al.*, 1999b), this region has been used extensively in characterizing lineages within the *Bostrychia radicans / B. moritziana* species complex and is congruent with all other molecular regions so far investigated in this group (Zuccarello *et al.*, 1999a; Zuccarello & West, 2003, 2006).

Maximum likelihood (ML) was used to construct the most-likely tree from the data set (10 random sequence additions) in PAUP*4.0b10 (Swofford, 2002). Previous studies have shown that all phylogenetic procedures give congruent and similar results with this data set (Zuccarello & West, 2003). The phylogeny was mid-point rooted as different rooting options (taxa) did not affect the ingroup topology. ML parameters were estimated using the hierarchical likelihood ratio test. The program Modeltest version 3.7 (Posada & Crandall, 1998) was used to find the model of sequence evolution that best fit each data set (alpha = 0.01). This evolution model was used in ML tree searches (HKY85, Hasegawa *et al.*, 1985; transition/transversion ratio = 2.3459; gamma distribution shape parameter = 0.3436). Support for individual internal branches was determined by bootstrap analysis (Felsenstein, 1985) as implemented in PAUP*. For bootstrap analysis, 100 bootstrap data sets were generated from resampled data (5 random sequence additions) using ML analysis. New unique sequences are deposited in GenBank (culture 3822, Lineage1, NC3 = DQ355983; culture 4165, Lineage 6 = DQ355984). GenBank accession numbers for all other previously published isolates can be found in Zuccarello *et al.* (1999a) and Zuccarello & West (2003).

Statistical parsimony (Templeton *et al.*, 1992), as implemented in TCS 1.21 (Clement *et al.*, 2000), was used to estimate gene genealogies and construct haplotype networks.

Table 1. Samples of *Bostrychia radicans /B. moritziana* species complex collected in New Caledonia; numbers of locations correspond to sites indicated in Fig. 1. AU, NC1, NC2, NC3 refer to haplotypes within Lineage 1, samples in the same row and same lineage have identical sequences, haplotype designations only applied to Lineage 1 samples. Samples 4-number code (beginning with E-) from field collections, otherwise JAW culture collection numbers. Rough "location" given on the island (west, east, upper, middle, lower).

	Location	*Latitude and Longitude*	*Haplotype / Lineage*	*Samples*	*Location*
1	Conception	21°14'S, 166°30'E	AU	3823[2]	west lower
			NC2	E983	
2	Poé Beach	21°36'S, 165°23'E	AU	4167[2], E682	west middle
3	Nera River	21°37S, 165°28'E	AU	4164[2], E684	west middle
			Lineage 6	4165	
4	Koumac	20°34'S, 164°16'E	NC3	3822[2]	west upper
5	Voh, Plage de Gatope	20°58'S, 164°39'E	AU	4366[1]	west upper
			NC2	4369[2]	
6	Plage de Foué	21°06'S, 164°49'E	AU	3819[2], 4168[2], 4169[2], 4170[2], 4171[2], 4172, 4173[2], 4174[3]	west upper
			NC1	3817[2]	
			NC2	3818[2], E680	
7	Hienghene	20°46'S, 164°11'E	NC2	4362[1]	east upper
8	Ponerihouen	21°08'S, 165°29'E	AU	4370[1]	east middle
			NC2	4371[1], E981	
			Lineage 2	E976	
9	Cape Bocage	21°10'S, 165°32'E	AU	4363	east middle
10	Kouaoua	21°24'S, 165°49'E	Lineage 7	4156, 4157, 4160, 4161, 4162, 4163[1], E676	east middle
			Lineage 6	E672, E673	
			Lineage 2	4158, 4159	

[1]= sexual reproduction in laboratory culture; [2]= asexual reproduction in laboratory culture; [3]= Asexual reproduction, but did form one female gametophyte. Others either uncultured field samples or vegetative in culture.

RESULTS

Samples were collected from 10 populations around the island of New Caledonia (Fig. 1). We analysed 21 samples from the west coast, and 17 from the east coast. The molecular data set consisted of 30 taxa (after the removal of most shared sequences that are listed in Table 1) and 285 characters, of which 22 were variable and parsimony-uninformative and 80 were parsimony-informative. ML analysis produced one tree of *–ln* score= 1264.4789. The results are consistent with previous analyses (Zuccarello & West, 2003). Seven distinct and moderately to well-supported lineages are seen (Fig. 2). New Caledonia samples are found in

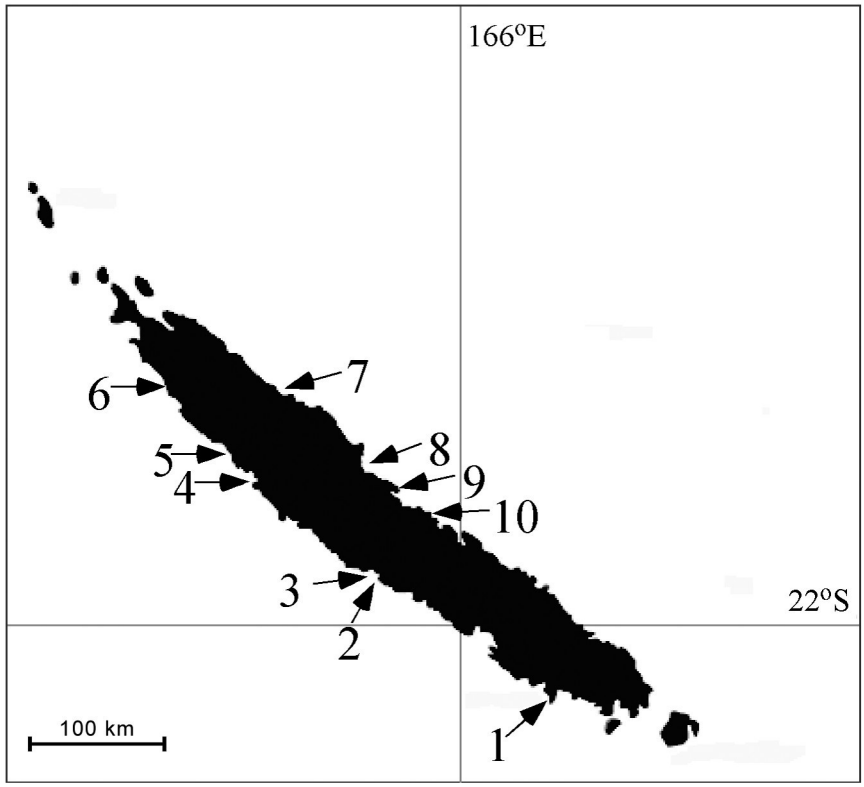

Fig. 1. Map indicating locations of collections around New Caledonia. Population numbers correspond to locations in Table 1.

four of these lineages. Only two unique sequences were found in this analysis. One belonged to a new haplotype (NC3) of Lineage 1, increasing the number of haplotypes in New Caledonia to four (Fig. 3). These haplotypes differ by only one basepair from each other and also differ by one basepair from haplotypes found in South Africa. Of the 21 samples collected on the west coast of New Caledonia all belonged to Lineage 1 except for one (culture 4165).

The only other unique haplotype, and the only Lineage 6 sample collected on the west coast (4165), is distinct from other Lineage 6 New Caledonian samples collected on the east coast. Sample 4165 forms a moderately supported sister group to the other Lineage 6 New Caledonian samples (E672, E673) and a sample from Queensland, Australia (3881).

Of the 17 samples collected on the east Coast of New Caledonia only four belong to Lineage 1, the others belong to lineages 2, 6, and 7. Lineage 6 samples were mentioned previously. Of samples collected within Lineage 2 all three were identical in sequence (4158, 4159, E976, two populations) and identical to a sample from Sulawesi, Indonesia (3453), they are different from a sample from the Northern Territory, Australia (3751).

The greatest sampling effort on the east coast was in Kouaoua, near the nickel ore shipping port. At this location no lineage 1 samples were found and

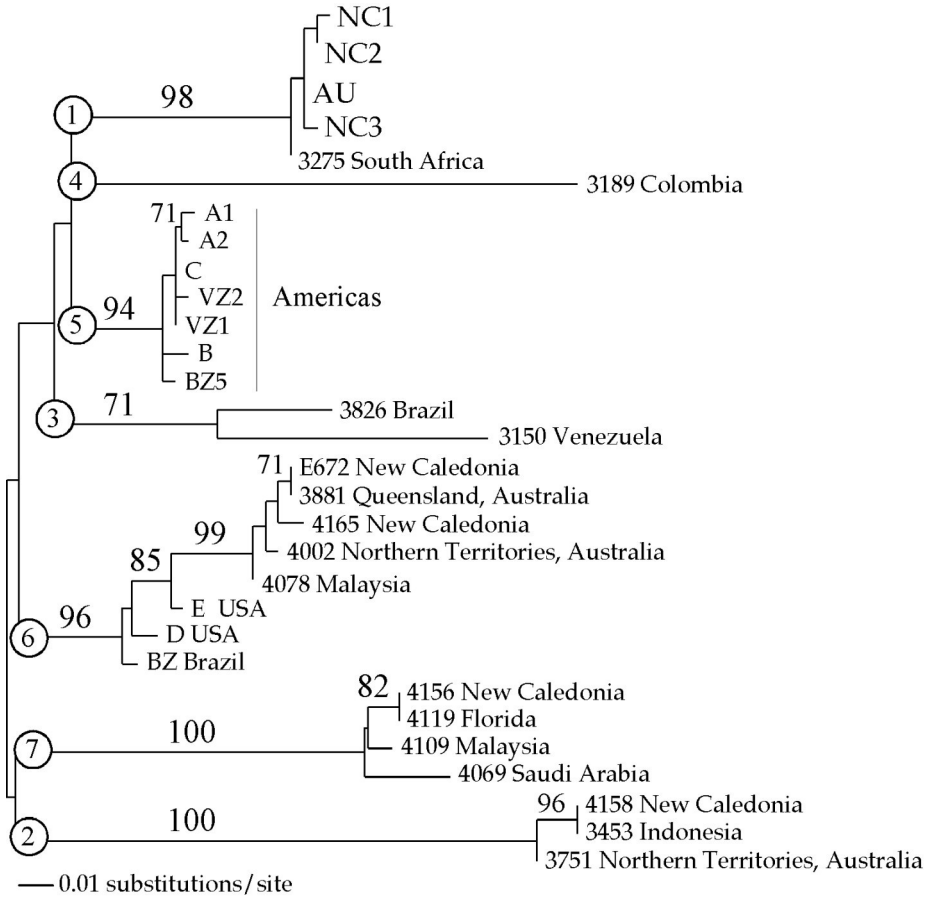

Fig. 2. Maximum likelihood tree topology for RuBisCo spacer DNA sequence data for samples of the *Bostrychia radicans / B. moritziana* species complex. Lineages 1-7 indicated. Exact location and Genbank accession numbers of non-New Caledonia samples, by culture number, found in Zuccarello and West (2003). Maximum likelihood bootstrap values (> 70%) shown above branch.

most samples were part of Lineage 7, the others were in lineages 2 and 6 mentioned previously. The Lineage 7 samples from the nickel ore port had an identical RuBisCo spacer sequence to a sample collected in Florida, USA (4119, Fort Myers), while it is different from a sample from Sabah, Malaysia (4109).

Reproductive data showed that most samples grown in laboratory culture of Lineage 1 had an asexual life cycle but not all (Table 1). Thirteen plants were asexual, with 12 of these from the west coast. Four plants had a sexual life cycle, three were found on the east coast with only one sexual plant collected from the west coast (4366 from Voh). One sample from the west coast (Plage de Foué 4174), is asexual in laboratory, tetrasporophytes recycling tetrasporophytes, though it did once produce one female gametophyte among many sporophytes. This rare development of gametophytes in an otherwise consistent asexual sporophyte recycling has been seen before (West & Zuccarello 1999). All samples from other lineages were completely vegetative in laboratory culture.

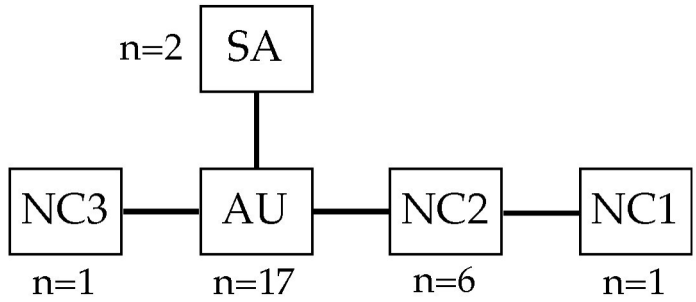

Fig. 3. Haplotype network of chloroplast RuBisCo spacer sequence data of samples within Lineage 1. n = number of samples of each haplotype from New Caledonia, except for South African samples. Haplotype designation from Zuccarello *et al.*, (1999): AU = Australia haplotype; NC1 = New Caledonia haplotype 1; NC2 = New Caledonia haplotype 2; NC3 = New Caledonia haplotype 3; SA = South Africa haplotype (cultures 3275, 3204).

DISCUSSION

The most striking result from this analysis of the diversity of the *Bostrychia radicans / B. moritziana* species complex in New Caledonia is the difference in biodiversity on either side of the island, again indicating that biodiversity of marine algae can not be properly determined solely by morphology (e.g. Zuccarello & West, 2003). Only one of the 21 samples collected on the west coast was not found in Lineage 1, while only four samples out of 17 were from Lineage 1 on the east coast. The east coast also contained Lineage 2 and Lineage 7 samples that were not found on the west coast. It is not known whether this difference in diversity, between the two sides of the islands, is reflected overall in algal species assemblages as not enough work has been done in this area addressing this issue (Garrigue & Tsuda, 1988; Millar & Prud'homme van Reine, 2005; Millar & Payri, 2006). There are known to be some differences in the sea temperatures around New Caledonia, with summer upwelling lowering sea surface temperatures by 5 °C on the west coast (Hénin & Cresswell, 2005). Whether this environmental difference or any other ecological differences lead to this variation in diversity on the two sides of the island is not known.

Lineage 1 samples are the most common in New Caledonia, as they are in Australia, New Zealand and Fiji (Zuccarello *et al.*, 1999a). Most samples of Lineage 1 from New Caledonia that were grown in culture proved to be asexual (recycling tetrasporophytes). This reproductive pattern is seen in most north Australian samples (central and northern New South Wales, Queensland, Northern Territory and Western Australia) but not in samples from other Pacific islands (Fiji, New Zealand) (West & Zuccarello, 1999). A single origin of asexuality in this lineage, and the stability of asexuality over evolutionary time scales (though they are stable in laboratory culture over seven years) is unlikely. Samples with haplotype AU are both asexual (the majority) and sexual (4362, 4366, 4370), as are samples with haplotypes NC2 (most asexual except for 4371). This would indicate that asexuality has been gained at least twice once in each of these two haplotypes. We assume a sexual ancestor as: i) the closest sister group

to the New Caledonia and Australian samples, from South Africa (3275), is sexual (West & Zuccarello, 1999); ii) samples from near-by Pacific islands are sexual [Fiji, New Zealand (West & Zuccarello, 1999)]; iii) asexuality has been shown to have been gained several times in other mangrove red algae (West *et al.*, 2001) and evidence is starting to accumulate that asexuality could be a consequence of hybridisation between genetically dissimilar sexual lineages (Kamiya, 2004; Zuccarello *et al.*, 2005b). The other less likely possibility is a non-homologous but identical base pair substitution occurred to produce the "same" haplotype in a sexual and an asexual lineage. Interestingly all the Lineage 1 samples investigated from the east coast are sexual while only one out of 12 is sexual from the west coast. Whether conditions (*i.e.* environmental stability, competition) are more favourable for asexual, or sexual, reproduction on one coast versus another is unclear. Further collections and culturing might indicate if this trend is continued, and ecological and/or ecophysiological experiments may indicate if there are differences in environments or variability on the east versus west coast and in asexual versus sexual plants.

Samples of the *Bostrychia radicans / B. moritziana* species complex from Lineage 7 from New Caledonia are only found in Kouaoua. The RuBisCo spacer sequence of these samples is identical to a sample from Florida. This is the second largest nickel ore port in New Caledonia (http://www.townsville-port.com.au). It is possible that these Lineage 7 samples are human mediated introductions to this busy port in the recent past. Though sampling was intensive at Kouaoua we did not find any Lineage 1 samples, the most common lineage in New Caledonia, at this site. It is possible that Lineage 1 samples are out competed at this site by the other lineages. Little is known about differences in the ecophysiology of *Bostrychia* samples from different lineages, though in *Caloglossa leprieurii* (Montagne) G. Martens, another alga associated with mangrove habitats, different haplotypes had significantly different physiologies and abundances (Zuccarello *et al.*, 2001).

Our data shows striking differences in the biodiversity of algae of the *Bostrychia radicans / B. moritziana* species complex in New Caledonia. Differences are seen in the distribution of lineages between the east and west coast and in the distribution of reproductive cycles between the east and the west coast. These differences could be due to the geological/ecological differences on these two sides of the island or human mediated differences, or could just be random. More research in this poorly explored island is necessary.

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