Review

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Insights into evolution and speciation in the red alga *Bostrychia*: 15 years of research

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Studies of the red algal genus *Bostrychia* over the last 15 years have made it a model system for many evolutionary processes within red algal species. The combination of newly developed, or first employed methods, in red algal species studies has made *Bostrychia* a pioneer genus in intraspecific studies. *Bostrychia* was the first genus in which a mitochondrial marker was used for intraspecific red algal phylogeny, and the first for which a 3-genome phylogeny was undertaken. The genus was the first red alga used to genetically show maternal plastid and mitochondria inheritance, and also to show correlation between cryptic species (genetically divergent intraspecific lineages) and reproductive incompatibility. The chemotaxonomic use, and physiological function of osmolytes, has also been extensively studied in *Bostrychia*. Our continuous studies of *Bostrychia* also highlight important aspects in algal species studies. Our worldwide sampling, and resampling in certain areas, show that intensive sampling is needed to accurately assess the genetic diversity and therefore phylogeographic history of algal species, with increased sampling altering evolutionary hypotheses. Our studies have also shown that long-term morphological character stability (stasis) and character convergence can only be correctly assessed with wide geographic sampling of morphological species. While reproductive incompatibility of divergent lineages supports the biological species nature of these lineages, reproductive incompatibility is also seen between isolates with little genetic divergence. It seems that reproductive incompatibility may evolve quickly in red algae and the unique early stages of fertilization (e.g., gametes covered by walls, active movement of spermatium nuclei to the distant egg nucleus), also well investigated in *Bostrychia*, may be key to our understanding of this process.

Key Words: evolution; molecular marker; organelle inheritance; osmolyte; phylogeny; phylogeography reproductive compatibility; speciation

INTRODUCTION

While studying model systems can be controversial, the prolonged study of a particular group of organisms (e.g., a species or genus) allows a level of insight that is difficult to achieve from one-off species studies. The study of model organisms allows several areas of research to converge to address significant questions that, hopefully, will be of interest outside the realm of the model organisms. Model organisms do have the drawback that evolutionary histories are species-specific (patterns of selection and stochastic events), so that they do not faithfully represent all similar taxa. Nevertheless, model organisms have proven very useful in elucidating particular biological phenomena. The green alga *Chlamydomonas reinhardtii* is one example that has been used to

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study flagellar functioning (Iomini et al. 2009) and plastid inheritance (Nishimura 2010). In brown algae, the work done by Müller and colleagues on life cycles and hybridization (e.g., Müller and Eichenberger 1995) and viruses (Müller et al. 1996) of *Ectocarpus* spp. has to the first brown algal genome sequence (Cock et al. 2010). In red algae, model organisms are less obvious. While research on *Porphyra* spp. has been impressive, the diversity within this one genus, and its polyphyletic sister genus *Bangia*, makes it difficult to compare between species that are distantly related (e.g., Broom et al. 2004).

One red alga that has had consistent research and a variety of approaches used to address its evolution and biology is the Rhodomelacean genus *Bostrychia* Montagne (Ceramiales). While this alga has had a long taxonomic history, our research in the last 15 years, addressing evolutionary and speciation questions, has made this a good example of the insights that are possible with prolonged study of a single genus. This genus also was the first in which several techniques, markers and discoveries of red algae were first made or developed. This review will try to summarize this work and indicate where this work has shed insight into general questions in red algal biology.

TAXONOMIC HISTORY

Bostrychia is usually found in the tropical eulittoral around the world, but is most closely associated with estuarine habitats. It is especially conspicuous in mangrove habitats, but is also found in cold temperate and subantarctic regions. The type species (*Bostrychia scorpioides* [Hudson] Montagne) is from Europe and is usually associated with estuaries.

The genus is distinct within the Rhodomelaceae, mainly by having a regular arrangement of tier cells (transverse divisions of the pericentral cells usually found in the Rhodomeleaceae). Only one other genus is known to have this arrangement of tier cells (*Rhodolachne*). Its morphological taxonomy has had two comprehensive reviews. The first was by Post (1936), who did the main taxonomic and biogeographic work on this genus in the first half of the twentieth century. She recognized a single genus in the subfamily Bostrychioideae, and commented that two groups could be recognized in the subfamily based on attachment structure types. She also proposed many varieties and subspecies within known or newly described species. The second monograph was by King and Puttock (1989), who supported a generic distinction first proposed by Falkenberg (1901) dividing the subfamily into two genera (*Bostrychia*, *Stictosiphonia* J. D. Hooker et Harvey) based on the number of tier cells per axial cell (a single transverse division producing two tier cells per axial cell in *Bostrychia*, and more than one division producing more than two tier cells per axial cell in *Stictosiphonia*). King and Puttock (1989) synonymized many of Post's species and sub-specific ranks, but also described new species.

DEVELOPMENT OF MOLECULAR MARKERS IN RED ALGAE

For intraspecific studies, and for studies of closely related species, markers of appropriate variability are needed. Fifteen years ago, these markers were few. The ribosomal RNA (rRNA) cistron was the most widely used marker in the early history of organismal phylogeny (Woese and Fox 1977). While the reasons for its use in bacterial phylogeny have been defended (Woese 2000), mostly its ease of use in the days before the development of the polymerase chain reaction (PCR) made its use ubiquitous. Along with the rRNA gene, very early in algal molecular systematics the ribosomal internal transcribed spacer (ITS) spacer1-5.8S rRNA-ITS spacer2, commonly just called ITS, was used as a marker. These primers, developed by White et al. (1990), appeared to be universal and were quickly used in red algae (Goff et al. 1994). While the ITS marker has appropriate levels of variation for inter- and intraspecific phylogenies, there are several drawbacks. Its size varies greatly in red algae making even PCR amplification difficult and results in a few cases of mis-amplification of contaminants (Bown et al. 2003). Alignment, especially if lengths are very variable, has made its use in determining the relationships between distantly related species, even of the same genus, problematic. Similar to the rest of the ribosomal cistron, the ITS is present in multiple and unlinked copies. While this has some advantages (e.g., ease of amplification of degraded DNA) at the level of intraspecific analysis, it can cause problems. Due to its large copy number and the effective population size of copies, and random nature of copy homogenization, copies can remain that were previously found in different ancestors or multiple copies still present within individuals (maintenance of ancestral polymorphisms). This can cause problems in producing reliable phylogenies and means that monophyly of groups (even species) will take longer to manifest than other single copy markers (i.e., organellar) (Álvarez and Wendel 2003).

Organelle markers have many advantages (and disad-

vantages) that include haploid complement, supposed uniparental inheritance and, therefore, a smaller effective population size (Palumbi et al. 2001). For intraspecific studies, non-functional DNA is often preferable to protein coding genes. One of the first markers to be developed that is near-universal in red algae and shows good levels of variation was the chloroplast encoded ribulose-1, 5-bisphosphate carboxylase oxygenase (RuBisCo) spacer (Destombe and Douglas 1991, 1992). These spacer primers also amplify the 5-prime end of the RuBisCo large subunit and 3-prime end of the RuBisCo small subunit. The amplicon is between 250 and 350 bp in length. The size of this spacer also makes it appropriate to use for single-stranded conformation polymorphism (SSCP). This method separates small DNA fragments based on sequence versus solely on size (Sunnucks et al. 2000). This is especially useful in population studies where many sequences are expected to be very similar, thus reducing sequencing costs substantially. SSCP analysis of amplified product variation was first used in red algae with *Bostrychia* (Zuccarello et al. 1999*b*).

The mitochondrial genome has always been considered to be slow-evolving in red algae. This is widely-known in higher plants and subsequently a similar situation was hypothesized in other photosynthetic organisms (Bakker et al. 2006). Therefore, the development of mitochondrial markers was not pursued in red algae. The first mitochondrial molecular marker used was also developed for the study of *Bostrychia*. An intergenic spacer using conserved regions of the *cox*2 and *cox*3 gene was developed and shown to be amplified in many red algal genera (Zuccarello et al. 1999*a*). This *cox*2-3 spacer showed greater intraspecific variation than the RuBisCo spacer, indicating that the mitochondrion was evolving at a rate that could make it more useful in phylogenetic and population studies. Subsequently, the cytochrome oxidase subunit 1 gene (*cox*1 or COI) has been proposed as a barcoding gene in red algae (Saunders 2005).

Organelle inheritance

While it was always suspected that organelles were inherited maternally in red algae, genetic confirmation had to wait for appropriate genetic markers to be developed, i.e., markers variable within isolates that were still able to cross. While electron microscopy revealed both mitochondria and chloroplasts in many red algal spermatia (e.g., Kugrens and West 1972), it was always assumed that the large size and cytoplasmically-rich carpogonium would at least lead to the dilution of any paternal organelles in offspring. The first confirmation of maternal inheritance of chloroplasts in red algae was with the use the RuBisCo spacer, SSCP and crosses of isolates of *Bostrychia radicans* (Montagne) Montagne (Zuccarello et al. 1999*b*). This study confirmed that while plastids were found in spermatia, tetrasporophytes grown from successful crosses possessed the RuBisCo spacer sequence of the maternal plant. A similar study was conducted using the *cox*2-3 spacer, and demonstrated that mitochondria are maternally inherited in *Bostrychia moritziana* (Sonder *ex* Kuetzing) J. Agardh (Zuccarello et al. 1999*a*). Of course, paternal leakage (inheritance of organelles from the other parent) could still be possible and only the screening of many isolates would indicate how consistent this pattern is within and between species of red algae. A recent study has shown that paternal and biparental inheritance of chloroplast and mitochondria, while rare, is possible in *Porphyra yezoensis* Ueda (Choi et al. 2008).

PHYLOGEOGRAPHY OF *BOSTRYCHIA*: *B. CALLIPTERA* AND *B. TENUISSIMA* AND *B. RADICANS*-*B. MORITZIANA*

Bostrychia calliptera

With the availability of several markers and samples from around the world, the very first red algal three-genome (nuclear, plastid, mitochondria) phylogenetic and phylogeographic study was undertaken on the species complex of *B. calliptera* (Montagne) Montagne and *B. pinnata* J. Tanaka et M. Chihara (Zuccarello and West 2002). This was also the first study to estimate mutation rate and divergence times using molecular markers in red algae.

Taxonomically, the two species did not form reciprocally monophyletic lineages and the older name *B. calliptera* is accepted as *per* the International Code of Botanical Nomenclature. All three genomes showed congruent topology with three well-supported lineages: one confined to the Atlantic, one specific to the Indo-Pacific, and a third found throughout the world. Also, samples collected close to the Isthmus of Panama, but on opposite geographic sides, were used to estimate the divergence rate. Molecular clock dating was only done on one lineage, as the other two lineages had not evolved in a clock-like manner, based on, at the time, recommended rate tests. Within the error of all clock estimates and calibrations using the last closure of the Isthmus (Knowlton and Weigt 1998), the analysis of one lineage suggested

that this species had been morphologically static for over 40 million years. The mutation rate of the genes tested indicated that ITS and the *cox*2-3 spacer had similar mutation rates, greater than the plastid spacer and the large-subunit rRNA gene.

Bostrychia tenuissima

A similar study (Zuccarello et al. 1999*c*) looking at variation in the RuBisCo spacer of isolates of *B. tenuissima* revealed that three lineages of *B. tenuissima* were found on the coast of New South Wales; these lineages were highly divergent and showed a north-south pattern. One lineage from southern Australia to New South Wales just south of Sydney (42-34° S), another from Sydney to northern New South Wales (34-33° S) and the last from 28° S northwards. Interestingly these lineages also showed different patterns of sorbitol content, with the southern lineage having only sorbitol while the other lineages had both sorbitol and dulcitol. A hypothesis proposed that the southern lineage lost its dulcitol synthesis pathway because it is more sensitive to cold temperatures (Karsten et al. 1995*a*).

Both these studies occurred before a comprehensive phylogeny of the genetic variants and species of the genus *Bostrychia* (Zuccarello and West 2006, more later). These later analyses indicated that certain assumptions of intra-species analysis were not fulfilled. These lineages, the three lineages in *B. calliptera* and the three lineages in *B. tenuissima*, were not monophyletic. While the relationships within the lineages were confirmed, certain hypotheses based on the previous analyses need reinterpretation. The morphological stasis seen between lineages in *B. calliptera* may be a case of convergence, while within lineages that are 40 million years old morphological stasis can still be invoked. Instead of ecological differentiation within a species, *B. tenuissima*, we have different non-monophyletic species with almost non-overlapping distributions and morphological convergence of the *B. simplisciuscula* / *B. tenuissima* morphology (Zuccarello and West 2006).

Bostrychia radicans-Bostrychia moritziana

By far the greatest amount of our research is on the species complex of *B. radicans* and *B. moritiziana*. Papers have reported its global phylogeography (Zuccarello et al. 1999*d*, Zuccarello and West 2003) and specific patterns in various regions of the United States (Zuccarello and West 2003, Zuccarello et al. 2006), Mexico (Zuccarello and West 2003, Zuccarello et al. 2011) and New Caledonia (Zuccarello et al. 2006). The morphological difference between these two species was determined to be the presence of monosiphonous laterals on *B. moritziana* (King and Puttock 1989). The first molecular study (Zuccarello and West 1997) showed that isolates mostly from the Americas formed two groups that correlated fairly well with their ability to hybridize, but not with presence of monosiphonous laterals. This was the first paper to show a correlation between sequence similarity and ability to hybridize in red algae. The paper also highlighted the probability of cryptic species within this species complex. Subsequent papers (Zuccarello et al. 1999*d*, Zuccarello and West 2003) revealed seven evolutionarily divergent and well-supported lineages in this species complex. The distribution of these lineages showed different patterns with some found in particular ocean basins, but many with world-wide distributions. More importantly, different lineages were found in the same locality, often meters from each other. Crossing experiments between isolates showed that they were reproductively incompatible. This indicates that these morphologically similar but divergent lineages fit the criteria of species, except for the absence of morphologically distinguishable characters associated with each lineage, so from two species we could identify seven cryptic species.

Factors driving morphological differentiation in red algae are not known, since many morphological characters used in algal taxonomy have no obvious selective advantage. For example, what is the advantage of one type of attachment structure over another, or light cortication versus heavier cortication, or monosiphonous lateral branches versus polysiphonous lateral branches? Therefore, it is possible that changes are driven by random processes. In a stable environment these changes may be rare, even constrained, so that change is difficult. It was suggested that the morphology of the red alga *Bangia* has been stable for over 1 billion years (Butterfield 2000). This indicates that we are overlooking much of the variation existing in populations and true species by basing our observations only on morphological criteria. There are many studies that confirm the abundance of cryptic species in red algae (e.g., Saunders 2005, Robba et al. 2006).

An advantage of repeat studies on a single species is highlighted by studies of the *B. radicans-B. moritziana* species complex from the east coast of the U. S. and from Pacific Mexico. The first study targeted plants along the east coast of the U. S. (Zuccarello et al. 1999*d*); as these plants were also going to be used for physiological and crossing experiments (Karsten et al. 1994*a*), only one

or two plants were collected from each site (Zuccarello et al. 1999*d*, Zuccarello and West 2003). They showed a pattern of a certain haplotype in the north (haplotype B), another common haplotype further south (haplotype C) and a further haplotype from another lineage in the most southerly areas (haplotype D). Suggested hypotheses for this pattern include physiological adaptation of haplotype B to cooler conditions and adaptation of haplotype D to warmer conditions. Collecting more extensively (average 10 samples per site) more that 10 years later, Zuccarello et al. (2006) found different patterns. Haplotype C was almost non-existent, haplotype D was found further north and haplotype B was found further south. There are two non-mutually exclusive explanations for this. First, increased sampling provided a more accurate pattern of the distribution of genetic variation within areas. Second, in the intervening 10 years climatic conditions and distribution patterns of haplotypes changed. The genetically well-structured populations of mangrove algae (Zuccarello et al. 2001) suggest that sampling in a single area (meters square) could easily miss detection of haplotypes in that area.

This re-evaluation of phylogeographic hypotheses was also driven by repeated studies in Pacific Central America. An initial study (Zuccarello et al. 1999*d*, Zuccarello and West 2003) showed low variation in Pacific Mexico, with one major haplotype (A1) along the whole coast from Baja California to Guatemala; also, samples from Pacific Mexico were all reproductively compatible (Zuccarello and West 1995, 2003). This uniformity led to the proposal of two scenarios that suggested either a recent introduction to the Pacific, and / or a more uniform environment leading to genetically connected populations and low differentiation between populations (Zuccarello and West 1995, 2003). The results from more intensive sampling (average 18 samples per site) suggested that the previous phylogeographic proposals of the species in this area were untenable. With sampling in areas from southern Mexico to El Salvador (Zuccarello et al. 2011), closer to the Isthmus of Panama, genetic variation was shown to be higher, with haplotypes previously only identified from the Atlantic, new haplotypes found plus the major northern haplotype (A1) found as far south as El Salvador. Again, further and more intensive sampling in an area already sampled, plus an area further south, has changed our hypotheses of the evolution of this species complex in the east Pacific.

Can these results be expanded to other groups? Certainly it is well known that other algae have populations genetically structured at small spatial scales (Faugeron et al. 2001, 2004, Zuccarello et al. 2001). This is one of the novel insights from population genetics data, as organisms that release spores in the environment would *a priori* be expected to have a more uniform genetic structure, at least on local scales. Many phylogeographic studies of red algae have been based on limited sampling within locations. These could all benefit from further study to comprehensively assess the existing variation and the evolutionary hypotheses derived from this data.

MOLECULAR PHYLOGENY OF *BOSTRYCHIA*: POLYPHYLY OF MORPHOLOGICAL SPECIES

The advent of molecular methodologies revolutionized our understanding of many aspects of algal evolution. The 'reverse' taxonomy provided by molecular data has also led to a taxonomy that, in theory, reflects the evolution of the organisms more accurately (Markmann and Tautz 2005). This 'reverse' taxonomy has proven especially useful in algal systematics, where phylogenetic relationships are based on few morphological synapomorphies and, therefore, homology is always in doubt.

Molecular phylogeny of all species recognized by King and Puttock was undertaken in two separate studies (Zuccarello and West 2006, 2008). The sampling of this global *Bostrychia* phylogeny was informed by previous work on separate species (Zuccarello et al. 1999*c*, 1999*d*, Zuccarello and West 2002, 2003), so that the phylogeny incorporated from the genetic diversity of the species under study. These species were often from disparate localities and / or from well-sampled populations in which diverse lineages were found. This *Bostrychia* phylogeny used two 'traditional' genetic markers. One was the chloroplast-encoded ribulose-bisphosphate carboxylase / oxygenase (*rbc*L). This marker has been used extensively in the Rhodophyta, from the very first comprehensive algal phylogeny (Freshwater et al. 1994) to the present day. *Rbc*L has several properties that make it useful: it is a fairly large gene (sequence length and various amplified products vary between 1,200 and 1,400 bp) and, for protein coding, alignment is straight-forward. As well, the partitioning of codons for phylogenetic analysis is easily achieved. A second marker is the nuclear-encoded rRNA gene, which has also been used extensively in the Rhodophyta. Although the small subunit was the first to be used extensively in algal systematics (Ragan et al. 1994), the increased length and many variable regions of the large-subunit rRNA gene have proven useful for phylogenetic analyses at all taxonomic levels (Harper and Saun-

ders 2001). Unfortunately, the variable regions can make alignment of the rRNA highly problematic.

The phylogeny of the Bostrychioidiae used both *rbc*L and nuclear-encoded partial sequence to the large-subunit RNA gene. The work highlighted two aspects related to the taxonomy of *Bostrychia* and more general aspects. One aspect was the polyphyly of the morphological character used to distinguish genera by King and Puttock (1989) (i.e., *Stictosiphonia* from *Bostrychia*). Tier cell numbers greater than two seems to have evolved more than once: in warm water clades "*Stictosiphonia*" *tangatensis*, "*Stictosiphonia*" *kelanensis*, and in a clade containing some cold water "*Stictosiphonia*" (*S. intricata*, *S. arbuscula*, *S. gracilis*, *S. vaga*). This led us to synonymize *Stictosiphonia* into *Bostrychia*. This application of molecular data again showed that the apparent homology and synapomorphic nature (shared tier cell number, in this case) of this easily distinguishable morphological character was unwarranted. This is just one example of the utility of 'reverse taxonomy' and, more significantly, the ability of molecular data to indicate supported relationships and, therefore, to highlight morphological convergence. Now, instead of hypothesizing an evolutionary scenario of character derivation to produce two generic groups, we must ask why this character is plastic and look more carefully at its development to identify any stages that may reflect this homoplasy.

Not only at this level were morphological characters misleading in determining the systematics of this tribe, but the data also revealed that characters that had been applied to identify new species were also not useful. For example, some samples identified as *B. simplisciuscula* showed no sequence variation from some isolates of *B. tenuissima*. These species were separated by King and Puttock (1989) based on monosiphonous branches in *B. simplisciuscula* and their absence in *B. tenuissima*. In a previous study (Zuccarello and West 2002), cortication was also shown not to distinguish monophyletic species in the species groups *B. calliptera* and *B. pinnata*. The presence or absence of cortication, and the presence or absence of a clear morphological character such as terminal branches being monosiphonous or polysiphonous would be used to distinguish species in many groups. It is clear that morphological characters such as these, at least in *Bostrychia*, cannot designate species.

Unfortunately, not only do these characters not support species but morphologically distinguishable 'species' are not even monophyletic. The previously mentioned species *B. simplicuiscula* and *B. calliptera* are found in three distinct lineages. Within the resolution of the genes employed, it would appear that these morphologies were produced or maintained in multiple, not closely related groups.

One other genus in the Rhodomelaceae has been reported with tier cells, the genus *Rhodolachne* Wynne. While the generitype was not sequenced, molecular analysis of *R. radicosa* Itono showed it to be a new species of *Bostrychia* (*B. radicosa* [Itono] West, Zuccarello *et* Hommersand). This previously under-reported species had been found throughout the Indo-Pacific (West et al. 2006*b*). Whether *Rhodolachne* will be confirmed as a distinct genus, with convergence of the morphological character of tier cells, requires collection and molecular analysis of a confirmed specimen of the generitype *Rhodolachne decussuta* M. J. Wynne.

So what does it mean for other groups and our understanding of species? It seems that we need to be very cautious with the characters we use and morphological diversity that we see within species. While molecular data is never foolproof (i.e., limited genes can give relationships that do not reflect the complexity of a species history), we must accept that both the number of morphological characters and the homology of morphological characters, especially in algal groups, is even more likely to be misleading both in phylogenetic reconstruction and in generic and species descriptions. Molecular studies within species and supported evolutionary clades, as exemplified in the research on *Bostrychia*, also highlight that, for many, a typological species concept is still in use. It is likely that classifying specimens based on morphology is both under-representing variation with morphologically similar but evolutionarily distinct groups merged, and on some occasions inflating diversity with morphological variation within species, with morphological variation that has been used to separate species being plastic within a species. It seems these problems are being addressed, with few species descriptions in algae presented these days without some molecular data supporting the relationships.

ASEXUALITY

As with many red algae (West et al. 2001), mechanisms of asexual propagation are found in *Bostrychia* (West and Zuccarello 1999). In the *B. radicans*-*B. moritziana* species complex, asexuality is manifest as tetrasporophytes that produce either bisporangia or tetrasporangia, and these spores recycle into tetrasporophytes. The first report of asexual plants of *B. moritziana* also led to its description

as a new species (*B. bispora*) (West et al. 1992*a*). However, later work using molecular markers demonstrated that this species did not differ genetically from sexual plants (Zuccarello et al. 1999*d*) and the name was synonymized with *B. moritziana*. The patterns of asexual populations were studied extensively around Australia (West and Zuccarello 1999, Zuccarello et al. 1999*d*). Sexual plants of *B. moritziana* from lineage 1 (Zuccarello et al. 1999*d*) were found in most southern areas of Australia (State of Victoria), while the number of populations of sexual plants diminished on the east coast of Australia and became very rare in populations north of Sydney, New South Wales. Asexual plants were also found in Fiji and both sexual and asexual plants in New Caledonia (12 of 16 plants in culture were asexual). *B. moritziana* from lineage 1 was also found in South Africa and these plants were also shown to be sexual. It would appear that southern populations, at least in this lineage, are sexual and northern populations are mainly asexual. There are many theories of the forces driving asexuality. In animals, geographic parthenogenesis is related to populations found in marginal habitats (Haag and Ebert 2004). This asexuality in marginal habitats could possibly be evoked for the mangrove-associated alga *Caloglossa vieillardii* (Kützing) Setchell in which asexual plants are found only in the extreme edge of its range in South Australia (West et al. 2001), with sexual plants found further north along the west and east coasts of Australia. But, this would be unusual for the mangrove associated *B. moritziana* lineage 1, where its most widely distributed life cycle is asexual, and asexuality seems mostly to be restricted to its northern limits. The causes and maintenance of asexuality need further investigation and simple theories have to take into account empirical observations.

POLYOLS

Bostrychia possesses two polyols, sorbitol and dulcitol, which are both hexan-1, 2, 3, 4, 5, 6-hexol, that are unique within the Florideophyceae. The distribution and function of these polyols have been extensively studied in algae and flowering plants.

Synthesis and function

The enzyme synthesis pathways of sorbitol and dulcitol in *Bostrychia* have not yet been investigated. Other enzymatic pathways in polyol synthesis are known for red algae (e.g., mannitol synthesis) (Karsten et al. 1997, Eggert et al. 2006).

Polyols are maintained as soluble compounds in the cells and apparently play multiple functions in red algae as they do in other organisms. As organic osmolytes they are important in regulating osmotic pressure, cell volume and fluid balance. When salinity increases, polyol synthesis also increases in the cells. These compatible solutes protect and stabilize organelle systems, protein synthesis and enzymatic functions. Manifold increases of polyol levels occur in *B. moritziana*, *B. radicans* and *B. simpliciuscula* (Karsten et al. 1993, 1994*a*, 1994*b*).

Polyols also serve as important antioxidants by scavenging harmful oxygen radicals, as heat protectants by stabilizing proteins and as quick energy sources for maintenance metabolism (Eggert and Karsten 2010).

Polyol distribution in different taxa

Polyols as well as other low molecular weight carbohydrates serve as important taxonomic characters at all levels from class to subspecies. For example, sorbitol is found in all genera of the class Stylonematophyceae that have been analyzed thus far. Dulcitol is also found in the class, but only in the genus *Rhodospora* (Karsten et al. 1999, 2003). The distribution of compatible solutes has been extensively investigated for *Bostrychia*. The following summarizes the state of knowledge.

***B. arbuscula* J. Hooker et Harvey.** Only one sample from New Zealand has been analyzed and was shown to contain sorbitol and digeneaside (Karsten et al. 1995*a*).

***B. calliptera* / *B. pinnata*.** Isolates from Australia, Brazil, Guatemala, Peru, Japan and Singapore contain sorbitol and dulcitol (Karsten et al. 1992, 1995*a*, Pedroche et al. 1995). Traces of digeneaside were evident in *B. calliptera* from Brazil but none was seen in *B. pinnata* from Japan (Karsten et al. 1995*a*).

***B. flagellifera* Post.** No isolates have been analyzed.

***B. harveyi* Montagne.** Only one sample (Tasmania, Australia) has been analyzed and showed only sorbitol and digeneaside (Karsten et al. 1995*a*).

***B. intricata* (Bory de Saint-Vincent) Montagne (as *Stictosiphonia hookeri*).** Isolates from Australia, New Zealand, Argentina, Chile and South Africa had only sorbitol with traces of digeneaside (Karsten et al. 1992, 1995*a*, 1996, West et al. 1996).

***B. kelanensis* Grunow ex Post.** Only two isolates from Australia were analysed and contained only sorbitol and digeneaside (Karsten et al. 1992, 1995*a*).

***B. montagnei* Harvey.** Samples from Bermuda, Brazil and the USA contains sorbitol and dulcitol (Karsten et al.

1992, 1995*a*). Traces of digeneaside were evident in one field sample from Brazil.

B. moritziana. Isolates from Venezuela, Brazil and Australia contained sorbitol and dulcitol (Karsten et al. 1992, 1993).

B. radicans. Strains from South Carolina to Connecticut (lineage 5, haplotype B, see Zuccarello and West 2003) were shown to produce only sorbitol whereas strains from all other regions (Florida, USA, Mexico, Venezuela, Peru, Brazil, Australia). Micronesia have sorbitol and dulcitol (Karsten et al. 1992, 1994*b*, 1995*a*, West et al. 1992*a*, Pedroche et al. 1995). Digeneaside was variably present in those samples with and without dulcitol (Karsten et al. 1994*b*).

***B. radicosa* (Itono) J. A. West, G. C. Zuccarello et M. H. Hommersand.** Isolates from Australia, Madagascar, Malaysia, New Caledonia and Thailand contained high levels of digeneaside and low levels of sorbitol. Traces of dulcitol were also detected in New Caledonia and Madagascar isolates (West et al. 2006*b*). This is the only species of *Bostrychia* with such a high concentration of digeneaside and very low concentration of polyols. This species is also common in Micronesia, but analyses are not completed.

***B. scorpioides* Montagne.** A sample from France contained sorbitol and dulcitol with traces of digeneaside according (Karsten et al. 1995*a*). Kremer (1976) demonstrated that both polyols were present in another sample from Brittany.

B. simpliciuscula* / *B. tenuissima. Samples of lineage H3, from Singapore and Australia contained sorbitol and dulcitol (Karsten et al. 1992). In Australia all the populations south of latitude 34° S and belonging to lineage H1, contained sorbitol and digeneaside, whereas in lineages H2 and H3 all those north of 34° S contained sorbitol and dulcitol (Karsten et al. 1992, 1995*b*, Zuccarello et al. 1999*c*).

***B. tangatensis* Post.** Only one sample from South Africa was analyzed and it contained sorbitol, dulcitol and digeneaside (Karsten et al. 1995*a*). It was also isolated from Madagascar (West et al. 2006*a*). It differed from the morphologically similar species, *B. kelanensis*, which contained only sorbitol and digeneaside.

***B. tenella* (J. V. Lamouroux) J. Agardh.** Many isolates from Puerto Rico, Belize, Panama, Brazil, Philippines, Indonesia and Australia contained sorbitol and dulcitol (Karsten et al. 1992). Only one isolate from the Philippines was analyzed for digeneaside and it was positive (Karsten et al. 1995*a*). Kremer (1976) showed several samples (Australia, Brazil, Venezuela) of *B. binderi* (Harvey) Kuntze (now *B. tenella*, King and Puttock 1989) also contained both polyols.

***B. vaga* J. D. Hooker et Harvey.** Only one sample from the Falkland Islands has been investigated by Kremer (1976) and that contained both sorbitol and dulcitol.

To have a full understanding of the taxonomic and ecological importance of polyols in species of *Bostrychia*, it is clear that more extensive acquisition and analyses of each species is needed throughout their geographic ranges.

Fertilization

The life cycle of *Bostrychia* plants can differ between asexual populations or sexual populations (see above), and the life cycle of red algae has been extensively studied in culture and *in situ*. While stages after fertilization (post-fertilization development) have been well-studied, especially for their utility in red algal systematics (Hommersand and Fredericq 1990), few studies have investigated the early stages of fertilization. While these processes are interesting in and of themselves, aspects of early gamete recognition may also be important in understanding speciation processes in red algae (Brodie and Zuccarello 2007). The cytological events occurring in the early stages of syngamy in red algae have been studied in *Bostrychia* in two seminal papers (Pickett-Heaps and West 1998, Wilson et al. 2002). The spermatial nuclei, after attachment to the trichogyne, divide without undergoing DNA synthesis. There is then cell wall dissolution between the spermatium and the trichogyne (egg extension). After dissolution, the two nuclei enter the trichogyne. Video-microscopy, time-lapse observations revealed that one nucleus traveled towards the carpogonium (egg cell body), while the other nucleus traveled in the opposite direction towards the trichogyne tip. Which nucleus moves in which direction was shown to be random and the first nucleus to emerge does not always move in one direction consistently. The mechanism of this spermatangial differentiation is not known, but the movement involves the actin cytoskeleton and myosin, and is inhibited by a range of specific cytoskeletal-inhibiting drugs. These processes seem to be very similar in another member of the Rhodomelaceae (*Murrayella periclados*) (Wilson et al. 2003), so the mechanism of nuclear movement may not be species specific; other stages (e.g., spermatium attachment, cell wall dissolution, movement of nuclei on cytoplasmic cytoskeleton) could be stages in which barriers to reproductive isolation occur. Intriguingly, the mechanism of spermatial nu-

clei differentiation in movement is unknown. This nature of the mechanism is an interesting cytological question.

Symbioses in *Bostrychia*

Oomycete parasites have been investigated in *Bostrychia*, with a new species of *Olpidiopsis* described (*O. bostrychiae*) (Sekimoto et al. 2009) and its host specificity has been investigated (West et al. 2006*a*, Sekimoto et al. 2009). Parasite specificity and development has also been investigated in two red algal parasites of *Bostrychia*, *Bostrychiocolax australis* Zuccarello and West and *Dawsoniocolax bostrychiae* (Joly et Yamaguishi-Tomita) Joly et Yamaguishi-Tomita (Zuccarello and West 1994*a*, 1994*b*). While red algal parasites are very common in the red algae (Goff 1982), only a few species have been investigated cytologically (e.g., Goff and Coleman 1985, Goff and Zuccarello 1994) and much more work needs to be done on this unique parasitism.

FUTURE DIRECTIONS

The study of different aspects of *Bostrychia* biology has lead to a great deal of understanding into its evolution. This long-running research shows what we can learn with concentrated research on a single genus, but also highlights what little we know.

The aspects of phylogeography discovered early in algal studies pointed out that many evolutionary lineages, which all appear similar morphologically, can be found in any particular population. While cryptic species are not new, and are becoming better known in studies applying molecular data and intensive sampling, its discovery in *Bostrychia* was one of the first in red algae. In *B. radicans* / *B. moritziana*, seven lineages are found and limited inter-lineage crosses suggest that they are reproductively isolated, as would be expected with long-term isolation and genetic drift of compatibility genes (Coyne and Orr 2004). So, it appears that phylogenetic species (supported distinct lineages) are also biological species, i.e., reproductively isolated. But, studies in one lineage in the U. S. also showed that samples within a lineage can be reproductively isolated (Zuccarello and West 2003). So, it may be that genetic data does not reveal all the 'species' possible. This also indicates that reproductive isolation occurs quickly in *Bostrychia*. Reproductively isolated lineages can show little variation in the molecular markers used (e.g., spacers of both the plastid and mitochondrion). Reproductive isolation (reproductive incompatibility and the genetic distinctness of populations) must be one of the major concepts if we wish to understand the production of diversity and the evolution of characters under selection. What controls this isolation is unknown, but results from an early study on the proteome of *B. radicans* / *B. moritziana* (Kim et al. 2008) has suggested useful approaches to investigating specific proteins that vary substantially between lineages or sexual stages of known reproductively compatible and incompatible isolates.

This research has been very fruitful over the years and further investigation in this genus in the coming years will greatly benefit from this accumulated knowledge. Applying new techniques (e.g., next-generation-sequencing) to address questions of speciation in *Bostrychia*, where a solid background of reproductive cycles, reproductive compatibility, genetic diversity and cryptic species has been established, promises to give us significant insights into the processes that produce the wide diversity of red algal species known in the world.

REFERENCES

Álvarez, I. & Wendel, J. F. 2003. Ribosomal ITS sequences and plant phylogenetic inference. Mol. Phylogenet. Evol. 29:417-434.

Bakker, F. T., Breman, F. & Merckx, V. 2006. DNA sequence evolution in fast evolving mitochondrial DNA *nad*1 exons in Geraniaceae and Plantaginaceae. Taxon 55:887-896.

Bown, P., Plumb, J., Sánchez-Baracaldo, P., Hayes, P. K. & Brodie, J. 2003. Sequence heterogeneity of green (Chlorophyta) endophytic algae associated with a population of *Chondrus crispus* (Gigartinaceae, Rhodophyta). Eur. J. Phycol. 38:153-163.

Brodie, J. & Zuccarello, G. C. 2007. Systematics of the species-rich algae: red algal classification, phylogeny and speciation. *In* Hodkinson, T. R. & Parnell, J. A. N. (Eds.) *Reconstructing the Tree of Life: Taxonomy and Systematics of Species Rich Taxa*. CRC Press, Boca Raton, pp. 323-336.

Broom, J. E. S., Farr, T. J. & Nelson, W. A. 2004. *Phylogeny* of the *Bangia* flora of New Zealand suggests a southern origin for Porphyra and Bangia (Bangiales, Rhodophyta). Mol. Phylogenet. Evol. 31:1197-1207.

Butterfield, N. J. 2000. *Bangiomorpha pubescens* n. gen., n. sp.: implications for the evolution of sex, multicellularity, and the Mesoproterozoic / Neoproterozoic radiation of eukaryotes. Paleobiology 26:386-404.

Choi, S. -J., Park, E. -J., Endo, H., Kitade, Y. & Saga, N. 2008.

Inheritance pattern of chloroplast and mitochondrial genomes in artificial hybrids of *Porphyra yezoensis* (Rhodophyta). Fish. Sci. 74:822-829.

Cock, J. M., Coelho, S. M., Brownlee, C. & Taylor, A. R. 2010. The *Ectocarpus* genome sequence: insights into brown algal biology and the evolutionary diversity of the eukaryotes. New Phytol. 188:1-4.

Coyne, J. A. & Orr, H. A. 2004. *Speciation*. Sinauer Associates, Sunderland, MA, 545 pp.

Destombe, C. & Douglas, S. E. 1991. Rubisco spacer sequence divergence in the rhodophyte alga *Gracilaria verrucosa* and closely related species. Curr. Genet. 19:395-398.

Destombe, C. & Douglas, S. E. 1992. Erratum: rubisco spacer sequence divergence in the rhodophyte alga *Gracilaria verrucosa* and closely related species. Curr. Genet. 22:173.

Eggert, A. & Karsten, U. 2010. Low molecular weight carbohydrates in red algae: an ecophysiological and biochemical perspective. *In* Seckbach, J. & Chapman, D. J. (Eds.) *Red Algae in the Genomic Age, Vol. 13*. Springer Academic Press, Dordrecht, pp. 443-456.

Eggert, A., Raimund, S., Van den Daele, K. & Karsten, U. 2006. Biochemical characterization of mannitol metabolism in the unicellular red alga *Dixoniella grisea* (Rhodellophyceae). Eur. J. Phycol. 41:405-413.

Falkenberg, P. 1901. *Die Rhodomelaceen des Golfes von Neapel und der angrenzenden Meeres-Abschnitte. Fauna und Flora des Golfes von Neapel, Monographie* 26. Friedlander, Berlin, 754 pp.

Faugeron, S., Martínez, E. A., Correa, J. A., Cardenas, L., Destombe, C. & Valero, M. 2004. Reduced genetic diversity and increased population differentiation in peripheral and overharvested populations of *Gigartina skottsbergii* (Rhodophyta, Gigartinales) in southern Chile. J. Phycol. 40:454-462.

Faugeron, S., Valero, M., Destombe, C., Martínez, E. A. & Correa, J. A. 2001. Hierarchical spatial structure and discriminant analysis of genetic diversity in the red alga *Mazzaella laminarioides* (Gigartinales, Rhodophyta). J. Phycol. 37:705-716.

Freshwater, D. W., Fredericq, S., Butler, B. S., Hommersand, M. H. & Chase, M. W. 1994. A gene phylogeny of the red algae (Rhodophyta) based on plastid *rbc*L. Proc. Natl. Acad. Sci. U. S. A. 91:7281-7285.

Goff, L. J. 1982. The biology of parasitic red algae. *In* Round, F. E. & Chapman, D. J. (Eds.) *Progress in Phycological Research, Vol. 1*. Elsevier Biomedical Press, New York, pp. 289-369.

Goff, L. J. & Coleman, A. W. 1985. The role of secondary pit connections in red algal parasitism. J. Phycol. 21:483-508.

Goff, L. J., Moon, D. A. & Coleman, A. W. 1994. Molecular delineation of species and species relationships in the red algal agarophytes *Gracilariopsis* and *Gracilaria* (Gracilariales). J. Phycol. 30:521-537.

Goff, L. J. & Zuccarello, G. 1994. The evolution of parasitism in red algae: cellular interactions of adelphoparasites and their hosts. J. Phycol. 30:695-720.

Haag, C. R. & Ebert, D. 2004. A new hypothesis to explain geographic parthenogenesis. Ann. Zool. Fenn. 41:539-544.

Harper, J. T. & Saunders, G. W. 2001. The application of sequences of the ribosomal cistron to the systematics and classification of the florideophyte red algae (Florideophyceae, Rhodophyta). Cah. Biol. Mar. 42:25-38.

Hommersand, M. H. & Fredericq, S. 1990. Sexual reproduction and cystocarp development. *In* Cole, K. M. & Sheath, R. G. (Eds.) *Biology of the Red Algae*. Cambridge University Press, Cambridge, pp. 305-345.

Iomini, C., Till, J. E. & Dutcher, S. K. 2009. Genetic and phenotypic analysis of flagellar assembly mutants in *Chlamydomonas reinhardtii*. Methods Cell Biol. 93:121-143.

Karsten, U., Barrow, K. D., Nixdorf, O., West, J. A. & King, R. J. 1997. Characterization of mannitol metabolism in the mangrove red alga *Caloglossa leprieurii* (Montagne) J. Agardh. Planta 201:173-178.

Karsten, U., Bock, C. & West, J. A. 1995*a*. ^{13}C-NMR spectroscopy as a tool to study organic osmolytes in the mangrove red algal genera *Bostrychia* and *Stictosiphonia* (Ceramiales). Phycol. Res. 43:241-247.

Karsten, U., Bock, C. & West, J. A. 1995*b*. Low molecular weight carbohydrate patterns in geographically different isolates of the eulittoral red alga *Bostrychia tenuissima* from Australia. Bot. Acta. 108:321-326.

Karsten, U., Koch, S., West, J. A. & Kirst, G. O. 1994*a*. The intertidal red alga *Bostrychia simpliciuscula* Harvey ex J. Agardh from a mangrove swamp in Singapore: acclimation to light and salinity. Aquat. Bot. 48:313-323.

Karsten, U., Koch, S., West, J. A. & Kirst, G. O. 1996. Physiological responses of the eulittoral macroalga *Stictosiphonia hookeri* (Rhodomelaceae, Rhodophyta) from Argentina and Chile: salinity, light and temperature acclimation. Eur. J. Phycol. 31:361-368.

Karsten, U., West, J. A. & Ganesan, E. K. 1993. Comparative physiological ecology of *Bostrychia moritziana* (Ceramiales, Rhodophyta) from freshwater and marine habitats. Phycologia 32:401-409.

Karsten, U., West, J. A. & Zuccarello, G. 1992. Polyol content of *Bostrychia* and *Stictosiphonia* (Rhodomelaceae, Rhodophyta) from field and culture. Bot. Mar. 35:11-19.

Karsten, U., West, J. A., Zuccarello, G. C., Engbrodt, R., Yokoyama, A., Hara, Y. & Brodie, J. 2003. Low molecular weight carbohydrates of the Bangiophycidae (Rhodophyta). J. Phycol. 39:584-589.

Karsten, U., West, J. A., Zuccarello, G. & Kirst, G. O. 1994*b*. Physiological ecotypes in the marine red alga *Bostrychia radicans* (Ceramiales, Rhodophyta) from the east coast of the USA. J. Phycol. 30:174-182.

Karsten, U., West, J. A., Zuccarello, G. C., Nixdorf, O., Barrow, K. D. & King, R. J. 1999. Low molecular weight carbohydrate patterns in the Bangiophyceae (Rhodophyta). J. Phycol. 35:967-976.

Kim, G. H., Shim, J. B., Klochkova, T. A., West, J. A. & Zuccarello, G. C. 2008. The utility of proteomics in algal taxonomy: *Bostrychia radicans* / *B. moritziana* (Rhodomelaceae, Rhodophyta) as a model study. J. Phycol. 44:1519-1528.

King, R. J. & Puttock, C. F. 1989. Morphology and taxonomy of *Bostrychia* and *Stictosiphonia* (Rhodomelaceae / Rhodophyta). Aust. Syst. Bot. 2:1-73.

Knowlton, N. & Weigt, L. A. 1998. New dates and new rates for divergence across the Isthmus of Panama. Proc. R. Soc. Lond. Series B Biol. Sci. 265:2257-2263.

Kremer, B. P. 1976. Distribution of alditols in the genus *Bostrychia*. Biochem. Syst. Ecol. 4:139-141.

Kugrens, P. & West, J. A. 1972. Ultrastructure of spermatial development in the parasitic red algae *Levringiella gardneri* and *Erythrocystis saccata*. J. Phycol. 8:331-343.

Markmann, M. & Tautz, D. 2005. Reverse taxonomy: an approach towards determining the diversity of meiobenthic organisms based on ribosomal RNA signature sequences. Philos. Trans. R. Soc. Lond. B Biol. Sci. 360:1917-1924.

Müller, D. G. & Eichenberger, W. 1995. Crossing experiments, lipid-composition, and the species concept in *Ectocarpus siliculosus* and *E. fasciculatus* (Phaeophyceae, Ectocarpales). J. Phycol. 31:173-176.

Müller, D. G., Sengco, M., Wolf, S., Bräutigam, M., Schmid, C. E., Kapp, M. & Knippers, R. 1996. Comparison of two DNA viruses infecting the marine brown algae *Ectocarpus siliculosus* and *E. fasciculatus*. J. Gen. Virol. 77:2329-2333.

Nishimura, Y. 2010. Uniparental inheritance of cpDNA and the genetic control of sexual differentiation in *Chlamydomonas reinhardtii*. J. Plant Res. 123:149-162.

Palumbi, S. R., Cipriano, F. & Hare, M. P. 2001. Predicting nuclear gene coalescence from mitochondrial data: the three-times rule. Evolution 55:859-868.

Pedroche, F. F., West, J. A., Zuccarello, G. C., Sentíes, A. & Karsten, U. 1995. Marine red algae of the mangroves in southern Pacific México and Pacific Guatemala. Bot. Mar. 38:111-119.

Pickett-Heaps, J. D. & West, J. 1998. Time-lapse video observations on sexual plasmogamy in the red alga *Bostrychia*. Eur. J. Phycol. 33:43-56.

Post, E. 1936. Systematische und pflanzengeographische Notizen zur *Bostrychia-Caloglossa*-Assoziation. Rev. Algol. 9:1-84.

Ragan, M. A., Bird, C. J., Rice, E. L., Gutell, R. R., Murphy, C. A. & Singh, R. K. 1994. A molecular phylogeny of the marine red algae (Rhodophyta) based on the nuclear small-subunit rRNA gene. Proc. Natl. Acad. Sci. U. S. A. 91:7276-7280.

Robba, L., Russell, S. J., Barker, G. L. & Brodie, J. 2006. Assessing the use of the mitochondrial *cox*1 marker for use in DNA barcoding of red algae (Rhodophyta). Am. J. Bot. 93:1101-1108.

Saunders, G. W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. Philos. Trans. R. Soc. Lond. B Biol. Sci. 360:1879-1888.

Sekimoto, S., Klochkova, T. A., West, J. A., Beakes, G. W. & Honda, D. 2009. *Olpidiopsis bostrychiae* sp. nov.: an endoparasitic oomycete that infects *Bostrychia* and other red algae (Rhodophyta). Phycologia 48:460-472.

Sunnucks, P., Wilson, A. C. C., Beheregaray, L. B., Zenger, K., French, J. & Taylor, A. C. 2000. SSCP is not so difficult: the application and utility of single-stranded conformation polymorphism in evolutionary biology and molecular ecology. Mol. Ecol. 9:1699-1710.

West, J. A., Klochkova, T. A., Kim, G. H. & Loiseaux-de Goër, S. 2006*a*. *Olpidiopsis* sp., an oomycete from Madagascar that infects *Bostrychia* and other red algae: host species susceptibility. Phycol. Res. 54:72-85.

West, J. A. & Zuccarello, G. C. 1999. Biogeography of sexual and asexual populations in *Bostrychia moritziana* (Rhodomelaceae, Rhodophyta). Phycol. Res. 47:115-123.

West, J. A., Zuccarello, G. & Calumpong, H. P. 1992*a*. *Bostrychia bispora* sp. nov. (Rhodomelaceae, Rhodophyta), an apomictic species from Darwin, Australia: reproduction and development in culture. Phycologia 31:37-52.

West, J. A., Zuccarello, G. C., Hommersand, M., Karsten, U. & Görs, S. 2006*b*. Observations on *Bostrychia radicosa comb. nov.* (Rhodomelaceae, Rhodophyta). Phycol. Res. 54:1-14.

West, J. A., Zuccarello, G. C. & Kamiya, M. 2001. Reproductive patterns of *Caloglossa* species (Delesseriaceae, Rhodophyta) from Australia and New Zealand: multiple origins of asexuality in *C. leprieurii*. Literature review on apomixis, mixed-phase, bisexuality and sexual compat-

ibility. Phycol. Res. 49:183-200.

West, J. A., Zuccarello, G. C. & Karsten, U. 1996. Reproductive biology of *Stictosiphonia hookeri* (Rhodomelaceae, Rhodophyta) from Argentina, Chile, South Africa and Australia in laboratory culture. Hydrobiologia 326/327:277-282.

West, J. A., Zuccarello, G. C., Pedroche, F. F. & Karsten, U. 1992*b*. Marine red algae of the mangroves in Pacific México and their polyol content. Bot. Mar. 35:567-572.

White, T. J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J. (Eds.) *PCR Protocols: A Guide to Methods and Applications*. Academic Press, San Diego, pp. 315-322.

Wilson, S. M., Pickett-Heaps, J. D. & West, J. A. 2002. Fertilization and the cytoskeleton in the red alga *Bostrychia moritziana* (Rhodomelaceae, Rhodophyta). Eur. J. Phycol. 37:509-522.

Wilson, S. M., West, J. A. & Pickett-Heaps, J. D. 2003. Time-lapse videomicroscopy of fertilization and the actin cytoskeleton in *Murrayella periclados* (Rhodomelaceae, Rhodophyta). Phycologia 42:638-645.

Woese, C. R. 2000. Interpreting the universal phylogenetic tree. Proc. Natl. Acad. Sci. U. S. A. 97:8392-8396.

Woese, C. R. & Fox, G. E. 1977. Phylogenetic structure of prokaryotic domain: the primary kingdoms. Proc. Natl. Acad. Sci. U. S. A. 74:5088-5090.

Zuccarello, G. C., Buchanan, J., West, J. A. & Pedroche, F. F. 2011. Genetic diversity of the mangrove-associated alga *Bostrychia radicans* / *B. moritziana* (Ceramiales, Rhodophyta) from southern Central America. *Phycological Research* (in press).

Zuccarello, G. C., Burger, G., West, J. A. & King, R. J. 1999*a*. A mitochondrial marker for red algal intraspecific relationships. Mol. Ecol. 8:1443-1447.

Zuccarello, G. C. & West, J. A. 1994*a*. Comparative development of the red algal parasites *Bostrychiocolax australis* gen. et sp. nov. and *Dawsoniocolax bostrychiae* (Choreocolacaceae, Rhodophyta). J. Phycol. 30:137-146.

Zuccarello, G. C. & West, J. A. 1994*b*. Host-specificity in the red algal parasites *Bostrychiocolax australis* and *Dawsoniocolax bostrychiae* (Choreocolacaceae, Rhodophyta). J. Phycol. 30:462-473.

Zuccarello, G. C. & West, J. A. 1995. Hybridization studies in *Bostrychia*: 1. *B. radicans* (Rhodomelaceae, Rhodophyta) from Pacific and Atlantic North America. Phycol. Res. 43:233-240.

Zuccarello, G. C. & West, J. A. 1997. Hybridization studies in *Bostrychia*: 2. Correlation of crossing data and plastid DNA sequence data within *B. radicans* and *B. moritziana* (Ceramiales, Rhodophyta). Phycologia 36:293-304.

Zuccarello, G. C. & West, J. A. 2002. Phylogeography of the *Bostrychia calliptera-B. pinnata* complex (Rhodomelaceae, Rhodophyta) and divergence rates based on nuclear, mitochondrial and plastid DNA markers. Phycologia 41:49-60.

Zuccarello, G. C. & West, J. A. 2003. Multiple cryptic species: molecular diversity and reproductive isolation in the *Bostrychia radicans* / *B. moritziana* complex (Rhodomelaceae, Rhodophyta) with focus on North American isolates. J. Phycol. 39:948-959.

Zuccarello, G. C. & West, J. A. 2006. Molecular phylogeny of the subfamily Bostrychioideae (Ceramiales, Rhodophyta): subsuming *Stictosiphonia* and highlighting polyphyly in species of *Bostrychia*. Phycologia 45:24-36.

Zuccarello, G. C. & West, J. A. 2008. *Bostrychia* (Rhodomelaceae, Rhodophyta) species of New Zealand, and relationships in the Southern Hemisphere. N. Z. J. Mar. Freshw. Res. 42:315-324.

Zuccarello, G. C., West, J. A. & Goer, S. L. D. 2006. Diversity of the *Bostrychia radicans* / *Bostrychia moritziana* species complex (Rhodomelaceae, Rhodophyta) in the mangroves of New Caledonia. Cryptogam. Algol. 27:245-254.

Zuccarello, G. C., West, J. A., Kamiya, M. & King, R. J. 1999*b*. A rapid method to score plastid haplotypes in red seaweeds and its use in determining parental inheritance of plastids in the red alga *Bostrychia* (Ceramiales). Hydrobiologia 401:207-214.

Zuccarello, G. C., West, J. A., Karsten, U. & King, R. J. 1999*c*. Molecular relationships within *Bostrychia tenuissima* (Rhodomelaceae, Rhodophyta). Phycol. Res. 47:81-85.

Zuccarello, G. C., West, J. A. & King, R. J. 1999*d*. Evolutionary divergence in the *Bostrychia moritziana* / *B. radicans* complex (Rhodomelaceae, Rhodophyta): molecular and hybridization data. Phycologia 38:234-244.

Zuccarello, G. C., Yeates, P. H., Wright, J. T. & Bartlett, J. 2001. Population structure and physiological differentiation of haplotypes of *Caloglossa leprieurii* (Rhodophyta) in a mangrove intertidal zone. J. Phycol. 37:235-244.