Fábio Nauer da Silva

Definindo espécies e sua distribuição: um estudo de caso em *Hypnea* (Gigartinales, Rhodophyta)

Defining species and their distribution: a case study with *Hypnea* (Gigartinales, Rhodophyta)

São Paulo
2018

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Orientadora: Prof[a] Dr[a] Mariana Cabral de Oliveira

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Prof(a). Dr(a).

“The sea, once it casts its spell, holds one in its net of wonder forever.”

Jacques Yves Cousteau

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***MUITO OBRIGADO*!!!**

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Resumo Geral

O gênero *Hypnea* Lamouroux (1813) e particularmente a espécie *H. musciformis* (Wulfen) J.V.Lamouroux apresentam grande importância ecológica e econômica como matéria prima para a produção de carragenana, com ampla distribuição geográfica em águas tropicais e sub-tropicais ao redor do mundo. No entanto, a identificação das espécies de *Hypnea* com base apenas em dados morfológicos é dificultada devido à plasticidade fenotípica presente neste grupo e sua morfologia relativamente simples. Em vista disso, diversas ferramentas de estudo para delimitar espécies dentro do gênero e sua distribuição geográfica foram utilizados neste trabalho, para testar duas hipóteses principais: 1) *Hypnea musciformis* forma um complexo de espécies proximamente relacionadas, mas com distribuição geográfica distinta e 2) Os variantes morfológicos "musciformis" e "nigrescens" de *H. pseudomusciformis* que ocorrem na costa Brasileira correspondem a uma única espécie biológica. Com base em marcadores moleculares e análises morfológicas, confirmamos a ocorrência de nove espécies diferentes na costa do Brasil: *H. brasiliensis*, *H. cervicornis*, *H. edeniana*, *H. flava*, *H. pseudomusciformis*, *H. platyclada*, *H. spinella*, *H. yokoyana* e *H. wynnei*. E duas espécies não descritas, *Hypnea* sp.1 e *Hypnea* sp2. Além disso, dados de cruzamento com linhagens distintas de *H. pseudomusciformis* indicam a separação em duas espécies, sendo *H. pseudomusciformis* restrita ao Sudeste e Sul do país e *H.* sp. 5 restrita ao Nordeste, elevando o número total de espécies do país para 12. Análises filogeográficas mostram que *H. musciformis* possui distribuição ao longo do Hemisfério Norte, enquanto que *H. pseudomusciformis* possui distribuição ao longo do Hemisfério Sul. Além disso, foram detectados fortes padrões de estrutura genética e foram reconhecidas distintas zonas filogeográficas marinhas: a província do Uruguai, a província do sul do Brasil, a província do Rio de Janeiro, a província do leste do Brasil e a província do norte do Brasil. O grau de isolamento genético e distinção entre essas províncias variou consideravelmente. A maior diversidade genética foi encontrada na região de ressurgencia de Cabo Frio, no estado do Rio de Janeiro, uma região conhecida pela presença de um número diversificado de habitats microclimáticos distintos. Esses resultados corroboram a hipótese 1. Para testar a hipótese 2, foram coletados indivíduos das duas variantes morfológicas de *H. pseudomusciformis*, "musciformis" e

“nigrescens” que foram cultivados *in vitro* nas mesmas condições. O histórico de vida de ambas as variantes morfológicas foi completado em aproximadamente 121 dias. Quando cultivados *in vitro*, as diferenças morfológicas entre as variantes "musciformis" e "nigrescens" foram atenuadas. O conteúdo de pigmentos fotossintetizantes (clorofila a, carotenóides e ficobiliproteínas), proteínas solúveis totais e capacidade antioxidante (DPPH, ABTS, capacidade de redução de Folin-Ciocalteu, FRAP e capacidade de quelação de ferro) também foram analisados para amostras dessas duas variantes morfológicas coletadas no campo e cultivadas *in vitro*. A variação "nigrescens" coletada no campo apresentou mais ficobiliproteínas, proteínas solúveis totais e apresentou maior atividade antioxidante para os ensaios ABTS e Folin-Ciocalteu do que a variação "musciformis". As amostras em cultura de ambas as variações morfológicas não mostraram diferenças significativas nos pigmentos e atividade antioxidante, exceto para aloficocianina, que foi maior em "musciformis". Estes dados indicam que as diferenças encontradas entre "musciformis" e "nigrescens" são principalmente devidas a pressões ambientais e representam um exemplo de plasticidade fenotípica. Além disso, foram realizados experimentos de cruzamento *in vitro* entre gametófitos de "musciformis" e "nigrescens". Esses cruzamentos resultaram na formação de cistocarpos, que após a liberação dos esporos, originaram novos tetrasporófitos, que posteriormente ficaram férteis liberando tetrásporos que se desenvolveram em novos gametófitos, confirmando os dados moleculares que mostram que essas variantes pertencem à mesma espécie biológica, corroborando a hipótese 2.

Abstract

The genus *Hypnea* Lamouroux (1813) and particularly *H. musciformis* (Wulfen) J.V.Lamouroux present great economic and ecological importance as source for the production of carrageenan, with a wide geographic distribution in tropical and sub-tropical waters around the world. However, the identification of *Hypnea* species based only on morphological data is hampered by phenotypic plasticity and its relatively simple morphology. In this work, several approaches were used to study species of the genus *Hypnea*, based on two hypothesis: 1) The species *H. musciformis* is a complex of closely related species, but with a distinct geographic distribution. 2) The morphological variants "musciformis" and "nigrescens" of *H. pseudomusciformis* occurring on the Brazilian coast correspond to a single biological species. Based on DNA barcode molecular markers and morphological analyzes, we confirmed the occurrence of nine different species for the genus *Hypnea* on the coast of Brazil: *H. brasiliensis*, *H. cervicornis*, *H. edeniana*, *H. flava*, *H. pseudomusciformis*, *H. platyclada*, *H. spinella*, *H. yokoyana* and *H. wynnei*. And two undescribed species, *H.* sp.1 and *H.* sp2. In addition, cross-breeding tests were made within two linaeges of *H. pseudomusciformis* , the resultus indicate the segregation in two distint species, with *H. pseudomusciformis* restrict to Southeast and South of the country and H. sp. 5 to Northeast, elevating the total number of species to 12. Phylogeographic analyzes show that *H. musciformis* is distributed in the Northern Hemisphere of the planet, while *H. pseudomusciformis* is distributed in the Southern Hemisphere. In addition, strong patterns of genetic structure were detected and distinct marine phytogeographic zones were recognized: the province of Uruguay, the province of southern Brazil, the province of Rio de Janeiro, the province of eastern Brazil and the province of northern Brazil. The degree of genetic isolation and distinction between these provinces varied considerably. The greatest genetic diversity was found in Cabo Frio, in the state of Rio de Janeiro, a upwelling region known for the presence of a diverse number of distinct microclimatic habitats. These results corroborate hypothesis 1. To test hypothesis 2, individuals of the two morphological variants of *H. pseudomusciformis*, "musciformis" and "nigrescens" were collected and kept under the same *in vitro* culture conditions. The life history of both morphological variants was completed in approximately 121 days. When cultured *in*

vitro, the morphological differences between the "musciformis" and "nigrescens" variants were attenuated. The content of photosynthetic pigments (chlorophyll a, carotenoids and phycobiliproteins), total soluble proteins and antioxidant capacity (DPPH, ABTS, reduction capacity of Folin-Ciocalteu, FRAP and iron chelation capacity) were also analyzed for samples of these two morphological variants collected in the field and from *in vitro* cultures. The "nigrescens" variation collected in the field showed more phycobiliproteins, total soluble proteins and presented greater antioxidant activity for ABTS and Folin-Ciocalteu than the "musciformis" variation. Samples maintained *in vitro* of both morphological variations did not show significant differences in pigment and antioxidant activity, except for allophycocyanin, which was higher in "musciformis". These data indicate that the differences found between "musciformis" and "nigrescens" are due to environmental pressures and represent an example of phenotypic plasticity. In addition, *in vitro* crossing experiments were performed between gametophytes of "musciformis" and "nigrescens". These crossings generated cystocarps and, after the spores were released, new tetrasporophytes were observed, which later produced tetraspores that germinated forming new gametophytes, confirming the molecular data that show that these variants belong to the same biological species, corroborating the hypothesis 2.

CAPÍTULO

Um

Introdução Geral

1.1. O gênero *Hypnea* J. V. Lamoroux 1813

O gênero *Hypnea* (Rhodophyta) foi nomeado por Lamouroux em 1813. Atualmente são descritas atualmente 121 espécies (Guiry & Guiry 2017). Conhecida por sua ampla distribuição geográfica, espécies do gênero *Hypnea* são encontradas em mares tropicais e subtropicais de todo o globo. O oceano Índico destaca-se por deter o maior número de espécies, seguido pelo oceano Pacífico e em terceiro lugar pelo Atlântico (Mshigeni & Chapman 1994).

Hypnea é caracterizada por possuir talo ereto (Figura 1), abundantemente ramificado com ramos curtos muitas vezes espinescentes. O crescimento se dá por uma célula apical, mas o formato dos ápices dos ramos é bastante variável, muitas vezes em formato de ganchos e gavinhas que essas algas utilizam para se enroscarem e crescerem de forma epifítica sobre outras algas. O histórico de vida é diplobionte trifásico, sendo duas fases diplóides e uma fase haplóide (Quadro 1).

Dentre todas as espécies do gênero, a espécie *H. musciformis* (Wulfen) J. V. Lamouroux destaca-se por ser um importante recurso marinho que pode ser utilizado como fonte de carragenana do tipo K (Reis et al. 2006), um polissacarídeo sulfatado presente na parede celular da alga que é largamente utilizado na indústria alimentícia e farmacêutica, como agente espessante, estabilizante e gelificante. Além disso, a carragenana apresenta atividades antivirais, inibindo, por exemplo, o vírus HSV e o vírus HIV (Neushul 1990). Estudos têm mostrado que *H. musciformis* produz lectinas, que são importantes substâncias utilizadas como agentes pró e anti-inflamatórios e no diagnóstico e terapia em oncologia (Nagano *et al.* 2002).

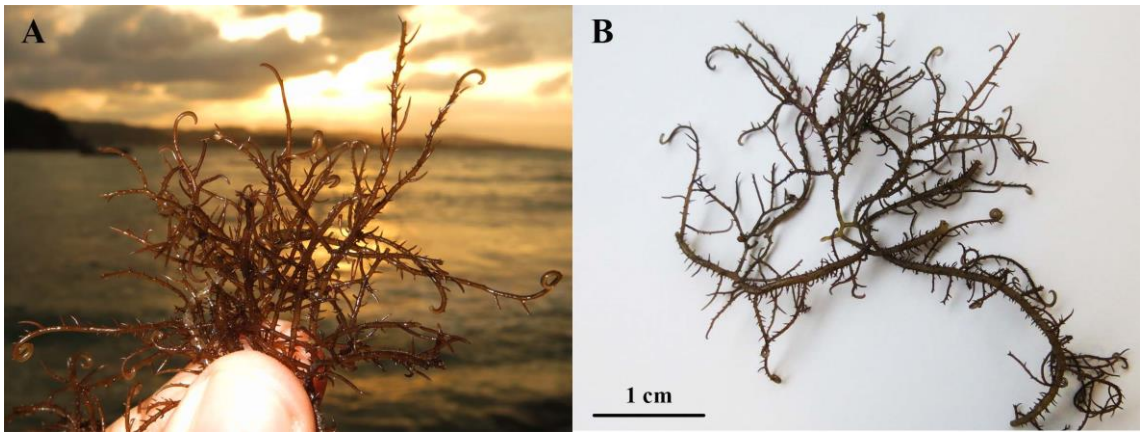


Figura 1. Hábito em campo de um exemplar de *Hypnea*. **A.** Indivíduo com gavinhas. **B.** Indivíduo onde é possível observar os inúmeros râmulos espinescentes e ápices terminando em ganchos e gavinhas.

Em 1999, o Brasil importou cerca de 1.000 toneladas de carragenana e produziu uma tonelada por mês (Furtado 1999). Em 2004, o consumo de carragenana passou para 100 toneladas por mês e a produção para 10 toneladas por mês, a partir de biomassa coletada em bancos naturais de *H. musciformis* da região nordeste do país (Furtado 2004). A preocupação com a diminuição da matéria algácea no ambiente levou a realização de estudos com populações de *H. musciformis* distribuídas ao longo da costa brasileira, que constataram que há uma ampla variação na biomassa e no teor de carragenanas ao longo do ano, algumas vezes relacionada à sazonalidade (Schenkman 1986, Faccini & Berchez 2000, Ramos *et al.* 2006). Uma vez que a produção de carragenana a partir de populações naturais é muito variável e com a finalidade de diminuir a exploração dessas populações, foram realizados estudos para determinar a viabilidade da maricultura de *H. musciformis* (Oliveira-Filho & Berchez 1987, Berchez & Oliveira-Filho 1990, Berchez *et al.* 1993, Lima *et al.* 1981, Câmara Neto 1987, Reis *et al.* 1998, Reis *et al.* 2006).

QUADRO 1: HISTÓRICO DE VIDA EM *Hypnea* (Fig.2)

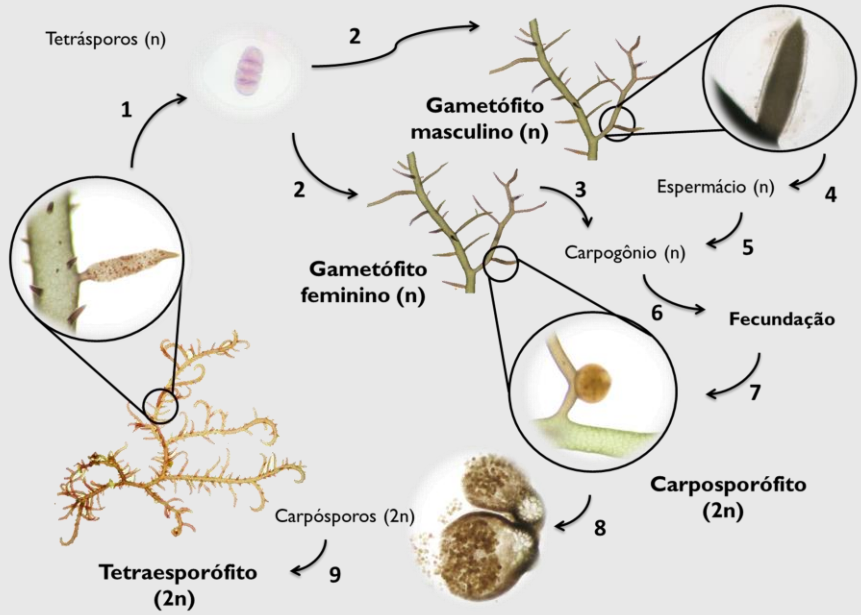


Figura 2. Histórico de vida em *Hypnea*.

O histórico de vida é diplobionte trifásico, típico das Florideofíceas, onde terasporófito é diploide e de vida livre. Os tetrasporângios são zonados, formados na região cortical dos râmulos dos tetraspróftos produzindo soros escuros e proeminentes. Os tetrasporângios podem ser encontrados envolvendo completamente ou parcialmente a base, o ápice ou o meio dos râmulos laterais. Os tetrásporos são produzidos através de meiose (meiose espórica) e liberados no ambiente. Os tetrásporos germinam dando origem a gametófitos haplóides masculinos e femininos. Gametófitos e tetrasporófitos são isomórficos.

QUADRO 1 (Continuação)

No gametófito masculino, os espermatângios são organizados em cadeia na região cortical dos râmulos e produzem espermácios por mitose. Já no gametófito feminino são produzidos carpogônios, também por mitose. Os espermácios são transportados passivamente até a tricogine no gametófito feminino e a fecundação é realizada no carpogônio. O zigoto desenvolve-se na terceira fase do ciclo, o carposporófito. O carposporófito cresce no interior do gametófito feminino e é tido como uma fase parasita, formando estruturas chamadas cistocarpos, onde o carposporófito diplóide é envolvido por uma camada de células haplóides do gametófito feminino, o pericarpo. Os cistocarpos das espécies de *Hypnea* são protuberantes e estão situados nos ramos e râmulos do gametófito feminino quase sempre visíveis a olho nú, podendo ser globosos ou subesféricos. Carpósporos diplóides são produzidos pelo carposporófito por mitose, que quando liberados originam o tetrasporófito, completando o histórico de vida (Masuda *et al.* 1997,Graham & Wilcox 2009).

Apesar da importância do gênero, sua taxonomia é bastante problemática e confusa (Price et al. 1992). Os problemas encontrados na discriminação das espécies de *Hypnea* devem-se ao fato dessas algas possuírem uma morfologia simples, muitas vezes influenciada pelas condições ambientais. Alguns táxons de *Hypnea* são tratados como complexos devido à difícil separação das espécies, a exemplo do complexo *H. charoides-valentiae* (Abbott 1999). Entretanto, alguns autores sugerem ainda que espécimes de *H. musciformis* são facilmente reconhecidos, dentre todas as demais espécies do gênero pela presença de gavinhas e ramos espinescentes abundantes (Price et al. 1992, Abbott 1999, Dawes & Mathieson 2008).

No Brasil, Schenkman (1986) diz que as espécies *H. musciformis*, *H. nigrescens* (Wulfen) J. V. Lamouroux e *H. valentiae* (Turner) Montagne podem ser confundidas

entre si, e somente a análise conjunta de habitat, morfologia, anatomia e crescimento permitem a correta identificação. Em 2011, Guimarães realizou o primeiro estudo do gênero, para a costa do estado de São Paulo, com base em marcadores moleculares (COI-5P, UPA e *rbc*L). A autora apontou que as amostras identificadas como *H. nigrescens* para o estado de São Paulo representariam, na verdade, uma variação morfológica de *H. musciformis*. Jesus (2012), ao contrário, em um estudo com o gênero para o estado da Bahia, com base na morfologia das espécies, corroborou os dados de Schenkman (1986) na separação de *H. musciformis* e *H. nigrescens*.

Nauer *et al.* (2015) analisaram espécimes provenientes do Mar Adriático, Itália, localidade tipo de *H. musciformis*, e encontraram divergências significativas nos marcadores moleculares utilizados em relação aos espécimes identificados como a mesma espécie coletados no litoral brasileiro. Os exemplares italianos apresentam hábito do talo semelhante a parte dos espécimes brasileiros, com talo longo, flexível, abundantemente ramificado e com presença de gavinhas nos ápices dos ramos. Entretanto, o resultado obtido com três marcadores moleculares (COI, UPA e *rbc*L) indicaram que os exemplares brasileiros identificados como *H. musciformis* possuem divergência genética com os espécimes da localidade-tipo, o que levou a descrição de uma espécie nova para a ciência, denominada *H. pseudomusciformis* Nauer, Cassano & M.C. Oliveira.

Além disso, as análises moleculares de Nauer et al. (2015) mostraram que os espécimes brasileiros identificados como *H. musciformis*, *H. nigrescens* e *H. valentiae* com base em dados morfológicos, correspondiam a variações morfológicas de *H. pseudomusciformis*. Além de apresentarem distinção morfológica, esses espécimes habitam zonas distintas no costão rochoso (Figura 3). Nos costões rochosos, geralmente observa-se uma transição das variações morfológicas *H. pseudomusciformis* do mesolitoral superior em direção ao infralitoral superior, principalmente em costões rochosos com alto hidrodinamismo.

Do mesolitoral superior em direção à linha d'água, os espécimes encontrados apresentaram variação morfológica cujas características levaram a identificá-los previamente como *H. valentiae*. Nesses espécimes, o talo apresenta um eixo principal cilíndrico com até 5 cm de altura, abundantemente ramificado, com ramificações em ângulo agudo ou reto. Râmulos espinescentes são abundantes e gavinhas estão ausentes;

os ápices dos ramos são retos ou recurvados. Podem ser epilíticos ou epífitos, principalmente de algas calcárias articuladas como *Amphiroa* e *Jania* em costões protegidos. Exemplares coletados nas regiões sudeste, sul e nordeste com essas características foram identificados como *H. valentiae* por Schenkman (1986) e Jesus (2012). Mas, usando dados dos marcadores moleculares, esses exemplares foram reagrupados dentro de *H. pseudomusciformis* (Guimarães 2011, Nauer et al. 2015). Além disso, esses espécimes não apresentam râmulos estrelaçados que podem estar presentes em *H. valentiae* (Schneider & Searles 1991, Mshigeni & Chapman 1994).

Outra variação morfológica, identificada previamente como *H. nigrescens*, é encontrada exclusivamente em costões com forte impacto de ondas, crescento epiliticamente na região entremarés. Espécimes coletados nessa região possuem colorações variando de vermelho-vinácea, vermelho-enegrecida, verde claro e verde escuro, o talo é mais robusto e rígido, formando tufos de múltiplos eixos principais com até 8 cm de altura, apresentando numerosos râmulos espinescentes longos e finos. Gavinhas podem estar presentes nos ápices dos ramos laterais, porém é mais comum a presença de ápices retos. Essa variação morfológica caracteriza-se por ser uma forma mais robusta que a variação morfológica anterior, apresentando ramos mais longos e uniformes, podendo chegar a 9 cm de altura, formando talos que lembram cerdas de um pincel. Os râmulos espinescentes também são mais longos e numerosos, não mais lembrando pequenos espinhos. Os ápices dos ramos podem ser retos, recurvados ou em forma de gavinhas. Formas epífitas não foram encontradas. Esses exemplares foram identificados por Schenkman (1986) e Jesus (2012) como *H. nigrescens*, porém os resultados com marcadores moleculares (Guimarães 2011, Nauer et al. 2015), colocam a espécie *H. nigrescens* como uma variação morfológica de *H. musciformis* para os estado de São Paulo.

Na região do infralitoral superior, principalmente em ambientes mais calmos, são encontrados indivíduos com a morfologia típica de *H. musciformis*, definida pela presença abundante de gavinhas, numerosos râmulos espinescentes e talo flexível, epífita de outras algas, podendo formar grandes populações. O tamanho e número de ramificações e de râmulos espinescentes variam em relação as regiões nordeste e sudeste e sul do país.

Figura 3. Praia de Subaúma, na Bahia, mostrando que é possível encontrar as variações morfológicas de *H. pseudomusciformis* crescendo proximamente, mas em diferentes microambientes sugeitos a diferentes condições ambientais.

Os exemplares da região nordeste apresentam quase o triplo do tamanho dos exemplares do resto do país, podendo chegar até 16 cm de altura, possuindo talo com maior número de ramificações e numerosos e longos râmulos espinescentes, sendo que um ramo espinescente pode chegar até 1,5 cm de comprimento (Jesus 2012). São facilmente encontrados epifitando espécies de diversos gêneros como *Cryptonemia* J.Agardh, *Gracilaria* Greville, *Palisada* K. W. Nam, *Sargassum* C. Agardh e *Ulva* Linnaeus (Jesus 2012). Os exemplares das regiões sudeste e sul são expressivamente menores em tamanho, podendo alcançar até 7 cm de altura, portando ramificações em menor número e râmulos espinescentes menos numerosos, em comparação aos exemplares do nordeste, porém mais abundantes do que em comparação com outras espécies do gênero. São encontrados epifitando gêneros como *Amphiroa* J.V.

Lamouroux, *Chondracanthus* Kützing, *Jania* J.V. Lamouroux, *Laurencia* J.V. Lamouroux, *Palisada*, *Sargassum*, *Ulva*, entre outros.

1.2. Definindo espécie: conceitos, plasticidade fenotípica e convergência morfológica.

Dentre os divesos grupos de organismos conhecidos, as algas estão entre os mais difíceis de serem estudados a nível taxonômico. O termo "alga" refere-se a um conjunto de organismos heterogêneo, que variam em tamanho desde pequenas células à seres vivos com mais de 50 m de comprimento e que representam linhagens distintas, muitas vezes sem relação filogenética. Segundo estimativas, o número total de espécies de algas pode variar de 36.000 (John & Maggs 1997) à mais de 10 milhões (Norton et al. 1996). A descrição de novas espécies é frequente e com o avanço das técnicas moleculares a taxonomia dos diferentes grupos de algas está em constante mudança. Um dos fatores que explicam essa confusão na quantidade e taxonomia de espécies de algas relaciona-se com a presença de alta plasticidade fenotípica que algumas espécies podem apresentar, incluindo aqui as espécies do gênero *Hypnea*.

Plasticidade fenotípica consiste na capacidade dos organismos de sofrer alterações de acordo com as condições do ambiente. Também pode ser definida como "a habilidade de um genótipo de produzir mais de um fenótipo quando exposto a diferentes condições ambientais". O termo plasticidade fenotípica abrange todos os tipos de mudanças induzidas pelo ambiente, sejam elas morfológicas, fisiológicas, comportamentais e fenológicas (Raven 2001).

O efeito (hipotético) da plasticidade fenotípica é que duas populações clonais (geneticamente idênticas) expostas cada uma à diferentes desafios ambientais, apresentam respostas (no decorrer das gerações) diferentes entre si a fim de sobreviver e se perpetuar em ambos os ambientes. Essas transformações podem ou não ser permanentes no ciclo de vida do indivíduo. Usado de forma ampla, descrevendo todas as mudanças fenotípicas em resposta à mudança ambiental, como a aclimatação e o aprendizado. O genoma codifica uma variedade potencial de fenótipos, e o ambiente

seleciona aquele fenótipo que usualmente é o mais adaptativo (Zuccarello *et al*., 1999; Engel *et al*., 2004).

Segundo Raven (2001), a capacidade do indivíduo de apresentar diferentes respostas ao ambiente pode ser ela própria um atributo desenvolvido. A plasticidade é maior em plantas (aqui incluindo-se também as algas vermelhas) do que em animais devido ao padrão de crescimento indeterminado e pode ser mais facilmente modificado para produzir expressões diferentes de um determinado genótipo. Outro fator que contribui para a maior abundância de plasticidade fenotípica em plantas do que em animais é o fato delas frequentemente estarem fixas ao substrato, ou seja, não apresentam capacidade de mudar de um ambiente caso as condições ambientais não forem favoráveis. As espécies que apresentam plasticidade fenotípica em caracteres relacionados à sobrevivência possuem vantagens adaptativas em ambientes heterogêneos, instáveis ou de transição, já que as mudanças podem facilitar a exploração de novos nichos, ocasionando o aumento da tolerância ambiental (Ford 1964, Innes 1984, Plastino & Guimarães, 2001).

A plasticidade fenotípica dentro de *Hypnea* é comum o que resultou em variações morfológicas de uma mesma espécie sendo descritas e catalogadas como espécies distintas, como exemplo de caso entre *H. musciformis*, *H. nigrescens* e *H. valentiae* relatados previamente nesse capítulo. Mas esse não é o único caso relatado para as espécies brasileiras do gênero. Jesus et al. (2016) colocou em sinonímia com *H. cervicornis* J. Agardh, outra espécie de ampla distribuição na costa brasileira, espécies como *H. aspera* Kützing, *H. boergesenii* e *H. flexicaulis*.

Para complicar o cenário taxonômico de *Hypnea*, com o uso das técnicas moleculares foram encontrados espécimes idênticos morfologicamente, mas que apresentavam grandes diferenças genéticas, ou seja, formando espécies crípticas. As espécies *H. musciformis* e *H. pseudomusciformis* são espécies proximamente relacionadas cuja a identificação só foi possível com base em marcadores moleculares, como o COI. O complexo *H. cornuta-stellulifera* é outro exemplo, onde *H. stellulifera* (J. Agardh) Yamagishi et Masuda foi descrita e separada de *H. cornuta* J. Agardh com base também no marcador COI, porém suas morfologias são idênticas. O mesmo vale para *H. edeniana* Nauer, Cassano & M.C. Oliveira e *H. flava* Nauer, Cassano & M.C.

Oliveira, duas novas espécies descritas para a costa brasileira com base em marcadores moleculares (Nauer et al. 2014).

Quantificar a biodiversidade e numerar e catalogar as espécies, não somente do gênero *Hypnea* mas de basicamente qualquer organismo vivo do planeta, é necessário levar em conta os diferentes conceitos e abordagens do que é, de fato, uma espécie. Partindo de Platão, passando por Aristóteles, Lineu, Darwin e outros, a definição do que vem a ser uma espécie ainda é um problema. O termo "espécie", em latim, significa simplesmente "tipo", ou seja, tipos diferentes de organismos. Foi graças a Lineu (séculos XVII – XVIII), que a classificação dos seres vivos passou a representar categorias taxonômicas, baseada numa rede de comparações entre eles, através da observação cuidadosa dos organismos, estabelecendo as bases do que é chamado atualmente como **Conceito Morfológico de Espécie** (Pereira & Soares-Gomes 2009). É deste período, também, o primeiro conceito de espécie propriamente dito, que incluía, além da simples semelhança, a ideia de continuidade através de gerações, o que ficou conhecido, posteriormente como **Conceito Biológico de Espécie**, onde espécies são populações que devem ser definidas pelo seu isolamento reprodutivo e não pelas suas semelhanças ou diferenças e que o isolamento reprodutivo deve ser primariamente intrínseco (genético) ao invés de extrínseco (barreira geográfica) (Pereira & Soares-Gomes 2009).

Um dos problemas de aplicação do Conceito Biológico de Espécie para alguns grupos de organismos ocorre justamente por este se basear no isolamento reprodutivo, já que o isolamento reprodutivo não é critério universal e a capacidade de intercruzamento da grande maioria das espécies é desconhecida. Com isso, conceitos alternativos têm sido propostos e, atualmente, existem dezenas de conceitos de espécies diferentes. Foi a partir das ideias de Darwin no século XIX, que a Taxonomia e a Sistemática passaram a incluir a Filogenia em suas classificações. Foi nesse período que a espécie passou a ser considerada uma unidade evolutiva, levando posteriormente ao **Conceito Filogenético de Espécie**, onde espécies representam grupos monofiléticos, compartilhando um ancestral comum. O status de espécie é decidido baseado principalmente na coesão fenotípica dentro dos grupos contra a descontinuidade fenotípica entre os grupos. Portanto, o conceito filogenético de espécie dá ênfase ao aspecto mais geral da diversidade biológica, o processo de diferenciação, não importando se este é seguido ou não de isolamento reprodutivo. A maior vantagem

deste conceito é a sua universalidade, podendo ser usado para espécies de reprodução assexuada e fósseis (Pereira & Soares-Gomes 2009).

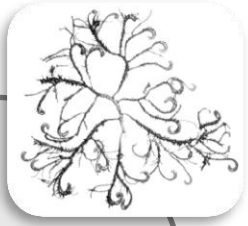
Comumente, caracteres morfológicos são usados para definir espécies, contudo é um desafio definir quais caracteres morfológicos são estáveis dentro de uma espécie e filogeneticamente informativos. Isto significa que a variação fenotípica dentro de grupos pode ser interpretada de maneira diversa, sendo, portanto, subjetiva, e a decisão sobre o que é significativo ou não para a definição de um espécie acaba dependendo do olhar do especialista (De Queiroz 2007). Além disto, o número de espécies reconhecidas pode ser dependente dos métodos usados e seu poder de resolução para identificar caracteres. No ambiente marinho, por exemplo, grupos taxonômicos como algas, esponjas e corais apresentam um número restrito de caracteres pelos quais podem ser classificados. Como consequência, grande número de espécies nestes grupos foi definido no passado como sendo cosmopolita (De Queiroz 2007). De modo geral, é desejável que um conceito de espécie apresente três aspectos chaves: I) Universalidade (se é aplicável a todos os tipos de espécie: sexuada e assexuada, fósseis e ainda vivas, etc.); II) Operacionalidade (aplicabilidade prática do conceito) e III) Significado biológico (o quanto o conceito é coerente com aquilo que entendemos do processo de especiação) (Mayden 1997; Hey 2006).

Nos dias atuais, é possível encontrar diversos conceitos de espécie na literatura científica, dos quais os principais estão no Quadro 2. Nem todos os conceitos foram igualmente bem caracterizados ou explicitamente definidos. Muitos deles foram concebidos para atender necessidades particulares, sendo alguns teorizados com base em dados empíricos, outros apenas em conceitos teóricos e outros criados com base nas características peculiares do organismo estudado (De Queiroz 2007). Muitos desses conceitos e suas definições associadas são incompatíveis na medida em que podem levar a conclusões diferentes sobre as delimitações e número de espécies (Figura 4). Portanto, o problema do conceito de espécie - isto é, os desentendimentos atuais sobre o conceito teórico da espécie – está estreitamente ligada à questão da delimitação de espécies – isto é, como determinar os limites e números de espécie de dados empíricos (Mayden 1997; Hey 2006).

Para Hey (2001) “Pesquisadores usam o conceito de espécie mais apropriado para o grupo de organismos que estudam no momento. Então, biólogos que estudam

animais, provavelmente compartilham de uma visão do que é uma espécie, enquanto que aqueles que estudam plantas que formam híbridos, compartilham de outra, e aqueles que estudam formas de vida que, raramente, se não nunca, trocam genes, também terão outra visão distinta". Já Rosselló-Mora (2003) defende a existência de diversos conceitos de espécie e diz que "A variedade dos conceitos de espécie é necessária para adequar a complexidade da variação de padrão na natureza. Subsumir essa variação dentro de qualquer conceito leva a confusão e tende a obscurecer importantes questões evolutivas. Nós precisamos resistir a urgência de impor uma falsa simplicidade. Se as "espécies" são diversas, então uma variedade de conceitos é necessária para refletir essa complexidade".

Rosselló-Mora (2003) encerra ilustrando a importância de se estudar não somente a biodiversidade, mas também os processos que a criam, dizendo que "Uma tendência histórica dos modernos conceitos de espécie tem sido mudar de uma visão de espécie como um sistema fechado, para uma visão mais flexível, onde as espécies são produtos da interação de inúmeras forças evolutivas em ação. Esta tendência reflete uma mudança de interesse da simples ordenação da biodiversidade para o entendimento do processo criador da diversidade biológica (especiação)".



QUADRO 2: PRINCIPAIS CONCEITOS DE ESPÉCIE

I. **Conceito Biológico de Espécie.** Este conceito refere-se a espécies como "grupos de populações naturais intercruzantes, que são isoladas reprodutivamente de outros grupos intercruzantes de populações" (Mayr 1977);

II. **Conceito Filogenético de Espécie.** Neste conceito, espécie representa "Um grupo irredutível de organismos que pode ser distinguido de outros grupos e dentro do qual existe um padrão de parentesco do tipo ancestral e descendente" (Cracraft 1983);

III. **Conceito de coesão de Espécie.** "A população mais inclusiva de indivíduos que possuem o potencial de coesão fenotípica, dado através de mecanismos intrínsecos de coesão" (Templeton 1989);

IV. **Conceito Evolutivo de Espécie** "Espécie é uma linhagem (uma sequência de populações ancestrais-descendentes) que evolui separadamente (ou seja, mantém sua identidade) a partir de outras espécies e que possui suas próprias tendências evolutivas e destino histórico" (Wiley 1989);

V. **Conceito genealógico de Espécie**. "Grupo basal de organismos cujos membros acumularam diferenças genéticas que permitem que sejam mais proximamente relacionados entre si do que organismos fora do grupo " (Hudson & Coyne 2002).

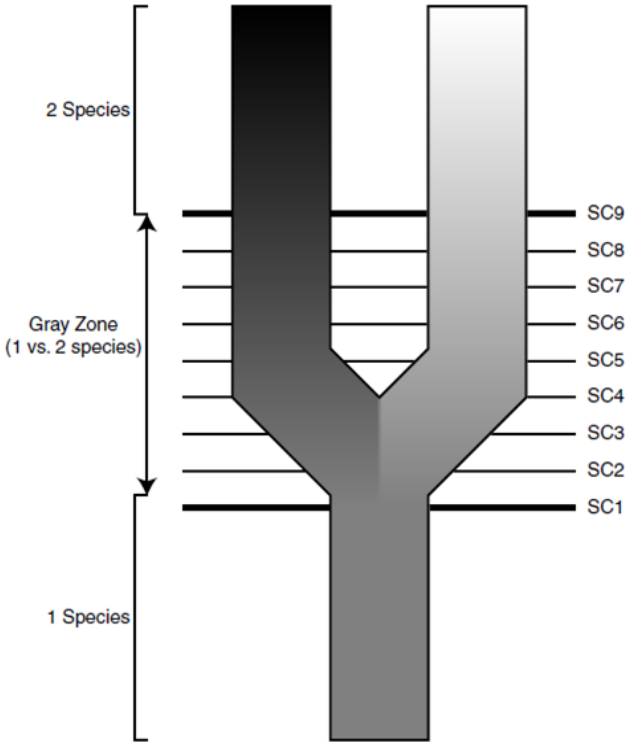


Figura 4. Separação e divergência de linhagens (especiação). Esta abordagem representa uma única linhagem (espécie) que se divide para formar duas linhagens (espécies). As gradações em tons de cinza representam as linhagens filhas divergindo ao longo do tempo, e as linhas horizontais marcadas com SC (critério de espécie) 1 a 9 representam os tempos em que adquirem propriedades diferentes (isto é, quando se tornam feneticamente distinguíveis, reciprocamente monofiléticas, reprodutivamente incompatíveis, ecologicamente distintos, etc.). Todo o conjunto de propriedades forma uma zona cinzenta dentro da qual os conceitos de espécies entram em conflito. Em ambos os lados da zona cinzenta, haverá um acordo unânime sobre o número de espécies. Antes da aquisição do primeiro critério (SC1), todos concordarão que há uma única espécie, e depois da aquisição da última propriedade (SC9), todos concordarão que existem duas. Entre SC1 e SC9, haverá discordância. A razão é que diferentes conceitos de espécies contemporâneas adotam diferentes propriedades (Representadas pelas linhas horizontais) como seus critérios de espécies – isto é, diferentes propriedades como seus pontos de corte para considerar que uma linhagem evoluiu e separou-se em uma nova espécie. (Adaptado de De Queiroz 2007).

1.3. Importância da Biodiversidade.

O termo "biodiversidade" pode ser definido como toda variação hereditária em qualquer nível de organização, de genes de uma única população ou espécie, à espécies compondo uma comunidade e por fim, todas as comunidades que compõem os

ecossistemas do planeta (Reaka-Kudla et al. 1997). O termo nasceu como "BioDiversidade" durante o "National Forum on BioDiversity" que ocorreu em Washington, D. C., nos E.U.A. em setembro de 1986. Os anais do fórum foram publicados em 1988 sob o título "BioDiversity" e se transformou em um best-seller, sendo que o tema passou a ser relevante em noticiários e discursos políticos, onde ficou conhecido apenas como "biodiversidade" e desde então se transformou em uma questão discutida por toda a sociedade (Barros 2011).

Desde o Fórum de 1986, o senso comum entre os cientistas é de que apenas uma pequena fração da biodiversidade na Terra foi descoberta e que, sua origem e manutenção estão entre os problemas mais fundamentais das ciências biológicas (Reaka-Kudla et al. 1997). Alguns cientistas acreditam que a magnitude da biodiversidade presente no planeta é tão grande, que o custo de sua exploração e documentação é tão elevado e que o número de biólogos qualificados para analisar os dados é tão pequena, que o objetivo de conhecer toda a biodiversidade de espécies do planeta é uma tarefa impossível no panorama atual (Reaka-Kudla et al. 1997, Carbayo & Marques, 2011).

Por outro lado, outros cientistas defendem justamente o contrário, que o potencial dos benefícios de se conhecer e preservar a biodiversidade são enormes e que o custo de sua perda são muito altos, e portanto, esforços devem ser feitos no sentido da documentação da biodiversidade existente no planeta (Reaka-Kudla et al. 1997). De fato, os benefícios de se conhecer a biodiversidade são inúmeros, já que ela é fonte de imenso valor econômico para o homem, fornecendo produtos para atividades agrícolas, pecuárias, pesqueiras, florestais e para a fabricação de remédios, cosméticos, enzimas industriais, entre outros (Barros 2011). Enquanto o número de espécies descritas está na ordem de 1.4 milhões, estimativas atuais sugerem que o número total de espécies no planeta está entre 10 e 100 milhões. Essas 1,2 milhões de espécies foram catalogadas nos últimos 250 anos de classificação taxonômica, ou seja, pelo menos 91% das espécies existentes ainda não foram descritas, considerando as estimativas mais baixas do total de espécies mencionados acima (Mora et al. 2011).

Para piorar o cenário, a atividade humana tem alterado amplamente o meio ambiente, mudando os ciclos biogeoquímicos, alterando o uso da terra e a mobilidade da biota. A queima de combustível fóssil e desmatamento aumentaram a concentração

de dióxido de carbono atmosférico (CO_2) em 30% nos últimos três séculos (com mais de metade deste aumento ocorrendo nos últimos 40 anos). A concentração de metano mais do que duplicou e o aumento de concentrações de outros gases contribuem para aquecimento global. Fixação industrial de nitrogênio para fertilizantes e outras atividades humanas mais do que duplicou as taxas de fixação terrestre de nitrogênio gasoso em sistemas biológicos. Eliminação de nutrientes na agricultura e sistemas urbanos prejudicou bacias hidrográficas, causando grandes mudanças ecológicas em estuários e zonas costeiras (Chapin III et al. 2000).

Os seres humanos transformaram 40-50% da superfície de terra livre de gelo, transformando pradarias, florestas e zonas úmidas em sistemas agrícolas e urbanos. A mobilidade das pessoas transportou organismos através de barreiras geográficas que mantinham regiões bióticas da Terra separadas, de modo que muitas das espécies de organismos foram introduzidos em diferente locais em tempos históricos e atuais (Chapin III et al. 2000). Juntas, essas mudanças vem alterando de forma bastante significativa a diversidade biológica da Terra. Muitas espécies foram eliminadas de áreas dominadas por influências humanas. Mesmo em áreas de preservação, espécies nativas competem ou são eliminadas por espécies introduzidas. Extinção é um processo natural, mas está ocorrendo em uma taxa anormal como consequência das atividades humanas. Estimas-se que o homem já tenha causado a extinção de 5 – 20% de muitos grupos de organismos e as taxas de extinção estão estimadas em 100 – 1.000 vezes maior do que as taxas de extinção pré-humanidade (Nunes & Van den Bergh 2001). O lento processo de descrição de espécies faz com que organismos desconhecidos possam ser extintos antes mesmo de terem sido descobertos (Mora et al. 2011).

1.4. Hipóteses e Objetivos

Considerando o conhecimento limitado a cerca da biodiversidade, particularmente a marinha, e a dificuldade no uso de diferentes conceitos de espécies aplicados aos seres vivos, neste trabalho damos prosseguimento ao estudo da biodiversidade de espécies de *Hypnea*, que apresentam diversos desafios como a ocorrência de plasticidade fenotípica, convergência morfológica e de espécies

consideradas cosmopolitas, algumas de grande importância ecológica e econômica. Com base nisso, esta pesquisa baseia-se em duas hipóteses:

1. Baseado em estudos moleculares, a espécie *Hypnea musciformis* forma um complexo de espécies proximamente relacionadas, mas com distribuição geográfica distinta.

2. Os variantes morfológicos "musciformis" e "nigrescens" de *Hypnea pseudomusciformis* que ocorrem na costa Brasileira correspondem a uma única espécie biológica.

Para testar estas hipóteses, esta pesquisa tem como objetivos:

Geral: investigar a biodiversidade espécies do gênero *Hypnea* que ocorrem no país e do complexo *Hypnea musciformis*/*H. pseudomusciformis* obtidos de diferentes regiões geográficas, no país e no exterior.

***Específicos*:**

- Revisar as espécies do gênero *Hypnea* de ocorrência na costa brasileira, bem como as taxas de divergências de marcadores moleculares do tipo "DNA barcode", além de definir quais são os caracteres morfológicos úteis na taxonomia (Capítulo 2);

- Obter amostras georeferenciadas das espécies do complexo *Hypnea musciformis/H. pseudomusciformis* de diferentes localidades do mundo, para investigar a ocorrência e distribuição das espécies com a união de dados moleculares, filogeográficos e morfológicos (Capítulo 3);

- Investigar duas variantes morfológicas de *Hypnea pseudomusciformis* obtidas em campo e cultivados *in vitro,* a fim de verificar se há a ocorrência

de plasticidade fisiológica, além da plasticidade morfológica, nessas variantes e se há indícios de um fator genético atuante nessas diferenças ou apenas fatores ambientais (Capítulo 4);

- Utilizar dados do ciclo de vida completo *in vitro*, além de dados de teste de crescimento e conteúdo pigmentar de duas variantes morfológicas de *H. pseudomusciformis*, afim de obter indícios que testem a hipótese 2 (Capítulo 5);

- Realizar experimentos de cruzamento *in vitro* com duas variantes morfológicas de *H. pseudomusciformis*, com ausência de divergência no "DNA barcode" 5P-COI, a fim de testar a hipótese 2 (Capítulo 6);

- Realizar experimentos de cruzamento *in vitro* de *H. pseudomusciformis* entre indivíduos de uma mesma variante morfológica, mas obtidos em regiões geográficas distintas e que apresentam divergência acima de 1,5 % no "DNA barcode" 5P-COI, a fim de utilizar o conceito biológico de espécie para testar os limites das divergências moleculares para este marcados no gênero *Hypnea* (Capítulo 7).

1.5. Referências bibliográficas.

Abbott, I.A., 1999. Marine red algae of the Hawaiian Islands. xv + 477 pp. *Honolulu Museum Press*, Honolulu, Hawaii.

Barros, H. L. 2011. Biodiversidade e renovação da vida. Claro Enigma. São Paulo. 94p.

Berchez, F.A.S. & Oliveira-Filho, E.C. 1990. Maricultural essays with the carragenophyte *Hypnea musciformis* in São Paulo, Brazil. In: OLIVEIRA, E.C.F. & KAUTSKY, N. (eds.) *Cultivation of seaweeds in Latin América*. São Paulo: Universidade de São Paulo, p.: 89-94.

Câmara-Neto, C. 1987. Seaweed culture in Rio Grande do Norte, Brazil. *Hydrobiologia*, 151/152: 363-367.

Cracraft, J. 1983. Species concepts and speciation analysis, in Current Ornithology, vol. 1, Plenum Press, New York, pp. 159-87.

Dawes, C.J. & Mathieson, A.C. 2008. The seaweeds of Florida. *University Press of Florida*. 591p.

De Queiroz, K. 2007. Species concepts and species delimitation. Systematic Biology, 56: 879–886.

Engel, K., Tollrian, R. & Jeschke, J.M. 2011. Integrating biological invasions, climate change and phenotypic plasticity, Communicative & Integrative Biology, 4:3, 247-250, DOI: 10.4161/cib.4.3.14885

Faccini, A.L. & Berchez, F. 2000. Management of natural beds and standing stock evaluation of *Hypnea musciformis* (Gigartinales, Rhodophyta) in south-eastern Brazil. *Journal of Applied Phycology* 12: 101–103.

Ford, E.B. 1964. Ecological genetics. Methuen, London; John Wiley, New York. 335p.

Furtado, M.R. 1999. Alta lucratividade atrai investimento – Hidrocolóides. *Química e Derivados* 377: 20-29.

Furtado, M.R. 2004. Desequilíbrio climático abre mercado para novos hidrocolóides. *Química e Derivados* 430: 1-4.

Graham, L.E. & Wilcox, L.W. 2009. Algae. *Prentice-Hall*, New Jersey. 640 pp.

Guimarães, N. 2011. Diversidade do gênero Hypnea (Gigartinales, Rhodophyta) do Estado de São Paulo baseada em marcadores moleculares e morfologia. Dis. Mestrado. *Inst. De Biociências.* USP.

Guiry, M.D. & Guiry, G.M. 2017. AlgaeBase. World-wide electronic publication. *National University of Ireland,* Galway. http://www.algaebase.org. Consultado em 11 de Junho de 2013.

Hey, J. 2006. On the failure of modern species concepts. Trends in Ecology and Evolution, 21: 447–450.

Hudson, R.R. & Coyne, J.A. 2002. Mathematical consequences of the genealogical species concept. Evolution, 56: 1557–1565.

Innes, D.J. 1994. Genetic differentiation among populations of marine algae. Helgolander Meeresunters. 38: 401-407.

Jesus, P. B. 2012. O gênero *Hypnea* J.V. Lamouroux (Gigartinales, Rhodophyta) no litoral do estado da Bahia, Brasil: aspectos morfológicos e anatômicos. Dissertação de Mestrado. *Universidade Feira de Santana*. Bahia. 195 p.

Jesus, P. B., Nauer, F., Lyra, G. d. M., Cassano, V., Oliveira, M. C., Nunes, J. M. d. C. and Schnadelbach, A. S. 2016). Species-delimitation and phylogenetic analyses of some cosmopolitan species of *Hypnea* (Rhodophyta) reveal synonyms and misapplied names to *H. cervicornis*, including a new species from Brazil. J. Phycol., 52: 774–792. doi:10.1111/jpy.12436

John, D.M. & Maggs, C.A. 1997. Species problems in eukaryotic algae: a modern perspective. Chapter 5, pp. 83-107. in Species The Units of Biodiversity. Ed. Claridge, M.F., Dawah, H.A. & Wilson, M.R. 1997. Chapman & Hall. London.

Lima, A.M.; Camara-Neto, C.; Oliveira-Filho, E.C. & Aaraujo, R.A., 1981. Cultivo experimental de *Hypnea musciformis* e *Gracilaria* sp. em áreas protegidas por antigas linhas de costa (recifes) no litoral do Rio Grande do Norte. In: *Projeto Algas. Estado do Rio Grande do Norte*. Série: Brasil. SUDENE. Estudos de Pesca, 9: 97-107.

Masuda, M., Yamagishi, Y., Chiang, Y.-M., Lewmanomont, K. & Xia, B. 1997. Overview of Hypnea (Rhodophyta, Hypneaceae). In Abbott, I. A. (Ed.) Taxonomy of Economic Seaweeds, Vol. 6. *California Sea Grant College System*, La Jolla, pp. 127–33.

Mayden, R.L. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. In Species: The Units of Biodiversity (Claridge, M.F., Dawah, H.A. & Wilson, M.R., editors), 381–424. Chapman and Hall, London.

Mayr, E. 1992. A local flora and the biological species concept. American Journal of Botany, 79, 222-38.

Mshigeni, K. E. & Chapman, D. J. 1994. *Hypnea* (Gigartinales,Rhodophyta). In: AKATSUKA, I. (ed.) *Biology of Economic Algae*. SPB Academic Publishing, The Hague, the Netherlands, p.: 245–281.

Nagano, C.S., Moreno, F.B.M.B., Bloch, C., Prates, M.V., Calvete, J.J., Saker-Sampaio, S., Farias, W.R.L., Tavares, T.D., Nascimento, K.S., Grangeiro, T.B., Cavada, B.S. & Sampaio, A.H. 2002. Purification and characterization of lectins from the red marine alga *Hypnea musciformis*. *Protein and Peptide Letters* 9: 159-165.

Nauer, F.; Cassano, V. & Oliveira, M. C. 2014. *Hypnea* species (Gigartinales, Rhodophyta) from the southeastern coast of Brazil based on molecular studies complemented with morphological analyses, including descriptions of *Hypnea edeniana* sp. nov. and *H. flava* sp. nov. *European Journal of Phycology* 49 :550-575. DOI:10.1080/09670262.2014.981589.

Nauer, F.; Cassano, V. & Oliveira, M. C. 2015. Description of Hypnea pseudomusciformis sp. nov., a new species based on molecular and morphological analyses, in the context of the H. musciformis complex (Gigartinales, Rhodophyta). Journal of Applied Phycology 27: 2405-2417. DOI: 10.1007/s10811-014-0488-y

Neushul, M. 1990. Antiviral carbohydrates from marine red algae. *Hidrobiologia* 204/205: 99-104.

Norton T.A, Melkonian M. & Andersen R.A. 1996. Algal biodiversity. *Phycologia* 35: 308-326.

Nunes, P. & Van den Bergh, J. 2001. Economic valuation of biodiversity: sense or nonsense? *Ecological Economics* 39, Issue 2: 203 – 222.

Oliveira-Filho, E.C. & Berchez, F.A.S. 1987. Ensayos sobre el cultivo del alga roja *Hypnea musciformis* (Rhodophyta, Gigartinales) en São Paulo, Brasil. In: VERRET, J.A.; CARILLO, M.; ZANUY, S. & HUISMAN, E.A. (eds.) Procedimientos del trabajo sobre acuicultura en America Latina. *International Foundation for Science*, p.: 399-409.

Pereira, R. C. & Soares-Gomes, A. (Orgs.) 2009. Biologia marinha, 2ª Edição. *Rio de Janeiro: Ed. Interciência.* 631 pp.

Plastino, E.M. & Guimarães, M. 2001. Diversidad intraespecifica. In: Alveal, K. & Antezana, T. (eds.). Sustentabilidad de la biodiversidad, un problema actual. Bases cientifico-tecnicas, teorizaciones y proyecciones. Universidad de Concepcion, Concepcion, pp. 19-27.

Price, J.H.; John, D.M. & Lawson, G.W. 1992. Seaweeds of the western coast of tropical Africa and adjacent islands: a critical assessment. IV. Rhodophyta (Florideae) 3. Genera H-K. *Bulletin of British Museum (Natural History – Botany Series)*, 22 (2): 123-146.

Ramos, M.B., Alves, J.P. & Yokoya, N.S. 2006. Variação temporal da biomassa, do teor de carragenana e da flora associada a populações de *Hypnea musciformis* (Rhodophyta, Gigartinales) da Praia da Enseada das Garças, Fundão, ES. *In*: *Livro de resumos do XI Congresso Brasileiro de Ficologia*, Sociedade Brasileira de Ficologia, Salvador, pp. 204.

Reis, R. P. & Yoneshigue-Valentin, Y. 1998. Variação espaço-temporal de populações de *Hypnea musciformis* (Rhodophyta, Gigartinales) na Baía de Sepetiba e Armação dos Búzios, RJ, Brasil. *Acta Botanica Brasilica*, 12 (3): 465-483.

Reis, R. P., Caldeira, A.Q., Miranda, A.N.S., Barros-Barreto, M.B. 2006. Mariculture potential of carragenophyta *Hypnea musciformis* (Wulfen) J.V. Lamouroux (Gigartinales - Rhodophyta) on Marambaia Island, Baía de Sepetiba, Rio de Janeiro State, Brazil. *Acta Bot. Bras.*, Oct./Dec. 2006, vol.20, no.4, p.763-769. ISSN 0102-3306.

Rosselló-Mora, R. 2003. Opinion: The Species Problem, Can We Achieve a Universal Concept? System. Appl. Microbiol. 26, 323–326.

Scheneider, C.W. & Searles, R.B. 1976. North Carolina marine algae. VII. New species of *Hypnea* and *Petroglossum* (Rhodophyta, Gigartinales) and additional records of other Rhodophyta. *Phycologia*, 15: 51-60.

Schenkman, R.P.F. 1986. Cultura de Hypnea (Rhodophyta) *in vitro* como subsídio para estudos morfológicos, reprodutivos e taxonômicos. *Tese de Doutorado*. Instituto de Biociências, Universidade de São Paulo. 81 pp.

Templeton, A.T. (1989) The meaning of species and speciation: a genetic perspective, in Speciation and Its Consequences (eds D. Otte and J. A. Endler), Sinauer Associates, Sunderland, Massachusetts, pp. 3--27.

Wiley, E.O. and Mayden RL. (1997) The evolutionary species concept, in Species Concepts and Phylogenetic Theory: A Debate (eds Q.D. Wheeler and R Meier), Columbia University Press, New York.

Zuccarello, G.C., URGER G., WEST J.A. & KING R. 1 999b. A mitochondrial marker for red algal intraspecific relationships: variable within populations and maternally inherited. Molecular Ecology 8 : 1.443-1.447.

CAPÍTULO *Dois*

Taxonomic revision of the genus Hypnea (Gigartinales, Rhodophyta) in Brazil based on DNA barcode and morphology.

Capítulo 2

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Taxonomic revision of the genus *Hypnea* (GIGARTINALES, RHODOPHYTA) in Brazil based on DNA barcode and morphology.

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Abbreviations: COI-5P, 5' end of the cytochrome c oxidase 1 gene; MP, maximum parsimony; NJ, neighbor joining; *rbc*L, gene encoding for the large subunit of the enzyme ribulose-1, 5-bisphosphate carboxylase-oxygenase; UPA, Universal Plastid Amplicon.

Abstract

The red algal genus *Hypnea* (Gigartinales) has a wide geographical distribution along tropical and subtropical coasts around the world. The relatively simple and plastic morphology, often influenced by the environment, complicates the identification of *Hypnea* species. Therefore, the number and status of some species remain in doubt.

Studies using molecular markers led to changes on the diversity of the genus in Brazil, where new species were described and others were placed in synonymy. In this study, morphological analysis and sequence data from COI-5P, UPA and *rbcL*-3P markers for representatives of *Hypnea* confirmed the existence of nine different species on the coast of Brazil: *H. brasiliensis*, *H. cervicornis*, *H. edeniana*, *H. flava*, *H. pseudomusciformis*, *H. platyclada*, *H. spinella*, *H. yokoyana* and *H. wynnei*. Furthermore, two other species, *Hypnea* sp1 and *Hypnea* sp2 remain undescribed. In this work, we verify that the following characteristics have taxonomic value considering the species analyzed: 1) Habit of the thallus (tufts erect or tufts densely intricate), 2) Main axis (single or multiple, persistent or not), 3) Apex shape (straight, bent, bifurcated, tendril), 4) Form of the branchlets (spinescent, starry, etc.), (5) Axial cell size in relation to periaxial cells and (6) Tetrasporangial sori position in the branches (basal, median, apical). However, the identification of the species based on morphological characteristics alone was imprecise, mainly due to the phenotypic plasticity present in the group, besides the existence of species with convergent morphologies. The DNA barcode technique, especially with respect to the COI-5P marker, proved to be essential for the identification and delimitation of the species, revealing scenarios that would go unnoticed by morphology alone.

Key words: COI-5P, DNA barcode, *Hypnea*, molecular phylogeny, morphology, *rbc*L, UPA.

Introduction

The genus *Hypnea* J.V. Lamouroux (1813) is well known for its economic importance for being a source for the production of carrageenan (Saito & Oliveira, 1990). The taxonomy of *Hypnea*, however, is still problematic and confusing. Regional revisions of the genus have been made in Brazil in the past few years, with new species being described, while other species were considered misapplied names (Jesus et al. 2015, 2016, Nauer et al. 2014, 2015, 2016).

Schenkman (1986) was the first taxonomic study of *Hypnea* in Brazil. Based on morphological characters, she recognized six species of *Hypnea*: *H. cervicornis* J. Agardh, *H. cornuta* (Kützing) J. Agardh, *H. musciformis* (Wulfen) J.V. Lamouroux, *H.*

nigrescens Greville ex J. Agardh, *H. spinella* (C. Agardh) Kützing and *H. valentiae* (Turner) Montagne. The author, however, pointed out difficulties for species identification, probably resulting from phenotypic plasticity.

Based on morphology, Yoneshigue (1985), Nunes (2005) and Guimarães (2006) cited *H. cenomyce* J. Agardh for Brazil. Jesus et al. (2013), also based on morphological studies, described a new species, *H. platyclada* Jesus & Nunes, the only *Hypnea* species found in Brazil with cylindrical estolon and stipe, but flattened upper portions and flattened fusiform branches. *Hypnea volubilis* Searles (1976: 53) was cited for Brazil in two floristic algal surveys: a survey from Laje de Santos Marine State Park, São Paulo State (Amado-Filho et al. 2006), which is the first citation of the species for the country, and a survey of Atol das Rocas (Villaça et al. 2010).

The first published molecular study with species of *Hypnea* occurring in Brazil was Nauer et al. (2014), using the plastid *rbc*L gene encoding the large subunit of the enzyme ribulose-1, 5-bisphosphate carboxylase-oxygenase and two DNA barcode markers, the 5' region of the gene *cox*1 that encodes cytochrome c oxidase subunit 1 (COI-5P) in the mitochondrial genome and the universal plastid amplicon (UPA) also in the plastid genome, alongside with morphological studies. The authors described two new species*: H. edeniana* Nauer, Cassano & M.C. Oliveira and *H. flava* Nauer, Cassano & M.C. Oliveira and was the first citation of *H. aspera* Kützing to the South Atlantic Ocean. Nauer et al. (2015) analyzing specimens from Adriatic Sea, Italy, type-locality of *H. musciformis*, pointed out molecular divergences with Brazilian specimens, therefore, they described a new species, *H. pseudomusciformis* Nauer, Cassano & M.C. Oliveira. Based on the analyses of COI-5P and *rbc*L, the authors also pointed out that, for Brazil, *H. nigrescens* and *H. valentiae* corresponded to two of the several morphotypes presented by *H. pseudomusciformis* whose identification based only on morphology is extremely complicated and confusing.

Jesus et al. (2015) made the first citation of *H. stellulifera* (J. Agardh) Yamagishi *et* Masuda for Brazil, extending the occurrence of this Asiatic species in the world. These authors affirmed that the species *H. cornuta* (Kützing) J. Agardh and *H. stellulifera*, constitute a complex to be investigated. Jesus et al. (2016), in a extended taxonomic revision of some *Hypnea* species, revealed some misapplied names, and confusion with the identification of *H. cervicornis*. In that work, *H. aspera*, *H.*

boergesenii and *H. flexicaulis* were considered synonyms of *H. cervicornis*. In addition, specimens previously identified as *H. cervicornis* in some works were described as new species, *H. brasiliensis* P.B. Jesus, Nauer & J.M.C. Nunes. At last, Nauer et al. (2016), during a DNA barcode survey, described two new species, *H. wynnei* Nauer, Cassano & M.C. Oliveira and *H. yokoyana* Nauer, Cassano & M.C. Oliveira (Table 1).

Table 1: Species of *Hypnea* cited for Brazil based on previous works.

Species	First citation for Brazil	Status
H. aspera Kützing	Nauer *et al.* (2014)	Synonymous of *H. cervicornis* (Jesus et al. 2016)
H. brasiliensis P.B. Jesus, Nauer & J.M.C. Nunes	Jesus et al. (2016)	**Accepted**
H. cenomyce J. Agardh	Yoneshigue (1985)	Needs taxonomic revision
H. cervicornis J. Agardh	J. Agardh (1852)	**Accepted**
H. cornuta (Kützing) J. Agardh	Joly et al. (1968)	Needs taxonomic revision
H. edeniana Nauer, Cassano & M.C. Oliveira	Nauer et al. (2014)	A**ccepted**
H. flava Nauer, Cassano & M.C. Oliveira	Nauer et al. (2014)	**Accepted**
H. musciformis (Wulfen) J.V. Lamouroux	Saint Hilaire (1833)	For Brazil, morphological variation of *H. pseudomusciformis* (Nauer et al. 2015)
H. nigrescens Greville ex J. Agardh	Schenkman (1986)	For Brazil, morphological variation of *H. pseudomusciformis* (Nauer et al. 2015)
H. platyclada Jesus &Nunes	Jesus et al. (2013)	**Accepted**
H. pseudomusciformis Nauer, Cassano & M.C. Oliveira	Nauer et al. (2015)	**Accepted**
H. spinella (C. Agardh) Kützing	Martens (1879)	**Accepted**
H. stellulifera (J. Agardh) Yamagishi et Masuda	Jesus et al. (2015)	**Accepted**
H. valentiae (Turner) Montagne	Schenkman (1986)	For Brazil, morphological variation of *H. pseudomusciformis* (Nauer et al. 2015)
H. volubilis Searles	Amado-Filho et al. (2006)	Needs taxonomic revision
H. wynnei Nauer, Cassano & M.C. Oliveira	Nauer et al. (2016)	**Accepted**
H. yokoyana Nauer, Cassano & M.C. Oliveira	Nauer et al. (2016)	**Accepted**

Therefore, considering the taxonomic challenges in *Hypnea*, the confusion with misapplied names, the descriptions of new species, the lack of a revision for the coast of Brazil and the lack of a common sense of which molecular marker is more useful to identify species and which is the intraspecific and interspecific divergence on delimiting those species, this study generated new sequence data to complement previous studies and for the first time, the *Hypnea* species diversity for the entire Brazilian coast is presented, along with discussion about the divergences of three different molecular markers and the taxonomy features used to identify species.

Materials and methods

We used 97 specimens collected along the Brazilian coast (ranging from 2°54’14.51"S to 27°08'42.44"S in latitude) in intertidal and subtidal zones. The specimens were the same used by Nauer et al. (2014, 2015, 2016). For molecular procedures, apical regions were separated from the rest of the thallus, cleaned and stored in silica gel. The remaining of the thalli was preserved in 4% formalin in seawater for taxonomic studies. Representatives were deposited at the Herbarium of University of São Paulo (SPF). Herbarium abbreviation follows the on-line *Index Herbariorum* (Thiers, 2017 continuously updated).

The algal material from silica gel was macerated in liquid nitrogen and the DNA was extracted using the NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany), following the kit protocol. Amplification of each marker was performed using a PCR Master Mix (Promega Corp., Madison, WI, USA). The primers and cycles used were: GazF1 and GazR1 for COI-5P (Saunders 2005), p23SrV_f1 and p23SrV_r1 for UPA (Sherwood & Presting 2007) and F993 - RrbcS for *rbc*L (Freshwater & Rueness 1994). The column GTX™ PCR DNA and Gel Band Purification Kit (GE Healthcare, Buckinghamshire, UK) were used for purification of PCR products and quantified using NanoDrop 2000 (Wilmington, DE, USA). The same PCR primers were used with a sequencing kit BigDye Terminator Cycle Sequencing Ready Reaction" (Applied Biosystems, Foster City, USA) according to the protocol of the supplier. The samples were sequenced in a 3730 DNA Sequencer (Applied Biosystems, Carlsbad, CA, USA). The sequences obtained were aligned in the BioEdit 7.0 sequence editor (Hall 1999).

For making the trees, a total of 65 COI-5P sequences from Brazil (9 new and 56 from GenBank), 65 *rbc*L-3P sequences (35 new and 30 from GenBank) and 63 UPA sequences (42 new and 21 from GenBank) were used in the analyses (Table S1, Supplementary material). For outgroups, *Calliblepharis ciliata* (Greville) Kützing (DQ442896) was used for COI-5P analyses, *Calliblepharis fimbriata* (AF385653) for *rbc*L analyses. Trees were constructed by Neighbour-joining (NJ) using the MEGA5 program (Molecular Evolutionary Genetics Analysis, Tamura et al. 2011) with 2000 bootstrap replicates. To calculate the interspecific and intraspecific divergences in base-pairs (bp) and % for the markers COI-5P, UPA and *rbc*L, we used 367 sequences among the samples of the genus *Hypnea* from Genbank and obtained in this study.

For morphological studies, the samples kept in 4% formalin were sectioned by hand with a razor blade and stained with 1% aniline blue acidified with 1N HCl. Habit and diagnostic characters of each species were obtained with digital capture and image analysis, using a compound microscope with Leica DM4000 digital camera (Solms, Germany). Vegetative and reproductive morpho-anatomical characters analyzed were based in the taxonomic studies of the genus *Hypnea* by Masuda et al. (1997).

Results

MOLECULAR MARKERS AND DIVERGENCE

The combined results for the three molecular markers and the morphological analysis indicate the occurrence of eleven taxa of *Hypnea* for the coast of Brazil: *H. brasiliensis*, *H. cervicornis*, *H. edeniana*, *H. flava*, *H. pseudomusciformis*, *H. spinella*, *H. wynnei* , *H. yokoyana* , *H. platyclada*, *Hypnea* sp. 1 and *Hypnea* sp. 2. *Hypnea platyclada* was the only species that we could not obtain molecular sequences, since this rare species was described based only on morphology of just one individual. The intraspecific variation for the three markers is shown in Table 2. The COI-5P matrix had 565 bp and the intraspecific divergence ranged from 0 to 1.7% (0 to 14 bp). Based on the results of the COI-5P analysis (Figure 1), each of the ten taxa formed monophyletic clades with full bootstrap support. The sequences of *Hypnea* sp1 and *Hypnea* sp. 2 formed a monophyletic clade with divergence of of 6.6 to 6.7%. The sequences of the Brazilian specimens of *H.* sp2 formed a monophyletic clade with sequence of *H. stellulifera* from the type locality, Philippines (EU345984) with divergence of 5.9 to 6.1%.

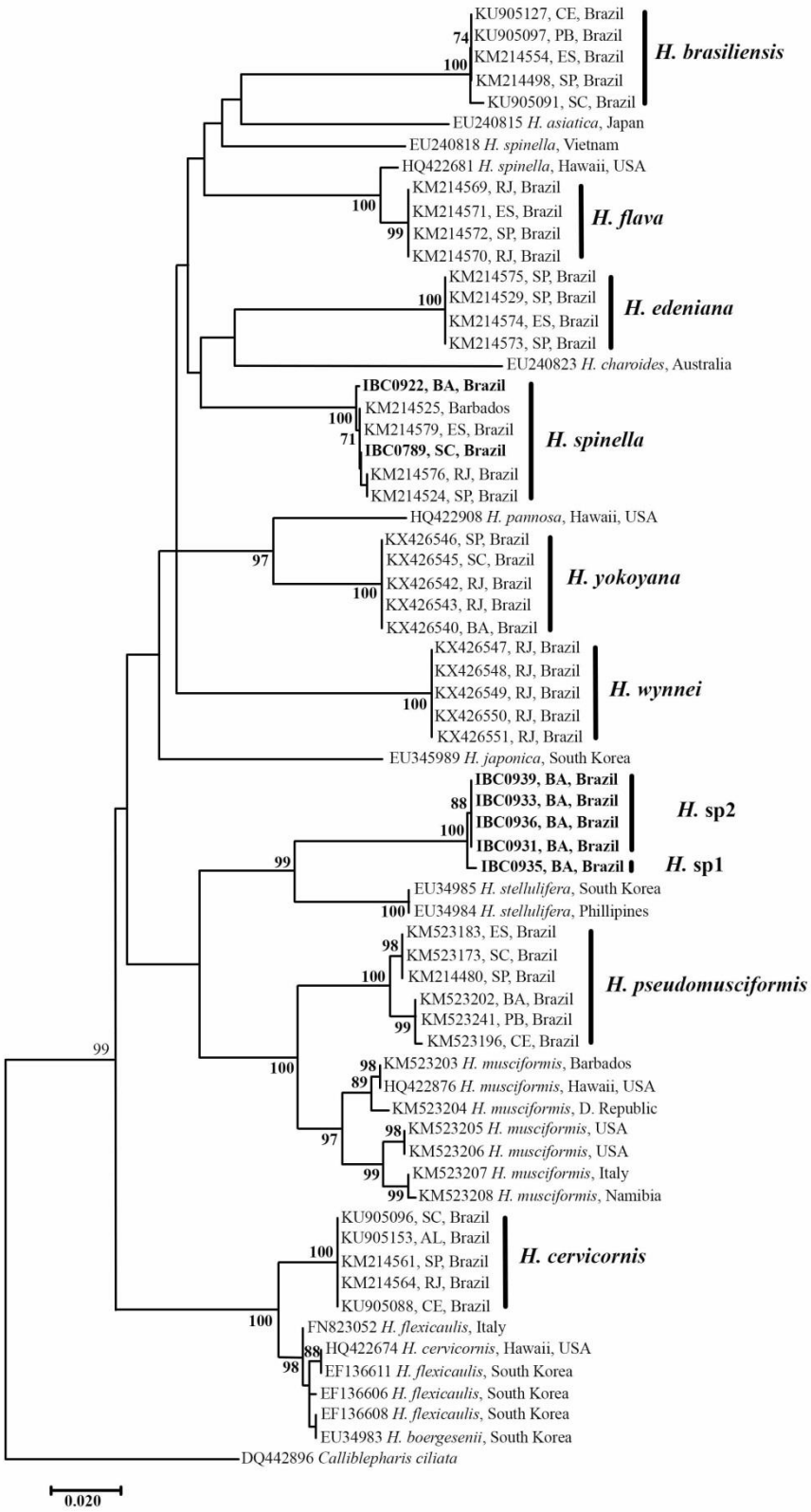

Figure 1. Neighbour-Joining (NJ) analysis for COI-5P sequences for *Hypnea* species. *Calliblepharis ciliata* was used as outgroup. On the branches are the bootstrap values for 2000 replicates. Sequences generated in this work are in bold. Accession numbers and country of origin, when available, are included for sequences obtained from GenBank.

For the UPA marker (Figure 2), each of the ten taxa mentioned above formed monophyletic clades, but only for *H. cervicornis* and *H. wynnei* full bootstrap support was observed. Excluding *H. brasiliensis* and *Hypnea* sp. 1 the other bootstrap supports ranged from 95 to 99. The *Hypnea* sp. 1 clade had a bootstrap support of only 56. The UPA matrix had 410 bp and the intraspecific divergence ranged from 0 to 0.8% (0 to 3 bp) (Table 3).

For the *rbc*L-3P analysis (Figure 3), each of the ten taxa formed monophyletic clades, most with full bootstrap support, with the exception of *H. pseudomusciformis* (99) and *H. yokoyana* (98). The sequences for *Hypnea* sp. 2 formed a monophyletic clade with sequence for *H. stellulifera* from the type locality (Philippines) with divergence of 2.0%. The *rbc*L-3P matrix had 450 bp and the intraspecific divergence ranged from 0 to 1.4% (0 to 6 bp) (Table 2).

The Table 3 provides a summary of the intraspecific and interspecific divergences found for the Brazilian samples of the genus *Hypnea* for the three markers studied. The interspecific variation was calculated using the same matrices only with *Hypnea* sequences from Brazil. For COI-5P marker, the interspecific variation ranged from 9.8 to 18.2% (69 to 130 bp). The lowest divergence found was between *H. edeniana* and *H. spinella*, 9.8%, and highest between *H.* sp. 2 and *H. wynnei*, 18.2%. For the UPA marker, the interspecific variation ranged from 1.8 to 6.9%(7 to 28 bp). The lowest divergence found was between *H. spinella* and *H. yokoyana*, 1.8%, and highest between *H.* sp. 2 and *H. wynnei*, 6.9%. For the *rbc*L-3P marker, the interspecific variation ranged from 3.4 to 8.7% (15 to 39 bp). The lowest divergence found was between *H. edeniana* and *H. flava*, 3.4%, and highest between *H. cervicornis* and *H.* sp. 2, 8.7%. For all three markers, there was no overlap of the intraspecific and interspecific variations.

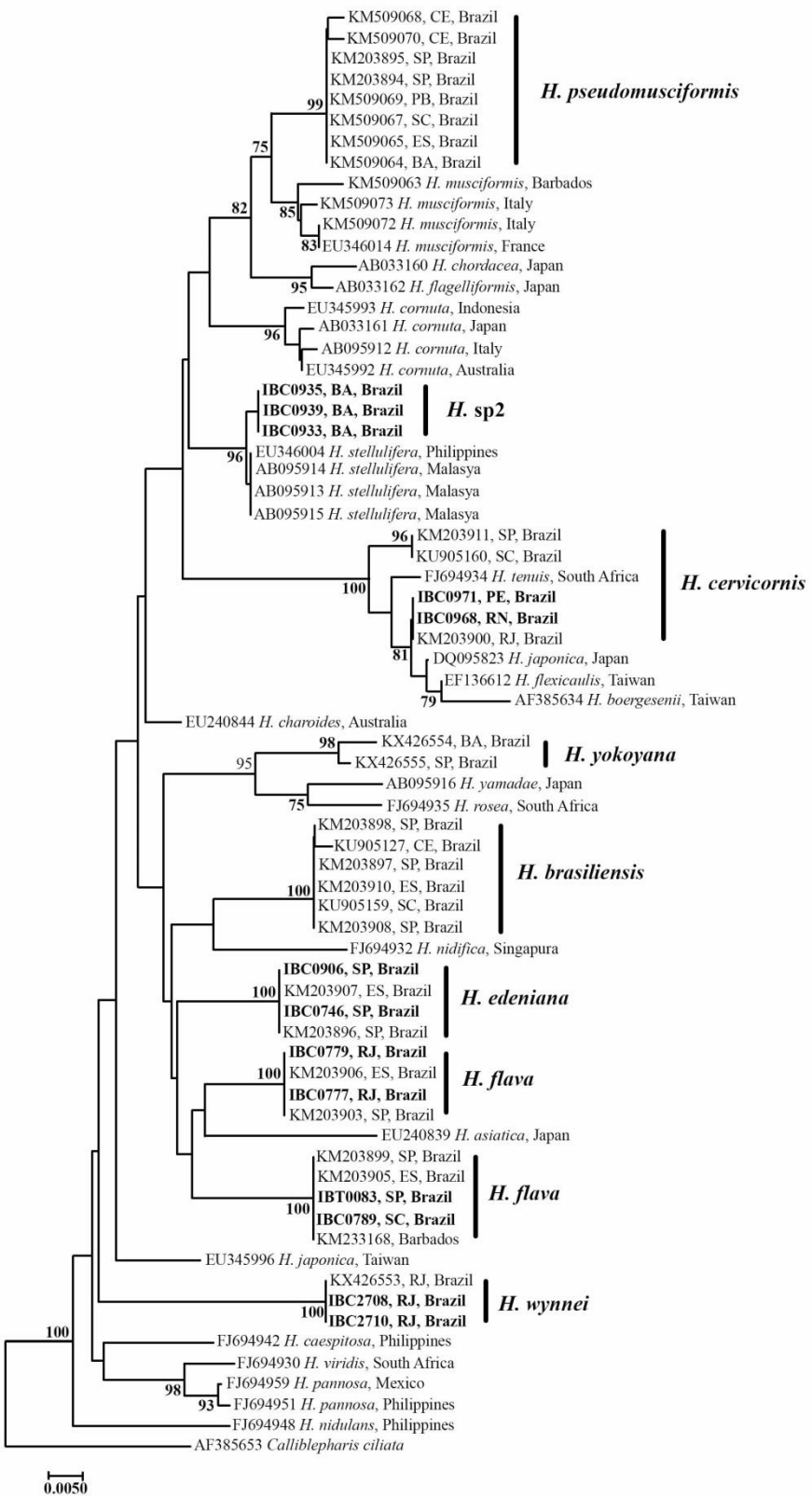

Figure 2. Neighbour-Joining (NJ) analysis for UPA sequences for *Hypnea* species. *Calliblepharis ciliata* was used as outgroup. On the branches are the bootstrap values for 2000 replicates. Sequences generated in this work are in bold. Accession numbers and country of origin, when available, are included for sequences obtained from GenBank.

Among the markers studied, the COI-5P was the most variable and efficient in the separation of species, with up to 18.2% interspecific divergence. The UPA and *rbc*L-3P markers, have a similar interspecific divergence rates, with up to 6.9% and 8.7%, respectively.

Table 2: Intraspecific variation found for the molecular markers COI-5P, UPA and *rbc*L-3P for Brazilian species of genus *Hypnea*, represented by the percentage (var.) and number of base pairs (bp). The number of sequences obtained for each species by molecular marker is also indicated (n^0).

Species	**COI-5P**			**UPA**			***rbc*L-3P**		
	n^0	**var.**	**pb**	**n^0**	**var.**	**pb**	**n^0**	**var.**	**pb**
H. brasiliensis	59	0 - 0.5%	0 - 3	34	0 - 0.3%	0 - 1	4	0 - 0.3%	0 - 4
H. cervicornis	17	0 - 0.3%	0 - 2	6	0%	0	6	0 - 0.4%	0 - 6
H. edeniana	4	0%	0	3	0 - 0.3%	0 - 1	4	0%	0
H. flava	3	0%	0	2	0 - 0.3%	0 - 1	4	0%	0
H. pseudomusciformis	109	0 - 1.7%	0 - 14	48	0 - 0.3%	0 - 1	12	0 - 0.7%	0 - 11
H. spinella	18	0 - 0.3%	0 - 2	8	0 - 0.8%	0 - 3	4	0 - 0.2%	0 - 2
H. wynnei	6	0%	0	4	0%	0	3	0%	0
H. yokoyana	7	0%	0	4	0%	0	3	0 - 0.4%	0 - 6
H. sp. 1	1	0 - 0.3%	0 - 2	-	-	-	3	0 - 0.3%	0 - 4
H. sp. 2	12	0 - 0.5%	0 - 3	7	0 - 0.8%	0 - 3	2	0 - 0.3%	0 - 4

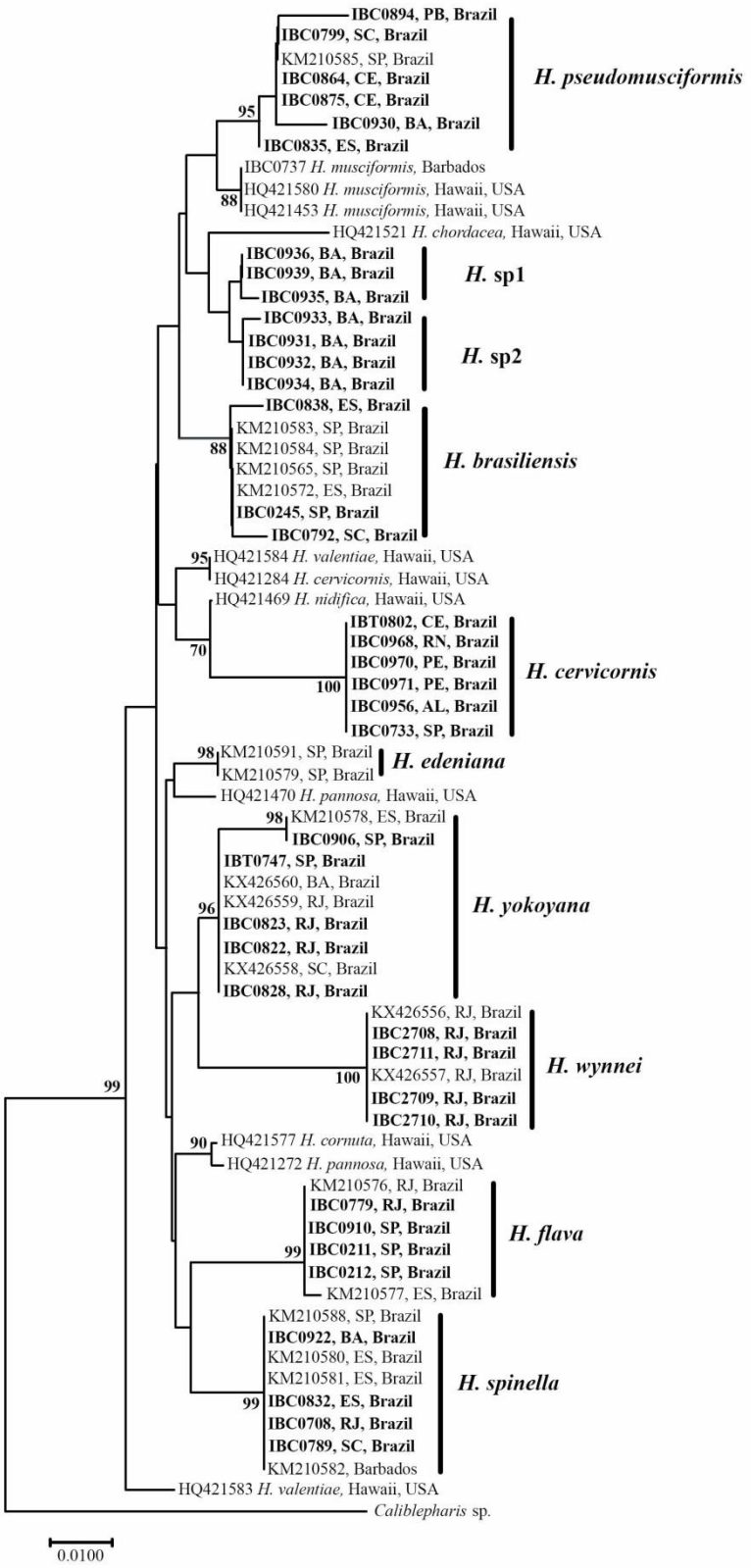

Figure 3. Neighbour-Joining (NJ) analysis for *rbc*L-3P sequences for *Hypnea* species. *Calliblepharis ciliata* was used as outgroup. On the branches are the bootstrap values for 2000 replicates. Sequences generated in this work are in bold. Accession numbers and country of origin, when available, are included for sequences obtained from GenBank.

Table 3. Interspecific and intraspecific divergences in bp and % for the markers COI-5P, UPA and *rbc*L-3P among the samples of the genus *Hypnea* used in this study.

Marker	Intraespecific	Interespecific
UPA	0 – 3 bp	7 – 28 bp
(115 sequences)	(0 – 0.8%)	(1.8 - 6.9 %)
COI-5P	0 – 14 bp	69 – 130 bp
(223 sequences)	(0 – 1.7%)	(9.8 – 18.2%)
*rbc*L-3P	0 – 7 bp	15 – 39 bp
(65 sequences)	(0 – 1.4%)	(3.4 – 8.7%)

TAXONOMY OF THE BRAZILIAN *HYPNEA* SPECIES

The vegetative and reproductive morpho-anatomical characters most used over time in the taxonomic studies of the genus were summarized by Masuda et al. (1997). However, due to the high phenotypic plasticity and the presence of cryptic species in the genus, the analysis of isolated morphological characters proved to be ineffective, where for almost all morphological characters observed there are overlaps between the species. For Brazilian specimens, of all 14 characteristics proposed by Masuda et al. (1997), the only ones that were taxonomically informative considering the species analyzed in this work are: 1) Habit of the thallus (tufts erect or tufts densely intricate); 2) Main axis (single or multiple, persistent or not); 3) Apex shape (straight, bent, bifurcated, tendril); 4) Form of the branchlets (spinescent, starry, etc.); 5) Axial cell size in relation to periaxial cells and 6) Tetrasporangial sori position in the branches (basal, median, apical) (Tables 4 and 5).

Table 4: Characteristics used in the separation of *Hypnea* species, summarized by Masuda et al. (1997) and useful in this work for delimiting Brazilian species.

Morpho-anatomical taxonomic characters	
Masuda et al. (1997)	This work
1. Basal system and algae habit	1. Habit of the thallus
2. Size of thallus	2. Main axis form
3. Texture of thallus	3. Apex shape
4. Color of herbal and freshly collected algae	4. Form of the branchlets
5. Iridescence	5. Axial cell size in relation to periaxial cells
6. Distinguished main axis	6. Position of the tetrasporangial sori in the branches
7. Shape of main axes in cross-section	
8. Abundance of lateral branches and proliferations	
9. Width of axes and branches	
10. Presence of special branches and branchlets	
11. Branch direction	
12. Frequency of lenticular thickening on the wall of cells	
13. Presence of small cells around the axial cell	
14. Position of the tetrasporangial sori in the fertile branches	

Table 5. Summary of morphological characteristics of *Hypnea* species based on the observation of Brazilian specimens.

Species / Reference	Habitat	Habit	Thallus height (cm)	Color	Texture	Main axis	Main axis diameter (µm)	Branching	Anastomoses
H. brasiliensis (Jesus *et al.* 2016)	epilithic or epiphytic	erect	1-4	reddish-pink to brownish	fleshy	distinguishable	350-700	irregular, alternate to dichotomous or subdichotomous with a horn-like aspect	absent
H. cervicornis (Jesus *et al.* 2016)	epilithic	erect	5-7	brownish-yellow, yellowish-green to brownish-red	cartilaginous	Multiple	560-1080	spirally alternate to irregular	present
H. edeniana (Nauer *et al.* 2014)	epilithic or epiphytic	sub-erect	3-6	red	rigid-cartilaginous	Multiple	500-860	irregular to alternate	present
H. flava (Nauer *et al.* 2014)	epilithic or epiphytic	prostrate	1-3	brownish-yellow	fleshy	multiple	230-570	irregular to alternate	present
H. platyclada **(**Jesus *et al.* 2013)	epiphytic	prostrate	1-3	pale-red	fleshy	often	99-644	irregular	present
H. pseudomusciformis **(**Nauer *et al.*(2015)	epilithic or epiphytic	erect	3-7	greenish to blackish-red	cartilaginous	often undistinguishable	525-925	irregular to alternate	absent
H. spinella (Nauer *et al.* 2014)	epilithic	sub-erect	2-4	pale-red to dark-red	fleshy	often indistinguishable	330-600	irregular to alternate or opposite	present
H. wynnei (Nauer *et al.* 2016)	epilithic	sub-erect	1-1.5	fleshy	fleshy	multiple	200-375	irregular to alternate	absent
H. yokoyana (Nauer *et al.* 2016)	epilithic	sub-erect	2-4	red	pale-red to dark-red	multiple	200-500	irregular to alternate	absent
H.* sp. *1 (Jesus *et al.* 2015 as *H. cornuta*)	epilithic	erect	3-7	brownish-yellow	cartilaginous	distinguishable	350-650	spirally alternate to irregular	absent
H. sp. 2 (Jesus *et al.* 2015 as *H. stellulifera*)	epilithic	erect	3-7	brownish-yellow	cartilaginous	distinguishable	350-650	spirally alternate to irregular	absent

Table 5. Summary of morphological characteristics of *Hypnea* species based on the observation of Brazilian specimens.

Species / Reference	Apices	Axial cell	Axial cell diam. (µm)	N° of periaxial cells	Periaxial cells diam. (µm)	Lenticular thickenings	Tetrasporangial sori	Tetrasporangia diam. (µm)
H. brasiliensis Jesus *et al.* (2016)	straight, often forked	rounded, smaller than periaxial cells	27-75	4-6	42-130	abundant, observed in all parts of the thalli	surrounding the basal swollen portions of simple or forked branchlets	17-27
H. cervicornis Jesus *et al.* (2016)	usually straight or curved outward, hooks rare	rounded, smaller than periaxial cells	50-72	4-6	72-107	present at basal	present at basal portions of main axes and first-order branches surrounding the basal swollen portions of branchlets	22-37
H. edeniana Nauer *et al.* (2014)	straight	rounded, smaller than periaxial cells	40-112	5-7	47-132	not observed	surrounding the basal and median swollen portions of branchlets	17-32
H. flava Nauer *et al.* (2014)	straight	rounded, smaller than periaxial cells	26-50	5-6	50-175	abundant in all parts of the thalli	not observed	not observed
H. platyclada Jesus *et al.* (2013)	straight	rounded, smaller than periaxial cells	19-76	5-6	90-174	frequently in the middle portions of the thalli	median portions of branchlets	41-47
H. pseudomusciformis Nauer *et al.* (2015)	Straight, hooks or tendrils	rounded to elliptical, equal, or larger than periaxial cells	40-137	5-8	42-150	not observed	surrounding the basal swollen portions of branchlets, or partially involving the middle portions of branchlets	15-27
H. spinella Nauer *et al.* (2014)	straight, rarely forked	rounded, smaller than periaxial cells	30-65	4-6	55-132	frequently at the middle or apical portions of the thalli	surrounding the basal, median and apical swollen portions of branchlets	12-25
H. wynnei Nauer *et al.* (2016)	straight	rounded, smaller than periaxial cells	30-45	4-5	80-162	not observed	surrounding the apical portions of branchlets	13-20
H. yokoyana Nauer *et al.* (2016)	straight	rounded, smaller than periaxial cells	45-57	4-5	50-62	not observed	not observed	not observed
H.* sp. *1 (Jesus *et al.* 2015 as *H. cornuta*)	straight	rounded, smaller than periaxial cells	20-25	4-5	37-100	present at basal	surrounding the basal and median swollen portions of branchlets	17-23
H. sp. 2 (Jesus *et al.* 2015 as *H. stellulifera*)	straight	rounded, smaller than periaxial cells	20-25	4-5	37-100	present at basal	surrounding the basal and median swollen portions of branchlets	17-23

Discussion

MOLECULAR MARKERS

COI-5P

The DNA barcode technique was first proposed by Hebert et al. (2003) for groups of animals through the use of the 5 'region of the mitochondrial marker *cox*1 also known as COI-5P. Saunders (2005) used 101 COI-5P marker sequences with 710 base pairs in length, representing 15 families of the class of red algae Florideophyceae. The intraspecific divergence found by the author was 0 to 2 bp (0 - 0.3%), and the interspecific divergence was 30 to 90 bp (4.5 - 13.6%), except for *Mazzaella linearis* - *M. splendens* (0.8 - 1.2%) and *Dilsea carnosa* - *D. integra* (1.1%).

For the genus *Hypnea*, the COI-5P marker was first used by Geraldino et. al (2006), who sequenced 1,422 bp of 21 samples in the study of *H. flexicaulis* for the coast of South Korea. Later, Geraldino et al. (2009) used the same marker in the study of *H. charoides* for the Asian region, 44 sequences of 1,362 bp were generated for four *Hypnea* species, in this case, the intraspecific variation found was up to 28 bp (2.06%) and the interspecific divergence of 157 to 165 bp (11.5 - 12.1%). In 2010, Geraldino et. al. extended the coverage of the study to 10 species of the genus, with a total of 27 sequences of 1,245 bp. The intraspecific divergence found was 0 to 17 bp (0 - 1.3%) and the interspecific divergence was 138 to 162 bp (11 - 13%).

The work of Nauer et al. (2014) was the first to use DNA barcode markers for the study of the genus in the Atlantic Ocean. The authors generated 115 COI-5P sequences with 466 bp for six species of *Hypnea* occurring on the Southeast coast of Brazil. The intraspecific divergence ranged from 0 to 5 bp (0 - 0.9%) and the interspecific divergence ranged from 62 to 100 bp (10.1 - 16.3%). In the present study, 65 COI-5P sequences of 565 bp were used for ten Brazilian taxa of the genus. The intraspecific divergence found was 0 to 14 bp (0 - 1.7%) and the interspecific divergence found was 69 to 130 bp (9.8 - 18.2%).

The values of the divergences obtained for the COI-5P marker are close to the values described by Nauer et al. (2014) for the Brazilian *Hypnea* sequences. The values are also relatively close to those proposed by Saunders (2005) for red algae and by Geraldino et al. (2009, 2010) for the Asian sequences of *Hypnea*. The exception is in

the maximum values of intraspecific divergences (1.7% against 1.3% of Geraldino et al. 2010) and the higher interspecific divergences (18.2% against 13.6% of Saunders 2005). Despite this, there were no overlapping divergences and the difference between intraspecific and interspecific variation is significantly high.

UPA

The UPA marker was initially proposed by Sherwood & Presting (2007). These authors used 107 sequences of 377 base pairs, representing the diversity of eukaryotic algae and cyanobacteria. Sherwood et al. (2010), in a study of the diversity of red algae in Hawaii, used three molecular markers, the mitochondrial COI-5P the plastidial gene, UPA and the nuclear gene LSUrDNA, representing 42 families of the class Florideophyceae, including Cystocloniaceae and the genus *Hypnea*. The results of the authors indicate an efficiency of 44.8 ± 28% in the amplification of the COI-5P marker, against 67.4 ± 25.8% success of UPA amplification. The authors further emphasize that despite the greater ease of amplification of UPA and the difficulty of amplification of the COI-5P, the mitochondrial marker is more efficient in the separation of species, being the plastidial marker more conserved.

Nauer et al. (2014) also sequenced the UPA marker for studies of the genus *Hypnea* for the coast of Brazil. The author obtained 33 sequences for the marker, representing six species found in the Southeast region, with intraspecific divergence of 0 to 1 bp (0 - 0.3%) and the interspecific divergence of 9 to 16 bp (2.5 - 4.4 %). In the present work, 63 sequences for ten species of the genus were used. The intraspecific divergence obtained was 0 to 3 bp (0 - 0.8%) and the interspecific divergence found was 7 to 28 bp (1.8 - 6.9%). The difference found in the values of intra- and interspecific divergence of this work in relation to those presented by Nauer et al. (2014) is probably due to the sampling difference in the number of sequences (63 against 33) and species (ten to six).

Clarkston & Saunders (2010) used the markers COI-5P and UPA in the study of the family Kallymeniaceae (Gigartinales, Rhodophyta). The intraspecific divergence for the COI-5P marker varied from 0 to 0.9%, while the divergence between species was greater than 4.5%. For the UPA marker, the intraspecific divergence was 0 to 0.25% and

the interspecific divergence was 0.81 to 6.2%. Therefore, the little difference between the maximum intraspecific divergence and the minimum of interspecific divergence can be considered a limiting factor of the UPA marker, indicating low efficiency in the separation of closely related species (Clarkson & Saunders 2010). The results obtained for the marker corroborate the data of Sherwood et al. (2010) in the sense that UPA is more successful in amplification than the COI-5P marker, but it has less variation and, therefore, less efficiency as a DNA barcode.

***rbc*L**

The *rbc*L marker, is widely used in the phylogenetic studies of photosynthetic organisms. *Hypnea* sequences generated for this marker usually had around 1,355 bp. The amplification success of this marker was less than the amplification of other markers. In addition, in some cases the sequencing had to be redone until good quality sequences were obtained. Saunders & Moore (2013) proposed the utility of the 3' region of this gene (*rbc*L-3P) as a DNA Barcode, reducing the size and effort of sequencing the marker. In relation to the interspecific divergence within *Hypnea*, the variation was from 48 to 92 bp (3.6 - 6.8%) (Nauer et al. 2014). Geraldino et al. (2010), in their study of the genus *Hypnea* for the Asian region, used 42 sequences of 1,356 bp representing 23 species of the genus. The interspecific divergence found for the marker was 18 to 93 bp (1.3 - 6.8%).

Of the three DNA barcodes used in this study for *Hypnea*, COI-5P was the most succeful considering the combined amplification and sequencing efficiency and the level of variation. Despite the efficiency of the COI-5P marker for the genus *Hypnea*, it may present complications for other algal groups and the lack of universal primers for the marker is an already known problem (Clarkson & Saunders 2010).

TAXONOMY OF THE BRAZILIAN *HYPNEA* SPECIES

The two undescribed species, *Hypnea* sp1 and *Hypnea* sp2 were both found only in the state of Bahia, on the beach of Coroa Vermelha, growing in tidal pools, partially buried in the sand, fixed to small buried rocks. Initially, all the samples were identified as *H. cornuta*, by its coloration, presence of starred branchlets and because they were

collected at the same location by Jesus (2013b). However, molecular analyzes revealed that Brazilian specimens were closer to *H. stellulifera* than to *H. cornuta*. *Hypnea stellulifera* originally was considered a variety of *H. cornuta*, described as *H. cornuta* var. *stellulifera* J. Agardh, which was raised to the species level by Yamagishi et al. (2003). *Hypnea stellulifera* differs from *H. cornuta*, according to Yamagishi et al. (2003), by the shorter thallus, with presence of star-shaped branchlet in greater abundance and quantity, whereas *H. cornuta* has a more elongated and narrow thallus, with less and sparse star-shaped branchlets. The environment in Brazil where the specimens were collected coincides with the environments described by Yamagishi et al. (2003), as well as measures of thallus and reproductive features. However, Brazilian specimens, although more closely related to *H. stellulifera*, still present significant molecular divergences, and may be a third and fourth species of the *Hypnea cornuta-stellulifera* complex.

Both *Hypnea* sp1 and *Hypnea* sp2 can be easily distinguished from the other species of the genus in the country by the light yellowish coloration, its habitat, presence of star-shaped sprites of up to 6 points and by the stalk with thick cell wall, visible in cross-section.

The anatomical characters useful in distinguishing species of the genus *Hypnea* were initially raised by Kutzing (1868), Okamura (1909) and Setchell (1924). However, the first extensive anatomical study was performed by Tanaka (1941), who used the cross section of the thallus in his analyzes, considering internal characteristics such as the main axis cells and the presence of lenticular thickening in the walls of the medullar cells to describe species of *Hypnea* from Japan. The vegetative and reproductive morpho-anatomical characters most used over time in the taxonomic studies of the genus were summarized by Masuda et al. (1997).

Studies with *Hypnea* emphasize that morphological characters undergo variation in relation to the environmental conditions and habitat of the specimen (Yamagishi & Masuda 2000, Lucio 2006, Geraldino *et al.* 2010, Guimarães 2011, Jesus 2013b, Nauer et al. 2015). The color of the thallus is highly variable for some species, but has been more homogeneous for others. Species such as *H. spinella*, *H. edeniana*, *H. flava*, *H. yokoyana* and *H. wynnei* presented uniform color of the thallus, and variation occurred only in relation to the tonality (lighter red or darker red, for example). Other species such as *H. brasiliensis*, *H. cervicornis* and *H. pseudomusciformis* showed great

variation in the color of the thallus, being yellow, brown, green or red, besides the variation in the tonality (light or dark), depending on the habitat.

As for the habit and height of the thallus, the species of Brazilian coast can be divided into two groups, one presenting a non-intricate tufted thallus and the other having a densely intricate thallus. In the first group are species such as *H. brasiliensis*, *H. cervicornis*, *H. pseudomusciformis*, *Hypnea* sp. 1 and *Hypnea* sp. 2. In these species, specimens larger than 10 cm in height are relatively common. Some morphological variations of *H. pseudomusciformis* may form tufts, but the branches are not intricate and do not form tangles, since there is no occurrence of anastomoses or presence of secondary spinning discs in these species (Nauer et al. 2014, 2015). The second group is formed by species such as *H. edeniana*, *H. flava*, *H. platyclada*, *H. spinella*, *H. yokoyana* and *H. wynnei*. These species have a considerably smaller size than the species of the first group, where it is common thalli of 2 or 3 cm in height. The appearance of the thallus in tangled tufts is due to the presence of numerous secondary fixation discs among the branches of the algae. Some species such as *H. edeniana*, *H. flava* and *H. spinella* have anastomoses that help the thallus to form tangles of difficult separation (Nauer et al. 2014, 2016; Jesus et al. 2016).

The texture of the algae varied between membranous or cartilaginous. The species *Hypnea* sp. 1 and *Hypnea* sp. 2 presented the most cartilaginous texture, which can be explained by the fact that the cell wall of this alga is noticeably thicker than the other species, a characteristic observed in anatomical sections. The main axis is present in all species, with the difference being only one or multiple and whether it is persistent to the apex of the branch or highly branched. The species *Hypnea* sp. 2 has the most visible persistent main axis between species and the main axis of *H. pseudomusciformis* has proved to be the most difficult to observe due to the amount of branching, but with careful examination it is possible to observe it through the diameter of the main axis, which is relatively larger than the diameter of the lateral branches (Nauer et al. 2015).

Species like *H. cervicornis*, *H. edeniana*, *H. flava* and *H. spinella* have multiple major axes, which are branches of similar size that start from the same point at the base of the thallus. Subsequent branches have a significantly smaller size and diameter than the multiple principal axes. All species have a cylindrical axis, with the exception of *H. wynnei* and *H. platyclada*, which presented a cylindrical base and flattened upper

portions. This characteristic, however, is not exclusive to those species, and were observed in other species of *Hypnea*, such as *H. volubilis* (Schneider & Searles 1976, Dawes & Mathieson 2008, Jesus et al. 2013).

Irregular branching is present in all species of the genus and is easily observed in a first analysis, where branches can be formed in any part of the thallus, in any plane. In a more detailed analysis of some species, however, some patterns may be observed, even if these patterns are not maintained throughout the length of the thallus. In *H. wynnei*, for example, it is more common to observe an alternate pattern of branching, but with parts of the thallus having irregular branching (Nauer et al. 2016). This pattern also occurs to a greater or lesser extent in the species *H. edeniana*, *H. flava* and *H. spinella* (Nauer et al. 2014). *Hypnea brasiliensis* presents irregular branching pattern throughout the extension of the thallus, but the apical region commonly presents dichotomic or subdicotomic ramifications (Jesus et al. 2016).

Almost all analyzed species, with the exception of *H. brasiliensis* and *Hypnea* sp. 2, exhibit spinescent branchlets along the entire length of the thallus. The shape of the spinous branchlets varies, and may be in the form of small horns, which can be curved upwards, as in *H. cervicornis* and *H. edeniana*, or in the form of tiny branches, as in other species. The spinous branchlets in *H. pseudomusciformis* are larger and more abundant compared to other species and may resemble small hairs covering the thallus. In addition to spinous branchlets, *H. brasiliensis* also has bifurcated branchlets, a characteristic that helps to distinguish this species from the others. Spinous branchlets may also be present in *Hypnea* sp. 2, but this species also has star-shaped branches, which may detach from the thallus (Jesus et al. 2015).

Straight apices are found in all species. In *H. cervicornis* and *H. pseudousciformis*, crooked, hook-shaped apices are common, but only in *H. pseudomusciformis* occurs the presence of tendrils. Tendrils, however, are not unique to this species. In Asian species, for example, the presence of tendrils in the apex of the branches is common (Masuda et al. 1994). We could observe in our samples a gradation in relation to the formation of hooks. Apices may be slightly recurved and others may be on a level that may resemble a tendril in formation. A specimen may present several levels of gradation of its curved apices, without uniformity.

Using anatomical characteristics of the genus, species of *Hypnea* can be divided into two groups in relation to the axial cell size compared to the periaxial cells, a feature observed in the transverse section of the thallus (Bodard 1968). In the first group, the axial cell is significantly smaller than the periaxial cells, while in the second group the axial cell is larger or equal to the periaxial cells.

H. pseudomusciformis has significantly larger axial cell size (reaching 137.5 μm in diameter) in comparison to the other species studied. In the other species it is more common that the axial cell has similar to that of the periaxial cells. The species *Hypnea* sp. 1 and *Hypnea* sp. 2 has the smallest axial cell among the species studied, followed by *H. wynnei*. Larger periaxial cells were found in the species *H. cervicornis*. The number of periaxial cells, in turn, varied uniformly among the species, being a characteristic that overlaps and is inefficient in the separation of the species. For example, while in most species it is common to find five or more periaxial cells in transversal sections, in *H. yokoyana* and *H. wynnei* is common to find only four periaxial cells, however, it is possible to find five periaxial cells in both species. In addition, though rare, four periaxial cells could also be observed in *H. cervicornis*, *H. spinella*, *Hypnea* sp. 1 and *Hypnea* sp. 2.

Another feature considered in the taxonomy and observed in the cross section of the thallus is the lenticular thickening, which occurs in the cell wall of the medullary cells, mainly in the periaxial cells, and are visible even without the aid of dyes. Although they are used in the characterization of *Hypnea* species, Ogawa & Lewmanomont (1981) stated that the presence of these thickenings depends on differences in ecological conditions in the various environments where they occur. The presence or absence of lenticular thickening on the walls of the medullary, periaxial and axial cells was also not useful in distinguishing the species. The presence of lenticular thickening may vary in specimens of the same species (Jesus et al. 2013b). In addition, Ogawa & Lewmanomont (1981) argue that this variation may be related to habitat and environmental conditions.

Regarding the reproductive structures, the position of the tetrasporangial sori in the branches (basal, median or apical) is the only characteristic that has been constant in the identification of the species. *Hypnea wynnei* is the only Brazilian species to have tetrasporangial sori at the apex of the branches. In species *H. cervicornis* and *H.*

edeniana, the sori were only found in the basal region of the branches, and in *Hypnea* sp2 were only found in the median portion. In the other species, the position varied and was found in both the mid and basal portions of the branches. It is common for sori to completely surround the branches where they are found, but in the species *H. cervicornis* and *H. pseudomusciformis* it is also common to find the sori on only one side of the branches, circling them incompletely (Figures 4, 5 and 6, Table 5).

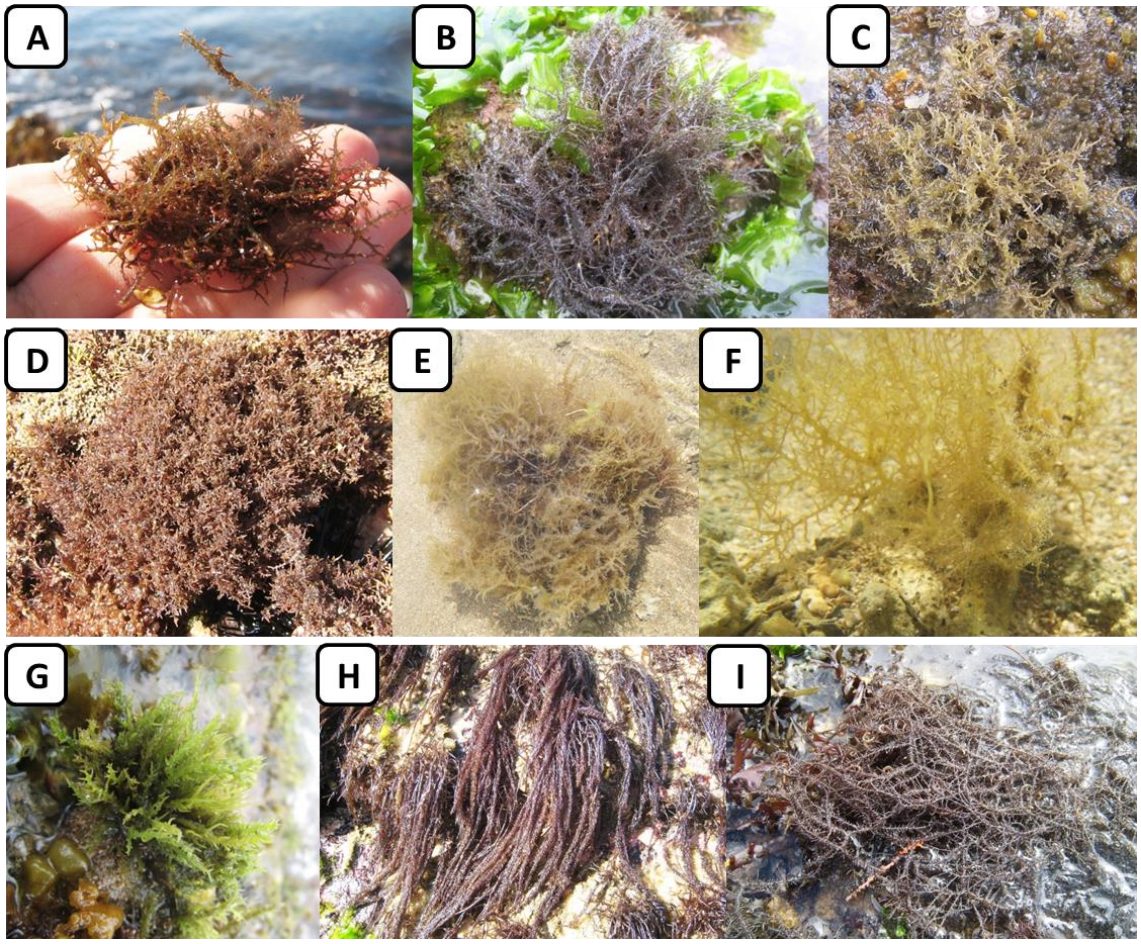

Figure 4: Habits of *Hypnea* on the field. **A.** *H. brasiliensis*. **B.** *H. cervicornis*. **C.** *H. flava*. **D.** *H. spinella*. **E.** Turf of *Hypnea* sp. 1. **F.** *Hypnea* sp. 2. **G**, **H** and **I.** Morphological variations of *H. pseudomusciformis*.

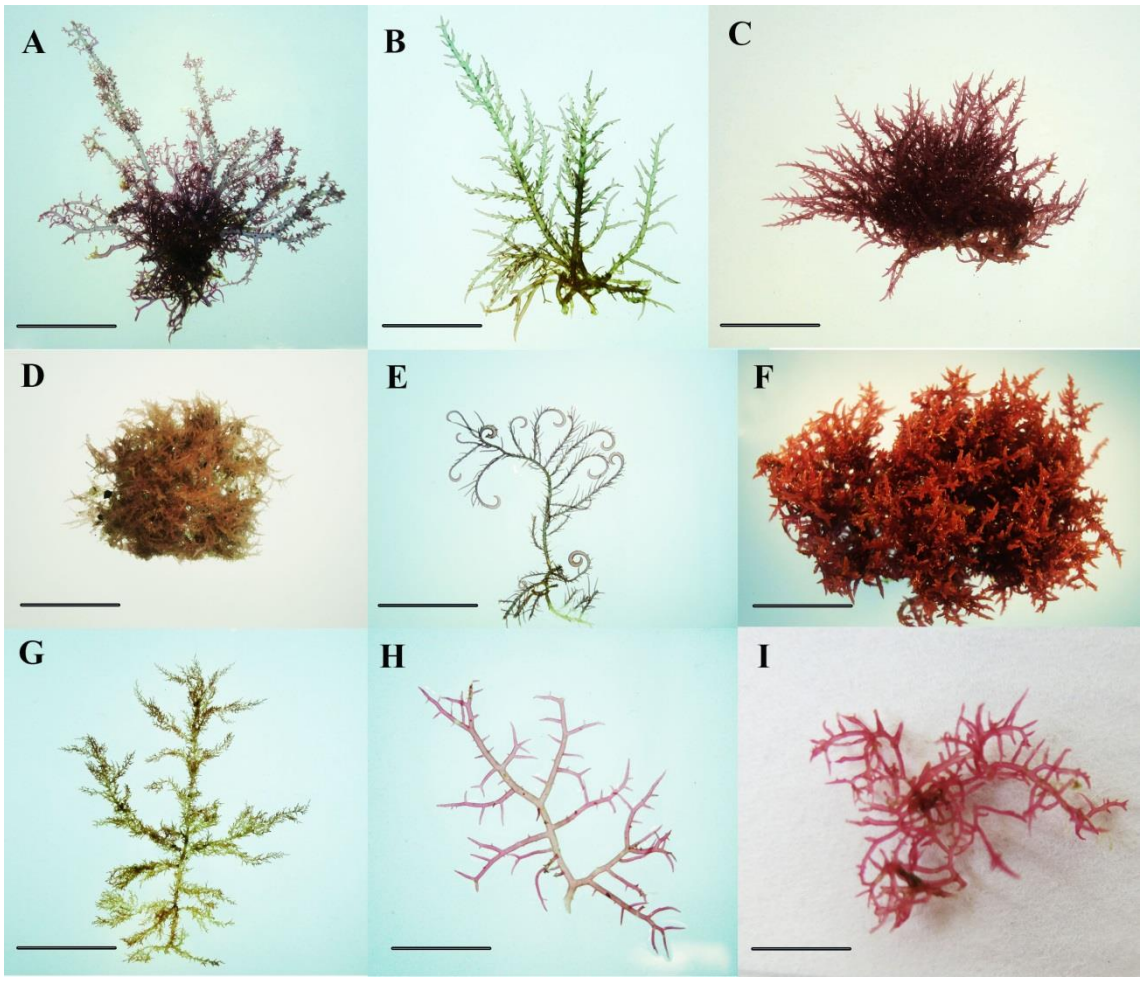

Figure 5: Habits of Brazilian *Hypnea* species. **A.** Habit of *H. brasiliensis*. **B.** Habit of *H. cervicornis*. **C.** Habit of *H. edeniana*. **D.** Habit of *H. flava*. **E.** Habit of *H. pseudomusciformis*. **F.** Habit of *H. spinella*. **G.** Habit of *Hypnea* sp. 2. **H.** Habit of *H. yokoyana* and **I.** Habit of *H. wynnei*. Scale bar: 1 cm.

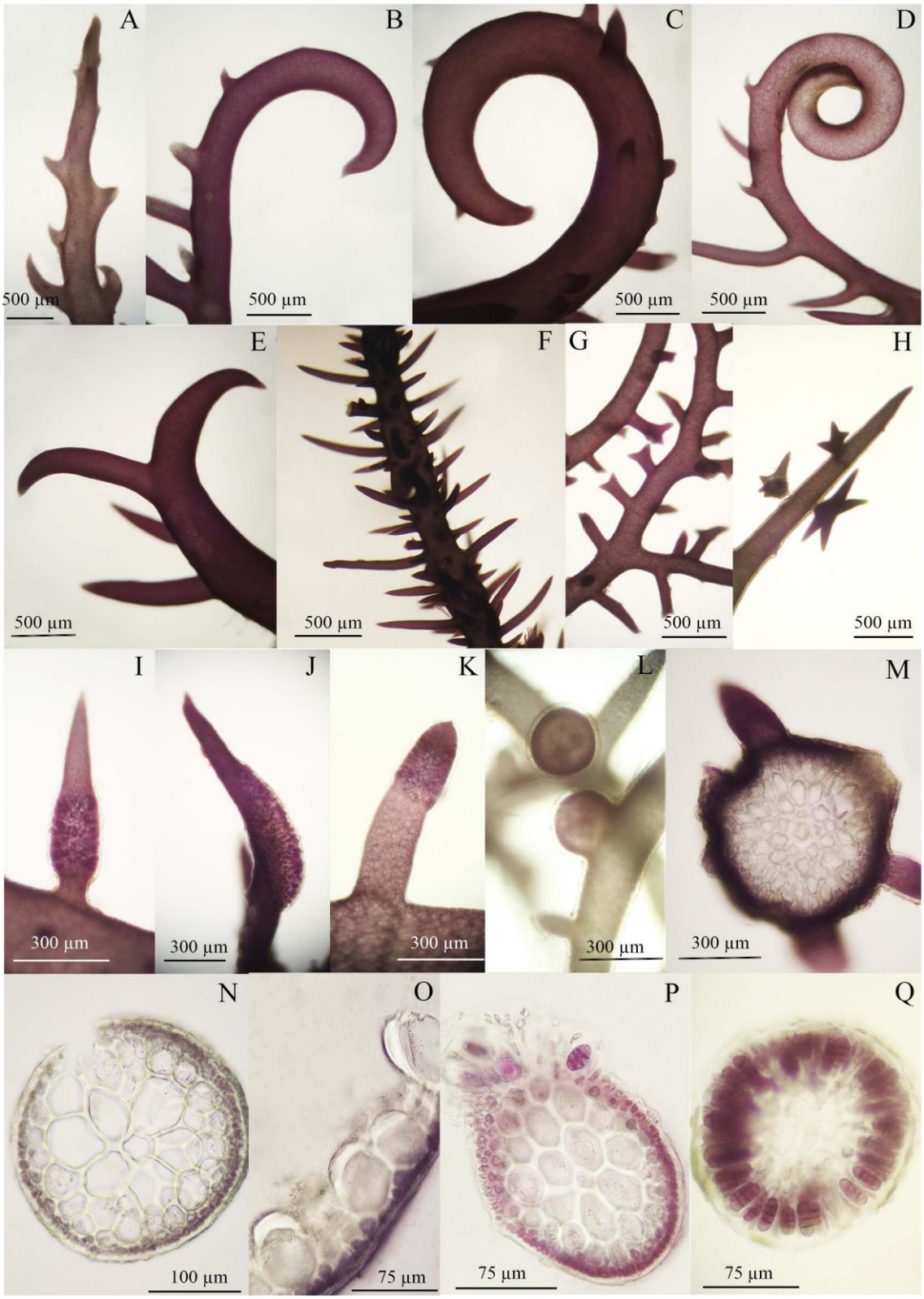

Figure 6: Variation of the main morpho-anatomical characters in *Hypnea*. **A - E.** Variety of apices from *H. pseudomusciformis*. **A.** Straight or acute apex. **B.** Recumbent apex. **C.** Apex in the shape of a hook. **D.** Apex in the shape of a tendril. **E.** Bifurcated apex. **F - H.** Variety of branchlets. **F.** Spinescent, from *H. pseudomusciformis*. **G.** Forked, from *H. brasiliensis*. **H.** Starlets, from *Hypnea* sp.2. **I - K.** Variation in the position of tetrasporangial sori. **I.** Tetrasporangia at the base of the branch, from *H.*

cervicornis. **J.** Tetrasporangia in the middle portion of the branch, from *H. pseudomusciformis*. **K.** Tetrasporangia at the apex of the branches, from *H. wynnei*. **L.** Cystocarps, from *H. brasiliensis*. **M and N.** Transverse section of the thallus of two specimens of *Hypnea* evidencing the basic types of axial organization, defined by Bodard (1968). **M.** *H. pseudomusciformis.* **N.** *H. spinella*. **O.** Lenticular thickening in the cells of the spinal cord. **P and Q.** Transverse section tetrasporangial sori, both from *H. pseudomusciformis*. **P.** Incompletely circling the radius. **Q.** Completely circling the radius. Adapted from Nauer (2013).

Identification key of species of the genus *Hypnea* for the Brazilian coast:

1a. Plant forming tufts densely intricate 2

1b. Plant forming tufts with upright main axes 8

2a. Thallus entirely flattened……..…...……*H. platyclada*

2b. Thallus erect with flattened portions or entirely cylindrical……3

3a. Thallus up to 1 cm in height, stalk with flattened portions, tetrasporangia may be located at the apex of the fertile branches .....*H. wynnei*

3b. Thallus more than 1 cm high, cylindrical stem, tetrasporangia located in the medial and / or basal portions of the thallus 4

4a. Anastomoses present and abundant, rare or absent secondary fixation discs 5

4b. Rare or absent anastomoses, secondary and abundant fixation discs 6

5a. Anastomoses present throughout the extension of the thallus ..... *H. spinella*

5b. Anastomoses present in the basal portions of the thallus, free apices ..... *H. flava*

6a. Bifurcated apexes, deer antler-like branching, abundant spinescent branchlets…….*H. brasiliensis*

6b. Simple apexes, branching differently, sparse spinescens 7

7a. Rare secondary branches .....*H. yokoyana*

7b. Secondary ramifications present and abundant .....*H. edeniana*

8a. Thick cell wall, may present starred branchlets 9
8b. Fine cellular wall, absent starry stars 10

9a. Thick cell wall, yellow color, branchlets star shaped absent…….. *Hypnea* sp1
9b. Thick cell wall, yellow to brown color, branchlets star shaped present. *Hypnea* sp2

10a. Straight and / or curved apices, short spinescent branches with broad base and fine apex, axial cell smaller than periaxial .....*H. cervicornis*
10b. Apex in the form of tendrils, straight and / or curved, long spinescent branches of base and apex of same diameter, axial cell greater or equal to periaxial cells ....*H. pseudomusciformis*

Conclusion

In red algae, convergent evolution and recent speciation have led to the emergence of several cryptic species that cannot be differentiated on the basis of morphology alone, but which have genetic differences. On the other hand, specimens of the same species may have a highly variable morphology depending on environmental conditions, and in this case genetic differences are not taxonomically significant (Cianciola et al. 2004). For the genus *Hypnea*, the use of isolated morphological characteristics alone was not sufficient for taxonomic identification of all specimens due to the great phenotypic plasticity of the genus, whose morphology varies depending on the environment, such as *H. pseudomusciformis*, where morphological variations of the species were previously identified as *H. nigrescens* and *H. valentiae* (Nauer et al. 2015). The morphology can also vary with geographic distribution, as an example of *H. brasiliensis*, where specimens from the northeast of the country have a distinct morphology of the specimens from the Southeast and South regions (Jesus et al. 2016).

On the other hand, due to a simple and convergent morphology with few taxonomically informative characters, other species are similar, which makes it difficult to detect new occurrences and new species for science, as an example of *H. yokoyana* and *H. wynnei* (Nauer et al. 2016). The detection of these species was only possible with the use of the DNA barcode technique in a wide range of samples. This technique proved to be fast and efficient in the separation of species, especially since many

specimens are found with only few branches, little biomass and no reproductive structures, especially those that inhabit the subtidal zone.

In this work, the Brazilian species of the *Hypnea* were identified based on the phylogenetic concept of species, separated primarily by genetic differences and representing evolutionary monophyletic lineages, a characteristic observed in the concordant topologies of the three molecular markers. Of the 16 species previously cited, we molecularly confirmed the occurrence of only ten: *H. brasiliensis*, *H. cervicornis*, *H. edeniana*, *H. flava*, *H. pseudomusciformis*, *H. spinella*, *H. yokoyana*, *H. wynnei*, *Hypnea* sp. 1 and *Hypnea* sp. 2. *Hypnea platyclada* was confirmed based on its unique morphology. The DNA barcode technique, especially with respect to the COI-5P marker, proved to be very useful and essential in the identification and delimitation of the species, revealing scenarios that would go unnoticed by morphology. However, after establishing a framework using the molecular data to infer the range of morphological variation and biogeographic distribution for each species, morphology can be usefull for field identification of *Hypnea* species on the coast of Brazil.

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References

Amado-Filho, G.M., Horta, P.A., Brasileiro, P.S., Barros-Barreto, M.B. & Fujii, M.T. 2006. Subtidal benthic marine algae of the Marine State Park of Laje de Santos (Sao Paulo, Brazil). *Brazilian Journal of Oceanography* 54: 225-234. http://dx.doi.org/10.1590/S1679-87592006000300006

Bodard, M., 1968. Les *Hypnea* au Sénégal (Hypnéacées, Gigartinales). Bulletin de l'institud Fondamental d'Afrique Noire, 3: 811-829.

Clarkston, B.E. & Saunders, G.W. 2010. A comparison of two DNA barcode markers for species discrimination in the red algal family Kallymeniaceae (Gigartinales, Florideophyceae), with a description of *Euthora timburtonii* sp. nov. Botany, 2010, 88(2): 119-131, https://doi.org/10.1139/B09-101

Cianciola, E.; Popolizio, T.; Schneider, C. W. & Lane, C. 2004. Using Molecular-Assisted Alpha Taxonomy to Better Understand Red Algal Biodiversity in Bermuda. Diversity 2010, 2, 946-958.

Dawes, C.J. & Mathieson, A.C. 2008. The seaweeds of Florida. University Press of Florida. 591p.

Freshwater, D.W. & Rueness, J. 1994. Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species based on *rbc*L nucleotide sequence analysis. *Phycologia* 33: 187-94. http://dx.doi.org/10.2216/i0031-8884-33-3-187.1

Geraldino, P.J.L., Yang, E.C. & Boo, S.M. 2006. Morphology and molecular phylogeny of *Hypnea flexicaulis* (Gigartinales, Rhodophyta) from Korea. *Algae* 21: 417-423.DOI 10.4490/ALGAE.2006.21.4.417

Geraldino, P.J.L., Yang, E.C, Kim, M.S. & Boo, S.M. 2009. Systematics of *Hypnea asiatica* sp. nov. (Hypneaceae, Rhodophyta) based on morphology and nrDNA SSU, plastid *rbc*L, and mitochondrial *cox*1. *Taxon* 58: 606-616.

Geraldino, P.J.L., Riosmena-Rodriguez, R., Liao, L.M. & Boo, S.M. 2010. Phylogenetic relationships within the genus *Hypnea* (Gigartinales, Rhodophya), with a description of *H. caespitosa* sp. nov. *Journal of Phycology* 46: 336-345. DOI: 10.1111/j.1529-8817.2009.00804.x

Guimarães, S.M.P.B. 2006. Checklist of Rhodophyta from the State of Espírito Santo. Boletim do Instituto de Botânica 17: 143-194.

Guimarães, N. 2011. Diversidade do gênero *Hypnea* (Gigartinales, Rhodophyta) do Estado de São Paulo baseada em marcadores moleculares e morfologia. Dis. Mestrado. Inst. De Biociências. USP.

Hall, T.A. 1999. BioEdit: a user-friendly biological alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95-98.

Herbert P.D.N., Cywinska A., Ball S.L. & deWaard J.R. 2003. Biological identifications through DNA barcoding. Proceedings of the Royal Society of London, B, 270: 313-322.

Jesus, P.B., Guimarães, S.M.P.B. & Nunes, J.M.C. 2013 a. *Hypnea platyclada*, a new species of red alga (Rhodophyta, Cystocloniaceae) from Brazil. *Phytotaxa* 85: 26-34. http://dx.doi.org/10.11646/phytotaxa.85.1.4

Jesus, P.B., Schnadelbach, A.S. & Nunes, J.M.C. 2013 b. O gênero *Hypnea* (Cystocloniaceae, Rhodophyta) no litoral do estado da Bahia, Brasil. *Sitientibus série Ciências Biológicas* 13: 1-21. DOI:10.13102/scb215

Jesus, P.B., Silva, M.S., Lyra, G.M., Nunes, J.M.C. & Schnadelbach, A.S. 2015. Extension of the distribution range of *Hypnea stellulifera* (Cystocloniaceae, Rhodophyta) to the South Atlantic: morphological and molecular evidence. *Aquatic Botany* 123: 26-36. DOI: 10.1016/j.aquabot.2014.12.003

Jesus, P.B., Nauer, F., Lyra, G.M., Cassano, V., Oliveira, M.C., Nunes, J.M.C. & Schnadelbach, A.S. 2016. Species delimitation and phylogenetic analyses of some cosmopolitan species of *Hypnea* (Rhodophyta) reveal synonyms and misapplied names to *H. cervicornis*, including a new species from Brazil. *Journal of Phycology (in press).* DOI: 10.1111/jpy.12436

Kützing, F.T. 1868. Tabulae phycologicae; oder, Abbildungen der Tange. Nordhausen: Gedruckt auf kosten des Verfassers (in commission bei W. Köhne)Vol. 18. p. 35.

Lamouroux, J.V. 1813. Essai sur les genres de la famille des Thalassiophytes non articulées. *Annales du Muséum d'Histoire Naturelle, Paris* 20: 21-47, 115-139, 267-293, pls 7-13.

Lucio, A. M. 2006. El género Hypnea Lamouroux (Gigartinales, Rhodophyta) en las costas del Océano Atlántico. Tesis Doctoral. Facultad de Ciencias Biológicas. Universidad Complutense de Madrid. 186 pp.

Masuda, M., Yamagishi, Y., Chiang, Y.-M., Lewmanomont, K. & Xia, B. 1997. Overview of *Hypnea* (Rhodophyta, Hypneaceae). In *Taxonomy of Economic Seaweeds* (Abbott, I.A., editor), 6: 127–33. California Sea Grant College System, La Jolla.

Nauer, F., Guimarães, N.R., Cassano, V., Yokoya, N.S. & Oliveira, M.C. 2014. *Hypnea* species (Gigartinales, Rhodophyta) from the southeastern coast of Brazil based on molecular studies complemented with morphological analyses, including descriptions of *Hypnea edeniana* sp. nov. and *H. flava* sp. nov. *European Journal of Phycology* 49: 550-575. DOI:10.1080/09670262.2014.981589

Nauer, F., Cassano, V. & Oliveira, M.C. 2015. Description of *Hypnea pseudomusciformis* sp. nov., a new species based on molecular and morphological analyses, in the context of the *H. musciformis* complex (Gigartinales, Rhodophyta). *Journal of Applied Phycology* 27: 2405-2417. DOI: 10.1007/s10811-014-0488-y

Nauer, F., Cassano, V. & Oliveira, M.C. 2016. *Hypnea wynnei* and *Hypnea yokoyana* (Cystocloniaceae, Rhodophyta), two new species revealed by a DNA barcoding survey on the Brazilian coast. Phytotaxa 268 (2): 123–134. http://dx.doi.org/10.11646/phytotaxa.268.2.3

Nunes, J. M. C. 2005. Rodoficeas marinhas bentonicas do estado da Bahia, Brasil. *Tese de Doutorado*. Instituto de Biociencias da Universidade de Sao Paulo. Departamento de Botanica. Sao Paulo. 410 p.

Ogawa, H. & Lewmanomont, K. 1981. Economic seaweeds of Thailand. I. The genus *Hypnea* in the vicinity of Si Racha, Chonburi Province. *Kasetsart University Fishery Research Bulletin* 12: 1-14.

Okamura, K. 1909. Icones of Japanese Algae. Tokyo: published by the author, vol. 2.

Saito, R.S. & Oliveira, E.C. 1990. Chemical screening of Brazilian marine algae producing carrageenans. *Hydrobiologia* 204/205**:** 585-588.

Saunders, G.W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philosophical Transactions of the Royal Society B.* 360: 1879-1888. DOI: 10.1098/rstb.2005.1719

Saunders, G.W. & Moore, T.E. 2013. Refinements for the amplification and sequencing of red algal DNA barcode and RedToL phylogenetic markers: a summary of current

primers, profiles and strategies. ALGAE Volume 28, *Issue1, p31-43. DOI: 10.4490/algae.2013.28.1.031*

Schenkman, R.P.F. 1986. Cultura de *Hypnea* (Rhodophyta) in vitro como subsídio para estudos morfológicos, reprodutivos e taxonômicos. Ph.D. thesis, Universidade de São Paulo, Brazil.

Scheneider, C.W. & Searles, R.B. 1976. North Carolina marine algae. VII. New species of *Hypnea* and *Petroglossum* (Rhodophyta, Gigartinales) and additional records of other Rhodophyta. *Phycologia* 15: 51-60.

Sherwood, A.R. & Presting, G.G. 2007. Universal primers amplify a 23S rDNA plastid marker in eukaryotic algae and cyanobacteria. *Journal of Phycology* 43: 605-608. DOI: 10.1111/j.1529-8817.2007.00341.x

Sherwood, A.R, Kurihara, A., Conklin, K.Y., Sauvage, T., Presting, G.G. 2010. The Hawaiian Rhodophyta Biodiversity Survey (2006-2010): a summary of principal findings. BMC Plant Biology 10:258.

Setchell, W.A. 1924. American Samoa: Part I. Vegetation of Tutuila Island. Part II. Ethnobotany of the Samoans. Part III. Vegetation of Rose Atoll. Publications of the Carnegie Institution of Washington, p. 341.

Tanaka, T. 1941. The genus *Hypnea* from Japan. *Scientific Papers of the Institute of Algological Research, Faculty of Science, Hokkaido University* 2: 227–250.

Thiers, B. 2017. *Index Herbariorum*: *a global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. http://www.nybg.org/bsci/ih/ih.html. [continuously updated].

Villaça, R., Fonseca, A.C., Jensen, V.K. & Knoppers, B. 2010. Species composition and distribution of macroalgae on Atol das Rocas, Brazil, SW Atlantic. Botanica Marina 53: 113-122. DOI: 10.1515/BOT.2010.013.

Yamagishi, Y & Masuda, M. 2000. A taxonomic revision of a *Hypnea charoides-valentiae* complex (Rhodophyta, Gigartinales) in Japan, with a description of *Hypnea flexicaulis* sp. nov. *Phycological Research* 48: 27-35. DOI: 10.1046/j.1440-1835.2000.00181.x

Yamagishi, Y., Masuda, M., Abe, T., Uwai, S., Kogami, K., Kawagushi, S. & Phang, S.M. 2003. Taxonomic notes on marine algae from Malaysia. XI. Four species of Rhodophyceae 1. *Botanica Marina* 46: 534-547. DOI: 10.1515/BOT.2003.056

Yoneshigue, Y. 1985. Taxonomie et ecologie des algues marines dans la region de Cabo Frio (Rio de Janeiro, Bresil). PhD Thesis - Faculté dês Sciences de Lumin. L'Universite D'aix – Marseille I. 466 p.

Table 1S. Samples used in this study for molecular analyses collected in the Brazilian coast. identified by species name. Project ID number; Collection information and GPS coordinates; Voucher number (SPF herbarium); COI-5P, UPA and *rbc*L GenBank accession numbers (xxxxxx, new sequences generated in this work; GenBank accession will be added once the paper is accepted for publication).

Species	ID number	Collection data	Voucher	COI-5P	UPA	*rbc*L
Hypnea cervicornis	IBT0802	Dois Coqueiros, Caucaia, Ceará, Brazil, 3°41’08.40’’S, 38°38’00.18’’W, leg. L. Soares, 28 Aug. 2011	SP365646	KU905088	xxxxxx	xxxxxx
	IBC0968	Rio do Fogo Beach, Rio do Fogo, Rio Grande do Norte, Brazil, 5°16’32.90’’S, 35°22’41.76’’W, coll. C. A. Azevedo, 12 Jan. 2013	SPF57641	KU905139	xxxxxx	xxxxxx
	IBC0970	Toquinho Beach, Ipojuca, Pernambuco, Brazil, 8°36’12.37’’S, 35°02’42.25’’W, coll. N. S. Yokoya, 14 Mar. 2013	SPF57643	KU905154	xxxxxx	xxxxxx
	IBC0971	Toquinho Beach, Ipojuca, Pernambuco, Brazil, 8°36’12.37’’S, 35°02’42.25’’W, coll. N. S. Yokoya, 14 Mar. 2013	SPF57644	KU905120	xxxxxx	xxxxxx
	IBC0956	Francês Beach, Marechal Deodoro, Alagoas, Brazil, 9°46’07.79’’S, 35°50’17.44’’W, coll. T. Vieira-Pinto et. al., 10 Mar. 2013	SPF57631	KU905153	xxxxxx	xxxxxx
	IBC0733	Cabras Island, Ubatuba, SP, -.23.310.141, -	SPF57443	KM214561	xxxxxx	KM203911

		45.022.936, C. Iha & B. Torrano				
	IBC0190	Saco do Mamanguá, Paraty, RJ, - 23.263.633, - 44.636.397, F. Nauer, C. Iha & B. Torrano	SPF56540	KM214530	KM210590	KM203900
	IBC0703	Farol Island, Arraial do Cabo, RJ, -22.595.515, - 42.001.113, F. Nauer, C. Iha & B. Torrano	SPF57418	KM214564	KM210574	xxxxxx
	IBC0801	Ponta das Canas, Florianópolis, Santa Catarina, Brazil, 27°23’38.79’’S, 48°26’08.75’’W, coll. F. Nauer et al., 26 Feb. 2012	SPF57498	KU905096	xxxxxx	KU905160
Hypnea brasiliensis	IBC0881	Canoa Quebrada, Aracati, Ceará, Brazil, 4°31’28.62’’S, 37°41’58.06’’ W; coll. F. Nauer, 05 Jun. 2012	SPF57566	KU905127	xxxxxx	KU905127
	IBC0888	Carapibus Beach, Conde, Paraíba, Brazil, 7°17’59.63’’S, 34°47’55.31’’ W; coll. F. Nauer & C. A. Azevedo, 19 Jul. 2012	SPF57571	KU905097	xxxxxx	KU905161
	P14	Mucugê Beach, Arraial D’Ajuda, Porto Seguro, Brazil; 16°29’51, 67”S, 39°04’10,47”W; coll. Jesus et al., 06 Nov. 2010	ALCB100234	KU905078	-	KU905157
	P42	Emissário Beach, Camaçari, Bahia, Brazil, 12°44’25,70”S, 38° 8’58,97”W; coll. P.B. Jesus et al., 23 Sep. 2010	ALCB100228	KU905118	-	KU905166
	IBC0028	Vermelha do Sul Beach, Ubatuba, SP, - 23.512.377, - 45.172.014, F. Nauer, C. Iha & B. Torrano	SPF56877	KM214498	KM210583	KM203898

	IBC0064	Dura Beach, Ubatuba,SP, - 23.495.159, -45.165.038, F. Nauer, C. Iha & B. Torrano	SPF56881	KM214500	KM210584	KM203897
	IBC0245	Bom Abrigo Island,Cananéia, SP, - 25.133.333,-47.966.667, F. Nauer, C. Iha & B. Torrano	SPF57139	KM214516	xxxxxx	KM203902
	IBC0254	Queimada Grande Island, Itanhaém, SP, -24.292.129, -46.403.462, F. Nauer, & B. Torrano	SPF57507	KM214539	KM210565	KM203908
	IBC0838	Agha Beach, Piúma, ES, -20.523.909, -40.453.588, F. Nauer, C. Iha & B. Torrano	SPF57528	KM214552	xxxxxx	KM203910
	IBC0843	Cruz Beach, Marataízes, ES, -21.020.168, -40.484.551, F. Nauer, C. Iha & B. Torrano	SPF57533	KM214554	KM210572	xxxxxx
	IBC0792	Arvoredo Island, Governador Celso Ramos, Santa Catarina, Brazil, 27°17’19.49’’S, 48°22’05.95’’W; coll. F. Nauer et al., 25 Feb. 2012	SPF57490	KU905091	xxxxxx	KU905159
Hypnea edeniana	BOT0003	Cigarras Beach, São Sebastião, SP, - 23.436.790, -45.157.910, F. Nauer, C. Iha & B. Torrano	SP390803	KM214529	KM210591	KM203896
	IBC0746	Eden Beach, Guaruja, SP, -23.590.510, -46.111.164, F. Nauer, C. Iha & A. Medeiros	SPF57454	KM214573	KM210579	xxxxxx
	IBC0906	Pacuiba Beach, Bela Island, SP, -23.453.580, -45.205.683, F. Nauer, C. Iha & A. Medeiros	SPF57587	KM214575	xxxxxx	xxxxxx
	IBC0842	Cruz Beach, Marataízes, ES, -21.020.168, -40.484.551, F. Nauer, C. Iha & A. Medeiros	SPF57532	KM214574	KM210578	KM203907

Hypnea flava	IBC0211	Picinguaba Beach, Ubatuba, SP, -23.380.032, -44.840.732, F. Nauer, C. Iha & A. Medeiros	SPF57044	KM214531	xxxxxx	xxxxxx
	IBC0212	Vermelha do Sul Beach, Ubatuba,SP, -23.506.713, -45.173.065, F. Nauer, C. Iha & A. Medeiros	SPF57045	KM214532	xxxxxx	KM203903
	IBC0910	Pinto Beach, Bela Island, SP, -23.442.511, -45.205.154, F. Nauer, C. Iha & A. Medeiros	SPF57590	KM214572	xxxxxx	xxxxxx
	IBC0777	Carro Beach, Saco do Mamangua, RJ, -23.142.195, -44.370.685, F. Nauer, C. Iha & A. Medeiros	SPF57477	KM214569	KM210576	xxxxxx
	IBC0779	Ponta do Costa, Saco do Mamangua, RJ, -23.153.217, -44.371.667, F. Nauer & B. Torrano	SPF5749	KM214570	xxxxxx	xxxxxx
	IBC0851	Namorados Beach, Guarapari, ES, -20.401.965, -40.294.722, F. Nauer, C. Iha & B. Torrano	SPF57539	KM214571	KM210577	KM203906
Hypnea pseudomusciformis	IBC0864	Beach Lagoinha. Paraipaba. CE, $3^0$20’42.37’’S, $39^0$08’09.24’’W, leg. F. Nauer, 02 Jun. 2012	SPF57554	KM523190	xxxxxx	KM509070
	IBC0875	Beach Mundaú. Trairi. CE, $3^0$10’44.22’’S/$39^0$22’21.81’’W, leg. F. Nauer, 03 Jun. 2012	SPF57560	KM523196	xxxxxx	KM509068
	IBC0894	Beach Coqueirinhos. Conde. PB, $7^0$19’18.86’’S/$34^0$47’52.28’’W, leg. F. Nauer & C. A. Azevedo, 19 Jul. 2012	SPF57577	KM523241	xxxxxx	KM509069
	IBC0930	Beach Coroa Vermelha. Santa Cruz de Cabrália. BA, $16^0$19’57.02’’S/$39^0$00’13.59’’W, leg. F. Nauer	SPF57608	KM523202	xxxxxx	KM509064

		& C. A. Azevedo, 15 Sep. 2012				
	IBC0835	Beach Itaoca, Itapemirim, ES, F. Nauer, C. Iha & B. Torrano-Silva. 06. V. 2012 $20^0$54’20.32’’S/$40^0$46’42.72’’O	SPF57526	KM523183	xxxxxx	KM509065
	IBC0040	Vermelha do Sul Beach, Ubatuba,SP, -23.506.713, -45.173.065	SPF56878	KM214480	KM210585	KM203894
	IBC0098	Costa Beach, Ubatuba, SP, - 23.775.581, -45.233.514	SPF56883	KM214483	KM210586	KM203895
	IBC0799	Ponta das Canas. Florianópolis. SC. F. Nauer, C. Azevedo, M. Sissini & B. Torrano-Silva. 26. II. 2012 27°23'38.79"S / 48°26'08.75"O	SPF57496	KM523173	xxxxxx	KM509067
Hypnea spinella	IBC0922	Praia de Mutá. Porto Seguro. F. Nauer & C. A. Azevedo. 14. IX. 2012 $16^0$21’19.07’’S/$39^0$00’46.95’’O	SPF57601	xxxxxx	xxxxxx	xxxxxx
	IBC0129	Saco do Poço, Ilhabela, SP, - 23.515.066,-45.166.797	SPF56884	KM214524	KM210588	KM203899
	IBC0708	Forno Beach, Arraial do Cabo, RJ, -22.580.566, -42.005.756	SPF57423	KM214576	xxxxxx	xxxxxx
	IBC0832	Castelhanos Beach, Anchieta, ES, -20.500.418, -40.372.157	SPF57523	KM214579	xxxxxx	KM203905
	IBC0844	Cruz Beach, Marataizes, ES, -21.020.168, -40.484.551	SPF57534	KM214588	KM210580	xxxxxx

	IBC0845	Cruz Beach, Marataizes, ES, -21.020.168, -40.484.551	SPF57535	KM214589	KM210581	xxxxxx
	IBC0789	Ilha do Xavier. Florianópolis. F. Nauer, C. Azevedo, M. Sissini & B. Torrano-Silva. 24. II. 2012 27°36'35.02"S / 48°23'10.32"O	SPF57486	xxxxxx	xxxxxx	xxxxxx
Hypnea sp.1	IBC0931	Praia de Coroa Vermelha. Santa Cruz de Cabrália. F. Nauer & C. A. Azevedo. 15. IX. 2012 $16^0$19’57.02’’S/$39^0$00’13.59’’O	SPF57609	xxxxxx	xxxxxx	xxxxxx
	IBC0932	Praia de Coroa Vermelha. Santa Cruz de Cabrália. F. Nauer & C. A. Azevedo. 15. IX. 2012 $16^0$19’57.02’’S/$39^0$00’13.59’’O	SPF57610	xxxxxx	xxxxxx	xxxxxx
	IBC0933	Praia de Coroa Vermelha. Santa Cruz de Cabrália. F. Nauer & C. A. Azevedo. 15. IX. 2012 $16^0$19’57.02’’S/$39^0$00’13.59’’O	SPF57611	xxxxxx	xxxxxx	xxxxxx
	IBC0934	Praia de Coroa Vermelha. Santa Cruz de Cabrália. F. Nauer & C. A. Azevedo. 15. IX. 2012 $16^0$19’57.02’’S/$39^0$00’13.59’’O	SPF57612	xxxxxx	xxxxxx	xxxxxx
	IBC0935	Praia de Coroa Vermelha. Santa Cruz de Cabrália. F. Nauer & C. A. Azevedo. 15. IX. 2012 $16^0$19’57.02’’S/$39^0$00’13.59’’O	SPF57613	xxxxxx	xxxxxx	xxxxxx
	IBC0936	Praia de Coroa Vermelha. Santa Cruz de Cabrália. F. Nauer & C. A. Azevedo. 15. IX. 2012 $16^0$19’57.02’’S/$39^0$00’13.59’’O	SPF57614	xxxxxx	xxxxxx	xxxxxx

	IBC0939	Praia de Coroa Vermelha. Santa Cruz de Cabrália. F. Nauer & C. A. Azevedo. 15. IX. 2012 $16^0$19’57.02’’S/$39^0$00’13.59’’O	SPF57617	xxxxxx	xxxxxx	xxxxxx
Hypnea wynnei	IBC0824	Ilha Grande Island, Angra dos Reis, RJ, 23.100°S, 044.200°E, 5-7 m depth, coll. F. Nauer, tetrasporangial plant, 18 April 2012	SPF57515	KX426547	KX426556	KX426553
	IBC0825	Ilha Grande Island, Angra dos Reis, RJ, 23.100°S, 044.200°E, 5-7 m depth, coll. F. Nauer, tetrasporangial plant, 18 April 2012	SPF57516	KX426548	KX426557	xxxxxx
	IBC2708	Ilha Grande Island, Angra dos Reis, RJ, 23.100°S, 044.200°E, 5-7 m depth, coll. F. Nauer, tetrasporangial plant, 26 April 2014	SPF57834	KX426549	xxxxxx	xxxxxx
	IBC2709	Ilha Grande Island, Angra dos Reis, RJ, 23.100°S, 044.200°E, 5-7 m depth, coll. F. Nauer, tetrasporangial plant, 26 April 2014	SPF57835	KX426550	xxxxxx	xxxxxx
	IBC2710	Ilha Grande Island, Angra dos Reis, RJ, 23.100°S, 044.200°E, 5-7 m depth, coll. F. Nauer, tetrasporangial plant, 26 April 2014	SPF57836	KX426551	xxxxxx	xxxxxx
	IBC2711	Ilha Grande Island, Angra dos Reis, RJ, 23.100°S, 044.200°E, 5-7 m depth, coll. F. Nauer, tetrasporangial plant, 26 April 2014	SPF57837	KX426552	xxxxxx	xxxxxx

Hypnea yokoyana	IBC0791	Ilha do Arvoredo Island, Governador Celso Ramos, SC, 27.171°S, 048.220°E, 5-7m depth, coll. F. Nauer, B. Torrano-Silva & C.A. Azevedo, 25 February 2012	SPF57488	KX426545	KX426558	xxxxxx
	IBC0822	Ilha Grande Island, Angra dos Reis, RJ, 23.100°S, 044.200°E, 5-7 m depth, coll. F. Nauer, tetrasporangial plant, 18 April 2012	SPF57513	KX426541	xxxxxx	xxxxxx
	IBC0823	Ilha Grande Island, Angra dos Reis, RJ, 23.100°S, 044.200°E, 5-7 m depth, coll. F. Nauer, tetrasporangial plant, 18 April 2012	SPF57514	KX426542	xxxxxx	xxxxxx
	IBC0828	Ilha Grande Island, Angra dos Reis, RJ, 23.100°S, 044.200°E, 5-7 m depth, coll. F. Nauer, tetrasporangial plant, 19 April 2012	SPF57519	KX426544	xxxxxx	xxxxxx
	IBC0829	Ilha Grande Island, Angra dos Reis, RJ, 23.100°S, 044.200°E, 5-7 m depth, coll. F. Nauer, tetrasporangial plant, 19 April 2012	SPF57520	KX426543	KX426559	xxxxxx
	IBC0928	Mutá Beach, Porto Seguro, BA, 16.211°S, 039.004°E, intertidal, coll. F. Nauer & C.A. Azevedo, 14 September 2012	SPF57602	KX426540	KX426560	KX426554
	IBT0747	Laje de Santos, Santos, SP, 24.172°S, 46.120°E, 5-7 m depth, coll. R. Rocha-Jorge. 24 February 2011	SP365689	KX426546	xxxxxx	KX426555

CAPÍTULO Três

Marine Phylogeography: a case study with the benthic macroalgae species complex Hypnea musciofrmis – Hypnea pseudomusciformis (Gigartinales, Rhodophyta).

Capítulo 3

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Marine Phylogeography: a case study with the benthic macroalgae species complex *Hypnea musciformis* - *Hypnea pseudomusciformis* (Gigartinales, Rhodophyta).

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Abstract

Populations of the marine benthic red macroalgae *Hypnea musciformis* and *H. pseudomusciformis* along the Atlantic and Pacific Oceans were tested for phylogeographic structure using the DNA barcode marker COI-5P. Strong patterns of genetic structure were detected across 174 sequences and 37 haplotypes were found, using multiple statistical approaches (Fst, AMOVA, SPN, IBD). For *H. pseudomusciformis* five distinct marine phylogeographic provinces were recognized: Uruguay, southern Brazil, Rio de Janeiro upwelling region, the eastern Brazil and the northern Brazil. The degree of genetic isolation and distinctness between these provinces varied considerably. The Uruguay province was the most genetically distinct, characterized by four unique haplotypes not shared with any of the Brazilian populations. The highest genetic diversity was found in the Cabo Frio upwelling region of Rio de Janeiro state, a region known for a presence of a diverse number of distinct microclimatic habitats. Regions were shifts in genetic structure occur coincide with the presence of unique geological and oceanographic processes known to produce an inprint on the genetic structure of organisms in other parts of the world, e.g. coastal splits in oceanic boundary currents, coastal upwelling, geological inflection of continental

masses, shifts in climatic zones. This study helped to map concordant patterns of marine genetic structure (phylogeography) along the Brazilian coast.

Abbreviations: AMOVA, analysis of molecular variance; bp, base pair; IDB, isolation by distance; SPN, statistical parsimony network

Key words: algae, COI, genetic structure, *Hypnea musciformis*, *Hypnea pseudomusciformis*, marine, phylogeography, *rbc*L, Rhodophyta, systematics.

Introduction

Knowledge on how different evolutionary lineages are biogeographically distribuited is paramount in conservation biology and conservation genetics, which attempt to identify and preserve the highest levels of overall diversity in space and time. Phylogeography aims to discover shared patterns of phylogeographic structure among molecular markers, genomes and species is (i.e. phylogeographic concordance, Avise 2000). Concordances can identify processes responsible for shaping genetic structure and driving speciation in the terrestrial or marine environment, including possible barriers that may lead to speciation.

Hypnea musciformis is a pantropical, carrageenan-producing benthic species, and one of the five most cultivated red algae in the world (Zemke-Ohno 1999). Wherever *H. musciformis* occurs, it becomes a conspicuous member of marine communities. When introduced into areas where it is not native, *H. musciformis* has the potential to become invasive. Nauer et al. (2015) carried out a study of the species based on molecular markers (5P-COI and *rbc*L) and morphology, including a few samples from the type locality in the Adriatic Sea, Italy, and found out that the species *Hypnea musciformis*, *H. nigrescens* (Wulfen) J.V. Lamouroux and *H. valentiae* (Turner) Montagne collected in Brazil were morphological variations of a new species named *H. pseudomusciformis* Nauer, Cassano & M.C. Oliveira. In fact, the authors argued that, based on molecular data, the specimens globally known as *H. musciformis* represent a complex of closely related species, rather than a single cosmopolitan species.

Based on 5P-COI marker, sequences of specimens from Brazil formed a monophyletic cluster with 5.9 % to 7.1 % divergence from the type locality of *H. musciformis* (Adriatic Sea). The Brazilian specimens were further divided into two major groups, one formed by sequences from specimens collected in the northeast region of the country and the other formed by sequences from the southeast and south regions, which also included a few samples collected in the northeast. The divergence between the two groups are up to 1.8%.

Jesus et al. (2016) investigated the evolutionary history of both *H. musciformis* and *H. pseudomusciformis* based on sequences of COI-5P and *rbc*L markers. Their results splitted the *H. musciformis* clade in two major lineages: from both sides of the Atlantic Ocean, and from both sides of the Panama Isthmus. *Hypnea pseudomusciformis*, on other hand, revealed evidences of recent population expansion.

Together, the Uruguayan and Brazilian coastlines extend across 9,000 Km mostly in a north-south direction from latitude 04º 26' north to 33º 45' south. Despite its extensive length and latitudinal breadth, most of its range is contained within tropical to subtropical zones. Contrary to both coasts of the Northern Atlantic Ocean (i.e. North America and Europe) where a plethora of marine phylogeographic studies have been conducted across multiple taxa (e.g Soltis et al. 2006, Wares 2002), very little is known about the presence of marine barriers to gene flow and the presence of concordant phylogeography patterns in the southern Atlantic Ocean. Lack of knowledge also includes how local historical or contemporary oceanographic, geological and climatological processes shape population genetic structure in the region.

The Uruguay-Brazilian coast presents a range of geographic and oceanographic processes that could act as barriers to gene flow across different marine species. Each of them represents a phylogeographic hypothesis waiting to be tested. For example, two main rivers, the Amazon River and the São Francisco River , could act as barriers to gene flow analogous to the way the Mississippi River acts separating populations east and west of northern Gulf of Mexico (e.g. the arrow squid *Loligo plei*, Herke and Foltz 2002; the thalassinidean crustacean, *Callichirus islagrande* Staton and Felder 1995, the red alga *Gracilaria tikvahiae*, Gurgel et al. 2004; and several fish species, Portnoy and Gold 2012). The Rio Grande do Norte Cape, i.e. the inflection of the continent at latitude 5º 09' south where the coastline assumes a north-south direction instead of continuing in northwest-southeast direction, is also the region where the South

Equatorial Current splits to form the southward Brazilian Current and the northward North Brazilian Current. This split and formation of distinct boundary currents with opposite directions could act as barrier to gene flow similarly to the way Cape Canaveral on the east side of the Florida Peninsula is known to promote genetic discontinuity in continuously distributed marine species in that region (reviewed in Avise 2000, Soltis et al. 2006), or the split of the Pacific Southern Equatorial Current at about latitude 17º-18º south at the Great Barrier Reef (GBR), Australia, to form the southward East Australian Current and the northward North Queensland Current (Brinkman *et al.* 2001, Choukroun et al. 2010). In the GBR, this region is known to coincide with the genetic disjunction for some marine species with restricted dispersal capabilities, particularly macroalgae (e.g. Howells et al. 2009).

Another process known to promote shifts in genetic signature for otherwise continuously distributed marine species refers to latitudinal gradients associated with changes in climatic zones followed by local adaptation (Adey & Steneck 2001). Furthermore, cases of restricted gene flow between geographically contiguous marine populations have been observed even in the absence of any noticeable obstacles to dispersal (Karl & Avise 1992, Burton and Lee 1994, Pogson et al. 1995, Knutsen et al. 2003, Taylor & Hellberg 2003). As such there could also be regions along the Uruguay-Brazilian coast promoting genetic isolation and differentiation where no apparent paleological, oceanographic or geological processes could be identified.

Even though some kelp species whose dislodged thalli are capable of floating, drift offshore and disperse for hundreds if not thousands of kilometers (e.g. Frazer et al. 2011), most benthic macroalgae are among the poorest dispersers in the marine environment, compared to benthic animals (Kilan & Gaines 2003). Consequently, genetic structure has been found in most species of red and brown algae (e.g. Gurgel et al. 2004, Frazer et al. 2010, 2011) and in many green algae (Shimada et al. 2009) making intertidal marine benthic macroalgae good model organisms to infer shared phylogeographic patterns.

In this study, the primary goal was to perform a phylogeographic analysis of the economically and ecologically important marine red macroalgae species complex *Hypnea musciformis- H. pseudomusciformis* along the Atlantic and Pacific Oceans and along the Uruguay-Brazil coastline to detect and locate the presence of genetic

structure. Secondly, we tested hypotheses related to marine barriers to gene flow along the same areas.

Materials and methods

TAXON SAMPLING AND MORPHOLOGY

Sequences of *H. musciformis* and *H. pseudomusciformis* from different locations were used in this study: Italy, France and Spain from the Mediterrean Sea; Mexico, Barbados, Dominican Republic, Jamaica and Colombia from the Caribbean Sea; Florida and North Carolina from the North Atlantic United States; Namibia and Ghana from Eastern Atlantic in Africa; Hawaii and Mexico from Pacific Ocean and Brazil and Uruguay from South Eastern Atlantic Ocean (Table 1S). Voucher specimens were housed at SPF and SP herbaria (abbreviations Thiers, 2017).

For new specimens, apical regions were separated from the thallus, cleaned and stored in silica gel for molecular analyses. The rest of the thallus was stored in 4% formalin solution for morphological studies, as follow: samples were sectioned by hand with a razor blade and stained with 1% aniline blue acidified with 1N HCl. Habit and diagnostic characters (following Masuda et al. 1997 and Nauer et al. 2015) of each species were obtained with digital capture and image analyses, using a compound microscope with Leica DM4000 digital camera (Solms, Germany).

DNA EXTRACTION, PCR AND SEQUENCING

DNA was extracted and purified using the NucleoSpin 96 Plant II kit (Macherey-Nagel, Düren, Germany). Specific primer pairs were used for the amplification and sequencing reactions of each gene: for *rbc*L, FrbcL-R753, F492-R1150 and F993–RrbcS start (Freshwater et al. 1994) and for 5P-COI, GazF1–GazR1 (Saunders 2005). All PCR amplifications were carried out in a Techne TC-4000 thermal cycler (Bibby Scientific Ltd., Staffordshire, UK).

The PCR products were purified by column PCR ™ GTX DNA and Gel Band Purification Kit (GE Healthcare, Pittsburgh, USA) according to the protocol of the supplier. Sequencing of 10 to 40 ng of purified PCR product was done using the same primers of the PCR reactions described above and the sequencing kit BigDye

Terminator Cycle Sequencing Ready Reaction" (Applied Biosystems, Foster City, USA) according to the protocol of the supplier. The samples were sequenced on 3100 ABI PRISM ™ automatic sequencer (Applied Biosystems, Carlsbad, CA, USA). Consensus sequences were generated for each marker per sample aligning forward and reverse sequences manually using the program BioEdit (Hall 1999). Inconsistencies in the sequences were reviewed checking the chromatograms.

A total of 174 *Hypnea* sequences for COI-5P (33 new and 141 from GenBank) and 86 sequences for *rbc*L (12 new and 74 from GenBank) were used in the analyses. For COI-5P analyses, *Calliblepharis ciliata* (Greville) Kützing (DQ442896) was used as outgroup. For *rbc*L analyses, *Calliblepharis fimbriata* (AF385653) was used as outgroup.

The 5P-COI cluster tree was constructed by Neighbour-Joining (NJ) using the MEGA5 program (Molecular Evolutionary Genetics Analysis, Tamura et al. 2011) with 2000 bootstrap replicates. For the *rbc*L phylogenetic analysis, the model of evolution was selected using the MrModeltest 2.2 (Nylander, 2004), using Akaike Information Criterion (AIC). The phylogenetic tree was constructed in MrBayes v3.1.2. (MB, two runs, 4,000,000 generations with sampling every 1,000 generations, the first 100,000 generations in both runs were discarded as burn-in to build the consensus tree) and PAUP 4.0b8 with 2000 bootstrap replicates both for Neighbor-joining (NJ, with substitution model Tamura and Nei 1993) and maximum parsimony (MP, with heuristic search).

Pairwise values of F_{ST} among locations were calculated and their significance were tested using 1000 permutations. Moreover, a nested Analysis of Molecular Variance (AMOVA) was implemented to test for the null hypotheses of no population structure within groups and between groups (Excoffier et al., 1992). The isolation by distance model (Mantel test) was used to test for a relationship between genetic distance (F_{ST}) and geographic distance (in km, measured among population pairs), and its significance was tested using 1000 permutations. The sequences also were tested for background selection using Tajima's D (Tajima, 1989) and Fu's FS statistics (Fu, 1997). These indexes are based on the observed pattern of allelic variation, which can be influenced by demographic processes such as a selective sweep or an expanding population (Ballard & Whitlock, 2004). All these analyses were performed in Arlequin (Excoffier & Lischer, 2010).

For each locality, four diversity indices were calculated for COI-5P marker using Arlequin v 3.5 (Excoffier & Lisher, 2010): the number of haplotypes (h), the number of polymorphic sites (S), gene diversity (H) (Nei, 1987), and nucleotide diversity (π) (Nei & Li, 1979). A Mann-Whitney U test was used to evaluate differences in genetic diversity among regions (northeast vs. southeast) with the STATISTICA.10 software.

Single factor designs were used to classify samples into different numbers of fixed level treatments to test for the presence of different marine barrier to gene flow hypotheses:

(a) Climatic zones hypothesis for South Atlantic ocean: temperate zone ≠ subtropical zone ≠ upwelling region ≠ tropical zone: (URU) ≠ (SC, SP) ≠ (RJ) ≠ (SC+SP) ≠ (ES, BA, AL, PB, RN, PI, CE), respectively.

(b) The split of the Southern Equatorial Current into the two boundary currents of opposite flow directions offshore Rio Grande do Norte state hypothesis: populations under the Brazilian Current influence (ES, BA, AL, PB) ≠ populations under the the Northern Brazilian Current influence (PI, CE).

c) Cape Seixas (PB) hypothesis, the easternmost point of the South American continent as the region of phylogeographic break for marine benthic populations: (CE, PI, RN) ≠ (PE, AL, BA, ES), respectively.

Results

In the *rbc*L phylogeny of *Hypnea* (Figure 1), *H. pseudomusciformis* sequences from South America (Brazil and Uruguay) specimens formed a monophyletic group with high support in all analyzes. Samples of *H. musciformis*, including the type locality, Trieste, Italy, also formed another monophyletic group with high support in all analyzes. Other two groups were formed based major on sequences from Caribbean sea. One incluing sequences from Jamaica, Barbados, Mexico and Dominican Republic and the other with few sequences from Dominican Republic only. These four groups within *H. musciformis-pseudomusciformis* complex formed a monophyletic clade in all analyzes, indicating that they are closely related.

The divergence of South America and Italian specimens is 1.7 % to 2.3 % for *rbc*L, and the maximum intraspecific divergence found for this marker was 0.7 %. These results support that the Brazilian samples, as a distinct species *H. pseudomusciformis*. Now, the divergence of Caribbean and Italian specimens is 1.6 % to 1.7 % for *rbc*L and the divergenc of Dominican Republic and Italian specimens is 1.1%.

Based on 5P-COI marker (Figure 2), sequences of *H. pseudomusciformis* from South Atlantic, including sequences from South America and Africa formed a monophyletic cluster with high bootstrap support, with 5.9 % to 7.1 % divergence of Italian specimens of *H. musciformis*. The South Atlantic specimens were further divided into three major groups, one formed by sequences from specimens collected in the northeast region of Brazil (Group I) with high bootstrap support, the other formed by sequences from the southeast and south regions of Brazil (Group II) and the last group formed with specimens from Uruguay. The divergence between the Brazilian groups is 1.5% to 1.7%, and the divergence of Uruguay specimens and the two Brazilian groups is up to 0.9 % (Group I) and 1.8 % (Group II).

The group from Caribbean Sea, which also included sequences from Hawaii in the COI-5P analysis, the divergence of this cluster and Italian specimens is 3.2% to 5.2% and the group formed only with Dominican Republic sequences has a divergence of 4.7% with Italian specimens of *H. muscifomis*. The divergence between Caribbean and Hawaii cluster and Dominican Republic cluster is up to 3.4%. Because of these results, the Caribbean and Hawaii specimens were indentified as *Hypnea* sp. 3 and D. Republic as *Hypnea* sp. 4.

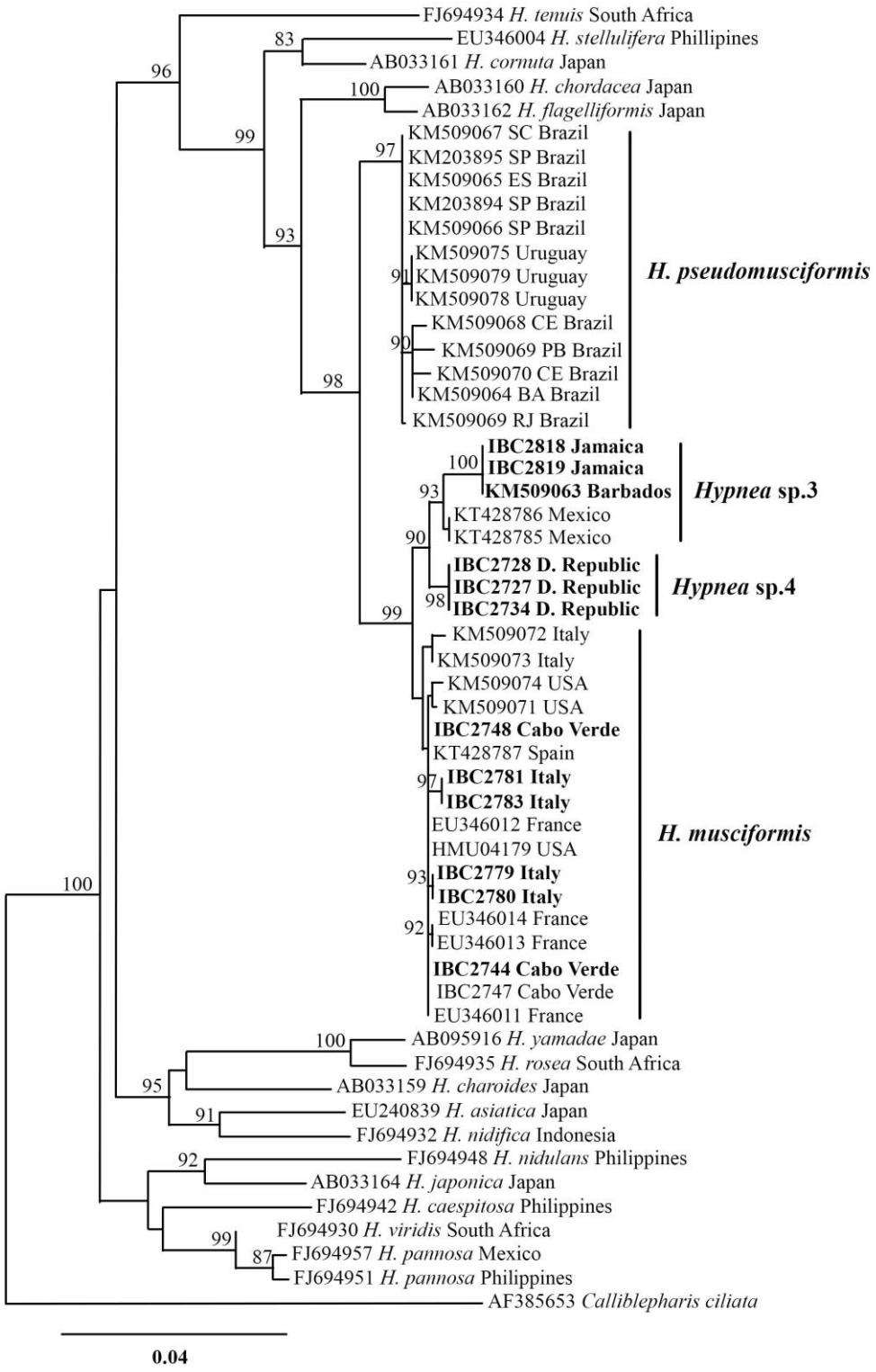

Figure 1. Bayesian analysis for *rbc*L sequences for *Hypnea musciformis-H. pseudomusciformis* complex. On the branches are the bootstrap values (only values ≥ 70 were shown). Accession numbers and country of origin, when available, are included for sequences obtained from GenBank. New sequences are in bold. Mexico-A, Atlantic coast fo Mexico; Mexico-P, Pacific coast fo Mexico.

For the samples analysed in this paper, intraspecific variation for marker 5P-COI (465 bp) was 0-9 bp (0-1.8%) and for marker *rbc*L (1355 bp) was 0-10 bp (0-0.7%). Interspecific variation (including also the sequences from GenBank used in the analyses) for 5P-COI, was 69-130 bp (3.2 to 18.2%) and for the *rbc*L was 48-92 bp (1.1 to 6.8%).

COI-5P PHYLOGOGRAPHY ANALYSIS

The final data set was 640 bp long, contained 174 DNA sequences and included 37 haplotypes, labeled h1 to h37 with an estimated total haplotype diversity of $h = 0.819 \pm 0.018$. Indexes of population genetic diversity are given in Table 1. Tajima's D neutrality test (Tajima 1989) for the overall dataset produced a value of $D = +1.01238$ which is not significantly different from 0 ($p > 0.10$) showing that the neutral mutation hypothesis can explain the COI-5P DNA polymorphism found among Brazilian and Uruguay populations (Table 1). A significant **IBD** correlation was found along the Uruguay-Brazilian coast (Mantel test, r=6.881, p=0.0007).

Despite the lack of statistical significance, positive value of **Tajima's D** was obtained for the combined dataset. Positive Tajima's D results is often observed when a subdivided population is pooled for analysis (Simonsen et al. 1995). Significant negative values of Tajima's D and **Fu and Li's F*** were obtained for the populations in Italy and Brazil for CE, SP, SC populations. Negative values typically result from population growth following a bottleneck, or a selective sweep (Tajima 1989; Simonsen et al. 1995).

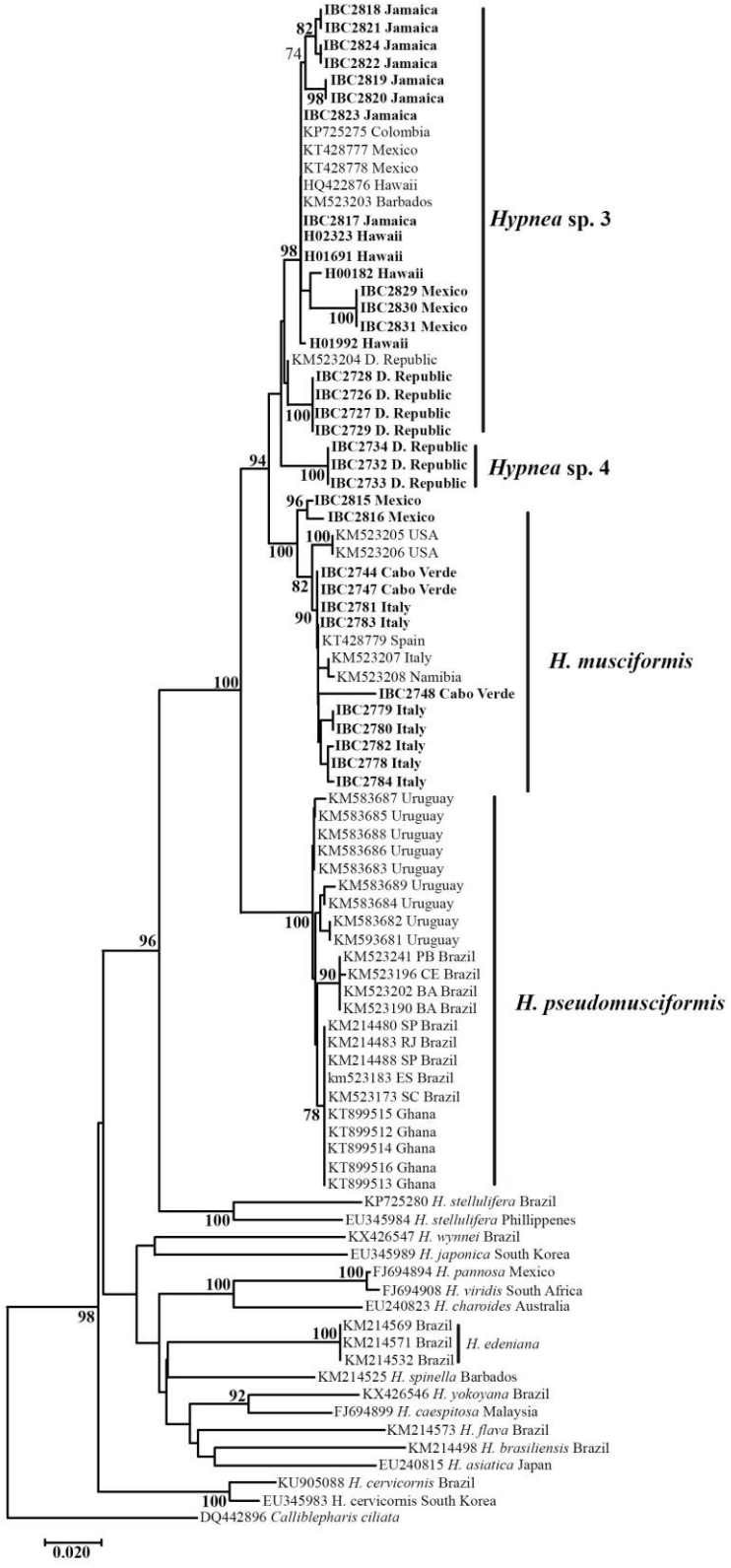

Figure 2. Neighbor-Joining (NJ) analysis for COI-5P sequences for *Hypnea musciformis-H. pseudomusciformis* complex. On the branches are the bootstrap values for 2000 replicates (only values ≥ 70 were considered). Accession numbers and country of origin, when available, are included for sequences obtained from GenBank. New sequences are in bold.

The haplotype network (Figure 3) for COI-5P sequences also presented four main haplogroups supporting the phylogenetic results separating the *H. musciformis* complex in four species and separating sequences from *H. pseudomusciformis* individuals from Northeast of Brazil from the ones obtained from Southeast and the ones in Uruguay. Haplogroups were separated from 7 to 17 bp while, within each haplogroup, pairs of haplotypes were separated by 1 to 6 bp. The *H. musciformis* haplogroup was found mainly on North hemisphere, with the exception of Namibia h24 haplotype (Figure 4) and in both in the Atlantic and Pacific Oceans. The *H. psudomusciformis* haplogroup was found only on South hemisphere, in both sides of the Atlantic Ocean. *Hypnea* sp. 3 haplogroup was found in the Caribbean Sea and Hawaii while the *Hypnea* sp. 4 haplogroup was limted to Dominican Repubic. Most of the haplotypes were limited among regions, while others were shared (Figure 4). The *H. musciformis* h1 haplotype was found in Italy, Spain and Cabo Verde. The *H. pseudomusciformis* h25 haplotype was found in Southeast region of Brazil and in Ghana, African continent. The *Hypnea* sp1 h14 haplotype was found all over the Caribbean Sea (Barbados, Colombia, D. Republic and Jamaica) and in Hawaii, Pacific Ocean.

The haplotype network detected the presence of strong genetic structure between Uruguay (i.e. temperate) and Brazilian populations (i.e. subtropical, upwelling and tropical) where the former is composed by four unique haplotypes not found anywhere else along the Brazilian coast (Figure 3 and 5). The Brazilian haplotypes formed two star-shaped clades: one central clade containing in its core the most abundant (**h25**, n=52) and broadly distributed haplotype in the entire dataset (and hence and most likely ancestor sequence). The haplotype **h25** is found from Santa Catarina (SC) to Rio Grande do Norte (RN) states (> 2800 km in distribution along the Brazilian coast). The second Brazilian major clade containing in its core the second most abundant haplotype (**h29**, n=40) and included four other haplotypes (**h31-h34**). Distributional range of h29 is from Bahia to Piauí state, ~ 1800 km).

A higher genetic diversity was observed in Italy, *H. musciformis* type locality (H =0.8545 and π (*10-2) =0.005787, Table 1), the combined AL/PE/PB population of *H. pseudomusciformis* in Northeast Brazil (H =0.8214 and π =0.007450, Table 1) and

Mexico, *Hypnea* sp1 population (H =0.8095 and π (*10-2) =0.025397, Table 1), although these differences were not significant (p >0.05). F_{ST} were significant between most population pairs, except for Spain, Namibia, Barbados and Colombia, regions with only one sequence (Table 2). The Analysis of Molecular Variance (AMOVA) indicated that total genetic variance was mainly explained by the variance among haplogroups (87,97 %, Table 3). The variance among populations within haplogroups (9,85 %) and the variance within populations (2,18 %) were much lower (Table 3).

Table 1: Sampling sites of *Hypnea musciformis* and associated genetic diversity (COI-5P). Molecular diversity indices calculated for COI (630 bp-long): number of sequences (N), number of haplotypes (nH), gene diversity (H), nucleotide diversity (π), number of polymorphic sites (S), Tajima's *D* (D), and Fu's F*S* statistics (F*S*). Standard deviations (SD) are noted between brackets. Ceará (CE), Rio Grande do Norte (RN), Paraíba (PB), Pernambuco (PE), Bahia (BA), Espírito Santo (ES), Rio de Janeiro (RJ) São Paulo (SP) and Santa Catarina (SC) States.

Species	**Region**	**N**	**nH**	**H (SD)**	**Π(*10^{-2}) (SD)**	**S**	**D**	**Fs**	**Npriv**
H. musciformis	Italy	11	6	0.8545 (0.0852)	0.005787 (0.003756)	7	0.51106	-0.80159	7
	Spain	1	1	1.0000 (0.0000)	0.0000 (0.0000)	0	0.000	-	0
	Cabo Verde	3	2	0.6667 (0.3143)	0.011470 (0.009462)	8	0.000	3,10109	8
	USA	2	1	0.0000 (0.0000)	0.0000 (0.0000)	0	0.000	-	0
	Namibia	1	1	1.0000 (0.0000)	0.0000 (0.0000)	0	0.000	-	0
H. pseudomusciformis	**Brazil – Group I**								
	CE	18	2	0.1176 (0.1012)	0.000253 (0.000469)	1	-1,16387	-0.74844	1
	RN	7	3	0.5238 (0.2086)	0.004301 (0.003144)	7	-1,55311	1,32063	0
	AL/PE/PB	18	4	0.8214 (0.1007)	0.007450 (0.004836)	7	1,34767	1,32061	1
	BA	9	2	0.5000 (0.1283)	0.001075 (0.001138)	1	0.98627	0.84950	1
	Brazil – Group II								
	ES	7	1	0.0000 (0.0000)	0.0000 (0.0000)	0	0.000	-	0
	RJ	18	3	0.5425 (0.0861)	0.001321 (0.001223)	2	0.13811	0.09974	1
	SP	11	4	0.7091 (0.0990)	0.002268 (0.001831)	3	0.10123	-0.62653	2
	SC	18	2	0.1111 (0.0964)	0.000239 (0.000454)	1	-1,16467	-0.79427	1
	Uruguay	9	4	0.7500 (0.1121)	0.004062 (0.002894)	4	1,15206	0.27032	3
	Ghana	5	1	0.0000 (0.0000)	0.0000 (0.0000)	0	0.000	-	0
***Hypnea* sp.3**	Barbados	1	1	1.0000 (0.0000)	0.0000 (0.0000)	0	0.000	-	0
	Colombia	1	1	1.0000 (0.0000)	0.0000 (0.0000)	0	0.000	-	0
	Mexico	7	4	0.8095 (0.1298)	0.025397 (0.015021)	24	1,16332	3,89154	18
	Jamaica	10	3	0.6444 (0.1012)	0.002915 (0.002219)	5	-0.93443	1,09090	5
	Hawaii	5	3	0.7000 (0.2184)	0.004301 (0.003378)	5	-1,12397	0.64355	3
***Hypnea* sp.4**	Dominican Republic	12	3	0.4394 (0.1581)	0.010459 (0.006176)	13	0.54987	5,47423	12
Total		174	37						

Table 2. Pairwise estimates of genetic differentiation (*F*ST) between populations of *Hypnea musciformis-H. pseudomusciformis* complex for COI-5P. Ceará (CE), Rio Grande do Norte (RN), Paraíba (PB), Pernambuco (PE), Bahia (BA), Espírito Santo (ES), Rio de Janeiro (RJ) São Paulo (SP) and Santa Catarina (SC) States.

Region	Italy	Spain	C. Verde	USA	Nam.	CE	RN	AL/PE/ PB	BA	ES	RJ	SP	SC	Uru.	Ghana	Barb.	Col.	Mex.	Jam.	Haw.	D. Rep
Italy																					
Spain	-0,345																				
C. Verde	**0,242**	-1,000																			
USA	**0,673**	0,000	0,553																		
Namibia	**0,395**	1,000	0,058	1,000																	
Brazil																					
CE	**0,966**	0,996	**0,978**	**0,996**	0,996																
RN	**0,925**	0,934	**0,913**	**0,942**	0,940	0,115															
AL/PE/PB	**0,952**	1,000	**0,963**	**1,000**	0,971	**0,607**	-0,123														
BA	**0,948**	0,984	**0,955**	**0,985**	0,985	**0,276**	0,087	0,256													
ES	**0,942**	1,000	**0,953**	**1,000**	1,000	**0,985**	**0,810**	**0,977**	**0,955**												
RJ	**0,952**	0,977	**0,961**	**0,978**	0,979	**0,942**	**0,821**	**0,907**	**0,915**	0,194											
SP	**0,936**	0,961	**0,939**	**0,963**	0,965	**0,928**	**0,762**	**0,855**	**0,886**	**0,205**	**0,207**										
SC	**0,962**	0,995	**0,976**	**0,996**	0,996	**0,981**	**0,878**	**0,974**	**0,962**	-0,065	**0,274**	**0,320**									
Uruguay	**0,915**	0,925	**0,906**	**0,932**	0,933	**0,886**	**0,677**	**0,747**	**0,823**	**0,576**	**0,620**	**0,545**	**0,695**								
Ghana	**0,934**	1,000	**0,939**	**1,000**	1,000	**0,984**	**0,778**	0,968	**0,947**	0,000	0,154	0,155	-0,103	**0,528**							
Barbados	0,820	1,000	0,659	0,000	1,000	0,994	0,907	0,955	0,977	1,000	0,970	0,948	0,994	0,902	1,000						
Colombia	0,820	1,000	0,659	0,000	1,000	0,994	0,907	0,955	0,977	1,000	0,970	0,948	0,994	0,902	1,000	0,000					
Mexico	**0,636**	0,248	**0,472**	**0,485**	0,369	**0,871**	**0,747**	**0,656**	**0,805**	**0,765**	**0,849**	**0,793**	**0,862**	**0,740**	**0,727**	-0,503	-0,503				
Jamaica	**0,870**	0,902	**0,868**	-0,506	0,919	**0,975**	**0,928**	**0,943**	**0,959**	**0,961**	**0,959**	**0,944**	**0,973**	**0,920**	**0,955**	-0,506	-0,506	**0,303**			
Hawaii	**0,845**	0,857	**0,810**	-1,000	0,882	**0,978**	**0,912**	**0,923**	**0,956**	**0,961**	**0,958**	**0,938**	**0,977**	**0,905**	**0,952**	-1,000	-1,000	0,141	**0,141**		
D. Republic	**0,806**	0,726	**0,757**	**0,787**	0,765	**0,920**	**0,852**	**0,833**	**0,886**	**0,871**	**0,905**	**0,876**	**0,916**	**0,840**	**0,856**	0,567	0,567	**0,472**	**0,732**	**0,678**	

Table 3. Analysis of molecular variance (AMOVA) within locations, among locations within haplogroup, and among haplogroups for both molecular markers. Haplogroups were defined according to the haplotype network (see Figure 3).

Source of variation	COI-5P				
	d.f.	SS	Variance components	% variation	p-value
Among haplogroups	6	1054,021	7,63120 Va	87,97	p< 0.05
Among populations within haplogroups	18	36,491	0,18879 Vb	2,18	p< 0.000
Within populations	154	131,628	0,85472 Vc	9,85	p< 0.000
Total	178	1222,14	8,67471	-	-

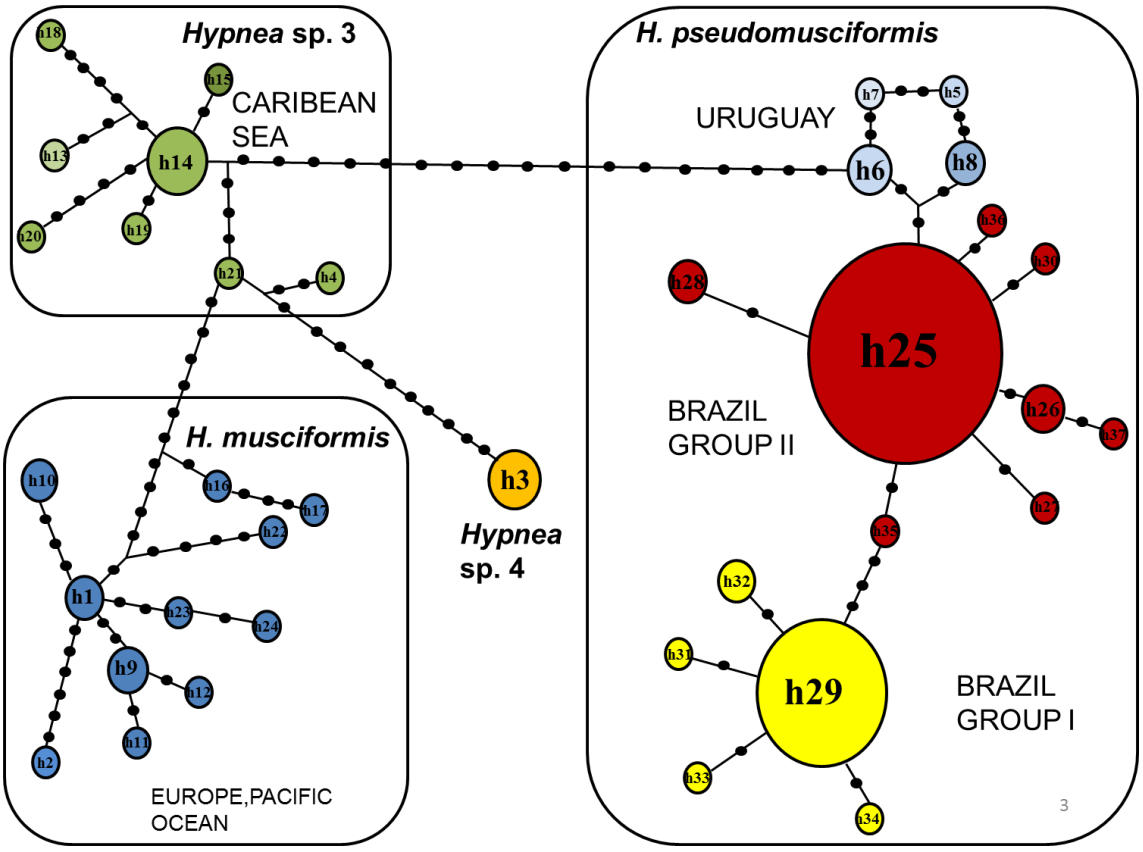

Figure 3. Median-joining network of t mtDNA haplotypes (COI-5P) *Hypnea musciformis-H. pseudomusciformis* complex. In the network, each circle represents a haplotype and its size is proportional to the frequency in which the haplotype was encountered in each population.

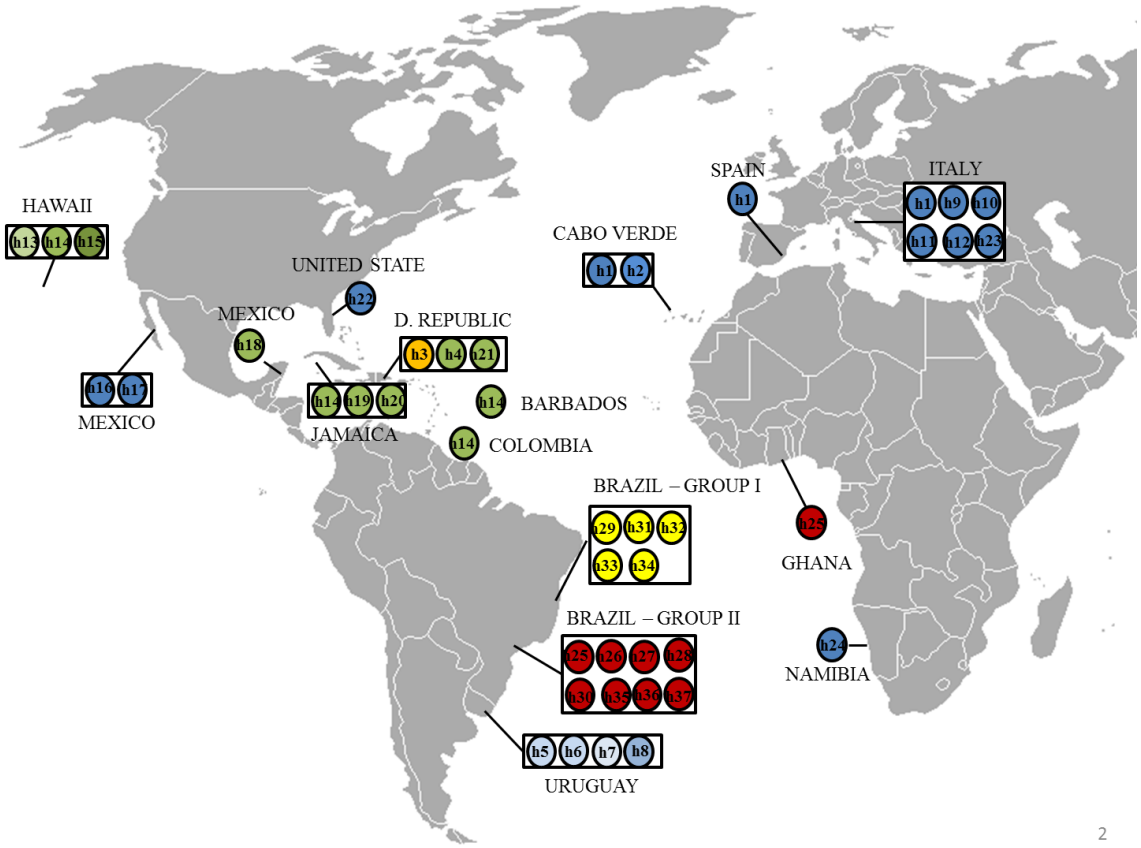

Figure 4. Median-joining network of t mtDNA haplotypes (COI-5P) *Hypnea musciformis- H. pseudomusciformis* complex and their geographic distribution in the world. Hplotypes colors as in Figure 3.

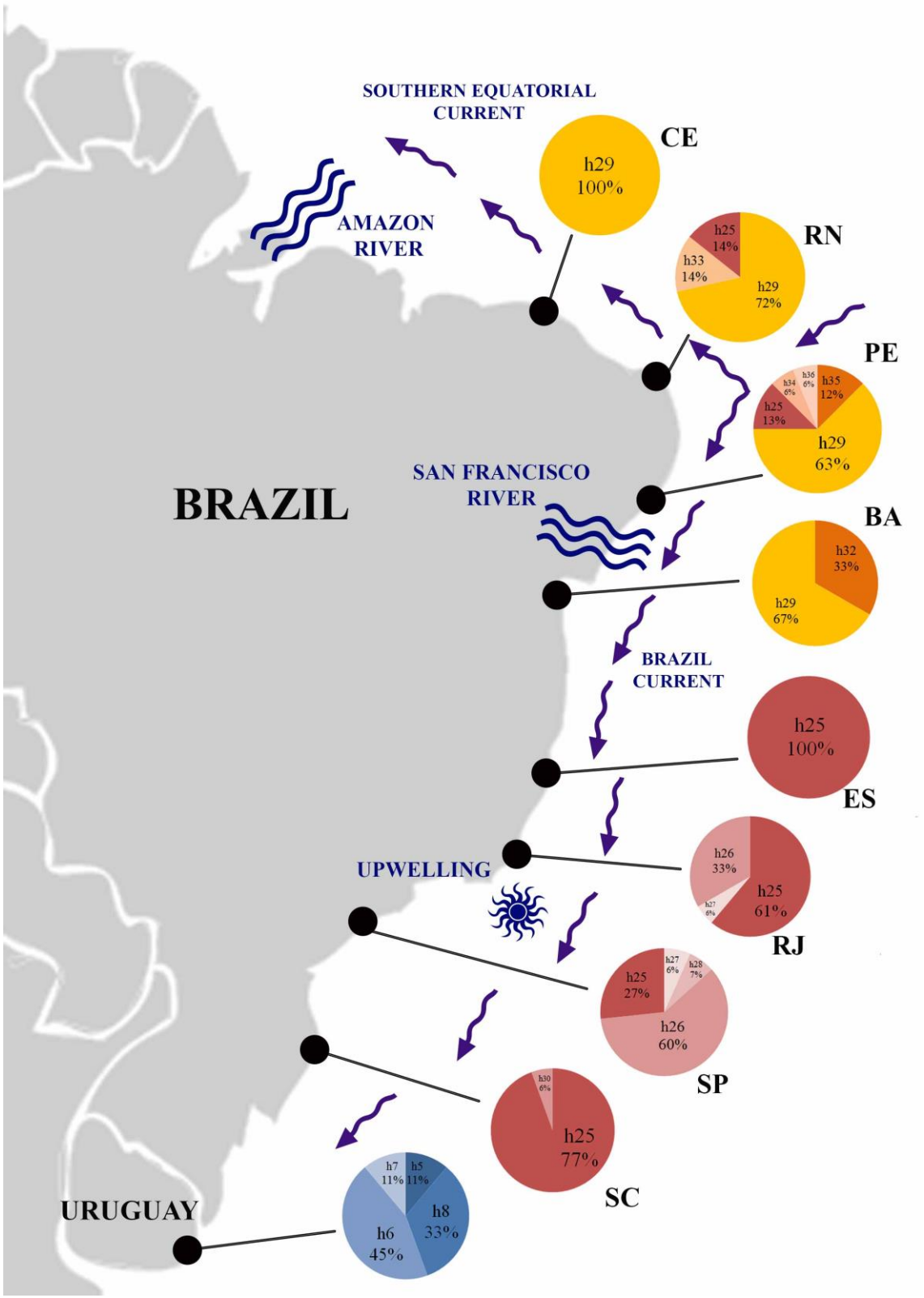

Figure 5. Map of Brazil and Uruguay coast showing the direction of major ocean currents: South Equatorial Current and Brazil Current. The Vitória-Trindade Ridge, a known biogeographical barrier and median-joining network of t mtDNA haplotypes (COI-5P) for *Hypnea pseudomusciformis*. Ceará (CE), Rio Grande do Norte (RN),

Paraíba (PB), Pernambuco (PE), Bahia (BA), Espírito Santo (ES), Rio de Janeiro (RJ) São Paulo (SP) and Santa Catarina (SC) States.

Based on our molecular results, two new species are described for the Caribbean sea:

Hypnea sp. 3 (Fig 6)

DESCRIPTION: Plants erect, epilithic or epiphytic, terete, cartilaginous in texture, forming tufts up to 16 cm tall, reddish, brownish, greenish when alive, attached to the substrate by discoid holdfast and stolon-like branches or often by tendrils when epiphytic. Irregular branching in various planes, in acute or right angles, with up to 3 orders of branches. Spiny-like branchlets numerous and irregularly arranged throughout the thalli with 225-1200 µm long. Anastomoses absent. Epiphytes often growing at the shallow subtidal zone, fixed to the host plant by abundant tendrils at the ends of branches and branchlets (Figs 6D), with branches entangled each other by tendrils of the same plant or of different plants of the same species. Branches and branchlets may also have straight or curved apices (Fig 6C). Thalli have substantially uniform diameter throughout its length. Main axis with many lateral branches with 1.5-9.0 cm long and 300-800 µm in diameter. In cross section in subapical region, thalli with one to two layers of pigmented cortical cells and two to three layers of colorless medullary cells, which decrease in size towards the cortex (Fig. 6F). Small cortical cells, elongated, 7.5-12.5 µm long and 5.0-12.5 µm in diameter. Large medullary cells, rounded to elliptical, 42.5-125 µm long and 37.5-137.5 µm in diameter (Fig. 6F-G). Axial cell rounded to elliptical, usually equal or less than to periaxial cells, or rarely larger. Each axial cell is surrounded by 5-7 periaxial cells (Fig. 6G), 37.5-175 µm long and 37.5-137.5 µm in diameter. Medullary cell walls uniformly thickened. Lenticular thickening frequent in periaxial cells and other medullary cells (Fig. 6F). Tetrasporangial branchlets distributed from the basal to the apical region of the branchlets, with 600-2000 µm long and 225-625 µm in diameter (Fig. 6H). Tetrasporangia zonately divided, 25-50 µm long and 15-55 µm in diameter.

HOLOTYPE: Jamaica, tetrasporic plant, F. Nauer (SPF****). (GBnumber) *rbc*L and (GBnumber) COI sequences of holotype, respectively.

HABITAT: The specimens were collected from the intertidal zone in protected rocky shores and reefs near the beaches to the shallow subtidal zone, up to 2 m deep, growing on *Sargassum* C. Agardh.

SPECIMENS EXAMINED: Jamaica. F. Nauer (SPF****).

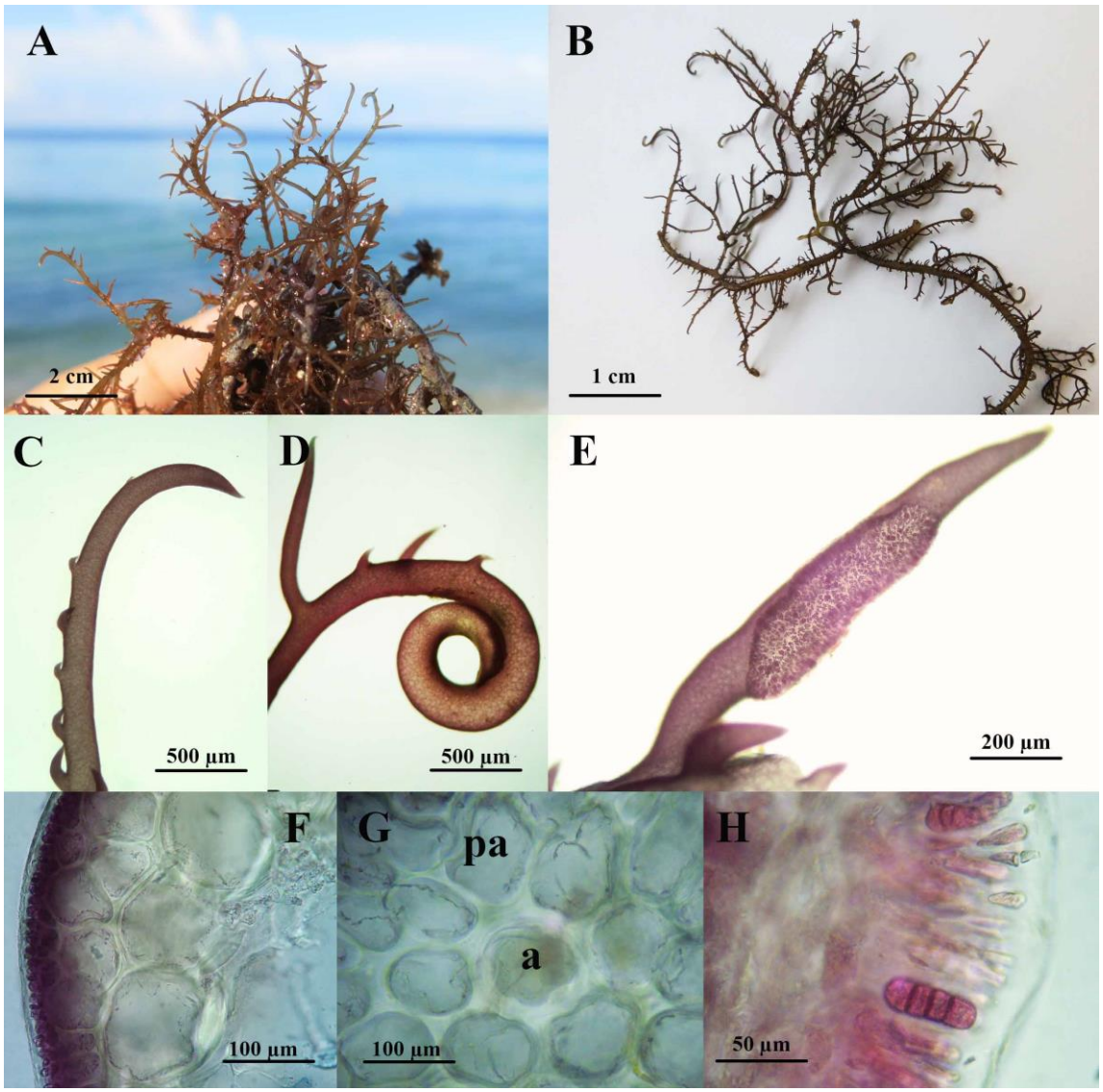

Figure 6. Habits of *Hypnea* sp. 3. **A.** Habit of Holotype on nature. **B.** Habit of a tetrasporangial thallus showing fertile branchlets densely grouped at the apical portions and branches with hooks and tendrils. **C.** Upper portion of branches. **D.** Upper portion of branches showing tendrils. **E.** Detail of a portion of the thallus showing tetrasporangial spiny-like branchlets disposed unilaterally on dorsal side of branch (arrow). **F.** Transverse section of the thallus with detail of lenticular thickening. **G.** Detail of medullary region showing an axial cell with seven periaxial cells. **H.**

Transverse section of a tetrasporangial branchlet showing tetrasporangia zonately divided.

Hypnea sp. 4 (Fig 7)

DESCRIPTION: Plants erect, epilithic, terete, cartilaginous in texture, forming tufts up to 16 cm tall, reddish, brownish, greenish when alive, attached to the substrate by discoid holdfast and stolon-like branches. Irregular branching in various planes, in acute or right angles, with up to 3 orders of branches. Spiny-like branchlets numerous and irregularly arranged throughout the thalli with 225-1200 µm long. Anastomoses absent. Epilithic plants forming tufts at the intertidal zone with numerous erect axes, 1.0-11 cm long and 500-1700 µm in diameter, arising from a discoid holdfast and from stolon-like branches (Fig. 7D). Tendrils are rare and the main and lateral branches are usually straight or curved. In some populations, all the main axes reach near the same height with the longer axis placed at the central portion of the tufts. This arrangement of the thalli resembles the bristles of a brush. In cross section in subapical region, thalli with one to two layers of pigmented cortical cells and two to three layers of colorless medullary cells, which decrease in size towards the cortex (Fig. 7I). Small cortical cells, elongated, 7.5-12.5 µm long and 5.0-12.5 µm in diameter. Large medullary cells, rounded to elliptical, 42.5-125 µm long and 37.5-137.5 µm in diameter (Fig. 7I). Axial cell rounded to elliptical, usually equal or less than to periaxial cells, or rarely larger. Each axial cell is surrounded by 5-7 periaxial cells (Fig. 7I), 37.5-175 µm long and 37.5-137.5 µm in diameter. Medullary cell walls uniformly thickened. Tetrasporangial branchlets distributed from the basal to the apical region of the branchlets, with 600-2000 µm long and 225-625 µm in diameter (Fig. 7G-J). Tetrasporangia zonately divided, 25-50 µm long and 15-55 µm in diameter. Cystocarps globose, solitary or in clusters, found in the apical portions of the branches, measuring up to 150 µm long and up to 187.5 µm in diameter (Fig. 7H, I). Male gametophytes not found.

HOLOTYPE: Dominican Republic (SPF****). (GBnumber) *rbc*L and (GBnumber) COI sequences of holotype, respectively.

HABITAT: The specimens were collected from the intertidal zone in protected rocky shores and reefs near the beaches to the shallow subtidal zone.

SPECIMENS EXAMINED: Dominican Republic. F. Nauer (SPF****).

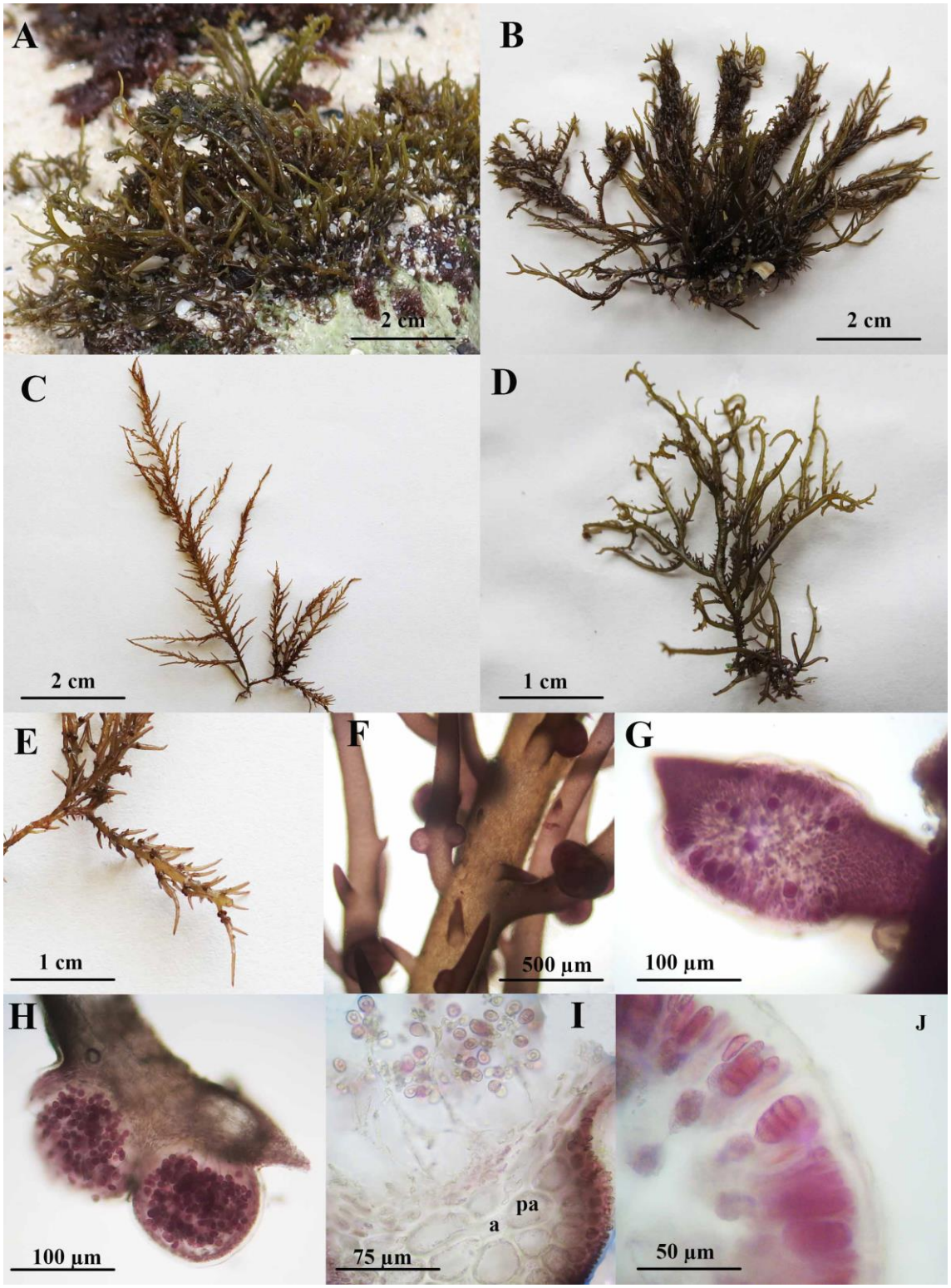

Figure 7. Habits of *Hypnea* sp. 4. **A.** Habit of Holotype on nature. **B.** Habit of a tetrasporangial thallus showing fertile branchlets densely grouped at the apical portions

and branches with hooks and tendrils. **C.** Habit of a female gametophyte thallus showing fertile branchlets with cistocarps. **D.** Detail of a tetrasporangial thallus. **E.** Detail of female gametophyte thallus with cistocarps. **F.** Detail of branches with several cystocarps. **G.** Detail of a portion of the thallus showing tetrasporangial spiny-like branchlets disposed unilaterally on dorsal side of branch. **H.** Transverse section of the thallus with two cystocarps with carpospores. **I.** Detail of the thallus with medullary region showing an axial cell with six periaxial cells and carpospores inside the cystocarp. **J.** Transverse section of a tetrasporangial branchlet showing tetrasporangia zonately divided.

Observations on the *Hypnea musciformis* from the type locality (Trieste, Italy) (Fig. 8)

The morphological analysis of the material from Trieste, Italy showed that the Italian specimens are somewhat similar to the epiphytic specimens of *H. pseudomusciformis* growing on upper subtidal, whose thallus is long, flexible, abundantly branched with tendrils at the apices of the branches and branchlets. However, the Italian material has fewer and shorter spiny branchlets scattered throughout the thallus (Fig. 8 A – C), and few tendrils are present in branches and branchlets (Fig. 8 D); the apices may be curved in hooks or right. In cross section, the thallus has one to two layers of pigmented cortical cells and two to three layers of colorless medullary cells (Fig. 8E). The axial cell, smaller than periaxial cells, is surrounded by 5-6 periaxial cells (Fig. 8F).

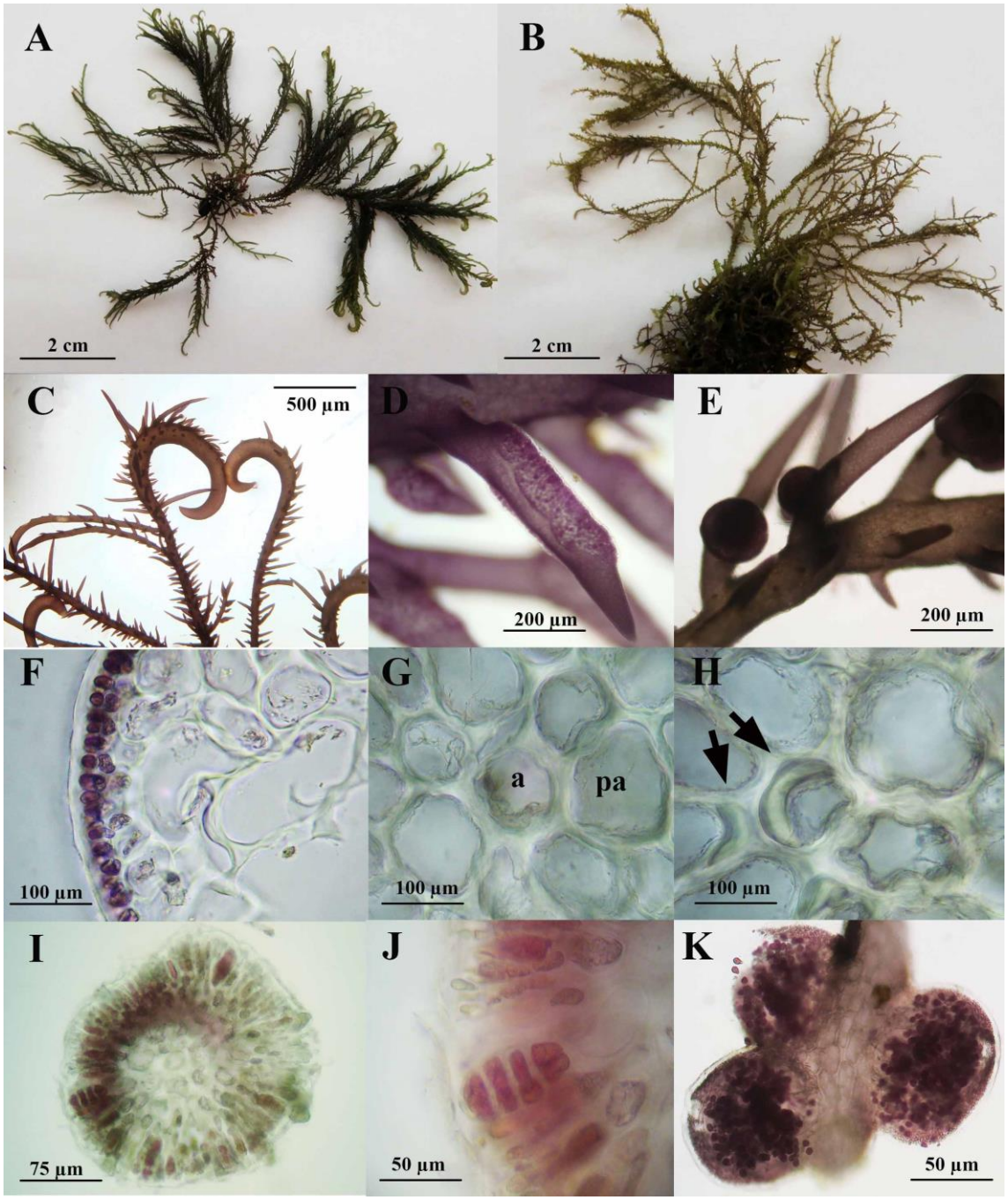

Figure 8. *Hypnea musciformis* from type locality, Italy. **A-B.** Habit of a tetrasporangial thallus. **C.** Upper portion of branches showing tendrils. **D.** Detail of a portion of the thallus showing tetrasporangial spiny-like branchlets disposed unilaterally on dorsal side of branch. **E.** Detail of branches with several cystocarps. **F.** Transverse section of the thallus. **G.** Detail of medullary region showing an axial cell with seven periaxial cells. **H.** Transverse section of the thallus with detail of lenticular thickening. **I.** Transverse section of a tetrasporangial branchlet. **J.** Detail of tetrasporangia zonately divided. **K.** Transverse section of the thallus with three cystocarps with carpospores.

Discussion

The haplotype network revelead four major lineages on the *Hypnea musciformis-H. pseudomusciformis* complex with 37 haplotypes of high diversity (Hd=0,8573). These finds corroborates with Jesus (2016) were three major lineages were observed for these complex of species: one of them was *H. pseudomusciformis* with 23 haplotypes of high diversity (Hd=0.8082); colocar as 2 outras.... In our paper, the same lineage was recovered with 17 haplotypes strongly strucutered in three internal groups. The second lineage in Jesus et al. (2016) included *H. musciformis* in two groups, one from Europe, Africa and North America with five haplotypes of high diversity (Hd=0.9048) and the other from Colombia, Mexico, Barbados and Hawaii, with three haplotypes with médium diversity (Hd=0.5556). In our work, *H. musciformis* (including type locality, Italy) presented 11 haplotypes. And the Caribbean specimens were subdivided into two lineages, one representing *Hypnea* sp. 1 from Caribbean Sea and Hawaii with eigth haplotypes and the other representing *Hypnea* sp. 2 from Dominican Republic, with one haplotype).

Nauer et al. (2015) was the first to mention that *H. musciformis* could be a complex of closely related species instead of being pantropical with wide distribution. In fact, our data shows this complex could be formed by, at least, four species with different geographic distribution. Overall, *H. musciformis* is restricted to the North Hemisphere and *H. pseudomsciformis* to the South Hemisphere, while *Hypnea* sp. 1 and *Hypnea* sp. 2 are mostly in the Caribbean Sea. The presence of Hypnea sp. 3 in Hawaii could indicate a event of introduction. For Jesus et al. (2016), the Panama Isthmus (complete closure ocurred around 3 mya, separating the waters of the tropical western Atlantic and tropical western Pacific) is the geographyc barrier responsible for the separation of *Hypnea* lineages in that region.

Significant levels of genetic discontinuity for the populations of *H. pseudomusciformis* were detected along the Uruguay-Brazilian coast. Even tough different methods were sensitive to different components of the molecular dataset, strong spatial genetic structure was observed with the recognition of three distinct genetic provinces (or phylogroups): the Uruguay province, South-Southeastern Brazil province and North-northeastern Brazil province. The Phylogeographic structure observed coincides with the most recent bio-regionalization of the Brazilian marine benthic macroalgal flora that also recognizes the presence of two distinct provinces

along the Brazilian coast separated to each other in the region between ES and BA (Horta et al. 2001). The region encompassing northern ES and southern BA has also been recognized as a place of major biogeographic break, or overalp, between two distinct coastal terrestrial floras (e.g. Santos et al. 2007). This region is also unique for aggregating high levels terrestrial plant endemism (Thomas et al. 1998) attributed to climatic stability over geological time (i.e. climatic refuge). There is therefore a concordance between the biogeography of coastal plant communites, benthic macrolagal communities and the Phylogeography of *H. pseudomusciformis* populations. If the phylogeorgaphy of other marine and terrestrial species will also concord with this pattern is yet to be observed as very few to no studies yet exist to further test these observations.

Values of F_{ST} between populations within the N-NE province varied from 0 (or even a negative number) representative of panmixia (e.g. RN x PE+AL), to 0.05 and 0.19 (BA x CE) indicative of very low to moderate levels of restricted gene flow, respectively. Same pattern was observed within the S-SE Braziianl Province (F_{ST}: 0.03 – 0.21). The highest F_{ST} values observed in South-Brazil province are from the population of ES and RJ, which are unique populations due to the presence of excessive of variation, including the presence of three unique haplotype in RJ (Figure 5) and the fixation of a unique haplotype in the ES population.

The Rio de Janeiro upwelling region and Bahia, the latter region with the longest coastline in the Brazil, presented the highest levels of population genetic diversity while Espírito Santo population presented the lowest. The low genetic diversity observed in the latter population can be a result of the small population size found during field work at that location.

Evidence of the presence of balancing natural selection was detected in RJ, BA, and SP populations due to the presence of excessive genetic variation above than expected under a model of neutral evolution (i.e. significant negative Fu and Li's D and Tajima D values). These populations, particularly RJ, are characterized by the presence of strong summer upwelling phenomenon, which lowers sea surface temperatures down to 12-13˚C compared to surround non-upwelling waters that remain at 22-26˚ (Rodriguez and Lorenzzetti 2001). Coastal upwelling can promote genetic diversity by two ways: increasing habitat heterogeneity leading to local adaptation and the maintenance of an overall high levels of genetic diversity (i.e. disruptive selection and

micro-scale radiation), or the more dynamic variation in temperature along the year can favour, at different times, differently adapted haplotypes decreasing the likelihood of exclusion of any particular thermo-adapted haplotype and hence promoting the preservation of higher overall diversity (i.e. balancing selection).

While SPN and AMOVA were able to detect genetic structure between Uruguay and Brazilian populations, furthermore, the genetic uniqueness of the RJ population given by the presence of four highly divergent haplotypes also suggests a distinct evolutionary history of this population. It is expected that macroalgae, particularly red macroalgae which do not present neither flagella nor relevant planktonic stages, display some degree of population genetic structure associated to distance alone. This has been evidenced in nearly all marine red macrolgal phylogeographic studies, e.g. *Gracilaria tikvahiae* (Gurgel et al. 2004), *Caulacanthus ustulatus* and *Spiridia filamentosa* (Zuccarello et al. 2002a,b).

Also corroborate the present of spatial genetic structuring along the Uruguay-Brazilian coast which would be established as a result of non-random genetic association resulted from spatial auto-correlation alone rather than the presence of any past or extant geological and oceanographic barriers to gene flow. However, patterns of genetic structure observed in this study coincide with known processes geological and oceanographic processes expected to promote isolation, restricted gene flow, local adaptation and differentiation.

Ayres-Ostrock (personal communication) investigated the diversity of *Gracilaria caudata* along the Brazilian coast, using a combination of mitochondrial (COI, *cox*2-3) and chloroplstic (*rbc*L) markers associated with a population genetics approach using 15 nuclear microsatellites. The results indicates two main gentic clusters of *G. caudata*, the Northeast (CE, RN, PB and PE) and Southeast (ES and SP), with a possible transition zone in the BA region. Additionally, a phylogeographic analysis of the Atlantic spiny lobsters *Panulirus argus* (Crustacea: Palinuridae) using the hypervariable domain of the mtDNA control region, found two genetically diverse groups along the Brazilian coast: one for the North (Pará State - 01oS) and the other for the Southeast population (ES) (Diniz et al., 2004). Our data with *Hypnea* showed similiar findings along the Brazilian coast, indicating that know biogeographic barriers, such as the São Francisco River mouth and the Vitória-Trindade Ridge play a importante role on the distibution and evolution of marine life in Brazil. The Vitória-

Trindade Ridge have been previously related to changes in the genetic diversity of tropical fishes (Joyeux et al., 2001) and deep-water shrimps (Pezzuto & Dias, 2009). Meanwhile, the discharge of the São Francisco River due to its large freshwater inflow could exert a barrier effect on propagules and spore's dispersion.

A pronounced genetic break in south BA region was also observed in a study with the king weakfish *Macrodon ancylodon* between tropical (from Venezuela to BA) and subtropical samples (from ES to Argentina) using mitochondrial DNA cytochrome b sequences (Santo et al., 2006). These authors suggest that, additional to the Vitoria-Trindade Ridge, differences in seawater temperature may also act as a barrier to gene flow between tropical (Venezuela to Bahia) and subtropical (Espírito Santo to Argentina) clades of *M. ancylodon* (Santos et al., 2006). Changes in seawater temperature have been previously suggested as a barrier to gene flow in the red macroalgae *Chondrus crispus* (Krueger-Hadfield et al., 2013) and *Fucus vesiculosus* (Nicastro et al., 2013). Temperature ecotypes have been previously described along the Brazilian coast for *Gracilaria birdiae* (Ursi et al, 2003).

Hypnea sp. 1, Hypnea sp. 2 and H. musciformis

Taken together, our results indicate the presence of two new species for the *H. musciformis* complex, supporting the hypothesis that this species forms a complex of close related species instead being cosmopolitan.

The morphological analysis does not show sufficient characteristics that distinguish the authentic *H. musciformis* from the type locality and other close related species. However, *H. musciformis*, *H. pseudomusciformis* and *Hypnea* sp. 1 and *Hypnea* sp. 2 can be distinguished based on molecular grounds. In red algae, convergent evolution and recent speciation have led to the emergence of several cryptic species that cannot be differentiated on the basis of morphology alone, but which have genetic differences. Due to a simple and convergent morphology with few taxonomically informative characters, other species are similar, which makes it difficult to detect new occurrences and new species for science. The detection of these species was only possible with the use of the DNA barcode technique in a wide range of samples. This technique proved to be fast and efficient in the separation of species, especially since many specimens are found with only few branches, few biomass and no reproductive structures, especially those that inhabit the infralittoral zone.

References

Avise, J. C. 2000. Phylogeography: the history and formation of species. Harvard University Press.

Brinkman, R., Wolanski, E., Deleersnijder, E., McAllister, F., & Skirving, W. 2002. Oceanic inflow from the coral sea into the great barrier reef. Estuarine, Coastal and Shelf Science, 54(4), 655-668.

Castelar, B., Reis, R. P., Azeredo, F., Mattos, P., & Berardinelli, G. 2015. *Hypnea musciformis*: alternative or complement to the production of *Kappaphycus alvarezii* introduced in tropical countries? Aquaculture Research 1-13.

Chase, M. W., & Hills, H. H. 1991. Silica gel: an ideal material for field preservation of leaf samples for DNA studies. Taxon, 215-220.

Choukroun, S., Ridd, P. V., Brinkman, R., & McKinna, L. I. 2010. On the surface circulation in the western Coral Sea and residence times in the Great Barrier Reef. Journal of Geophysical Research: Oceans (1978–2012), 115(C6).

Clement, M., Posada, D. C. K. A., & Crandall, K. A. 2000. TCS: a computer program to estimate gene genealogies. Molecular ecology, 9(10), 1657-1659.

Diniz, F. M., Maclean, N., Ogawa, M., Cintra, I. H., & Bentzen, P. 2005. The hypervariable domain of the mitochondrial control region in Atlantic spiny lobsters and its potential as a marker for investigating phylogeographic structuring. Marine Biotechnology, 7(5), 462-473.

Excoffier, L., Smouse, P. E., & Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, 131(2), 479-491.

Fraser, C. I., Thiel, M., Spencer, H. G., & Waters, J. M. (2010). Contemporary habitat discontinuity and historic glacial ice drive genetic divergence in Chilean kelp. BMC evolutionary biology, 10(1), 203.

Fraser, C. I., Nikula, R., & Waters, J. M. (2011). Oceanic rafting by a coastal community. Proceedings of the Royal Society B: Biological Sciences, 278(1706), 649-655.

Fraser, C. I., Nikula, R., & Waters, J. M. 2011. Oceanic rafting by a coastal community. Proceedings of the Royal Society B: Biological Sciences, 278(1706), 649-655.

Gurgel, C. F. D., Fredericq, S., & Norris, J. N. 2004. Phylogeography of *Gracilaria tikvahiae* (Gracilariaceae, Rhodophyta): a study of genetic discontinuity in a continuously distributed species based on molecular evidence. Journal of Phycology, 40(4), 748-758.

Herke, S., & Foltz, D. 2002. Phylogeography of two squid (*Loligo pealei* and *L. plei*) in the Gulf of Mexico and northwestern Atlantic Ocean. Marine Biology, 140(1), 103-115.

Howells, E. J., Van Oppen, M. J. H., & Willis, B. L. 2009. High genetic differentiation and cross-shelf patterns of genetic diversity among Great Barrier Reef populations of Symbiodinium. Coral Reefs, 28(1), 215-225.

Jensen, J. L., Bohonak, A. J., & Kelley, S. T. 2005. Isolation by distance, web service. BMC genetics, 6(1), 13.

Joyeux, J. C., Floeter, S. R., Ferreira, C. E. L., & Gasparini, J. L. 2001. Biogeography of tropical reef fishes: the South Atlantic puzzle. Journal of Biogeography, 28(7), 831-841.

Kim, K. M., Hoarau, G. G., & Boo, S. M. 2012. Genetic structure and distribution of *Gelidium elegans* (Gelidiales, Rhodophyta) in Korea based on mitochondrial cox1 sequence data. Aquatic Botany, 98(1), 27-33.

Kinlan, B. P., & Gaines, S. D. 2003. Propagule dispersal in marine and terrestrial environments: a community perspective. Ecology, 84(8), 2007-2020.

Krueger-Hadfield, S. A., Roze, D., Mauger, S., & Valero, M. 2013. Intergametophytic selfing and microgeographic genetic structure shape populations of the intertidal red seaweed Chondrus crispus. Molecular ecology, 22(12), 3242-3260.

Librado, P., & Rozas, J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25(11), 1451-1452.

Maddison, D. R., & Maddison, W. P. 2005. MacClade v. 4.08. Sinauer Assoc.

Melo Santos, A. M., Cavalcanti, D. R., Silva, J. M. C. D., & Tabarelli, M. 2007. Biogeographical relationships among tropical forests in north- eastern Brazil. Journal of Biogeography, 34(3), 437-446.

Oliveira, E.C. 1998. The seaweeds resources of Brazil. In: Critchley AT, Ohno M (eds) Seaweeds resources of the world. Japan International Cooperation Agency, Japan, pp 366–371.

Pezzuto, P. R., & Coachman Dias, M. 2009. Reproductive cycle and population structure of the deep-water shrimp Aristeus antillensis A. Milne Edwards & Bouvier, 1909 (Decapoda: Aristeidae) on southeast Brazilian continental slope. Latin American Journal of Aquatic Research, 37(3).

Posada, D., Crandall, K. A., & Templeton, A. R. 2000. GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. Molecular Ecology, 9(4), 487-488.

Portnoy, D. S., & Gold, J. R. 2012. Evidence of multiple vicariance in a marine suture-zone in the Gulf of Mexico. Journal of Biogeography, 39(8), 1499-1507.

Rodrigues, R. R., & Lorenzzetti, J. A. 2001. A numerical study of the effects of bottom topography and coastline geometry on the Southeast Brazilian coastal upwelling. Continental Shelf Research, 21(4), 371-394.

Russell, D. J. 1992. The ecological invasion of Hawaiian reefs by two marine red algae, *Acanthophora spicifera* (Vahl) Boerg. and *Hypnea musciformis* (Wulfen) J. Ag., and their association with two native species, Laurencia nidifica. In J. Ag. and *Hypnea cervicornis* J. Ag. ICES Mar. Sci. Symp 194: 110-125.

Santos, S., Hrbek, T., Farias, I. P., Schneider, H., & Sampaio, I. 2006. Population genetic structuring of the king weakfish, *Macrodon ancylodon* (Sciaenidae), in

Atlantic coastal waters of South America: deep genetic divergence without morphological change. Molecular Ecology, 15(14), 4361-4373.

Saunders, G. W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. Philosophical transactions of the Royal Society B: Biological sciences, 360(1462), 1879-1888.

Shimada, S., Yokoyama, N., Arai, S., & Hiraoka, M. 2009. Phylogeography of the genus *Ulva* (Ulvophyceae, Chlorophyta), with special reference to the Japanese freshwater and brackish taxa. In Nineteenth International Seaweed Symposium (pp. 529-539). Springer Netherlands.

Soltis, D. E., Morris, A. B., McLachlan, J. S., Manos, P. S., & Soltis, P. S. 2006. Comparative phylogeography of unglaciated eastern North America. Molecular ecology, 15(14), 4261-4293.

Staton, J. L., and D. L. Felder. 1995. Genetic variation in populations of the ghost shrimp genus *Callichirus* (Crustacea, Decapoda, Thalassinidea) in the western Atlantic and Gulf of Mexico. Bull. Mar. Sci. 56:523–536.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics, 123(3), 585-595.

Wares, J. P. 2002. Community genetics in the Northwestern Atlantic intertidal. Molecular Ecology, 11(7), 1131-1144.

Zuccarello, G. C., West, J., & Rueness, J. 2002. Phylogeography of the cosmopolitan red alga *Caulacanthus ustulatus* (Caulacanthaceae, Gigartinales). Phycological research, 50(2), 163-172.

Zuccarello, G., Sandercock, B., & West, J. 2002. Diversity within red algal species: variation in world-wide samples of *Spyridia filamentosa* (Ceramiaceae) and *Murrayella periclados* (Rhodomelaceae) using DNA markers and breeding studies. European Journal of Phycology, 37(3), 403-417.

Table 1S: Detailed list of data collection of specimens analyzed in this work. GB – GenBank accession numbers (**, to be added), - not sequenced.

Voucher	Species	Collection Site. Collector and Date	Country	Ocean	Hemisphere	GPS	COI GB Number	*rbc*L GB number
****	*H. musciformis*	Quebra Canela Beach. Cidade Praia. E. C. Oliveira. 04.20.2014	Cabo Verde	Atlantic	North		**	-
****	*H. musciformis*	Quebra Canela Beach. Cidade Praia. E. C. Oliveira. 04.20.2014	Cabo Verde	Atlantic	North		**	-
****	*H. musciformis*	Quebra Canela Beach. Cidade Praia. E. C. Oliveira. 04.20.2014	Cabo Verde	Atlantic	North		**	-
****	*H. musciformis*	Quebra Canela Beach. Cidade Praia. E. C. Oliveira. 04.20.2014	Cabo Verde	Atlantic	North		**	-
****	*H. musciformis*	Quebra Canela Beach. Cidade Praia. E. C. Oliveira. 04.20.2014	Cabo Verde	Atlantic	North		**	-
****	*H. musciformis*	Passetto Beach. Ancona. F. Nauer & F. Rindi. 08.19.2014	Italy	Adriatic Sea	North		**	-
****	*H. musciformis*	Passetto Beach. Ancona. F. Nauer & F. Rindi. 08.19.2014	Italy	Adriatic Sea	North		**	-
****	*H. musciformis*	Passetto Beach. Ancona. F. Nauer & F. Rindi. 08.19.2014	Italy	Adriatic Sea	North		**	-
****	*H. musciformis*	Passetto Beach. Ancona. F. Nauer & F. Rindi. 08.19.2014	Italy	Adriatic Sea	North		**	-
****	*H. musciformis*	Passetto Beach. Ancona. F. Nauer & F. Rindi. 08.19.2014	Italy	Adriatic Sea	North		**	-

****	*H. musciformis*	Passetto Beach. Ancona. F. Nauer & F. Rindi. 08.19.2014	Italy	Adriatic Sea	North	**	-
****	*H. musciformis*	Passetto Beach. Ancona. F. Nauer & F. Rindi. 08.19.2014	Italy	Adriatic Sea	North	**	-
****	*H. musciformis*	Passetto Beach. Ancona. F. Nauer & F. Rindi. 08.19.2014	Italy	Adriatic Sea	North	**	-
****	*Hypnea* sp. 3	Quebra Canela Beach. Cidade Praia. F. Nauer. 02.20.2016	Jamaica	Caribbean Sea	North	**	-
****	*Hypnea* sp. 3	Quebra Canela Beach. Cidade Praia. F. Nauer. 02.20.2016	Jamaica	Caribbean Sea	North	**	-
****	*Hypnea* sp. 3	Quebra Canela Beach. Cidade Praia. F. Nauer. 02.20.2016	Jamaica	Caribbean Sea	North	**	-
****	*Hypnea* sp. 3	Quebra Canela Beach. Cidade Praia. F. Nauer. 02.20.2016	Jamaica	Caribbean Sea	North	**	-
****	*Hypnea* sp. 3	Quebra Canela Beach. Cidade Praia. F. Nauer. 02.20.2016	Jamaica	Caribbean Sea	North	**	-
****	*Hypnea* sp. 3	Quebra Canela Beach. Cidade Praia. F. Nauer. 02.20.2016	Jamaica	Caribbean Sea	North	**	-
****	*Hypnea* sp. 3	Quebra Canela Beach. Cidade Praia. F. Nauer. 02.20.2016	Jamaica	Caribbean Sea	North	**	-
****	*Hypnea* sp. 3	Quebra Canela Beach. Cidade Praia. F. Nauer. 02.20.2016	Jamaica	Caribbean Sea	North	**	-
****	*Hypnea* sp. 4	Playa Dominicus Beach. Bayahibe. F. Nauer. 05.09.2014	D. Republic	Caribbean Sea	North	**	-

****	*Hypnea* sp. 4	Playa Dominicus Beach. Bayahibe. F. Nauer. 05.09.2014	D. Republic	Caribbean Sea	North	**	-
****	*Hypnea* sp. 4	Playa Dominicus Beach. Bayahibe. F. Nauer. 05.09.2014	D. Republic	Caribbean Sea	North	**	-
****	*Hypnea* sp. 4	Playa Dominicus Beach. Bayahibe. F. Nauer. 05.09.2014	D. Republic	Caribbean Sea	North	**	-
****	*Hypnea* sp. 4	Isla Saona. Bayahibe. F. Nauer. 05.11.2014	D. Republic	Caribbean Sea	North	**	-
****	*Hypnea* sp. 4	Isla Saona. Bayahibe. F. Nauer. 05.11.2014	D. Republic	Caribbean Sea	North	**	-
****	*Hypnea* sp. 4	Isla Catalina. Bayahibe. F. Nauer. 05.11.2014	D. Republic	Caribbean Sea	North	**	-
****	*Hypnea* sp. 4	Isla Catalina. Bayahibe. F. Nauer. 05.11.2014	D. Republic	Caribbean Sea	North	**	-
****	*Hypnea* sp. 4	Isla Catalina. Bayahibe. F. Nauer. 05.11.2014	D. Republic	Caribbean Sea	North	**	-

CAPÍTULO *Quatro*

Life history, growth and pigments of two morphological variations of Hypnea pseudomusciformis (Gigartinales, Rhodophyta).

Capítulo 4

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Life history, growth and pigments of two morphological variations of *Hypnea pseudomusciformis* (Gigartinales, Rhodophyta).

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Abstract

Hypnea musciformis has been used as a economically important resource for the production of carrageenan on the coast of Brazil. Specimens previously described as *Hypnea musciformis* and *H. nigrescens* in Brazil were shown to be morphological variants of a new species named *Hypnea pseudomusciformis.* In this study, individuals of both of those morphological variants were collected, and cultivated in the same unialgal culture conditions. The life history of both morphological variants of *H. pseudomusciformis*, was completed in 121 days. Tetrasporophytes produced tetrasporangia in 30 days. Released tetraspores germinated and around 15 days later, an erect cylindrical axis developed from basal disk. At the age of 45 days, spermatangial conceptacles and cystocarps (indicating that fertilization occurred) could be observed in different gametophytes. Finally, carpospores released from cystocarps germinated and originated new tetrasporophytes in 31 days. When cultured *in vitro*, the morphological diferences between the variants "musciformis" and "nigrescens" were attenuated. Futhermore, both morphological variations presented similar growth rates and pigment content. These results corroborates that, for Brazil, specimens previously identified as *H. musciformis* and *H. nigrescens* are variations of *H. pseudomusciformis*, a high plastic

species. These results indicate that the morphological variation “nigrescens” could also be selected for commercial cultivation.

Keywords *Hypnea*, Physiological Plasticity, Pigments, Growth Rates

Introduction

Nauer et al. (2015) showed that three species cited in the past for the Brazilian coast (*H. musciformis*, *H. nigrescens* Greville ex J. Agardh and *H. valentiae* (Turner) Montagne) were morphological variations of a new species, *H. pseudomusciformis* Nauer, Cassano & M.C. Oliveira. Those morphological variations of *H. pseudomusciformis* have distinct morphology and grow on different zones on the rocky shore. The variation “musciformis” is very common on Brazilian shore and can be easily found forming populations with high biomass. This variant has an epiphytic habit, using numerous tendrils present at the apex of the branches to curl up on other macroalgae, and usually occur in the lower intertidal zone in protected environments (Reis et al. 2000). The morphological variation “nigrescens*”* is characterized by its epilithic habit and is usually found growing in mid intertidal zone in wave-impacted environments. Its morphology is characterized by having numerous long branches that resemble bristles of a brush and a red-blackish color (Nauer et al. 2015).

The *in vitro* cultivation of *Hypnea pseudomusciformis* (as *H. musciformis*) has been used as a tool for different studies in Brazil. Bravin & Yoneshigue-Valentin (2002) studied the influence of environmental factors on the species growth. Yokoya et al. (2007) studied somatic mutations in color variants and concluded that both color strains (brown and green) have similar growth rates (although in diferent optimal light conditions). Martins et al. (2009) and Fernandes et al. (2012) also conducted studies related to two color variants, were they showed that both of those variants could be selected for acquaculture. But, the only study using *in vitro* culture of *Hypnea* species aiming taxonomy was held by Schenkman (1986). This author also made the first citation of “nigrescens” (as *H. nigrescens*) for Brazil based on the morphological diferences compared to “musciformis” (as *H. musciformis*). In fact, Schenkman (1986)

made the only citation of *H. nigrescens* outside the Indian Ocean and, until today, studies with this species are quite rare.

The life history of *Hypnea* is an alternation between two diploid phases and one haploid phase (Masuda et al. 1997) (Figure 1). Gametophytes and tetrasporophytes are isomorphic. The tetrasporangium sori are formed in the cortical region of branchlets. Cystocarps are located in female gametophyte branches, usually visible to the naked eye. Spermatangium sori are organized in the cortical region of branchlets and is also visible to the naked eye (Masuda et al. 1997).

Species of *Hypnea* are considered an important carrageenan-producing red alga in Brazil (Oliveira 1998) and, for this reason, studies on aquacultural essays, as well as biomass production, phenology and carrageenan yield of natural populations of the variation "musciformis" of *H. pseudomusciformis* (as *H. musciformis*) have been done (Berchez and Oliveira 1990; Wallner et al. 1992; Berchez et al. 1993; Reis & Yoneshigue-Valentin 1998, 2000; Reis et al. 2003; Mouradi et al. 2008, Caires et al. 2013a). For "nigrescens", Jesus (2016) made first phenology studied based on samples form Northeast of Brazil, and both "musciformis" and "nigrescens" displays a prevalence of tetrasporophytes and absence of male gametophytes on natural populations.

Here we describe the life history of *Hypnea pseudomusciformis*, for the two morphological variants ("musciformis" and "nigrescens"). Furthermore, we compare the growth rates and pigment content of the tetrasporophytic and gametophytic phases of those variants. The physiological characterization of those two morphological variants could provide basic information about frequency of life phases on natural populations and to select suitable sites for comercial cultivation of *Hypnea*.

Materials and methods

ALGAL MATERIAL

The material used in our experiments were derived from three tetrasporophytic plants of *Hypnea pseudomusciformis* presenting the "nigrescens" morphology, collected in Cibratel, SP ($24^0$12'05.68''S/$46^0$48'38.72''O) and three tetrasporophytic plants of "musciformis" morphology, collected in Cigarras beach, São Sebastião, SP ($23^0$43'41.38''S/$45^0$23'53.84''O) (Figure 2A-B, 3A-B). Branches of these specimens were isolated and kept in unialgal culture in the germoplasm bank at the "Edison J. de

Paula" Laboratory of Marine Algae, University of São Paulo. Voucher specimens were deposited in the SPF herbarium of the Institute of Bioscience at the University of São Paulo.

CULTURE CONDITIONS

The standard culture conditions were: von Stosch-enriched seawater with modification (Ursi and Plastino 2001) and diluted to 50 % with sterile seawater (32 psu), photoperiod of 14h, 24±1°C, 70±5 µmol photons $m^{-2}s^{-1}$ (Osram 40W day light fluorescent tubes). Cultures were aerated for 30 min h^{-1}. The medium was renewed and thalli observed weekly. Specimens were incubated in the standard culture conditions in Erlenmeyer vials with 500 ml of medium.

GROWTH RATES

Three apical branches (1 cm length each and fresh weight (FW) ca. of 1 mg total) of unfertile female gametophytes and of tetrasporophytes (n=3) were cultivated for 4 weeks in 500 ml of enriched seawater in the standard conditions. Branches were gently blotted dry and weighted weekly on an analytical balance Mettler – Toledo AE 200S. Growth rates (GR) were assessed weekly according to Lignell and Pedersén (1989).

PIGMENTS AND TOTAL SOLUBLE PROTEINS

The analyzis of pigments and total soluble proteins (TSP) were done from samples of 70 mg FW of unfertile female gametophytes and of tetrasporophytes (n=3). Extraction of pigments was performed according to Kursar et al. (1983), adapted by Wanderley (2009). Initially, the samples were ground in liquid nitrogen and extracted in 1 mL of 50 M sodium phosphate buffer pH 5.5 for 1 h at 4 °C, protected from the light. After extraction, the samples were centrifuged and the supernatant transferred to a new microtube. This extract was called a buffered extract and used for phycobiliprotein and TSP analyzes. Absorbance readings (400 nm – 700 nm) were measured in a 96-well

microplates with a final volume of 400 µL in a UV-vis spectrophotometer (Epoch 2-BioTek, USA). The levels of phycobiliproteins (mg.g FW^{-1}) were calculated according to Beer & Eshel (1985).

In the second stage of extraction, from the residue (pellet) obtained from the buffered extract, chlorophyll *a* and carotenoids were extracted by adding 1.5 ml of methanol for 3 h at 4 °C protected from light. Absorbance readings (400 nm – 700 nm) were measured in a 96-well microplates with a final volume of 400 µL in a UV-vis spectrophotometer (Epoch 2-BioTek, USA). The levels of chlorophyll *a* and carotenoids (mg.g FW^{-1}) were calculated according to Lichtenthaler & Buschmann (2001).

The TSP quantification method, established by Bradford (1976) and used in this study, is based on the colorimetric reaction with the Coomassie Brilliant Blue G-250 acid. The buffered strata had the determined PST content and was made using the Bio-Rad colorimetric protein assay solution (Bio Rad, USA). The Bio Rad solution was prepared fresh using 1:4 v/v ratio in ultrapure water. The reaction was prepared by adding 40 L of ultrapure water, 90 µL of the Bio Rad solution and 60 µL of the buffered extract. The TSP content was expressed in g.g.FW^{-1} and for the standard bovine serum albumin (BSA, Sigma, Brazil) was used. The standard curve was constructed in the concentration range of 0-16 g.ml. Absorbance readings of both standard and samples were conducted in 96-well microplates with a final volume of 400 µL at 595 nm wavelength in a UV-vis spectrophotometer (Epoch 2-BioTek, USA).

DATA ANALYSIS

For GR, repeated-measures analyses of variance (RMANOVA) were performed using the software STATISTICA 10.0 (StatSoft, Inc.), considering time and life-history stages as independent variables and the growth rates as dependent variable. This analysis was followed by a Newman-Keuls a posteriori test. The data of TSP were tested for normality (Kolmogorov-Smirnov) and homoscedasticity (Bartlett's test) and subsequent one-way or two-way ANOVA ($p < 0.05$). To determine statistical differences was used the *a posteriori* Newman-Keuls test ($p < 0.05$). Before the analysis of variance, the data in percentage were transformed into arc sen [square root (x/100)].

Results

LIFE HISTORY

The life history of *Hypnea pseudomusciformis* for both morphological variants, "nigrescens" and "musciformis", were completed in 121 days (Figure 1). Tetrasporophytic branches cultivated in standard culture conditions produced tetrasporangia in ca. 40 days (Figure 2C for "musciformis" and 3C for "nigrescens"). Tetrasporangia are scattered on the córtex, and are produced from the most external subcortical cells on lateral branches spread around the entire thallus (Figure 2D for "musciformis" and 3D for "nigrescens"). They can be found surrounding completly or partially the base or the middle of those branches. They are ovoid (24-33 x 45-48 µm) and zonated (Figure 2G for "musciformis" and 3G for "nigrescens"). Released tetraspores sunk, attached to the bottom of the vial, produced a cell wall and started the germination process. Around 15 days later, an erect cylindrical axis developed from basal disk. Erect axes were initially unbranched, but later became sparsely branched. Secondary erect axes were formed from the disc-like base. At the age of 35 days, spermatangium sori could be observed in the cortical region of branchlets of the male gametophytes (Figure 2E for "musciformis" and 3E for "nigrescens"). Spermatangium sori are thinner and lighter than tetrasporangium sori (Figure 2H for "musciformis" and 3H for "nigrescens"). After spermacium began to be released, in two/three days the first cystocarps in the female gametophytes were visible to the naked eye (Figure 2F for "musciformis" and 3F for "nigrescens"). The cystocarps are protruding and commonly they form clusters of three or more, but can also be found isolated. They may be globular or sub-spherical, and the release of carpospores occurs by a small ostiole (Figure 2I for "musciformis" and 3I for "nigrescens"). In about 10 days cystocarps start to release carpospores. From the release of carpospores to the development of tetraporophytic plants with diferentiated tetrasporangium sori took about 35 to 40 days.

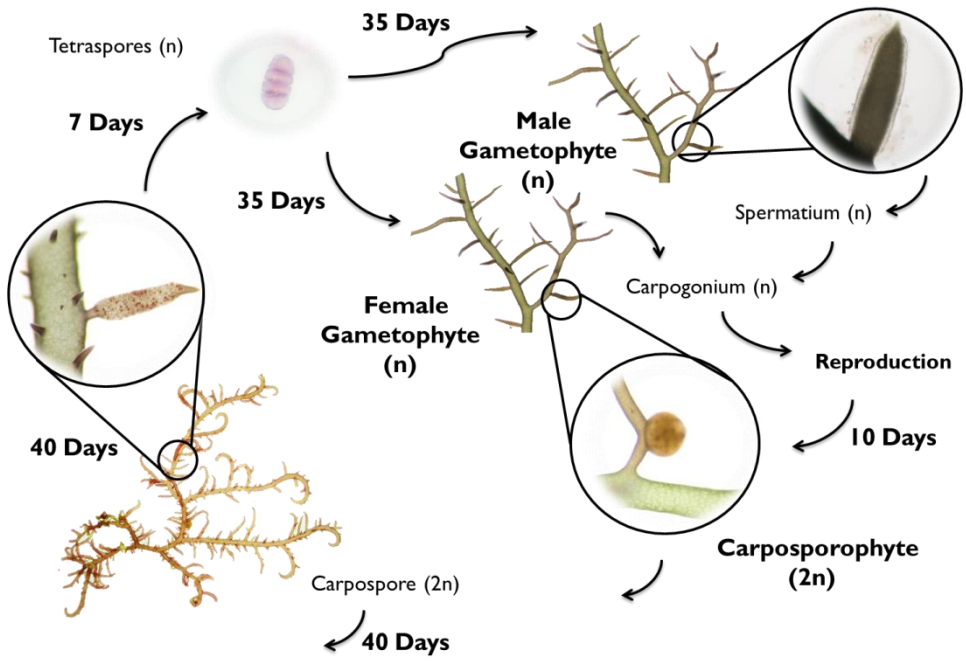

Figure 1. The life history of *H. pseudomusciformis*. Gametophytes and tetrasporophytes are isomorphic. The tetrasporangium sori are formed in the cortical region of branchlets. Cystocarps are located in female gametophyte branchs, usually visible to the naked eye. Spermatangia sori are organized in the cortical region of branchlets. The life history was completed in 121 days, subdivided in each life phase, indicated with arrows.

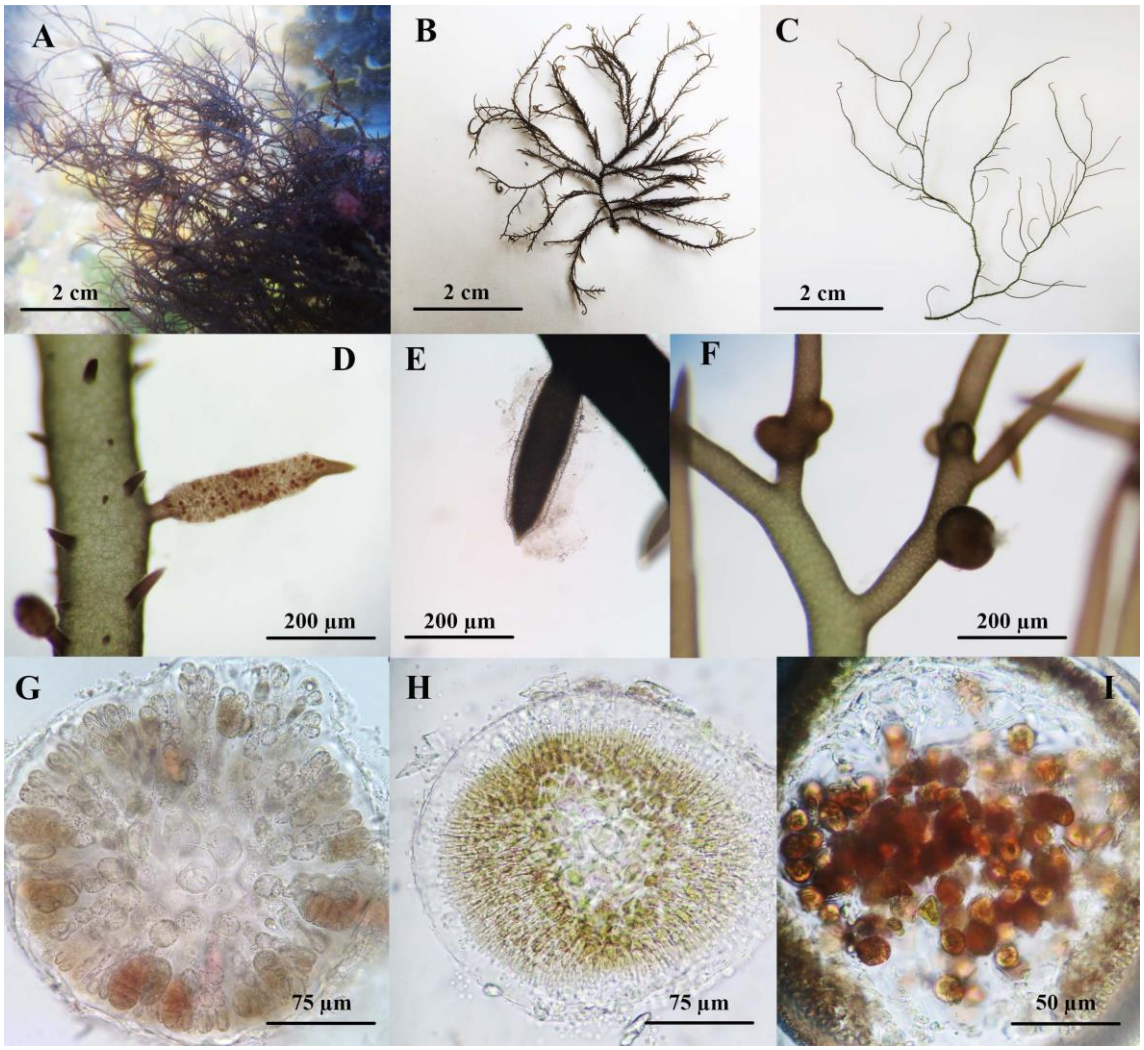

Figure 2. Life phases of *H. pseudomusciformis* morphological variant "musciformis". **A.** Plant growing in natural habitat. **B.** Habit of tetrasporophyte specimen from the field. **C.** Habit of the tetraporophyte plant after 40 days in culture. **D.** Detail of a tetrasporophytic thallus with tetrasporangia. **E.** Detail of a gametophyte thallus with spermatangia. **F.** Detail of a gametophyte thallus with cystocarps. **G.** Tetrasporangia in cross section. **H.** Spermatangia in cross section. **I.** Cystocarps in cross section.

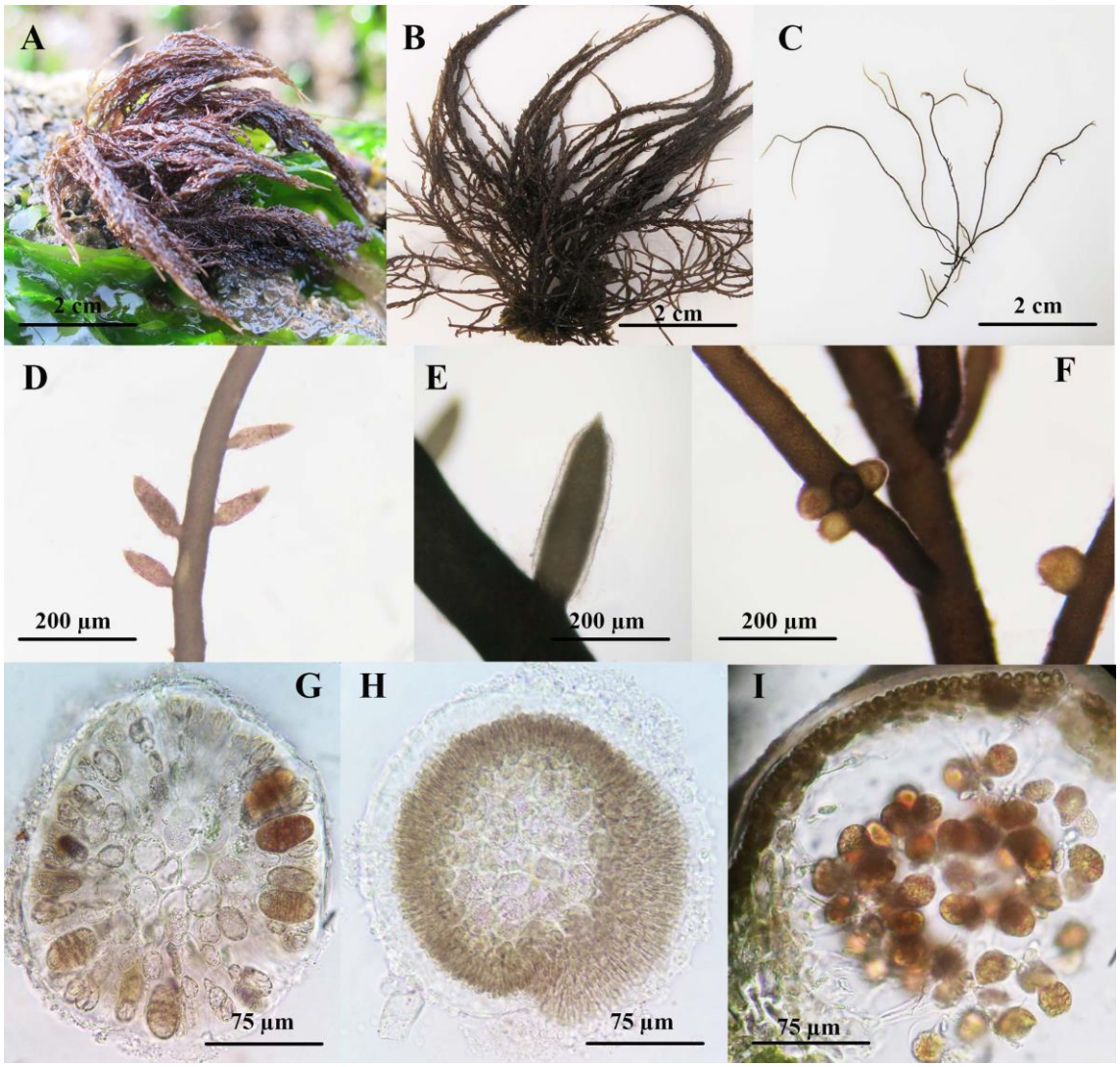

Figure 3. Life phases of *H. pseudomusciformis* morphological variant "nigrescens". **A.** Plant growing in natural habitat. **B.** Habit of tetrasporophyte specimen from the field. **C.** Habit of the tetraporophyte plant after 40 days in culture. **D.** Detail of a tetrasporophytic thallus with tetrasporangia. **E.** Detail of a gametophyte thallus with spermatangia. **F.** Detail of a gametophyte thallus with cystocarps. **G.** Tetrasporangia in cross section. **H.** Spermatangia in cross section. **I.** Cystocarps in cross section.

GROWTH RATES

Comparison of growth rates of tetrasporophytes (T) and female gametophytes (FG) of both variants found little and punctual differences. In "musciformis", T growth rate was higher only during the 3rd week of cultivation, when compared to FG ($F= 11.50$, $p= 0.000$); within each reproductive phase, absolute values of growth rates ranged from 6 to 16% FW.day^{-1} (female gametophytes) and from 8 to 15 % FW.day^{-1} (tetrasporophytes). In "nigrescens", T growth rate was higher only during the 1st week

of cultivation, when compared to FG (F= 25.8, p= 0.000); within each reproductive phase, absolute values of growth rates ranged from 6 to 11% FW.day^{-1} (female gametophytes) and from 4 to 13 % FW.day^{-1} (tetrasporophytes).

Comparison of both morphological variants found higher growth rate in "musciformis" tetrasporophytes only in the 3rd week of cultivation, when compared to the tetrasporophytes of "nigrescens" (F= 22.738, p= 0.003). On the other hand, growth rate was higher in the female gametophytes of "musciformis" in the 1st and last week of cultivation, when compared to the female gametophytes of "nigrescens" (F= 57.415, p≤ 0.017). Nevertheless, in the 3rd week of cultivation, the female gametophytes of "nigrescens" presented a higher growth rate (F= 57.415, p= 0.041).

The two life phases of both morphological variations present similar morphology when kept *in vitro*, having similar thallus architecture, differently from specimens from the field. However some differences still could be noticed in the branching pattern and apex shape. "Musciformis" (tetrasporophytes and female and male gametophytes) usually grows on all sides and planes, the growth of the lateral or spinescent branches occurs in a very curved way which confers a more tangled aspect of the thallus. Most branches, lateral or main, have their apical portion very curved, but formation of tendrils was very rare under the culture conditions. On other hand, "nigrescens" (tetrasporophytes and female and male gametophytes) grows erect, with few spinescent branches. As the plant develops in culture, new branches form in the median-inferior region of the thallus and take the same dimensions as the main axis, with irregular branching. The angle of the branches does not follow a very defined pattern, they can be very closed, with 50 degrees or less in the apical and very open in the medial or basal region. As the branches grow, they tend to be straight. There is no tendrils formation, but it is common for the apical region of the branches to have a slight curvature. Regarding reproduction, plants from both variations became fertile and formed cystocarps around the same time.

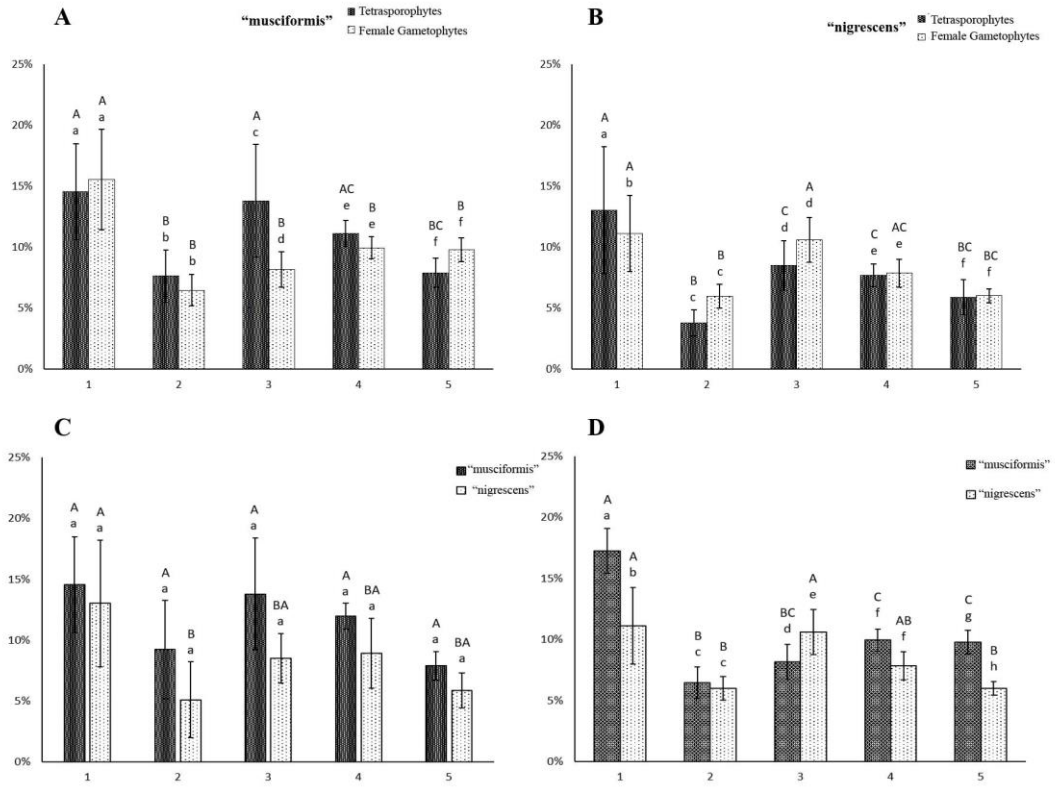

Figure 4. Growth rates of *H. pseudomusciformis*. **A.** "musciformis", tetrasporophyte compared with female gametopyte. **B.** "nigrescens", tetrasporophyte compared with female gametopyte **C.** "musciformis" tetrasporophyte compared with "nigrescens" tetrasporophyte. **D.** "musciformis" female gametopyte compared with "nigrescens" female gametopyte. A = comparison between weeks within the same life phase. b = comparing life phase each week.

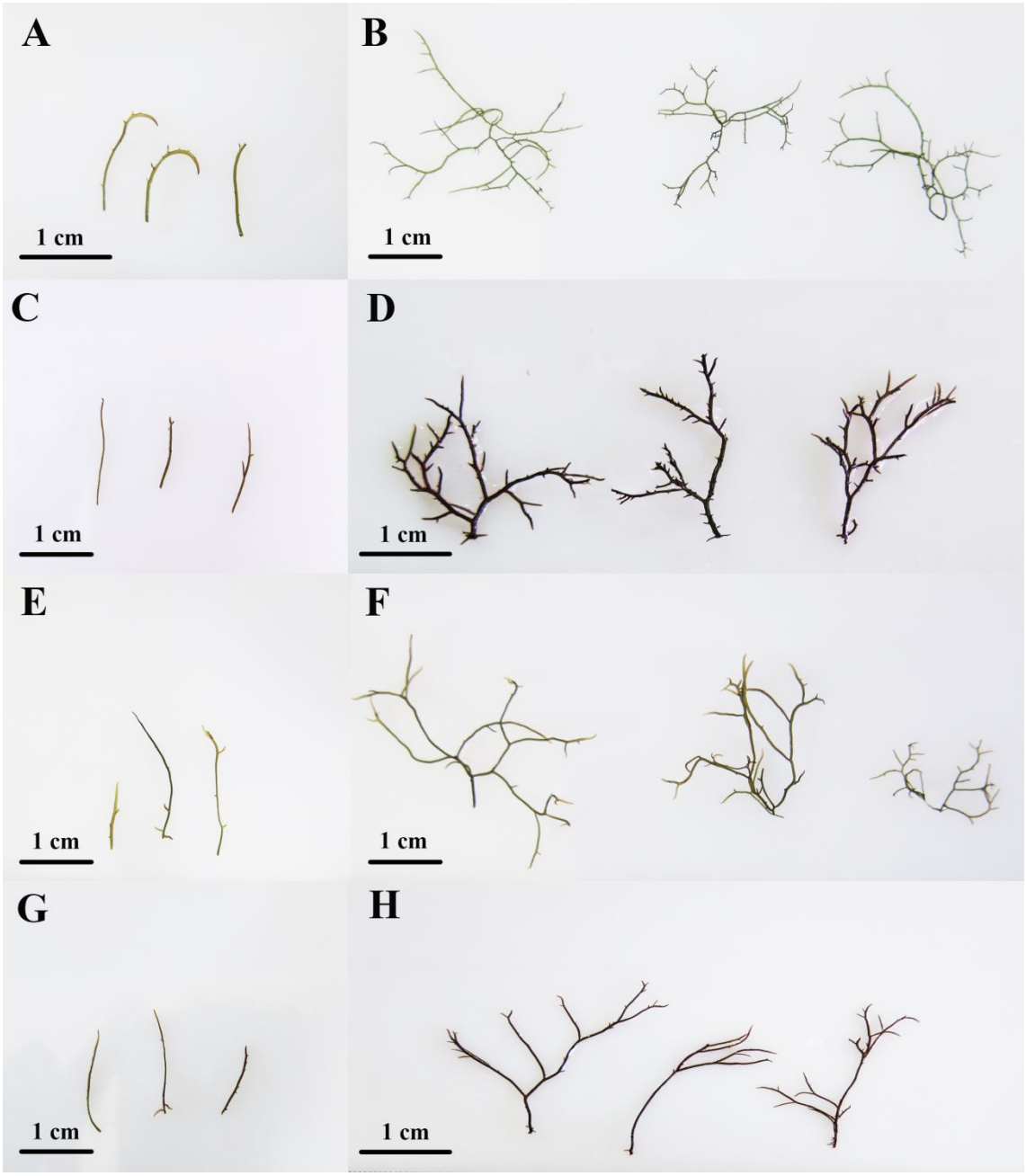

Figure 5. Growth rate test for samples of *H. pseudomusciformis*. **A to D.** Morphological variation "musciformis". **A.** Tetrasporophytic apices on the beginning of the first week. **B.** Tetrasporophytic plants on the end of the fifth week. **C.** Female gametophytic apices on the beginning of the first week. **D.** Female gametophytic plants on the end of the fifth week. **E to H.** Morphological variation "nigrescens". **E.** Tetrasporophytic apices on the beginning of the first week. **F.** Tetrasporophyic plants on the end of the fifth week. **G.** Female gametophytic apices on the beginning of the first week. **H.** Female gametophytic plants on the end of the fifth week.

PIGMENTS AND TOTAL SOLUBLE PROTEINS

Phycobiliprotein contents varied both in the morphological variants and in the life history phases of *H. pseudomusciformis* (Figures 6-9). Overall, "musciformis" tetrasporophytes showed higher concentration for phycoerythrin (PE), allophycocyanin (APC) and total soluble proteins when compared to "musciformis" gametophytes. However, the male gametophyte showed highest concentration of chlorophyll *a* when compared to female gametophytes and tetrasporophytes. For "nigrescens", female gametophytes showed higher concentration of PE, phycocyanin (PC) and total soluble proteins when compared to tetrasporophytes and male gametophytes. The male gametophyte had the highest concentration of chlorophyll *a*.

For phycoerythrin (PE), both male and female "musciformis" gametophytes showed no significant differences (208,01 - 548,19 µg.g FW^{-1}; 297,36 - 528,70 µg.g FW^{-1}, respectively), but tetrasporophytes showed higher concentration (437,97 - 758,64 µg.g FW^{-1}) (Figure 6A). However, for variation "nigrescens" the female gametophytes showed differences for this pigment (194,47 and 307,30 µg.g FW^{-1}), while the male gametophyte and the tetrasporophyte showed no difference (364,85 and 463,75 µg.g FW^{-1}; 223,68 and 459,96 µg.g FW^{-1}, respectively) (Figure 6B). For phycocyanin (PC), the life phases of "musciformis" showed no difference (200,66 and 583,94 µg.g FW^{-1} for male gametophyte; 200,45 and 363,85 µg.g FW^{-1} for female gametophyte; 287,31 and 519,39 µg.g FW^{-1} for tetrasporophyte), while for "nigrescens", the female gametophyte (183,11 and 289,44 µg.g FW^{-1}) showed difference compared to the other two life phases (343,96 and 441,52 µg.g FW^{-1} for male gametophyte; 162,15 and 359,56 µg.g FW^{-1} for tetrasporophyte). For the latter, the allophycocyanin (APC) content was higher for tetrasporophyte phase (167,05 and 248,19 µg.g FW^{-1}) compared to male and female gametophytes (106,29 and 249,54 µg.g FW^{-1}; 87,08 and 143,92 µg.g FW^{-1} , respectively), for variation "musciformis". For "nigrescens", the female gametophyte showed difference (82,50 and 170,37 µg.g FW^{-1}) while the male gametophyte and the tetrasporophyte showed no difference (168,91 and 208,65 µg.g FW^{-1}; 124,90 and 183,96 µg.g FW^{-1} , respectively).

The results for chlorophyll *a* content was similar for both variations were all life phases showed significant difference (Figure 7A and 7B). The male gametophyte was the life phase with highest concentration of this pigment (306,20 µg.g FW^{-1} for "musciformis" and 354,79 µg.g FW^{-1} for "nigrescens"), followed by female

gametophyte (274,65 µg.g FW^{-1} for "musciformis" and 214,16 µg.g FW^{-1} for "nigrescens") and for the last, the tetrasporophyte (157,79 µg.g FW^{-1} for "musciformis" and 158,47 µg.g FW^{-1} for "nigrescens") . Carotenoids concentration were higher on male gametophytes too for both variations (43,22 µg.g FW^{-1} for "musciformis" and 64,79 µg.g FW^{-1} for "nigrescens"), but for this pigment, female gametophyte (36,48 µg.g FW^{-1} for "musciformis" and 33,92 µg.g FW^{-1} for "nigrescens") and tetrasporophyte (39,05 µg.g FW^{-1} for "musciformis" and 32,41 µg.g FW^{-1} for "nigrescens") showed no differences.

The amount of total soluble proteins presented significant difference between the life phases for the two variations. For "musciformis", the tetrasporophyte was the life phase with highest concentration (3188,39 µg.g FW^{-1}), significant different compared with male and female gametophytes (1423,92 µg.g FW^{-1} and 1916,85 µg.g FW^{-1}, respectively) (Figure 7C). For "nigrescens", the female gametophyte showed significant difference (1442,52 µg.g FW^{-1}), while the male gametophyte (2665,55 µg.g FW^{-1}) and the tetrasporophyte (2213,91 µg.g FW^{-1}) showed no difference (Figure 7D).

When we compared the phycobiliproteins content for the life phases of "musciformis" with the life phases of "nigrescens", there were different results. For phycoerythrin (Figure 8A), the "musciformis" tetrasporophyte showed difference compared with "nigrescens" tetrasporophyte, while the gametophytes showed no differences. For phycocyanin (Figure 8B), all life phases showed no differences. For allophycocyanin (Figure 8C), the "musciformis" tetrasporophyte showed difference compared with "nigrescens" tetrasporophyte, while the gametophytes showed no differences.

When we compared the chlorophyll a content for life phases of "musciformis" with the life phases of "nigrescens", the results were opposite of phycobiliproteins, with "musciformis" tetrasporophyte showed no difference compared with "nigrescens" tetrasporophyte, while both gametophytes showed differences when compared (Figure 9A). The results for the comparison of carotenoids showed that the "musciformis" male gametophytes was significantly different than the "nigrescens" male gametophyte, while the female gametophyte and tetrasporophyte showed no difference (Figure 9B).

At last, the comparison of total soluble proteins between life phases of "musciformis" with the life phases of "nigrescens", showed that both phases were different from each other, with tetrasporophytes, male gametophytes and female gametophytes having differences (Figure 9C).

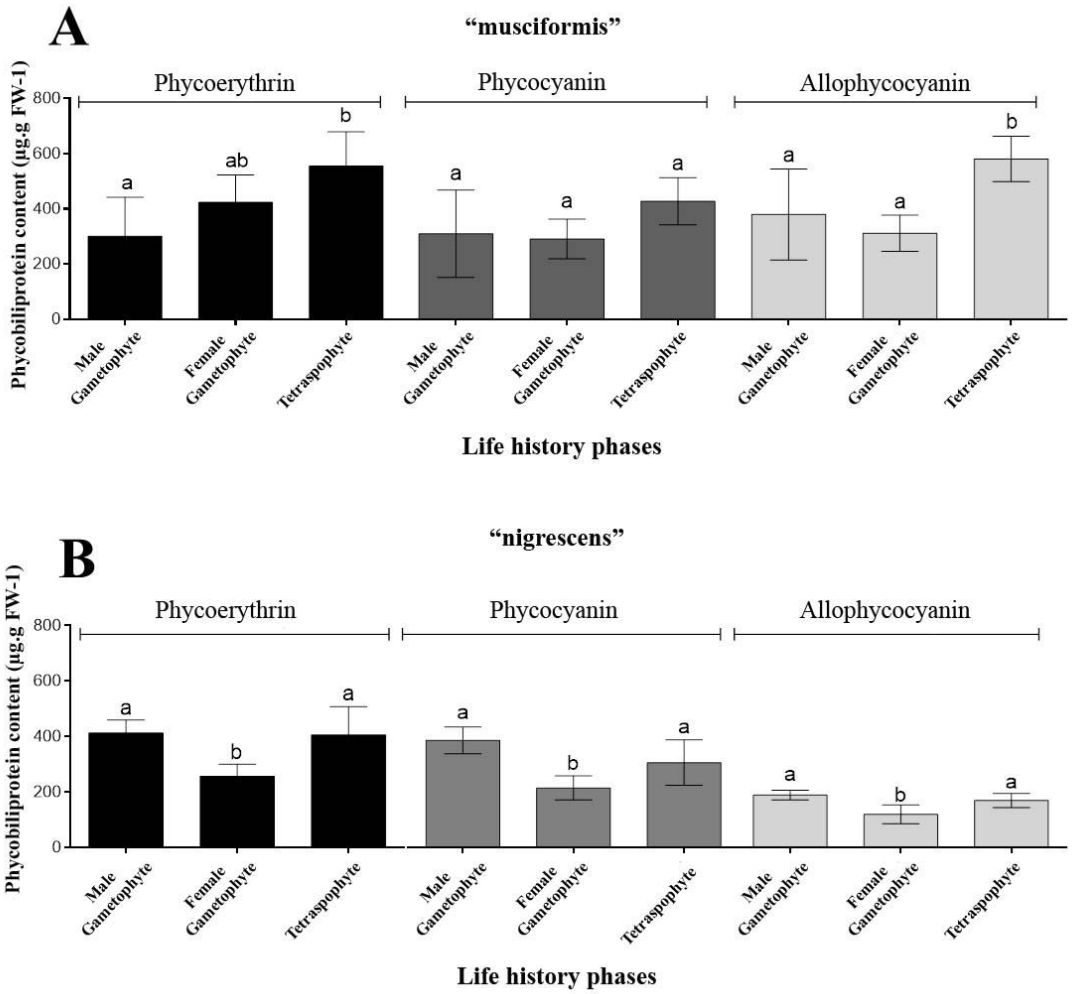

Figure 6. Quantification of pigments content of different life history phases of two morphological variations of *H. pseudomusciformis*, "musciformis" and "nigrescens". **A.** Phycobiliprotein (phycoerythrin, phycocyanin and allophycocyanin) content of variation "musciformis". **B.** Phycobiliprotein (phycoerythrin, phycocyanin and allophycocyanin) content of variation "nigrescens". The bars correspond to the mean ± standard deviation (n = 5). Different letters represent significant differences ($p < 0.05$).

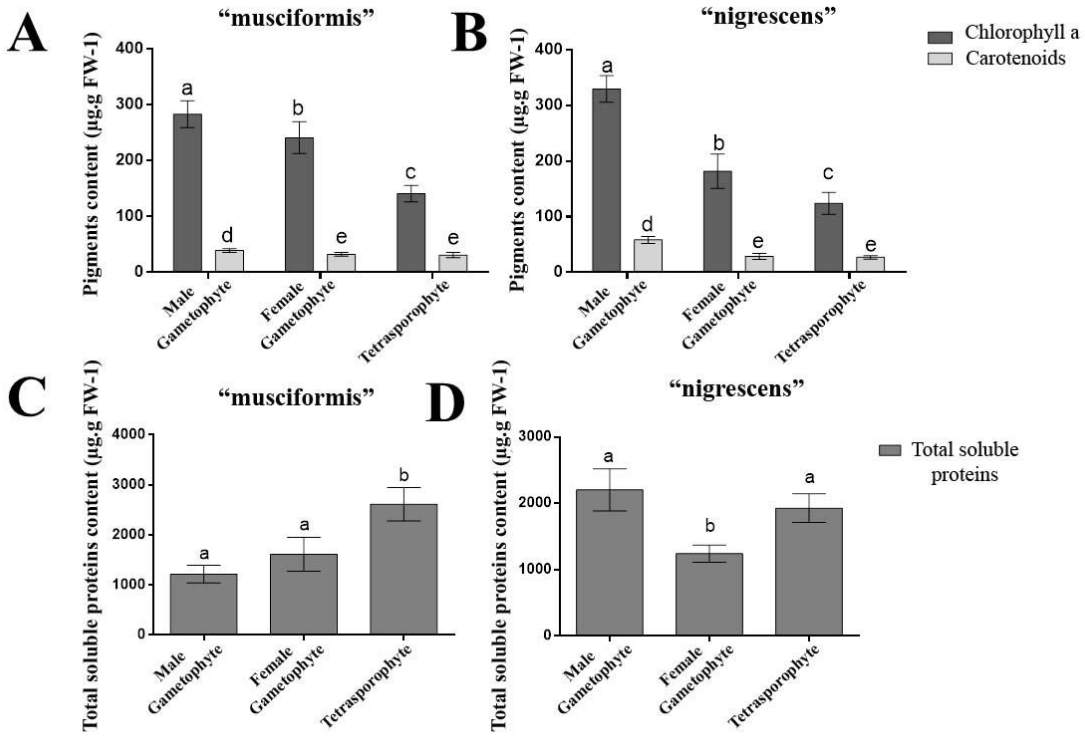

Figure 7. Quantification of pigments content of different life history phases of two morphological variations of *H. pseudomusciformis,* "musciformis" and "nigrescens",. **A.** Chlorophyll a and carotenoids content of variation "musciformis". **B.** Chlorophyll *a* and carotenoids content of variation "nigrescens". **C.** Soluble Proteins of variation "musciformis". **D.** Soluble Proteins of variation "nigrescens". The bars correspond to the mean ± standard deviation (n = 5). Different letters represent significant differences ($p < 0.05$).

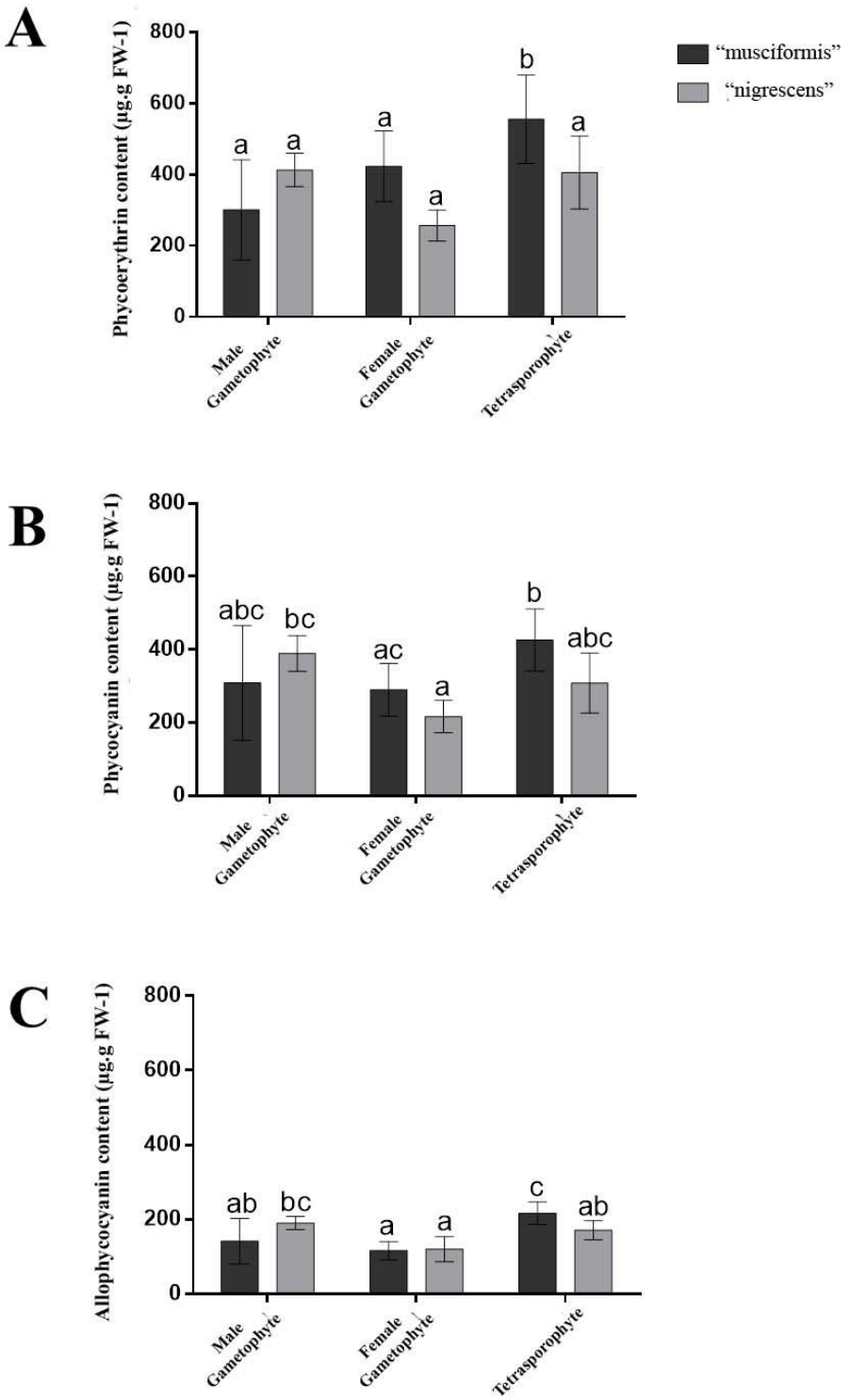

Figure 8. Comparing phycobiliprotein content of different life history phases between two morphological variations of *H. pseudomusciformis*, "musciformis" and "nigrescens". **A.** Phycoerythrin. **B.** Phycocyanin. **C.** Allophycocyanin. The bars correspond to the mean ± standard deviation (n = 5). Different letters represent significant differences ($p < 0.05$).

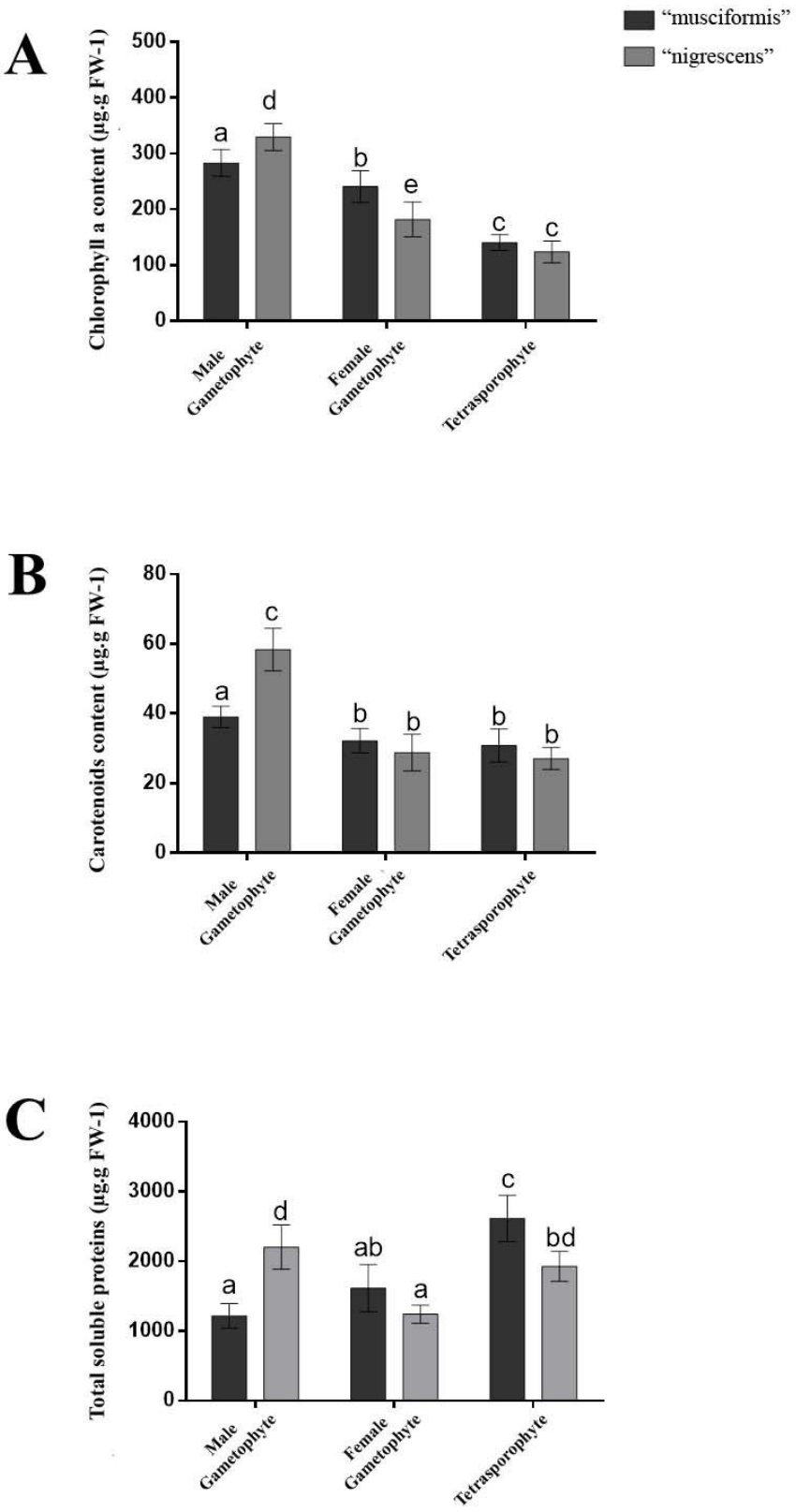

Figure 9. Comparing chlorophyll a, carotenoids and soluble proteins of different life history phases between two morphological variations of *H. pseudomusciformis*, "musciformis" and "nigrescens",. **A.** Chlorophyll *a*. **B.** Carotenoids. **C.** Soluble

Proteins. The bars correspond to the mean ± standard deviation (n = 5). Different letters represent significant differences ($p < 0.05$).

Discussion

LIFE HISTORY

H. pseudomusciformis morphological variations "musciformis" and "nigrescens" present differences in morphology on natural populations to the point they were identified as different species (Nauer et al. 2015). However, morphological and anatomical diferences between "musciformis" and "nigrescens" could not be distinguished when they were kept *in vitro*. The life phases of both variations presented similar morphology and similar life histories, which were completed around 121 days. While on field variation "musciformis" present numerous tendrils and hooks at the apex of the branches and with numorous spinescent branchlets throughout the entire thallus and variation "nigrescens*"* present numerous long branches that resemble bristles of a brush that usually lacks tendrils and hooks and spinescent branchlets are more abundant on the apex, both variations in *in vitro* lacks tendrils and, hooks and spinescent branchlets, were more rare.

Tetrasporophytes of both variations became fertile around 40 days, while the male gametophyte became fertile around 35 days. These results goes in agreement with Schenkman (1986), were "musciformis" and "nigrescens" tetrasporophytes (as *H. musciformis* and *H. nigrescens*) became fertile in the laboratory after 4 weeks, but sometimes could take around 8 weeks for the appearence of the first tetrasporangia sori. The male gamethophytes of "musciformis" and "nigrescens" (as *H. musciformis* and *H. nigrescens*), became fertile within 1 to 3 weeks (Schenkman 1986). The authors sugested these difference in fertility time between tetrasporophytes and male gametophytes could be due to some other factor, besides irradiance, responsible for the induction of fertility in tetrasporophytes. Comparing the results obtained for the two phases, it is observed that gametophytes require less irradiance than the tetraporophytes to form reproduction structures and these are formed more quickly and in a more defined time interval.

The genus *Hypnea* has a Polysiphonia-type life history with isomorphic gametophytic and tetrasporophytic generations (Masuda et al. 1997). The life history of

the genus was completed for the first time *in vitro* in this study. In the laboratory the life history phases could be evaluated under controlled conditions, thereby possibly leading to a better interpretation of their performance. The duration of *Hypnea* life history (121 days) was almost the same found for *Gracilaria verrucosa* (Hudson) Papenfuss (about 120 days) (Ogata et al. 1972), but very short when compared to *Chondracanthus chamissoi* (about 608 days or 20 months) (Avila et al. 2010). It is difficult to compare these results as diferent culture conditions were used.

The absence of male and the rare occurrence of female gametophytes for *H. pseudomusciformis* were reported in several studies (Schenkman 1989; Faccini & Berchez 2000; Reis & Yoneshigue-Valentin 2000; Mouradi et al. 2008; Caires et al. 2013a, as *H. musciformis*, and Jesus, 2016, as *H. nigrescens*), but in the laboratory conditions devolopment of male gametophytes from tetraspores and the spermatangium differentiation was regularly observed.

In natural populations of red algae, however, the domination of tetrasporophytes over gametophytes have been reported, althought the reasons for this fenomena are unknow (Cecere et al. 2000). Several factors could explain this scenario, such as tetraspores (n) having lower viability than carpospores (2n) leading to a prevalence of tetrasporophytes (Rão 1977, Mathieson 1989) or replacement of sexual reproduction by asexual reproduction via vegetative reproduction or apomixis that can occur by apogamy or apomeiosis (Reis & Yoneshigue-Valentin 2000, Kong & Ang Jr. 2004). Reis & Yoneshigue-Valentin (2000, as *H. musciformis*) suggests a prevalence of asexual reproduction over sexual reproduction in populations of *H. pseudomusciformis* in Brazil. The vegetative reproduction may be an important reproductive strategy, which enables its maintenance throughout difficult environmental conditions for growth.

Schenkman (1986) observed a great mortality of female gametophytes of "musciformis" and "nigrescens" (as *H. musciformis* and *H. nigrescens*) when the irradiance was increased. In one experiment, of twenty gametophytes plants of "nigrescens", originated from the germination of tetraspores, in the laboratory, twelve formed spermatangia and eight formed carpogonium. Among the eight female gametophytes obtained, only one remained alive, while, of the male ones, ten survived, being kept in the same light conditions and frequency of culture change as the female gametophytes. From female gametophyte of "musciformis", the clones always died within a week when transferred to higher irradiance, while those maintained in the same

irradiance survived. The same, however, was not observed for male gametophytes and tetrasporophytes and, apparently, both were more resistant to culture (Schenkman 1986). In this scenario, the author suggested that the high frequency of tetrasporophytes in nature is related to a greater adaptation of tetrasporophyte to field conditions, favoring the tetrasporic phase in the process of competition and selection, mainly related to ambient light conditions. However, based on our growtth rates data and pigments content (see below), this could be truth for "musciformis" but not for "nigrescens", were only punctual differences were found comparing tetrasporophytes and gametophytes.

In the case of *Hypnea* species, another problem is the difficulty to recognize male plants (Schenkman 1986; Reis & Yoneshigue-Valentin 2000; Jesus et al. 2013, Nauer et al. 2015) and while descriptions of sexual structures have been made for "nigrescens" (Jesus et al. 2013) for "musciformis" they are rare. However, our results demonstrate the morphology, anatomy and development of the life phases of both "musciformis" and "nigrescens" are mostly identical. Our data corroborates the findings of Nauer et al. (2015) were specimens previously described for Brazil as *H. musciformis* and *H. nigrescens* actually belongs to the same species. Also, the data of this work could provide usefull information on future phenology studies of *H. pseudmusciformis* and its morphological variations.

GROWTH RATES

Overall, our data indicates no difference in GR between "musciformis" and "nigrescens". Taxonomic studies of *Hypnea* from Brazil often reported differences in size between "musciformis" and "nigrescens", with the first reaching thallus size up to 20 cm, while the second usually have a thallus size of 10 cm (Jesus et al. 2013, Nauer et al. 2014, as "*H. musciformis*" and "*H. nigrescens*", Nauer et al. 2015) these data could lead to expect a higher growth rate of "musciformis" when compared to "nigrescens". In controled conditions, however, growth rates of both morphological variations were similar, along with the thallus size (about 5 cm in 4 weeks of cultivation and 15 cm in two months). The greater size of the thallus of "musciformis" on the field could be a reflection of the environment in which it is found, usually in the lower intertidal zone in protected environments as an epiphyte on other macroalgae (Reis & Yoneshigue-Valentin 2000, Nauer et al. 2015), while "nigrescens*"* is usually found growing in mid

intertidal zone in wave-impacted environments, that could afect its growth (Nauer et al. 2015).

When comparing GR between tetrasporophytes and gametophytes, only punctual differences were found. Usually, tetrasporophytes presented higher growth rates than gametophytes in red algae (Destombe et al. 1993, Barufi et al., 2010, Araujo et al. 2013). However, Ursi and Plastino (2001) and Gupta et al. (2011) verified that female gametophytes presented higher growth rates than tetrasporophytes for species of *Gracilaria*. This apparent inconsistency in phase performance, as reported in the literature, can be attributed to experimental cultivation conditions, such as those related to the quality and quantity of light and nutrient concentrations (Araujo et al. 2013). Possibly, in nature, small variations in environmental conditions may favor one or another phase of life history, thereby attributing benefits to the maintenance of the species in a given environment (Destombe et al. 1993). These differences in physiological performance in the isomorphic phases are pointed out as advantageous for the maintenance of this type of life history in nature (Destombe et al. 1993, Hughe and Otto 1999, Araujo et al. 2013).

Our GR data also corroborate the findings of Yokoya et al. (2006). The authors verified that *Hypnea* GR were higher under 20-25°C and became sensitive under high temperatures (30°C). They also verified the GR reamined almost unchanged from 40 to 120 μmol photons m−2 s−1. However, Yokoya et al. (2006) discovered the photoperiod (long, 14:10 h light:dark cycle or short, 10:14 h light:dark cycle) plays a important role in colour variant strains of *H. pseudomusciformis* (as *H. musciformis*), with the green strain being more sensitive to the short photoperiod. Our samples did not present differences in colour, but its well know green strains of both "musciformis" and "nigrescens" can be found on natural populations (Nauer et al. 2014, Nauer et al. 2015).

PIGMENTS AND TOTAL SOLUBLE PROTEINS

Under *in vitro* cultures, phycobiliproteins had the highest concentration for both morphological variations of *H. pseudomusciformis* when compared to chlorophill a and carotenoids, with "musciformis" tetrasporophyte presenting higher concentration of PE and APC over "nigrescens" tetrasporophyte. Apart from using phycobiliproteins as a nitrogen source (García-Sánchez et al. 1993; Talarico and Maranzana 2000), their production is a photoprotective mechanism against increased irradiances (Sinha et al.

1996). Phycobiliproteins, especially PE, located on the periphery of phycobilisomes, are promptly affected by UV radiation (Sinha et al. 1995). These pigments play an important role in the acclimatization mechanism in red algae (Figueroa et al. 1997; Roleda et al. 2004a, b; Barufi et al. 2012). These results could indicate that "musciformis" tetrasporophyte could have a greater hability of acclimatize to different environmental conditions over "nigrescens".

The pigment content of our samples is in acordance with Yokoya et al. (2006) for the brown strain of "musciformis" (as *H. musciformis*), were the phycoerythrin was the phycobiliprotein with highest concentration, followed by phycocyanin and allophycocyanin. However, Yokoya et al. (2006) verified the phycobiliproteins were higher under short photoperiod compared to long photopheriod and the green strain was phycoerythrin defficient on both photoperiods. On other hand, chlorophyll *a* showed no difference between brown and green strains or between long or short photoperiod. For Yokoya et al. (2006) chlorophyll *a* concentrantions were up to 150 µg.g FW-1, these values were similar with our concentrations for "musciformis" and "nigrescens" tetrasporophyte, however, the chlorophyll *a* concentration of our gametophytes were almost double (up to 306,20 µg.g FW-1).

Considering chorophyll *a*, the male gametophyte presented highest concentration for both morphological variations of *H. pseudomusciformis,* while tetrasporophytes presented the lowest concentrations. Between "musciformis" and "nigrescens", we found differences only on gametophyte life phases. Yokoya et al. (2006) pointed that the brown strain of "musciformis" (as *H. musciformis*") exhibits characteristics of shade-adapted plants, whereas the green strain does that of sun-adapted plants, which could be related to the pigment concentration, and the higher phycoerythrin content (Sagert et al. 1997).

Male gametophytes also presented highest concentration of carotenoids and also differ between "musciformis" and "nigrescens" while the others life phases were similar. Carotenoids constitute an important compound in photoprotective mechanisms (Carnicas et al. 1999;CEsteban et al. 2009; Figueroa et al. 2008) and were the pigments found with the lowest concentration. Araujo et al. (2013) reported for *Gracilaria caudata* that tetrasporophytes showed the highest concentrations of chlorophyll *a*, carotenoids, PE, PC, and APC than gametophytes cultivated under controlled conditions. The author suggested the higher pigment content in tetrasporophytes could contribute to higher viability of this diploid phase when compared to the haploid phase.

For “musciformis” the higher phycobiliproteins content compared to gametophytes could favor the tetrasporic phase in the process of competition and selection in the field conditions, as suggested by Schenkman (1980, 1986). Its interesting to note the same scenario could not be applied for “nigrescens”, since the pigment content was similar between tetrasporophytes and gametophytes.

Conclusion

Hypnea pseudomusciformis has isomorphic gametophytes and tetrasporophytes which, when considering the parameters analyzed, responded similarly under the controled culture conditions, with punctual differences. The results of the present study indicate that both “musciformis” and “nigrescens” morphological variations of *H. pseudomusciformis* could be selected for cultivation since growth rates are similar. Furthermore, field experiments are needed to access whether these data obtained under controlled laboratory conditions would remain in the wild. Moreover, these results on the life history, combined with pigment content are important for a cultivation on a commercial scale, to choose wich morphological variation and which life history phase have desirable characteristics for mariculture and carragenaan extraction.

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References

Araújo, F.O., Ursi, S. & Plastino, E.M. 2013. Intraspecific variation in *Gracilaria caudata* (Gracilariales, Rhodophyta): growth, pigment content, and photosynthesis. J. Appl. Phycol. DOI 10.1007/s10811-013-0184-3.

Avila, M., Piel, M.I., Caceres, J.H. & Alveal, K. 2010. Cultivation of the red alga *Chondracanthus chamissoi*: sexual reproduction and seedling production in culture under controlled conditions. J Appl Phycol (2011) 23:529‑536. DOI 10.1007/s10811-010-9628-1.

Barufi, J.B., Oliveira, E.C., Plastino, E.M. & Oliveira, M.C. 2010. Life history, morphological variability and growth rates of the life phases of *Gracilaria tenuistipitata* (Rhodophyta: Gracilariales) in vitro. Scientia Marina 74(2): 297-303.

Barufi, J.B., Mata, M.T., Oliveira, M.C. & Figueroa, F.L. 2012. Nitrate reduces the negative effect of UV radiation on photosynthesis and pigmentation in *Gracilaria tenuistipitata* (Rhodophyta): the photoprotection role of mycosporine-like amino acids. Phycologia 51:636‑648.

Beer, S. & Eshel, A. 1985. Determining phycoerythrin and phycocyanin concentrations in aqueous crude extracts of red algae. Mar. Freshw. Res. 36(6):785-792.

Berchez, F.A.S. & Oliveira-Filho, E.C. 1990. Maricultural essays with the carragenophyte *Hypnea musciformis* in São Paulo, Brazil. In: OLIVEIRA, E.C.F. & KAUTSKY, N. (eds.) *Cultivation of seaweeds in Latin América*. São Paulo: Universidade de São Paulo, p.: 89-94.

Berchez, F.A.S., Pereira, R.T.L. & Kamiya, N.F.1993. Culture of *Hypnea musciformis* (Rhodophyta, Gigartinales) on artificial substrates attached to linear ropes. *Hydrobiologia*, 260/261: 415-420.

Bravin, I.C. & Yoneshigue-Valentin, Y. 2002. The influence of environmental factors on in vitro growth of *Hypnea musciformis* (Wulfen) Lamouroux (Rhodophyta). Ver. Bras. Bot. 25: 469-474.

Caires, T.A., I.O. Costa, M.R.B. Matos, G.M. Lyra and J.M.C. Nunes. 2013a. Phenological studies in populations of Hypnea musciformis (Rhodophyta: Gigartinales) in a tropical region of Brazil. Braz. J. Bot 36: 135-140.

Caires, T.A., I.O. Costa, P.B. Jesus, M.R.B. Matos, G.H. Pereira-Filho and J.M.C. Nunes. 2013b. Evaluation of the stocks of Hypnea musciformis (Rhodophyta: Gigartinales) on two beaches in Bahia, Brazil. Braz. J. Oceanogr. 61: 65-71.

Caires, T.A., M.R.B. Matos, G.M. Lyra, J.G.A. Assis and J.M.C. Nunes. 2013c. Proposal of a fertility index of Hypnea for ecophysiological studies. Rev. Biol. Mar. Oceanogr. 48: 635-639.

Carneiro, M.A.A., Marinho-Soriano, E. & Plastino, E.M. 2011. Phenology of an agarophyte *Gracilaria birdiae* Plastino and E.C. Oliveira (Gracilariales, Rhodophyta) in Northeastern Brazil. Rev. Bras. Farmacogn. 21:317‑322.

Carnicas, E., Jiménez, C. & Niell, F.X. 1999. Effects of changes of irradiance on the pigment composition of *Gracilaria tenuistipitata* var. *liui* Zhang et Xia. J. Photochem. Photobiol. B. Biol. 50:149‑158.

Destombe, C., Godin, J., Nocher, M. Richerd, S. & Valero, M. 1993. Differences in response between haploid and diploid isomorphic phases of *Gracilaria verrucosa* (Rhodophyta: Gigartinales) exposed to artificial environmental conditions. Hydrobiologia 260/261: 131-137.

Esteban, R., Martínez, B., Fernández-Marín, B., Becerril, J.M. & García-Plazaola, J.I. 2009. Carotenoid composition in Rhodophyta: insights into xantophyll regulation in *Corallina elongata*. Eur J Phycol 44:221‑230.

Faccini AL & Berchez F **.**2000. Management of natural beds and standing stock evaluation of *Hypnea musciformis* (Gigartinales, Rhodophyta) in south-eastern Brazil. *Journal of Applied Phycology* 12, p. 101–103.

Fernandes, D.R.P., Caetano, V.S., Tenório, M.M.B., Reinert, F. & Yoneshigue-Valentin, Y. 2012. Characterization of the photosynthetic conditions and pigment profiles of the colour strains of *Hypnea musciformis* from field collected and *in vitro* cultured samples. Brazilian Journal of Pharmacognosy 22(4): 753-759.

Figueroa, F.L.; Salles, S.; Aguilera, J.; Jiménez, C.; Mercado, J.; Viñegla, B.; Flores-Moya, A. & Altamirano, M. 1997. Effects of solar radiation on photoinhibition and pigmentation in the red alga *Porphyra leucosticta*. Mar. Ecol. Prog. Ser. 151: 81-90.

Figueroa, F.L., Bueno, A., Korbee, N., Santos, R., Mata, L. & Schuenhoff, A. 2008. Accumulation of mycosporine-like amino acids in *Asparagopsis armata* grown in tanks with fishpond effluents of gilthead sea bream Asparus aurata. J. World. Aquacult. Soc 39: 692‑699.

García-Sánchez, M.J., Fernández, J.A. & Niell, F.X. 1993. Biochemical and physiological responses of *Gracilaria tenuistipitata* under two different nitrogen treatments. Physiol. Plantarum 88:631‑637.

Gupta, V., Baghel, R.S., Kumar, M., Kumari, P., Mantri, V.A., Reddy, C.R.K. & Jha, B. 2011. Growth and agarose characteristics of isomorphic gametophyte (male and female) and sporophyte of *Gracilaria dura* and their marker assisted selection. Aquaculture 318:389‑396.

Guimarães, M.; Plastino, E.M. & Destombe, C. 2003. Green mutant frequency in natural populations of *Gracilaria domingensis* (Gracilariales, Rhodophyta) from Brazil. Europ. J. Phycol. 38(2): 165-169.

Hughe, J.S. & Otto, S.P. 1999. Ecology and the evolution of biphasic life cycles. Am. Natural. 154: 306-320.

Jesus, P.B., A.S. Schnadelbach and J.M.C. Nunes. 2013a. O gênero Hypnea (Cystocloniaceae, Rhodophyta) no litoral do estado da Bahia, Brasil. Sitientibus Sér. Ci. Biol. 13: 1-21.

Jesus, P.B. 2016. Estudos biossistemáticos em espécies do gênero Hypnea J.V. Lamouroux (Gigartinales, Rhodophyta). Tese de doutorado. Feira de Santana. 221 p.

Kong, C.S.L. and P.O. Ang Jr. 2004, Seasonal occurrence and reproduction of Hypnea charoides (Rhodophyta) in Tung Ping Chau, N.T., Hong Kong SAR, China. Hydrobiologia 512: 63–78.

Kursar, T.A., van der Meer, J.P. & Alberte, R.S. 1983a. Lightharvesting system of the red alga *Gracilaria tikvahiae*. I. Biochemical analysis of pigment mutations. Plant. Physiol. 73:353‑360.

Kursar, T.A., van der Meer, J.P. & Alberte, R.S. 1983b. Lightharvesting system of the red alga *Gracilaria tikvahiae*. II. Phycobilisome characteristics of pigment mutants. Plant. Physiol. 73:361‑369.

Lamouroux, J.V. 1813. Essai sur les genres de la famille des Thalassiophytes non articulées. *Annales du Muséum d'Histoire Naturelle, Paris* 20: 21-47, 115-139, 267-293, pls 7-13.

Lignell, A. & Pedersén, M. 1989. Agar composition as a function of morphology and growth rate. Studies on some morphological strains of *Gracilaria secundata* and *Gracilaria verrucosa* (Rhodophyta). Bot. Mar. 32: 219 – 227.

Lichtenthaler, H.K. & Buschmann, C. 2001. Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy. Current Protocols in Food Analytical Chemistry F4.3.1-F4.3.8. DOI: 10.1002/0471142913.faf0403s01

Martins, A.P., Chow, F. & Yokoya, N.S. 2009. Ensaio in vitro da enzima nitrato redutase e efeito da disponibilidade de nitrato e fosfato em variantes pigmentares de *Hypnea musciformis* (Wulfen) J. V. Lamour. (Gigartinales, Rhodophyta). Revista Brasil. Bot. 32(4): 635-645.

Masuda, M., Yamagishi, Y., Chiang, Y.M., Lewmanomont, K. & Xia, B. 1997. Overview of *Hypnea* (Rhodophyta, Hypneaceae). In Abbott, I. A. (Ed.) Taxonomy of Economic Seaweeds, Vol. 6. *California Sea Grant College System*, La Jolla, pp. 127–33.

Mathieson, A. C. 1989. Phenological patterns of New England seaweeds. Bot. Mar. 32: 419-438.

Nauer, F., Cassano, V. & Oliveira, M. C. 2015. Description of *Hypnea pseudomusciformis* sp. nov., a new species based on molecular and morphological analyses, in the context of the H. musciformis complex (Gigartinales, Rhodophyta). Journal of Applied Phycology 27: 2405-2417. DOI: 10.1007/s10811-014-0488-y

Ogata, E., Matsui, T. & Nakamura, H. 1972. The life cycle of *Gracilaria verrucosa* (Rhodophyceae, Gigartinales) *in vitro*. Phycologia, 11: 75-85.

Oliveira, E.C. 1998. The seaweed resources of Brazil. In: Critchley AT, Ohno M (eds) Seaweed resources of the world. Japan International Cooperation Agency, Yokosuka, pp 366‑371.

Rao, R. K. 1970. Studies on growth cycle and phycocolloid content in *Hypnea musciformis* (Wulf.) Lamour. Botanica Marina 13: 163-165.

Reis, R.P. & Yoneshigue-Valentin, Y. 1998. Variação espaço-temporal de populações de *Hypnea musciformis* (Rhodophyta, Gigartinales) na Baía de Sepetiba e Armação dos Búzios, Rio de Janeiro, Brasil. Acta Bot Bra 12:465-483.

Reis, R.P. & Yoneshigue-Valentin, Y. 2000. Phenology of *Hypnea musciformis* (Wulfen) Lamouroux (Rhodophyta, Gigartinales) in three populations from Rio de Janeiro State, Brazil. Bot Mar 43: 299-304.

Reis, R.P.,Leal, M.C.R., Yoneshigue-Valentin, Y. & Belluco, F. 2003 Efeito de fatores bióticos no crescimento de *Hypnea musciformis* (Rhodophyta - Gigartinales). Acta Bot Bras 17:279-286.

Reis, R.P., Barros-Barreto, M.B., Caldeira, A.Q. & Miranda, A.P.S. 2005 Cultivo de algas vermelhas de interesse comercial na Ilha da Marambaia. In: Sociedade Brasileira de Ficologia (org) Formação de Ficólogos: um compromisso com a sustentabilidade dos recursos aquáticos: Anais da X Reunião Brasileira de Ficologia, Salvador. Rio de Janeiro: Museu Nacional, Série Livros; 10, pp 289-300.

Reis, R.P., Caldeira, A.Q., Miranda, A.P.S. & Barros-Barreto, M.B. 2006. Potencial para maricultura da carragenófita *Hypnea musciformis* (Wulfen) J.V. Lamour. (Giagartinales - Rhodophyta) na Ilha da Marambaia, Rio de Janeiro, Brasil. Acta Bot Bras 20:763-769.

Roleda, M.Y.; Halnet, D.; Kräbs, G. & Wiencke, C. 2004a. Morphology, growth, photosynthesis and pigments in Laminaria ochroleuca (Laminariales, Phaeophyta) under ultraviolet radiation. Phycol. 43(5): 603-613.

Roleda, M.Y.; van der Poll, W.H.; Halnet, D. & Wiencke, C. 2004b. PAR and UVBR effects on photosynthesis, viability, growth and DNA in different life stages of two coexisting Gigartinales: implications for recruitment and zonation pattern. Mar. Ecol. Prog. Ser. 281: 37-50.

Schenkman, R.P.F .1986. Cultura de Hypnea (Rhodophyta) *in vitro* como subsídio para estudos morfológicos, reprodutivos e taxonômicos. *Tese de Doutorado*. Instituto de Biociências, Universidade de São Paulo. 81 pp.

Sinhá, R.P.; Singh, N.; Kumar, A.; Kumar, H.D.; Hader, M. & Hader, D.P. 1996. Effects of UV irradiation on certain physiological and biochemical processes in cyanobacteria. J. Photochem. Photobiol. B: Biol. 32(1-2): 107-113.

Talarico, L. & Maranzana, G. 2000. Light and adaptative responses in red macroalgae: an overview. J. Photochem. Photobiol. B: Biol. 56: 1-11.

Ursi, S. & Plastino, E.M. 2001. Crescimento in vitro de linhagens de coloração vermelha e verde clara de *Gracilaria* sp. (Gracilariales, Rhodophyta) em dois meios de cultura: análise de diferentes estádios reprodutivos. Rev. Bras. Bot. 4: 585-592.

Wallner, M., Lobo, S., Boccanera, N. & Silva, E.M. 1992. Biomass, carrageenan yield and reproductive state of *Hypnea musciformis* (Rhodophyta: Gigartinales) under natural and experimental cultivated conditions. *Aquaculture Fisheries Manage*, 23: 443-451.

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

Yokoya, N.S., Plastino, E.M. & Artel, R. 2003. Physiological responses and pigment characterization of two colour strains of the carrageenophyte Hypnea musciformis (Rhodophyta). *In*: A.R.O.

Yokoya, N.S., Necchi, O., Martins, A.P., Gonzalez, S.F. & Plastino, E.M. 2007. Growth responses and photosynthetic characteristics of wild and phycoerythrin-deficient strains of *Hypnea musciformis* (Rhodophyta). J. Appl. Phycol. 19: 197-205.

CAPÍTULO Cinco

Physiological plasticity in the morphological plastic red seaweed Hypnea pseudomusciformis (Gigartinales, Rhodophyta).

Capítulo 5

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Physiological plasticity in the morphologically plastic red seaweed *Hypnea pseudomusciformis* (Gigartinales, Rhodophyta).

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Abstract

Hypnea pseudomusciformis, a morphologically plastic species used as carrageenan source in Brazil, was recently described based on molecular markers. Two species previously described for the Brazilian coast (*H. musciformis* and *H. nigrescens*) are currently considered morphological variations of *H. pseudomusciformis* that are found in different intertidal regions. The hypothesis of this work is that these two morphological variations of *H. pseudomusciformis* have different physiological responses due to environmental pressures and/or genetic differences. To test this hypothesis, the content of photosynthetic pigments (chlorophyll *a*, carotenoids and phycobiliproteins), total soluble proteins and antioxidant capacity (DPPH, ABTS, Folin-Ciocalteu reductive capacity, FRAP and iron chelating capacity) were analyzed for samples of the two morphological variations collected directly from the field or grown *in vitro*. Physiological differences between the two morphological variants from field samples were observed for all the antioxidant assays, with the exception of DPPH, for phycoerithrin pigments and for total soluble protein, while between *in vitro* samples, differences were found in relation to phycocyanin, phycoerythrin and total soluble proteins. Overall, the two morphological variants have a similar physiological behavior

when grown under the same culture conditions, corroborating its classification under one species. These results indicate that the physiological differences found between these variants are mostly due to environmental pressures and represent an example of phenotypic plasticity, which allows occurrence in different environments and may lead to different morphologies.

Keywords: acclimation, *Hypnea*, in vitro culture, physiological plasticity, seaweed

Introduction

The genus *Hypnea* J.V. Lamouroux (1813) has been widely studied because of its worldwide distribution in marine tropical regions (Geraldino et al. 2009) and its economic importance as a source of carrageenan (Reis & Yoneshigue-Valentin 2000, Reis et al. 2003, Reis et al. 2006). Despite its importance, discrimination of *Hypnea* species is difficult due to a simple and plastic morphology, often influenced by environmental conditions (Schenkman 1986; Reis et al. 2003). Some taxa in the genus are well known to form species complexes, such as the *H. charoides-valentiae* complex (Price et al. 1992, Abbott 1999, Dawes & Mathieson 2008). Nauer et al. (2015) discovered using molecular markers that Brazilian specimens of *H. musciformis* (Wulfen) J.V. Lamouroux differed from specimens collected on the type locality in Italy. For this reason, these Brazilian specimens were described as a distinct species, named *Hypnea pseudomusciformis* Nauer, Cassano & M.C. Oliveira.

Nauer et al. (2015) also demonstrated that three species cited in the past for the Brazilian coast (*H. musciformis*, *H. nigrescens* Greville ex J. Agardh and *H. valentiae* (Turner) Montagne), showed no molecular divergence for the markers COI-5P and *rbc*L. These authors concluded that those species do not occur in Brazil and their specific morphologies were considered variations of *H. pseudomusciformis*. In this sense, the morphological variation of *H. pseudomusciformis* before described as *H. musciformis* is characterized by its epiphytic habit, using numerous tendrils present at the apex of the branches to curl up on other macroalgae, been found in upper subtidal zone in protected environments. The morphological variation previously described as *H. nigrescens* is characterized by its epilithic habit, growing in mid intertidal zone in wave-

impacted environments. Its morphology is characterized by having numerous long branches that resemble bristles of a brush and a red-blackish color.

Since the morphological variations of *H. pseudomusciformis* occurs in different environments as described above, they are subjected to different abiotic and biotic conditions, such as solar radiation, desiccation, herbivory pressure among others. It is well know that rocky shores are systems of high environmental complexity and the incidence of light, in quantity and quality, is a key factor affecting the marine environment (Houvinen & Gómez 2011). Macroalgae have several mechanisms of photoprotection, such as energy dissipation through specific accessory pigments, including the xanthophyll cycle for some species, or even dynamic photoinhibition by reversible changes in photosynthetic capacity and efficiency, accumulation of UV-absorbing compounds and increase of antioxidant activity (Gómez et al. 2009, Cox et al. 2010, Goss & Jakob 2010).

The evaluation of the photosynthetic pigment (chlorophyll, carotenoids and phycobiliproteins) as physiological responses to stress conditions have been studied in red algae (Levy & Gantt 1988, Cunnigham et al. 1989, Bernanrd et al. 1996, Tala & Chow 2014, Faria & Plastino 2015, Schmidt et al. 2015, Torres et al. 2015, Wu et al. 2015, Schmidt *et al*. 2016). Other common physiological response to stress conditions is the increase in antioxidant molecules (Sies 1993).

In this scenario, the hypothesis of this work is that the two morphological variants of *H. pseudomusciformis* ("musciformis" and "nigrescens") present distinct physiological responses. To test this hypothesis, we quantified the concentrations of photosynthetic pigments (chlorophyll *a*, carotenoids and phycobiliproteins) and evaluated the antioxidant capacity of these two morphological variants, comparing samples collected directly from the field and samples that were cultured *in vitro*.

Materials and methods

COLLECTION AND SAMPLING

Tetrasporophytes of the morphological variant "musciformis" were collected on Cigarras Beach (23°43'41.38"S / 45°23'53.84"O), São Sebastião City, in the upper-subtidal in a protected rocky shore (Fig . 1A-B). Tetrasporophytes of the morphological

variant “nigrescens” were collected on Cibratel Beach (24°12’05.68”S / 46°48’38.72”O), Itanhaém City, 120 km South of Cigarras Beach, in the mid-intertidal in an exposed rocky shore (Fig. 1C-D), with high hydrodynamism. Both cities are in southeastern Brazil, in São Paulo State.

Figure 1. Collection sites of *Hypnea pseudomusciformis*. **A.** Cigarras beach, São Sebastião city, protected rocky shore. **B**. morphological variation “musciformis”. **C**. Cibratel beach, Itanhaém city, exposed rocky shore. **D**. morphological variation “nigrescens”.

For each morphological variant, about 3 kg of fresh weight (FW) of algal material was collected at low tide, conditioned in plastic bags and transported in thermic boxes to the laboratory. In the laboratory, the algae were cleaned of macroepiphytes and other associated organisms and washed with filtered seawater to remove sand and salt residues. Five apical branches (1 cm) from different individuals of each morphological variation (Fig. 2) were conditioned separately in silica gel for molecular analysis by

DNA barcoding technique. From the same individuals used above, others three apical branches (1 cm) were conditioned separately in sterile seawater for *in vitro* culture under controlled conditions (see below) and then, after acquiring enough biomass, samples were frozen in liquid nitrogen (apical portions totalizing samples of 150 and 300 mg FM; 5 individuals for each variant) and stored at -80°C for further analysis of photosynthetic pigments, total soluble proteins and antioxidant potential (see bellow). The remaining portions of the same individuals from each variant were used for confection of exsiccates and deposited at the SPF Herbarium of the University of São Paulo.

For the *in vitro* culture under controlled conditions apical branches (length 1 cm, total fresh mass ca. 1 mg) of unfertile tetrasporophytes (n = 5) were cultivated for 10 weeks in 500 mL of sterilized seawater (32 psu) enriched with 50% von Stosch medium (Ursi & Plastino 2001), 14 h light: 10 h dark, 24 ± 1°C and 70 ± 5 µmol photons $m^{-2}s^{-1}$ (Osram 40W day light fluorescent tubes). The cultures were bubbled with compressed air for 30 min h^{-1}, and the medium was renewed weekly.

Figure 2. Samples of *Hypnea pseudomusciformis*. **1 to 5.** Tetrasporophytes of morphological variation "nigrescens". **6 to 10**. Tetrasporophytes of morphological variation "musciformis". Scale bar = 1 cm.

MOLECULAR MARKERS AND SEQUENCING

Samples stored in silica-gel were ground in liquid nitrogen and total DNA was extracted using the NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany), following the manufacturers protocol. Amplification for COI-5P DNA barcode was

performed using a PCR Master Mix (Promega Corp., Madison, WI, USA) using the primers GazF1 and GazR1 and cycles described by Saunders (2005). The PCR products were purified using the column GTX™ PCR DNA and Gel Band Purification Kit (GE Healthcare, Buckinghamshire, UK), quantified using NanoDrop 2000 (Wilmington, DE, USA), and directly sequenced with the same primers used in PCR amplification in a 3730 DNA Sequencer (Applied Biosystems, Carlsbad, CA, USA). The sequences obtained were aligned in the BioEdit 7.0 sequence editor (Hall, 1999). Neighbor-Joining (NJ) analysis was performed under heuristic search with 2,000 bootstrap replicates (Felsenstein 1985), using PAUP 4.0b10 (Swofford 2002).

ANTIOXIDANT POTENTIAL

Antioxidant potential was evaluated from of frozen samples from the field and from the culture. The samples (300 mg each) were ground in liquid nitrogen, extracted in 1 mL of methanol by maceration for 3 h at room temperature and protected from light, and centrifuged (Hettich Mikro 22R, UK) at 14,000 rpm for 15 min at 25°C. The antioxidant capacity of the supernatant was analyzed at final concentration of 40 $mg.mL^{-1}$ for each antioxidant assay.

Five different antioxidant assays were tested: a) ABTS radical scavenger activity (Torres *et al*. (2017) modified from Rufino *et al*. (2007) and Yang *et al*. (2011)), b) DPPH radical scavenger capacity (Pires et al. (2017a) modified from Brand-Williams et al. (1995) and Rufino et al. (2007)), c) total phenolic compounds by the Folin-Ciocalteu reagent, or also reducing power (Pires et al. (2017b) modified from Singleton & Rossi (1965) and Mole & Waterman (1994)), d) FRAP assay (Ferric Reducing Antioxidant Power, Urrea-Victoria et al. (2016) modified from Brito et al. (2006)), and e) metal chelation activity (Harb et al. (2016) modified from Min et al. (2011)). The antioxidant assays were conducted in 96-well microplates with a final volume of 300 µL. The absorbances were measured with an UV-vis spectrophotometer (Epoch 2-BioTek, USA) and the results expressed as percentage of antioxidant activity and gallic acid equivalent (GAE; mg GAE.g FW^{-1}).

In general, the analytical tests for the determination of the antioxidant potential are based on two mechanisms of reaction that are the transference of a Hydrogen Atom Transfer (HAT) and the transfer of an Single Electron Transfer (SET). Methods based

on the HAT mechanism investigate the ability of antioxidants to donate hydrogen atoms to block the action of peroxyl radicals (ROO •). The methods based on the SET mechanism detect the ability of the sample to reduce the oxidant, which does not need to be strictly a free radical, unlike the HAT mechanism tests (Castelo-Branco & Torres, 2011).

The DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical sequestration assay is one of the most widely used for investigating the antioxidant potential. This assay is based primarily on the SET mechanism and, marginally, on the HAT mechanism. The substance with antioxidant potential acts as a donor of a hydrogen atom when it is added to the DPPH solution. This mixture causes the reduction of the DPPH radical in hydrazine, which promotes the simultaneous change in coloration, from violet to light yellow.

The sequestration method of the ABTS (2,2'-azino-bis- (3-ethyl-benzothiazoline) -6-sulphonic radical) is based on the SET and HAT mechanisms. The ABTS (ABTS +) radical sequestration assay estimates the antioxidant's ability to capture the ABTS chromophore radical, which has its absorbance reduced as it reacts with antioxidants. In the test, ABTS is chemically oxidized by the addition of potassium leading to the formation of the ABTS + reactive species, which has intense green coloration (λmax = 734 nm); the antioxidant activity is measured from the loss of this green coloration.

The antioxidant power reduction iron test (FRAP) is based on the SET mechanism and quantifies the ability of the antioxidant to reduce the ferric iron tripyridyltriazine (Fe + 3-TPTZ) complex to the ferrous tripyridyltriazine iron complex (Fe + 2- TPTZ) at pH 3.6. In the FRAP assay, the Fe + 2-TPTZ complex exhibits blue staining and the absorbance of the solution is measured at the wavelength of 595 nm, allowing monitoring of the reducing ability of the sample. This assay has been effective in quantifying the antioxidant potential mainly of samples containing phenolic substances and having a more acidic behavior (Gorinstein et al., 2003; Szydowska-Czerniak et al., 2008).

The ability to chelate the iron ion is also widely used to examine the antioxidant potential. This assay evaluates how much the algal extracts block generation of the OH radical by the bond with the transition metal Fe. This method also mainly follows the mechanism of SET.

The Folin Ciocalteu reagent consists of the mixture of phosphomolybdic and phosphotungstic acids, in which molybdenum and tungsten are in the oxidation state +6, but in the presence of reducing agents such as phenolic compounds, so-called blue molybdenum and tungsten, the color of which allows the determination of the reducing substances under basic pH conditions, which is based on the study of the phenolics (Hou et al., 2003, Ikawa et al., 2003, Bonoli et al., 2004).

PHOTOSYNTHETIC PIGMENTS AND TOTAL SOLUBLE PROTEINS

Photosynthetic pigments and total soluble proteins (TSP) were analyzed from 70 mg FW from both field and culture samples following Harb et al. (2018). The material was ground in liquid nitrogen, extracted in 1 mL of cold 50 mM sodium phosphate buffer (pH 5.5) for 1 h at 4°C and protected from light, and centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant was used for determining phycobiliproteins and TSP while the pellet was re-extracted by adding 1.5 mL of methanol for 3 h at 4°C and protected from light, and centrifuged again. The supernatant was used for determining chlorophyll *a* and total carotenoids. The absorbances (from 400 nm to 750 nm) were measured with an UV-vis spectrophotometer from aliquots of 400 µL and the levels of phycobiliproteins, chlorophyll *a* and carotenoids (µg.g FW^{-1}) were calculated as described in Harb et al. (2018). Total soluble proteins (µg.g.FW^{-1}) was measured by Bradford (1976) using a colorimetric protein assay solution (Bio Rad, USA) as reported in Harb et al. (2018) and the bovine serum albumin (BSA, Sigma, Brazil) was used as standard.

DATA ANALYSIS

The data were tested for normality (Kolmogorov-Smirnov test) and homoscedasticity (Bartlett's test) and subsequent one-way or two-way ANOVA. To determine significant differences the *post-hoc* Newman-Keuls test ($p < 0.05$) was used. Before the analysis of variance the data in percentage were transformed into the square root arcsine to normalize the data. Principal components analysis (PCA) was also performed to evaluate the correlation between the main driver parameters.

Results

MOLECULAR DATA

Based on 5P-COI DNA barcode (Figure 3), sequences of *H. pseudomusciformis* field samples for both “musciformis” and "nigrescens" variants formed a monophyletic cluster with high bootstrap support. The sequence of the type locality of *H. musciformis* from Italy was used as outgroup. The divergence between Brazilian sequences and the sequence from the type locality of *H. musciformis* was 5.8 to 6.2%. Intraspecific variation for marker 5P-COI was 0.2 to 0.6% and this variation did not correlate with morphological variations or collection sites.

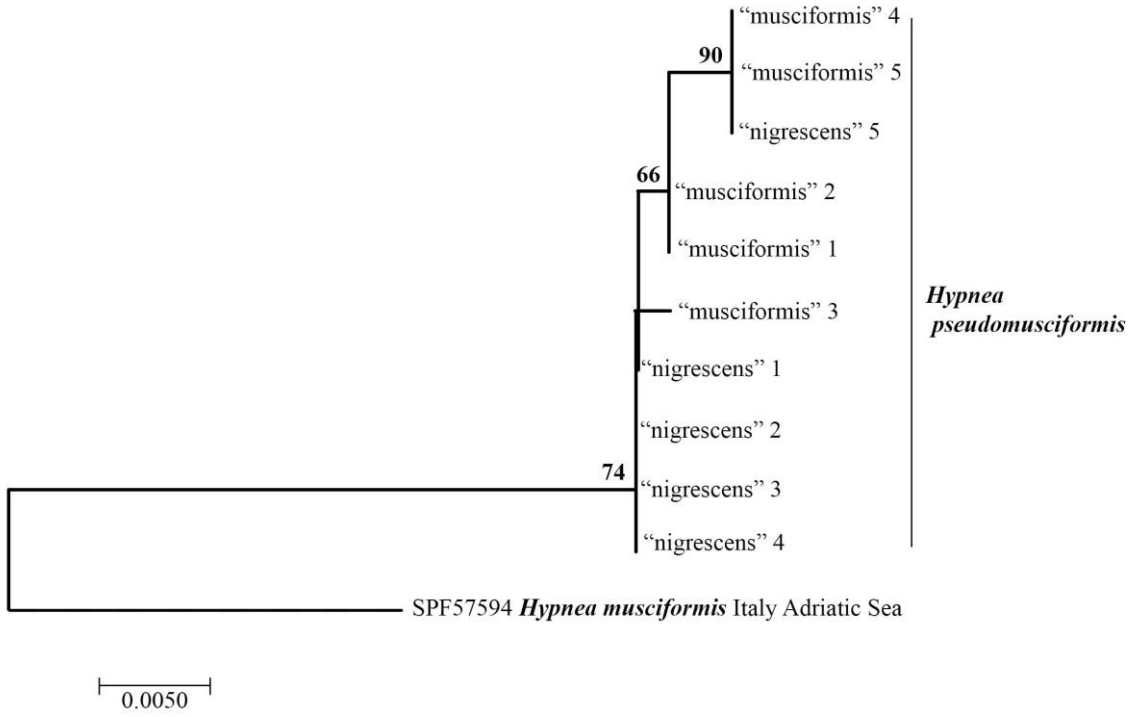

Figure 3. Neighbor-Joining (NJ) analysis for COI-5P sequences for *H. pseudomusciformis* using *H. musciformis* (SPF57594) as outgroup. Boostrap numbers (when above 70) are shown in the branches.

ANTIOXIDANT POTENTIAL

Considering the comparison between the morphological variants "musciformis" and "nigrescens", differences were observed for all the analyzed antioxidant activities from field samples (Fig. 4A, C-E), with the exception of DPPH assay (Fig. 4B). On the

other hand, samples kept under *in vitro* culture conditions for 10 weeks showed no significant differences between the morphological variants. Considering the comparison between field and *in vitro* culture samples, no clear tendencies were observed for the antioxidant assays, except for DPPH activity, which showed higher scavenger power for *in vitro* samples and for metal chelation activity that showed higher values for field samples for both variants.

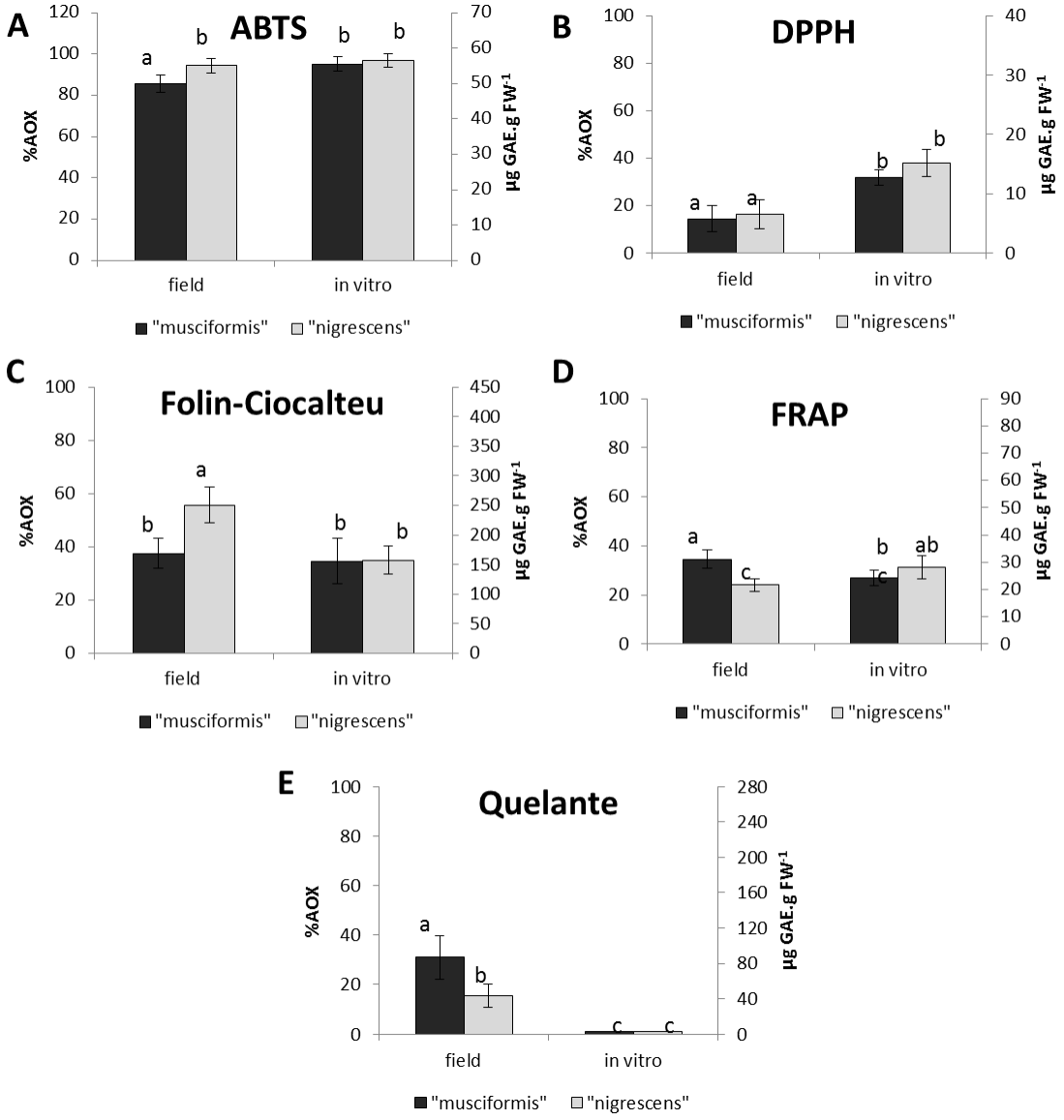

Figure 4. Antioxidant activity of crude extracts of two variants "musciformis" and "nigrescens" from *H. pseudomusciformis*. **A.** ABTS radical scavenger assay. **B.** DPPH radical scavenger assay. **C.** Reducing power of the Folin-Ciocalteu reagent. **D.** FRAP

assay. **E.** Metal chelation activity. The bars correspond to the mean ± standard deviation (n = 5). Different letters represent significant differences for $p < 0,05$.

PHOTOSYNTHETIC PIGMENTS AND TOTAL SOLUBLE PROTEINS

Results for pigment content and total soluble proteins of both morphological variants from field and *in vitro*-cultured samples are presented in Figure 5.

For phycobiliproteins (Fig. 5A) significant differences between the two morphological variants were observed for phycocyanin (PC) for the both field and *in vitro* cultures. For phycoerythrin (PE) significant differences between the two morphological variants were observed only for *in vitro* samples. The content of chlorophyll *a* (Fig. 5B) and total carotenoids (Fig. 5C) showed no significant differences between the two morphological variants within the same type of sample, field or *in vitro*. However, chlorophyll *a* and carotenoids were more than two-fold higher for *in vitro* samples than for in field samples.

Based on the data of pigments, "nigrescens" showed no significant differences in phycobiliproteins content between field samples and *in vitro* samples, while "musciformis" presented a significant increase for *in vitro* culture samples for all three phycobiliproteins pigments.

The amount of total soluble proteins presented significant difference between the two morphological variants for both in field and *in vitro* samples (Fig. 5D). The field sample "musciformis" presented higher amount of soluble proteins than field "nigrescens", but the opposite was observed for *in vitro*-cultured samples.

A Principal Component Analysis (PCA) was performed to identify the main drivers which explain possible correlations (Fig. 6). The Component 1 (PC 1) explains 63.4% of the data set variation and the Component 2 (PC 2) 23.0%. The variables DPPH, Chlorophyll *a*, Carotenoids, Phycoerythrin, Phycocyanin, Allophycocyanin, Proteins and ABTS present a positive correlation with PC 1. Based on this component, two groups were observed, one group formed from the field samples that have a negative correlation with PC 1 and a second group formed by the *in vitro* samples with a positive correlation with PC 1. Additionally, the variables FRAP, DPPH, Chlorophyll *a* and Carotenoids present a positive correlation with PC 2. The samples "musciformis" from field, "musciformis" *in vitro*-cultures and "nigrescens" *in vitro*-cultured presented a positive correlation with these components as well. In summary, this PCA analysis

show that for PC 1 field and *in vitro* culture samples have different behaviors, independent of the morphological variant, while for PC 2 field samples of "nigrescens" are different in relation to the others. All antioxidant potential and pigments content data comparing "musciformis" with "nigrescens" are summarized in Table 1.

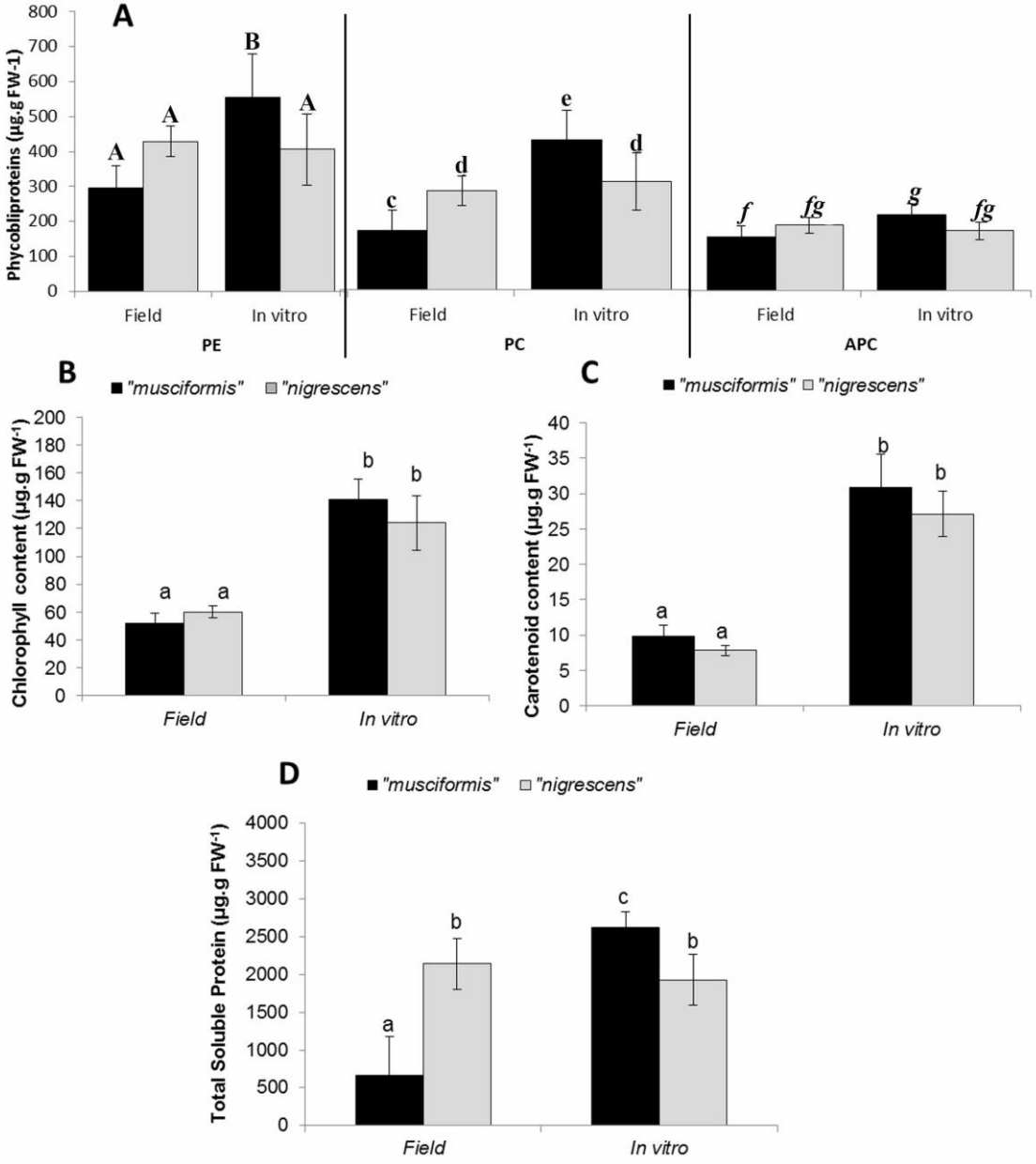

Figure 5. Quantification of pigments content of two morphological variants of *H. pseudomusciformis*, "musciformis" and "nigrescens". **A.** Phycobiliprotein (PE,

phycoerythrin, PC, phycocyanin and APC, allophycocyanin). **B.** Chlorophyll a. **C.** Carotenoids. **D.** Total soluble proteins. The bars correspond to the mean ± standard deviation (n = 5). Different letters represent significant differences ($p < 0.05$).

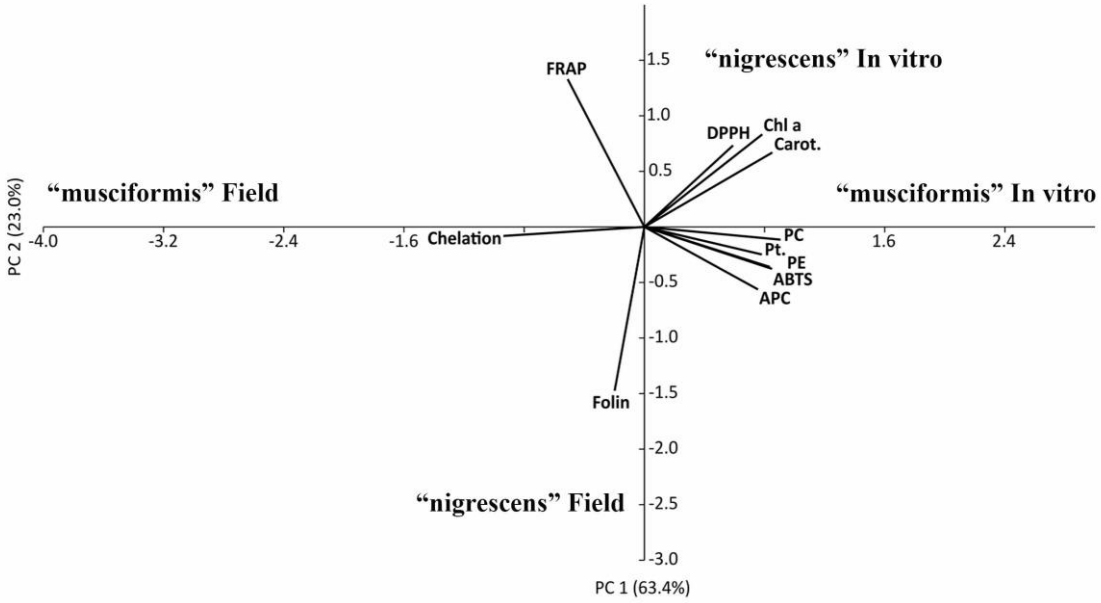

Figure 6. Principal Component Analysis for the analyzed parameters measured for *H. pseudomusciformis*.

Table 1. Distinct physiological responses for field and *in vitro* samples for the two morphological variants of *H. pseudomusciformis,* "musciformis" and "nigrescens". NS – No significant differences.

	FIELD SAMPLES		***IN VITRO* SAMPLES**	
	"*musciformis*"	"*nigrescens*"	"*musciformis*"	"*nigrescens*"
ABTS	lower	higher	NS	NS
DPPH	NS	NS	NS	NS
Folin-Ciocalteu	lower	higher	NS	NS
FRAP	higher	lower	lower	higher
Metal Chelation	higher	lower	NS	NS
Phycoerythrin	NS	NS	higher	NS
Phycocyanin	lower	higher	higher	NS
Allophycocyanin	NS	NS	NS	NS
Chlorophyl *a*	NS	NS	NS	NS
Carotenoids	NS	NS	NS	NS
Soluble Proteins	lower	higher	higher	NS

Discussion

MOLECULAR DATA AND MORPHOLOGY

Sequences of *Hypnea pseudomusciformis* for both "musciformis" and "nigrescens" variants formed a monophyletic cluster with high bootstrap support. These results corroborates the findings of Nauer et al. (2015), where, based on molecular markers, showed that what was previously described for Brazil as *H. musciformis* and *H. nigrescens* belonged to *H. pseudomusciformis*, a species with plastic morphology. Phenotypic plasticity consists of the ability of organisms to change their phenotype

according to environmental conditions. The term phenotypic plasticity covers all types of changes induced by the environment, including morphological, physiological, and phenological changes (Plastino & Guimarães 2001, Plastino et al. 2004).

ANTIOXIDANT POTENTIAL

Hypnea pseudomusciformis variants "musciformis" and "nigrescens" display a great morphological plasticity, and are also found in different sectors of the shoreline, therefore this species is an interesting model to study the drivers for this plasticity and if it extends to more than the morphology. Considering the differences observed for most of the analyzed antioxidant activities (with the exception of DPPH assay) from field samples between the morphological variants, and that under *in vitro* culture conditions, those differences were not observed between the variants, we can presume the environment is playing an important role.

Macroalgae occurring in the rocky shores, mainly in the mid-coast, are frequently subject to abrupt changes in solar and UV radiation, drying due to exposure to the atmosphere and variations in temperature and salinity, which may change in the short (daily), medium (seasonally) or long term (annually). In order to avoid the damage caused by oxidative stress, macroalgae need mechanisms, among them antioxidants, to combat these adversities efficiently (Matsukawa et al. 1997, Rocha et al. 2007). Aguilera et al. (2002) evaluated the activity of antioxidant enzymes of algae that occupied different portions in the zonation of rock shores. Algae from the upper intertidal, exposed to a higher incidence of light and exposure to air, had higher activity of antioxidant enzymes compared to lower intertidal species, indicating more efficient biochemical protection in algae exposed to higher stress conditions *in situ*. Similar results were described by Choo et al. (2004), in which the authors verified that the increase in the production of antioxidants in two green macroalgae was positively correlated with the increase in luminous intensity.

Morphological variation "musciformis", which grows mostly in lower intertidal zone, presented higher antioxidant potential than "nigrescens" for FRAP and Metal Chelation assays. On other hand, "nigrescens", which is found in the middle intertidal zones and under the impact of waves, presented higher antioxidant potential for ABTS and Folin-Ciocalteu (Table 1), assays with the highest antioxidant potentials.

Antioxidant methods measure the potential of the extracts tested, but each assay has a particular chemical principle and affinity with the components of each extract. Thus, to evaluate the antioxidant potential using several assays allows more complete understanding of the antioxidative responses of the studied species (Matanjun et al., 2008).

Our data were similar to those found by Harb (2016) for the red algae *Palisada capillacea* Santelices & Hommersand, were ABTS, FRAP and Folin-Ciocalteu presented the highest antioxidant activity. Kiran & Murugesan (2014) tested antioxidant potential of the brown algae *Colpomenia sinuosa* (Mertens ex Roth) Derbés & Solier and of the red algae *Halymenia poryphyroids* C. Agardh, and the authors verified higher activity for DPPH and ABTS assays. An explanation for the higher antioxidant potential of these extracts may be related to structural characteristics of macroalgal polysaccharides such as sugar type, glycosidic branching and degree and position of sulphations, and these characteristics may vary between algal groups and between species. In the work proposed by Jiménez-Escrig et al. (Roth) with *Gigartina pistillata* (SGGmelin) Stackhouse, *Mastocarpus stellatus* (Stackhouse) Guiry, *Nemalion helminthoides* (Velley) Batters and *Osmundea pinnatifida* (Hudson) Stackhouse, observed a strong correlation between the degree of sulphation of red algae polysaccharides and antioxidant activity.

According to Castelo Branco & Torres (2011) and Floegel et al. (2011), the ABTS radical sequestration it is most common assay used for red algae because is compatible with hydrophilic and lipophilic substances. However, the assay has some limitations, such as the ability of a sample to react with ABTS radical, instead of inhibiting the oxidative process, or the slow reaction of many phenolic compounds. Therefore, it is necessary to use other complementary antioxidant tests to evaluate the actual antioxidant response of an extract (Roginsky & Lissi 2005).

The Folin-Ciocalteu method quantifies substances with reducing power under basic pH conditions, while FRAP is widely used to evaluate the reducing power of substances in acidic environment, and phenolic substances are included in this group (Castelo Branco & Torres 2011). Guarino (2003) pointed that no changes were observed in the polyphenols contents in relation to the increase of irradiance in studies with *Stypopodium zonale* (J.V.Lamoroux) Papenfuss. However, Torres et al. (2014) evaluated that the phenolic content in the red macroalgae *Gracilaria tenuifrons* C.J.

Bird & E.C. Oliveira responded directly to the increase in light intensity. Phenolic substances are responsible for most antioxidant activities in extracts and vegetable oils, and because of the redox potential similar to that of Fe + 3 and the ABTS radical the results of these methods should be positively correlated. However, in the present study it was not possible to observe this trend. The absence of a similar trend between the FRAP and ABTS assays for the present study may indicate that there are other classes of substances that contribute to the antioxidant potential of the species studied (Matanjun et al., 2008).

The samples tested in the present work did not present high potential against the metal chelating assay and DPPH. An explanation for this, according to Sabeena Farvin & Jacobsen (2013), is the fact that phenols may not be the only ones involved in this activity. From the multivariate analysis of PCA, the authors observed that the chelating activity did not correlate with the phenolic content and suggested that there may be other compounds acting synergistically or antagonistically, orchestrating the intensity of this activity. Our data for *H. pseudomusciformis* was similar with others species of red algae, such *Gracilaria verrucosa* (Hudson) Papenfuss (Yan et al. 1998), *Kappaphycus alvarezii* Doty (Doty) ex P.C. Silva (Kumar et al. 2008), *Chondria crassicaulis* Harvey (Huang & Wang 2004) and species of the genus *Laurencia* J.V. Lamouroux and *Chondrophycus* (J. Tokida & Y. Saito) Garbary & J.T. Harper (Yan et al. 1998, Kim et al. 2005, Zubia & Robledo 2007), which also did not present high antioxidant activity for DPPH.

When we compare our data from field samples with *in vitro* samples, the differences in antioxidant activity between the two morphological variants of *H. pseudomusciformis* were not observed and no discernable pattern of antioxidant activity could be detected. Our data could indicate the antioxidant defenses are relatively constant between the variants to the point be able to maintain flexibility between the phenotypic expression, which allows the ecological occurrence in different environments, what may lead to different morphologies.

PHOTOSYNTHETIC PIGMENTS AND TOTAL SOLUBLE PROTEINS

In general, only punctual differences were found between “musciformis” and “nigrescens” in relation to photosynthetic pigments and total soluble proteins.

Comparing “musciformis” and “nigrescens” from field, the only difference found was in relation to phycocyanin and total soluble proteins. On other hand, comparing “musciformis” and “nigrescens” from *in vitro* cultures, differences were found in relation to phycoerythrin, phycocyanin and total soluble proteins.

Pigment degradation is a natural process reported for algae (Figueroa et al. 1997) and is related to the decrease of photosynthetic rates in response to high solar radiation. Carotenoids, present in a variety of macroalgae groups, have a known antioxidant capacity and confer protection against algae against UV radiation. However, the nitrogen balance, signaled by the protein content, apparently, is quite different between the variants. This could represent a more adapted metabolism for extreme conditions, since an internal pool of N in red algae may represent better physiological conditions to protect against adverse conditions. And in this case, “nigrescens” has a higher protein content than “musciformis” (for field samples).

The phycobiliproteins are more susceptible to the radiation when compared to the other pigments and carotenoids are less sensitive than chlorophylls (Bischof et al., 2006). Comparing our data, the greater increase in *in vitro* culture was related to chlorophylls, having an increase of 265% for “nigrescens” and 250% for “musciformis” but the phycobiliproteins was the most abundant pigment, which agrees with previous works with *Hypnea* and others red algae (Costa 2000, Plastino et al. 2004, Donato 2005, Barufi 2004). This could indicate an adaptation to an environment with less irradiance, meaning those algae could be submitted to more irradiance in field than in culture. In fact, Yokoya et al. (2006) reported that variation “musciformis” (as *H. musciformis*) exhibits characteristics of shade-adapted plants. The authors also verified that the change in phycoerythrin concentration seems to provide an advantage to acclimate to the short photoperiod, so the increase in phycoerythrin in “musciformis” when we compare field and *in vitro* samples, could also indicate less irradiance in culture than in the field.

In the aquatic environment, the water column acts as a natural protection against the radiation (Kirk, 1994; Franklin & Foster, 1997). In algae, mechanisms of protection from the exposure to UV radiation include the accumulation of carotenoids and detoxifying enzymes and antioxidants that fight against reactive oxygen species (ROS) (Franklin & Foster, 1997). In the mid-intertidal in higher hydrodynamic zones of the

shore, “nigrescens” is subjected not only to intense impact from waves, but greater UV radiation and longer periods of desiccation, compared to “musciformis”, found in the transition between the low-intertidal, as epiphyte of other algae in protected environments (Nauer et al. 2015). From the results of pigments (and total soluble proteins) it seems that “nigrescens” does not have as much ability to adjust their phycobilins, whereas “musciformis” seems to have more ability to adjust pigments according to environmental conditions.

Conclusion

Based on the habitat and morphological differences observed for those two variants, we proposed the hypothesis that “nigrescens” and “musciformis” would also present physiological differences. For the parameters analyzed in this work, significant physiological differences between the two morphological variants from field samples were observed for all the antioxidant assays, with the exception of DPPH, for phycoerithrin pigments and for total soluble protein, corroborating the hypothesis that physiological differences could also occur between “musciformis” and “nigrescens” variants. On the other hand, those physiological differences in antioxidant activity disappear when we compare *in vitro* samples, and the only differences found, in this case, was between phycocyanin, phycoerythrin and total soluble proteins. Overall, the two morphological variants have a similar physiological behavior when grown under the same culture conditions, corroborating its classification under one species. These results indicate that the physiological differences found between these variants are mostly due to environmental pressures and represent an example of phenotypic plasticity, which allows occurrence in different environments and may lead to different morphologies.

Acknowledgements

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References

Abbott, I.A. 1999. Marine red algae of the Hawaiian Islands. Bishop Museum Press, Honolulu, Hawaii.

Aguilera, J., Dummermuth, A., Karsten, U., Schriek, R. & Wiencke, C. 2002. Enzymatic defenses against photooxidative stress induced by ultraviolet radiation in Artic marine macroalgae. Polar Biol. 25: 432-441.

Bernard, C., Etienne, A. L., & Thomas, J. C. 1996. Synthesis and binding of phycoerythrin and its associated linkers to the phycobilisome in Rhodella violacea (Rhodophyta): compared effects of high light and translation inhibitors. J. Phycol., 32: 265-271.

Bonoli, M., Verardo, V., Marconi, E., & Caboni, M. F. 2004. Antioxidant phenols in barley (Hordeum vulgare L.) flour: comparative spectrophotometric study among extraction methods of free and bound phenolic compounds. J. Agri. Food Chem., 52(16): 5195-5200.

Brand-Williams, W., Cuvelier, M. E. & Berset, C. L. W. T. 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol., 28(1): 25-30.

Castelo-Branco, V. N. & Torres, A. G. 2011. Capacidade antioxidante total de óleos vegetais comestíveis: determinantes químicos e sua relação com a qualidade dos óleos. Rev. Nutr., 24(1): 173-187.

Cox, S., Abu-Ghannam, N., Gupta, S. 2010. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. Inter. Food Res. J., 17: 205-220.

Choo, K-S., Snoeijs, P. & Pedersén, M. 2004. Oxidative stress tolerance in the filamentous green algae *Cladophora glomerata* and *Enteromorpha ahlneriana*. J. Exp. Mar. Biol. Ecol. 298: 111-123.

Cunningham, F. X., Dennenberg, R. J., Mustardy, L., Jursinic, P. A. & Gantt, E. 1989. Stoichiometry of photosystem I, photosystem II, and phycobilisomes in the red alga Porphyridium cruentum as a function of growth irradiance. Plant physiol., 91(3): 1179-1187.

Dawes, C.J. & Mathieson, A.C. 2008. The seaweeds of Florida. University Press of Florida. 591p.

Faria, A. V. & Plastino, E. M. 2015. Physiological assessment of the mariculture potential of a Gracilaria caudata (Gracilariales, Rhodophyta) variant. J. Appl. Phycol., 28(4): 2445-2452.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution,* **39:** 783-791.

Geraldino PJL, Yang EC, Kim MS & Boo SM (2009) Systematics of *Hypnea asiatica* sp. nov. (Hypneaceae, Rhodophyta) based on morphology and nrDNA SSU, plastid *rbc*L, and mitochondrial *cox*1. *Taxon* 58(2): 606-616.

Gómez, I., Wulff, A., Roleda, M. Y., Huovinen, P., Quartino, M. L, Dunton, K., Christian, W. 2009. Light and temperature demands of marine benthic microalgae and seaweeds in polar regions. Bot. Mar., 52: 593-608.

Gorinstein, S., Martin-Belloso, O., Katrich, E., Lojek,, A., Ciz, M. & Gligelmo-Miguel, N. 2003. Comparison of the contents of the main biochemical compounds and the antioxidant activity of some Spanish olive oils as determined by four different radical scavenging tests. J. Nutr. Biochem. 14: 154-59.

Goss, R. & Jakob, T. 2010. Regulation and function of xanthophyll cycle-dependent photoprotection in algae. Photosynth. Res., 106: 103−122.

Guarino, A.G. 2013. Variabilidade e atuação defensiva de polifenóis em macroalgas pardas. Tese de Doutorado. Universidade Federal Fluminense. Rio de Janeiro. 122p.

Harb, T.B., Torres, P.B., Pires, J.S., Dos Santos, D.Y.A.C. & Chow, F. 2016. Ensaio em microplaca do potencial antioxidante através do sistema quelante de metais para extratos de algas. Instituto de Biociências, Universidade de São Paulo.

Hall, T.A. 1999. BioEdit: a user-friendly biological alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series,* 41: 95-98.

Hou, W. C., Lin, R. D, Cheng, K. T., Hung, Y. T., Cho, C. H, Chen, C. H, Hwang, S. Y & Lee, M. H. 2003. Free radical scavenging activity of Taiwanese native plants. Phytomed., 10: 170-175.

Houvinen, P. & Gómez, I. 2011. Spectral attenuation of solar radiation in Patagonian fjord and coastal waters and implications for algal photobiology. Cont. Shelf. Res. 31: 254-259.

Huang, H.L. & Wang, B.G. 2004. Antioxidant capacity and lipophilic content of seaweeds collected from the Qingdao coastline. J. Agric. Food Chem. 52: 4993-4997.

Ikawa, M., Schaper, T.D., Dollard, C. A. & Sasner, J. J. 2003. Utilization of Folin-Ciocalteu phenol reagent for the detection of certain nitrogen compounds. J. Agric. Food Chem. 51: 1811-1815.

Jesus, P. B., Nauer, F., Lyra, G. d. M., Cassano, V., Oliveira, M. C., Nunes, J. M. d. C. and Schnadelbach, A. S. (2016), Species-delimitation and phylogenetic analyses of some cosmopolitan species of *Hypnea* (Rhodophyta) reveal synonyms and misapplied names to *H. cervicornis*, including a new species from Brazil. J. Phycol., 52: 774–792. doi:10.1111/jpy.12436

Kim, S.J., Woo, S., Yun, H., Yum, S., Choi, E., Do, J.R., Jo, J.H., Kim, D., Lee, S. & Lee, T.K. 2005. Total phenolic contentes and biological activities of Korean seaweed extracts. Food Sci. Biotechnol. 14: 798-802.

Kiran, V.M. & Murugesan, S. 2014. Antioxidant activity of silver nano-particles from *Colpomenia sinuosa* and *Halymenia poryphyroids*. World. J. Pharm. Sci. 2: 817-820.

Kumar, K.S., Ganesan, K. & Rao, P.V.S 2008. Antioxidant potential of solvent extracts of Kappaphycus alvarezii (Doty) Doty – An edible seaweed. Food Chem. 107: 289-295.

Lamouroux JV, (1813). Essai sur les genres de la famille des Thalassiophytes non articulées. *Annales du Muséum d'Histoire Naturelle, Paris* 20: 21-47, 115-139, 267-293, pls 7-13.

Levy, I. & Gantt, E. 1988. Light acclimation in Porphyridium purpureum (Rhodophyta): growth, photosynthesis, and phycobilisomes. J. Phycol., 24: 452-458.

Matanjun, P., Mohamed, S., Mustapha, N.M., Muhammad, K. & Ming, C.H. 2008. Antioxidant activities and phenolics content of eight species of seaweed from North Borneo. J. Appl. Phycol. 20: 367-373.

Matsukawa, R., Dubinsky, Z., Kishimoto, E., Masakki, K., Masuda, Y. & Takeuchi, T. A comparison of screening methods for antioxidant activity in seaweeds. J. Appl. Phycol. 9: 29-35.

Min, B., McClung, A.M. & Chen, M.-H. 2011. Phytochemicals and antioxidant capacities in rice brans of different color. J. Food Sci., 76(1): 117–26.

Nauer, F.; Cassano, V. & Oliveira, M. C. (2015). Description of Hypnea pseudomusciformis sp. nov., a new species based on molecular and morphological analyses, in the context of the H. musciformis complex (Gigartinales, Rhodophyta). Journal of Applied Phycology 27: 2405-2417. DOI: 10.1007/s10811-014-0488-y.

Pires. J., Torres, P.B., dos Santos, D.Y.A.C. & Chow, F. 2017. Ensaio em microplaca do potencial antioxidante através do método de sequestro do radical livre DPPH para extatos de algas. Instituto de Biociências. Universidade de São Paulo. ISBN 978-85-85658-71-7.

Pires. J., Torres, P.B., dos Santos, D.Y.A.C. & Chow, F. 2017. Ensaio em microplaca de substâncias redutoras pelo método do Folin-Ciocalteudo para extatos de algas. Instituto de Biociências. Universidade de São Paulo. ISBN 978-85-85658-70-0.

Plastino, E.M. & Guimarães, M. 2001. Diversidad intraespecifica. In: Alveal KV, Antezana TJ (eds) Sustentabilidad de la Biodiversidad. Universidad de Concepción, Concepción, pp 19‑27.

Plastino, E.M., Ursi, S. & Fujii, M.T. 2004. Color inheritance, pigment characterization, and growth of a rare light green strain of *Gracilaria birdiae* (Gracilariales, Rhodophyta). Phycol Res 52:45‑52.

Price, J.H.; John, D.M. & Lawson, G.W. 1992. Seaweeds of the western coast of tropical Africa and adjacent islands: a critical assessment. IV. Rhodophyta (Florideae) 3. Genera H-K. *Bulletin of British Museum (Natural History – Botany Series)*, 22 (2): 123-146.

Reis, R.P. & Yoneshigue-Valentin, Y. 2000. Phenology of *Hypnea musciformis* (Wulfen) Lamouroux (Rhodophyta, Gigartinales) in three populations from Rio de Janeiro State, Brazil. Bot Mar 43: 299-304.

Reis, R.P., Leal, M.C.R., Yoneshigue-Valentin, Y. & Belluco, F. 2003. Efeito de fatores bióticos no crescimento de *Hypnea musciformis* (Rhodophyta - Gigartinales). Acta Bot Bras 17:279-286.

Reis, R.P., Caldeira, A.Q., Miranda, A.P.S. & Barros Barreto, M.B. 2006. Potencial para maricultura da carragenófita *Hypnea musciformis* (Wulfen) J.V. Lamour. (Giagartinales - Rhodophyta) na Ilha da Marambaia, Rio de Janeiro, Brasil. Acta Bot Bras 20:763-769.

Rocha, F.D., Pereira, R.C., Kaplan, M.A.C. & Teixeira, V.L. 2007. Natural products of marine seaweeds and their antioxidant potential. Rev. Bras. J. Farmacogn. 17:631-639.

Roginsky, V. & Lissi, E.A. 2005. Review of methods to determine chain-breaking antioxidant activity in food. Food Chem. 92: 235-254.

Rufino, M. S. M., Alves, R. E., Brito, E. S., Morais, S. M., Sampaio, C. G., Pérez-Jiménez, J. & Saura-Calixto, F. D. 2006. Metodologia científica: Determinação da atividade antioxidante total em frutas pelo método de redução do ferro (FRAP). Comunicado Técnico, 125. Disponível em: http://www.cnpat.embrapa.br/cnpat/down/index.php?pub/cot_125.pdf>

Saunders, G.W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philosophical Transactions of the Royal Society B.,* 360: 1879-1888.

Schenkman RPF (1986) Cultura de Hypnea (Rhodophyta) *in vitro* como subsídio para estudos morfológicos, reprodutivos e taxonômicos. *Tese de Doutorado*. Instituto de Biociências, Universidade de São Paulo. 81 pp.

Schmidt, É.C., Kreusch, M., Felix, M.R.L, Pereira, D.T., Costa, G.B., Simioni, C., Ouriques, L.C., Farias-Soares, F.L., Steiner, N., Chow, F., Ramlov, F., Maraschin, M. & Bouzon, Z.L. 2015. Effects of ultraviolet radiation (UVA+UVB) and copper on the morphology, ultrastructural organization and physiological responses of the red alga *Pterocladiella capillacea*. *Photochemistry and Photobiology* 91: 359 – 370.

Schmidt, É.C., Felix, M.R.L., Kreusch, M.G., Pereira, D.T., Costa, G.B., Simioni, C., Ouriques, L.C., Steiner, N., Chow, F., Floh, E.S.L., Ramlov, F., Maraschin, M. & Bouzon, Z.L. 2016. Profiles of carotenoids and amino acids and total phenolic compounds of the red alga *Pterocladiella capillacea* exposed to cadmium and different salinities. *J. Appl. Phycol.* 28: 1955 – 1963.

Sies, H. 1993. Strategies of antioxidant defence: review. Eur. J. Biochem., 215: 213-219.

Singleton, V. & Rossi, J. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Ame. J. Enol. Viticul., 16: 144–158.

Swofford, D.L. 2002. PAUP: Phylogenetic Analysis Using Parismony, version 4.08a. *Computer program distributed by the Illinois Natural History Survey, Champaign, Ill.*

Szydowska-Czerniak, A., Karlovits, G., Dianoczki, C., Recseg, K. & SzByk, E. 2008. Comparison of two analytical methods for assessing antioxidant capacity of rapeseed and olive oils. J. Am. Oil Chem. Soc., 85: 141-149.

Tala, F. & Chow, F. 2014. Ecophysiological characteristics of *Porphyra* spp. (Bangiophyceae, Rhodophyta): seasonal and latitudinal variations in northern-central Chile. *J. Appl. Phycol.* 26: 2159 – 2171.

Thiers, B. (2018) *Index Herbariorum*: *a global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. http://www.nybg.org/bsci/ih/ih.html. [continuously updated].

Torres, P.B. 2012. Análise de pigmentos fotossintetizantes e substâncias fenólicas em Gracilariopsis tenuifrons (C.J. Birdi & E.C. Oliveira) Fredericq & Hommersand em diferentes intensidades de luz. Dissertação de mestrado. Instituto de Biociências, Universidade de São Paulo. 104p.

Torres, P.B., Chow, F. & Santos, D.Y.A.C. 2015. Growth and photosynthetic pigments of *Gracilariopsis tenuifrons* (Rhodophyta, Gracilariaceae) under high light *in vitro* culture. J. Appl. Phycol. 27: 1243 – 1251.

Torres, P.B., Pires, J, dos Santos, D.Y.A.C. & Chow, F. 2017. Ensaio de potencial antioxidante de extratos de algas através do sequestro do ABTS em microplaca. Instituto de Biociências. Universidade de São Paulo. ISBN 978-85-85658-69-4.

Urrea-Victoria, V., Pires, J,, Torres, P.B., dos Santos, D.Y.A.C. & Chow, F. 2016. Ensaio antioxidante em microplaca de poder de redução de ferro (FRAP) para extratos de algas. Instituto de Biociências. Universidade de São Paulo. ISBN 978-85-85658-62-5.

Ursi, S. & Plastino, E.M. 2001. Crescimento in vitro de linhagens de coloração vermelha e verde clara de *Gracilaria* sp. (Gracilariales, Rhodophyta) em dois meios de cultura: análise de diferentes estádios reprodutivos. Rev. Bras. Bot. 4: 585-592.

Yan, X.J., Nagata, T. & Fan, X. 1998. Antioxidant activities in some common seaweeds. Plant Food Hum. Nutr. 52: 253-262.

Yang, H., Dong, Y., Du, H., Shi, H., Peng, Y. & Li, X. 2011. Antioxidant compounds from propolis collected in Anhui, China. Molecules (Basel, Switzerland), 16 (4): 3444–3455.

Wu, H., Jiang, H., Liu, C. & Deng, Y. 2015. Growth, pigment composition, chlorophyll fluorescence and antioxidant defenses in the red alga *Gracilaria lemaneiformis* (Gracilariales, Rhodophyta) under light stress. South African J. Bot., 100: 27-32.

Zubia, M. & Robledo, D. 2007. Antioxidant activities in tropical marine macroalgaes from the Yucatan Peninsula, Mexico. J. Appl. Phycol.,19:449-458.

Table 1S. Antioxidant response for the two morphological variations from field and *in vitro* within the antioxidant methods used in this study.

		FIELD		***IN VITRO***	
		"musciformis"	"nigrescens"	"musciformis"	"nigrescens"
ABTS	Avarage (%)	85,47	94,38	95,18	96,88
	Deviation (%)	4,23	3,50	3,52	3,50
DPPH	Avarage (%)	14,30	16,15	31,74	37,97
	Deviation (%)	5,57	6,17	3,45	5,71
FOLIN	Avarage (%)	37,42	55,72	34,48	34,98
	Deviation (%)	5,65	6,86	8,52	5,18
FRAP	Avarage (%)	34,57	24	27,06	31,24
	Deviation (%)	3,81	2,49	3,21	4,68
METAL CHELATION	Avarage (%)	30,96	15,45	-	-
	Deviation (%)	8,74	4,74	-	-

Table 2S. Pigments content for the two morphological variations from field and *in vitro.*

		FIELD		***IN VITRO***	
		"musciformis"	"nigrescens"	"musciformis"	"nigrescens"
PE	Concentration (µg.g FW^{-1})	390,63	467,93	575,44	459,56
PC	Concentration (µg.g FW^{-1})	268,23	315,72	450,39	359,56
APC	Concentration (µg.g FW^{-1})	199,16	221,68	248, 19	183,96
Chlor. *a*	Concentration (µg.g FW^{-1})	62,9	59,67	157,79	158,47
Carotenoids	Concentration (µg.g FW^{-1})	8,5	11,5	39,05	32,4
TSP	Concentration (µg.g FW^{-1})	661,79	2.139,66	1927,30	2.614,21

CAPÍTULO *Seis*

Crossing experiments of two morphological variants of Hypnea pseudomusciformis (Gigartinales, Rhodophyta) corroborate molecular data.

Capítulo 6

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Crossing experiment of two morphological variations of *Hypnea pseudomusciformis* (Gigartinales, Rhodophyta) corroborates molecular data.

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***Abbreviations*:** COI-5P, 5' end of the cytochrome c oxidase 1 gene; NJ, neighbor joining; *rbc*L, gene encoding for the large subunit of the enzyme ribulose-1, 5-bisphosphate carboxylase-oxygenase.

Abstract

Hypnea musciformis is a red macroalga widely distributed in tropical and sub-tropical coasts around the world and an economic resource for industrial production of carrageenan. Previously, the DNA barcode marker 5P-COI and the plastid *rbc*L gene in addition to morphological studies were used to investigate the species on the coast of Brazil and to compare it with specimens from other countries, including the type locality in Italy. This study led to the description of a new species *Hypnea pseudomusciformis* Nauer, Cassano & M.C. Oliveira for the coast of Brazil to accommodate three morphologically divergent species occurring on the Brazilian coast, *H. musciformis*, *H. nigrescens* and *H. valentiae*. Here, alga material was isolated and kept in unialgal culture in the germoplasm bank and *in vitro* cross-breeding tests were conducted between specimens with the typical morphologies for "musciformis*"* and for "nigrescens*"*. Three male gametophyte and three female gametophyte individuals each

were used and cystocarps were present in all gametohpytes. Carpospores were released, generated tetrasporophytes that became fertile and after the release of tetraspores, new gametophytes were observed. Along with growth rates and pigments content data, our results confirm the molecular data that show that those morphological variant belong to the same species. The identification of specimens based only on morphological characteristics proved to be unsatisfactory mainly due to phenotypic plasticity in this species. The technique of DNA barcoding was essential for the identification and definition of species, revealing scenarios that would go unnoticed by using only morphology.

Keywords *Hypnea*, DNA Barcode, Crossing experiments, Pigments, Growth Rates

Introduction

Commonly, morphological characters are used to define species, yet it is a challenge to define which morphological characters are stable within a species and phylogenetically informative (De Queiroz 2007). Furthermore, phenotypic variation within groups can be interpreted in different ways and is therefore subjective, and the decision about what is significant or not for the definition of a species ends up depending on the specialist's view (Mayden 1997; Hey 2001). In addition, the number of recognized species may be dependent on the methods used and their resolution power to identify characters. In the marine environment, for example, taxonomic groups such as algae, sponges and corals have a restricted number of characters by which they can be classified (Queiroz 2007). As a consequence, large numbers of species in these groups have been defined in the past as being cosmopolitan (with extensive geographic and ecological distribution) (Mayden 1997; Hey 2001).

The taxonomy of genus *Hypnea* J.V. Lamouroux (1813) is quite problematic and confusing, since these algae possess a simple morphology, often influenced by environmental conditions. Some taxa in the genus are treated as complexes due to the difficulty in the delimitation of species, such as the complex *H. musciformis-H. pseudomusciformis* (Nauer et al. 2015; see chaper X). The species *H. musciformis* (Wulfen) J.V. Lamouroux is widely studied because of its economic importance as a

source of carrageenan (Reis et al. 1998, Reis et al. 2003, Reis et al. 2006). Nauer *et al*. (2015) discovered using molecular markers that Brazilian specimens of *H. musciformis* differ from specimens from the type locality, Italy. For this reason, the Brazilian specimens were elevated to a distinct species, named *H. pseudomusciformis* Nauer, Cassano & M.C. Oliveira.

Schenkman (1986) made the first mention of *H. nigrescens* (Wulfen) J.V. Lamouroux and *H. valentiae* (Turner) Montagne for the Brazilian coast, but the author argued that these two species could be easily confused with *H. musciformis*, and only the joint analyses of habitat, morphology, anatomy and growth allowed the correct identification. Based on molecular markers, sequences from those three species showed no molecular divergence for markes COI-5P and *rbc*L, being considered then as morphological variations of *H. pseudomusciformis* (Nauer et al. 2015).

Hebert et al. (2003) proposed the use of the 5' end of the mitochondrial gene *cox*1, coding for the subunit 1 of cytochrome oxidase, as a standard molecular marker (COI-5P) for species identification. The term DNA barcode for those short species-specific markers was proposed by Stoeckle (2003). Saunders (2005) validated the COI-5P marker for Rhodophyta, presenting levels of intra and interspecific divergence sufficient to delimit species. This marker was first used for *Hypnea* by Geraldino et al. (2006) for delimiting Asiatic species. For Brazilian specimens, the DNA barcode technique was used in several studies for the genus, which led to the proposal and redefinition of several new species (Nauer et al. 2014, 2015, 2016; Jesus et al. 2015, 2016).

Crossing experiments with Rhodophyta are still scarce, the first study of this type was conducted by Edwards (1970) and later some others used the technique to study the taxonomic relationships among species of red algae (Rueness 1973, Guiry 1992, Brodie *et al.* 1993). In Brazil, Plastino & Oliveira (1988a and 1988b) used the technique with taxonomic study of the genus *Gracilaria* Greville, and Guimarães *et al*. (1999) in studies related to *G. domingensis* biology. Zuccarello & West (1995) used crossing experiments in the biogeographic study of the *Bostrychia radicans* (Montagne) Montagne species complex. Later, Zuccarello & West (2002) used molecular and reproductive isolation approaches to study the *Bostrychia* species complex and concluded that the combined use of the two techniques proved very effective in studies of this kind.

In this study, individuals of both morphological variations of *H. pseudomusciformis* ("musciformis" and "nigrescens") were collected from the field and placed in the same culture conditions. Crossing experiments between the two morphological variations were carried out in order to verify the species genetically compatibility according to the biological species concept (see chapter 1 for species concept introduction, pg X). DNA barcode sequences, growth rates and photosynthetic pigments content were also analysed. We utilize the joint analyses of habitat, morphology, growth, pigment content, DNA barcode and cross experiments to understand species delimitation in intraspecific diversity of *Hypnea pseudomusciformis*.

Materials and methods

ALGAL MATERIAL

The material used in our experiments (Table 1) derived from two populations, from state of São Paulo, southeast region of Brazil. Three tetrasporophytic plants of each morphological variation were collected, "musciformis" in Cigarras beach, São Sebastião and "nigrescens" in Cibratel, Itanhaém (Figure 1). Apical branches (length 1 cm, total fresh mass ca. 1 mg) were isolated from each individual and kept in unialgal culture under controlled conditions in 500 mL of sterilized seawater (32 psu) enriched with 50% von Stosch medium (Ursi & Plastino 2001), 14 h light: 10 h dark, 24 ± 1°C and 70 ± 5 µmol photons $m^{-2}s^{-1}$ (Osram 40W day light fluorescent tubes). The cultures were bubbled with compressed air for 30 min h^{-1}, and the medium was renewed weekly. *Hypnea* life cycle for both morphological variations was completed in 121 days (see Chapter 4) and tetrasporophytic, female and male gametophytes were kept isolated in unialgal culture.

CROSSING EXPERIMENT

For the crossing experiment, between individuals of "musciformis" and "nigrescens" morphologies, three male gametophytes and three females of each variation were used (IBC2806, IBC2807, IBC2808 for "musciformis" and IBC2809, IBC2810, IBC2811 for "nigrescens", Table 1). For each female gametophyte (♀), apical segments were incubated in Erlenmeyer flasks with 500 ml of medium in three distinct

experimental settings: 1) Three ♀ alone, to check for the formation of unfertilized cystocarps (negative control). 2) Three ♀ with three apical segments of male gametophytes (♂) of the same morphotype (positive control). 3) Three ♀ with three ♂ of different morphotype (crossing test). Each scenario was repeated three times, one for each female gametophyte, resulting 18 experimental flasks (Figure 2).

Table 1. Collection information of specimens analyzed in this work. *GB – GenBank accession numbers (to be added upon publication), - not sequenced.

Samples	Morphological Variation	ID number	Collection site, Latitude, Longitude	Voucher	COI-5P*
Southeast region.	"musciformis"	IBC2806	Cigarras Beach. São Sebastião. SP. F. Nauer & L. M. Ayres. 30. Setpember. 2016. $23^0$43'41.38''S/$45^0$23'53.84''O	***	***
	"musciformis"	IBC2807	Cigarras Beach. São Sebastião. SP. F. Nauer & L. M. Ayres. 30. Setpember. 2016. $23^0$43'41.38''S/$45^0$23'53.84''O	***	***
	"musciformis"	IBC2808	Cigarras Beach. São Sebastião. SP. F. Nauer & L. M. Ayres. 30. Setpember. 2016. $23^0$43'41.38''S/$45^0$23'53.84''O	***	***
	"nigrescens"	IBC2809	Cibratel Beach. Itanhaém. SP. F. Nauer & M. C. Oliveira. 02 July 2015. $24^0$12'05.68''S/$46^0$48'38.72''O	***	***
	"nigrescens"	IBC2810	Cibratel Beach. Itanhaém. SP. F. Nauer & M. C. Oliveira. 02 July 2015. $24^0$12'05.68''S/$46^0$48'38.72''O	***	***
	"nigrescens"	IBC2811	Cibratel Beach. Itanhaém. SP. F. Nauer & M. C. Oliveira. 02 July 2015. $24^0$12'05.68''S/$46^0$48'38.72''O	***	***
Resulting from Crossing Experiments	-	IBC2812	Cross between IBC2806 and IBC289	***	***
	-	IBC2813	Cross between IBC2806 and IBC289	***	***
	-	IBC2814	Cross between IBC2806 and IBC289	***	***

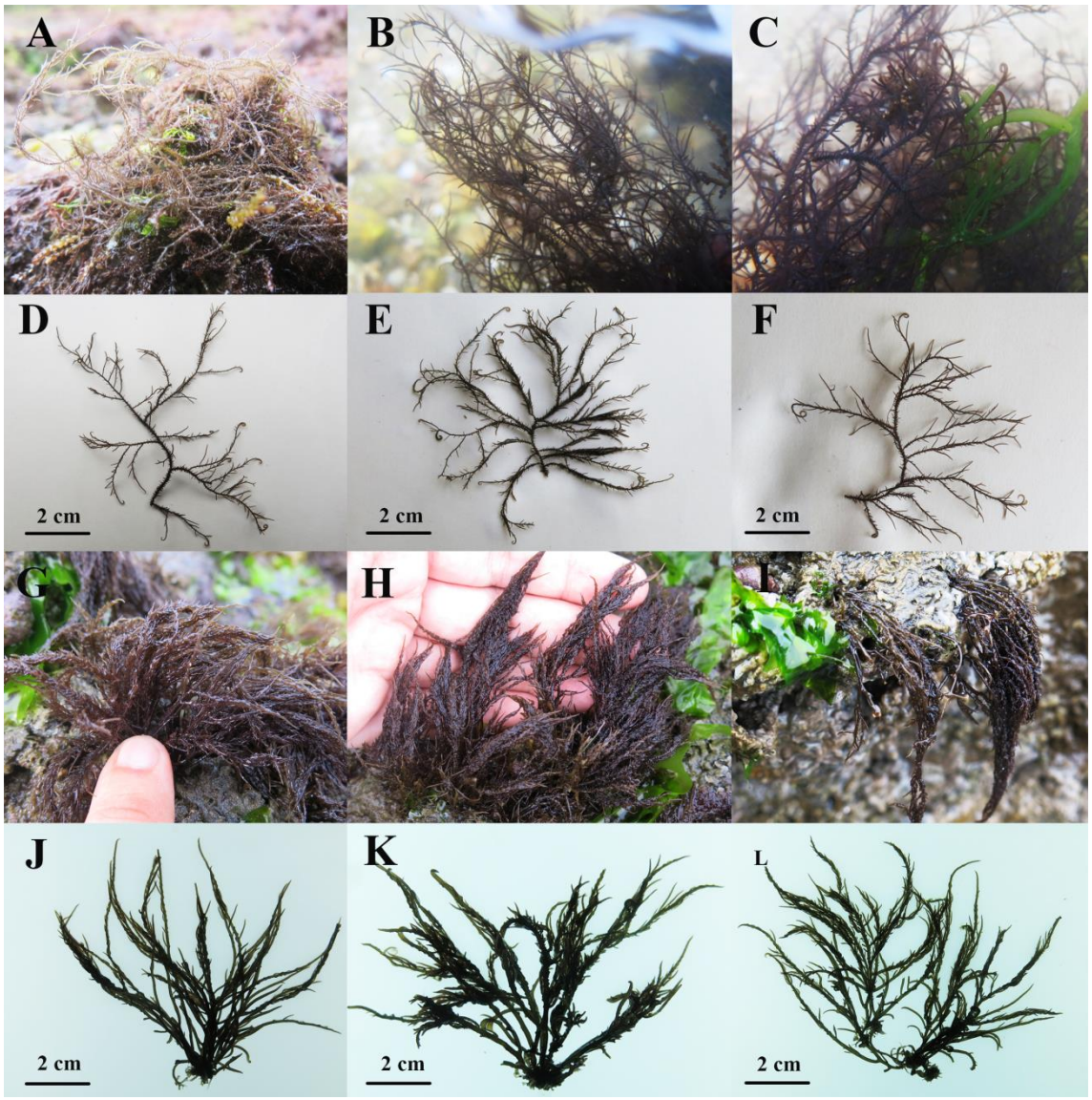

Figure 1. *Hypnea pseudomusciformis* tetrasporophytes from São Paulo populations. A-F. "musciformis" morphological variants. **A-C.** individuals growing in the natural habitat. **D-F.** Habit of specimens collected from the field. G-L. "nigrescens" morphological variants. **G- I.** individuals growing in the natural habitat. **J, K and L.** Habit of specimens from the field.

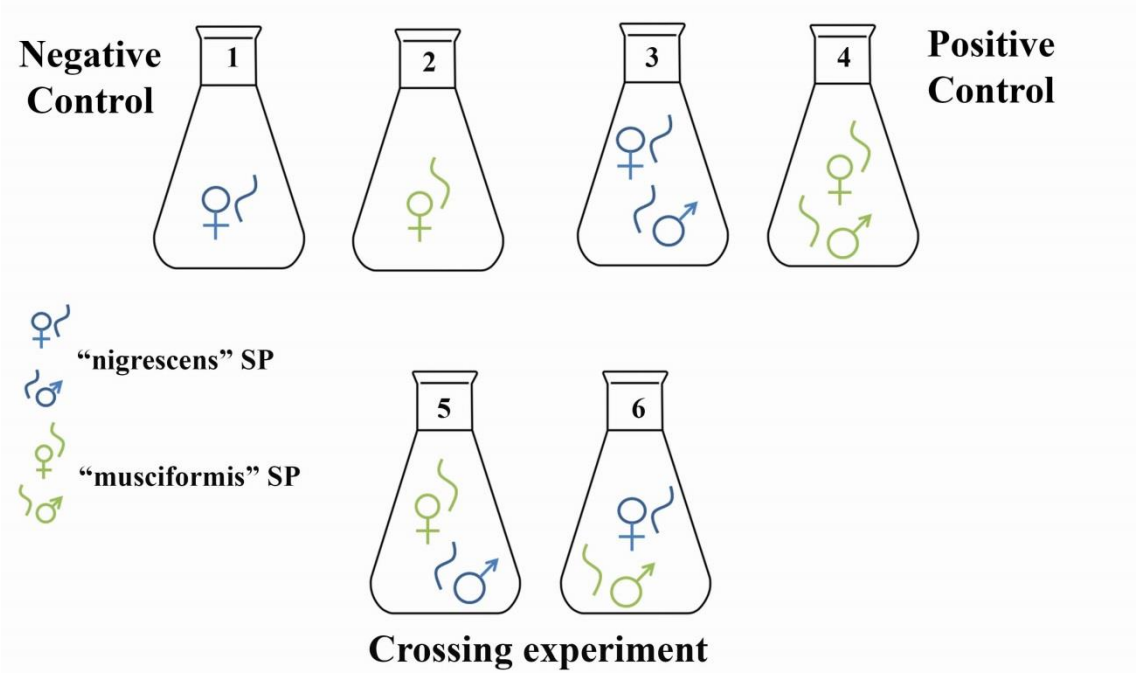

Figure 2. Experimental settings for crossing between morphological variations "musciformis" and "nigrescens".

GROWTH RATES

Apical branches (length 1 cm, total fresh weight ca. 1 mg) of female gametophytes and of unfertile tetrasporophytes (n=3) for both variations and tetrasporophytes resulting from the crossing experiments (n=3) were cultivated for 4 weeks in 500 ml of enriched seawater in the standard conditions described above. The biomass was recorded weekly. Branches were gently blotted dry and weighted weekly on an analytical balance Mettler – Toledo AE 200S. Growth rates (GR) were assessed weekly according to Lignell and Pedersén (1989). A two-way analysis of variance was performed using the software STATISTICA 7.0 (StatSoft, Inc.), considering the time and life-history stages as independent variables and the growth rates as dependent variables. This analysis was followed by a Newman-Keuls *a posteriori* test.

PHTOSYNTHETIC PIGMENTS

The content analyzes of photosynthetic pigments and total soluble proteins (TSP) were performed from of 70 mg FW of unfertile tetrasporophytic samples from

both morphological variantions and unfertile tetrasporophytic samples from crossing experiment. Extraction of photosynthetic pigments was performed according to Kursar et al. (1983), adapted by Wanderley (2009). In the first step, the samples were ground in liquid nitrogen and extracted in 1 mL of 50 M sodium phosphate buffer pH 5.5 for 1 h at 4 °C, protected from light. After extraction, the samples were centrifuged and the supernatant transferred to a new microtube. This extract was called a buffered extract and used for phycobiliprotein and TSP analyzes. Absorbance readings (400 nm – 700 nm) were measured in a 96-well microplates with a final volume of 400 µL in a UV-vis spectrophotometer (Epoch 2-BioTek, USA). The levels of phycobiliproteins (mg.g FW^{-1}) were calculated according to Beer & Eshel (1985).

In the second stage of extraction, from the residue (pellet) obtained from the buffered extract, chlorophyll *a* and carotenoids were extracted by adding 1.5 ml of methanol for 3 h at 4 °C protected from light. Absorbance readings (400 nm – 700 nm) were measured in a 96-well microplates with a final volume of 400 µL in a UV-vis spectrophotometer (Epoch 2-BioTek, USA). The levels of chlorophyll *a* and carotenoids (mg.g FW^{-1}) were calculated according to Lichtenthaler & Buschmann (2001).

MOLECULAR PROCEDURES

Fragments of each plant used in growth rate and cross experiments, were macerated in liquid nitrogen, and DNA was extracted using the NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany), following the kit protocol. Amplification for COI-5P marker was performed using a PCR Master Mix (Promega Corp., Madison, WI, USA) with the primers GazF1 and GazR1 and cycles described by Saunders (2005). The PCR products were purified using the column GTX™ PCR DNA and Gel Band Purification Kit (GE Healthcare, Buckinghamshire, UK), quantified using NanoDrop 2000 (Wilmington, DE,USA), and directly sequenced with the same primers used in PCR in a 3730 DNA Sequencer (Applied Biosystems, Carlsbad, CA, USA).

DATA ANALYSIS

The data were tested for normality (Kolmogorov-Smirnov) and homoscedasticity (Bartlett's test) and subsequent one-way or two-way ANOVA ($p <$

0.05). To determine significant differences was used th *post-hoc* Newman-Keuls test ($p < 0.05$). Before the analysis of variance the data in percentage were transformed into arcsen [square root (x/100)].

Results

CROSSING EXPERIMENT

For the cross experiment, between the morphological variations “musciformis” and “nigrescens” and from two different populations, cystocarps were observed (Figure 3) in all positive control and crossing flasks (Table 2). Carposporophytes resulting from those crossings released carpospores, which developed into tetrasporophytes. These F1 tetrasporophytes became fertile in 40 days showing branchlets with tetrasporangia (Figure 7), subsequently releasing tetraspores that developed into viable gametophytes. No reduction in fertility was observed. Crosses between F1 gametophytes, however, were not carried out. The sequences from F1 tetrasporophytes (IBC2812, IBC2813 and IBC2814) had no divergence with parentals sequences. With these results, we confirm, using the biological species concept, that for Brazil, the species *H. nigrescens* and *H. musciformis* belong to the same biological species, *H. pseudomusciformis*, corroborating the results with COI-5P DNA barcode marker. Intraspecific variation among individuals used in this experiments was up to 0.6% and this variation did not correlate with morphology or collection sites.

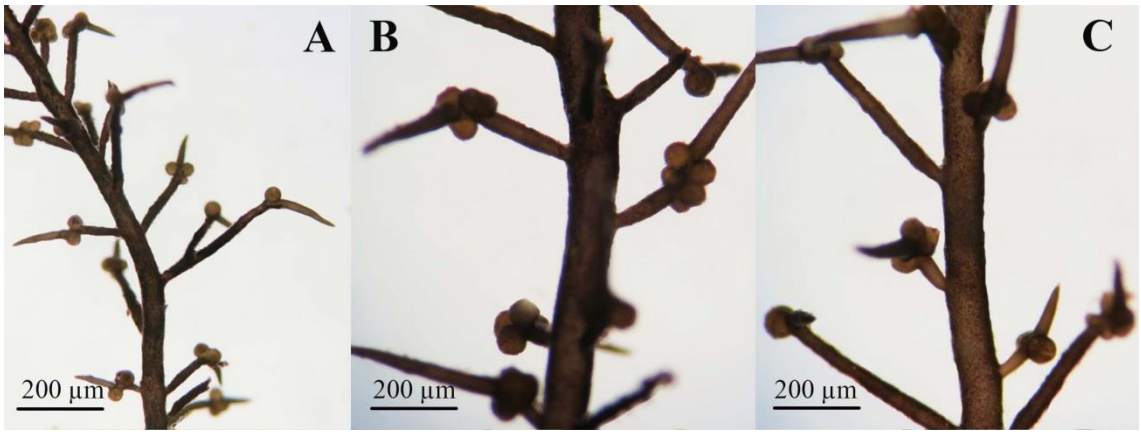

Figure 3. Cystocarps formed in the crossings between “musciformis” and “nigrescens”. A, B and C are from different crossings.

Table 2. Crossing experiments results, including negative controls (only female gametophytes), positive controls (male and female gametophytes of the same morphology) and between "musciformis" and "nigrescens" gametophytes.

	CROSS EXPERIMENTS "***musciformis***" x "***nigrescens***"			
	Flasks	**Gametophytes**	**Cystocarp**	**Carpospores status**
Negative Control	**1**	♀**N1**	Absent	-
	2	♀**N2**	Absent	-
	3	♀**N3**	Absent	-
	4	♀*M1*	Absent	-
	5	♀*M2*	Absent	-
	6	♀*M3*	Absent	-
Positive Control	**7**	♀**N1** x **N1**♂	**Present**	**Viable**
	8	♀**N2** x **N2**♂	**Present**	**Viable**
	9	♀**N3** x **N3**♂	**Present**	**Viable**
	10	♀*M1* x *M1*♂	**Present**	**Viable**
	11	♀*M2* x *M2*♂	**Present**	**Viable**
	12	♀*M3* x *M3*♂	**Present**	**Viable**
Crossings	**13**	♀**N1** x *M1*♂	**Present**	**Viable**
	14	♀**N2** x *M2*♂	**Present**	**Viable**
	15	♀**N3** x *M3*♂	**Present**	**Viable**
	16	♀*M1* x **N1**♂	**Present**	**Viable**
	17	♀*M2* x **N2**♂	**Present**	**Viable**
	18	♀*M3* x **M3**♂	**Present**	**Viable**

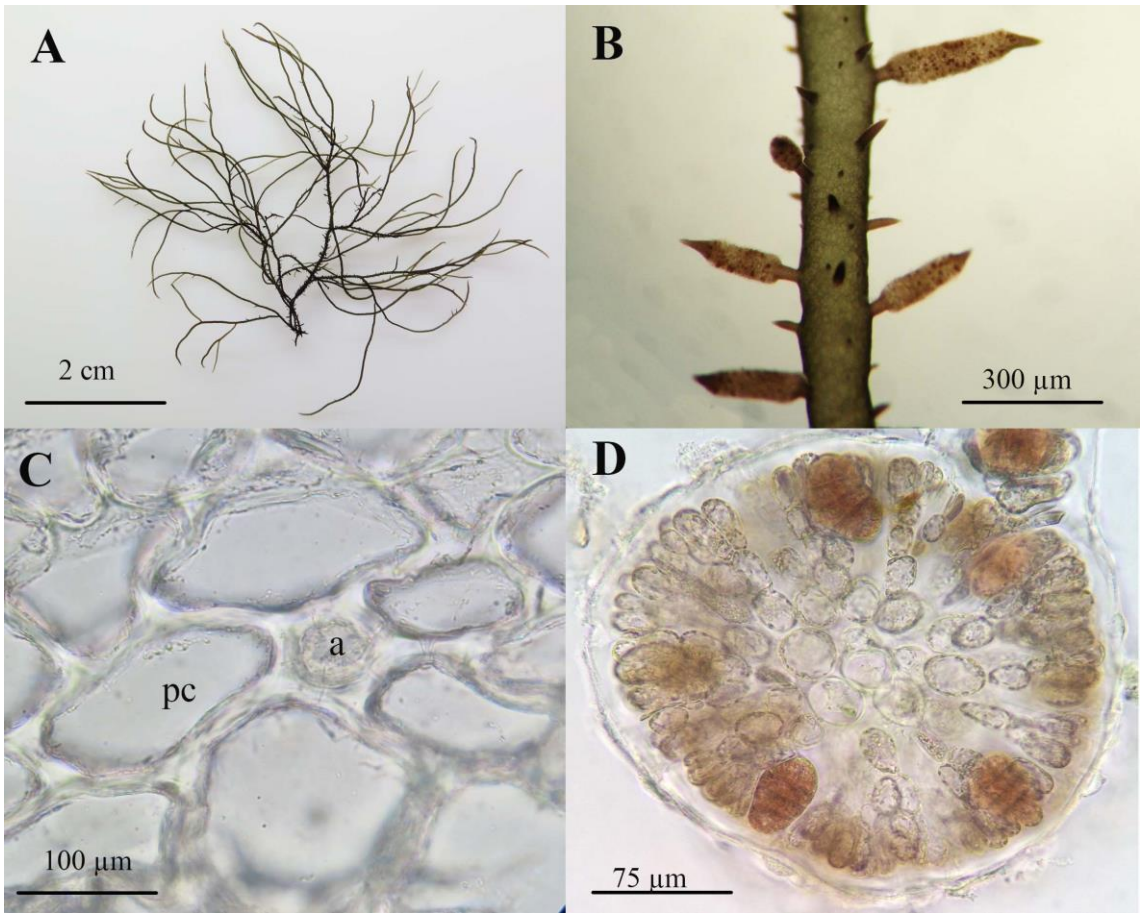

Figure 4. Tetrasporophyte plant of *H. pseudomusciformis* resulting from crossing experiment between morphological variations "musciformis" and "nigrescens". **A.** Habit of fertile tetrasporophyte specimen after 6 weeks in culture. **B.** Detail of a tetrasporophytic thallus with tetrasporangia. **C.** Cross section of the thallus with axial cell and five periaxial cells. **D.** Tetrasporangia in cross section.

GROWTH RATES

An analysis comparing growth rates of F1 generation tetrasporophyte and the "musciformis" tetrasporophyte that was originally isolated from the field and that gave rise to the gametophyte that was used in the crossings, found no significant differences. In "musciformis", growth rates ranged from 8 to 15 % FW.day^{-1}. In F1 generation, growth rates ranged from 8 to 17 % FW.day^{-1} (Figure 5A). However, an intraspecific analysis comparing growth rates of F1 generation tetrasporophyte and the "nigrescens" original tetrasporophyte found statistical differences, but they were little and punctual. In "nigrescens", growth rates ranged from 4 to 13 % FW.day^{-1}(Figure 5B).

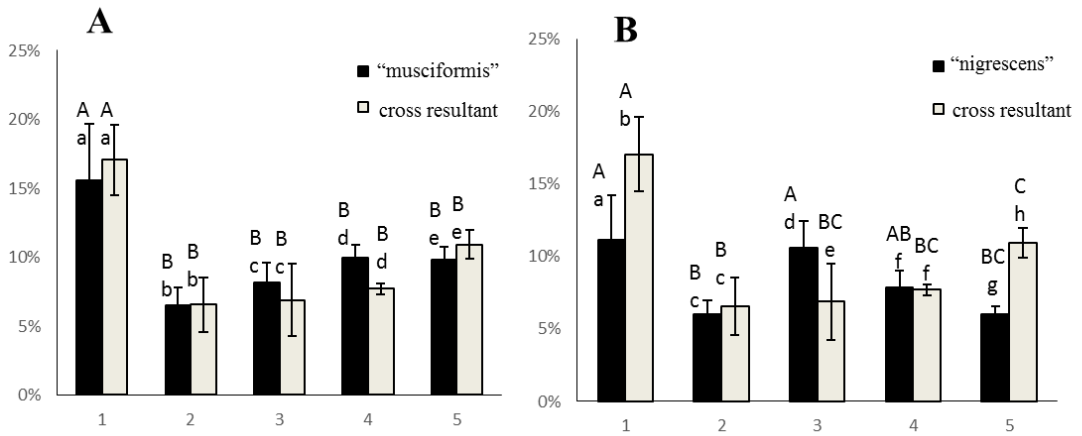

Figure 5. Growth rates (%, Y axis) of *H. pseudomusciformis* over 5 weeks (1 to 5 in the X axis). **A.** F1 generation tetrasporophyte compared with "*musciformis*" tetrasporophytes isolated from the field. **B.** F1 generation tetrasporophyte compared with "*nigrescens*" tetrasporophytes isolated from the field. A = comparison between weeks. b = comparing each week.

PHTOSYNTHETIC PIGMENTS

Results for pigment content between F1 tetrasporophytes resultanting from the crossings between the different morphological variants and both morphological variants "musciformis" and "nigrescens" tetrasporophytes isolated from the field and cultured in the lab under the same conditions are presented in Figure 6.

The F1 terasporophytes presented significant differences from "musciformis" terasporophytes for phycoerythrin (PE) (Fig. 6A) and phycocyanin (PC) (Fig. 6B) while for allophycocyanin (APC) was similar (Fig. 6C). Compared to "nigrescens", phycobiliproteins were similar for the three pigments. The content of chlorophyll *a* and carotenoids (Fig. 6D) showed no significant differences between the F1 and "musciformis" terasporophytes. However, significant differences were observed between F1 and "nigrescens" terasporophytes.

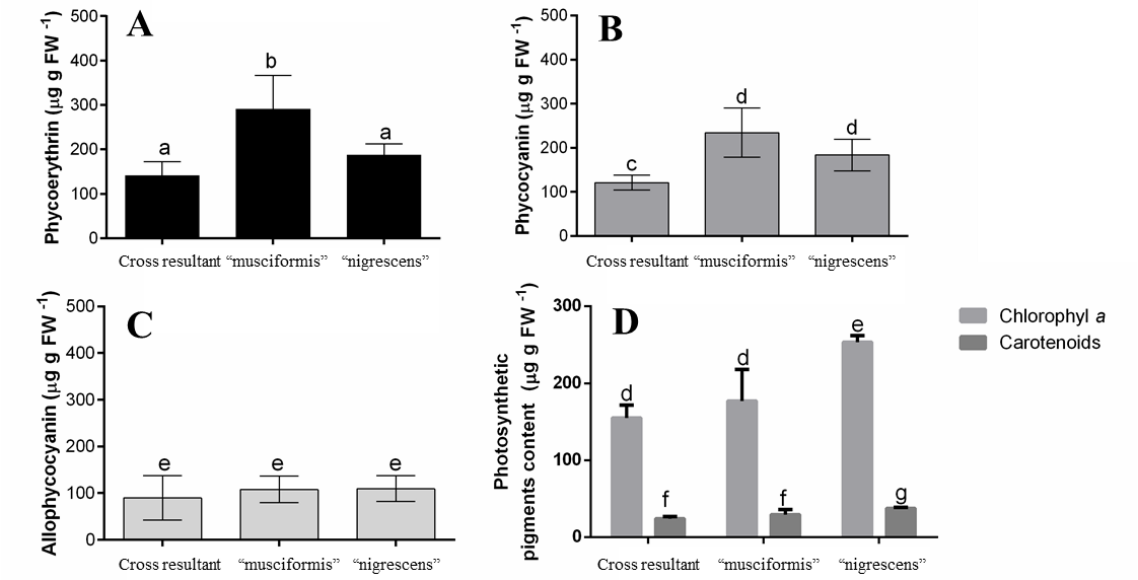

Figure 6. Quantification of pigments content of two morphological variants of *H. pseudomusciformis*, "musciformis" and "nigrescens" tetrasporophytes and F1 tetrasporophyte resulting from crossings between gametophytes derived from the former tetrasporophytes. **A.** Phycoerythrin. **B.** Phycocyanin. **C.** Allophycocyanin. **D.** Quantification of chlorophyll a and carotenoids. The bars correspond to the mean ± standard deviation (n = 5). Different letters represent significant differences ($p < 0.05$).

Discussion

In 2015, Nauer et al. proposed based on the use of molecular markers a new species *Hypnea pseudomusciformis* to three morphologically divergent species occurring on the coast fo Brazil, *H. musciformis, H. nigrescens* and *H. valentiae*. Therefore, *H. pseudomusciformis* was established based on the phylogenetic species concept. In this work we aimed to test if the biological species concept could also be applied to *H. pseudomusciformis*. For that we crossed *in vitro* individuals with the typical morphologies for "musciformis" and "nigrescens" isolated from two different locations. We also compared some basic physiological parameters such as growth rates and photosynthesis pigments of field-derived "musciformis" and "nigrescens" and F1 tetrasporophytes.

The results of our crossing experiments between the two morphological variations resulted in viable carpospores which developed into new tetrasporophytes

(F1). These F1 tetrasporophytes released viable tetraspores which formed new F1 gametophytes. These results corroborate the proposal of *H. pseudomusciformis* based on the use of molecular markers (Nauer et al. 2015).

The morphological species concept uses discontinuities in morphological variation to distinguish species. This concept has, for practical reasons, dominated algal systematics for many decades (John & Maggs, 1997; Mann, 1999) and it's easy to understand why the confusion with "musciformis" and "nigrescens" identification as two species were made. The detection that these morphological divergence was due to plasticity within a single species was only possible with the use of the DNA barcode technique in a wide range of samples (Nauer et al. 2015). This technique proved to be fast and efficient in the separation of species, especially since many specimens are found with only few branches, little biomass and no reproductive structures, especially those that inhabit the infralittoral (Nauer et al. 2014). Morphological characters are used to define species, however it is a challenge to define which morphological characters are important and phylogenetically informative. This means that phenotypic variation within groups can be interpreted differently, and therefore it is subjective to decide on what is important or not.

To overcome difficulties associated with morphological data, the biological species concept has been applied to assess species boundaries in some algal groups, including diatoms (Mann, 2010), and some red, green and brown algae (Coleman, 1977; Zuccarello & West, 2003; Hiraoka et al., 2011; Maggs et al., 2011). The biological species concept is closely allied to the evolutionary species concept and focuses on reproductive isolation to distinguish species. Although many taxonomists grew dissatisfied with this concept because it did not give a clear indication of how strong reproductive isolation between populations had to be for them to qualify as different species (Brodie et al., 1993; Coyer et al., 2002; Kamiya, 2004; Casteleyn et al., 2009; Zardi et al., 2011) we belive it is still a value method to understand phenotypic plasticity and DNA divergences.

In vitro crossings have been used to test species boundaries in different genera of Rhodophyta by other authors (Rueness 1973, Guiry 1992, Brodie et al. 1993, Zuccarello & West 1995 and Zuccarello & West 2002). In Brazil, few cross-breeding studies were made. Plastino & Oliveira (1988a and 1988b), attempted hybridization of five different species of *Gracilaria* growing sympatricatly, as well as crossings among allopatric

populations of the same species. In that study, monospecific crossings were positive even between isolated populations, with production of cystocarps and carpospores, similar to the results found in this work with *Hypnea*, were "musciformis" and "nigrescens" were collected from different populations, about 120 km apart. Nauer et al. (2015) reported that was it was uncommon to find both of these variations of *H. pseudomusciformis* growing on the same rock shore, therefore we don't know if in field conditions these morphological variants do cross. It would be interesting to study if "musciformis" and "nigrescens" have the hability to cross while in nature and if its resulting morphology is derived from the environment where the spores settles.

For the genus *Hypnea*, most times, the use of morphological characteristics alone is not sufficient for precise taxonomic identification due to the high phenotypic plasticity in the species of the genus. One example of plastic morphology is *H. pseudomusciformis*, whose morphology varies with the environment (Nauer et al. 2015). The use of molecular markers have been a valuable tool to aid in the correct identification of *Hypnea* species (Nauer et al. 2014, 2015, 2016).

The phenotypic plasticity present in many species of red algae (Rhodophyta) is described as a consequence of intraspecific diversity, which comprises the set of possible phenotypic expressions, which may be manifested in a species by means of acclimatization and / or adaptation processes (Plastino 2004).

Schenkman (1986) made not only the first citation of *H. nigrescens* for Brazil, but the first citation of this species outside Indic Ocean. Even then, the author claimed that only the joint analysis of habitat, morphology, anatomy and growth allowed the correct identification of *H. musciformis* and *H. nigrescens*. Besides displaying great morphological differences on natural population, when put in culture those differences attenuate. In fact, the morphology of "musciformis", "nigrescens" and the F1 tetrasporophyte resultant from the crossing between these tow morphological variants are very similar in culture. Those differences also attenuate in terms of the physiological response, (see chapter 5). The growth rates showed no differences between "musciformis" and F1 tetrasporophytes, and, despite some punctual differences in some of the weeks, the growth rate between "nigrescens" and F1 tetrasporophytes were similar in general.

Schenkman (1986) claimed that "nigrescens" (as *H. nigrescens*) in culture showed to be distinct from the other species because both of spermatangium and

tetrasporangium are formed only on one side of the branches without enveloping it. As the plant develops, new nematocyst can be formed in younger branches, as in other species, or they can be formed in the same branches that already contained nematocyst. Nauer et al. (2015), however, based on specimnes from nature, observed tetrasporangium on just one side of the branches and totally involving it. Our data from specimens in culture correlates with Nauer et al. (2015) were both morphological variations "musciformis" and "nigrescens" and F1 tetrasporophytes could have nematocysts on one side of the branch and/or completing involving it, without an apparent pattern.

Regarding physiological responses, the F1 tetrasporophyte showed no differences in growth rate compared to "musciformis" and "nigrescens" parental tetrasporophytes. On the other hand, pigment content presented differences for PE and PC compared to "musciformis" while for "nigrescens" the differences were observed for chlorophyll a and carotenoids. Schenkman (1986) observed that irradiance was the main factor that interfered with the morphology and fertility of the thallus of *Hypnea* in culture. With respect to morphology, irradiance affected growth in general, height and thickness of the thallus, number of branches, general appearance of plants, branching pattern and color. Differences in irradiance between nature and culture, could explain differences in pigments observed between "musciformis" and "nigrescens". These results reinforce the environment as a selection agent, which would indicate the direction of selection for the expansion of one morphological variation or another. It's interesting to note, however, the F1 tetrasporophytes have less pigment content in general, compared to the parental tetrasporphytes. These differences could be due to intraspecific variation, or that the F1 generation was never submitted to high light intensities found in field conditions or some genetic factor. It's has been showed that pigment deficient mutants from *H. musciformis* have similar growth rates, but lower concentrations of phycobiliproteins (specially phycoerithrin) compared to wild strains (Yokoya et al. 2003, 2006). Phycoerithrin is composed of two subunits encoded by two genes in the plastid genome (Apt et al. 2001). The structure and expression of these two proteins are associated with environmental factors, particularly irradiance levels (Waaland et al. 1974), while some photosynthetic characteristics such as photosynthetic efficiency (α) are also related to the phycoerythrin content (Sagert et al. 1997).

Conclusion

Our results indicate that the Biological Species Concept and crossing experiment are still is a viable resource to resolve taxomic issues in delimiting species boundaries, and the material to conduct this kind of experiment is much cheaper when compared to the DNA barcode technique. Our results from the crossing experiments confirms that, for Brazil, *H. nigrescens* and *H. musciformis*, belong to the same species, *H. pseudomusciformis*.

Aknowledgements

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References

Apt, K.E., Metzner S. & Grossman, A.R. 2001. The ɤ subunits of phycoerythrin from a red alga: position in phycobilisomes and sequence characterization. J. Phycol. 37:64-70

Beer, S. & Eshel, A. 1985. Determining phycoerythrin and phycocyanin concentrations in aqueous crude extracts of red algae. Mar. Freshw. Res. 36(6):785-792.

Brodie, J., Guiry, M.D. & Masuda, M. 1993. Life history, morphology and crossability of *Chondrus ocellatus* forma *ocellatus* and *C. ocellatus* forma *crispoides* (Gigartinales, Rhodophyta) from the north-western Pacific. Eur. J. Phycol. 28:183-196.

Coleman, A.W. 1977. Sexual and genetic isolation in cosmopolitan algal species Pandorina morum. American Journal of Botany, 64: 361–368.

Casteleyn, G., Adams, N.G., Vanormelingen, P., Debeer, A.E., Sabbe, K. & Vyverman, W. 2009. Natural hybrids in the marine diatom *Pseudo-nitzschia pungens* (Bacillariophyceae): genetic and morphological evidence. Protist, 160: 343–354.

Coyer, J.A., Peters, A.F., Hoarau, G., Stam, W.T. & Olsen, J.L. 2002. Hybridization of the marine seaweeds, *Fucus serratus* and *Fucus evanescens* (Heterokontophyta: Phaeophyceae) in a 100-year-old zone of secondary contact. Proceedings of the Royal Society B–Biological Sciences, 269: 1829–1834.

De Queiroz, K. 2007. Species concepts and species delimitation. Systematic Biology, 56: 879–886.

Edwards, P. 1970. Attempted hybridization in the red alga Polysiphonia. Nature 226: 467-468.

Geraldino, P.J.L., Yang, E.C. & Boo, S.M. 2006. Morphology and molecular phylogeny of *Hypnea flexicaulis* (Gigartinales, Rhodophyta) from Korea. *Algae* 21: 417-423.DOI 10.4490/ALGAE.2006.21.4.417

Guimarães, M., Plastino, E. M. & OLIVEIRA, E. C. 1999. Life-History, reproduction, and growth of *Gracilaria domingensis* (Gracilariales, Rhodophyta) from Brazil. Botanica Marina, v. 42, p. 481-486.

Guiry, M.D. 1992. Species concepts in marine red algae. In Round, F.E. & Chapam, D.J. (Eds). Progress in Phycological Research. Volume 8. Biopress Ltd Bristol., pp 251-278.

Herbert P.D.N., Cywinska A., Ball S.L. & deWaard J.R. 2003. Biological identifications through DNA barcoding. Proceedings of the Royal Society of London, B, 270: 313-322.

Hey, J. 2006. On the failure of modern species concepts. Trends in Ecology and Evolution, 21: 447–450.

Hiraoka, M., Ichihara, K., Zhu, W., Ma, J. & Shimada, S. 2011. Culture and hybridization experiments on an *Ulva clade* including the Qingdao strain blooming in the Yellow Sea. PLoS One, 6:e19371.

Jesus, P.B., Silva, M.S., Lyra, G.M., Nunes, J.M.C. & Schnadelbach, A.S. 2015. Extension of the distribution range of *Hypnea stellulifera* (Cystocloniaceae, Rhodophyta) to the South Atlantic: morphological and molecular evidence. *Aquatic Botany* 123: 26-36. DOI: 10.1016/j.aquabot.2014.12.003

Jesus, P. B., Nauer, F., Lyra, G. d. M., Cassano, V., Oliveira, M. C., Nunes, J. M. d. C. and Schnadelbach, A. S. 2016. Species-delimitation and phylogenetic analyses of some cosmopolitan species of *Hypnea* (Rhodophyta) reveal synonyms and misapplied names to *H. cervicornis*, including a new species from Brazil. J. Phycol., 52: 774–792. doi:10.1111/jpy.12436

John, D.M. & Maggs, C.A. 1997. Species problems in eukaryotic algae: a modern perspective. Chapter 5, pp. 83-107. in Species The Units of Biodiversity. Ed. Claridge, M.F., Dawah, H.A. & Wilson, M.R. 1997. Chapman & Hall. London.

Kamiya, M. 2004. Speciation and biogeography of the *Caloglossa leprieurii* complex (Delesseriaceae, Rhodophyta). Journal of Plant Research, 117: 421–428.

Kursar, T.A., van der Meer, J.P. & Alberte, R.S. 1983b. Lightharvesting system of the red alga *Gracilaria tikvahiae*. II. Phycobilisome characteristics of pigment mutants. Plant. Physiol. 73:361‑369.

Lichtenthaler, H.K. & Buschmann, C. 2001. Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy. Current Protocols in Food Analytical Chemistry F4.3.1-F4.3.8. DOI: 10.1002/0471142913.faf0403s01

Maggs, C.A., Fletcher, H.L., Fewer, D., Loade, L., Mineur, F. & Johnson, M.P. 2011. Speciation in red algae: members of the Ceramiales as model organisms. Integrative and Comparative Biology, 51: 492–504.

Mann, D.G. 1999. The species concept in diatoms. Phycologia, 38:437–495.

Mann, D.G. 2010. Discovering diatom species: is a long history of disagreements about species-level taxonomy now at an end? Plant Ecology and Evolution, 143: 251–264.

Mayden, R.L. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. In Species: The Units of Biodiversity (Claridge, M.F., Dawah, H.A. & Wilson, M.R., editors), 381–424. Chapman and Hall, London.

Nauer, F.; Cassano, V. & Oliveira, M. C. 2014. *Hypnea* species (Gigartinales, Rhodophyta) from the southeastern coast of Brazil based on molecular studies complemented with morphological analyses, including descriptions of *Hypnea edeniana* sp. nov. and *H. flava* sp. nov. *European Journal of Phycology* 49 :550-575. DOI:10.1080/09670262.2014.981589.

Nauer, F.; Cassano, V. & Oliveira, M. C. 2015. Description of Hypnea pseudomusciformis sp. nov., a new species based on molecular and morphological analyses, in the context of the H. musciformis complex (Gigartinales, Rhodophyta). Journal of Applied Phycology 27: 2405-2417. DOI: 10.1007/s10811-014-0488-y

Nauer, F., Cassano, V. & Oliveira, M.C. 2016. *Hypnea wynnei* and *Hypnea yokoyana* (Cystocloniaceae, Rhodophyta), two new species revealed by a DNA barcoding survey on the Brazilian coast. Phytotaxa 268 (2): 123–134. http://dx.doi.org/10.11646/phytotaxa.268.2.3

Pereira, R. C. & Soares-Gomes, A. (Orgs.) 2009. Biologia marinha, 2ª Edição. *Rio de Janeiro: Ed. Interciência.* 631 pp.

Plastino, E.M. 2004. Diversidade intraespecífica em gracilarióides. Tese de Livre-Docência. Universidade de São Paulo. São Paulo.

Plastino, E.M., Oliveira, E.C. 1988a. Deviation in the life-history of *Gracilaria* (Rhodophyta, Gigartinales) from Coquimbo, Chile, Under Different Culture Conditions. Hydrobiologia (The Hague), v. 164, p. 67-74.

Plastino, E.M., Oliveira, E.C. 1988b. Sterelity barriers among species of *Gracilaria* (Rhodophyta, Gigartinales) from the São Paulo Littoral, Brazil - (BRITISH PHYCOLOGICAL JOURNAL). European Journal of Phycology, Glasgow, v. 23, p. 267-271.

Reis, R. P. & Yoneshigue-Valentin, Y. 1998. Variação espaço-temporal de populações de *Hypnea musciformis* (Rhodophyta, Gigartinales) na Baía de Sepetiba e Armação dos Búzios, RJ, Brasil. *Acta Botanica Brasilica*, 12 (3): 465-483.

Reis, R. P., Caldeira, A.Q., Miranda, A.N.S., Barros-Barreto, M.B. 2006. Mariculture potential of carragenophyta *Hypnea musciformis* (Wulfen) J.V. Lamouroux (Gigartinales - Rhodophyta) on Marambaia Island, Baía de Sepetiba, Rio de Janeiro State, Brazil. *Acta Bot. Bras.*, Oct./Dec. 2006, vol.20, no.4, p.763-769. ISSN 0102-3306.

Rueness, J. 1973. Speciation in *Polysiphonia* (Rhodophyceae, Ceramiales) in view of hybridization experiments. *P. hemisphaerica* and *P. boldii*. Phycologia 12: 107-109.

Sagert, S., Forster, R.M., Feuerpfeil, P. & Schubert, H. 1997. Daily course of photosynthesis and photoinhibition in *Chondrus crispus* (Rhodophyta) from different shore levels. Eur. J. Phycol. 32:363‑371.

Saunders, G.W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philosophical Transactions of the Royal Society B.* 360: 1879-1888. DOI: 10.1098/rstb.2005.1719

Schenkman, R.P.F. 1986. Cultura de *Hypnea* (Rhodophyta) *in vitro* como subsídio para estudos morfológicos, reprodutivos e taxonômicos. *Tese de Doutorado*. Instituto de Biociências, Universidade de São Paulo. 81 pp.

Ursi, S. & Plastino, E.M. 2001. Crescimento in vitro de linhagens de coloração vermelha e verde clara de *Gracilaria* sp. (Gracilariales, Rhodophyta) em dois meios de cultura: análise de diferentes estádios reprodutivos. Rev. Bras. Bot. 4: 585-592.

Waaland, J.R., Waaland, S.D. & Bates, G. 1974. Chloroplast structure and pigment composition in the red alga *Griffithsia pacifica*. J. Phycol. 10:193‑199.

Yokoya, N.S., Plastino, E.M. & Artel, R. 2003. Physiological responses and pigment characterization of two colour strains of the carrageenophyte *Hypnea musciformis* (Rhodophyta). In: Chapman ARO, Anderson RJ, Vreeland VJ, Davison IR (eds) Proc 17th Int Seaweed Symp, Oxford University Press, New York, pp 425‑433.

Zardi, G.I., Nicastro, K.R., Canovas, F., Ferreira Costa, J., Serrão, E.A. & Pearson, G.A. 2011. Adaptive traits are maintained on steep selective gradients despite gene flow and hybridization in the intertidal zone. PLoS One, 6: e19402.

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

Zuccarello, G.C. & West, J. A. 2002. Phylogeography of the *Bostrychia calliptera-B. pinnata* complex (Rhodomelacea, Rhodophyta) and divergence rates based on nuclear, mitochondrial and plastid DNA markers. Phycologia 41(1): 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. Journal of Phycology, 39: 948–959.

CAPÍTULO *Sete*

Species dilemma: a case study on the genus Hypnea (Gigartinales, Rhodophyta) using multiple approaches

Capítulo 7

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Species dilemma: a case study on the genus *Hypnea* (Gigartinales, Rhodophyta) using multiple approaches.

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***Abbreviations*:** COI-5P, 5' end of the cytochrome c oxidase 1 gene; NJ, neighbor joining; *rbc*L, gene encoding for the large subunit of the enzyme ribulose-1, 5-bisphosphate carboxylase-oxygenase.

Abstract

Hypnea pseudomusciformis was recently described based on DNA barcode marker COI-5P and the plastid *rbc*L gene in addition to morphological studies. In Brazil, *H. pseudomusciformis* is divided in two subclades, one with specimens from northeast region and the second with specimens from southeast, south and some specimens from northeast region. In this work, we use multiple approaches to understand the relationship between these two lineages. Samples from two populations, Bahia (Northeast region of Brazil) and São Paulo (Southeast region) were collected and kept in unialgal cultures. I*n vitro* crossing tests were conducted between then, along with growth rates and pigment content. Growth rates were similar between Bahia and São Paulo specimens, but differences were observed in the content of all photosynthetic pigments, along with divergence for COI-5P marker (X – Y%). Crossing tests among gametophytes of the two lineages resulted in the formation of cystocarps, carpospores

were released but did not germinate. We conclude that a post-fertilization barrier is present for those two lineages and that they represent two different species concerning the Biological Species Concept. Furthermore, relatively lower divergences in the DNA barcode COI-5P, which have been considered intraspecific variation, have to be seen with caution for other red algae groups. If speciation is occurring under similarly lower divergence values for COI-5P, we are greatly underestimating red algae diversity.

Keywords *Hypnea*, DNA Barcode, Crossing experiments, Pigments, Species delimitation

Introduction

Defining and delimiting species have been long debated in the literature, particularly for algae these have been complex and difficult issues (Guiry 1992, John & Maggs 1997, Mann 1999, Hey, 2006; Wilkins, 2009, Mann 1999, 2010). Many algal species are known to exhibit substantial intraspecific morphological variation, either as a result of genetically controlled polymorphism or environmentally induced plasticity (Lurling 2003, Logares et al. 2007). Ignoring this intraspecific morphological variation may result in an overestimation of species diversity (Trainor 1998, Macaya & Zuccarello 2010). On the other hand, morphological differences between two species will often only emerge after enough time has passed since lineage divergence, and therefore recently diverged species are likely to remain undetected (Leliaert et al. 2014).

Nowadays, species delimitation on macroalgae is based on molecular data combined with at least some information about the morphology, ecology or (eco-) physiology of the organisms. Whereas molecular and phylogenetic techniques are usually explained in detail, the criteria or methods used to delimit species based on sequence data are rarely specified (Leliaert et al. 2014).

The DNA barcode technique, especially with respect to the COI-5P marker, proved to be very useful and essential in the identification and delimitation of the species, revealing scenarios that would go unnoticed by morphology alone (Hebert et al. 2003, Saunders 2005). In that technique, species are delimited based on a ratio of intra

versus interspecific divergence, and the distance between the intraspecific and the iterspecific divergences is called the barcode gap (Hebert et al. 2004).

A good example on how species delimitation is made based on DNA barcode is the taxonomy of Brazilian species of the red algae *Hypnea* J.V. Lamouroux (1813). Like many other algae, *Hypnea* exhibit substantial intraspecific morphological variation (Jesus et al. 2015, 2016; Nauer et al. 2014, 2015, 2016) and some taxa in the genus are treated as complex due to the difficult separation of species, such as the complex *H. musciformis-pseudomusciformis* (Nauer et al. 2015, Jesus 2017). While others taxa, such *H. edeniana* and *H. yokoyana* are morphologically indistinguishable from others species of *Hypnea* (Nauer et al. 2014, 2016).

Nauer *et al.* (2015) described that the Brazilian specimens of *H. pseudomusciformis* were further divided into two groups, one formed by sequences from specimens collected in the northeast region of the country and the other formed by sequences from the southeast and south regions, which also includes a few samples collected in the northeast. The divergence for COI-5P marker between these groups varied from 1% to 1.8 %. These level of divergence in COI-5P has been considered within ranges of intraspecific variation (REFs)

Among closely related species, levels of overlap between intra- and interspecific genetic distances are likely to be significant (Hamsher et al. 2011, Hoef- Emden 2012) and with increased geographic sampling, barcoding gaps usually blur (Meyer & Paulay, 2005; Bittner et al., 2010; Bergsten et al., 2012). As a result, distance and tree-based methods often rely on intuition to decide whether sequence divergence is high enough or branches are long enough to consider a clade a distinct species.

To overcome difficulties associated with morphological and molecular data, the biological species concept has been applied to assess species boundaries in some algal groups, including diatoms (Mann, 2010), and some red, green and brown algae (Coleman, 1977; Zuccarello & West, 2003; Hiraoka et al., 2011; Maggs et al., 2011). The biological species concept is closely allied to the evolutionary species concept and focuses on reproductive isolation to distinguish species.

In this study, individuals for both molecular subclades (group I- northeast region and group I - southeast and south regions) were collected and placed in the same culture conditions. Crossing experiments between the molecular subclades were carried out in order to verify if the lineages were genetically compatible according to the biological

species concept. DNA barcode sequences, growth rates and photosynthetic pigments content were also analysed. We used the joint analyses of habitat, morphology, growth, pigment content, DNA barcode and crossing experiments to understand species delimitation in intraspecific diversity of *H. pseudomusciformis*.

Materials and methods

ALGAL MATERIAL

The material used in our experiments (Table 1) derived from two populations, one from the state of Bahia (BA), northeast region of Brazil, and the other from state of São Paulo (SP), southeast region. Three tetrasporophytic plants of *H. pseudomusciformis* were collected in Subaúma beach, north area of Salvador, BA, (group I) (Figure 1). And three tetrasporophytic plants of *H. pseudomusciformis* were collected in Cigarras beach, São Sebastião, SP (group II) (the same speciemens used in the crossing experiment from chapter 6).

Apical branches (length 1 cm, total fresh mass ca. 1 mg) of this material were isolated and kept in unialgal culture under controlled conditions in 500 mL of sterilized seawater (32 psu) enriched with 50% von Stosch medium (Ursi & Plastino 2001), 14 h light: 10 h dark, 24 ± 1°C and 70 ± 5 µmol photons $m^{-2}s^{-1}$ (Osram 40W day light fluorescent tubes). The cultures were bubbled with compressed air for 30 min h^{-1}, and the medium was renewed weekly. *Hypnea* life cycle for both morphological variations was completed in 121 days and tetrasporophytic, female and male gametophytes were kept isolated in unialgal culture.

Table 1. Collection information of specimens analyzed in this work. *GB – GenBank accession numbers (to be added), - not sequenced.

Molecular Group	ID number	Collection site, Latitude, Longitude	Voucher	COI-5P*
Group I Northeast region	IBC2800	Subauma Beach. Subauma. BA. F. Nauer & L. M. Ayres. 01 October 2016. 16°09'22.85''S/38°56'53.80''O	***	***
	IBC2801	Subauma Beach. Subauma. BA. F. Nauer & L. M. Ayres. 01 October 2016. 16°09'22.85''S/38°56'53.80''O	***	***
	IBC2802	Subauma Beach. Subauma. BA. F. Nauer & L. M. Ayres. 01 October 2016. 16°09'22.85''S/38°56'53.80''O	***	***
Group II Southeast region.	IBC2806	Cigarras Beach. São Sebastião. SP. F. Nauer & L. M. Ayres. 30. Setpember. 2016. $23^0$43'41.38''S/$45^0$23'53.84''O	***	***
	IBC2807	Cigarras Beach. São Sebastião. SP. F. Nauer & L. M. Ayres. 30. Setpember. 2016. $23^0$43'41.38''S/$45^0$23'53.84''O	***	***
	IBC2808	Cigarras Beach. São Sebastião. SP. F. Nauer & L. M. Ayres. 30. Setpember. 2016. $23^0$43'41.38''S/$45^0$23'53.84''O	***	***

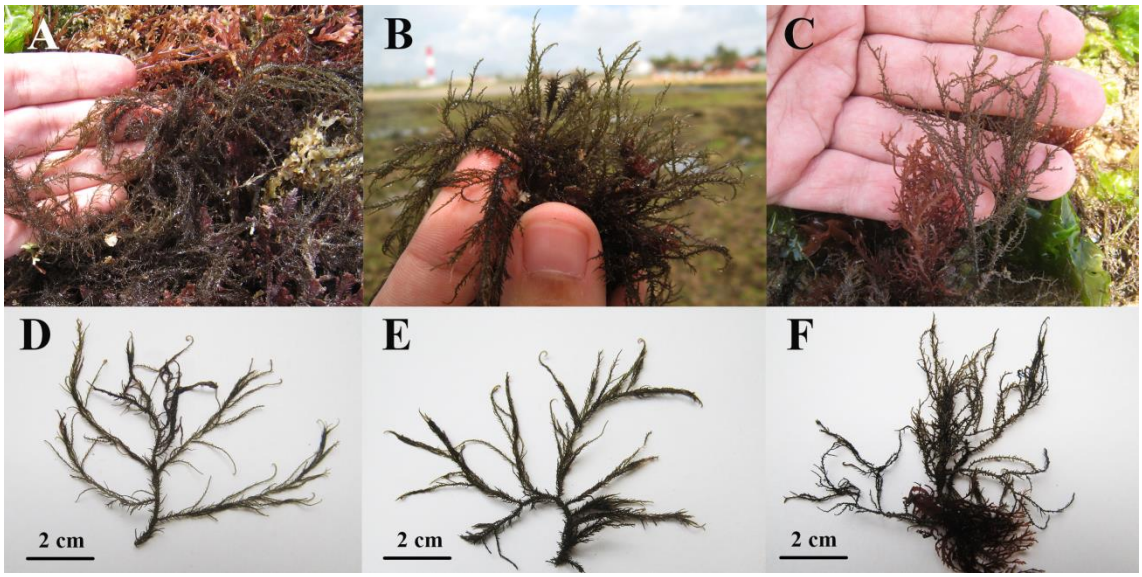

Figure 1. *Hypnea pseudomusciformis* from Bahia population. **A, B and C.** Three specimens growing in natural habitat. **D. E and F.** Habit of tetrasporophyte specimens from field.

CROSSING EXPERIMENT

For the crossing experiment, between BA and SP populations, three male gametophytes and three females of each population were used (IBC2806, IBC2807, IBC2808 from SP population and IBC2800, IBC2801, IBC2802 from BA population). For each female gametophyte (♀), apical segments were incubated in Erlenmeyer flasks with 500 ml of medium in three distinct experimental settings: 1) Three ♀ alone, to prevent the formation of unfertilized cystocarps (negative control). 2) Three ♀ with three apical segments of male gametophytes (♂) of the same group (positive control). 3) Three ♀ with three ♂ of different group (crossing test). Each setting was repeated three times, one for each female gametophyte, resulting in the use of 18 flasks (Figure 2).

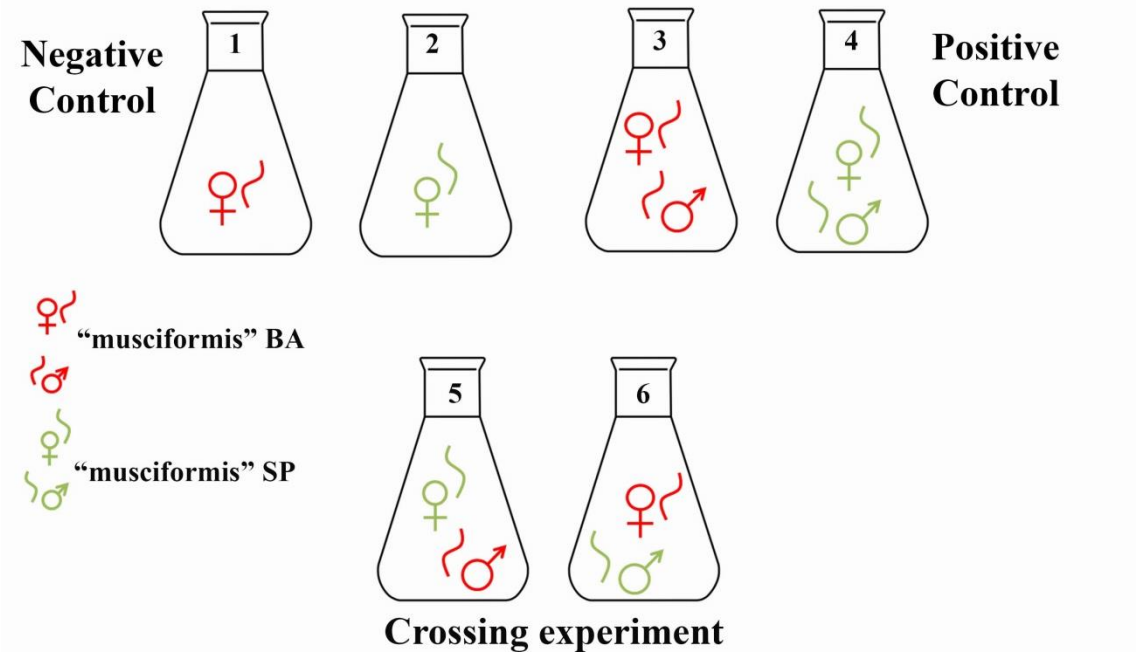

Figure 2. Crossing experiment between *H. pseudomusciformis* from Bahia and São Paulo.

GROWTH RATES

Apical branches (length 1 cm, total fresh weight ca. 1 mg) of female gametophytes and of unfertile tetrasporophytes (n=3) were cultivated for 4 weeks in 500 ml of enriched seawater in the standard conditions described above. The biomass was recorded weekly. Branches were gently blotted dry and weighted weekly on an analytical balance Mettler – Toledo AE 200S. Growth rates (GR) were assessed weekly according to Lignell and Pedersén (1989). A two-way analysis of variance was performed using the software STATISTICA 7.0 (StatSoft, Inc.), considering the time and life-history stages as independent variables and the growth rates as dependent variables. This analysis was followed by a Newman-Keuls a posteriori test.

PHTOSYNTHETIC PIGMENTS AND TOTAL SOLUBLE PROTEINS

The content analyzes of photosynthetic pigments and total soluble proteins (TSP) were performed from samples of 70 mg FW. Extraction of photosynthetic pigments was performed according to Kursar et al. (1983), adapted by Wanderley (2009). In the first step, the samples were ground in liquid nitrogen and extracted in 1 mL of 50 M sodium phosphate buffer pH 5.5 for 1 h at 4 °C, protected from light. After extraction, the samples were centrifuged and the supernatant transferred to a new microtube. This extract was called a buffered extract and used for phycobiliprotein and TSP analyzes. Absorbance readings (400 nm – 700 nm) were measured in a 96-well microplates with a final volume of 400 µL in a UV-vis spectrophotometer (Epoch 2-BioTek, USA). The levels of phycobiliproteins (mg.g FW^{-1}) were calculated according to Beer & Eshel (1985).

In the second stage of extraction, from the residue (pellet) obtained from the buffered extract, chlorophyll *a* and carotenoids were extracted by adding 1.5 ml of methanol for 3 h at 4 °C protected from light. Absorbance readings (400 nm – 700 nm) were measured in a 96-well microplates with a final volume of 400 µL in a UV-vis spectrophotometer (Epoch 2-BioTek, USA). The levels of chlorophyll *a* and carotenoids (mg.g FW^{-1}) were calculated according to Lichtenthaler & Buschmann (2001).

The method of quantification of TSP established by Bradford (1976) and used in this work, is based on the colorimetric reaction with the Coomassie Brilliant Blue G-250 acid. The buffered strata had the determined PST content based on the method proposed by Bradford (1976) and was made using the Bio-Rad colorimetric protein assay solution (Bio Rad, USA). The Bio Rad solution was prepared at the time of the 1: 4 v/v ratio in ultrapure water. The reaction was prepared by adding 40 L of ultrapure water, 900 L of the Bio Rad solution and 60 L of the buffered extract. The TSP content was expressed in g.g.FW^{-1} and the standard used was bovine serum albumin (BSA, Sigma, Brazil). The standard curve was constructed in the concentration range of 0-16 g.ml. Absorbance readings of both standard and samples were conducted in 96-well microplates with a final volume of 400 µL at 595 nm wavelength in a UV-vis spectrophotometer (Epoch 2-BioTek, USA).

MOLECULAR PROCEDURES

Fragments of each plant used in growth rate and crossing experiments, were macerated in liquid nitrogen, and DNA was extracted using the NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany), following the kit protocol. Amplification for COI-5P marker was performed using a PCR Master Mix (Promega Corp., Madison, WI, USA) with the primers GazF1 and GazR1 and cycles described by Saunders (2005). The PCR products were purified using the column GTX™ PCR DNA and Gel Band Purification Kit (GE Healthcare, Buckinghamshire, UK), quantified using NanoDrop 2000 (Wilmington, DE,USA), and directly sequenced with the same primers used in PCR in a 3730 DNA Sequencer (Applied Biosystems, Carlsbad, CA, USA).

A total of 60 COI-5P sequences with 466 nucleotides (nt) of *Hypnea pseudomusciformis* species (15 new and 45 from GenBank), were aligned using Clustal-W within BioEdit 7.0 sequence editor (Hall, 1999) and checked visually. Neighbor-Joining (NJ) analysis was performed under heuristic search with 2000 bootstrap replicates (Felsenstein 1985), using PAUP 4.0b10 (Swofford 2002). *Calliblepharis ciliata* (Greville) Kützing (DQ442896) were used as outgroup for COI-5P.

DATA ANALYSIS

The data was tested for normality (Kolmogorov-Smirnov) and homoscedasticity (Bartlett's test) and subsequent one-way or two-way ANOVA ($p < 0.05$). To determine significant differences was used th *post-hoc* Newman-Keuls test ($p < 0.05$). Before the analysis of variance the data in percentage were transformed into arcsen [square root (x/100)].

Results

DNA BARCODE DIVERGENCES

The COI-5P (446 bp) analysis of 60 samples of *H. musciformis – H. pseudomusciformis* complex (Table 1S) from the entire coast of Brazil and several other countries, incuding the type locality of *H. musciformis*, Italy showed the interspecific divergence of 5.9 to 7.1 %. Intraspecific variation was 0-9 bp (0-1.8%). Therefore, no overlap between intraspecific and interspecific variation was observed.

The Brazilian specimens were further divided into two groups (Figure 3), one formed by sequences from specimens collected in the northeast region of the country (group I) with high bootstrap support and the other formed by sequences from the southeast and south regions, which also includes a few samples collected in the northeast (group II). The divergence between group I and group II was from X% to 1.8 %. The divergence within group I was 0.2 to 0.3% and within group II was 0.3 to 0.5%.

The samples from Subaúma, Bahia state, used in the crossing experiments are in group I, and the divergence found between these samples and others samples from northeast region of Brazil was 0.3%. The samples from São Paulo state, also used in the crossing expriments, grouped with group II, and the divergence found between these samples and others from southeast and south regions was also 0.3%. However, between samples from Bahia and São Paulo the divergence in COI-5P was 1.3 to 1.8%.

CROSSING EXPERIMENT

For the crossing experiment, between BA and SP populations of H. *pseudomusciformis*, cystocarps were observed after X days (Figure 4) in all posivite controls and crossing flasks (Table 2). Carpospores were released, however, the carpospores from the crosses between gametophytes from group I and II did not survived and F1 tetrasporophytes plants were not observed. For the positive controls (crosses within each group) F1 tetrasporophytes were formed and kept *in vitro*.

GROWTH RATES

Analysis comparing tetrasporophytes from lineages from both SP and BA populations found no significant statistical differences (Figure 5).

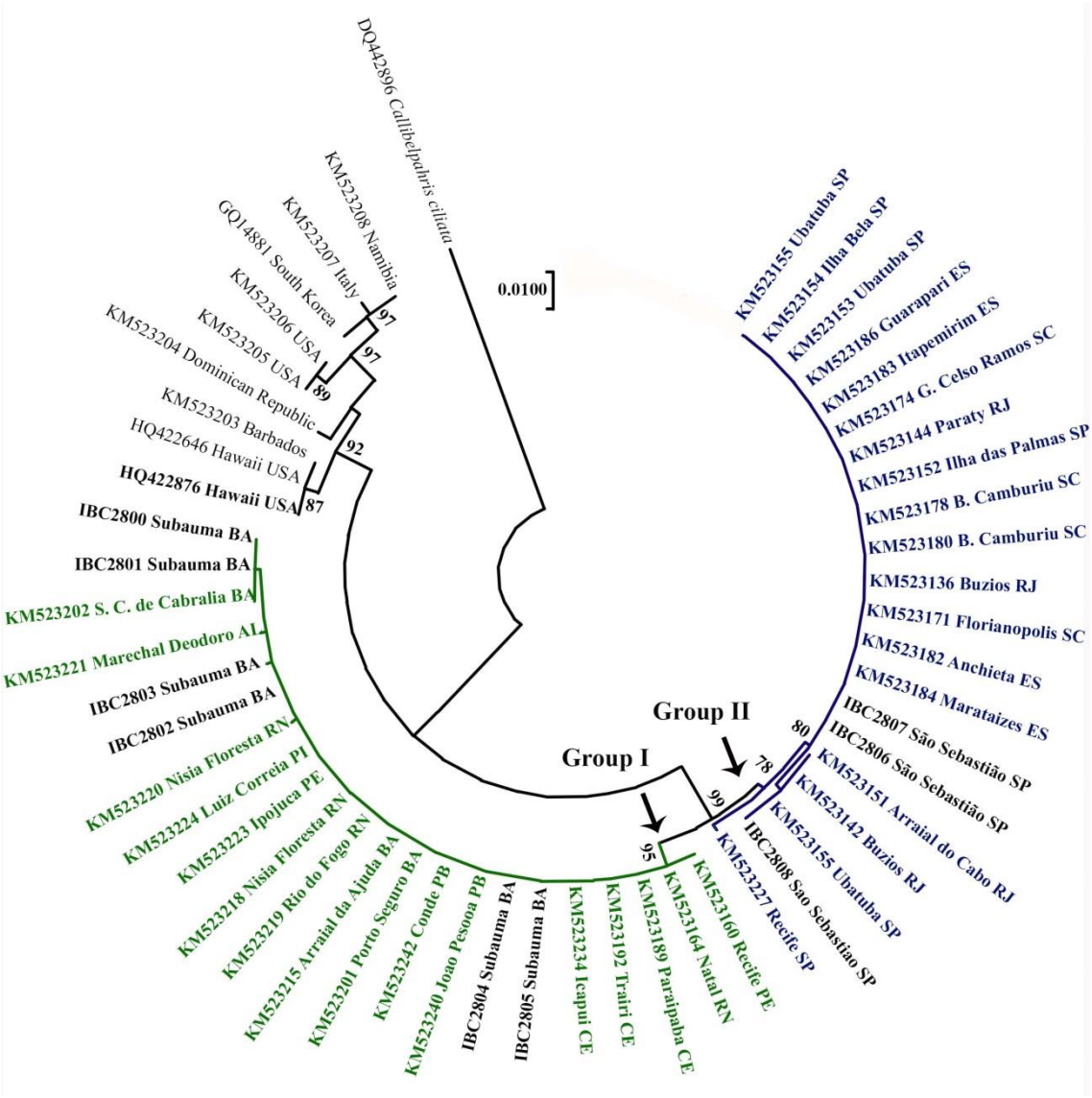

Figure 3. Neighbor-Joining (NJ) analysis for COI-5P sequences for *Hypnea musciformis-H. pseudomusciformis* complex. *Calliblepharis ciliata* was used as outgroup. On the branches are the bootstrap values for 2000 replicates. Sequences generated in this work are in bold and in black. Accession numbers and country of origin, when available, are included for sequences obtained from GenBank.

Table 2. Crossing experiment results between group X from São Paulo with group Y Bahia. **M1** = BA population and *M1*=SP population.

	CROSS EXPERIMENTS			
	Bahia x São Paulo			
	Flasks	**Gametophytes**	**Cystocarp**	**Carpospores status**
Negative Control	**1**	♀**M1**	Absent	-
	2	♀**M2**	Absent	-
	3	♀**M3**	Absent	-
	4	♀*M1*	Absent	-
	5	♀*M2*	Absent	-
	6	♀*M3*	Absent	-
Positive Control	**7**	♀**M1** x **M1**♂	**Present**	viable
	8	♀**M2** x **M2**♂	**Present**	viable
	9	♀**M3** x **M3**♂	**Present**	viable
	10	♀*M1* x *M1*♂	**Present**	viable
	11	♀*M2* x *M2*♂	**Present**	viable
	12	♀*M3* x *M3*♂	**Present**	viable
Crossing	**13**	♀**M1** x *M1*♂	**Present**	Not viable
	14	♀**M2** x *M2*♂	**Present**	Not viable
	15	♀**M3** x *M3*♂	**Present**	Not viable
	16	♀*M1* x **M1**♂	**Present**	Not viable
	17	♀*M2* x **M2**♂	**Present**	Not viable
	18	♀*M3* x **M3**♂	**Present**	Not viable

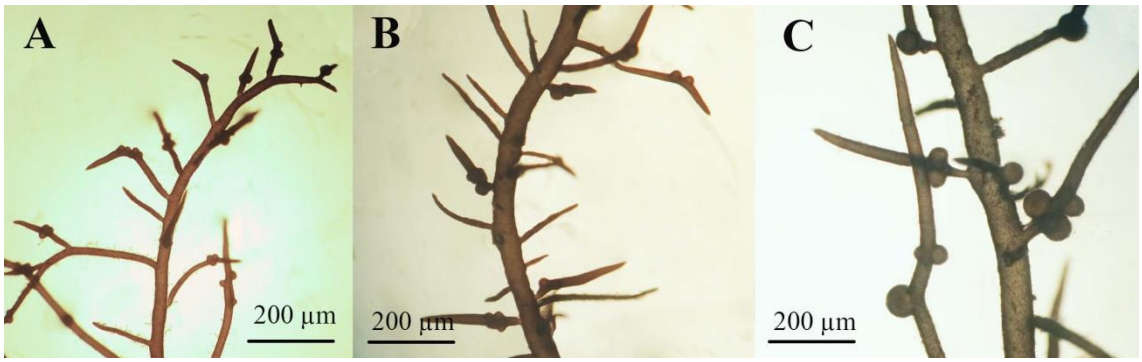

Figure 4. Crossing experiment results. A, B and C. Cystocarps formed in the crosses between group I and group II gametophytes.

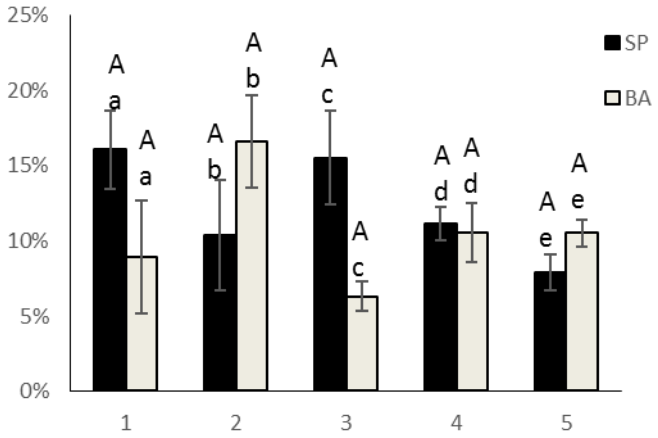

Figure 5. Growth rates of *H. pseudomusciformis* tetrasporophytes from Bahia compared with tetrasporophytes from São Paulo.

PHTOSYNTHETIC PIGMENTS

Photosynthetic pigments content varied between tetrasporophytes of BA population and tetrasporophytes of SP population. For phycobiliproteins (Figure 6), tetrasporophytes from SP showed higher concentration for phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC). While tetrasporophytes from BA showed higher concentration for chlorophyll *a* and carotenoids (Figure 7).

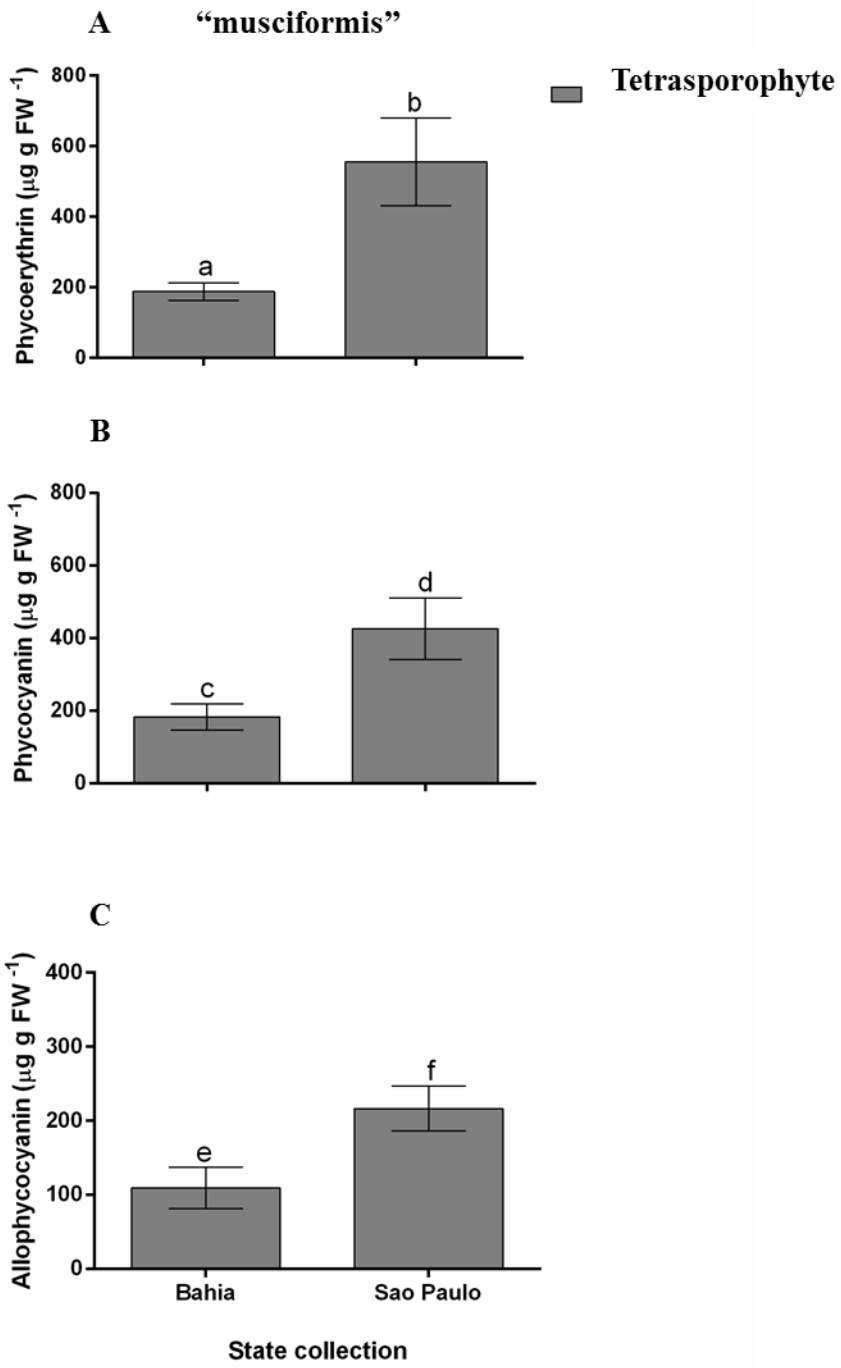

Figure 6. Comparing phycobiliprotein of *H. pseudomusciformis* from BA and SP. **A.** Phycoerythrin. **B.** Phycocyanin. **C.** Allophycocyanin. The bars correspond to the mean ± standard deviation (n = 5). Different letters represent significant differences ($p < 0.05$).

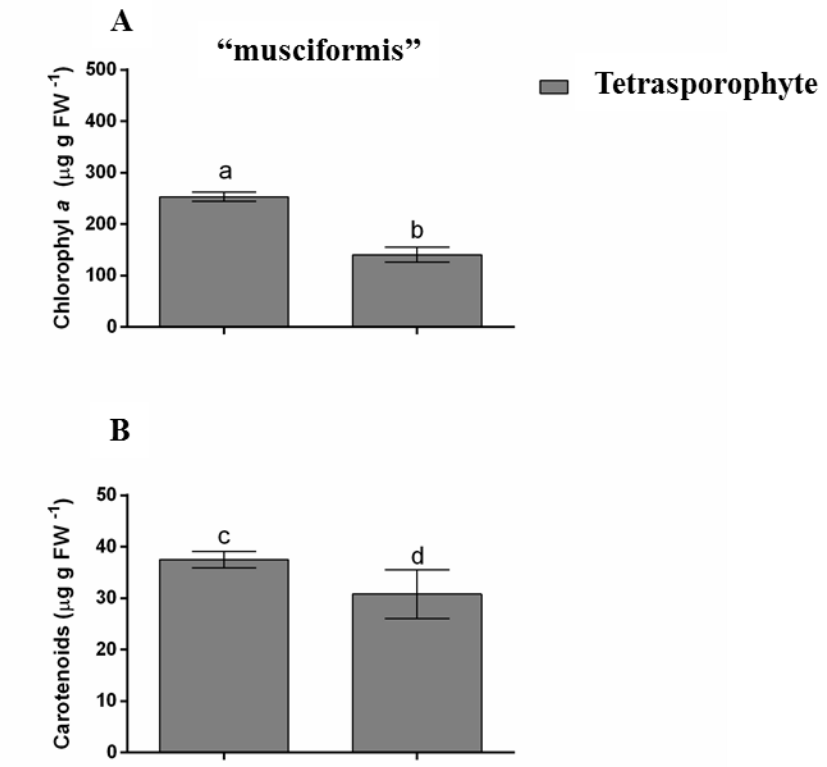

Figure 7. Comparing chlorophyll a, carotenoids and soluble proteins of *H. pseudomusciformis* from BA and SP. **A.** Chlorophyll a. **B.** Carotenoids. The bars correspond to the mean ± standard deviation (n = 5). Different letters represent significant differences ($p < 0.05$).

Discussion

Divergence between Bahia, representing the Group I from northeast region of Brazil, and São Paulo, representing Group II from southeast and south, was 1.3 to 1.8% for COI-5P marker, the same values found by Nauer et al. 2015. For the genus *Hypnea*, the COI-5P marker was first used by Geraldino et al. (2006), who sequenced 1,422 bp of 21 samples in the study of *H. flexicaulis* for the coast of South Korea. Subsequently, Geraldino et al. (2009) used the marker in the study of *H. charoides* for the Asian region, 44 sequences of 1,362 bp were generated for four species of *Hypnea*, in this case, the intraspecific variation found was up to 28 bp (2.06%) and interspecific divergence of 157 to 165 bp (11.53 - 12.12%). In 2010, Geraldino et al. expanded the coverage of the study to 10 species of the genus, with a total of 27 sequences of 1,245 bp. The intraspecific divergence found was 0 to 17 bp (0 - 1.3%) and the interspecific divergence was 138 to 162 bp (11 - 13%).

Nauer et al. (2014, 2015 and 2016) obtained a total of COI-5P sequences of 710 bp for nine Brazilian species of the genus. The intraspecific divergence found was 0 to 14 bp (0 - 1.7%) and the interspecific divergence found was 69 to 130 bp (9.8 - 18.2%). The greatest intraspecific divergence of 1.7%, found refers to the divergence between the Northeastern and Southeastern and Southern lineages of *H. pseudomusciformis*, similar to the values of the divergences obtained for the COI marker in this study. These values are also relatively close to those proposed by Saunders (2005) for red algae and by Geraldino et. al. (2009, 2010) for the *Hypnea* Asian sequences. The exception is in the maximum values of intraspecific divergences (1.7% vs. 1.3% by Geraldino et al. 2010) and interspecific differences (18.2% versus 13.6% by Saunders 2005).

Based on DNA barcode gap for *Hypnea*, this divergence of 1.8% between group I and group II of *H. pseudomusciformis* could be considered intraspecific variation. However, the results from the crossing experiment between BA (group I) and SP (group II) which led to unviable carpospores puts in check this barcoding gap values. The establishement of reproduction isolation between these 2 lineages, even with relatively lower values for COI-5P divergences, indicate that group I and group II could be considered distinct species, based on biologial species concept.

Clarkston & Saunders (2010) used the COI-5P marker in the study of the family Kallymeniaceae (Gigartinales, Rhodophyta) and the intraspecific divergence for this marker ranged from 0 to 0.9%, while the divergence between species were greater than 4.5%. If speciation is occurring under similarly lower divergence values for COI-5P, we are greatly underestimating red algae diversity

Crossing experiments resulting in cystocarps formation, but unviable carpospores, have been documented for Rhodophyta before. Similar results were found by Brodie et al. (1993) in crossing tests between several strains of *Chondrus ocellatus* f. *ocellatus* Okamura and *C. ocellatus* f. *crispoides* Mikami. In one cross, they found dark structures superficially resembling immature cystocarps, but these did not develop into mature carposporophytes and no carpospores were released. In another cross, developed cystocarps were obtained, but no spores were released and the cystocarps eventually degenerated. However, occasionally, some crossing could yield carpospores that sometimes developed into viable tetrasporophytes.

The formation of structures resembling cystocarps that do not reach maturity to form carpospores has been reported in crosses of other red algae. Bird & McLachlan

(1982), in an investigation of crossability between strains and species of *Gracilaria*, referred to them as 'pseudocystocarps', but did not consider these structures to indicate affinities between species as they sometimes formed parthenogenetically on isolated females. Similar structures were also recorded by Guiry et al. (1987) when a female strain of *Gigartina intermedia* Suringar from Japan was crossed with male plants of *Gigartina teedii*. Guiry et al. (1987) suggested that the formation of such structures was indicative of an evolutionary link between the two species, perhaps at the generic level. Hommersand et al. (1993) have since proposed the transfer of these two entities to the genus *Chondracanthus*.

Plastino & Oliveira (1988a and 1988b) attempted hybridization of five diferent species of *Gracilaria* growing sympatricatly, as well as crossings among allopatric populations of the same species. In that study, monospecific crossings were positive even between isolated populations, with production of cystocarps and carpospores. Interspecific crossings were negative. However a pseudocystocarp was produced by female *G. mammillaris* in the presence of male branches of *G. cervicornis.* These cystocarpic-like structures did not produce carpospores and a section showed only an empty cavity surrounded by a pericarp. The authors concluded that the presence of sterility barriers in species of *Gracilaria* was expected for many of the Brazilian species that occur sympatrically. Such sterility barriers were also noticed in the Caribbean (Hay & Norris, 1984). These results, including the ones presented here indicate, show that crosses of different morphs or lineages can be a valuable tool in recognizing specific entities in difficult genus.

Plastino & Oliveira (1996) were not able to cross *Gracilaria verrucosa* from Argentina with a species cultured from Baja California, known locally as *G. verrucosa* (Norris, 1985). However, populations separated by thousands of kilometers, for example, from southeastern Brazil and Venezuela, can cross and produce viable F1 generation. Having in mind the results of our second crossing experiment, between specimens distant kilometers from each other (distance between SP and BA is about 1500 km), were negative despite the appearence of cystocarps, could be a indicator that the molecular subclades of *H. pseudomusciformis* represent two different lineages in process of speciation.

Reproductive barriers between sexually reproducing algal species are not always complete (Brodie et al., 1993; Coyer et al., 2002; Kamiya, 2004; Casteleyn et al., 2009; Zardi et al., 2011). Moreover, laboratory crosses also only take into account intrinsic

reproductive barriers and not extrinsic barriers, such as differences in timing of sexual reproduction or ecological preferences (Leliaert et al. 2014). Because of this, studies in physiological responses between specimens from BA and SP were made and, while the comparison of growth rates results showed similar growth rates of BA and SP populations; differences were found in all photosynthetic pigments content varied between BA and SP populations, despite growing under the same culture conditions. Specimens of SP showed higher concentration for phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC). While BA specimens showed higher concentration for chlorophyll *a* and carotenoids.

Our molecular, cross-breeding and pigment content data indicate that the two subclades of *H. pseudomusciformis* are under speciation and are separately evolving population lineages. What is important here is if those lineages could be named as different species already. Although many of the species concepts are related, none is completely satisfactory (Leliaert et al. 2014), and most are at least partially incompatible in that they can lead to different conclusions regarding species boundaries (Mallet, 1995). There is fairly general agreement that species are principal units in biology, and that there is a common evolutionary idea underlying them: speciation results from isolation of populations by interrupted gene flow, resulting in divergence due to selection and drift, and ultimately in separately evolving metapopulation lineages (Coyne & Orr, 2004). When two lineages separate, they will eventually acquire different properties (e.g. become sexually incompatible or morphologically distinct), which may serve as diagnostic evidence relevant to species delimitation. During the process of speciation these secondary properties do not necessarily arise at the same time or in a regular order, or may not arise at all, so conflict will occur between species concepts in many cases, especially between recently formed species (Leliaert et al. 2014).

While the unviability of carpospores could indicate a barrier in reproduction between group I and II, its importante to notice the group II also contains some sequence/specimens from northeast region (Nauer et al. 2015) and information about gene flow between those two groups in nature are unknow. Incomplete lineage sorting is associated with the persistence of ancestral alleles in recently diverging lineages, gene trees will inevitably go through phases where individuals of a species are polyphyletic and paraphyletic before becoming monophyletic as alleles become fixed through time (Avise & Ball, 1990; Klein et al., 1998; Rosenberg & Nordborg, 2002). Clearly, this

process has important consequences for delimitation of recently diverged species. Most importantly, it implies that reciprocal monophyly is not a necessary property of species, especially for recently diverged species (Kizirian & Donnelly, 2004; Knowles & Carstens, 2007). Simulation studies have indicated that a substantial amount of time (i.e. number of generations) may be required after lineage divergence before there will be a high probability of observing monophyly for a given locus, which is considered to depend on its effective population size (Avise, 2000; Hudson & Coyne, 2002; Hickerson et al., 2006).

Because speciation is a process and not a single event in time (Hey & Pinho, 2012), uncertainty about species boundaries is inevitable in recently diverged lineages (Leliaert et al. 2014). We believe that joint analysis of molecular, physiology and cross data lead us to a further refinements in our understanding of the scenario of *H. pseudomusciformis* as two close related species instead of one (being *H. pseudomusciformis* and *Hypnea* sp. 5), consequently, the barcoding gap for the genus *Hypnea* based on COI-5P marker reach very low values, with 1.3% enough to separate species. This results affects *Hypnea* species diversity and integration of population genetic, phylogenetic methods and cross-breeding are required to disentangle complications caused by processes like incomplete lineage sorting and polymorphism.

Conclusion

Several researchers have advocated that adequate delimitation of species should be based on additional lines of evidence, including morphological, ultrastructural, biochemical, geographic, ecological and/or breeding data (e.g. Pröschold et al. 2001, Walker et al. 2009, Bendif et al. 2011, Milstein & Saunders 2012). Based on our molecular, cross-breeding and pigment content results bewteen two linaeges of *H. pseudomusciformis* on Brazilian coast, we conclude those linaeges are already two separately evolved species that suffered recent speciation. To be better understand *Hypnea* species diversity, multimarker datasets, and integration of population genetic and phylogenetic methods are necessary to delimate species boundaries on genus *Hypnea*, especially in recently diverged lineages.

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References

Apt, K.E., Metzner S. & Grossman, A.R. 2001. The ɤ subunits of phycoerythrin from a red alga: position in phycobilisomes and sequence characterization. J. Phycol. 37:64-70

Avise, J.C. & Ball, R.M. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Oxford Surveys in Evolutionary Biology, 7: 45–67.

Beer, S. & Eshel, A. 1985. Determining phycoerythrin and phycocyanin concentrations in aqueous crude extracts of red algae. Mar. Freshw. Res. 36(6):785-792.

Bendif, E.M., Probert, I., Herve, A., Billard, C., Goux, D., Lelong, C., Cadoret, J.-P. & Veron, B. 2011. Integrative taxonomy of the Pavlovophyceae (Haptophyta): a reassessment. Protist, 162: 738–761.

Bergsten, J., Bilton, D.T., Fujisawa, T., Elliott, M., Monaghan, M.T., Balke, M., Hendrich, L., Geijer, J., Herrmann, J., Foster, G.N., Ribera, I., Nilsson, A.N., Barraclough, T.G. & Vogler, A.P. 2012. The effect of geographical scale of sampling on DNA barcoding. Systematic Biology, 61: 851–869.

Bittner, L., Halary, S., Payri, C., Cruaud, C., De Reviers, B., Lopez, P.& Bapteste, E. 2010. Some considerations for analyzing biodiversity using integrative metagenomics and gene networks. Biology Direct, 5: 47.

Brodie, J., Guiry, M.D. & Masuda, M. 1993. Life history, morphology and crossability of *Chondrus ocellatus* forma *ocellatus* and *C. ocellatus* forma *crispoides* (Gigartinales, Rhodophyta) from the north-western Pacific. Eur. J. Phycol. 28:183-196.

Coleman, A.W. 1977. Sexual and genetic isolation in cosmopolitan algal species Pandorina morum. American Journal of Botany, 64: 361–368.

Casteleyn, G., Adams, N.G., Vanormelingen, P., Debeer, A.E., Sabbe, K. & Vyverman, W. 2009. Natural hybrids in the marine diatom *Pseudo-nitzschia pungens* (Bacillariophyceae): genetic and morphological evidence. Protist, 160: 343–354.

Clarkston, B.E. & Saunders, G.W. 2010. A comparison of two DNA barcode markers for species discrimination in the red algal family Kallymeniaceae (Gigartinales,

Florideophyceae), with a description of *Euthora timburtonii* sp. nov. Botany 88: 119–131 (2010). doi:10.1139/B09-101

Coyer, J.A., Peters, A.F., Hoarau, G., Stam, W.T. & Olsen, J.L. 2002. Hybridization of the marine seaweeds, *Fucus serratus* and *Fucus evanescens* (Heterokontophyta: Phaeophyceae) in a 100-year-old zone of secondary contact. Proceedings of the Royal Society B–Biological Sciences, 269: 1829–1834.

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

De Queiroz, K. 2007. Species concepts and species delimitation. Systematic Biology, 56: 879–886.

Edwards, P. 1970. Attempted hybridization in the red alga Polysiphonia. Nature 226: 467-468.

Geraldino, P.J.L., Yang, E.C. & Boo, S.M. 2006. Morphology and molecular phylogeny of *Hypnea flexicaulis* (Gigartinales, Rhodophyta) from Korea. *Algae* 21: 417-423.DOI 10.4490/ALGAE.2006.21.4.417

Geraldino, P.J.L., Yang, E.C., Kim, M.S. & Boo, S.M. 2009. Systematics of *Hypnea asiatica* sp. nov. (Hypneaceae, Rhodophyta) based on morphology and nrDNA SSU, plastid rbcL, and mitochondrial cox1. Taxon 58:606–16.

Geraldino, P. J. L., Riosmena-Rodriguez, R., Liao, L. M. & Boo, S.M. 2010. Phylogenetic relationships within the genus *Hypnea* (Gigartinales, Rhodophyta), with a description of *Hypnea caespitosa* sp. nov. J. Phycol. 46:336–45.

Guimarães, M., Plastino, E. M. & Oliveira, E. C. 1999. Life-History, reproduction, and growth of *Gracilaria domingensis* (Gracilariales, Rhodophyta) from Brazil. Botanica Marina, v. 42, p. 481-486.

Guiry, M.D. 1992. Species concepts in marine red algae. In Round, F.E. & Chapam, D.J. (Eds). Progress in Phycological Research. Volume 8. Biopress Ltd Bristol., pp 251-278.

Hamsher, S.E., Evans, K.M., Mann, D.G., Poulickova, A. & Saunders, G.W. 2011. Barcoding diatoms: exploring alternatives to COI-5P. Protist, 162: 405–422.

Herbert P.D.N., Cywinska A., Ball S.L. & deWaard J.R. 2003. Biological identifications through DNA barcoding. Proceedings of the Royal Society of London, B, 270: 313-322.

Hey, J. 2006. On the failure of modern species concepts. Trends in Ecology and Evolution, 21: 447–450.

Hey, J. & Pinho, C. 2012. Population genetics and objectivity in species diagnosis. Evolution, 66: 1413–1429.

Hickerson, M.J., Meyer, C.P. & Moritz, C. 2006. DNA barcoding will often fail to discover new animal species over broad parameter space. Systematic Biology, 55: 729–739.

Hiraoka, M., Ichihara, K., Zhu, W., Ma, J. & Shimada, S. 2011. Culture and hybridization experiments on an *Ulva clade* including the Qingdao strain blooming in the Yellow Sea. PLoS One, 6:e19371.

Hoef-Emden, K. (2012). Pitfalls of establishing DNA barcoding systems in protists: the Cryptophyceae as a test case. PLoS One, 7: e43652.

Hudson, R.R. & Coyne, J.A. 2002. Mathematical consequences of the genealogical species concept. Evolution, 56: 1557–1565.

Jesus, P.B., Silva, M.S., Lyra, G.M., Nunes, J.M.C. & Schnadelbach, A.S. 2015. Extension of the distribution range of *Hypnea stellulifera* (Cystocloniaceae, Rhodophyta) to the South Atlantic: morphological and molecular evidence. *Aquatic Botany* 123: 26-36. DOI: 10.1016/j.aquabot.2014.12.003

Jesus, P. B., Nauer, F., Lyra, G. d. M., Cassano, V., Oliveira, M. C., Nunes, J. M. d. C. and Schnadelbach, A. S. 2016. Species-delimitation and phylogenetic analyses of some cosmopolitan species of *Hypnea* (Rhodophyta) reveal synonyms and misapplied names to *H. cervicornis*, including a new species from Brazil. J. Phycol., 52: 774–792. doi:10.1111/jpy.12436

John, D.M. & Maggs, C.A. 1997. Species problems in eukaryotic algae: a modern perspective. Chapter 5, pp. 83-107. in Species The Units of Biodiversity. Ed. Claridge, M.F., Dawah, H.A. & Wilson, M.R. 1997. Chapman & Hall. London.

Kamiya, M. 2004. Speciation and biogeography of the *Caloglossa leprieurii* complex (Delesseriaceae, Rhodophyta). Journal of Plant Research, 117: 421–428.

Klein, J., Sato, A., Nagl, S. & O'huigín, C. 1998. Molecular trans-species polymorphism. Annual Review of Ecology and Systematics, 29: 1–21.

Kizirian, D. & Donnelly, M. 2004. The criterion of reciprocal monophyly and classification of nested diversity at the species level. Molecular Phylogenetics and Evolution, 32: 1072–1076.

Knowles, L.L. & Carstens, B.C. 2007. Delimiting species without monophyletic gene trees. Systematic Biology, 56: 887–895.

Kursar, T.A., van der Meer, J.P. & Alberte, R.S. 1983b. Lightharvesting system of the red alga *Gracilaria tikvahiae*. II. Phycobilisome characteristics of pigment mutants. Plant. Physiol. 73:361‑369.

Leliaert, F., Verbruggen, H., Vanormelingen, P., Steen, F., López-Bautista, J.M., Zuccarello, G.C. & De Clerck, O. 2014. DNA-based species delimitation in algae, European Journal of Phycology, 49:2, 179-196, DOI: 10.1080/09670262.2014.904524

Logares, R., Rengefors, K., Kremp, A., Shalchian-Tabrizi, K., Boltovskoy, A., Tengs, T., Shurtleff, A. & Klaveness, D. 2007. Phenotypically different microalgal morphospecies with identical ribosomal DNA: a case of rapid adaptive evolution? Microbial Ecology, 53: 549–561.

Lichtenthaler, H.K. & Buschmann, C. 2001. Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy. Current Protocols in Food Analytical Chemistry F4.3.1-F4.3.8. DOI: 10.1002/0471142913.faf0403s01

Macaya, E.C. & Zuccarello, G.C. 2010. DNA barcoding and genetic divergence in the giant kelp macrocystis (Laminariales). Journal of Phycology, 46: 736–742.

Maggs, C.A., Fletcher, H.L., Fewer, D., Loade, L., Mineur, F. & Johnson, M.P. 2011. Speciation in red algae: members of the Ceramiales as model organisms. Integrative and Comparative Biology, 51: 492–504.

Mallet, J. 1995. A species definition for the modern synthesis. Trends in Ecology and Evolution, 10: 294–299.

Mann, D.G. 1999. The species concept in diatoms. Phycologia, 38:437–495.

Mann, D.G. 2010. Discovering diatom species: is a long history of disagreements about species-level taxonomy now at an end? Plant Ecology and Evolution, 143: 251–264.

Mayden, R.L. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. In Species: The Units of Biodiversity (Claridge, M.F., Dawah, H.A. & Wilson, M.R., editors), 381–424. Chapman and Hall, London.

Meyer, C.P. & Paulay, G. 2005. DNA barcoding: error rates based on comprehensive sampling. PLoS Biology, 3: 2229–2238.

Milstein, D. & Saunders, G.W. 2012. DNA barcoding of Canadian Ahnfeltiales (Rhodophyta) reveals a new species – Ahnfeltia borealis sp. nov. Phycologia, 51: 247–259.

Nauer, F.; Cassano, V. & Oliveira, M. C. 2014. *Hypnea* species (Gigartinales, Rhodophyta) from the southeastern coast of Brazil based on molecular studies complemented with morphological analyses, including descriptions of *Hypnea edeniana* sp. nov. and *H. flava* sp. nov. *European Journal of Phycology* 49 :550-575. DOI:10.1080/09670262.2014.981589.

Nauer, F.; Cassano, V. & Oliveira, M. C. 2015. Description of Hypnea pseudomusciformis sp. nov., a new species based on molecular and morphological analyses, in the context of the H. musciformis complex (Gigartinales, Rhodophyta). Journal of Applied Phycology 27: 2405-2417. DOI: 10.1007/s10811-014-0488-y

Nauer, F., Cassano, V. & Oliveira, M.C. 2016. *Hypnea wynnei* and *Hypnea yokoyana* (Cystocloniaceae, Rhodophyta), two new species revealed by a DNA barcoding survey on the Brazilian coast. Phytotaxa 268 (2): 123–134. http://dx.doi.org/10.11646/phytotaxa.268.2.3

Pereira, R. C. & Soares-Gomes, A. (Orgs.) 2009. Biologia marinha, 2ª Edição. *Rio de Janeiro: Ed. Interciência.* 631 pp.

Plastino, E.M. 2004. Diversidade intraespecífica em gracilarióides. Tese de Livre-Docência. Universidade de São Paulo. São Paulo.

Plastino, E.M., Oliveira, E.C. 1988a. Deviation in the life-history of *Gracilaria* (Rhodophyta, Gigartinales) from Coquimbo, Chile, Under Different Culture Conditions. Hydrobiologia (The Hague), v. 164, p. 67-74.

Plastino, E.M., Oliveira, E.C. 1988b. Sterelity barriers among species of *Gracilaria* (Rhodophyta, Gigartinales) from the São Paulo Littoral, Brazil - (BRITISH PHYCOLOGICAL JOURNAL). European Journal of Phycology, Glasgow, v. 23, p. 267-271.

Proschold, T., Marin, B., Schlosser, U.G. & Melkonian, M. 2001. Molecular phylogeny and taxonomic revision of *Chlamydomonas* (Chlorophyta). I. Emendation of *Chlamydomonas Ehrenberg* and *Chloromonas* Gobi, and description of *Oogamochlamys* gen. nov. and *Lobochlamys* gen. nov. Protist, 152: 265–300.

Reis, R. P. & Yoneshigue-Valentin, Y. 1998. Variação espaço-temporal de populações de *Hypnea musciformis* (Rhodophyta, Gigartinales) na Baía de Sepetiba e Armação dos Búzios, RJ, Brasil. *Acta Botanica Brasilica*, 12 (3): 465-483.

Reis, R. P., Caldeira, A.Q., Miranda, A.N.S., Barros-Barreto, M.B. 2006. Mariculture potential of carragenophyta *Hypnea musciformis* (Wulfen) J.V. Lamouroux (Gigartinales - Rhodophyta) on Marambaia Island, Baía de Sepetiba, Rio de Janeiro State, Brazil. *Acta Bot. Bras.*, Oct./Dec. 2006, vol.20, no.4, p.763-769. ISSN 0102-3306.

Rosenberg, N.A. & Nordborg, M. 2002. Genealogical trees, coalescente theory and the analysis of genetic polymorphisms. Nature Reviews Genetics, 3: 380–390.

Rueness, J. 1973. Speciation in *Polysiphonia* (Rhodophyceae, Ceramiales) in view of hybridization experiments. *P. hemisphaerica* and *P. boldii*. Phycologia 12: 107-109.

Sagert, S., Forster, R.M., Feuerpfeil, P. & Schubert, H. 1997. Daily course of photosynthesis and photoinhibition in *Chondrus crispus* (Rhodophyta) from different shore levels. Eur. J. Phycol. 32:363‑371.

Saunders, G.W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philosophical Transactions of the Royal Society B.* 360: 1879-1888. DOI: 10.1098/rstb.2005.1719

Schenkman, R.P.F. 1986. Cultura de *Hypnea* (Rhodophyta) *in vitro* como subsídio para estudos morfológicos, reprodutivos e taxonômicos. *Tese de Doutorado*. Instituto de Biociências, Universidade de São Paulo. 81 pp.

Trainor, F.R. 1998. Biological aspects of *Scenedesmus* (Chlorophyceae) phenotypic plasticity. Nova Hedwigia Beiheft, 117: 1–367.

Ursi, S. & Plastino, E.M. 2001. Crescimento in vitro de linhagens de coloração vermelha e verde clara de *Gracilaria* sp. (Gracilariales, Rhodophyta) em dois meios de cultura: análise de diferentes estádios reprodutivos. Rev. Bras. Bot. 4: 585-592.

Waaland, J.R., Waaland, S.D. & Bates, G. 1974. Chloroplast structure and pigment composition in the red alga *Griffithsia pacifica*. J. Phycol. 10:193‑199.

Walker, R.H., Brodie, J., Russell, S., Irvine, L.M. & Orfanidis, S. 2009. Biodiversity of coralline algae in the northeastern Atlantic including *Corallina caespitosa* sp. nov. (Corallinoideae, Rhodophyta). Journal of Phycology, 45: 287–297.

Wilkins, J.S. 2009. Species: A History of the Idea. University of California Press, Berkeley, CA.

Yokoya, N.S., Plastino, E.M. & Artel, R. 2003. Physiological responses and pigment characterization of two colour strains of the carrageenophyte *Hypnea musciformis* (Rhodophyta). In: Chapman ARO, Anderson RJ, Vreeland VJ, Davison IR (eds) Proc 17th Int Seaweed Symp, Oxford University Press, New York, pp 425‑433.

Zardi, G.I., Nicastro, K.R., Canovas, F., Ferreira Costa, J., Serrão, E.A. & Pearson, G.A. 2011. Adaptive traits are maintained on steep selective gradients despite gene flow and hybridization in the intertidal zone. PLoS One, 6: e19402.

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

Zuccarello, G.C. & West, J. A. 2002. Phylogeography of the *Bostrychia calliptera-B. pinnata* complex (Rhodomelacea, Rhodophyta) and divergence rates based on nuclear, mitochondrial and plastid DNA markers. Phycologia 41(1): 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. Journal of Phycology, 39: 948–959.

Table 1S. Sequences used in this work. GB, GenBank accession numbers.

Voucher	Species	Collection Site. Collector and Date	Country	GPS	COI GB Number
SPF57638	*H.* pseudo*musciformis*	Beach Peito de Moça. Luíz Correia. PI. R. Petti. 16. I. 2013	Brazil	$2^{o}54'14.51''S/41^{o}32'40.65''O$	KM523224
SPF57553	*H.* pseudo*musciformis*	Beach Lagoinha. Paraipaba. CE. F. Nauer. 02. VI. 2012	Brazil	$3^{o}20'42.37''S/39^{o}08'09.24''O$	KM523189
SPF57556	*H.* pseudo*musciformis*	Beach Flecheiras. Trairi. CE. F. Nauer. 03. VI. 2012	Brazil	$3^{o}13'05.62''S/39^{o}16'07.07''O$	KM523192
SPF57562	*H.* pseudo*musciformis*	Beach Ponta Grossa. Icapuí. CE. F. Nauer. 05. VI. 2012	Brazil	$4^{o}37'36.24''S/37^{o}29'57.87''O$	KM523234
SPF57448	*H.* pseudo*musciformis*	Beach Ponta Negra. Natal. RN. M.C. Oliveira & E. C. Oliveira. 17. V. 2011	Brazil	$5^{o}52'55.23''S/35^{o}09'52.39''O$	KM523164
SPF57625	*H.* pseudo*musciformis*	Beach Tabatinga. Nísia Floresta. RN. C. A. Azevedo & R. Menezes. 12. I. 2013	Brazil	$6^{o}04'12.15''S/35^{o}05'51.40''O$	KM523218
SPF57626	*H.* pseudo*musciformis*	Beach Rio do Fogo. Rio do Fogo. RN. C. A. Azevedo & R. Menezes. 12. I. 2013	Brazil	$5^{o}16'32.90''S/35^{o}22'41.76''O$	KM523219
SPF57627	*H.* pseudo*musciformis*	Beach Camurupim. Nísia Floresta. RN. C. A. Azevedo & R. Menezes. 12. I. 2013	Brazil	$6^{o}04'12.15''S/35^{o}05'51.40''O$	KM523220
SPF57570	*H.* pseudo*musciformis*	Beach Cabo Branco. João Pessoa. PB. F. Nauer. 18. VII. 2012	Brazil	$7^{o}08'44.75''S/34^{o}48'28.90''O$	KM523240
SPF57573	*H. pseudomusciformis*	Beach Carapibus. Conde. PB. F. Nauer & C. A. Azevedo. 19. VII. 2012	Brazil	$7^{o}17'59.63''S/34^{o}47'55.31''O$	KM523242
SPF57466	*H.* pseudo*musciformis*	Cabo de Santo Agostinho. Recife. PE. N. S. Yokoya & S. M.P.B. Guimarães. 27. IX. 2011	Brazil	$8^{o}18'13.10''S/34^{o}56'35.99''O$	KM523160
SPF57635	*H.* pseudo*musciformis*	Beach do Toquinho. Ipojuca. PE. N. S. Yokoya. 13.III.2013	Brazil	$8^{o}36'12.37''S/35^{o}02'42.25''O$	KM523223

SPF57628	*H.* pseudo*musciformis*	Beach do Francês. Marechal Deodoro. AL. T. Vieira-Pinto, C. A. Azevedo & B. Torrano-Silva. 10.III.2013	Brazil	$9^{o}46'07.79''S/35^{o}50'17.44''O$	KM523221
SPF57608	*H.* pseudo*musciformis*	Beach Coroa Vermelha. Santa Cruz de Cabrália. BA. F. Nauer & C. A. Azevedo. 15. IX. 2012	Brazil	$16^{0}19'57.02''S/39^{0}00'13.59''O$	KM523202
SPF57618	*H.* pseudo*musciformis*	Ponta Grande. Porto Seguro. BA. F. Nauer & C. A. Azevedo. 16. IX. 2012	Brazil	$16^{0}22'33.05''S/39^{0}00'33.86''O$	KM523201
SPF57624	*H.* pseudo*musciformis*	Beach Mucugê. Arraial D'Ajuda. BA. F. Nauer & C. A. Azevedo. 18. IX. 2012	Brazil	$16^{0}30'09.69''S/39^{0}04'17.62''O$	KM523215
SPF57521	*H.* pseudo*musciformis*	Beach dos Castelhanos. Anchieta. ES. F. Nauer, C. Iha & B. Torrano-Silva. 05. V. 2012	Brazil	$20^{0}50'04.18''S/40^{0}37'21.57''O$	KM523182
SPF57526	*H. pseudomusciformis*	Beach Itaoca. Itapemirim. ES. F. Nauer, C. Iha & B. Torrano-Silva. 06. V. 2012	Brazil	$20^{0}54'20.32''S/40^{0}46'42.72''O$	KM523183
SPF57530	*H. pseudomusciformis*	Ponta das Raias. Marataízes. ES. F. Nauer, C. Iha & B. Torrano-Silva. 07. V. 2012	Brazil	$21^{0}01'57.43''S/40^{0}48'44.43''O$	KM523184
SPF57537	*H.* pseudo*musciformis*	Beach Setiba. Guarapari. ES. F. Nauer, C. Iha & B. Torrano-Silva. 08. V. 2012	Brazil	$20^{0}38'06.83''S/40^{0}26'13.25''O$	KM523186
SPF57417	*H.* pseudo*musciformis*	Prainhas do Atalaia. Arraial do Cabo. RJ. F. Nauer, C. Iha & B. Torrano-Silva. 28. IX. 2011	Brazil	$22^{0}59'17.09''S/42^{0}00'46.14''O$	KM523151
SPF57426	*H.* pseudo*musciformis*	Beach do Forno. Búzios. RJ. F. Nauer, C. Iha & B. Torrano-Silva. 25. IX. 2011	Brazil	$22^{0}45'42.56''S/41^{0}52'29.11''O$	KM523136
SPF57436	*H.* pseudo*musciformis*	Beach Brava. Búzios. RJ. F. Nauer, C. Iha & B. Torrano-Silva. 27. IX. 2011	Brazil	$22^{0}45'14.35''S/41^{0}52'24.45''O$	KM523142
SPF57476	*H.* pseudo*musciformis*	Beach do Carro. Saco do Mamanguá. Paraty. RJ. F. Nauer, C. Iha & B. Torrano-Silva. 10. XII. 2011	Brazil	$23^{0}14'21.95''S/44^{0}37'06.85''O$	KM523144
SPF57441	*H.* pseudo*musciformis*	Ilha das Palmas. Ubatuba. SP. A. Medeiros, C. Iha & B.	Brazil	$23^{0}32'45.30''S/45^{0}01'44.85''O$	KM523152

		Torrano-Silva. 29.VIII.2011			
SPF57442	*H.* pseudo*musciformis*	Ilha das Cabras. Ubatuba. SP. A. Medeiros, C. Iha & B. Torrano-Silva. 30.VIII.2011	Brazil	$23^0$31’01.41’’S/$45^0$02’29.36’’O	KM523153
SPF57473	*H.* pseudo*musciformis*	Beach Lagoinha. Ubatuba. SP. F. Nauer & F. Kino. 22. X. 2011	Brazil	$23^0$31’20.35’’S/$45^0$11’26.65’’O	KM523155
SP365649	*H.* pseudo*musciformis*	Beach Jabaquara. Ilha Bela. SP. M. T. Fujii. 20. V. 2008	Brazil	$23^0$44’07.14’’S/$45^0$17’36.48’’O	KM523154
SPF57494	*H.* pseudo*musciformis*	Beach do Sambaqui. Florianópolis. SC. F. Nauer, C. Azevedo, M. Sissini & B. Torrano-Silva. 26. II. 2012	Brazil	27°29'24.55"S / 48°32'18.89"O	KM523171
SPF57499	*H.* pseudo*musciformis*	Beach Palmas. Governador Celso Ramos. SC. F. Nauer, C. Azevedo, M. Sissini & B. Torrano-Silva. 27. II. 2012	Brazil	27°18'47.90"S / 48°32'23.01"O	KM523174
SPF57505	*H.* pseudo*musciformis*	Beach Laranjeiras. Balneário Camburiu. SC. F. Nauer, C. Azevedo & B. Torrano-Silva. 28. II. 2012	Brazil	27°18'46.30"S / 48°34'41.93"O	KM523178
SPF57506	*H.* pseudo*musciformis*	Beach Laranjeiras. Balneário Camburiu. SC. F. Nauer, C. Azevedo & B. Torrano-Silva. 28. II. 2012	Brazil	27°59'47.31"S / 48°35'39.02"O	KM523179
SPF57507	*H. pseudomusciformis*	Beach Laranjeiras. Balneário Camburiu. SC. F. Nauer, C. Azevedo & B. Torrano-Silva. 28. II. 2012	Brazil	27°59'47.31"S / 48°35'39.02"O	KM523180
SPF57447	*H. musciformis*	Needham's Point. M. C. Oliveira.	Barbados	13.04'41".00/ 59.36'43".66	KM523203
SPF57585	*H. musciformis*	Punta Cana. F. Nauer	D. Republic		KM523204
SPF57598	*H. musciformis*	North Carolina. M. Hommersand	USA		KM523205
SPF57597	*H. musciformis*	Florida. M. Hommersand	USA		KM523206
SPF57596	*H. musciformis*	M. Hommersand	Namibia		KM523208
SPF57594	*H. musciformis*	Ancona. Gulf of Trieste. Fabio Rindi	Italy		KM523207

CAPÍTULO

Oito

Considerações finais

Considerações finais

Baseado nos principais resultados obtidos nesse trabalho, discutimos aqui os objetivos propostos:

Revisar as espécies do gênero *Hypnea* de ocorrência na costa brasileira, bem como as taxas de divergências de marcadores moleculares do tipo "DNA barcode", além de definir quais são os caracteres morfológicos úteis na taxonomia (Capítulos 2 e 7).

Ao todo, foram obtidas 97 espécimes coletados ao longo da costa brasileira e 109 sequencias novas de *Hypnea* foram obtidas para três marcadores do tipo "DNA barcode": COI-5P, *rbc*L-3P e UPA. Além disso, todos os caracteres morfológicos propostos por Masuda et al. (1997) para delimitar espécies do gênero foram estudados para os espécimes brasileiros. Com isso, foram reconhecidas para a costa do Brasil um total de 11 espécies: *H. brasiliensis*, *H. cervicornis*, *H. edeniana*, *H. flava*, *H. pseudomusciformis*, *H. spinella*, *H. yokoyana*, *H. wynnei*, *H.* sp. 1, *H.* sp. 2 e *H. platyclada* (baseada em dados morfológicos apenas) (Figura 1). Com os dados do cruzamento do capítulo 7, acreditamos que *H. pseudomusciformis* deve ser dividia em duas espécies, sendo *H. pseudomusciformis* restrita ao Sudeste e Sul do país e *Hypnea* sp. 5 restrita ao Nordeste do país, elevando, assim, o total de espécies do Brasil para 12. Além disso, apenas seis caracteres morfológicos foram úteis na taxonomia das espécies estudadas nesse trabalho: 1) Hábito do talo (tufos eretos ou densamente intricados); 2) Eixo principal (único ou múltiplo, persistente ou não); 3) Formato do ápice (reto, recurvado, bifurcado, gavinhas); 4) Forma dos râmulos (espinescentes, estrelados, etc.); 5) Tamanho da célula axial em relação às células periaxiais; 6) Posição dos tetrasprângios nos râmulos (basal, mediano, apical).

A técnica de "DNA barcode", principalmente usando o marcador molecular COI-5P, mostraram-se eficientes na identificação e delimitação das espécies do gênero *Hypnea*, revelando um cenário de diversidade despercebido pela taxonomia baseada em caracteres morfológicos. Porém, apesar da escassez de caracteres morfológicos e a

dificuldade de visualização de algumas dessas características, elas são úteis para a identificação de espécies em campo, levando-se em conta a distribuição das mesmas ao longo da costa Brasileira.

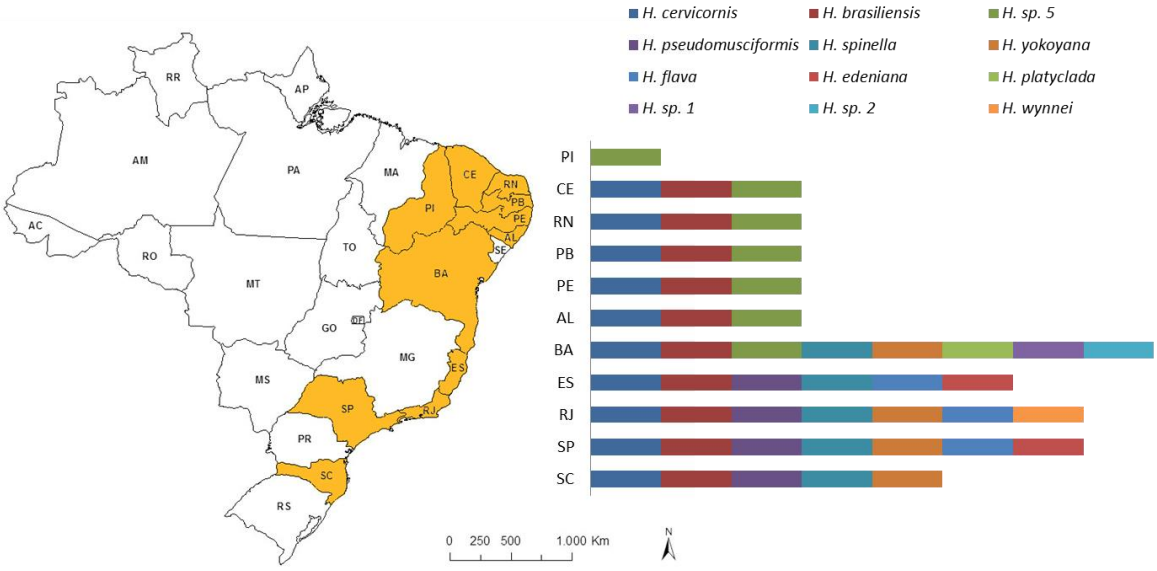

Figura 1. Mapa de distribuição das espécies de *Hypnea* por estados na costa brasileira (atualizado de Nauer 2013).

Obter amostras georeferenciadas das espécies do complexo *Hypnea musciformis/H. pseudomusciformis* de diferentes localidades do mundo, para investigar a ocorrência e distribuição das espécies com a união de dados moleculares, filogeográficos e morfológicos (Capítulos 3 e 7).

Um total de 174 sequências de *Hypnea*, provenientes de 14 países foram utilizados nas análises filogeográficas. Com isso, uma rede com 37 haplótipos foi obtida. Nossos dados indicam que a espécie *H. musciformis*, cuja localidade tipo é a Itália, predomina no Hemisfério Norte (Figura 2). Entretanto, nossos dados indicam a possível presença de duas espécies novas de ocorrência no Mar do Caribe, que, apesar de não apresentarem diferenças morfológicas com o material italiano de *H. musciformis*,

apresentam divergências para o "DNA barcode" 5P-COI da ordem de 3.2% (*H.* sp. 1) à 5.2% (*H.* sp. 2). Já a espécie *H. pseudomusciformis*, cuja localidade tipo é o Brasil, predomina no Hemisfério Sul. Além disso, os haplótipos dessa espécies formaram três grandes subgrupos: um subgrupo formado por haplótipos do Nordeste brasileiro, outro formado por haplótipos do Sudeste e Sul do Brasil, mas incluindo alguns do Nordeste, e um terceiro formado por haplótipos do Uruguai. O subgrupo do Nordeste possui divergências de até 1.7% com relação ao subgrupo do Sudeste e Sul, e a transição desses subgrupos podem estar relacionados com zonas biogeográficas brasileiras conhecidas, como a região do Espítiro Santo e Bahia, onde há transição do clima tropical para o subtropical, com diferenças nas temperaturas oceânicas que podem afetar o fluxo gênico.

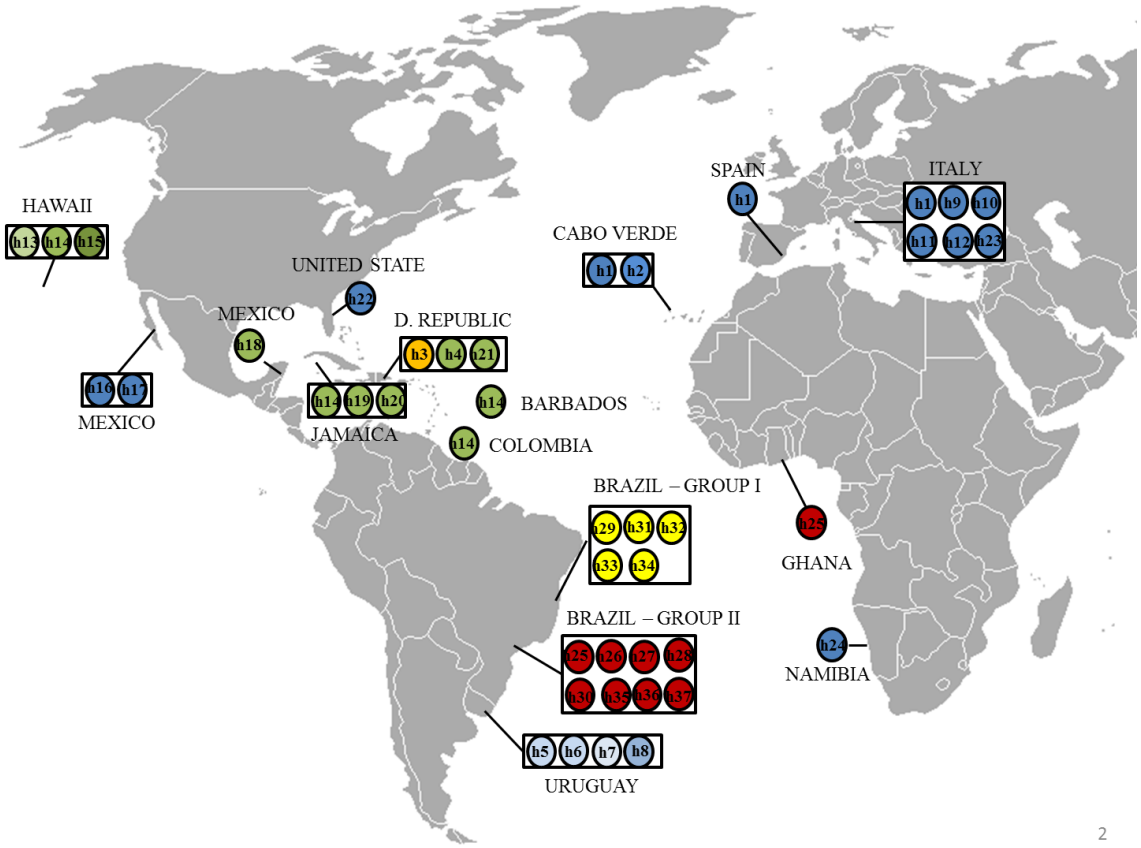

Figura 2. Mapa de distribuição das espécies do complexo *Hypnea musciformis-H. pseudomusciformis* (ver capítulo 3).

O marcador COI-5P se mostrou, mais uma vez, eficiente na identificação e delimitação, não somente de espécies, como também de haplótipos e diversidade intraspecífica do gênero *Hypnea*, mesmo que alguns espécimes apresentassem apenas alguns ramos, pouca biomassa e nenhuma estrutura reprodutiva. Além disso, os dados com esse marcador revelam possíveis zonas biogeográficas marinhas na costa do Brasil onde estudos são escassos, e indicam a presença de possíveis barreiras biogeográficas que afetam a distribuição de organismos marinhos. Com isso, estudos futuros sobre barreiras biogeográficas marinhas brasileiras e fluxo gênico entre organismos é de suma importância para estudar a distribuição de espécies, sua ecologia e prever possíveis alterações nesse cenário com base em mudanças climáticas e ações humanas na costa Brasileira.

Utilizar dados do ciclo de vida completo *in vitro*, além de dados de teste de crescimento e conteúdo pigmentar de duas variantes morfológicas de *H. pseudomusciformis*, afim de obter indícios que testem a hipótese 2 (Capítulo 4).

O histórico de vida de *Hypnea* é trifásico, onde o tetrasporófito e o carposporófito são diploides e os gametófitos masculinos e femininos são haploides. Na natureza, gametófitos masculinos de "musciformis" são raros (Schenkman 1986, Reis et al. 2003), enquanto que para "nigrescens" o balanço entre as fases de vida na natureza é levemente mais equilibrado (Jesus 2016). O ciclo de vida de *H. pseudomusciformis* foi completado *in vitro* em aproximadamente 121 dias para ambos variantes morfológicos. Esta foi a primeira vez que o ciclo de vida foi completado em laboratório, onde se foi possível observar todas as fases de vida, incluindo gametófitos masculinos de "musciformis". Não houve diferenças entre "musciformis" e "nigrescens" em nenhuma fase de vida e ambos variantes morfológicos apresentam taxas de crescimento e conteúdo pigmentar semelhantes.

Nossos dados indicam que ausência de diferenças fisiológicas entre "musciformis" e "nigrescens" se mantém por todo o ciclo de vida. Além disso, mais

estudos em campo são necessários para verificar se a ausência de gametofitos na natureza de "musciformis" seja devido ao habito epífito dessa planta, que pode dificultar o assentamento e desenvolvimento dos carpósporos dos tetrasporóftios epífitos férteis e que por possuírem crescimento apical, a capacidade de se reproduzir assexuadamente por fragmentação pode explicar a diferença na quantidade e biomassa dessa fase do histórico de vida em relação as demais.

Investigar duas variantes morfológicas de *Hypnea pseudomusciformis* obtidos em campo e cultivados *in vitro*, a fim de verificar se há a ocorrência de plasticidade fisiológica, além da plasticidade morfológica, nessas variantes e se há indícios de um fator genético atuante nessas diferenças ou apenas fatores ambientais (Capítulo 5).

Nauer et al. (2015) observou que, as espécies previamente identificadas, com base na morfologia, *Hypnea musciformis* e *H. nigrescens* não possuim divergências com base em marcadores moleculares e que seriam, na verdade, variações morfológicas de *H. pseudomusciformis*. Nossos dados com base no perfil de pigmentos fotossintetizantes e respostas antioxidantes indicam que a plasticidade morfológica de amostras em cultura corroboram essa hipótese. Além disso, os dados referente as respostas antioxidantes das amostras de campo, juntamente com as diferenças morfológicas apresentadas indicam que as pressões do ambiente em que são encontradas são responsáveis por essas diferenças. Comparando os dados de "musciformis" e "nigrescens" do campo, há respostas antioxidantes diferentes, além de diferenças no conteúdo dos pigementos, ou seja, perfis diferentes de respostas fisiológicas. *In vitro*, porém, essas diferenças são atenuadas. Houve um aumento de conteúdo pigmentar com relação as amostras de campo, que provavelmente se devem as diferenças de irradiância entre o ambiente natural e o cultivo.

Em resumo, os dados de nossos experimentos fornecem subsídios aos dados moleculares, onde, além da grande diferença morfológica existente entre "musciformis" e "nigrescens", há uma divergência fisiológica. Essas diferenças são atenuadas sob

mesmas condições de cultivo que, juntamente com dados moleculares, indicam que se tratam de uma mesma espécie cuja ampla plasticidade fenotípica possibilita que a planta se adapte a diferentes habitats, com diferentes condições ambientais. Além disso, dados de respostas fisiológicas se mostraram úteis para estudar organismos altamente plásticos, podendo ser uma ferramenta de pesquisa para auxiliar a taxonomia, principalmente em cenários onde as divergências entre espécies enquadra-se na "zona cinza" da técnica "DNA barcode".

Realizar experimentos de cruzamento *in vitro* com duas variantes morfológicas de *H. pseudomusciformis*, com ausência de divergência no "DNA barcode" 5P-COI, a fim de testar a hipótese 2 (Capítulo 6).

Nesse estudo, além da obtenção de dados sobre respostas fisiológicas com base em atividade antioxidante, conteúdo de pigmentos fotossintetizantes, ciclo de vida *in vitro* e testes de crescimento das variações morfológicas "musciformis" e "nigrescens" da espécie *H. pseudomusciformis*, testes de cruzamento foram realizados para verificar o conceito biológico de espécie. Nesse cenário, dois organismos que possuem morfologias distintas, a ponto de anteriormente terem sido consideradas duas espécies distintas, mas que não apresentam divergências nos marcadores moleculares, foram colocados foram colocados juntos em cultura para verificar presença ou ausência de reprodução e desenvolvimento de descendentes férteis.

O teste de crescimento foi baseado em três situações: 1) Isolamento do gametófito feminino para garantir que não ocorreu autofecundação, ou seja, controle negativo; 2) Gametófitos femininos e masculinos da mesma variação morfológica postos juntos para verificar a presença da reprodução, ou seja, controle positivo e 3) pareamento de gametófitos de diferentes variações morfológicas visando testar o conceito biológico de espécie. Esse cenário foi repetido três vezes e em todas as vezes foi observado a presença de cistorcapos no cruzamento entre gametófitos de "musciformis" com gametófitos de "nigrescens". Os tetrasporófitos resultantes desses cruzamentos se desenvolveram normalmente e foi possível observar a presença de tetrasporângios contendo tetrásporos. Esses tetrásporos foram liberados e deram origem

a novos gametófitos. Além disso, testes de crescimento e perfil de pigmentos foram realizados com as variações morfológicas "musciformis", "nigrescens" e com o tetrasporófito resultante da fecundação e não foram observadas diferenças significativas. Com isso, os testes de cruzamento corroboram os dados moleculares do marcador COI-5P, unificando os conceitos biológico e filogenético para a espécie *H. pseudomusciformis.* No caso de espécies com grande plasticidade fenotípica como *H. pseudomusciformis*, a utilização de caracteres morfológicos pode levar a interpretação errônea da taxonomia e são necessárias outras ferramentas (marcadores moleculares) para a correta identificação das mesmas.

Realizar experimentos de cruzamento *in vitro* de *H. pseudomusciformis* entre indivíduos de uma mesma variante morfológica, mas obtidos em regiões geográficas distintas e que apresentam divergência acima de 1,5 % no "DNA barcode" 5P-COI, a fim de utilizar o conceito biológico de espécie para testar os limites das divergências moleculares para este marcados no gênero *Hypnea* (Capítulo 7).

O cenário do teste de cruzamento realizado nesse capítulo é o oposto do capítulo 6. Aqui, a variação é encontrada na divergência de marcadores moleculares enquanto que a morfologia se mantém. Nesse caso, a variação morfológica "musciformis" de *H. pseudomusciformis* foi utilizada, porém de duas populações distintas que apresentam divergência de 1.7% para o marcador COI-5P. Esse valor de divergência encontra-se na chamada "zona cinza" onde existe dúvida quanto a separação em duas espécies distinta. Além disso, essas duas populações representam os subgrupos de haplótipos encontrados com base nos dados do capítulo 3. Indivíduos de "musciformis" de São Paulo, representando o subgrupo de haplótipos do Sudeste e Sul do Brasil foram colocados em cultura com indivíduos de "musciformis" da Bahia, representando o subgrupo de haplótipos do Nordeste do Brasil.

Enquanto que nos experimentos de cruzamento do capítulo 6 foi possível observar a fecundação, o surgimento de cistocarpos e o desenvolvimento dos carposporos em novos tetrasporofitos férteis, nos experimentos de cruzamento entre haplótipos de SP e BA isto não ocorreu. O experimento foi repetido duas vezes com três replicas cada e apesar do surgimento de cistocarpos, em ambos os testes e em todas as réplicas, os carpósporos não se desenvolveram e novos tetrasporófitos não foram formados. Com isso foi possível verificar a presença de um isolamento reprodutivo e uma barreira em estágios após a fecundação. Com base no conceito biológico de espécie, dois indivíduos que apresentam uma barreira reprodutiva são considerados espécies distintas e, nesse cenário, a divergência de 1.7% para o marcador COI-5P já seria suficiente para a separação de espécies do gênero *Hypnea*. Nesse cenário, *H. pseudomusciformis* deve ser dividia em duas espécies, sendo *H. pseudomusciformis* restrita ao Sudeste e Sul do país e *Hypnea* sp. 5 restrita ao Nordeste do país. Esses dados levantam a questão de suma importância de se reavaliar o cuidado na identificação e separação de espécies com base em divergências moleculares que possam levar uma interpretação equivocada da biodiversidade do grupo estudado.

Baseado nos principais resultados obtidos nesse trabalho, discutimos as hipóteses levantadas:

1. **Baseado em estudos moleculares, a espécie *Hypnea musciformis* forma um complexo de espécies proximamente relacionadas, mas com distribuição geográfica distinta.**

Com base nos dados de filogeografia do capítulo 3, essa hipótese foi corroborada. Nossos dados confirmam que o complexo *H. musciformis* é formado por espécies/ linhagens com distribuição geográfica distintas, sendo que *H. musciformis* predomina no Hemisfério Norte e *H. pseudomusciformis* predomina no Hemisfério sul do globo. Os dados do capítulo três levantam a questão se há duas espécies novas nesse complexo, de ocorrência no Mar do Caribe. Além disso, dados do capítulo 7 levantam a questão se a espécie *H. pseudomusciformis* consiste, na verdade, em duas espécies com uma divergência molecular de 1.7% para o marcador COI-5P e que apresentam um isolamento reprodutivo após a formação do cistocarpo, com carposporófitos inviáveis.

Todas as espécies do complexo são intimamente relacionadas, ou seja, divergiram recentemente devido a presença de barreiras geográficas marinhas do globo que promoveram um isolamento das populações do ancestral comum. Além disso, trabalhos futuros são necessários para se estudar a origem e padrões de distribuição dessas espécies, estudos importantes para conservação e manejo de *H. musciformis*, espécie de grande importância comercial.

2. Os variantes morfológicos "musciformis" e "nigrescens" de *Hypnea pseudomusciformis* que ocorrem na costa Brasileira correspondem a uma única espécie biológica.

A análise conjunta dos dados fisiológicos, moleculares e cruzamento dos capítulos 4, 5 e 6 corroboram essa hipótese. Apesar da grande diferença morfológica entre "musciformis" e "nigrescens", semelhanças no ciclo de vida e aspectos da sua fisiologia quando cultivadas *in vitro* nas mesmas condições forma observadas. Além disso, foi possível a observar cistocarpos no cruzamento entre essas variações morfológicas, bem como o desenvolvimento de tetrasporófitos férteis e viáveis. Novos estudos são necessários para testar o conteúdo e qualidade da carregenana das demais variações morfológicas de *H. pseudomusciformis* e verificar se apresentam a mesma qualidade da variação "musciformis", de grande importância para a indústria. Estudos ecológicos em campo são necessários para verificar se há fluxo gênico entre as variantes morfológicas, que habitam regiões distintas no costão rochoso.

Referências

Jesus, P.B. 2016. Estudos biossistemáticos em espécies do gênero Hypnea J.V. Lamouroux (Gigartinales, Rhodophyta). Tese de doutorado. Feira de Santana. 221 p.

Nauer, F. 2013. Filogenia molecular e diversidade do gênero *Hypnea* (Giagertinales, Rhodophyta) na costa brasileira. Dissertação de Mestrado. Universidade de São Paulo. Brasil. 180 p.

Nauer, F., Cassano, V. & Oliveira, M.C. 2015. Description of *Hypnea pseudomusciformis* sp. nov., a new species based on molecular and morphological analyses, in the context of the *H. musciformis* complex (Gigartinales, Rhodophyta). *Journal of Applied Phycology* 27: 2405-2417. DOI: 10.1007/s10811-014-0488-y

Reis, R.P., Leal, M.C.R., Yoneshigue-Valentin, Y. & Belluco, F. 2003. Efeito de fatores bióticos no crescimento de *Hypnea musciformis* (Rhodophyta - Gigartinales). Acta Bot Bras 17:279-286.

Schenkman, R.P.F. 1986. Cultura de *Hypnea* (Rhodophyta) in vitro como subsídio para estudos morfológicos, reprodutivos e taxonômicos. Tese de Doutorado. Universidade de São Paulo, Brasil.

Apêndice I - Matriz de sequências do marcador COI-5P de *Hypnea* sequenciadas neste estudo.

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                ....|....| ....|....| ....|....| ....|....| ....|....|
                        10         20         30         40         50
H00182          ATGATTTTTT TTATGGTAAT GCCCGTTATG ATAGGAGGAT TTGGCAATTG
H01691          ATGATTTTTT TTATGGTAAT GCCCGTTATG ATAGGAGGAT TTGGCAATTG
H01992          ATGATTTTTT TTATGGTAAT GCCCGTTATG ATAGGAGGAT TTGGCAATTG
H02323          ATGATTTTTT TTATGGTAAT GCCCGTTATG ATAGGAGGAT TTGGCAATTG
IBC2726         ATGATTTTTT TCATGGTAAT GCCAGTTATG ATAGGAGGAT TTGGCAATTG
IBC2727         ATGATTTTTT TCATGGTAAT GCCAGTTATG ATAGGAGGAT TTGGCAATTG
IBC2728         ATGATTTTTT TCATGGTAAT GCCAGTTATG ATAGGAGGAT TTGGCAATTG
IBC2729         ATGATTTTTT TCATGGTAAT GCCAGTTATG ATAGGAGGAT TTGGCAATTG
IBC2730         ATGATTTTTT TCATGGTAAT GCCAGTTATG ATAGGAGGAT TTGGCAATTG
IBC2731         ATGATTTTTT TCATGGTAAT GCCAGTTATG ATAGGAGGAT TTGGCAATTG
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IBC2826         ATGATTTTTT TTATGGTAAT GCCCGTTATG ATAGGAGGAT TTGGCAATTG
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H02323      GTTGGTTCCT ATAATGATAG GAAGTCCTGA TATGGCATTT CCTCGTTTAA
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IBC2730     GTTGGTTCCT ATAATGATAG GAAGTCCTGA TATGGCATTT CCCCGTTTAA
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IBC2825     GTTGGTTCCT ATAATGATAG GAAGTCCTGA TATGGCATTT CCTCGTTTAA
IBC2826     GTTGGTTCCT ATAATGATAG GAAGTCCTGA TATGGCATTT CCTCGTTTAA
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H01691          ATAATATTTC TTTCTGATTA TTACCGCCCT CATTATGTTT ATTATTATTA
H01992          ATAATATTTC TTTCTGATTA TTACCGCCCT CATTATGTTT ATTATTATTA
H02323          ATAATATTTC TTTCTGATTA TTACCGCCCT CATTATGTTT ATTATTATTA
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IBC2826         ATAATATTTC TTTCTGATTA TTACCGCCCT CATTATGTTT ATTATTATTA
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H01992      TCAGCTTTAG TAGAGGTGGG AGTAGGTACA GGTTGAACGG TTTATCCACC
H02323      TCAGCTTTAG TAGAGGTGGG AGTAGGTACA GGTTGAACGG TTTATCCACC
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H01691       ACCTTTATTT GTGTGGTCGA TTTTCGTAAC AGCATTTTTA TTATTATTAG
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H01691       CGGTACCTGT TTTAGCAGGT GCAATTACAA TGCTTTTAAC AGATCGTAAT
H01992       CGGTACCTGT TTTAGCAGGT GCAATTACAA TGCTTTTAAC AGATCGTAAT
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                          460
H00182       TTTAATACAT CTTTT
H01691       TTTAATACAT CTTTT
H01992       TTTAATACAT CTTTT
H02323       TTTAATACAT CTTTT
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IBC2726	TTTAATACAT CTTTT
IBC2727	TTTAATACAT CTTTT
IBC2728	TTTAATACAT CTTTT
IBC2729	TTTAATACAT CTTTT
IBC2730	TTTAATACAT CTTTT
IBC2731	TTTAATACAT CTTTT
IBC2732	TTTAATACAT CTTTT
IBC2733	TTTAATACAT CTTTT
IBC2734	TTTAATACAT CTTTT
IBC2735	TTTAATACAT CTTTT
IBC2736	TTTAATACAT CTTTT
IBC2743	TTTAATACAT CTTTT
IBC2744	TTTAATACAT CTTTT
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IBC2778	TTTAATACAT CTTTT
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IBC2799	TTTAACACAT CTTTT
IBC2800	TTTAACACAT CTTTT
IBC2801	TTTAACACAT CTTTT
IBC2802	TTTAACACAT CTTTT
IBC2803	TTTAACACAT CTTTT
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IBC2808	TTTAACACAT CTTTT
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IBC2810	TTTAACACAT CTTTT
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IBC2813	TTTAACACAT CTTTT
IBC2814	TTTAACACAT CTTTT
IBC2814	TTTAACACAT CTTTT
IBC2814	TTTAACACAT CTTTT
IBC2817	TTTAATACAT CTTTT
IBC2818	TTTAATACAT CTTTT
IBC2819	GCTAGTACAT CTTTT
IBC2820	TTTAATACAT CTTTT
IBC2821	TTTAATACAT CTTTT
IBC2822	TTTAATACAT CTTTT
IBC2823	TTTAATACAT CTTTT
IBC2824	TTTAATACAT CTTTT
IBC2825	TTTAATACAT CTTTT
IBC2826	TTTAATACAT CTTTT

Apêndice II - Matriz de sequências do marcador UPA de *Hypnea* sequenciadas neste estudo.

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                         10         20         30         40         50
IBC0211         CTTTACTGTA GCTTGGAATT GAATTTGGGT ATATTTTGTG CAGTATAGGT
IBC0212         CTTTACTGTA GCTTGGAATT GAATTTGGGT ATATTTTGTG CAGTATAGGT
IBC0245         CTTTACTGTA GCTTGGAATT GAATTTGGGT ATACTTTGCG CAGTATAGGT
IBC0708         CTTTACTGTA GCTTGGAATT GAATTTGGGT ATACTTTGCG CAGTATAGGT
IBC0733         CTTTTCTGTA GTTTGGAATT GAATTTGGGT ATGCTTTGCG CAGTATAGGT
IBC0737         CTTTACTGTA GCTTGGAATT GGATTTGGGT ATGCTTTGCG CAGTATAGGT
IBC0779         CTTTACTGTA GCTTGGAATT GAATTTGGGT ATATTTTGTG CAGTATAGGT
IBC0789         CTTTACTGTA GCTTGGAATT GAATTTGGGT ATACTTTGCG CAGTATAGGT
IBC0792         CTTTACTGTA GCTTGGAATT GAATTTGGGT ATACTTTGCG CAGTATAGGT
IBC0799         CTTTACTGTA GCTTGGAATT GGATTTGGGT ATGCTTTGCG CAGTATAGGT
IBC0822         CTTTACTGTA GCTTGGAATT GAATTTGGGT ATACTTTGCG CAGTATAGGT
IBC0823         CTTTACTGTA GCTTGGAATT GAATTTGGGT ATACTTTGCG CAGTATAGGT
IBC0828         CTTTACTGTA GCTTGGAATT GAATTTGGGT ATACTTTGCG CAGTATAGGT
IBC0832         CTTTACTGTA GCTTGGAATT GAATTTGGGT ATACTTTGCG CAGTATAGGT
IBC0835         CTTTACTGTA GCTTGGAATT GGATTTGGGT ATGCTTTGCG CAGTATAGGT
IBC0838         CTTTACTGTA GCTTGGAATT GAATTTGGGT ATACTTTGCG CAGTATAGGT
IBC0864         CTTTACTGTA GCTTGGAATT GGATTTGGGT ATGCTTTGCG CAGTATAGGT
IBC0875         CTTTACTGTA GCTTGGAATT GGATTTGGGT ATGCTTTGCG CAGTATAGGT
IBC0894         CTTTACTGTA GCTTGGAATT GGATTTGGGT ATGCTTTGCG CAGTATAGGT
IBC0906         CTTTACTGTA GCTTGGAATT GAATTTGGGT ATACTTTGCG CAGTATAGGT
IBC0910         CTTTACTGTA GCTTGGAATT GAATTTGGGT ATATTTTGTG CAGTATAGGT
IBC0922         CTTTACTGTA GCTTGGAATT GAATTTGGGT ATACTTTGCG CAGTATAGGT
IBC0930         CTTTTCTGTA GCTTGGAATA GGATTTGGGT ATGCTTTGCG CAGCATAGGT
IBC0931         CTTTACTGTA GCTTGAAATT GGATTTGGGT ATGCTTTGCG CAGTATAGGT
IBC0932         CTTTACTGTA GCTTGAAATT GGATTTGGGT ATGCTTTGCG CAGTATAGGT
IBC0933         CTTTACTGTA GCTTGAAATT GGATTTGGGT ATGCTTTGCG CAGTATAGGT
IBC0934         CTTTACTGTA GCTTGAAATT GGATTTGGGT ATGCTTTGCG CAGTATAGGT
IBC0935         CTTTACTGTA GCTTGGAATT GGATTTGGGT ATGCTTTGCG CAGTATAGGT
IBC0936         CTTTACTGTA GCTTGGAATT GGATTTGGGT ATGCTTTGCG CAGTATAGGT
IBC0939         CTTTACTGTA GCTTGGAATT GGATTTGGGT ATGCTTTGCG CAGTATAGGT
IBC0956         CTTTTCTGTA GTTTGGAATT GAATTTGGGT ATGCTTTGCG CAGTATAGGT
IBC0968         CTTTTCTGTA GTTTGGAATT GAATTTGGGT ATGCTTTGCG CAGTATAGGT
IBC0970         CTTTTCTGTA GTTTGGAATT GAATTTGGGT ATGCTTTGCG CAGTATAGGT
IBC0971         CTTTTCTGTA GTTTGGAATT GAATTTGGGT ATGCTTTGCG CAGTATAGGT
IBC2708         CTTTTCTGTA GCTTGGAATT AAATTTGGGT ATACTTTGCG CAGTATAGGT
IBC2709         CTTTTCTGTA GCTTGGAATT AAATTTGGGT ATACTTTGCG CAGTATAGGT
IBC2710         CTTTTCTGTA GCTTGGAATT AAATTTGGGT ATACTTTGCG CAGTATAGGT
IBC2711         CTTTTCTGTA GCTTGGAATT AAATTTGGGT ATACTTTGCG CAGTATAGGT
IBT0747         CTTTACTGTA GCTTGGAATT GAATTTGGGT ATACTTTGCG CAGTATAGGT
IBT0802         CTTTTCTGTA GTTTGGAATT GAATTTGGGT ATGCTTTGCG CAGTATAGGT


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IBC0211         GGGAGGCAAA GATCATATAT TTGCGGATAT ATAGGAGCCA ACAGTGAGAT
IBC0212         GGGAGGCAAA GATCATATAT TTGCGGATAT ATAGGAGCCA ACAGTGAGAT
IBC0245         GGGAGGCAAA AATAATATAT TTGCGGATAT ATAGTAGCCA ATAGTGAGAT
IBC0708         GGGAGGCAAA GATTATATAT TTGTGGATAT ATAGGAGCCA ACAGTGAGAT
IBC0733         GGGAGGCAAA GATAATATAT TTGCGGATAT ATGGGAGCCA ACAGTGAGAT
IBC0737         GGGAGGCAAA GATAATATAT TTGCGGATAT ATATTAGCCA ATAGTGAGAT
IBC0779         GGGAGGCAAA GATCATATAT TTGCGGATAT ATAGGAGCCA ACAGTGAGAT
IBC0789         GGGAGGCAAA GATTATATAT TTGTGGATAT ATAGGAGCCA ACAGTGAGAT
IBC0792         GGGAGGCAAA AATAATATAT TTGCGGATAT ATAGTAGCCA ATAGTGAGAT
IBC0799         GGGAGGCAAA GATAATATAT TTGCGGATAT ATATTAGCCA ACAGTGAGAT
IBC0822         GGGAGG-AAA GATAATATAT TTGCGGATAT ATAGGAGCCA CCAGTGAGAT
IBC0823         GGGAGG-AAA GATAATATAT TTGCGGATAT ATAGGAGCCA CCAGTGAGAT
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IBC0828      GGGAGG-AAA GATAATATAT TTGCGGATAT ATAGGAGCCA CCAGTGAGAT
IBC0832      GGGAGGCAAA GATTATATAT TTGTGGATAT ATAGGAGCCA ACAGTGAGAT
IBC0835      GGGAGGCAAA GATAATATAT TTGCGGATAT ATATTAGCCA ACAGTGAGAT
IBC0838      GGGAGGCAAA AATAATATAT TTGCGGATAT ATAGTAGCCA ATAGTGAGAT
IBC0864      GGGAGGCAAA GATAATATAT TTGCGGATAT ATATTAGCCA ACAGTGAGAT
IBC0875      GGGAGGCAAA GATAATATAT TTGCGGATAT ATATTAGCCA ACAGTGAGAT
IBC0894      GGGAGGCAAA GATAATATAT TTGCGGATAT ATATTAGCCA ACAGTGAGAT
IBC0906      GGGAGGCAAA GATAATATAT TTGCGGATAT ATAGGAGCCA CCAGTGAGAT
IBC0910      GGGAGGCAAA GATCATATAT TTGCGGATAT ATAGGAGCCA ACAGTGAGAT
IBC0922      GGGAGGCAAA GATTATATAT TTGTGGATAT ATAGGAGCCA ACAGTGAGAT
IBC0930      GGGAGGCAAA GATAATATAT TTGCGGATAT ATATTAGCCA ACAGTGAGAT
IBC0931      GGGAGGCAAA GATAATATAT TTGCGGATAT ATAGTAGCCA ATAGTGAGAT
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IBC0935      GGGAGGCAAA GATGATATAT TTGCGGATAT ATAGTAGCCA ATAGTGAGAT
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IBC0939      GGGAGGCAAA GATGATATAT TTGCGGATAT ATAGTAGCCA ATAGTGAGAT
IBC0956      GGGAGGCAAA GATAATATAT TTGCGGATAT ATGGGAGCCA ACAGTGAGAT
IBC0968      GGGAGGCAAA GATAATATAT TTGCGGATAT ATGGGAGCCA ACAGTGAGAT
IBC0970      GGGAGGCAAA GATAATATAT TTGCGGATAT ATGGGAGCCA ACAGTGAGAT
IBC0971      GGGAGGCAAA GATAATATAT TTGCGGATAT ATGGGAGCCA ACAGTGAGAT
IBC2708      GGGAGGCAAA GATAATATAT TTGCGGATAT ATAGTAGCCC CCAGTGAGAT
IBC2709      GGGAGGCAAA GATAATATAT TTGCGGATAT ATAGTAGCCC CCAGTGAGAT
IBC2710      GGGAGGCAAA GATAATATAT TTGCGGATAT ATAGTAGCCC CCAGTGAGAT
IBC2711      GGGAGGCAAA GATAATATAT TTGCGGATAT ATAGTAGCCC CCAGTGAGAT
IBT0747      GGGAGG-AAA GATAATATAT TTGCGGATAT ATAGGAGCCA CCAGTGAGAT
IBT0802      GGGAGGCAAA GATAATATAT TTGCGGATAT ATGGGAGCCA ACAGTGAGAT


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IBC0211      ACCACTCTAA ATATGCTAGG ATTCTAACTT TGAAAGCCGT TAGCCGGCCA
IBC0212      ACCACTCTAA ATATGCTAGG ATTCTAACTT TGAAAGCCGT TAGCCGGCCA
IBC0245      ACCACTCTAA ATATACTAGA ATTCTAACTT TGAAAGCCGT TAACCGGCCA
IBC0708      ACCACTCTAA TTATACTAGG ATTCTAACTT TGAATGCCGT TAACCGGCCA
IBC0733      ACCACTCTAA GTATACTAGG ATTCTAACTT TGAAAGTCGT TAACCGGCCA
IBC0737      ACCACTCTAA GTATACTAGA ATTCTAACTT TGAAAGCCGT TAACCGGCCA
IBC0779      ACCACTCTAA ATATGCTAGG ATTCTAACTT TGAAAGCCGT TAGCCGGCCA
IBC0789      ACCACTCTAA TTATACTAGG ATTCTAACTT TGAATGCCGT TAACCGGCCA
IBC0792      ACCACTCTAA ATATACTAGA ATTCTAACTT TGAAAGCCGT TAACCGGCCA
IBC0799      ACCACTCTAA GTATACTAGA ACTCTAACTT TGAAAGCCGT TAACAGGCCA
IBC0822      ACCACTCTAA GTATACTAGA ATTCTAACTT TGAATGCCGT TAACCGGCCA
IBC0823      ACCACTCTAA GTATACTAGA ATTCTAACTT TGAATGCCGT TAACCGGCCA
IBC0828      ACCACTCTAA GTATACTAGA ATTCTAACTT TGAATGCCGT TAACCGGCCA
IBC0832      ACCACTCTAA TTATACTAGG ATTCTAACTT TGAATGCCGT TAACCGGCCA
IBC0835      ACCACTCTAA GTATACTAGA ACTCTAACTT TGAAAGCCGT TAACAGGCCA
IBC0838      ACCACTCTAA ATATACTAGA ATTCTAACTT TGAAAGCCGT TAACCGGCCA
IBC0864      ACCACTCTAA GTATACTAGA ACTCTAACTT TGAAAGCCGT TAACAGGCCA
IBC0875      ACCACTCTAA GTATACTAGA ACTCTAACTT TGAAAGCCGT TAACAGGCCA
IBC0894      ACCACTCTAA GTATACTAGA ACTCTAACTT TGAAAGCCGT TAACAGGCCA
IBC0906      ACCACTCTAA GTATACTAGA ATTCTAACTT TGAATGCCGT TAACCGGCCA
IBC0910      ACCACTCTAA ATATGCTAGG ATTCTAACTT TGAAAGCCGT TAGCCGGCCA
IBC0922      ACCACTCTAA TTATACTAGG ATTCTAACTT TGAATGCCGT TAACCGGCCA
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IBC0931      ACCACTCTAA GTATACTAGA ATTCTAACTT TGAAAGTCGT TAACCGGCCA
IBC0932      ACCACTCTAA GTATACTAGA ATTCTAACTT TGAAAGTCGT TAACCGGCCA
IBC0933      ACCACTCTAA GTATACTAGA ATTCTAACTT TGAAAGTCGT TAACCGGCCA
IBC0934      ACCACTCTAA GTATACTAGA ATTCTAACTT TGAAAGTCGT TAACCGGCCA
IBC0935      ACCACTCTAA GTATACTAGA ATTCTAACTT TGAAAGTCGT TAACCGGCCA
IBC0936      ACCACTCTAA GTATACTAGA ATTCTAACTT TGAAAGTCGT TAACCGGCCA
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IBC0939      ACCACTCTAA GTATACTAGA ATTCTAACTT TGAAAGTCGT TAACCGGCCA
IBC0956      ACCACTCTAA GTATACTAGG ATTCTAACTT TGAAAGTCGT TAACCGGCCA
IBC0968      ACCACTCTAA GTATACTAGG ATTCTAACTT TGAAAGTCGT TAACCGGCCA
IBC0970      ACCACTCTAA GTATACTAGG ATTCTAACTT TGAAAGTCGT TAACCGGCCA
IBC0971      ACCACTCTAA GTATACTAGG ATTCTAACTT TGAAAGTCGT TAACCGGCCA
IBC2708      ACCACTCTAA TTATACTAGA ATTCTAACTT TGAAAGCCGT TATCCGGCCA
IBC2709      ACCACTCTAA TTATACTAGA ATTCTAACTT TGAAAGCCGT TATCCGGCCA
IBC2710      ACCACTCTAA TTATACTAGA ATTCTAACTT TGAAAGCCGT TATCCGGCCA
IBC2711      ACCACTCTAA TTATACTAGA ATTCTAACTT TGAAAGCCGT TATCCGGCCA
IBT0747      ACCACTCTAA GTATACTAGA ATTCTAACTT TGAATGCCGT TAACCGGCCA
IBT0802      ACCACTCTAA GTATACTAGG ATTCTAACTT TGAAAGTCGT TAACCGGCCA


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                       160        170        180        190        200
IBC0211      AAGAACAGTT TCAGGTGGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0212      AAGAACAGTT TCAGGTGGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0245      AAGAACAGTT TCAGGTGGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0708      AAGAACAGTT TCAGGTGGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0733      AAGAACAGTT TCAGGTGGGC AGTTGGACTG GGGCGGTCGC CTCCTAAAAA
IBC0737      AAGAACAGTT TCAGGTGGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0779      AAGAACAGTT TCAGGTGGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0789      AAGAACAGTT TCAGGTGGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0792      AAGAACAGTT TCAGGTGGGC AGTTTTAATG GGGCGGTCGC CTCCTAAAAA
IBC0799      AAGAACAGTT TCAGGTGGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0822      AAGAACAGTT TCAGGTGGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0823      AAGAACAGTT TCAGGTGGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0828      AAGAACAGTT TCAGGTGGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0832      AAGAACAGTT TCAGGTGGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0835      AAGAACAGTT TCAGGTGGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0838      AAGAACAGTT TCAGGTGGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0864      AAGAACAGTT TCAGGTAGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0875      AAGAACAGTT TCAGGTAGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0894      AAGAACAGTT TCAGGGGGGA AAGTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0906      AAGAACAGTT TCAGGTGGGC AGTTTGACTG GGGCGGTCGC CTCTCATCAA
IBC0910      AAGAACAGTT TCAGGTGGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0922      AAGAACAGTT TCAGGTGGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
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IBC0968      AAGAACAGTT TCAGGTGGGC AGTTGGACTG GGGCGGTCGC CTCCTAAAAA
IBC0970      AAGAACAGTT TCAGGTGGGC AGTTGGACTG GGGCGGTCGC CTCCTAAAAA
IBC0971      AAGAACAGTT TCAGGTGGGC AGTTGGACTG GGGCGGTCGC CTCCTAAAAA
IBC2708      AAGAACAGTT TCAGGTGAAC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC2709      AAGAACAGTT TCAGGTGAAC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
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IBC2711      AAGAACAGTT TCAGGTGAAC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0747      AAGAACAGTT TCAGGTGGGC AGTTTGACTG GGGCGGTCGC CTCCTAAAAA
IBC0802      AAGAACAGTT TCAGGTGGGC AGTTGGACTG GGGCGGTCGC CTCCTAAAAA


                ....|....| ....|....| ....|....| ....|....| ....|....|
                       210        220        230        240        250
IBC0211      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA
IBC0212      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA
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IBC0245      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA
IBC0708      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCATA
IBC0733      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA
IBC0737      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTTTTA
IBC0779      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA
IBC0789      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCATA
IBC0792      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA
IBC0799      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTCA
IBC0822      GTAACGGAGG CGTACAAAGG TTTCTTCAGG CTGGTCGGAA ATCAGTCTTA
IBC0823      GTAACGGAGG CGTACAAAGG TTTCTTCAGG CTGGTCGGAA ATCAGTCTTA
IBC0828      GTAACGGAGG CGTACAAAGG TTTCTTCAGG CTGGTCGGAA ATCAGTCTTA
IBC0832      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCATA
IBC0835      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA
IBC0838      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA
IBC0864      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTCA
IBC0875      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTCA
IBC0894      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTCA
IBC0906      GTAACGGAGG CGTACAAAGG TTTCTTCAGG CTGGTCGGAA ATCAGTCTTA
IBC0910      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA
IBC0922      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCATA
IBC0930      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTCA
IBC0931      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA
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IBC0933      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA
IBC0934      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA
IBC0935      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA
IBC0936      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA
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IBC0956      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA
IBC0968      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA
IBC0970      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA
IBC0971      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA
IBC2708      GTAACGGAGG CGCACAAAGG TTTCTTCAGG CTGGTCGGAA ATCAGTTTTA
IBC2709      GTAACGGAGG CGCACAAAGG TTTCTTCAGG CTGGTCGGAA ATCAGTTTTA
IBC2710      GTAACGGAGG CGCACAAAGG TTTCTTCAGG CTGGTCGGAA ATCAGTTTTA
IBC2711      GTAACGGAGG CGCACAAAGG TTTCTTCAGG CTGGTCGGAA ATCAGTTTTA
IBT0747      GTAACGGAGG CGTACAAAGG TTTCTTCAGG CTGGTCGGAA ATCAGTCTTA
IBT0802      GTAACGGAGG CGTACAAAGG TTTCTTCAGA CTGGTCGGAA ATCAGTCTTA


             ....|....| ....|....| ....|....| ....|....| ....|....|
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IBC0212      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACTTACA AGTCGAGCAG
IBC0245      GAGTATAAAG GCAAAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
IBC0708      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
IBC0733      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
IBC0737      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
IBC0779      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACTTACA AGTCGAGCAG
IBC0789      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
IBC0792      GAGTATAAAG GCAAAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
IBC0799      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
IBC0822      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
IBC0823      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
IBC0828      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
IBC0832      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
IBC0835      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
IBC0838      GAGTATAAAG GCAAAAGGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
IBC0864      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
IBC0875      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
IBC0894      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
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IBC0922      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
IBC0930      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
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IBC2711      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
IBT0747      GAGTATAAAG GCATAAAGAA GCTTGACTGC AAGACCTACA AGTCGAGCAG
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IBC0733      AGACGAAAGT CGGTCTTAGT GATCCGACAG TCCTGAGTGG AAAGGCTGCC
IBC0737      AGACGAAAGT CGGTCTTAGT GATCCGACAG TTCTGAGTGG AAAGGCTGTC
IBC0779      AGACGAAAGT CGGTCTTAGT GATCCGACAG TTCTGAGTGG AAAGGCTGTC
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IBC0792      AGACGAAAGT CGGTCTTAGT GATCCGACAG TTCTGAGTGG AAAGGCTGTC
IBC0799      AGACGAAAGT CGGTCTTAGT GATCCGACAG TTCTGAGTGG AAAGGCTGTC
IBC0822      AGACGAAAGT CGGTCTTAGT GATCCGACAG TTCTGAGTGG AAAGGCTGTC
IBC0823      AGACGAAAGT CGGTCTTAGT GATCCGACAG TTCTGAGTGG AAAGGCTGTC
IBC0828      AGACGAAAGT CGGTCTTAGT GATCCGACAG TTCTGAGTGG AAAGGCTGTC
IBC0832      AGACGAAAGT CGGTCTTAGT GATCCGACAG TGCTGAGTGG AAAGGCTGTC
IBC0835      AGACGAAAGT CGGTCTTAGT GATCCGACAG TTCTGAGTGG AAAGGCTGTC
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IBC0864      AGACGAAAGT CGGTCTTAGT GATCCGACAG TTCTGAGTGG AAAGGCTGTC
IBC0875      AGACGAAAGT CGGTCTTAGT GATCCGACAG TTCTGAGTGG AAAGGCTGTC
IBC0894      AGACGAAAGT CGGTCTTAGT GATCCGACAG TTCTGAGTGG AAAGGCTGTC
IBC0906      AGACGAAAGT CGGTCTTAGT GATCCGACAG TTCTGAGTGG AAAGGCTGTC
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IBC0922      AGACGAAAGT CGGTCTTAGT GATCCGACAG TGCTGAGTGG AAAGGCTGTC
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IBC0931      AGACGAAAGT CGGTCTTAGT GATCCGACAG TTCTGAGTGG AAAGGCTGTC
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IBC0956      AGACGAAAGT CGGTCTTAGT GATCCGACAG TCCTGAGTGG AAAGGCTGCC
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IBC0970      AGACGAAAGT CGGTCTTAGT GATCCGACAG TCCTGAGTGG AAAGGCTGCC
IBC0971      AGACGAAAGT CGGTCTTAGT GATCCGACAG TCCTGAGTGG AAAGGCTGCC
IBC2708      AGACGAAAGT CGGTCTTAGT GATCCGACAG TTCTGAGTGG AAAGGCTGTC
IBC2709      AGACGAAAGT CGGTCTTAGT GATCCGACAG TTCTGAGTGG AAAGGCTGTC
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IBT0802      AGACGAAAGT CGGTCTTAGT GATCCGACAG TCCTGAGTGG AAAGGCTGCC


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IBC0212      GCTCAACGGA TAAAAGTTA
IBC0245      GCTCAACGGA TAAAAGTTA
IBC0708      GCTCAACGGA TAAAAGTTA
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IBC0737      GCTCAACGGA TAAAAGTTA
IBC0779      GCTCAACGGA TAAAAGTTA
IBC0789      GCTCAACGGA TAAAAGTTA
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IBC2709      GCTCAACGGA TAAAAGTTA
IBC2710      GCTCAACGGA TAAAAGTTA
IBC2711      GCTCAACGGA TAAAAGTTA
IBT0747      GCTCAACGGA TAAAAGTTA
IBT0802      CCTCACCGGA TAAAAGTTC
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Apêndice III - Matriz de sequências do marcador COI-5P de *Hypnea* sequenciadas neste estudo.

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IBC0731         TGGGATCCTG AATATGTAAT TAAAGATACA GATGTATTAG CTTTATTTCG
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IBC0737         TGGGATCCTG AATATGTAAT TAAAGATACA GATGTATTAG CTTTGTTTCG
IBC0789         TGGGATCCTG ATTATGTAAT TAAAGATACA GATGTACTAG CTTTATTTCG
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IBC0835         TGGGATCCTG AATATGTAAT TAAAGATACA GATGTATTAG CTTTATTTCG
IBC0864         TGGGATCCTG ACTATGAAAA AAAAGATACA GATGTATTAG CTTTATTTCG
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KM509073      TGGGATCCTG AATATGTAAT TAAAGATACA GATGTATTAG CTTTATTTCG
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IBC0731       TGTAACTCCA CAACCAGGAG TAGATCCCGT TGAAGCTTCA GCTGCTGTTG
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IBC0737       TGTAACTCCA CAACCAGGAG TAGATCCGGT TGAAGCTTCA GCTGCTGTTG
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IBC0875       TGTAACCCCA CAACCAGGAG TAGATCCCGT TGAAGCTTCA GCTGCTGTTG
IBC0894       TGTAACCCCA CAACCAGGAG TAGATCCCGT TGAAGCTTCA GCTGCTGTTG
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IBC0916      ACTGCTTGCG ACTTATATAG AGCAAAAGCA TACAAAGTAG ATGCTGTACC
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KX426554     TAATACATCT GACCAATATT TTGCTTTTAT TGCATATGAT ATTGATCTTT
KX426555     TAATACATCT GACCAATATT TTGCTTTTAT TGCATATGAT ATTGATCTTT


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IBC0040      TGAATTTCCG AGTAATTTGT AAGTGGATGC GTATGTCTGG TGTAGATCAT
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KM203894      CTCAAGGAAT TTTCTTTGAA CAAGATTGGG CATCTTTACG TAAAGTTACT
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