PATRICIA MARÍA GONZÁLEZ SÁNCHEZ

Diversidade e filogenia do complexo *Laurencia* (Ceramiales, Rhodophyta) no arquipélago cubano

Tese apresentada ao Instituto de Pesquisas Ambientais, da Secretaria de Infraestrutura e Meio Ambiente, como parte dos requisitos exigidos para a obtenção do título de DOUTOR em BIODIVERSIDADE VEGETAL E MEIO AMBIENTE, na Área de Concentração de Plantas Avasculares e Fungos em Análises Ambientais.

SÃO PAULO

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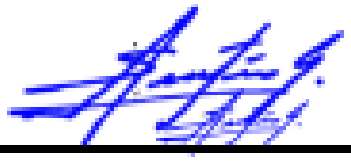

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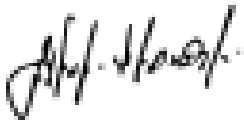

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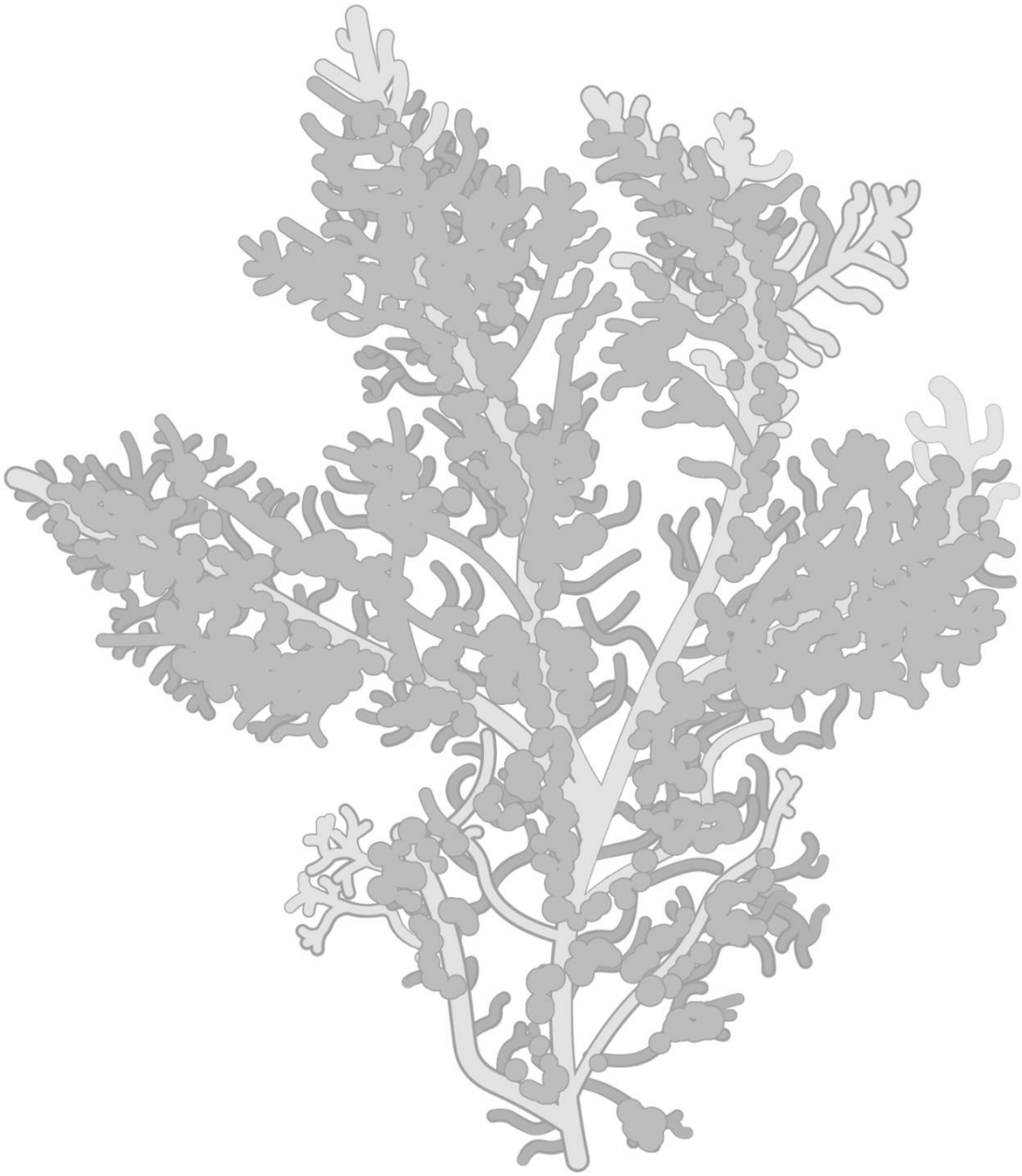

Esta tesis está dedicada a mis padres, pero principalmente a mi segunda madre Magalys Sánchez y mi papá por siempre guiarme con su sabiduría y persistencia por el camino que debía seguir para alcanzar mis metas y sueños. ¡Gracias por nunca desistir! Los quiero y son un orgullo para mí.

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Pois o que de Deus *se pode conhecer é manifesto entre eles, porque* Deus *lhes manifestou.*

Pois desde a criação do mundo os atributos invisíveis de Deus*, seu eterno poder e sua natureza divina, têm sido vistos claramente, sendo compreendidos por meio das coisas criadas, para que eles fiquem inescusáveis.*
Romanos 1:19-20

Ah! Soberano Senhor*, tu fizeste os céus e a terra pelo teu grande poder e por teu braço estendido. Nada é difícil demais para ti.*
Jeremias 32:17

Antes de nascerem os montes
e de criares a terra e o mundo,
de eternidade a eternidade tu és Deus.
Salmo 90:2

Ele fez tudo apropriado ao seu tempo. Também pôs no coração do homem o anseio pela eternidade; mesmo assim ele não consegue compreender inteiramente o que Deus *fez.*
Eclesiastes 3:11

Ele constrói suas câmaras altas,
e firma a abóbada sobre a terra;
ele reúne as águas do mar e as espalha
sobre a superfície da terra.
Senhor *é o seu nome.*
Amós 9:6

Pois toda casa é construída por alguém, mas Deus *é o edificador de tudo.*
Hebreus 3:4

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RESUMO

O complexo *Laurencia* (Ceramiales, Rhodophyta) apresenta uma alta diversidade e "complexidade" tornando quase impossível identificar seus representantes apenas pela morfologia. Ultimamente, pesquisadores tem modificado substancialmente a taxonomia do complexo *Laurencia* em várias regiões do mundo e, particularmente no Atlântico Ocidental tropical e subtropical, o uso de ferramentas moleculares tem revelado um número significativo de novos táxons. Na região do Caribe, o arquipélago cubano é uma das áreas reconhecidas por sua alta diversidade de macroalgas marinhas, no entanto, estudos moleculares de seus espécimes são praticamente inexistentes, representando uma lacuna no conhecimento taxonômico atual. Neste contexto, a diversidade do complexo *Laurencia* foi investigada pela primeira vez em Cuba sob uma abordagem molecular, complementada com dados morfológicos e padrões de distribuição das espécies. As coletas abrangeram os litorais norte e sul e as três regiões em que se divide o arquipélago (ocidental, central e oriental). Foram utilizados os marcadores mitocondrial do tipo DNA Barcode COI-5P e o plastidial *rbc*L para inferências filogenéticas. Cinco métodos de delimitação de espécies (ABGD, ASAP, PTP, GMYCs e GMYCm) foram aplicados para avaliar a diversidade do complexo em um conjunto de dados do COI-5P. Nossos resultados confirmaram a presença de 13 táxons para a plataforma cubana: *Laurencia caduciramulosa, L. catarinensis, L. dendroidea, L. microcladia¸ Laurenciella namii, Palisada corallopsis, P.* aff. *perforata* 3, *Palisada* sp. 1, *Palisada* sp. 2, *Yuzurua iridescens, Y. poiteaui, Yuzurua* sp. 1 e *Yuzurua* sp. 2. *Laurencia catarinensis* constitui um novo registro para Cuba e região do Caribe, ampliando sua área de distribuição. *Laurenciella namii*, além de ser outro novo registro para Cuba, aumenta o número de gêneros do complexo *Laurencia* reconhecidos até agora para o país. Das quatro espécies não identificadas em nível específico (duas de *Palisada* e duas de *Yuzurua*), três coincidem com sequências do GenBank, porém mais estudos são necessários para esclarecer seus status taxonômico. Das espécies citadas para Cuba, *Laurencia brongniartii*, *L. caraibica*, *L. chondrioides*, *L. intricata*, *L. minuscula*, *L. obtusa*, *Osmundea coelenterata*, *Palisada cervicornis*, *P. flagellifera* e *Yuzurua poiteaui* var. *gemmifera*, não foram encontradas nas nossas coletas e, não puderam ser confirmadas neste estudo. Nossas análises moelculares com o COI-5P, incluindo os métodos de delimitação de espécies, mostraram que *L. dendroidea* e *P. perforata* formam complexos de espécies. As análises moleculares e a comparação morfológica detalhada de *Palisada corallopsis* e *P. furcata* de suas localidades tipo apóiam a coespecificidade desses táxons, tendo *P. corallopsis* prioridade sobre o primeiro. O marcador COI-5P mostrou ser mais eficiente na distinção de espécies do que o *rbc*L, revelando a necessidade de revisão taxonômica para alguns táxons,

como *L. dendroidea* e *P. perforata*. As espécies do complexo *Laurencia* apresentaram um padrão de distribuição heterogêneo, com o maior número de espécies e indivíduos encontrados no litoral norte. A Análise de Parcimônia de Endemicidade (PAE, sigla em inglês) revelou áreas endêmicas determinadas pela presença de espécies raras ou incomuns do complexo para o arquipélago cubano dentro e fora das Áreas Marinhas Protegidas. *Laurencia dendroidea*, *Palisada perforata* e *Yuzurua poiteaui* foram as espécies mais comuns e frequentes registradas na costa cubana. Nosso estudo representa um primeiro esforço para relevar a diversidade do complexo *Laurencia* em Cuba com base em dados moleculares, fornecendo informações valiosas sobre a biodiversidade de algas no arquipélago e no Atlântico ocidental tropical, e destaca a importância de gerar um conjunto de dados de referência preciso para futuros esforços de monitoramento. No entanto, os resultados obtidos indicam que a diversidade do complexo pode estar superestimada e amostragens mais amplas são necessárias, incorporando novos marcadores às análises visando esclarecer sua diversidade e melhor inferir as relações intra- e interespecíficas deste complexo de algas.

Palavras-chave: Taxonomia, COI-5P, *rbc*L, métodos de delimitação de espécies, PAE

ABSTRACT

The *Laurencia* complex (Ceramiales, Rhodophyta) presents a high diversity and "complexity" making it almost impossible to identify its representatives only by morphology. Lately, researchers have substantially modified the taxonomy of the *Laurencia* complex in several regions of the world and, particularly in the tropical and subtropical Western Atlantic, the use of molecular tools has revealed a significant number of new taxa. In the Caribbean region, the Cuban archipelago is one of the areas recognized for its high diversity of marine macroalgae, however, molecular studies of its specimens are practically non-existent, representing a gap in current taxonomic knowledge. In this context, the diversity of the *Laurencia* complex was investigated for the first time in Cuba under a molecular approach, complemented with morphological data and species distribution patterns. The collections covered the north and south coasts and the three regions into which the archipelago is divided (western, central and eastern). Mitochondrial DNA Barcode COI-5P and plastid *rbc*L markers were used for phylogenetic inferences. Five species delimitation methods (ABGD, ASAP, PTP, GMYCs and GMYCm) were applied to assess complex diversity in a COI-5P dataset. Our results confirmed the presence of 13 taxa for the Cuban shelf: *Laurencia caduciramulosa*, *L. catarinensis*, *L. dendroidea*, *L. microcladia*¸ *Laurenciella namii*, *Palisada corallopsis*, *P.* aff. *perforata* 3, *Palisada* sp. 1, *Palisada* sp. 2, *Yuzurua iridescens*, *Y. poiteaui*, *Yuzurua* sp. 1, and *Yuzurua* sp. 2. *Laurencia catarinensis* constitutes a new record for Cuba and the Caribbean region, expanding its distribution area. *Laurenciella namii*, in addition to being another new record for Cuba, increases the number of genera of the *Laurencia* complex recognized so far for the country. Of the four species not identified at specific level (two from *Palisada* and two from *Yuzurua*), three match GenBank sequences, but further studies are needed to clarify their taxonomic status. Of the species cited for Cuba, *Laurencia brongniartii*, *L. caraibica*, *L. chondrioides*, *L. intricata*, *L. minuscula*, *L. obtusa*, *Osmundea coelenterata*, *Palisada cervicornis*, *P. flagellifera* and *Yuzurua poiteaui* var. *gemmifera*, were not found in our collections and could not be confirmed in this study. Our molecular analyzes with COI-5P, including the species delimitation methods, showed that *L. dendroidea* and *P. perforata* form species complexes. Molecular analyzes and detailed morphological comparison of *Palisada corallopsis* and *P. furcata* from their type localities support the cospecificity of these taxa, with *P. corallopsis* having priority over the former. The COI-5P marker proved to be more efficient in distinguishing species than *rbc*L, revealing the need for a taxonomic revision for some taxa, such as *L. dendroidea* and *P. perforata*. Species from the *Laurencia* complex showed a heterogeneous distribution pattern, with the highest number of species and individuals found on the north coast. The Endemicity Parsimony Analysis (PAE) revealed endemic areas determined by the presence of rare or unusual species of the complex for the Cuban archipelago inside and outside the Marine Protected Areas. *Laurencia dendroidea*, *Palisada perforata* and *Yuzurua poiteaui* were the most common and frequent species recorded on the Cuban coast. Our study represents a first effort to reveal the diversity of the *Laurencia* complex in Cuba based on molecular data, providing valuable information on algal biodiversity in the archipelago and the tropical western Atlantic, and highlights the importance of generating an accurate reference dataset. for future monitoring efforts. However, the results indicate that the complex's diversity may be overestimated and broader sampling is needed, incorporating new

markers to the analysis to clarify its diversity and better infer the intra- and interspecific relationships of this algae complex.

Key-words: Taxonomy, COI-5P, *rbc*L, species delimitation methods, PAE

LISTA DE ABREVIATURAS

%	Percentagem
˚C	Graus Celsius
®	Marca registrada
18S	18S RNA ribossomal
ABGD	Automatic Barcode Gap Discovery
ASAP	Assemble Species by Automatic Partitioning
BI	Inferência Bayesiana
bp	Base pair ou pares de base
cm	Centímetro
COI-5P	Cytochrome c oxidase subunit 1 (5P) ou região 5' (barcode) do gene cox1 que codifica a subunidade 1 da enzima citocromo c oxidase
DNA	Deoxyribonucleic Acid ou Ácido desoxirribonucleico
GMYC	Generalized Mixed Yule Coalescent Approach
GTR	General time-reversible
ITS	Internal Transcribed Spacer ou espaçador interno transcrito
km²	Quilômetro quadrado
LSU	Large subunit
m	Metro
m^2	Metro quadrado
MCMC	Markov Chain Monte Carlo method ou Cadeia de Markov Método de Monte Carlo
mg	Miligramo
ML	Maximum-likelihood
MPA	Marine protected area ou Área marinha protegida
SDM	Species delimitation methods ou Métodos para delimitar espécies
NJ	Neighbor Joining
PAE	Parsimony analysis of endemism ou Análise de parcimônia de endemismo
PP	Probabilidade posterior
PCR	Polymerase Chain Reaction ou reação em cadeia da polimerase
PTP	Poisson Tree Processes
*rbc*L	Ribulose bisphosphate carboxylase (Large subunit) ou gene que codifica a subunidade grande da Rubisco
*rbc*L-S	região espaçadora entre o *rbc*L e o *rbc*S
rRNA	Ribosomal Ribonucleic Acid ou Ácido ribonucléico ribossômico
Rubisco	Ribulose-1,5-bifosfato carboxilase/oxygenase

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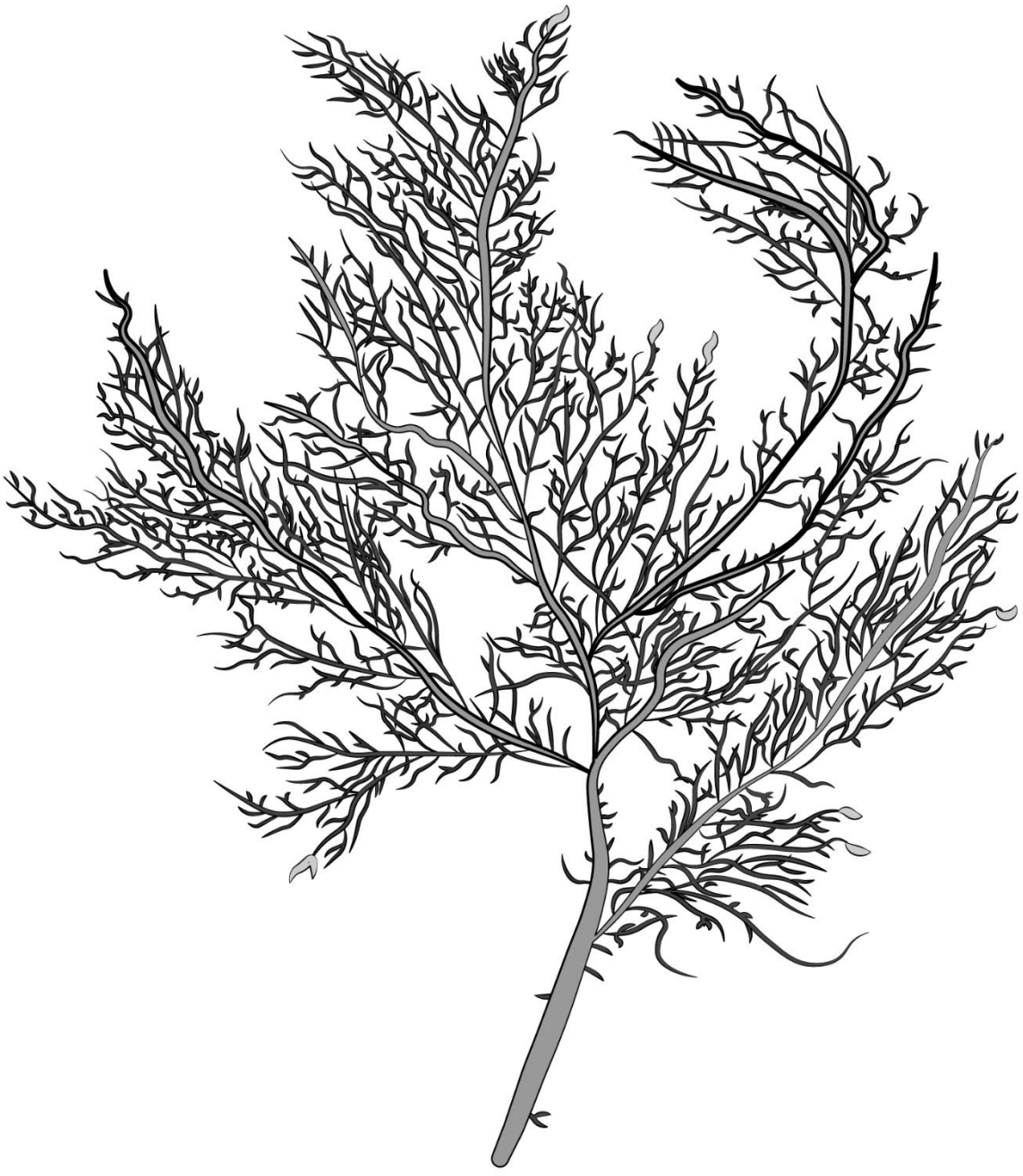

INTRODUÇÃO GERAL

CAPÍTULO 1. INTRODUÇÃO

1.1 Do filo Rhodophyta ao complexo *Laurencia* (Rhodomelaceae, Ceramiales)

Características gerais

O filo de algas vermelhas, Rhodophyta de Wettstein (1901) contém 7.565 espécies em todo o mundo (Guiry & Guiry 2022), e são membros-chave do ambiente aquático comumente colonizando e dominando habitats bentônicos de ecossistemas marinhos, estuarinos e de água doce em latitudes tropicais, subtropicais, temperadas e de águas frias (Sheath 1984, Freshwater & Rueness 1994, Lee 1999, Chapman 2013). Dentro do filo, alguns grupos unicelulares são conhecidos. No entanto, a maioria das espécies é macroscópica e pode ser encontrada em substratos rochosos. As células são eucarióticas, com um a muitos núcleos e carecem estruturalmente de flagelos e centríolos em todas as fases do seu ciclo de vida (Gurgel & López-Batista 2007). Além da clorofila *a* verde e do β-caroteno, esses organismos também possuem pigmentos à base de proteínas azuis e vermelhas chamadas ficobiliproteínas: a aloficocianina azul-esverdeada, a ficocianina azul e a ficoeritrina vermelha (Yoon et al. 2006). Sabe-se que os complexos de pigmentos estão na superfície das membranas para capturar a energia da luz do sol para a atividade fotossintetizante (Yoon et al. 2010). Além da celulose, as paredes geralmente contêm polímeros sulfatados de galactana, alguns dos quais são extratos de ficocolóides economicamente importantes (por exemplo, ágar, carragenina). Mais de 300 espécies de algas vermelhas também são usadas como fonte direta de alimento, conhecidas como vegetais marinos (Gurgel & López-Batista 2007). Conexões citoplasmáticas ("pit-connections"/ "pit-plugs") são características das paredes celulares de algas vermelhas (principalmente na classe Florideophyceae) e geralmente visíveis ao microscópio óptico. As conexões primárias resultam da divisão de duas células irmãs, enquanto as secundárias são formadas através do contato de células vizinhas (Pueschel 1980). Várias espécies deste grupo são calcificadas impregnadas de carbonato de cálcio na forma de calcita, que muitas vezes desempenha um papel importante na formação de recifes tropicais (Littler & Littler 2000). Seus membros também compartilham uma combinação de caracteres que não ocorrem juntos em nenhum outro eucarioto, como uma completa falta de estágios flagelados, incluindo ausência de centriolos e corpos basais flagelares (Adl et al. 2005).

Rhodophyta exibe também uma grande variedade de morfologias e histórias de vida, e atingiram essa diversidade sem ter evoluído a verdadeira diferenciação tecidual (Hommersand & Fredericq 1990). A distinta oogamia, a retenção e o desenvolvimento do zigoto no gametófito feminino e a intercalação de uma geração característica de esporos entre as fases gametofítica

e meiosporangial na história de vida da maioria das algas vermelhas formaram historicamente a base para definir as ordens e muitas famílias da classe Florideophyceae (Hommersand & Fredericq 1990, Saunders & Hommersand 2004).

Florideophyceae é a classe mais complexa e elaborada em Rhodophyta (Freshwater 2000, Guiry & Guiry 2022). Os membros de Florideophyceae apresentam conexões citoplasmáticas, crescimento apical e reprodução sexual com um ciclo de vida trifásico (Dixon 1973, Lee 1999). Algumas espécies são usadas diretamente pelos humanos como alimento, enquanto polissacarídeos extraídos da parede celular são usados como géis e aditivos em alimentos e produtos cosméticos (Freshwater 2000).

Falkenberg (1901) estabeleceu as bases da atual taxonomia de Rhodomelaceae. Sua excelente monografia forneceu o detalhe abundante e integrador da família. Posteriormente, Kylin (1956) e Hommersand (1963) forneceram mais detalhes sobre as estruturas reprodutivas e classificação das Rhodomelaceae. Kylin (1956) reconheceu as Nemaliales, Gelidales, Cryptonemiales, Gigartinales, Rhodymeniales e Ceramiales - seis ordens separadas conforme o corpo de frutificação (cistocarpo) que se originou do óvulo fertilizado (carpogônio) ou de outras células (células auxiliares) que foram contatadas diretamente pelo carpogônio ou indiretamente através de células de conexão ou filamentos conectivos. Análises filogenéticas moleculares no nível ordinal destacam a evolução dos primeiros estágios no desenvolvimento do aparelho feminino e células associadas e corroboram a relevância do sistema sexual de classificação de Kylin (Gavio et al. 2005).

Dentro de Rhodophyta, Ceramiales Oltmanns (1904) é um grupo altamente bem sucedido em termos de número de espécies e padrões de distribuição mundial, compreendendo quase metade dos gêneros em Florideophyceae e o mais diverso morfologicamente entre as algas vermelhas (Kraft & Woelkerling 1990, Maggs & Hommersand 1993, Lin et al. 2001). Ceramiales são caracterizadas pela a estrutura do talo uniaxial, a presença de células periaxiais e a formação pós-fertilização da célula auxiliar diretamente da célula de suporte (Oltmanns 1904, Maggs & Hommersand 1993, Womersley 1998). A ordem Ceramiales foi classificada tradicionalmente em quatro famílias de acordo com a morfologia. As primeiras filogenias baseadas em um ou dois marcadores moleculares foram mal suportadas e falharam em resolver essas famílias como monofiléticas (Lin et al. 2001, Choi et al. 2002, 2008). Estudos recentes realizados por Díaz-Tapia et al. (2019), reconhecem nove famílias para a ordem Ceramiales, usando genomas plastidiais para espécies representativas das principais linhagens. Três das famílias previamente reconhecidas foram resolvidas como linhagens monofiléticas independentes (Ceramiaceae, Wrangeliaceae e Rhodomelaceae) e as outras seis famílias precisam ser reclassificadas (Díaz-Tapia et al. 2019). Perspectivas taxonômicas entre as

famílias reconhecidas dentro de Ceramiales foram desafiadas por filogenética molecular e várias mudanças taxonômicas em nível familiar, subfamiliar e tribal foram propostas (Díaz-Tapia et al. 2019, Wynne 2022).

Rhodomelaceae é a maior família de algas vermelhas com uma grande diversidade na construção vegetativa, variando de filamentos polissifônicos (célula axial circundada por 2-4 pericentrais (Nam 2006) ou várias células pericentrais até 24 células, formadas em sequência alternada) a talos crostosos, folhosos, carnudos ou coriáceos de tamanho considerável (Hommersand, 1963). Os membros da família exibem crescimento monopodial; tricoblastos vegetativos incolores e decíduos; ramos monossifônicos holoblásticos que se desenvolvem a partir das células axiais e geralmente estão presentes nas células subapical; tetrasporângios divididos tetraedricamente desenvolvidos a partir de células pericentrais, ou a partir de células corticais em alguns gêneros; órgãos espermatangiais em tricoblastos modificados, que são ramos cilíndricos ou laminares; um ramo carpogonial com (3-)4 células, com um grupo lateral estéril; célula auxiliar que é cortada da célula de suporte após a fertilização (Hommersand 1963, Womersley 2003). Existe uma importante característica anatômica em Rhodomelaceae que apoia a monofilia encontrada nos estudos moleculares, a ramificação simpodial do gonimoblasto após o primeiro carpogônio formado (Kylin 1956, Hommersand 1963, Choi et al. 2002, Díaz-Tapia et al. 2019).

Biotecnologicamente, uma infinidade de estudos confirma o potencial de seus integrantes, principalmente na indústria farmacêutica de espécies das tribos Laurencieae, Amansieae e Polysiphonieae (Wang et al. 2013, Blunt et al. 2015). Entre eles, o gênero *Laurencia* J.V. Lamouroux representa uma fonte notável e amplamente investigada de compostos bioativos (Teixeira 2013).

Atualmente, a família Rhodomelaceae é composta por 21 tribos, com mais de mil espécies e aproximadamente 150 gêneros reconhecidos (Díaz-Tapia et al. 2017, Guiry & Guiry 2022). Tradicionalmente, as relações entre gêneros e espécies na família têm sido inferidas a partir da comparação da morfologia vegetativa e, em menor grau, da morfologia reprodutiva como consequência da uniformidade anatômica das estruturas reprodutivas (Phillips 2001). No entanto, Jong et al. (1998) sugeriram que caracteres vegetativos são mais abundantes que caracteres reprodutivos e provavelmente contêm mais informações filogenéticas do que se pensava anteriormente. Relações de simetria, morfologia dorsiventral versus radial, desempenharam um papel fundamental no delineamento de relações intrafamiliares em Rhodomelaceae (Scagel 1953).

O número atual de espécies e gêneros provavelmente está subestimado, uma vez que novos gêneros e novas espécies dentro da família têm sido continuamente propostos com base

em dados morfológicos e moleculares, por exemplo, *Laurenciella* Cassano, Gil-Rodríguez, Sentíes, Díaz-Larrea, M.C.Oliveira & M.T.Fujii (Cassano et al. 2012a), *Lampisifonia* H.-G. Choi, Díaz-Tapia & Barbara (Barbara et al. 2013), *Ohelopapa* F. Rousseau, Martin-Lescanne, Payri & L.Le Gall (Rousseau et al. 2017), *Wilsonosiphonia* D. Bustamante, Won & T.O. Cho (Bustamante et al. 2017), *Neochondria* S.Sutti, M.Tani, Y.Yamagishi, T.Abe & K.Kogame (Sutti et al. 2018). O grande número de espécies de Rhodomelaceae reflete sua alta diversidade morfológica, principalmente nas estruturas vegetativas. Apesar das revisões taxonômicas conduzidas com base em análises de filogenia molecular, bem como em análises morfológicas, a família Rhodomelaceae ainda inclui os grupos de táxons que estão incompletos na sistemática de algas vermelhas.

1.1.2 Complexo *Laurencia*

Laurencieae F. Schmitz é uma das tribos de Rhodomelaceae e, atualmente, contém os oito gêneros do complexo *Laurencia*, o gênero parasita *Janczewskia* Solms Laubach e *Rodriguezella* F.Schmitz (Guiry & Guiry 2022). O gênero tipo da tribo é *Laurencia* (Schmitz & Falkenberg 1897).

O nome *Laurencia* foi usado pela primeira vez em 1813 pelo botânico francês J.V. Lamouroux para descrever um grupo diversificado de oito algas vermelhas na ordem que ele chamou de *Floridées* com base em sua organização coralóide e coloração púrpura a avermelhada (Lamouroux 1813). O autor não designou um tipo para o gênero, porém isso foi corrigido posteriormente por Schmitz (1889), que estabeleceu a tribo *Laurenciae* e atribuiu formalmente a espécie-tipo *Laurencia obtusa* (Hudson) J.V.Lamouroux ao gênero. Deve-se notar que na mesma época que Lamouroux, o botânico inglês John Stackhouse propôs os gêneros *Osmundea* (1809) e *Pinnatifida* (1816), que compartilhavam algumas semelhanças morfológicas com *Laurencia*. *Osmundea* foi baseado na espécie-tipo *Osmundea expansa* Stackhouse *nom. ileg.*, que foi posteriormente sinonimizada com *Laurencia osmunda* (S.G. Gmelin) Maggs & Hommersand (Silva 1952) (atualmente *Osmundea osmunda* S.G.Gmelin) K.W.Nam & Maggs), enquanto *Pinnatifida vulgaris* Stackhouse, a espécie-tipo de *Pinnatifida*, é considerada sinônimo de *Laurencia pinnatifida* (Hudson) J.V. Lamouroux (1813) (atualmente *Osmundea pinnatifida* (Hudson) Stackhouse). Ambos os gêneros de Stackhouse foram rejeitados por Papenfuss (1947) - *Osmundea* como sinônimo heterotípico anterior de *Laurencia*, e *Pinnatifida* como sinônimo posterior de *Osmundea* - e *Laurencia sensu lato* (*s.l.*) de Lamouroux (1813) foi proposto como *nomen conservandum* por Papenfuss (1947), segundo Nam et al. (1994).

Entre o trabalho de Lamouroux (1813) e a designação da espécie-tipo de *Laurencia* por Schmitz (1889), várias espécies foram adicionadas ao gênero por vários autores, incluindo Gaillon (1828), Greville (1830), Agardh (1841), Sonder (1845), Hooker & Harvey (1847), Kützing (1849, 1865), Harvey (1855), Zanardini ex Fraudenfeld (1855), Crouan & Crouan in Schramm & Mazé (1865) e Martens (1871). Em seguida, autores como Kylin (1923, 1928), Yamada (1931), Saito (1967), Saito & Womersley (1974), Nam et al. 1994 e Garbary & Harper (1998) foram fundamentais no estabelecimento das principais características morfológicas (e posteriormente anatômicas) para a classificação de *Laurencia*. Por exemplo, Saito (1967) notou pela primeira vez o significado da presença ou ausência de conexões citoplasmáticas secundárias entre as células corticais adjacentes e arranjo diferencial dos tetrasporângios nos ramos e propôs os subgêneros *Laurencia* e *Chondrophycus* (J. Tokida & Y. Saito) Garbary & Harper, movendo todas as espécies de *Laurencia* sem conexões secundárias e arranjo em ângulo reto dos tetrasporângios em relação ao eixo longitudinal do ramo para o subgênero *Chondrophycus*, enquanto aquelas com conexões secundárias e arranjo paralelo dos tetrasporângios foram colocadas no subgênero *Laurencia*. A utilização de novas características vegetativas e reprodutivas levaram ao restabelecimento do gênero *Osmundea* por Nam et al. (1994), à elevação do subgênero *Chondrophycus* em nível genérico por Garbary & Harper (1998) e o estabelecimento do gênero *Palisada* por Nam (2006, 2007) baseado na seção *Palisadae* de Yamada (1931). Os trabalhos citados acima formaram as bases sobre as quais estudos posteriores expandiram a compreensão de *Laurencia* como um gênero e, mais recentemente, como um complexo de oito gêneros (complexo *Laurencia*).

O advento de métodos moleculares para a delineação de espécies e sua aplicação na taxonomia de *Laurencia* deu forte suporte às distinções anatômicas destacadas pelos autores acima e contribuiu significativamente para a caracterização e compreensão taxonômica do complexo *Laurencia*. Isso é visto mais claramente na transição do complexo de três gêneros (*Laurencia sensu stricto* (*s.s.*), *Osmundea* e *Chondrophycus*) (Nam et al. 1994, Garbary & Harper 1998) e apoiado pela filogenia molecular utilizando o marcador *rbc*L, gene que codifica a subunidade grande da Rubisco (McIvor et al. 2002, Abe et al. 2006, Fujii et al. 2006), para quatro gêneros (*Laurencia s.s.*, *Chondrophycus*, *Osmundea* e *Palisada* K.W. Nam) suportados pela análise cladística de Nam (2006) e análises filogenéticas com o *rbc*L de Díaz-Larrea et al. (2007).

O complexo foi então aumentado para cinco gêneros com a adição de *Yuzurua* (K.W. Nam) Martin-Lescanne conforme reconhecido por Martin-Lescanne et al. (2010) com base no gene *rbc*L e numa combinação de características vegetativas, como a produção de duas células pericentrais por célula axial vegetativa e células corticais mais externas não arranjadas como

paliçada, além da presença esporádica de conexões secundárias compartilhadas com *Laurencia s.s.* e *Osmundea*.

Posteriormente, o complexo englobou *Laurenciella* Cassano, Gil-Rodríguez, Sentíes, Díaz-Larrea, M.C. Oliveira & M.T. Fujii (Cassano et al. 2012a) e, mais recentemente, outros dois gêneros: *Ohelopapa* F. Rousseau, Martin-Lescanne, Payri & L.Le Gall (Rousseau et al. 2017) e *Corynecladia* J. Agardh (= *Coronaphycus* Metti) (Cassano et al. 2019), com suporte aportado pelas análises filogenéticas e de DNA barcoding, associadas a estudos morfológicos.

Assim, *Laurencia s.l.* é atualmente considerada um complexo (Garbary & Harper 1998), composto por estes oito gêneros intimamente relacionados: *Laurencia s.s.*, *Osmundea*, *Chondrophycus*, *Palisada*, *Yuzurua*, *Laurenciella*, *Ohelopapa* e *Corynecladia*. Seus membros se distinguem por possuírem células apicais localizadas no fundo da depressão terminal do talo, construção uniaxial cuja fileira de células só é discernível nas proximidades da célula apical, extensa corticação do talo (Falkenberg 1901, Kylin 1956) e duas ou quatro células pericentrais dependendo do gênero, além do desenvolvimento dos ramos espermatangiais do tipo "tricoblasto" ou "filamento", bem como a origem dos tetrasporângios e dos espermatângios a partir de células pericentrais ou base do tricoblastos, presença ou ausencia de *corps en cerise* (Nam 2006). As características como as conexões secundárias entre as células corticais externas e presença ou ausência de espessamentos lenticulares não são exclusivas deste complexo, mas muitas veces complementam a identificação em nivel de especie (Nam 2006).

1.2 O arquipélago e a plataforma marinha de Cuba

O arquipélago cubano é formado pela ilha de Cuba (com uma área de 105.007 km^2), a Isla de la Juventud (2.200 km^2) e aproximadamente 4.195 ilhas, cayos e cayuelos (pequenas ilhas rasas, arenosas, formadas na superfície de um arrecife de coral) (Núñez Jiménez 1982), cujas dimensões variam de algumas centenas de m^2 até 770 km² (Cayo Romano). O arquipélago está localizado numa plataforma insular pouco profunda (menos de 30 m), limitada por um acentuado declive insular. Ao lado da plataforma há uma zona elevada de cayos e recifes (Figura 1). O arquipélago é cercado pelas profundas bacias e trincheiras do Mar do Caribe, Golfo do México e Estreitos da Flórida e das Bahamas, que, em certa medida, atuam como barreiras ecológicas à distribuição e migração da biota nerítica.

A costa da ilha de Cuba estende-se por 5.746 km (3.209 ao norte e 2.537 ao sul), mais 229 km da Isla de la Juventud. A plataforma insular apresenta o relevo de uma planície submersa que cobre uma superfície que, incluindo os cayos e ilhas (com exceção da Ilha de Cuba), cobre uma área de 67.831 km² (Núñez Jiménez 1982). A área marinha cobre uma superficie de 53.126 km^2. Do ponto de vista geológico, tectônico e geomorfológico, a

plataforma pode ser considerada como uma única área com quatro regiões rasas independentes (Ionin et al. 1977), onde se encontram ilhas, cayos, cayuelos e recifes, que a bordeiam pela parte externa, formando grandes macrolagoas. Essas áreas rasas são favoráveis ao desenvolvimento de vários ecossistemas, como manguezais, gramas marinhas e recifes de coral, que abrigam uma grande diversidade de organismos.

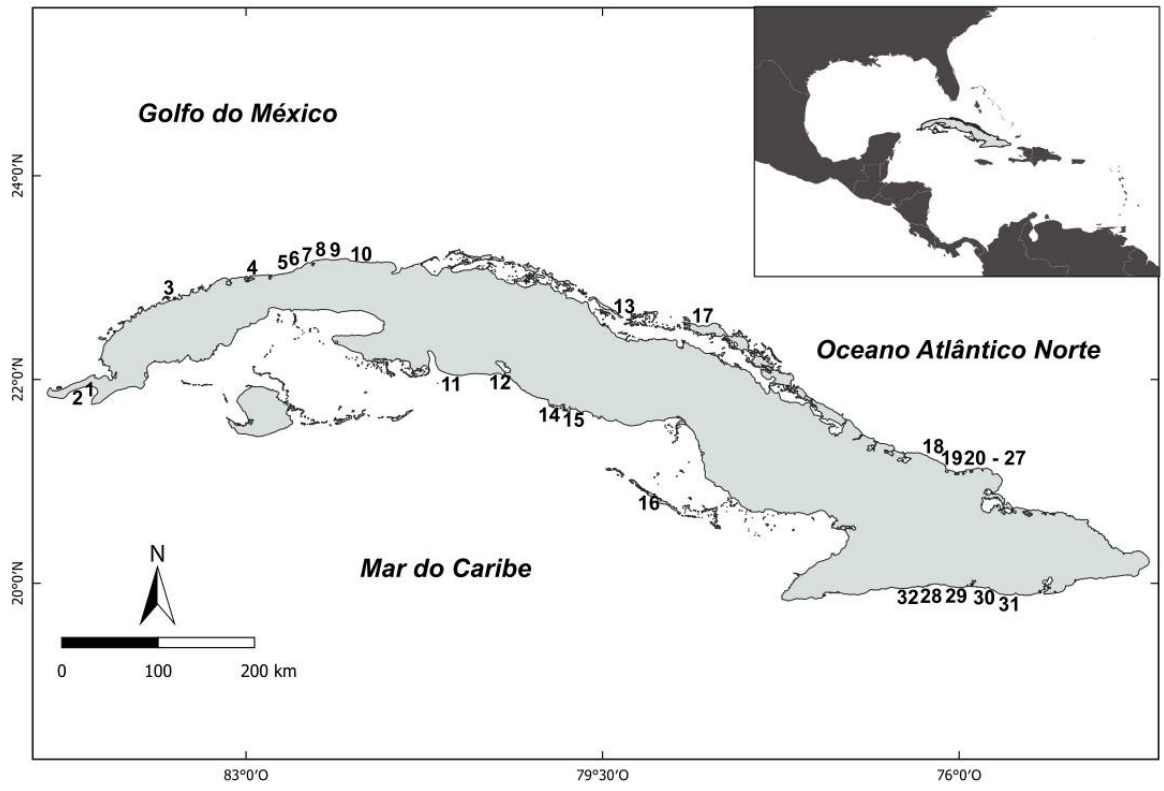

Figura 1. Mapa do arquipélago cubano (ver nomes dos locais de coleta na Tabela 1 do Capítulo 2).

Em termos gerais, o clima de Cuba pode ser considerado como uma savana semi-continental úmida com invernos amenos (Núñez Jiménez 1965). O que contribui para o aquecimento do arquipélago está o fato de estar quase totalmente rodeado por uma corrente marinha de águas quentes, cuja influência termal é reforçada por outras duas correntes da mesma natureza que, depois de atravessarem o Mar do Caribe, entram no Golfo do México através do canal de Yucatán (García et al. 1991, Figura 2).

No ritmo anual das precipitações atmosféricas, há duas estações do ano claramente expressas: a seca (de novembro a abril), com precipitações médias de cerca de 300 mm; e a estação chuvosa (de maio a outubro) com médias pluviométricas superiores a 1000 mm. Como consequência, exercem uma influência notável no regime hidrológico de algumas regiões costeiras, embora devido a represamentos e secas intensas, o fluxo de água doce para os ecossistemas costeiros tenha sido limitado nos últimos anos (Claro 2006).

A temperatura, em sua distribuição espacial, geralmente varia menos de 1-2ºC. Segundo Lluis-Riera (1972, 1977, 1981a, 1981b), foram registradas temperaturas de 19 a 31,6ºC, embora em corpos d´ água semifechados possam ser bem mais altas.

Segundo Lluis-Riera (1984), nas décadas de 1960 e 1970, as concentrações de nutrientes em geral não eram elevadas. No entanto, nas áreas costeiras próximas à foz dos rios, durante a estação chuvosa, as concentrações de nutrientes aumentaram até atingir valores comparáveis às de regiões relativamente férteis, como o banco de Campeche, México (Bessonov & González 1971, Bessonov et al. 1971). No entanto, esses altos valores de nutrientes geralmente são acompanhados por uma turbidez considerável, o que influencia negativamente a produtividade primária. Em algumas regiões, o aumento de nutrientes está relacionado à reciclagem de nutrientes dentro e acima do lodo de fundo.

As águas oceânicas adjacentes ao arquipélago cubano são, pelas suas características químicas e físicas, mais uniformes na sua distribuição horizontal e menos variáveis sazonalmente, pelo que a sua influência na plataforma é relativamente estável (Lluis-Riera 1983b). No entanto, o grau de influência de cada região depende da intensidade do intercâmbio, da topografia da plataforma e da dinâmica das águas, tanto interiores como oceânicas.

A circulação das águas na plataforma cubana parece ser determinada principalmente pelo vento, pela maré e pela influência das correntes no mar aberto adjacente (Emilson & Tápanes 1971). Dada a largura das macrolagoas da plataforma e a estreiteza das passagens entre os cayos que a ligam ao oceano, as correntes, principalmente provocadas pela maré, podem atingir velocidades consideráveis em alguns canais e suas imediações. Nas zonas interiores da plataforma, as correntes, embora muito variáveis em função da maré e dos ventos, têm uma componente principal a oeste (Emilson & Tápanes 1971, Blázquez-Echandi & Romeu 1982). As marés são geralmente de pouca amplitude, com valores médios inferiores a 50 cm, exceto no trecho entre Isabela de Sagua e Bahía de Nipe, onde podem ultrapassar 1 m.

O sistema de correntes nas águas oceânicas que circundam o arquipélago cubano é extraordinariamente complexo e variável. Ao sul do arquipélago, interagem a Corrente do Caribe, em direção a oeste, e a contracorrente cubana, em direção a leste (Figura 2). Como resultado desses fluxos, é produzido um complicado sistema de giros ciclônicos e anticiclônicos de intensidade e localização variáveis (Sukhovey et al. 1980, García et al. 1991, Victoria & Penié 1998, Gallegos et al. 1998) oferecendo condições favoráveis para a retenção de matéria em suspensão e aporte biológico das áreas circundantes. A existência destes giros ao redor do arquipélago constitui um fator importante para o transporte, dispersão e manutenção da diversidade biológica na plataforma cubana e sua influência em outras regiões do Grande Caribe.

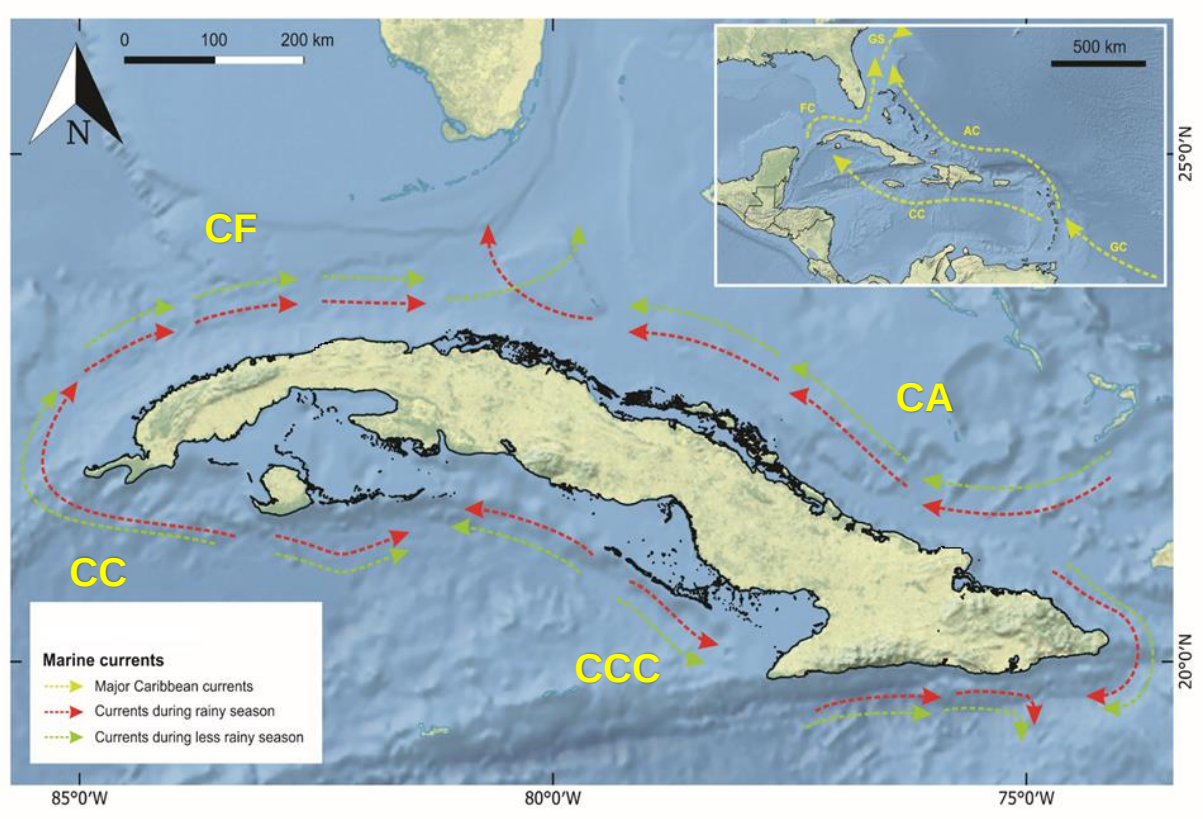

Figura 2. Padrão geral das correntes marinhas em Cuba. CF: Corrente da Flórida, CA: Corrente das Antilhas, CC: Corrente do Caribe e CCC: Contracorrente cubana (Autor: Otávio Luis Marques da Silva).

Numerosas zonas da costa cubana contam com infra-estrutura para o desenvolvimento turístico do país que, juntamente com os ecossistemas de manguezais e gramas marinhas, constituem um notável aporte de matéria orgânica ao ecossistema aquático. Às vezes se torna um problema para ambientes adjacentes quando os níveis de entrada de nutrientes são altos, pois contribui para a proliferação de microalgas, algas filamentosas e oportunistas que competem com outros organismos por recursos e espaço (Diez et al. 2019).

1.3 Inventários do complexo *Laurencia* nas Antilhas em relação ao Atlântico tropical e subtropical ocidental

Para a região antilhana onde se situa o arquipélago cubano, a informação disponível referente aos representantes do complexo *Laurencia* baseia-se em levantamentos locais generalizados em nível do país ou província geográfica e enquadram-se em três tipologías: compêndios florísticos com descrições de espécies e chaves para sua identificação, trabalhos monográficos e inventários da ficoflora regional.

Quatro trabalhos merecem menção na primeira categoria. O monumental compêndio elaborado por Taylor (1960) sobre a ficoflora tropical e subtropical do Atlântico americano. Síntese de trabalhos realizados entre 1924 e 1957, constitui um deles. Nele, o autor relatou dez

espécies: *L. microcladia* Kützing, *L. chondrioides* Børgesen, *L. intricata* J.V.Lamouroux, *L. obtusa* (Hudson) J.V.Lamouroux, *L. filiformis* (C.Agardh) Montagne (como *L. scoparia* J. Agardh), *Yuruzua poiteaui* var. *poiteaui* (J.V. Lamouroux) Martin-Lescanne (como *L. poitei* (Lamouroux) Howe), *Yuruzua poiteaui* var. *gemmifera* (Harvey) M.J.Wynne (como *L. gemmifera* Harvey), *Palisada perforata* (Bory) K.W.Nam (como *L. papillosa* (C.Agardh) Greville), *Palisada corallopsis* (Montagne) Sentíes, Fujii & Díaz-Larrea (como *L. corallopsis* (Montagne) M.Howe), e *L. caraibica* P.C. Silva (como *L. nana* Howe), sinalizando também a possível existência de outras sete, *L. brongniartii* J. Agardh (como *L. concinna* Montagne), *L. corymbosa* J. Agardh, *L. obtusa* var. *divaricata* Yamada (como *L. divaricata* J. Agardh), *L. heteroclada* Harvey, *Osmundea hybrida* (A.P. de Candolle) K.W. Nam (como *L. hybrida* A.P. de Candolle), *Palisada thuyoides* (Kützing) Cassano, Sentíes, Gil-Rodríguez & M.T. Fujii (como *L. paniculata* (C. Agardh) J. Agardh), *L. pinnatifida* (Gmelin) Lamouroux e *L. vaga* Kützing (atualmente como *Palisada perforata* (Bory de Saint-Vincent) K.W. Nam), considerados por ele na época como registros incertos na área.

O segundo trabalho publicado é o estudo de Chapman (1963) sobre a ficoflora de Jamaica. Com exceção de *Yuruzua poiteaui* var. *gemmifera* (como *L. gemmifera*), *L. chondrioides, L. dendroidea* (como *L. scoparia*) e *L. caraibica* (como *L. nana*), este autor corrobora a presença da espécie referida por Taylor (1960) e coloca *P. perforata* (como *L. perforata* (Bory) Montagne) como registro incerto. As duas obras restantes são compostas por guias ilustrados da flora dos recifes caribenhos preparados por Littler et al. (1989) e Littler & Littler (2000). Na primeira, os autores mencionam *L. intricata*, *Yuruzua poiteaui* var. *poiteaui* (como *L. poitei*), *Laurencia obtusa* e *Palisada perforata* (como *L. papillosa*). Na segunda, ambos os autores acrescentam *Yuzurua iridescens* (Wynne e Ballantine) K.W. Nam (como *L. iridescens* Wynne & Ballantine) e *Palisada cervicornis* Harvey (como *L. cervicornis*) às oito espécies encontradas por Taylor (1960).

Com a introdução e implementação de ferramentas moleculares no século 20, vários novos resultados sobre a taxonomia e sistemática deste complexo de algas foram adicionados à área. Três países próximos às Antilhas se destacam por suas contribuições às mudanças instauradas nas últimas décadas. Como primeiras referências podemos citar os estudos realizados por Díaz-Larrea et al. (2007) no Caribe mexicano, onde registraram *Chondrophycus gemmiferus* (Harvey) Garbary & J. Harper como uma variedade de *Chondrophycus poiteaui* (J.V. Lamouroux) K.W. Nam (atualmente *Yuzurua poiteaui* var. *gemmifera*) com base em dados moleculares e semelhanças morfológicas. Um ano depois, Sentíes & Díaz-Larrea (2008) propuseram a transferência desta espécie e de *Chondrophycus corallopsis* para o gênero *Palisada*. Posteriormente, Sentíes et al. (2015), fizeram a nova combinação de *Palisada*

iridescens (M.J. Wynne & D.L. Ballantine) K.W. Nam (Nam 2007) para *Yuzurua iridescens* (M.J. Wynne & D.L. Ballantine) Sentíes & M.J. Wynne.

À medida que novas sequências são incorporadas ao banco de dados GenBank e mais estudos são realizados sobre o grupo na região tropical do Atlântico Ocidental, novos registros são encontrados para a área, assim como novas espécies também são identificadas e descritas. Por exemplo, *Chondrophycus anabeliae* Sentíes, M.T. Fujii, Cassano & Dreckmann, uma espécie nova descrita por Sentíes et al. (2016), a primeira deste gênero relatada para esta região Atlântica (México), posteriormente, encontrada na Venezuela (Cassano et al. 2020), e *Laurencia natalensis* Kylin refereida por García-Soto & López-Bautista (2019) e *Laurencia digitata* Francis, Bolton, Mattio & R.J.Anderson referida por Cassano et al. (2020), ambas registradas como novas ocorrências para a Venezuela, e cuja localidade tipo de ambas é a África do Sul. Em direção ao norte das Antilhas e Caribe, temos os relatos de *Laurencia caduciramulosa* Masuda & S. Kawaguchi para a Flórida por Collado-Vides et al. (2014), uma espécie descrita do Vietnã (Masuda et al. 1997). Novos estudos realizados para o complexo *Laurencia* apoiaram a transferência de *Laurencia cervicornis* Harvey para *Palisada cervicornis* (Harvey) Collado-Vides, Cassano & M.T.Fujii (Collado-Vides et al. 2017) e a descrição de novas espécies do gênero *Laurenciella* para os Estados Unidos, *Laurenciella mayaimii* Collado-Vides, Cassano & M.T.Fujii (Collado-Vides et al. 2018) e Bermudas, *Laurenciella namii* Popolizio, C.W.Schneider & C.E.Lane (Popolizio et al. 2022) juntamente com uma espécie nova de *Chondrophycus*, *C. planiparvus* Popolizio, C.W.Schneider & C.E.Lane (Popolizio et al. 2022).

Até o momento, as contribuições monográficas sobre o complexo no Caribe Ocidental foram feitas por Sentíes (2000), Sentíes & Fujii (2002) e Areces & Sentíes (2002). Deles, apenas neste último estudo aparecem informações sobre as espécies do complexo no arquipélago cubano. Os autores relataram para Cuba um total de 11 espécies e duas variedades. Dez delas também foram encontradas no Caribe mexicano: *L. brongniartii*, *L. caraibica*, *L. intricata*, *L. microcladia*, *L. obtusa*, *P. corallopsis* (como *L. corallopsis*), *P. perforata* (como *L. papillosa*), *Yuzurua iridescens* (como *L. iridescens*), *Y. poiteaui* var. *gemmifera* (como *L. gemmifera*), e *Y. poiteaui* var. *poiteaui* (como *L. poiteaui*), mas apesar dessa proximidade geográfica, neste estudo quase um número equivalente de espécies apareceu em apenas uma dessas regiões: dois no arquipélago cubano *Palisada furcata* (como *Chondrophycus furcatus*), *Osmundea coelenterata* (D.L. Ballantine & Aponte) M.T. Fujii, Sentíes & Areces (como *L. coelenterata* Ballantine & Aponte) e outros três na costa maia: *Laurencia venusta* Yamada, *L. filiformis* e *Palisada flagellifera* (como *L. flagellifera*).

Os inventários de espécies para a região têm como foco as listas gerais feitas por Wynne (1986, 1998, 2005, 2011, 2017, 2022) para o Atlântico Ocidental Tropical e Subtropical. Outras listas como as de Dreckmann et al. (1996), Zayas et al. (2002) e Herrera-Moreno & Betancourt (2022) têm um caráter local ou derivam de caracterizações ecológicas. Por sua vez, Suárez et al. (2015), em seu último inventário de macroalgas para Cuba, referiram 19 espécies e uma variedade deste complexo distribuídas em três gêneros: *Laurencia s.s.* (12), *Palisada* (5) e *Yuzurua* (com uma variedade). No entanto, estudos posteriores incorporam o gênero *Osmundea* no complexo *Laurencia* para águas cubanas com a transferência de *Laurencia coelenterata* (Ballantine & Aponte 1995) como *Osmundea coelenterata* com base em análise molecular e morfológica (Fujii et al. 2016).

Apesar de sua extensão e posição privilegiada nas Antilhas, poucos estudos do complexo *Laurencia* foram realizados em Cuba (Montagne 1842, Díaz-Piferrer 1957, Díaz-Piferrer & López 1959, Brito & Suárez 1994, Areces 1999, Areces & Sentíes 2002, Zayas et al. 2002, Areces et al. 2003, Sentíes et al. 2010, Fujii et al. 2016) em comparação com os encontrados para a região, e o reconhecimento da maioria das espécies tem sido feito por meio da identificação tradicional, baseada em características morfo-anatômicas, resultando em descrições e inventários florísticos. Apesar do esforço considerável para classificar taxonomicamente a flora de algas nesta área (Suárez et al. 2015), muitas classificações aplicadas morfologicamente não são suportadas por dados moleculares. Até agora, existem apenas o trabalho de Fujii et al. (2016) e algumas amostras cubanas sequenciadas por Cassano et al. (2012b).

Recentemente, Wynne (2022) em sua lista de espécies para o Atlântico ocidental tropical e subtropical, elevou o número total de espécies do complexo *Laurencia* para 43 espécies e cinco variedades. Algumas das quais podem ser tratadas como nomes mal aplicados para determinadas regiões. Um exemplo disso pode ser a espécie *Laurencia obtusa* relatada para Cuba e região do Caribe por Taylor (1960). Em estudos recentes do complexo nas Bermudas, Popolizio et al. (2022) apresentaram evidências moleculares de que o que havia sido historicamente identificado como *Laurencia obtusa* corresponde a duas espécies conhecidas, a saber, *Laurencia dendroidea* e *L. catarinensis*. Esses autores corroboram a sugestão de Cassano et al. (2012b) de que *L. obtusa* (localidade-tipo na Inglaterra) não ocorre no Atlântico ocidental. Isso confirma que o uso de técnicas moleculares constitui uma ferramenta fundamental para os estudos da biodiversidade e das inter-relações entre as espécies do complexo *Laurencia*, buscando esclarecer as entidades taxonômicas e inferir suas relações filogenéticas (Cassano 2009).

Segundo a compilação feita no checklist de Wynne (2022), os 43 representantes do complexo no Atlântico ocidental tropical e subtropical estão distribuídos da seguinte forma: seis espécies de *Palisada*, cinco de *Osmundea*, três de *Laurenciella*, duas de *Yuzurua*, duas de *Chondrophycus* e 24 de *Laurencia*. Este último é o gênero mais bem representado na área (Wynne 2022). Destas, apenas *Laurencia obtusa* e *Yuzurua poiteaui* possuem variedades (Wynne 2022).

1.4 Marcadores moleculares no complexo *Laurencia*

Considerando a alta diversidade de espécies descritas, sua extensa distribuição geográfica e a grande dificuldade envolvida na taxonomia do grupo, a comparação de sequências de DNA tem sido fundamental para estudos de biodiversidade, sistemática, ecologia e até química de espécies do complexo *Laurencia* a fim de elucidar as entidades taxonômicas e inferir suas relações filogenéticas (Cassano 2009, Fujii et al. 2011, Popolizio et al. 2022).

Vários marcadores moleculares foram estudados em algas vermelhas (Hillis & Dixon 1991, Freshwater et al. 1994, Reddy et al. 2018). Especificamente para o complexo *Laurencia*, foram utilizadas sequências do gene que codifica a subunidade grande (*rbc*L) da enzima ribulose 1,5-bifosfato-carboxilase-oxigenase e a região espaçadora Rubisco (*rbc*L-S) (Díaz-Larrea 2008, Cassano et al. 2012a,b, Collado-Vides et al. 2014, 2017, 2018, Sentíes et al. 2015).

Outros marcadores usados citados na literatura são o SSU rDNA que codifica o rRNA da subunidade grande do ribossomo (“small subunit rRNA gene” ou 18S), o LSU rDNA que codifica o rRNA da grande subunidade do ribossomo (“large subunit rRNA gene” ou 28S), ITS1 e ITS2, espaçadores nucleares internamente transcritos de genes para RNA ribossômico (“internal transcribed spacers”), incluindo o gene 5.8S (Díaz-Larrea 2008, Lewis et al. 2008, Kurihara et al. 2010), e COI-5P (região 5' do gene mitocondrial que codifica a subunidade I da citocromo c oxidase ou *cox*1).

O COI-5P é um marcador molecular do tipo "DNA Barcoding", denominado em analogia ao sistema de código de barras utilizado em produtos manufaturados (Stoeckle 2003). Atualmente é usado como uma ferramenta apropriada para a identificação de algas vermelhas em nível de espécie (Robba et al. 2006, Saunders 2009, Milstein & Saunders 2012, Costa et al. 2012). A utilização do sistema "DNA Barcoding" é muito prática e oferece grandes vantagens, pois os dados gerados têm ampla aplicação, com grande utilidade em levantamentos de biodiversidade, filogeografia, resolução de estrutura populacional e na conservação de espécies (Yang et al. 2008). O DNA Barcoding é uma técnica baseada na amplificação por PCR (“Polymerase Chain Reaction”) de um segmento relativamente curto de DNA (~400-700 pb) que pode ser completamente sequenciado com os mesmos dois "primers" usados na PCR.

O COI-5P tem sido utilizado em várias ordens do filo Rhodophyta com bons resultados (Saunders 2009, Conklin et al. 2009, Saunders & McDonald 2010, Geraldino et al. 2006, 2010, Robba et al. 2006, Yang et al. 2008, Le Gall & Saunders 2010, Costa et al. 2012, Milstein & Saunders 2012, Reddy et al. 2018). Especificamente para a ordem Ceramiales, Cassano (2009) obteve sequências de COI-5P para os gêneros *Laurencia s.s.* e *Palisada*, além de comprovar a eficiência deste gene na identificação e delimitação de espécies intimamente relacionadas e sua utilização para "DNA Barcoding".

A região do gene do cloroplasto que codifica o RNA da grande subunidade do ribossomo (23SRNAr), conhecida como "universal plastid amplicon – UPA", é outro dos marcadores propostos como "DNA Barcoding" alternativa para eucariotos fotossintetizantes (Presting 2006). Sherwood & Presting (2007), Conklin et al. (2009) e Sherwood et al. (2010) usaram o UPA para diferenciar espécies dos diferentes grupos de algas, incluindo cianobactérias e diatomáceas.

Apesar da variedade de marcadores existentes, os comumente utilizados em estudos do complexo *Laurencia*, com bons resultados são o *rbc*L e o COI-5P, sendo observado um uso cada vez mais frequente de ambos os marcadores em estudos do complexo (Machín -Sanchéz et al. 2014, 2016, Rousseau et al. 2017, Collado-Vides et al. 2018, Cassano et al. 2019, 2020, Sentíes et al 2019, Serio et al. 2020, Hughey & Miller 2021, Popolizio et al. 2022). Análises filogenéticas recentes baseadas nas sequências dos genes *rbc*L e COI-5P por Rousseau et al. (2017), testaram a eficácia desses marcadores na identificação e delimitação de espécies dentro do complexo, mostrando que *Laurencia flexilis* Setch consistia em uma linhagem independente e propuseram sua elevação em nível genérico *Ohelopapa* F. Rousseau, Martin-Lescanne, Payri e L.Le Gall. Algo semelhante foi encontrado por Cassano et al. (2019) em seu exame de espécimes de *L. clavata* da localidade tipo (Austrália), onde esses espécimes formaram um clado bem suportado com espécies de *Coronaphycus*, sendo transferidas com as espécies deste gênero para *Corynecladia* por prioridade.

1.5 Importância do complexo *Laurencia*

O conhecido caráter cosmopolita de algumas de suas espécies (Saito & Womersley 1974, McDermid 1988), bem como a grande diversidade morfológica, a ampla distribuição do grupo em ambos os hemisférios e as controvérsas taxonômicas têm motivado os estudos detalhados atualmente disponíveis sobre muitos de seus representantes. O interesse causado por este grupo combina apreciações acadêmicas e econômicas. Seus membros constituem uma parte significativa da ficoflora tropical e subtropical (Taylor 1960, Cribb 1983) e através do processo de bioconversão desempenham um papel importante na cadeia alimentar, seja

diretamente como fonte de alimento ou indiretamente, através da cadeia alimentar do detrito. São também potenciais hospedeiros de numerosas macro e microalgas (Fujii 1990), dando origem à formação de um microcosmo que garante alimento e abrigo tanto para a mesofauna epibiótica como para os juvenis de alguns importantes recursos pesqueiros, entre os quais, da lagosta espinhosa *Panulirus argus* Latreille no oeste do Caribe (Lalana et al. 1989). Aparecem também como componentes essenciais em não poucas biocenoses marinhas, tipificando-as, o que tem permitido a sua utilização como indicadores de qualidade ambiental (Areces et al. 2015).

Mesmo que ainda não tenham sido explorados em escala industrial, também é notável o valor econômico que pode ser derivado do uso de seus membros. Algumas espécies têm sido utilizadas como alimento, diretamente (Setchell 1905, Abbott 1984), ou como condimento (Zaneveld 1959, Hoppe 1969, Chapman & Chapman 1980, Abbott 1984). Muitos são importantes como fonte de ficocolóides (O' Colla 1962, Bowker & Turver 1968, Zablackis & McDermid 1988) e pela extraordinária diversidade química dos metabólitos secundários que carregam (Fenical & Norris 1975, Faulkener 1984, 1986, 1987, 1990, 1994, Fujii et al. 2011), dos quais conservadoramente existem 26 classes estruturais diferentes e das quais pelo menos 16 são exclusivas do grupo (Faulkner 1994, Hay 1996), motivando-os a serem do ponto de vista fitoquímico as macroalgas mais estudadas (Erikson 1983). Essa característica química do complexo *Laurencia* também tem sido utilizada por taxonomistas na separação das espécies a partir da distinção de seus metabólitos secundários, principalmente do gênero *Laurencia s.s.* (Fenical & Norris 1975, Masuda et al. 1996, Pietra 2002, Gil-Rodríguez et al. 2009, Manchín-Sánchez et al. 2014).

Tudo isso determina que os representantes do complexo, especialmente de *Laurencia s.s.*, sejam considerados um grupo promissor como fonte de fármacos e substâncias biologicamente ativas (Chapman 1970, Díaz-Piferrer 1979, García-Alonso et al. 1994), ou para serem utilizados em pesquisas biológicas (Switzer-Dunlap & Hadfield 1977).

1.6 JUSTIFICATIVA E RELEVÂNCIA

A maioria das espécies do complexo *Laurencia* apresenta grande plasticidade morfológica, o que dificulta a taxonomia do grupo. Portanto, o uso de marcadores moleculares é essencial na identificação e delimitação das espécies do complexo e na interferência de suas afinidades filogenéticas.

As análises moleculares realizadas nos últimos anos geraram informações fundamentais para revelar potenciais novos táxons para o complexo *Laurencia*, mostrando que é altamente diverso. No entanto, é necessário realizar em conjunto estudos morfológicos e moleculares mais

profundos para sua definição, complementados com informações ecológicas relevantes existentes para a área de estudo no Atlântico tropical e subtropical. A obtenção de sequências gênicas, essenciais para a resolução dos táxons do complexo, ainda deve abranger um número maior de representantes para refletir de forma mais robusta as relações filogenéticas e a evolução do grupo.

O complexo *Laurencia* vem sendo estudado no Atlântico ocidental tropical e subtropical de forma colaborativa como parte de um esforço internacional para um melhor entendimento da taxonomia, filogenia molecular e química de produtos naturais deste complexo e envolve pesquisadores das Universidade de São Paulo (USP-Brasil), Instituto de Pesquisas Ambientais (IPA-Brasil), Universidade Autônoma Metropolitana-Iztapalapa (UNAM-México), Florida International University (FIU-Estados Unidos) e Universidade Central da Venezuela, Caracas (UC-Venezuela). Cuba aderiu a esse projeto, permitindo a expansão dos trabalhos até agora realizados no Atlântico ocidental tropical e subtropical. No entanto, toda a cadeia antilhana ainda precisa ser melhor examinada para identificar os limites de distribuição norte e sul da flora do Caribe. O arquipélago cubano, nas Grandes Antilhas, é uma das áreas menos estudadas do ponto de vista da sistemática molecular e representa uma lacuna no entendimento da diversidade de espécies, relações filogenéticas e distribuição geográfica do complexo *Laurencia* no Atlântico. Apenas um artigo sobre o complexo incluía dados moleculares para representantes de Cuba (Fujii et al. 2016).

Assim, este estudo permitirá ampliar a área de estudo na região Atlântica, proporcionando maior representatividade das espécies do complexo, bem como uma revisão morfológica detalhada dos táxons, fundamentalmente complementada por estudos moleculares, inéditos para o complexo *Laurencia* em Cuba. As informações obtidas serão comparadas com os dados encontrados recentemente para a região como os de Suárez et al. (2015), permitindo um melhor entendimento da filogenia e distribuição geográfica das espécies do complexo *Laurencia* e evitando assim a perpetuação de problemas na taxonomia do grupo.

Com este estudo pretende-se ainda elaborar, possivelmente, o primeiro “barcoding” de algas vermelhas do complexo *Laurencia* para Cuba e integrando dados morfológicos, moleculares e biogeográficos, identificar as espécies do complexo para as águas cubanas.

Com exceção de quatro sequências de *rbc*L depositadas no GenBank, breves descrições de espécies e inventários realizados (Areces & Sentíes, 2002, Areces et al., 2003, Sentíes et al. 2010, Cassano et al. 2012b, Suárez et al. 2015, Fujii et al. 2016), muito pouco foi feito sobre a taxonomia e filogenia do complexo *Laurencia* em Cuba, e sua real diversidade ainda precisa ser desvendada. Considerando as mudanças significativas que ocorreram na classificação do complexo na última década e a importância de *Laurencia* tanto ecológica quanto químicamente,

o presente estudo espera confirmar e delimitar as espécies apresentadas na literatura para o arquipélago cubano e propor hipóteses filogenéticas para as espécies do complexo. Assim, pretende-se atingir os seguintes objetivos:

1.7 OBJETIVO GERAL:

Investigar a diversidade do complexo *Laurencia* no arquipélago cubano por meio de estudos morfológicos e moleculares visando contribuir para o conhecimento do complexo no Atlântico ocidental tropical.

Objetivos Específicos:

1. Analisar o material histórico referido para o arquipélago cubano em adição às espécies coletadas no presente estudo.
2. Revisar a diversidade das espécies do complexo *Laurencia* do arquipélago cubano com base em dados morfológicos e moleculares, gerando e comparando sequências do gene plastidial *rbc*L e do mitocondrial do tipo DNA barcoding COI-5P para fins filogenéticos e para a correta identificação das espécies.
3. Analisar o padrão de distribuição geográfica dos membros do complexo *Laurencia* no arquipélago cubano.

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CAPÍTULO 2

DIVERSITY AND PHYLOGENY OF THE *LAURENCIA* COMPLEX (CERAMIALES, RHODOPHYTA) IN THE CUBAN ARCHIPELAGO

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CAPÍTULO 2

Diversity and phylogeny of the *Laurencia* complex (Ceramiales, Rhodophyta) in the Cuban archipelago

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Abstract:

The Caribbean region is characterized by its high diversity of organisms, including marine phycoflora. Particularly, the species of the *Laurencia* complex (Ceramiales, Rhodophyta) have achieved notoriety in recent years and in different parts of the world after the numerous recent taxonomic changes. In Cuba, molecular studies of marine macroalgae are very scarce or practically null. The present study used recent collections to investigate the diversity and phylogenetic relationships within the *Laurencia* complex in the Cuban archipelago. The study was based on the morphological analysis allied with molecular tools of mitochondrial COI-5P, and plastidial *rbc*L genes, and species delimitation methods.The results confirmed the presence of four of the eight previously recognized genera in the *Laurencia* complex: *Laurencia s.s.*, *Laurenciella*, *Palisada* and *Yuzurua*. The genus *Osmundea* reported before for the Cuban archipelago was not confirmed. *Laurencia catarinensis* and *Laurenciella namii* represent new occurrences for the Cuban archipelago and for the western Caribbean. We found a high genetic divergence between the specimens of *Laurencia dendroidea* and *Palisada perforata*, which form a species complexes that requires further studies.

Keywords: COI-5P, Cuba, diversity, *rbc*L, Rhodomelaceae, taxonomy

INTRODUCTION

Laurencia complex, as it has been named, includes eight genera closely related, which share morphological characters such as apical cells located at the bottom of the terminal depression of the thallus, surrounded by branching trichoblasts, an uniaxial construction whose row of cells is only discernible near the apical cell, extensive cortication of the thallus (Falkenberg 1901, Kylin 1923, 1956), and two or four pericentral cells originated by each vegetative axial segment depending on the genus (Nam 2007). The members of *Laurencia* complex are: *Laurencia sensu stricto* (*s.s.*) J.V. Lamouroux (1813), *Chondrophycus* (Tokida & Saito) Garbary & Harper (1998), *Osmundea* Stackhouse (Nam et al. 1994), *Palisada* (Yamada) K.W. Nam (2007), *Yuzurua* (K.W. Nam) Martin-Lescanne (Martín-Lescanne et al. 2010), *Laurenciella* (Cassano, Gil-Rodríguez, Sentíes, Díaz-Larrea, M.C. Oliveira, & M.T. Fujii) Cassano, Gil-Rodríguez, Sentíes, Díaz-Larrea, M.C. Oliveira, & M.T. Fujii (2012a), *Ohelopapa* F. Rousseau, Martin-Lescanne, Payri & L. Le Gall (2017) and *Corynecladia* J. Agardh (Cassano et al. 2019).

The morphological distinction among the genera is based on a combination of vegetative and reproductive characters: the number of pericentral cells per vegetative axial segment, the presence or absence of secondary pit-connections between outermost cortical cells, the origin of the spermatangial branches and the shape of the male receptacles, number of pericentral cells in the segment of the branch that gives rise to the procarps, origin of the tetrasporangia and their arrangement on the fertile branches, number of sterile pericentral cells in the tetrasporangial branches, and the origin and type of spermangial branches (Nam et al. 2000, Nam 2006, Martin-Lescanne et al. 2010, Cassano et al. 2012a).

The high diversity of this complex has generated confusion regarding the classification, identification, synonymy, and nomenclature of this polymorphic species group (Yamada 1931, Saito 1967, 1969a, 1969b, Saito & Womersley 1974, McDermid 1988, Calumpong 1989, Fujii 1990, 1998). The lack of consistency of some of the traditionally analyzed characters, the difficulty in observing others of proven validity, the loss of type specimens or scarcity of topotype material sequences, and works with poorly preserved material should not be omitted as factors that have an impact on this appreciation (Sentíes 2000, Sentíes & Fujii 2002, Cassano et al. 2012a). This circumstance was perhaps the main reason why, in more than two centuries of study,

various supraspecific groupings have been proposed for its members (Areces & Sentíes 2002), giving their taxonomic study a dynamism that is difficult to match.

In his most recent checklist of macroalgal species for the tropical and subtropical western Atlantic, Wynne (2022) reports 43 species and five varieties for the *Laurencia* complex (four from *Laurencia s.s.* and one from *Yuzurua*). The members of the complex show a wide representation in the Atlantic Ocean, with the exception of *Corynecladia* not yet registered for the area. From the *Laurencia* complex, five of its genera are reported for Cuba with 18 species and one variety: *Laurencia* is the most speciose with nine species (Suárez et al. 2015, Wynne 2022), *Laurenciella* with one species (González-Sánchez et al. 2022), *Palisada* with five, *Osmundea* with one species and *Yuzurua* with two species and one variety (Suárez et al. 2015). *Laurencia filiformis* cited in the catalog of Cuban marine macroalgae by Suárez et al. (2015) was considered a synonym of *L. dendroidea* by Wynne (2022).

Studies carried out on the *Laurencia* complex using molecular tools in recent decades for the tropical and subtropical western Atlantic region have revolutionized the systematics of the complex with the reorganization of the taxonomic position of some of its members and the emergence of new species (Collado-Vides et al. 2018, Cassano et al. 2019, 2020, Medeiros 2022, Popolizio et al. 2022). However, species of uncertain affinities remain, due to faulty taxonomic identification (Popolizio et al. 2022, Wynne 2022). Species identification and description using morphological characters generate primary species hypotheses (PSHs) that need to be tested and confronted with other sources of information to delineate robust secondary species hypotheses (SSHs) and potentially reveal cryptic species (Postaire et al. 2016). The use of molecular markers in recent years has become a common tool for species delimitation, both to propose candidate species and to describe new species (Postaire et al. 2016, Amador et al. 2018).

The Cuban archipelago, despite its privileged position and extension in the Caribbean, has few molecular studies of its marine flora. Works that considered members of the *Laurencia* complex are only that by Fujii et al. (2016) who transferred *Laurencia coelenterata* D.L.Ballantine & Aponte to the genus *Osmundea* based on morpho-anatomical characters and phylogenetic analyses, and Cassano et al. (2012a,b) and Popolizio et al. (2022) using three *rbc*L sequences from Cuban species. In contrast, the information available in floristic compendia with descriptions of species and keys for their identification, monographic works, and inventories referring to the representatives

of this complex in the Antilles is much richer and more abundant (Taylor 1960, Littler & Littler 2000, Sentíes & Fujii 2002, Suárez et al. 2015).

It is a proven fact that taxonomic studies of the *Laurencia* complex require phylogenetic confirmation for the identification of its species, due to the high plasticity observed in its representatives. Despite the abundant and recent phylogenetic studies in the region (García-Soto & López-Bautista 2014, Sentíes et al. 2016, Collado-Vides et al. 2017, 2018, Cassano et al. 2019, 2020, Medeiros 2022, Popolizio et al. 2022), Cuba constitutes a gap in the knowledge of the diversity and phylogenetic affinities of species of the *Laurencia* complex. For this reason, in this study, we carried out an extensive review of the complex and updated its taxonomy for Cuba based on detailed morpho-anatomical studies combined with molecular analyses and species delimitation methods. Molecular data banks were produced, and phylogenetic hypotheses inferred that will serve as a baseline for future comparisons and will enrich the knowledge of the complex in the Caribbean and tropical and subtropical regions of the Atlantic.

MATERIALS AND METHODS

Study area

The Cuban archipelago has approximately 4000 islands, keys, and islets (Núñez Jiménez 1982, Miloslavich et al. 2010) distributed on an insular shelf less than 30 m deep, whose marine segment occupies 56.126 km^2 of extension (Claro 2006). It is bathed by oligotrophic waters whose minimum average temperature does not fall below 24.7º C (Blázquez-Echandi 1981). The completely clear days vary on average for the coastal strip between 60 to 80 per year and no less than 100 days have 10 or more daylight hours (Claro 2006). The combination of high temperatures and strong insolation with low levels of nutrients in the environment during most of the year often gives rise to limiting situations that can generate well-defined floristic gradients even in the face of small variations in nutrient fluxes. This fact, associated with the geomorphological peculiarities of each place, notably diversifies their benthic habitats, of which 39 different types have been recognized only at the mesoscale (Areces 2002). In the Cuban archipelago, the entire mosaic of coastal and marine ecosystems of the northwest Tropical Atlantic can also be appreciated, considered the most extensive biogeographic province and with the greatest development of the reef ecosystem in the Americas (Sullivan & Bustamente 2000).

Morphological observations

The material of the present study was collected between 2017 and 2021 in 33 sites in Cuban archipelago (Table S1, Figure 1). Specimens were collected manually with the aid of a spatula in the intertidal region of rocky shores or, in apnea or by SCUBA up to 10 m deep.

The material intended for the morphological study was kept free of formaldehyde and only dried in the shade. For the molecular study, apical regions, usually free of epiphytes, were cut off, dried in paper towels, and dehydrated in silica gel. The material was packed in plastic bags and kept at room temperature.

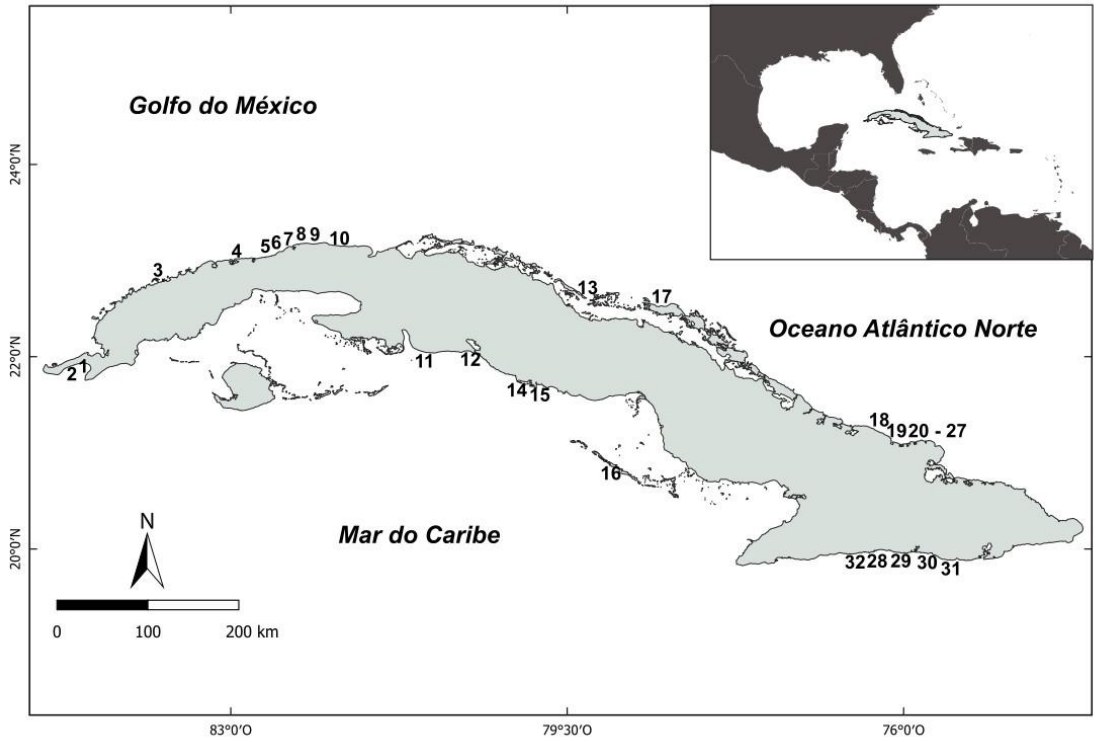

Figure 1. Map with the localities of the sampling sites on the Cuban archipelago coast (see names of collection sites in Table S1).

For analysis of the vegetative and reproductive characteristics, the material was rehydrated and free-hand cuts were made using a stainless-steel razor blade, under a stereoscopic microscope and stained with 0.5% aniline blue, acidified with 1N HCl (Tsuda & Abbott 1985). The images and photomicrographs of the thalli were obtained using a Panasonic Lumix DMC-FH4 camera (Panasonic Corporation, Osaka, Japan) coupled to a Zeiss Stemi 2000-C stereomicroscope and a Zeiss Primo Star microscope (Carl Zeiss Microscopy, Göttingen, Germany). All vouchers were deposited in the herbarium (SP) of the Environmental Research Institute, in São Paulo, Brazil.

Molecular analysis

Total DNA was extracted from approximately 5 mg of dry thalli in liquid nitrogen with the NucleoSpin® Plant II Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. The extracted DNA was stored at -20°C and used to amplify COI-5P and *rbc*L.

For the COI-5P gene amplification and sequencing reaction, specific primer pairs were used as follows: GAZF1-GAZR1 and GWSFn-GWSRx (Saunders 2005), and for the *rbc*L gene: FrbcLstart - R492, FrbcLstart - R753, F492 - R1150, F753-R1150, F492 - RrbcS, F492 - RrbcS, F577 - RrbcS, F993 - RrbcS, F753 - RrbcS, and F993 - rbcLrevNew (Freshwater & Rueness 1994, Saunders & Moore 2013). Both markers were amplified for a final volume of 25µL following the polymerase chain reaction (PCR) conditions described in Cassano et al. (2019) using GoTaq® Green Master Mix (Promega, Madison, Wisconsin, USA) in a Techne TC-512 thermocycler (Bibby Scientific Ltd, Staffordshire, UK). The successfully purified PCR products were sequenced (Biosciences Institute of the São Paulo University, Research Center on the Human Genome and Stem Cells, São Paulo University) with the same set of primers.

The forward and reverse sequences obtained were aligned manually using the BioEdit program (Hall 1999) to build a consensus sequence for each sample. These consensus sequences were aligned in Clustal W (within the BioEdit program) for the construction of matrices for phylogenetic analyses. All alignments were manually inspected.

In addition to the sequences generated in this study, sequences from Brazil, Venezuela, and Spain were included for analysis, and sequences from other species in the complex were downloaded from GenBank (Table S2). The species chosen as an outgroup in the alignments were: *Chondria baileyana* (Montagne) Harvey (KU564345), *Chondria collinsiana* M.Howe (GU330225), *Chondria dasyphylla* (Woodward) C.Agardh (U04021) and *Chondria acrorhizophora* Setchell & N.L.Gardner [as *C. californica* (Collins) Kylin) (AY172578)].

Phylogenetic analyses

Five matrices were created: a single matrix for COI-5P (175 sequences with 664 bp), a single matrix for *rbc*L (170 sequences with 1448 bp), and three matrices consisting of COI-5P sequences of *Laurencia* (87 sequences with 664 bp), *Palisada* (47 sequences with 664 bp) and *Yuzurua* (8 sequences with 664 bp). jModelTest 2.1 was used to estimate

the best evolutionary model of the Akaike information criterion parameters (Darriba et al. 2012). The selected phylogenetic model (GTR+I+G) for COI-5P and *rbc*L was used to complete the maximum likelihood (ML) and Bayesian (BI) analyses.

Phylogenetic trees were obtained using maximum likelihood and Bayesian inference. ML trees were inferred in IQ-TREE (Nguyen et al. 2015). Nodal support was assessed with 2,000 ultrafast snatch repeats (Minh et al. 2013). The pool of matched sequences from each array was contracted into single haplotypes using FaBox (v 1.41; Villesen 2007). For Bayesian analysis, trees were built using BEAST v1.8.4 (Suchard et al. 2018), with four MCMC chains with 100 million generations and sampling every 1000 generations. We discard the first 100.000 generations in both runs as burned to build the consensus tree. Clades with ML values equal to or greater than 70% and posterior probability (PP) values equal to or greater than 0.95 were considered strongly supported. Pairwise distances were calculated using the uncorrected "p" distances in PAUP.

Methods for delimiting species

We applied five DNA-based delimitation methods using separate data sets of COI-5P in order to make rational taxonomic decisions on this species complex. The methods used were the Automated Barcode Gap Discovery (ABGD) method of Puillandre et al. (2012a); Assemble Species by Automatic Partitioning (ASAP) by Puillandre et al. (2020); the Poisson Tree Process (PTP) model of Zhang et al. (2013), with a maximum likelihood (ML) implementation; and the general mixed Yule-coalescent model (GMYC) presented by Pons et al. (2006) that can be run using single (GMYCs) and multiple (GMYCm) delimiting thresholds.

The ABGD proposes the grouping of the input sequences into several hypothetical species automatically with the sole use of pair-wise distances (Puillandre et al. 2012a). The method was implemented using the Web interface available at http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html. A fasta alignment was used as input file using ABGD default parameters (pmin = 0.001, pmax = 0.10, number of bins = 10), except the relative gap width (X) was set to 1.0 (Puillandre et al. 2012a).

ASAP is the implementation of a hierarchical clustering algorithm that uses only paired genetic distances, avoiding the computational burden of phylogenetic reconstruction. This method uses partitions of species classified by a new scoring system, which does not use a prior biological view of intraspecific diversity.

The analyzes were performed on the full alignments using the default configuration of your online server (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html). The following parameters were used: p distances as a model to compute the distances, groups were split below the probability of 0.01, and the 10 best scores were saved.

PTP is a newly developed method that can delimit species by means of non-ultrametric phylogenies by using branch lengths to estimate the mean expected number of substitutions per site between two branching events (Zhang et al. 2013). ML tree files (obtained in the same way as for the phylogenetic analyses described below) were used as input files in the implementation of PTP analyses. The Web server of the Exelixis Lab (http://species.h-its.org/ptp) was used to run the analyses with default settings.

Finally, the GMYC method combines models of stochastic lineage growth (Yule models) with coalescence theory to determine the point of transition from species-level (speciation and extinction) to population-level (coalescence) evolutionary processes on a time-calibrated ultrametric tree by maximizing the likelihood score of the model (Pons et al. 2006, Zhang et al. 2013). Both the GMYCs and GMYCm versions were implemented on the Exelixis Lab web server (http://species.h-its.org/gmyc/).

All single-marker species delineation methods were compared with phylogenetic results, detailed morphological and anatomical analyses of several specimens, including a broad survey of the historical literature. This multiple-faceted approach allowed us to turn PSHs into conclusive secondary species hypotheses (SSHs) and draw taxonomic conclusions.

RESULTS

Phylogenetic analysis and species delimitation

COI-5P marker analysis

For DNA barcode analysis (COI-5P), a matrix was constructed with 175 sequences; 18 of these were generated for this study from specimens collected in Cuba, and sequences from Venezuela (38), Brazil (6), Spain (1) were also included, and the rest were downloaded from GenBank (Table S2). Among the complex genera, only *Yuzurua* and *Laurenciella* had high support for all analyses. All other genera have high to moderate support for ML and BI analysis (Figure S1).

The values of intraspecific and interspecific divergence for COI-5P ranged from 0 to 4.58% and 2.41 to 15.51%, respectively.

The species delimitation methods (SDM) were applied for each genus separately, each generic grouping being formed by our sequences from *Palisada*, *Yuzurua*, and *Laurencia s.s.* and those closely related (Figure 2).

The SDM generated different numbers of primary hypotheses of species (PHS). PTP was the most conserved method, generating 11 PHS, while ABGD, ASAP, and GMYC generated 19 to 29 PHS. Analyzing the congruence between the methods, we elaborated the species sequence hypothesis (SSH) with 19 SSH, of which 10 SSHs are from Cuba (Figure 2).

PSHs
SSHs
ABGD
ASAP
PTP
GMYCs
GMYCm
P. perforata Venezuela 12
P. perforata Venezuela 4
P. perforata Venezuela 5
P. perforata Dominica OM460643
P. perforata USA MH388699
P. perforata Bermuda OK209891
P. perforata Venezuela 16
P. perforata Venezuela MH388716
P. perforata Venezuela 21
P. perforata Venezuela SR2
P. perforata Venezuela AG1
P. perforata Venezuela T14
P. perforata Venezuela MH388710
P. perforata Venezuela T18
P. perforata Venezuela CV4
P. perforata Venezuela CV5
P. perforata Spain KF492773
P. perforata Cuba FSC3
P. perforata Cuba SO2
P. perforata Cuba SO3
P. perforata Cuba GH1
P. perforata Cuba RL0
P. furcata Brazil CE34
P. corallopsis Bermuda OK209890
P. corallopsis Cuba
P. sp. 1 Florida, USA OK209889
P. sp. Cuba CSC1
P. cervicornis Bermuda OK209888
Y. sp. Cuba BPC6
Y. sp. 1 Bermuda OK209893
Y. poiteaui Cuba G7
Y. poiteaui Bermuda OK209894
Y. poiteaui USA OK209895
Y. sp. Guadeloupe KX258843
Y. sp. Cuba RGbo3
Y. iridescens Bermuda OK209902
L. dendroidea Venezuela 14
L. dendroidea Venezuela 15
L. dendroidea Venezuela CH15
L. dendroidea Venezuela CH3
L. dendroidea Venezuela MH388711
L. dendroidea Spain KF492725
L. dendroidea Spain KF492728
L. dendroidea Brazil LVParati
L. dendroidea Brazil LfilAngra
L. dendroidea Brazil LobtRasa
L. dendroidea Venezuela 1
L. dendroidea Cuba AB
L. dendroidea Bermuda OK209900
L. dendroidea Venezuela 9
L. dendroidea Venezuela CM1905
L. dendroidea Venezuela 3
L. dendroidea Cuba CG2
L. dendroidea Molokai, USA GU223887
L. dendroidea Australia MT822859
L. dendroidea Australia MT822855
L. dendroidea Australia MT822856
L. dendroidea Australia MT822857
L. dendroidea Australia MT822858
L. caduciramulosa Cuba B1
L. caduciramulosa Brazil LCPV
L. caduciramulosa Spain LCTE
L. microcladia Virgin Islands OK209896
L. microcladia Cuba PCSC1
L. microcladia Bermuda OK209905
L. microcladia Venezuela LC3
L. microcladia Venezuela PA8
L. catarinensis Cuba CH3
L. catarinensis Spain KF492718
L. catarinensis Virgin Islands OK209898
L. catarinensis Brazil VC
L. catarinensis Bermuda OK209904
L. catarinensis USA OK209903
L. catarinensis Venezuela LC2
L. catarinensis Venezuela PA1
L. catarinensis Venezuela N2
P. aff. *perforata* 1
P. aff. *perforata* 2
P. perforata
P. aff. *perforata* 3
P. corallopsis
P. sp. 1 USA
P. sp. Cuba
P. cervicornis
Y. sp. Cuba
Y. sp. 1 Bermuda
Y. poiteaui
Y. sp.
Y. iridescens
L. dendroidea
L. aff. *dendroidea*
L. caduciramulosa
L. microcladia
L. aff. *microcladia* 1
L. catarinensis
0.009

Figure 2. Phylogenetic relationships of *Palisada*, *Yuzurua* and *Laurencia* species based on COI-5P sequences. Outgroups were removed. The tree summarizes the results of Maximum Likelihood (ML) and Bayesian Inference (BI) analyses. Black stars indicate tree nodes with a posterior probability (PP) ≥ 0.95 and Maximum Likelihood support (ML) > 70%; grey stars indicate tree nodes with a PP ≥ 0.90; blank stars indicate tree nodes with ML > 70%. Grey vertical bars represent singletons and clusters identified by species delimitation methods (from left to right: the ABGD, the ASAP, the PTP, the single (GMYCs) and multiple threshold models (GMYCm)), and represent also the primary species hypotheses (PSH).

For the genus *Palisada*, two groups of sequences were formed in this study for COI-5P (Figure S1). The first group with moderate to high support (76% NJ, 95% ML, 1.00 PP), which includes sequences of *P. perforata* (Figure S1). This grouping was divided into two lineages: one formed by the sequences of *P. perforata* from the type locality (Tenerife, Canary Islands, Spain, Bermuda, USA, Dominica, and Venezuela) and another by the sequences of Cuba (Figure S1). The second grouping also with moderate to high support (89% NJ, 92% ML, 1.00 PP), was formed by sequences of *P. corallopsis* (Cuba and Bermuda), *P. furcata* (Brazil), an unidentified species of *Palisada* from Florida, and another unidentified species of *Palisada* from Cuba that joined with *P. cervicornis* from Bermuda (Figure S1).

The variation of intraspecific divergence found in the genus *Palisada* was from 0 to 2.96% and interspecific was from 3.05 to 10.04% (Table S3). The application of the SDM to the *P. perforata* separated the sequences into four distinct lineages of SSH: *P.* aff. *perforata* 1, 2, and 3, and the topotype of *P. perforata* from Spain (Tenerife, Canary Islands) (Figure 2). However, the most conserved PTP method considered them all as a single entity (Figure 2). Due to the high divergence verified by the COI-5P and the results of the SDM, we named the Cuban specimens as *P.* aff. *perforata* 3 as they are morphologically similar to *P. perforata* as currently circumscribed.

The divergence found between the sequences of all groups of *P. perforata* varied from 0 to 6.59% (Table S4). The lineage of *P.* aff. *perforata* 1 *sensu* Medeiros (2022) included sequences from Venezuela, Dominica, the USA, and Bermuda with high support for most analyses (Figures 2, S1). The divergence found between these sequences was 0 to 1.93%, indicating that they all correspond to the same taxonomic entity.

The lineage of *P.* aff. *perforata* 2 *sensu* Medeiros (2022) included only sequences from Venezuela (Medeiros 2022, unpublished, and from GenBank, MH388710,

MH388716) with high support for most analyses (Figures 2, S1). The divergence found between these sequences ranged from 0 to 3.91% (Table S4).

The sequence from Spain (KF492773) corresponding to the type locality, was considered as an independent lineage by ABGD and ASAP (Figure 2) and presented high values of divergence from the other sequences of its grouping (3.66 to 5.06%, Table S4). When comparing the divergences of *P*. aff. *perforata* 1 and *P*. aff. *perforata* 2 showed high divergence between them, 3.16 to 4.87%, being even higher when we compare both groups with the sequence from Spain (3.66 to 5.06%) (Table S4).

Cuban specimens *P*. aff. *perforata* 3 formed an independent lineage recognized by the SDM, except PTP, and with moderate to high support (97% NJ, 89% ML, 1.00 PP; Figures 2, S1). The divergence found between the Cuban sequences ranged from 0 to 1.52% (Table S4).

The group formed by *P. corallopsis* (Cuba and Bermuda), *P. furcata* (Brazil), *P. cervicornis* (Bermuda), and the two unidentified species from Florida and Cuba had moderate to high support (89% NJ, 92% ML, 1.00 PP; Figure S1). For this grouping, the application of the SDM generated different numbers of PSH. The PTP method considered them as a single entity. On the other hand, GMYC generated three PHS, while the other methods generated four or more PHS (Figure 2).

The western Atlantic *P. corallopsis* sequences represented by Cuba (type locality) and Bermuda diverged from each other by 2.18%, but compared with the topotype of *P. furcata* (Brazil), they diverged by 1.09% for Bermuda and 1.65% for Cuba (Table S5). The unidentified *Palisada* species from Cuba diverged from *P. cervicornis* from Bermuda and from another species from Florida by 2.83% and 3.49%, respectively.

Three sequences of the genus *Yuzurua* (G7, BPC6, and RGbo3) were generated in this study (Figure S1). G7 grouped with the sequences of COI-5P from *Y. poiteaui* from Bermuda and the USA, whereas RGbo3 grouped with the unidentified species from Guadeloupe. There was no genetic match with any COI-5P sequence available from GenBank for BPC6. The divergence of the genus *Yuzurua* ranged from 0.15 to 8.07%, finding an intraspecific variation from 0.15 to 0.87% and interspecific variation from 5.24 to 8.07% (Table S6). Among the specimens of *Y. poiteaui* (Cuba, Bermuda, and Florida, USA) we recorded a divergence of 0.43 to 0.87%. Our RGbo3 sequence had a divergence from the Guadeloupe species of 0.15%, indicating that it is the same species, and the BPC6 sequence diverged from the other taxa from 5.67 to 8.07%, suggesting that it is a different taxon from the others. All applied SDM managed to distinguish BPC6 as a single

and independent entity. On the other hand, the ABGD, ASAP and PTP methods were able to recognize the G7 and RGbo3 sequences with their corresponding taxa, while single/multiple GMYC methods considered the *Yuzurua* taxa as two PHS (Figure 2).

For the genus *Laurencia*, three groups of the sequences generated in this study were obtained (Figures 2, S1). The first group with moderate to high support (97% NJ, 91 ML, 1.00 PP) grouped *L. dendroidea* (Bermuda, USA, Virgin Islands, Cuba, Venezuela, Brazil, and Spain) and sequences identified as *L.* aff. *dendroidea* (Australia and Hawaii, USA); the second group with moderate to high support (88% NJ, 1.00 PP), included sequences of *L. microcladia* (Bermuda, Virgin Islands, Cuba, and Venezuela) and sequences of *L. caduciramulosa* (Brazil, Spain, and Cuba); and the third, with full support, grouped sequences from *L. catarinensis* (Cuba, Brazil, Venezuela, Spain, Florida, USA, and Bermuda; Figure S1).

The intraspecific variation recorded for the genus *Laurencia* was from 0 to 2.93% (4.58% for *L. dendroidea*) and the interspecific variation was from 3.46 to 11.79% (Table S3).

In the COI-5P analysis, the *L. dendroidea* assemblage was divided into three lineages, most with high support, represented by sequences from the Atlantic and Pacific (Australia and Hawaii, USA). The Atlantic sequences were subdivided into two groups: one with sequences from Bermuda, USA, Virgin Islands, Cuba, and Venezuela, and another with sequences from Spain, Brazil, and Venezuela (Figure S1).

The SDM gave different PSH results for the grouping of *L. dendroidea* from Atlantic and Pacific Oceans (Figure 2). All methods applied separated the Pacific and Atlantic sequences. The ASAP and GMYC (single/multiple) methods generated three to four PHS, and two by ABGD for the Atlantic sequences. PTP separated the Pacific and Atlantic sequences as unique PSH. Part of the methods (ASAP and GMYC single/multiple) separated the specimens of *L. dendroidea* from Brazil, the type locality, as distinct lineage. On the other hand, the most conserved method PTP considered all taxa as a single entity, whereas the ABGD pointed to two lineages: one that included sequences from Venezuela, Spain, and Brazil, and another that included sequences from Venezuela, Bermuda, and Cuba (Figure 2).

All *L. dendroidea* sequences (n=35) diverged from 0 to 7.2%, recording the highest divergence (7.2%) between *L. dendroidea* from Atlantic (Spain) and Pacific (Australia), and also of the latter with various sequences from Venezuela (Table S7). The representatives of *L. dendroidea* from the Atlantic (Bermuda, USA, Virgin Islands,

Venezuela, Brazil, Spain, and our specimens from Cuba, n=4), presented a divergence from 0 to 4.58% (Table S7, Figures 2, S1). The Cuban samples showed genetic divergence, 0 to 0.30%, and a lowest divergence, 0 to 0.21%, between them and sequences from Bermuda, USA, Virgin Islands, and part of Venezuelan sequences, clustered in the same assemblage (Table S7). However, the divergence between Cuban and Venezuelan sequences reached up 3.39%. Similar divergence was observed between our sequences from Cuba and the topotypes from Brazil, 2.56 to 3.46%. For the analyzed Atlantic sequence set, a highest divergence was found between sequences from Cuba and those from the eastern Atlantic (Spain), 3.66 to 4.18 % (Table S7). For their part, the sequences that made up the Pacific group (Australia and Hawaii, USA) had a divergence of 0.17 to 7.21%, and the specimens from Cuba diverged from the Australian and Hawaiian specimens by 5.45 to 6.33% and from 5.57 to 5.87%, respectively (Table S7).

Two sequences of *L. microcladia* from Cuba grouped with *L. microcladia* from US Virgin Islands (type locality) and Bermuda with moderate support only for BI (0.92 PP), and it was closest to sequences of *L.* aff. *microcladia sensu* Medeiros (2022) from Venezuela (Figure S1). One sequence of *L. caduciramulosa* from Cuba grouped with sequences of this species from Brazil and Spain with moderate to high support (Figure S1), showing an intraspecific divergence of 0.64 to 0.80% (Table S8). Two of the SDM used (ABGD and PTP) resolved all these sequences (*L. microcladia*, *L.* aff. *microcladia* and *L. caduciramulosa*) as a single PSH, whereas ASAP and GMYC (single/multiple) separated them into four distinct PSHs (Figure 2). The Cuban sequences of *L. microcladia* diverged by 0.43% from the topotype sequence (US Virgin Islands), and diverged by 1.52% and 2.62 to 2.71% from the Bermuda and Venezuela sequences, respectively (Table S8). *Laurencia microcladia* and the closely related species, *L. caduciramulosa* showed an interspecific divergence of 1.52 to 2.77% (Table S8).

The sequences of *Laurencia catarinensis* from Cuba and Venezuela grouped with the sequences of this species from Santa Catarina, Brazil (type locality), Spain, Florida, USA, Virgin Islands, and Bermuda with full support (Figure S1). The genetic divergence between the sequences ranged from 0 to 0.66% (Table S9). All the SDM used resolved this grouping as a single PHS (Figure 2), confirming that the Cuban and Venezuelan specimens correspond to the authentic *L. catarinensis*.

*rbc*L marker analysis

For the *rbc*L phylogeny, seven new sequences were generated in this study, six from Cuba and one from Brazil. Sequences from Venezuela (11) and Brazil (1) were included in the analyses. A matrix with 170 sequences was constructed by adding sequences from GenBank (Table S2). We were unable to obtain sequences of *L. dendroidea*, *L. catarinensis*, *Yuzurua* sp. (BPC6), *Y. poiteaui*, *Palisada* sp. (CSC1), and *P.* aff. *perforata*. The maximum intraspecific divergence for *rbc*L ranged from 0 to 1.98%, whereas the interspecific variation ranged from 2.0 to 14.54%.

The complex was divided into the eight current monophyletic genera, most with full support, except for *Chondrophycus*, *Corynecladia*, and *Palisada* for which support for NJ and ML was moderate to high, and *Ohelopapa* for which there is only one sequence available in the GenBank (Figure 3). Although the genera have moderate to full support, the phylogenetic relationships among them were not supported by any analyses (Figure 3).

The studied taxa of the complex were divided into four distinct clades formed by the genera *Laurencia*, *Laurenciella*, *Yuzurua* and *Palisada* (Figure 3). The *Laurencia* clade included sequences of *L. caduciramulosa* (Spain, Cuba, Brazil, and USA), *L. laurahuertana* and *L. venusta* from Mexico, *L. microcladia* from the Virgin Islands and Bermuda, and *L.* aff. *microcladia* from Venezuela with high support (98% NJ, 100% ML, 1.00 PP). The *Laurenciella* clade was formed by *Laurenciella namii* from Bermuda, Cuba, and Florida, USA with full support. The *Yuzurua* clade included our sequence of *Yuzurua* sp. which grouped with the unidentified sequence from Guadeloupe, also with full support plus a sequence of *Y. iridescens* and an unidentified species from Bermuda. The *Palisada* clade grouped our sequences of *Palisada corallopsis* from Cuba with the other sequences of this species from GenBank (Bermuda, Mexico, and Florida, USA), plus *P. furcata* from Brazil, *P. cervicornis* from Bermuda and USA, and the unidentified species of *Palisada* from Florida, USA, and Cuba with a high support (100% NJ, 99% ML, 1.00 PP; Figure 3).

Chondria collinsiana Brazil (GU330225)
Chondria dasyphylla USA (U04021)
Chondria acrorhizophora USA (AY172578)
Ch. anabeliae Mexico (KX815262)
Ch. anabeliae Venezuela (MN597441)
Ch. sp. Australia (KY120337)
Ch. dotyi Hawaii (KX815263)
Ch. sp. South Africa (KY927795)
Ch. cf. *undulatus* New Caledonia (FJ785307)
Ch. sp. New Caledonia (KX146174)
Ch. tronoi Philippines (AF489864)
Ch. sp. USA (AF465817)
Chondrophycus
O. hybrida France (FJ785317)
O. osmunda France (FJ785318)
O. osmunda Ireland (AF281877)
O. pinnatifida Ireland (JX828140)
O. pinnatifida France (AF259495)
O. silvae Portugal (KU566561)
O. oederi Ireland (AF281880)
O. sp. Spain (JF781519)
O. truncata Spain (JF781523)
O. blinksii USA (AY172575)
O. splendens USA (KU564461)
O. spectabilis USA (AY172572)
O. spectabilis Mexico (AY172574)
O. sinicola USA (AY588407)
O. sanctarum Brazil (KC012601)
O.caspica Azerbaijan (KX146188)
Osmundea
Oh. flexilis Tahiti (KX146187)
Ohelopapa
C. clavata Australia (MF094079)
C. elatus Australia (KY120339)
C. novus Australia (KY120340)
Corynecladia
L. aldingensis Brazil (JF810351)
L. catarinensis Brazil (VC)
L. catarinensis Bermuda (OK209872)
L. catarinensis USA USVI (OK209874)
L. catarinensis USA (OK209873)
L. catarinensis Spain (KF492776)
L. catarinensis Venezuela (Lint2EV)
L. cf. *majuscula* New Caledonia (FJ785312)
L. cf. *mariannensis* New Caledonia (FJ785313)
L. mcdermidiae New Caledonia (FJ785314)
L. multiclavata South Africa (KY927789)
L. cf. *nidifica* New Caledonia (FJ785315)
L. digitata South Africa (KY927748)
L. digitata Venezuela (MN597443)
L. cf. *kuetzingii* New Caledonia (FJ785322)
L. karachiana Pakistan (MK796228)
L. sp. Sri Lanka (KX146183)
L. pumila South Africa (KY927765)
L. pumila var. *dehoopiensis* South Africa (KY927757)
L. alfredensis South Africa (KY927749)
L. flexuosa South Africa (AF465815)
L. caduciramulosa Spain (JF781525)
L. caduciramulosa Cuba (FJ904933)
L. caduciramulosa Brazil (KJ700865)
L. caduciramulosa USA (KJ700867)
***L. caduciramulosa* Cuba (B1)**
L. laurahuertana Mexico (KF279401)
L. microcladia USA USVI (OK209881)
L. venusta Mexico (EF061655)
***L. microcladia* Cuba (PCSC1)**
L. microcladia Bermuda (OK209880)
L. aff. *microcladia* Venezuela (LC3)
L. aff. *microcladia* Venezuela (PA20)
L. aff. *microcladia* Venezuela (LPA8PAE)
L. yamadana USA (GQ252550)
L. filiformis Australia (MH704449)
L. dichotoma South Africa (KY927786)
L. glomerata South Africa (KY927763)
L. natalensis South Africa (AF465816)
L. natalensis Venezuela (MH388523)
L. natalensis Sri Lanka (KX146176)
L. decussata Australia (KY120344)
L. rigida Australia (AY920852)
L. oliveirana Brazil (JF810352)
L. pacifica USA (AY588411)
L. corymbosa South Africa (KY927728)
L. stegengae South Africa (KY927743)
L. tasmanica Australia (MF094081)
L. sp. 2 Australia (MK125395)
L. intricata Cuba (GU330238)
L. intricata Mexico (AF465809)
L. intricata Bermuda (OK209879)
L. intricata USA (OK209878)
L. intricata USA USVI (OK209882)
L. longiramea Brazil (MH704451)
L. obtusa France (KX146185)
L. obtusa Ireland (AF281881)
L. viridis Portugal (KF492794)
L. viridis Spain (EF685999)
L. pyramidalis France (FJ785316)
L. mutueae Mexico (MK159177)
L. venusta Australia (KY120345)
L. sp. New Caledonia (KX146181)
L. dendroidea Brazil (AF465810)
L. dendroidea Brazil (GU330223)
L. dendroidea Venezuela (MH388528)
L. dendroidea Venezuela (T1)
L. dendroidea Mexico (GU330220)
L. dendroidea Bermuda (OK209876)
L. dendroidea USA (OK209875)
L. dendroidea Venezuela (10)
L. dendroidea USA USVI (OK209877)
L. dendroidea Venezuela (01)
L. dendroidea Guadeloupe (AF465811)
L. obtusa Venezuela (AF465812)
L. dendroidea Spain (EF686000)
L. majuscula Oman (KX146184)
L. sp. 1 Australia (MK125344)
L. sp. New Caledonia (KX146182)
L. cf. *brongniartii* New Caledonia (KX146178)
L. brongniartii Australia (KY120341)
L. complanata South Africa (KY927738)
L. sp. New Caledonia (KX146177)
L. brongniartii Taiwan (AF465814)
L. caraibica Mexico (EF658642)
L. caraibica Venezuela (MH388533)
Laurencia
La. marilzae Portugal (KF492798)
La. marilzae South Africa (KY927758)
La. marilzae Brazil (GU938189)
La. marilzae Spain (EF686001)
La. marilzae Croatia (KX146186)
La. sp. 1 Bermuda (OK209869)
La. namii Bermuda (OK209870)
***La. namii* Cuba (L10)**
La. namii USA (MG004184)
La. mayaimii USA (MG004182)
Laurenciella
Y. iridescens Bermuda (OK209858)
Y. sp. Guadeloupe (KX146198)
***Y.* sp. Cuba (RGbo3)**
Y. sp. 1 Bermuda (OK209862)
Y. poiteaui var. *gemmiferus* Cuba (EF061650)
Y. poiteaui var. *poiteaui* Mexico (EF061653)
Yuzurua poiteaui var. *poiteaui* USA (EF061652)
Y. poiteaui USA (OK209861)
Y. poiteaui var. *gemmiferus* Mexico (EF061648)
Y. poiteaui Bermuda (OK209860)
Yuzurua
P. cervicornis Bermuda (OK209863)
P. cervicornis USA (MG030375)
P. sp. 1 Florida USA (OK209864)
***P.* sp. Cuba (RPatricia)**
***P. corallopsis* Cuba (B)**
P. corallopsis Bermuda (OK209865)
P. corallopsis Mexico (EF061646)
P. corallopsis USA (MG020477)
***P. furcata* 1 Ceara Brazil**
P. flagellifera Spain (EF685998)
P. flagellifera Brazil (GU330227)
P. flagellifera Bermuda (OK209866)
P. cf. *perforata* New Caledonia (FJ785320)
P. papillosa Spain (EU256325)
P. perforata Spain (EU256327)
P. perforata Brazil (EU256331)
P. perforata Venezuela (MH388525)
P. aff. *perforata* Venezuela (SR2)
P. aff. *perforata* Venezuela (T22)
P. perforata USA (OK209867)
P. aff. *perforata* Venezuela (5)
P. perforata Bermuda (OK209868)
P. aff. *perforata* Venezuela (12)
P. perforata Mexico (EF658641)
P. sp. South Africa (KY927798)
P. patentiramea Philippines (AF489862)
P. sp. Hawaii (KX146190)
P. cf. *robusta* New Caledonia (FJ785321)
P. parvipapillata New Caledonia (KX146194)
P. crustiformans Hawaii (KX146196)
Palisada
0.04

Figure 3. Consensus tree derived from Maximum likelihood analyses of *rbc*L sequences with bootstrap values (≥70), maximum likelihood (≥70) and Bayesian posterior probabilities (≥0.95) appended, respectively. Samples generated in this study in bold, added from Venezuela and Brazil in blue; (–) indicates no boot support or values below 70; (*) indicates full support.

The sequence of *L. caduciramulosa* obtained in this study was grouped with a sequence from USA, whereas the other ones from GenBank (Brazil, Spain and Cuba) formed a subclade well-supported. The sequences of *L. caduciramulosa* showed a genetic divergence from 0 to 0.76%. As a sister group, it had the sequences of *L. laurahuertana* and *L. venusta* from Mexico, our sequence of *L. microcladia* from Cuba plus those from Bermuda, and the US Virgin Islands (type locality), and *L.* aff. *microcladia* from Venezuela. Our sequence of *L. microcladia* from Cuba diverged from the sequences from the Caribbean (Virgin Islands and Bermuda) by 0.11 to 0.43% (Table S10) confirming what was found by COI-5P. The genetic divergence of the entire clade was low (0-1.47%, Table S10), although the maximum value is within the interspecific variation for the genus *Laurencia* (Table S3). Likewise, the divergence observed in our study between *L. venusta* from Mexico and *L. microcladia* from the Virgin Islands and Bermuda was low, 0.16% between *L. venusta* and the topotype of *L. microcladia*, and 0.41% between *L. venusta* and *L. microcladia* from Bermuda (Table S10).

The *Laurenciella namii* clade grouped the sequence obtained in this study with the sequences from its type locality (Bermuda, OK209870) and from Florida, USA with full support. The divergence observed between the sequences ranged from 0.08 (between the Cuban sequence and the holotype) to 0.25%, confirming the identification of our specimen as *Laurenciella namii*. Its sister clade was formed by the sequences of *Ll. marilzae* from GenBank plus an unidentified species from Bermuda.

Our sequence of *Yuzurua* sp. was grouped with the unidentified species from Guadeloupe, with a genetic divergence of 0.09%, confirming to be the same species. *Y. iridescens* seem to be the closest taxa as the COI-5P results, but this relationship was not supported by *rbc*L gene. Our sequence showed a divergence of 3.55% with *Y. iridescens* from Bermuda (Table S11).

Our *Palisada* sequences formed two groups within the clade. The unidentified species from Cuba joined with full support to species from Florida (OK209864); both were 100% identical. These taxa were sister to *P. cervicornis* clade with sequences from the USA and Bermuda. The genetic divergence between *Palisada* sp. from Cuba and *P.*

cervicornis ranged from 2.71 to 2.79%, and between *Palisada* sp. 1 from Florida and *P. cervicornis*, 2.38% (Table S12).

Our sequence of *P. corallopsis* whose type locality is Cuba was grouped within a clade with the sequences of *P. corallopsis* from the USA, Mexico, Bermuda and also with a topotype of *P. furcata* from Brazil. The genetic divergence of *P. corallopsis* varied from 0.41 to 1.46% (Table S12), and between *P. corallopsis* from Cuba and *P. furcata* from Brazil, it was 0.95%. Despite registering a slightly high value for the intraspecific divergence of the members of *P. corallopsis*, the results of the genetic divergence between *P. corallopsis* from Cuba and *P. furcata* from Brazil for the COI-5P and *rbc*L, combined with the morphological analysis confirm that these species are conspecific.

DISCUSSION

Species delimitation when morphology is highly conserved or variable is a systematic challenge. Therefore, it has been recommended that multiple criteria (e.g., genetic, morphological, ecological, biogeographic) be used to delimit species (De Queiroz 2007, Leliaert et al. 2014, Gomes et al. 2020). This study revealed the existence of a wide cryptic diversity among some of the commonly identified species within the *Laurencia* complex. Combining analysis of two molecular markers, *rbc*L and COI-5P, five methods for DNA-based species boundary delimitation, together with morphological evidence of all taxa led us to a consensus to convert PSH in 19 SSH and 10 candidate species for Cuba (Figure 2). A conservative approach was followed to avoid an overestimation of species diversity and the creation of new unwarranted species names (Puillandre et al. 2012b).

Species delimitation methods, as well as the influence of the choice of molecular markers in phylogenetic analyses, is related to the restricted availability of sequences available in GenBank because they depend on the number of specimens to provide an estimate of intraspecific and interspecific relationships (Puillandre et al 2012b, Zhang et al 2013, Leliaert et al 2014). The *rbc*L gene is one of the most used to infer phylogenies and distinguish species in Rhodophyta, having an expressive number of sequences deposited in GenBank, often representing the only sequenced marker for certain species, which necessarily demands its use for comparative purposes (Medeiros 2022).

In general, the species delimitation methods recovered the same partitions in the clades of the complex, with greater inconsistencies being observed in the clade of *P. perforata* and *L. dendroidea*. Both clades comprised several specimens collected in Spain

and Brazil (type localities). It was included published sequences of taxa that have been extensively sampled by Cassano et al. (2012b), Machín-Sánchez et al. (2014), Popolizio et al. (2022), and also unpublished sequences generated by Cassano (2009) and Medeiros (2022). The number of genetic PSH observed was greater than the number of species identified based on morphology. Similar results were previously found for the *Laurencia* complex by Medeiros (2022), who pointed out the power of molecular tools to identify cryptic species within the complex.

For the genus *Palisada*, we were able to obtain topotype sequences of *Palisada corallopsis* from Cuba and *P. furcata* from Brazil, besides two unidentified species. One of them bound to the COI-5P sequence of *P. cervicornis* from Bermuda, and the other to *rbc*L bound to an unidentified species from Florida. We decided not to use the *rbc*L sequence of *P. furcata* from Brazil available in GenBank (GU330226) due to the high number of ambiguities (14) that the sequence present. *P. corallopsis* specimens had an intraspecific divergence of 2.18% for COI-5P and 0.41-1.46% for *rbc*L. When comparing the sequences of *P. corallopsis* and *P. furcata* for COI-5P and *rbc*L, we found a divergence of 1.6% and 0.95%, respectively. Despite our divergence values for *Palisada* goes beyond the limits found in previous studies by Cassano et al. (2012a), Popolizio et al. (2022) and Medeiros (2022), detailed morphological analyses of the type specimens and newly collected topotype specimens from both species, combined with the molecular results, indicated that these taxa are conspecific, with *P. corallopsis* having priority over *P. furcata* (Table 1). More detailed information on the description of *P. corallopsis* can be found in the Chapter 3 of the present thesis (González-Sánchez et al. unpublished).

Table 1. Comparison of morphological features between *Palisada corallopsis*, *P. furcata* and *P. cervicornis* from the western Atlantic Ocean. Based on Medeiros (2022). Species from the type locality in bold.

Species	Branching pattern	Lenticular thickenings	Outer cortex cell projections	Reference
P. corallopsis **Cuba**	Subdichotomous to dichotomous below; irregular, opposite to alternate above becoming subcorymbose	Absent	Absent	González-Sánchez et al. (unpublished)
P. corallopsis Bermuda	Irregularly cervicorn; apices often appearing dichotomous; some adventitious branching	Absent	Absent	Popolizio et al. (2022)

P. furcata **Brazil**	Di- to trichotomous	Absent	Absent	Cordeiro-Marino et al. (1994), Fujii & Sentíes (2005)
P. cervicornis **Florida, USA**	Irregularly branched to subdichotomous; secondary branches curved upwards	Absent (but medullary cell walls uniformly thickened)		Collado-Vides et al. 2017
P. cervicornis Bermuda	Irregularly palmate; branches varying in width/length at each node; to 10 branches per node	Uniform cell wall thickening present	Absent	Popolizio et al. (2022)

Unidentified *Palisada* species showed a divergence of 2.83% for COI-5P between *Palisada* sp. from Cuba (CSC1) and *P. cervcornis* from Bermuda, and 0% between the two unidentified species from Cuba (R) and Florida (OK209864) for *rbc*L. *Palisada* sp. (CSC1) was collected in Cazonal, Santiago de Cuba, the same collection site of *O. coelenterata* (Fujii et al. 2016). Despite that the external morphology of our material collected in Cazonal is similar to that of *O. coelenterata*, we consider that this species requires a revision since in our study we were unable to obtain the sequence of *rbc*L, and that of COI-5P that we generated it is grouped with the *Palisada* specimens. The *O. coelenterata* sequence of *rbc*L deposited in GenBank was not used because it is not of good quality, so it was not possible to compare it with our material.

Palisada perforata as well as *L. dendroidea* were the most common and abundant member of *Laurencia* complex, belonging to the genus *Palisada* and *Laurencia*, respectively. Our molecular data for these samples were pooled with the *P. perforata* sequences from GenBank and those used from Venezuela. However, they formed a separate lineage from the rest with a divergence between them from 0 to 1.52% and with the other sequences from 4.21 to 6.59%. The maximum value of divergence recorded within the clade of *P. perforata* corresponds to the interspecific level for the genus *Palisada* (Popolizio et al. 2022, 3.9-6.7%; Medeiros 2022, 3.16-9.27%) (Table S4).

The sequence from Spain (type locality) formed an independent lineage despite being only identified by two SDM (ABGD and ASAP) and presented high values of divergence with respect to the other sequences of its grouping (3.66 to 5.06%, Table S4), finding also within the limits of interspecific variation for the genus *Palisada* (3.9-6.7%, Popolizio et al. 2022, 3.16-9.27%; Medeiros 2022, 3.05-10.04%, this study) (Table S4).

Within the grouping of *P. perforata* from Spain, two more groups of sequences were joined, one formed by specimens from Bermuda, USA, Venezuela, and Dominica and another represented by sequences only from Venezuela (Figure 2, S1). These groups had moderate to high support and the intraspecific divergence of the lineages was low from 0 to 1.93% in the group from Bermuda, USA, Venezuela, and Dominica, indicating that they all correspond to the same taxonomic entity, whereas in the other group formed by sequences from Venezuela was from 0 to 3.24%. However, when comparing the divergences of both groups, a high divergence was observed between them, 3.16-4.87%, being even higher when we compare both groups with that of Spain (3.66-5.06%, Table S4). The other lineage formed by the Cuban species had a divergence with respect to the Spain sequence of 4.53-5.23% and to the groups of nearby localities it was 4.66 to 5.6% with the clade of Bermuda, USA, Venezuela, and Dominica; and with the clade formed by sequences from Venezuela from 4.21 to 5.64% (Table S4).

Although in our study only two SDM (ABGD and ASAP) managed to distinguish between the four lineages formed in *P. perforata*, we rely on the high genetic divergence results obtained from the groups, and on the criteria used by Medeiros (2022) to establish the SSHs, corresponding to *P. perforata* (topotype, Spain), *Palisada* aff. *perforata* 1 (Bermuda, USA, Venezuela, and Dominica), 2 (Venezuela), and 3 (Cuba) (Figure 2), since morphologically we were unable to differentiate them as entities other than the currently recognized *P. perforata*.

Medeiros (2022), in her study of the *Laurencia* complex from Venezuela, distinguished two different lineages from the topotype specimen from the Canary Islands, named them *Palisada* aff. *perforata* 1 and *Palisada* aff. *perforata* 2. This author reported that the first taxon is similar to *P. intermedia sensu* Rodríguez de Ríos (1979), whereas the second one to *P.* "*papillosa*" based on its morphology. However, Medeiros (2022) highlighted the need for further studies to define these taxa, including a review of the conspecificity of *P. papillosa* and *P. perforata,* which depends on obtaining sequences of the topotype of *P. papillosa* (C. Agardh) K.W. Nam (Red Sea).

The Cuban specimens named as *Palisada* aff. *perforata* 3, morphologically presented the characteristics described for *P.* "*papillosa*" by Taylor (1960), Littler & Littler (2000) and Cassano et al. (2009), but molecularly diverged from the Spanish topotype by 4.53-5.23%, these values being within the interspecific limits of *Palisada* for the COI-5P reported by Popolizio et al. (2022) and Medeiros (2022). Four of the SDM (ABGD, ASAP, GMYCs/m) used recognized this clade as an independent lineage

separating it from the rest of the *P. perforata* complex grouping. Our results confirm what was found by Medeiros (2022) for this species complex in Venezuela, being essential to obtain sequences of the type localities of *P. papillosa* (Red Sea) and *P. intermedia* (Japan) for different markers, with the objective of a better clarification of their taxonomic status.

Similar results for COI-5P were obtained by Cassano (2009). However, the relationships between sequences of the topotype of *P. perforata* from the Canary Islands, Spain, Brazil, Mexico, and the USA, including sequences identified as *P. papillosa*, were not resolved by the *rbc*L gene, whose divergence varied only by 0-0.3%. The phylogenetic position and the low divergence between these two taxonomic entities led Cassano et al. (2009) to propose the conspecificity between them, with *P. perforata* having priority over *P. papillosa*. In contrast, the divergence between COI-5P sequences from Brazilian and Canarian specimens was much higher, ranging from 0 to 4.1% (Cassano 2009). The conspecificity proposal made by Cassano et al. (2009) was based only on *rbc*L, a gene widely adopted at the time to identify species and infer phylogenetic relationships. As COI-5P has consolidated itself as a Barcode-type marker and greater sampling has been done for the *Laurencia* complex, taxonomic decisions should be revised using more markers or even multi-genetic and phylogenomic analyses. Furthermore, the definition of *P. papillosa* depends on the sequence comparison of its type locality (Red Sea), not available in GenBank, with the topotypes of *P. perforata* in order to clarify its taxonomic status, i.e., if it should remain reduced to a synonym *P. perforata* or whether it should be raised again to the specific category, as an independent species (Medeiros 2022).

Material referring to the genus *Yuzurua* was collected and sequenced in our study. The sequence of *Y. poiteaui* from Cuba was grouped with those of this species from the USA and Bermuda and whose divergence varied from 0.43 to 0.87% (Table S6). We were able to obtain RGbo3 sequence for COI-5P and *rbc*L which clustered with the sequence of an unidentified species from Guadeloupe, and by genetic divergence (0.15% for COI-5P and 0.08% for *rbc*L; Table S6, S11) appears to be the same species. We also found a third species of *Yuzurua* that did not group with any of the sequences present in GenBank and had a divergence with the other taxa from 5.67 to 8.16%, indicating it to be a different species.

Individuals of *Y. poiteaui* were first reported for Cuba by Taylor (1941) as *Laurencia poiteaui* (J.V. Lamouroux) M. Howe and within the genus, it is one of the common species on the Cuban coast (Suárez et al. 2015). Apparently, there is an area in

the Caribbean where the presence and development of *Y. poiteaui* are favored; several authors refer to its presence and abundance in places such as the Florida Keys and the US Virgin Islands (Popolizio et al. 2022) and Mexico (Pedroche & Sentíes 2020, García-García et al. 2020). Despite this, the genus was not recorded in the updated revision of the complex for Venezuela by Medeiros (2022). *Y. poiteaui* is considered rare in Bermuda by Popolizio et al. (2022) and was disregarded from the collections of Brazil and Spain due to incorrect identifications (Fujii & Sentíes 2005, Gil-Rodríguez et al. 2012). It was also possible to confirm the presence of *Y. iridescens* in Cuba (Figures S41-44, S45-50). Despite not having obtained a sequence for any of the markers used, the morpho-anatomical characteristics corresponded to those described by Wynne and Ballantine (1991), Littler and Littler (2000), and Sentíes et al. (2015) for the Caribbean specimens (Table 2).

Table 2. Comparison of morphological features between *Yuzurua iridescens* and *Y. poiteaui* from the western Atlantic Ocean. Based on Medeiros (2022). Species from the type locality in bold.

Species	Branching pattern	Outer cortex cell projections	Reference
Y. iridescens **Guadeloupe and Puerto Rico**	Thallus creeping or sprawling, branching irregular	Yes	Wynne & Ballantine (1991), Littler & Littler (2000)
Y. iridescens Mexico	Erect branches irregularly alternate and spirally arranged, usually with 1 (-2) orders of branches.	Yes	Sentíes et al. (2015)
Y. iridescens Cuba	Thallus creeping or sprawling, branching is irregularly alternate with 1(-2) orders of branches.	Yes	Present study
Y. poiteaui Mexico	Branching is irregularly alternate, with 2-3 orders of branching	No	Fujii et al. (1996), Sentíes & Fujii (2002)
Y. poiteaui Cuba	Branching is irregularly alternate, with 2-3 orders of branching	No	Present study

Specimens of *L. dendroidea* were grouped by COI-5P into one large clade that was subdivided into two main subclades determined by Atlantic and Pacific species. The

divergence verified between the sequences of all grouping of *L. dendroidea* varied from 0 to 7.21% between the species from Australia with those from Spain and some from Venezuela (14, 15, CH12, CH13, CH15, CH16, CH17, CH18, and T1), whose maximum value corresponds to the interspecific level for the genus *Laurencia* (Cassano et al. 2019, Popolizio et al. 2022) (Table S3).

Our sequences were grouped with the sequences from Bermuda, the USA, the Virgin Islands, Venezuela, Spain, and Brazil (type locality), with a divergence between them from 0 to 4.58% (Table S7, Figures 2, S1). Similar to observed by Cassano (2009) and Medeiros (2022), the highest values of genetic divergence for *L. dendroidea* samples were verified between the western and eastern Atlantic, being within the ranges reported for the genus *Laurencia* by Cassano et al. (2019, 2.6-10.2%), Popolizio et al. (2022, 2.8-9.3%) and Medeiros (2022, 1.74-13.87%) (Table S3).

Laurencia dendroidea was referred to Cuba by Díaz-Piferrer (1957) as *Laurencia scoparia* J. Agardh and later cited by Suárez (1973, 1984, 2005) and Suárez & Pérez (1989). The material studied is in agreement with the morphological characteristics of *L. dendroidea* described by Cassano et al. (2012b) for specimens from Brazil and Medeiros (2022) from Venezuela (Figures S19-24, S25-30).

We consider *L. dendroidea* as a species complex as already pointed out by Medeiros (2022) given the high divergence found among its specimens and the lack of morphological characters that allowed us to separate the specimens into different taxa. Based on our results and the previous detailed studies done for this species by Cassano (2009), Cassano et al. (2012b), Gíl-Rodríguez et al. (2012) and Medeiros (2022), we decided to consider the studied samples of *L. dendroidea* from the Atlantic as a single taxonomic entity. These results together with those of Medeiros (2022) demonstrate the need to use other markers and molecular analyzes to unequivocally define the taxonomic status of specimens from the Atlantic.

Similar to the results found by Medeiros (2022) when she compared the divergences of *L. dendroidea* from Venezuela with the subclade from Pacific, we also found high values with the specimens from Cuba (5.45 to 6.16%), from Venezuela (4.69 to 7.21%), from the USA, Virgin Islands and Bermuda (5.24 to 5.67%), from Spain (4.53 to 7.21%) and Brazil (5.27 to 7.04%), indicating that they can represent different taxonomic entities from the Atlantic (Table S7).

As well Medeiros (2022) explains in her analysis of the conspecificity between *L.* "*majuscula*" and *L. dendroidea* proposed by Metti et al. (2013), that the taxonomic

treatment of *L. majuscula* has been unstable over many years, leading to the integration and separation of both species. Therefore, *L. dendroidea* needs a broad revision for a better definition and delimitation of these taxa and we also agree with Medeiros (2022) that the conspecificity between *L.* "*majuscula*" and *L. dendroidea* needs to be reassessed, using and other molecular markers and, especially, phylogenomic studies. Because of this, we preferred to name the species of the Pacific as *L.* aff. *dendroidea*.

L. dendroidea and *L. obtusa* are two species cited for Cuba. In the catalog of macroalgae from Cuba, Suárez et al. (2015) mention that *L. dendroidea* is not common in Cuban waters, contrary to what is cited for *L. obtusa* where they refer to it as common and can become abundant. However, in our study, we did not find specimens of *L. obtusa* but of *L. dendroidea* in several collection sites, whose morphological characteristics correspond to the description made by Montagne (1842), Littler & Littler (2000) and Areces & Sentíes (2002) for *L. obtusa*. Recently, Popolizio et al. (2022) and Medeiros (2022) verified that specimens attributed to *L. obtusa* in Bermuda and Venezuela turned out to be *L. dendroidea* by molecular results and argued that, possibly, *L. obtusa* is not present in the western tropical Atlantic. These results confirmed those previously found by Cassano et al. (2012b) for *L. obtusa* and other poorly identified taxa for Brazil corresponding to *L. dendroidea*. Unfortunately, we were unable to obtain *rbc*L sequences of *L. dendroidea* from Cuba, but we believe that by obtaining sequences from this material in future work, our results may be similar to those found by the aforementioned authors.

Laurencia microcladia was first reported for Cuba by Montagne (1842). From our collected material we were able to obtain COI-5P and *rbc*L sequences for this species. Our sequences were pooled together with sequences of *L. microcladia* from the US Virgin Islands (type locality) and Bermuda, with sequences of *L.* aff. *microcladia* from Venezuela, plus sequences of *L. caduciramulosa* from Cuba, Brazil and Spain (Figures 2, S1). The variation found for the COI-5P (0.43-2.71%) is within the intraspecific values reported for the genus *Laurencia* by Medeiros (2022). The same was verified for the *rbc*L (0.1-0.36%) when compared with the intraspecific values found by Cassano et al. (2012a), Metti et al. (2013) and Medeiros (2022) (Table S3). Three of the SDM (ASAP and GMYCs/m) used identified *L. microcladia* from Bermuda as a different entity from the authentic *L. microcladia* from the US Virgin Islands (type locality) and Cuba (Figure 2). However, based on our intraspecific results and those of Popolizio et al. (2022) (Table

S10), we consider these specimens as a single taxon, and confirmed by molecular data the occurrence of *L. microcladia* for Cuba.

Our results showed that *Laurencia caduciramulosa* from the Atlantic is closely related to *L. microcladia* and *L.* aff. *microcladia*, as previously verified by Medeiros (2022). When comparing our genetic divergence results for COI-5P and *rbc*L genes (Tables S8, S10) with those reported in the literature for the intra- and interspecific divergences (Cassano et al. 2019, Popolizio et al. 2022, Medeiros 2022), our values between these three taxa are within the ranges determined by those authors for intraspecific divergence. However, when reviewing the morphology of our specimens of *L. caduciramulosa* and *L. microcladia* from Cuba and comparing them with those in the literature (Table 3, Figures S1-4, S5-9, S31-35, S36-40), we observe that there are morphological differences between them, such as the typical branching pattern of *L. microcladia*, i.e., irregular and with a whorled appearance in the upper third of the plant and the way of propagation of *L. caduciramulosa* from the fall of the deciduous branchlets that function as vegetative propagules. In the case of *L. microcladia*, the difference observed in our material regarding the described from Bermuda by Popolizio et al. (2022) and Venezuela by Medeiros (2022), was the presence of lenticular thickenings in the medullary cells (Table 3). Also, three of the SDM (ASAP and GMYCs/m) recognized *L. caduciramulosa* as a different entity from *L. microcladia*. Based on these results, we decided to maintain *L. caduciramulosa* and *L. microcladia* as independent taxa.

Table 3. Comparison of morphological features between *Laurencia microcladia*, *L.* aff. *microcladia* and *L. caduciramulosa* from the western Atlantic Ocean and Viet Nam. Based on Medeiros (2022). Species from the type locality in bold.

Species	**Branching pattern**	**Fixation**	**Epiphyte**	**Lenticular thickenings**	**Reference**
L. microcladia US Virgin Islands and Bermuda	Alternately to irregularly branched, dense distally; some branches clustered, appearing whorled	Discoidal holdfasts	-	Absent	Popolizio et al (2022)
L. microcladia Cuba	Alternately to irregularly branched, dense distally; some branches clustered, appearing whorled	Discoidal holdfasts with stoloniferous branches	No	Present	Present study
L. aff. *microcladia* Venezuela	Opposite to unilateral with a pyramidal appearance and clustered	Stoloniferous branches	No	Absent	Medeiros (2022)

	branches with a verticillate appearance				
***L. caduciramulosa* Viet Nam**	Irregularly alternate and spirally arranged	Discoid holdfast and stolon-like	No	Present	Masuda et al. 1997
L. caduciramulosa USA	Irregularly alternate and spirally arranged, usually with 2-3 orders of branches	Conspicuous discoid holdfast	Yes	Present	Collado-Vides et al. 2014
L. caduciramulosa Brazil	Irregularly alternate and spirally arranged, usually with 2-3 (4) orders of branches	Discoid holdfast and stolon-like branches	Yes	Abundant	Cassano et al. 2006
L. caduciramulosa Spain	Irregularly alternate and spirally arranged, usually with 2-3 (-4) orders	Discoid holdfast and stolon-like branches	No	Abundant	Cassano et al. 2008
L. caduciramulosa Cuba	Irregularly alternate and spirally arranged, usually with 2-3 orders of branches	Conspicuous discoid holdfast	Yes	Absent	Present study

The sequence of COI-5P of *Laurencia catarinensis* generated in this study grouped with *L. catarinensis* from Santa Catarina, Brazil (type locality), Venezuela, Spain, Virgin Islands, Florida, USA, and Bermuda (Figures S1, S10-14, S15-18). The genetic divergence between the sequences varied from 0 to 0.65%, corresponding to the values of intraspecific variation for the genus (Table S9). It was recognized as a single entity by all SDM applied (Figure 2). The molecular results were supported by the morphological observations made to our material and compared with that of the type locality (Brazil), Venezuela, and the Canary Islands (Table 4).

Table 4. Comparison of morphological features between *Laurencia catarinensis* from the western Atlantic Ocean. Based on Medeiros (2022). Species from the type locality in bold.

Species	**Habit**	**Branching pattern**	**Anastomoses between branches**	***Corps en cerise***	**Reference**
***L. catarinensis* Brazil**	Entangled, cushion-like tuft formation	Sparse, alternately spiral, to irregular,	Absent/Present	2 to 3 (cortical)	Cordeiro-Marino & Fujii (1985,

		sometimes unilateral			2005), Cassano (2009)
L. catarinensis Canary Islands, Spain	Entangled, cushion-like tuft formation	Sparse, alternate-spiral to irregular, and includes up to three orders of branches	Present	1 to 2 (3)	Machín-Sánchez et al. (2012)
L. catarinensis Venezuela	Entangled, cushion-like tuft formation	Alternate-spiral, to irregular	Absent	up to 2	Medeiros (2022)
L. catarinensis Cuba	Entangled, cushion-like tuft formation	Sparse, alternate-spiral to irregular	Present	Not observed	Present study

Laurenciella namii was a species recently described by Popolizio et al. (2022) for Bermuda. Our *rbc*L sequence grouped with the sequences of *Ll. namii* from Bermuda (type locality) and Florida, USA, with low genetic divergence (0.08%, Cuba and Bermuda; 0.21%, Cuba and Florida, USA), indicating that they are the same species (Figure 3). However, when comparing the morphological results, our specimen from Cuba showed differences in the coloration of the thallus with those from Bermuda and Florida (Table 5). This genus is reported for the first time for Cuba and its distribution area is extended to the western Atlantic.

Table 5. Comparison of morphological features between *Laurenciella* species from the western Atlantic Ocean. Based on Medeiros (2022). Species from the type locality in bold.

Species	**Thallus color**	**Branching pattern**	**Outer cortex cell projections**	**Reference**
***La. namii* Bermuda** and Florida, USA	Main axes yellow to orange, ultimate branches deep rosy red	Subopposite to spirally alternate	Present	Popolizio et al. (2022), Collado-Vides et al. 2018
La. namii Cuba	Main axes red, ultimate branches rosy red	Subopposite to spirally alternate	Present	Present study
La. mayaimii Florida, USA	Main axes brownish-yellow, ultimate branches rosy red branches rosy red	Subopposite to spirally alternate	Present	Collado-Vides et al. 2018
La. marlizae Spain	Main axes yellow-orange, ultimate branches rosy red	Irregularly alternate and spiral	Present	Gil-Rodríguez et al. 2009

CONCLUSIONS

We present the first approach using COI-5P sequences for specimens of the *Laurencia* complex in Cuba, together with new *rbc*L sequences generated in this study. The presence of six species were confirmed for Cuban archipelago: *L. caduciramulosa*, *L. dendroidea*, *L. microcladia*, *Palisada corallopsis*, *Y. poiteaui* and *Y. iridescens* besides two new records, *Laurenciella namii* and *Laurencia catarinensis*. The latter is referred for the first time for Cuba and also for the Caribbean area. The conspecificity of *Palisada corallopsis* and *P. furcata* was confirmed, the first having priority over the latter. For the other genetic groups found were assigned provisional names: *P*. aff. *perforata* 3, *Palisada* sp. CSC1, *Yuzurua* sp. BPC6, and RGbo3) pending further study. We did not have genetic groups that morphologically matched *Laurencia brongniartii*, *L. caraibica*, *L. chondrioides*, *L. intricata*, *L. minuscula*, *L. obtusa*, *Osmundea coelenterata*, *Palisada cervicornis*, *P. flagellifera* and *Yuzurua poiteaui* var. *gemmifera* species that are also reported for the Cuban archipelago.

Our results provide an overview of the current status of the *Laurencia* complex in Cuba and the tropical western Caribbean and at the same time denote the importance of increasing the number of samples and collection regions. It is also essential to use an integrative approach that encompasses morpho-anatomical analysis, phylogenomic, multigenic, biogeographical studies, speciation time analysis, reproductive strategies and gene flow verification for a precise delimitation, since the consensus of the methods only indicate how different approaches interpret the same data set, not being enough to precisely determine the number of species, especially when using only one marker.

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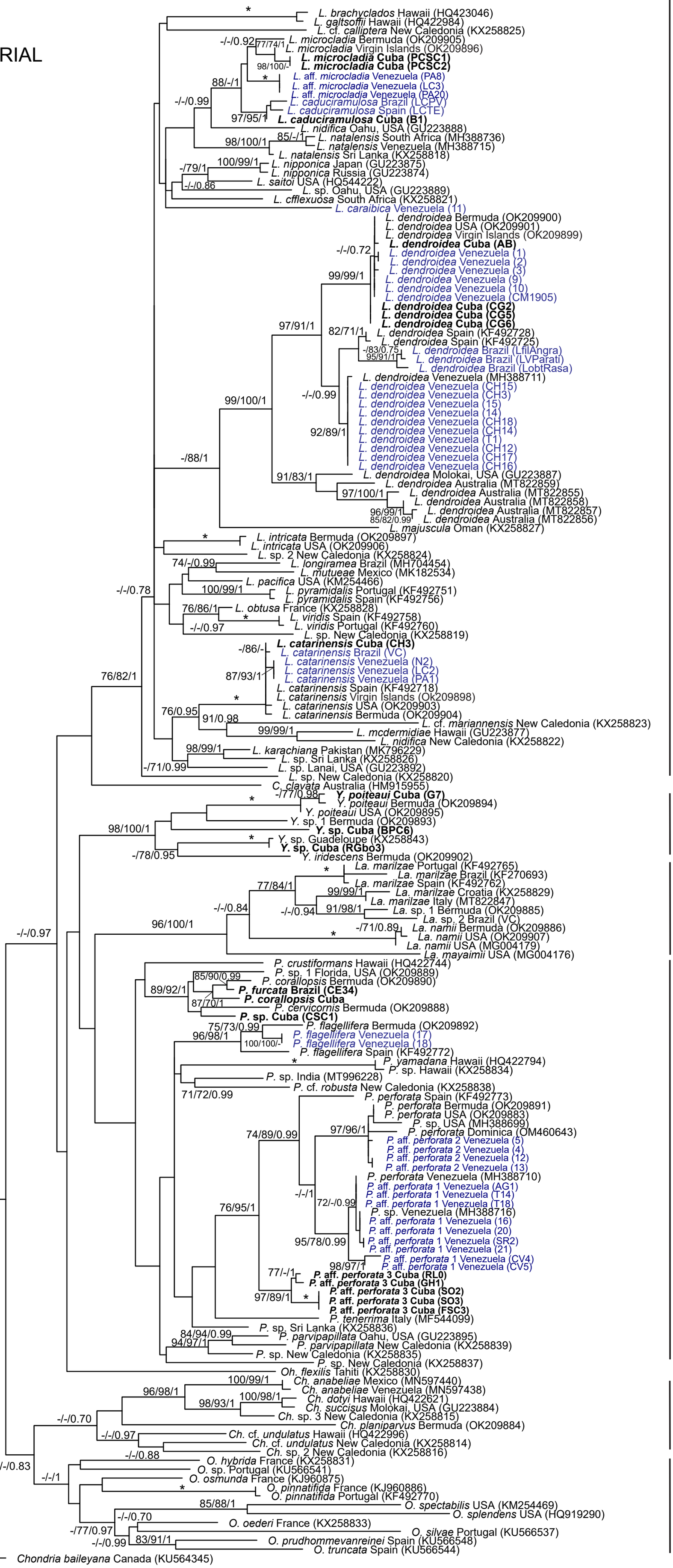
SUPPLEMENTARY MATERIAL



SUPPLEMENTARY MATERIAL

Figure S1. Consensus tree derived from Maximum likelihood analyses of COI-5P sequences with bootstrap values (≥70), maximum likelihood (≥70) and Bayesian posterior probabilities (≥0.95) appended, respectively. Samples generated in this study in bold, added from Venezuela and Brazil in blue; (–) indicates no boot support or values below 70; (*) indicates full support.

Table S1. Sample collection locations used in the study.

Country	Province/State	Local	Coordinates	Date	Coletor
CUBA	Pinar del Río	1. Cabezo Marcel, Guanahacabibes*	84°28'39"W, 21°51'05"N	March/2018 March/2019 April /2019	Y. Alfonso
		2. Laberinto, Guanahacabibes*	84°29'03"W, 21°54'30"N	March/2018 November/2018	Y. Alfonso
		3. Puerto Esperanza*	83°43'38"W, 22°46'32"N	April /2019	P. González Sánchez
	Artemisa	4. El Salado, Bauta	82°36'20"W, 23°02'25"N	May /2019	P. González Sánchez
		5. Playa Baracoa*	82°33'06"W, 23°03'16"N	May /2019 January /2021	P. González Sánchez
	La Habana	6. Bajo de Santa Ana, Santa Fe	82°31'38"W, 23°04'12"N	April /2019	P. González Sánchez, B. Martínez Daranas
		7. Playa La Puntilla, Santa Fe	82°30'28"W, 23°05'00"N	May /2019	P. González Sánchez
		8. La Francesita-Club Habana*	82°28'38"W, 23°05'32"N	January /2021	P. González Sánchez
		9. Litoral del Acuario Nacional de Cuba*	82°26'12"W, 23°06'59"N	January /2019 May /2019	Y. Alfonso Y. Alfonso, P. González Sánchez

	10. Rincón de Guanabo*	82°05'52"W, 23°10'21"N	April /2019	P. González Sánchez, A. Areces, D. Reyes
			February /2020	A. Areces, D. Reyes
Matanzas	11. Caleta Buena, Playa Girón*	80°57'20"W, 22°02'38"N	May /2019	P. González Sánchez
Cienfuegos	12. Rancho Luna*	80°25'32"W, 22°02'14"N	May /2019	P. González Sánchez, Ángel Moreira
Villa Clara	13. Cayo Santa María*	79°04'18"W, 22°38'48"N	May /2019	P. González Sánchez, Á. Moreira
Sancti Spíritus	14. Playa La Boca, Trinidad	80°01'59"W, 21°48'02"N	May /2019	P. González Sánchez
	15. Playa Ancón, Trinidad*	80°00'43"W, 21°44'15"N	May /2019	B. Martínez Daranas
Ciego de Ávila	16. Cayo Anclitas, Punta Prácticas, Jardines de la Reina*	78° 57' 57" W, 20° 48' 44" N	June /2017	P. González Sánchez, D. Hanisak
	17. Playa Flamenco, Cayo Coco	78° 26' 20" W, 22° 32' 36" N	July /2018	Á. Moreira
Holguín	18. Caletones, Gibara*	76°14'39"W, 21°12'38"N	May /2019	P. González Sánchez, E. Reynaldo de la Cruz
	19. Litoral de la ciudad de Gibara*	76°07'46"W, 21°07'03"N	May /2019	P. González Sánchez, E. Reynaldo de la Cruz
	20. Playa Cayuelo, Guardalavaca	75°40'32.2"W, 21°07'34.9"N	August /2019	E. Reynaldo de la Cruz
	Esmeralda	75°52'18"W, 21°06'51"N	August /2019	E. Reynaldo de la Cruz

		21. Playa las Guanas, Bahía Naranjo	75°52'32.8"W, 21°06'34.7"N	August /2019	E. Reynaldo de la Cruz
		22. Playa Yuraguanal	75°53'33"W, 21°06'39.7"N	August /2019	E. Reynaldo de la Cruz
		23. Playa Pesquero	75°56'17.5"W, 21°05'45.3"N	August /2019	E. Reynaldo de la Cruz
		24. Playa Don Lino	75°59'41.8"W, 21°05'16.8"N	August /2019	E. Reynaldo de la Cruz
		25. Gibara Turbinas Eólicas o Primer parque Eólico en Gibara	76°07'36.4"W, 21°07'48.7"N	August /2019	E. Reynaldo de la Cruz
		26. Playa Curita, Gibara	76°07'48.5"W, 21°06'57.8"N	August /2019	E. Reynaldo de la Cruz
		27. Gibara frente al punto guarda frontera	76°07'40.0"W, 21°07'34.6"N	August /2019	E. Reynaldo de la Cruz
	Santiago de Cuba	28. La Socapa*	75°52'20"W, 19°58'19"N	May /2019	P. González Sánchez, E. Reynaldo de la Cruz
		29. Cazonal*	75°28'32"W, 19°53'11"N	May /2019	
		30. Pedro el cojo*	75°30'40"W, 19°53'08"N	May /2019	
		31. Playa de Juraguá	75°40'19"W, 19°56'17"N	May /2019	
		32. Playa El Francés*	76°13'47"W, 19°59'40"N	May /2019	
VENEZUELA	Vargas (La Guaira)*	Puerto Azul	66°44' 50"W, 10°37' 19"N	June /2014	A. Brito, B. Vera.

		Los Caracas	66°34’17"W, 10°37’40"N	May /2011	B. Vera, C. Moreno
				June /2011	B. Vera, C. Moreno
		Taguao	67°06’07"W, 10°34’37"N	August /2014, 2015	A. Brito, B. Vera.
		Chichiriviche de la Costa	67°14’27"W, 10°33’01"N	July /2014	A. Brito, B. Vera.
		Naiguatá	66°44’21"W, 10°37’02"N	June /2011	B. Vera, C. Moreno.
	Falcón*	Cayo Muerto	68°16’38"W, 10°55’46"N	May /2015	S. Ardito *et al.*
		Punta Brava	68°17'51"W, 10°47'42"N	May /2015	B. Vera, S. Ardito
		Cayo Sal	68°15'00"W, 10°56'00"N	May /2015	S. Ardito *et al.*
		Puerto Escondido	69°63’14"W, 12°73’44"N	May /2015	S. Ardito *et al.*
		Tumatey	69°56'15"W, 12°10'34"N	May /2015	Ardito *et al.* 20/05/2015
		Santa Rosa	69°18´26"W, 11°31´01"N	July /2011	B. Vera, S. Ardito.
	Miranda*	Playa Corrales	66°09´49,76"W, 10°36´29,09"N	June /2015	S. Ardito *et al.*
BRAZIL	Rio de Janeiro*	Baía da Ilha Grande	44°28´58"W, 23°00´32"S	2005	V. Cassano
SPAIN	Canary Islands, Tenerife*	Punta del Hidalgo	16°19´56"W, 28°33´59"N	July /2006	M.T. Fujii, A. Sentíes, M.C. Gil-Rodríguez

* Sites that had samples sequenced for molecular study.

Table S2. Taxa used in this study for molecular analysis.

		GenBank accession numbers	
Samples	**Collection data**	**COI-5P**	***rbc*L**
Chondria baileyana (Montagne) Harvey	Canada, Nova Scotia, Pomquet (far on Monks Head Road), 16 Aug. 2012, G.W. Saunders, A. Savoie, C. Longtin, K. Dixon, M. Bruce	KU564345	-
Chondria collinsiana M.A. Howe	Brazil, Rio de Janeiro, Armação dos Búzios, Praia Rasa, 13 Jan. 2005, V. Cassano, J.C. De-Paula	-	GU330225
Chondria dasyphylla (Woodward) C. Agardh	United States, North Carolina, Carteret Co., Bogue Sound	-	U04021
Chondria acrorhizophora Setchell & N.L.Gardner	United States, California, San Diego Co., Beach Club Reef, La Jolla Shores, 1 Jul. 1996, M. Volovsek	-	AY172578
Chondrophycus anabeliae Sentíes, M.T.Fujii, Cassano & Dreckmann	Mexico, Quintana Roo, Isla Mujeres, Garrafón de Castilla, 12 Feb. 2007, A. Sentíes, M.C. Gil-Rodríguez	MN597440	KX815262
Ch. anabeliae	Venezuela, Estado Falcón, Parque Nacional Morrocoy, Cayo Muerto, 19 May 2015, S. Ardito. M.T. Fujii, A. Sentíes, V. Cassano	MN597438	MN597441
Chondrophycus dotyi	United States, Hawaii, A. Sherwood USA, Hawaii, Oahu, Sandy Beach, 31 May 2015, E.M. Stein	HQ422621	KX815263
Chondrophycus cf. *undulatus* (Yamada) Garbary & J.T. Harper	New Caledonia, Loyalty Is., Maré, 22 Mar. 2005, C. Payri	-	FJ785307
Ch. cf. *undulatus*	USA, Hawaii	HQ422996	-
Chondrophycus planiparvus Popolizio, C.W.Schneid & C.E.Lane	Bermuda, Gurnet Rock, mouth of Castle Harbour, 13 Mar. 2012, T. Popolizio	OK209884	-
Chondrophycus succisus (A.B. Cribb) K.W. Nam	USA, Molokai, 11 Feb. 2007	GU223884	-
Chondrophycus sp.	United States, Texas, Flower Garden Banks Sanctuary, Feb. 2000, S. Fredericq	-	AF465817
Chondrophycus sp.	South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Point, 9 Jun. 2010, R. Anderson & J. Bolton	-	KY927795
Chondrophycus sp.	New Caledonia, Loyalty Islands, New Caledonia, Beautemps/ Beaupre, 06 Apr. 2005, Payri, C.	KX258815 KX258816	KX146174
Chondrophycus sp.	Australia, Norfolk Island, Collins Head, 21 Mar. 2005, Y. Metti, A. Millar	-	KY120337
Chondrophycus tronoi (E. Ganzon-Fortes) K.W. Nam	Philippines, A.O. Lluisma	-	AF489864
Ch. cf. *undulatus*	New Caledonia, Loyalty Islands, New Caledonia, Beautemps/ Beaupre, 01 Jan. 2005, Payri, C.	KX258814	-
Corynecladia clavata J. Agardh	Australia, Victoria, Walkerville, 20 Jan. 2015, P. Díaz-Tapia, M. Brookes	-	MF094079
C. clavata	Australia, Tasmania, 24 Jan. 2004, G.W. Saunders, R. Withall	HM915955	-
C. elata (C. Agardh) Cassano, M.C. Oliveira & M.T. Fujii	Australia, Western Australia, Rottnest Island, 15 Nov. 2008, J. Eu	-	KY120339
C. nova (Metti) Cassano, M.C. Oliveira & M.T. Fujii	Australia, NSW, Jervis Bay, Plantation Point, 15 Feb. 2005, Y. Metti, A. Millar	-	KY120340
Laurencia aldingensis Y.Saito & Womersley	Brazil, Rio de Janeiro, Armação dos Búzios, Praia Rasa, 13 Jan. 2005, V. Cassano, J.C. De-Paula	-	JF810351
Laurencia alfredensis Francis, Bolton, Mattio & Anderson	South Africa, 04 Jul. 2008, R.J. Anderson, J.J. Bolton South Africa, Western Cape, Grootbank, 4 Jul. 2008, R. Anderson & J. Bolton	-	KY927749

Samples	Collection data	GenBank accession numbers	
		COI-5P	*rbc*L
Laurencia brongniartii J.Agardh	Australia, Norfolk Island, Kingston lagoon, Slaughter Bay, 16 Mar. 2005, Yola Metti & Alan Millar	-	KY120341
L. cf. *brongniartii*	New Caledonia: Loyalty Islands, New Caledonia, Mare, 21 Mar. 2005, Payri, C.	-	KX146178
L. cf. *brongniartii*	Taiwan, Makang Harbour, 11 Jul. 1993, S. Fredericq	-	AF465814
Laurnecia brachyclados Pilger	USA, Hawaii	HQ423046	-
Laurencia caduciramulosa Masuda & S.Kawaguchi	Spain, Canary Islands, Tenerife, Punta del Hidalgo, 6 May 2008, M. Gil-Rodríguez, M. Fujii, V. Cassano & J. Díaz-Larrea	-	JF781525
L. caduciramulosa	United States, Florida, Key Biscayne, Crandon Park, 13 Aug. 2013, L. Collado-Vides, A. Duran, M. Fujii, V. Cassano & J. Díaz-Larrea	-	KJ700867
L. caduciramulosa	Brazil, Rio de Janeiro, Angra dos Reis, Praia do Velho, 19 Apr. 2006, V. Cassano & J.C. De-Paula	-	KJ700865
L. caduciramulosa	Cuba, Havana, Rincón de Guanabo, 2002, A. Areces	-	FJ904933
L. caduciramulosa (B1)	Cuba, Artemisa, Playa Baracoa, 06 May 2019, P. González-Sánchez	XXX	XXX
L. caduciramulosa (LCPV)	Brazil, Rio de Janeiro, Bahía de Ilha Grande, 2001, V. Cassano	XXX	-
L. caduciramulosa (LCTE)	Spain, Canary Islands, Tenerife, Punta del Hidalgo, Jul. 2006, M.T. Fujii, A. Sentíes, M.C. Gil-Rodríguez	XXX	-
Laurencia cf. *calliptera* Kütz	New Caledonia, Ile des Pins, 2 Dec. 2005, C. Payri	KX258825	-
Laurencia caraibica P.C. Silva	Mexico, Quitana Roo, Cancún, Isla Mujeres, 2006, A. Sentíes	-	EF658642
L. caraibica	Venezuela, Falcon, Cabo San Roman, 06 Oct. 2012, G. Garcia-Soto	-	MH388533
L. caraibica (11)	Venezuela, Estado Falcón, Cayo Muerto, 19 May 2015, S. Ardito et al.	XXX	-
L. catarinensis Cordeiro-Marino & M.T.Fujii	Brazil, Rio Grande do Norte, Maracajau, 24 Jun. 2006, M. Fujii & I. Silva	-	OK209873
L. catarinensis (VC)	Brazil, Santa Catarina, Florianópolis, Prainha da Barra da Lagoa, 16 Jul. 2008, P.A. Horta	XXX	-
L. catarinensis	United States, Florida, Key West, East Dry Rocks Reef, 30 May 2013, C. Schneider, C. Lane & T. Popolizio	OK209903	-
L. catarinensis	Bermuda, St. David's I., Fort Hill Bay, Cooper's Island, 27 May 2012, T. Popolizio	OK209904	OK209872
L. catarinensis	US Virgin Islands, St. Croix, Fredericksted Pier, 24 Nov. 2013, T. Popolizio & E. Salomaki	OK209898	OK209874
L. catarinensis	Spain, Canary Islands, Lanzarote, Pechigueras, 15 Jan. 2013, M. Gil-Rodriguez & M. Machín-Sánchez Spain, Canary Islands, Tenerife, El Pris, 3 Feb. 2011, M. Machín-Sánchez	KF492718	KF492776
L. catarinensis (CH3)	Cuba, Havana, Litoral del Acuario Nacional de Cuba	XXX	-
L. catarinensis (N2)	Venezuela, Estado Vargas (La Guaira), Naiguatá, 28 Jun. 2011, B. Vera, C. Moreno.	XXX	-
L. catarinensis (LC2)	Venezuela, Estado Vargas (La Guaira), Los Caracas, 26 May 2011, B. Vera, C. Moreno.	XXX	-
L. catarinensis (PA1)	Venezuela, Estado Vargas (La Guaira), Puerto Azul, 17 Jun. 2014, A. Brito, B. Vera.	XXX	-

		GenBank accession numbers	
Samples	**Collection data**	**COI-5P**	***rbc*L**
Laurencia complanata (Suhr) Kützing	South Africa, 09 Dec. 2010, R.J. Anderson, J.J. Bolton	-	KY927738
Laurencia corymbosa J.Agardh	South Africa, Eastern Cape, Double Mouth, 14 Jul. 2010, R. Anderson & J. Bolton	-	KY927728
Laurencia crustiformans K.J.McDermid	USA, Hawaii	HQ422744	-
Laurencia dendroidea J.Agardh	Brazil, Sao Paulo, Ubatuba, Praia do Felix, 31 Aug. 2000, M. Fujii	-	AF465810
L. dendroidea	Brazil, Rio de Janeiro, Ponta do Caioba, Angra dos Reis, 2004, V. Cassano	-	GU330223
L. dendroidea	Bermuda, Middle buoy, Eastern Blue Cut channel, north of Daniel's Head, Somerset Is., 20 Aug. 2010, C. Schneider, C. Lane & T. Popolizio	OK209900	OK209876
L. dendroidea	United States, Florida, Key West, Fort Zachary Taylor, 28 May 2013, C. Schneider, C. Lane, D. McDevit & T. Popolizio	OK209901	OK209875
L. dendroidea	US Virgin Islands, St. Croix, Salt River Bay, 21 Nov. 2013, C. Lane, T. Popolizio & E. Salomaki	OK209899	OK209877
L. dendroidea	Guadeloupe, Pointe de la Verdure, 20 Mar. 94, S. Fredericq	-	AF465811
L. dendroidea (as *L. majuscula*)	USA, Molokai, 10 Feb. 2007	GU223887	-
L. dendroidea	Mexico, Isla Mujeres, Quintana Roo, 2006, Díaz-Larrea & Sentíes	-	GU330220
L. dendroidea	Venezuela, Falcon, Playa Buchuacos, 06 Oct. 2012, G. García-Soto	MH388711	MH388528
L. dendroidea	Spain, Canary Islands, Tenerife, Puerto Cruz, 13 Jul. 2006, M. GilRodriguez, M. Fujii & M. Manchín-Sánchez	-	EF686000
L. dendroidea	Spain, Canary Islands, La Gomera, Punta de La Dama, 21 Sep. 2009, E. Aylagas & M. Manchín-Sánchez	KF492725	-
L. dendroidea	Spain, Canary Islands, Lanzarote, Pechigueras, 15 Jan. 2013, M.C. Gil-Rodríguez, M. Machín-Sánchez	KF492728	-
L. dendroidea	Australia, New South Wales, Batehaven, Batemans Bay, Observation Point, 30 May 2005, Y. Metti	MT822855	-
L. dendroidea	Australia, New South Wales, Batehaven, Batemans Bay, Observation Point, 25 Out. 2005, Y. Metti & A. Millar	MT822856	-
L. dendroidea	Australia, Norfolk Island, Little Organ off Captain Cook Memorial, 18 Mar. 2005, Y. Metti	MT822857	-
L. dendroidea	Australia, New South Wales, Arrawara Headland, 28 Jul. 2004, Y. Metti	MT822858	-
L. dendroidea	Australia, Western Australia, Little Turtle, 15 May. 2008, J. Huisman	MT822859	-
L. dendroidea (AB)	Cuba, Sancti Spiritu, Playa Ancón, Trinidad, 30 May 2019, B. Martínez Daranas	XXX	-
L. dendroidea (CG2, CG5, CG6)	Cuba, Holguín, Caletones, Gíbara, 18 May 2019, P. González-Sánchez, E. Reynaldo de la Cruz	XXX	-
L. dendroidea (1, 2, 3, 9, 10)	Venezuela, Estado Falcón, Punta Brava, 18 May 2015 S. Ardito et al.	XXX	-

Samples	Collection data	GenBank accession numbers	
		COI-5P	*rbc*L
L. dendroidea (14)	Venezuela, Estado Falcón, Cayo Sal, 19 May 2015, S. Ardito et al.	XXX	
L. dendroidea (15)	Venezuela, Estado Falcón, Puerto Escondido, 20 May, 2015, S. Ardito et al.	XXX	
L. dendroidea (10, CM1905)	Venezuela, Estado Falcón, Cayo Muerto, 19 May 2015, S. Ardito et al.	XXX	XXX
L. dendroidea (CH3, CH12, CH14, CH15, CH16, CH17, CH18)	Venezuela, Estado Vargas (La Guaira), Chichiriviche de la Costa, 21 Jul. 2014, A. Brito, B. Vera	XXX	-
L. dendroidea (T1)	Venezuela, Estado Vargas (La Guaira), Taguao, 11 Aug. 2014, A. Brito, B. Vera	-	XXX
L. dendroidea (LfilAngra)	Brazil, Rio de Janeiro, Angra dos Reis, Praia do Velho, 20 Jul. 2006 V. Cassano & J.C. De-Paula	GU330232	-
L. dendroidea (LVParati)	Brazil, Rio de Janeiro Parati Praia da Lulaion, 25 Feb. 2007, V. Cassano	GU330229	-
L. dendroidea (LobtRasa)	Brazil, Rio de Janeiro, Armação dos Búzios Praia Rasa, 01 Feb. 2006, V. Cassano	GU330235	-
Laurencia dichotoma Francis, Bolton, Mattio & Anderson	South Africa, 22 Mar. 2011, J.J. Bolton	-	KY927786
Laurencia digitata Francis, Bolton, Mattio & Anderson	South Africa, 04 Jul. 2008, R.J. Anderson, J.J. Bolton	-	KY927748
L. digitata	Venezuela, Estado Falcón, Parque Nacional Morrocoy, Cayo Muerto, 19 May 2015, S. Ardito, M.T. Fujii, A. Sentíes, V. Cassano	-	MN597443
Laurencia filiformis (C.Agardh) Mont.	Australia, Western Australia, Tarcoola Beach, 21 Sep. 1995, M. Hommersand & F. Hommersand	-	MH704449
Laurencia flexuosa Kützing	South Africa, S. KwaZulu-Natal, Palm Beach, 07 Feb. 2001, S. Fredericq	-	AF465815
*L.*cf. *flexuosa*	South Africa, Eastern Cape Province, 15 Jun. 2003, O. De Clerck	KX258821	-
Laurencia galtsoffii M.A. Howe	USA, Hawaii	HQ422984	-
Laurencia glomerata (Kützing) Kützing	South Africa, 03 Mar. 2009, R.J. Anderson, J.J. Bolton	-	KY927763
L. heteroclada Harvey f. decussata Cribb	Australia, NSW, Arrawarra headland, 28 Jul. 2004, Y. Metti	-	KY120344
Laurencia intricata J.V. Lamouroux	Cuba, Ciego de Ávila, Cayo Coco, 25 Sept. 2005, M.T. Fujii	-	GU330238
L. intricata	Bermuda, Bermuda I., Fairyland Creek, 25 Aug. 2010, C. Schneider, C. Lane & T. Popolizio	OK209897	OK209879
L. intricata	United States, Florida, Key West, White Street Pier, 29 May 2013, C. Schneider, C. Lane, D. McDevit & T. Popolizio	OK209906	OK209878
L. intricata	US Virgin Islands, St. Croix, Deep Wrecks, 19 Nov. 2013, C. Lane, T. Popolizio & E. Salomaki	-	OK209882
L. intricata	Mexico, Campeche Bay, Yucatan, 14 Feb. 99, C. F. Gurgel	-	AF465809
Laurencia karachiana Bibi, Cassano & Rasheed	Pakistan, Karachi, French Beach (Buleji), 13 Aug. 2018, R. Bibi	MK796229	MK796228

Samples	Collection data	GenBank accession numbers	
		COI-5P	*rbc*L
Laurencia cf. *kuetzingii* A.Millar	New Caledonia, Loyalty Is., Ouvea, 31 Mar. 2005, C. Payri	-	FJ785322
Laurencia laurahuertana Mateo-Cid, Mendoza-González, Sentíes & Díaz-Larrea	Mexico, Quintana Roo, Punta Herrero, 12 Apr. 2012, A.C. Mendoza González, L.E. Mateo-Cid	-	KF279401
Laurencia longiramea Cassano, G.N. Santos, J.M.C. Nunes, M.C. Oliveira & M.T. Fujii	Brazil, Espírito Santo, Anchieta, Ilhote de Ubu, 30 Jun. 2007, E.M. Stein	MH704454	MH704451
	Brazil, Rio de Janeiro, Armação dos Búzios, Praia Rasa, 13 Jan. 2005, V. Cassano, J.C. De-Paula		
Laurencia majuscula (Harv.) A.H.S.Lucas	Oman, Dhofar, Sep. 2001, M. Wynne	KX258827	-
Laurencia cf. *majuscula*	New Caledonia, Ile des Pins, 2 Dec. 2005, C. Payri	-	FJ785312
Laurencia cf. *mariannensis* Yamada	New Caledonia, Lagon Sud-Ouest, Ilot Laregnere, 11 Jul. 2003, C. Payri	KX258823	FJ785313
Laurencia mcdermidiae I.A. Abbott	USA, Oahu, 08 Apr. 2007	GU223877	-
L. mcdermidiae	New Caledonia, Ile des Pins, 09 Nov. 2005, C. Payri	-	FJ785314
Laurencia microcladia Kützing	US Virgin Islands, St. Croix, Turtle Deli Beach, 20 Nov. 2013, C. Lane, T. Popolizio & E. Salomaki	OK209896	OK209881
L. microcladia	Bermuda, south shore Bermuda I., Capt. Williams' Bay, 15 Jan. 2012, C. Schneider, C. Lane & T. Popolizio	OK209905	OK209880
L. microcladia (PCSC1, PCSC2)	Cuba, Santiago de Cuba, Pedro el cojo, 21 May 2019, P. González-Sánchez & E. Reynaldo de la Cruz	XXX	XXX
L. aff. *microcladia* (LC3)	Venezuela, Estado Vargas (La Guaira), Los Caracas, 28 Jun. 2011, B. Vera	XXX	XXX
L. aff. *microcladia* (PA8, PA20)	Venezuela, Estado Vargas (La Guaira), Puerto Azul, 17 Jun. 2014, A. Brito, B. Vera.	XXX	XXX
Laurencia multiclavata C.Francis, J.J.Bolton, Mattio & R.J.Anderson	South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Point, 22 Mar. 2011, J. Bolton, R. Anderson & C. Francis	-	KY927789
Laurencia mutueae Sentíes, Cassano & Dreckmann	Mexico, Acapulco, Guerrero, Isla la Roqueta, 7 Jun. 2017, A. Sentíes & K. Dreckmann	MK182534	MK159177
Laurencia natalensis Kylin	South Africa, Natal, Reunion Rocks, Isipingo, 24 Jul. 1993, M.H. Hommersand	MH388736	AF465816
	South Africa, S. KwaZulu-Natal, Palm Beach, 07 Feb. 2001, S. Fredericq		
L. natalensis	Venezuela, Falcón, Cabo San Roman, 06 Oct. 2012, G. García-Soto	MH388715	MH388523
L. natalensis	Sri Lanka, Odayapiti lagoon, 8 Nov. 2006, E. Coppejans	KX258818	-
Laurencia nidifica J.Agardh	United States, Hawaii, Oahu, 20 May 2007, A. Sherwood	GU223888	-
L. cf. *nidifica*	New Caledonia, Ile des Pins, 30 Nov. 2005, C. Payri	-	FJ785315
Laurencia nipponica Yamada	Japan, Hokkaido, Muroran, 5 Jun. 2003	GU223875	-
L. nipponica	Russia, Sakhalin, 23 Jun. 2003	GU223874	-
Laurencia obtusa (Hudson) J.V. Lamouroux	Venezuela, Isla Pelone, 26 Jun. 1999, C. F. Gurgel	-	AF465812

Samples	Collection data	GenBank accession numbers	
		COI-5P	*rbc*L
L. obtusa	France, Languedoc-Roussillon, Pyrenees-Orientales, Cap Beart, Banyuls-sur-Mer, 11 Jul. 2007, L. Bittner	KX258828	KX146185
L. obtusa	Ireland, County Donegal, Fanad Head, C.A. Maggs	-	AF281881
Laurencia oliveirana Yoneshigue	Brazil, Rio de Janeiro, Arraial do Cabo, Ponta da Cabeça, Praia Grande, 07 Jul. 2008, V. Cassano, J.C. De-Paula	-	JF810352
L. pacifica Kylin	United States, California, Stillwater Cove, Pebble Beach, 20 May 2010, B. Clarkston, K. Hind, S. Toews United States, California, Moss Beach, Central Beach, 17 Feb. 1992, S. Fredericq	KM254466	AY588411
Laurencia pumila (Grunow) Papenfuss	South Africa, 10 Jun. 2009, R.J. Anderson, J.J. Bolton	-	KY927765
L. pumila var. *dehoopiensis* Francis, Bolton, Mattio & Anderson	South Africa, 19 Aug. 2008, R.J. Anderson	-	KY927757
Laurencia pyramidalis Bory ex Kützing	France, Brittany, Roscoff, 05 Dec. 2002, F. Rousseau	-	FJ785316
Laurencia pyramidalis Bory ex Kützing	Portugal, Azores, Sao Miguel, Mosteiros, 27 Jun. 2011, M. Fujii, A. Prestes, A. Pacheco & M. Machín-Sánchez	KF492751	-
L. pyramidalis	Spain, Canary Islands, Fuerteventura, Garcey, 10 Sept. 2012, M. Machín-Sánchez	KF492756	-
L. rigida J. Agardh	Australia, NSW, Botany Bay, 11 May 2000, G.C. Zuccarello, J.A. West	-	AY920852
Laurencia saitoi Perest.	United States, California, Monterey, McAbee Beach, 21 May 2010, B. Clarkston, K. Hind & S. Toews	HQ544222	-
Laurencia stegengae (Stegenga, Bolton & Anderson) Francis, Bolton, Mattio & Anderson	South Africa, 18 Mar. 2010, R.J. Anderson	-	KY927743
Laurencia sp.	USA, Oahu	GU223889	-
Laurencia sp.	Sri Lanka, Odayapiti lagoon, 08 Nov. 2006, E. Coppejans Sri Lanka, Odayapiti lagoon, 16 Aug. 2006, E. Coppejans	KX258826	KX146176 KX146183
Laurencia sp.	Oman, Dhofar, Sep. 2001, M. Wynne	-	KX146184
Laurencia sp.	USA, Lanai	GU223892	
Laurencia sp.	New Caledonia, Lagon Sud, Goro, 20 Nov. 2007, Martin-Lescanne, J.	-	KX146177
Laurencia sp. 1	New Caledonia, Loyalty Is., Maré, 19 Mar. 2005, C. Payri	KX258819	-
Laurencia sp.	New Caledonia, Loyalty Islands, Maré, 21 Mar. 2005, C. Payri	KX258820	KX146182
Laurencia sp. 2	New Caledonia , Lagon Sud-Ouest, Ilot Larégnère, 31 Jan. 2008, J. Martin-Lescanne	KX258824	KX146181
Laurencia sp. 2	Australia, Victoria, Mallacoota, H. Verbruggen, K. Dixon	-	MK125395 MK125344
Laurencia tasmanica J.D. Hooker & Harvey	Australia, Victoria, Tween Reef, between Cape Paterson & Inverloch, P. Díaz-Tapia	-	MF094081

Samples	Collection data	GenBank accession numbers	
		COI-5P	*rbc*L
Laurencia venusta Yamada	Australia, Lord Howe Island, Sylphs Hole, 26 Oct. 2005, Y. Metti & A. Millar	-	KY120345
L. venusta	Mexico, Quintana Roo, Puerto Morelos, Punta Brava, 18 Apr. 2004, J. Díaz-Larrea & A. Sentíes	-	EF061655
Laurencia viridis Gil-Rodríguez & Haroun	Spain, Canary Islands, Tenerife, Punta del Hidalgo, 13 Jan. 2012, M. Gil-Rodríguez & M. Machín-Sánchez	KF492758	EF685999
L. viridis	Spain, Canary Islands, Tenerife, Punta del Hidalgo, Roca Negra, 06 Oct. 2005, M.C. Gil-Rodríguez		
	Portugal, Azores, Santa Maria, Boca de Ribeira Seca, 2 Jul. 2011, M. Fujii, A. Neto, J. Pombo, M. Machín-Sánchez	KF492760	KF492794
Laurencia yamadana M.Howe	United States, Hawaii, Maui, Kihei, 5 Apr. 2006, A. Carlile	-	GQ252550
Laurenciella marilzae (Gil-Rodríguez, Sentíes, Díaz-Larrea, Cassano & M.T.Fujii) Gil-Rodríguez, Sentíes, Díaz-Larrea, Cassano & M.T.Fujii	Spain, Canary Islands, Lanzarote, Pechigueras, 15 Jan. 2013, M.C. Gil-Rodríguez, M. Machín-Sánchez	KF492762	EF686001
La. marilzae	Spain, Playa Paraiso Tenerife-Islas Canarias, 14 July 2006, Gil-Rodríguez, Fujii & Sentíes		
	Portugal, Azores, Sao Miguel, Cerco da Caloura-Baia, 26 Jun. 2011, E. Nogueira, V. Cassano & A. Sentíes	KF492765	KF492798
La. marilzae	Italy, Sicily, San Vito Lo Capo, Trapani, 09 July 2017, D. Serio, G. Furnari et Y. Metti	MT822847	-
La. marilzae	Croatia, Scedro, 11 Jun. 2007, J. Utge, L. Le Gall	KX258829	KX146186
La. marilzae	Brazil, São Paulo, Laje de Santos Marine State Park, Parcel do Sul, 25 Mar. 2007, R. Rocha-Jorge	KF270693	GU938189
La. marilzae	South Africa, 19 Aug. 2008, RJ Anderson & JJ Bolton	-	KY927758
Laurenciella mayaimii L. Collado-Vides, Cassano & M.T. Fujii	United States, Florida, Biscayne Bay, Deering Estate, 12 Aug. 2013, L. Collado-Vides, V. Cassano, M.T. Fujii	MG004176	MG004182
Laurenciella namii Popolizio, C.W.Schneid & C.E.Lane	Bermuda, north shore Bermuda I., Spanish Point Park, 25 Aug. 2010, C. Schneider, C. Lane & T. Popolizio	OK209886	OK209870
La. namii	United States, Florida, Key West, White Street Pier, 29 May 2013, C. Schneider, C. Lane, D. McDevit & T. Popolizio	OK209907	-
La. namii	United States, Florida, Key Biscayne, Crandon Park, 12 Aug. 2013, L. Collado-Vides, V. Cassano, M.T. Fujii	MG004179	MG004184
La. namii (L10)	Cuba, Ciego de Ávila, Jardines de la Reina, Punta Practicas, Cayo Anclitas, Jun. 2017, P. González-Sánchez & D. Hanisak	-	XXX
Laurenciella sp. 1Bda	Bermuda, Gibbet I., mouth of Flatts Inlet, 2 Feb. 2012, T. Popolizio	OK209885	OK209869
Ohelopapa flexilis (Setchell) F. Rousseau, Martin-Lescanne, Payri & L. Le Gall	French Polynesia, Tahiti, Tahara reef, 24 Mar. 2007, A. Apham	KX258830	KX146187
Osmundea blinksii (Hollenberg & I.A.Abbott) K.W. Nam	United States, California, San Mateo Co., Año Nuevo, Greyhound Rock, 17 Jul. 1996, M.H. Hommersand	-	AY172575
Osmundea caspica (Zinova & Zaberzhinskaya) Maggs & L.M. McIvor	Azerbaijan, Sangachal Bay, 01 Sept. 2003	-	KX146188

Samples	Collection data	GenBank accession numbers	
		COI-5P	*rbc*L
Osmundea hybrida (A.P. de Candolle) K.W. Nam	France, Brittany, Roscoff, Finistere, 12 May 2002, F. Rousseau France, Brittany, St. Lunaire, 20 Mar. 1999, F. Rousseau	KX258831	FJ785317
Osmundea oederi (Gunnerus) G. Furnari	Ireland, Co. Donegal, St. John´s Point, 12 Oct. 1999, C.A. Maggs	-	AF281880
O. oederi	France, Normandy, Manche, Grand Colombier nord, les Chausey, 16 Apr. 2008, L. Le Gall	KX258833	
Osmundea osmunda (S.G.Gmel.) K.W.Nam	France, Brittany, Le Loup, 19 May 2011, L. Couceiro, M. Robuchon France, Brittany, Roscoff, 5 Dec. 2002, F. Rousseau	KJ960875	FJ785318
O. osmunda	Ireland, County Donegal, St John's Point, 12 Oct. 1999, C. Maggs	-	AF281877
Osmundea pinnatifida (Huds.) Stackh.	Ireland, Galway, Black Head, H.-G. Choi & M. Guiry	-	JX828140
O. pinnatifida	France, Brittany, Le Loup, 08 Mar. 2012, L. Couceiro, M. Robuchon France, Brittany, Penmarch	KJ960886	AF259495
O. pinnatifida	Portugal, Azores, Pico, Lajes do Pico-Fabrica da Baleia, 29 June 2011, E. Nogueira, V. Cassano, A. Sentíes	KF492770	-
Osmundea prudhommevanreinei Machín-Sánchez & Gil-Rodríguez	Spain, Canary Islands, Tenerife, Playa Paraiso, 04 May 2011, M.C. Gil-Rodríguez & M. Machín-Sánchez	KU566548	-
Osmundea sanctarum M.T. Fujii & R. Rocha-Jorge	Brazil, São Paulo, Laje de Santos Marine State Park, Parcel do Sul, 19 Aug. 2012, R. Rocha-Jorge, M.B. Barros-Barreto	-	KC012601
Osmundea sinicola (Setchell & N.L. Gardner) K.W. Nam	United States, California, Orange Co., Crescent Beach, 28 May 2002, S. Murray	-	AY588407
Osmundea silvae M.Machín-Sánchez & M.C.Gil-Rodríguez	Portugal, Madeira, Madeira, Porto da Cruz, 06 Jul. 2011, M.T. Fujii, A. Neto, H. Encarnação & M. Machín-Sánchez	KU566537	KU566561
Osmundea spectabilis (Postels & Rupr.) K.W.Nam	United States, California, Pacific Grove, Bird Rock, 22 May 2010, B. Clarkston, K. Hind & S. Toews United States, California, San Diego Co., Point Loma, College of the Nazarenes, 7 Jul. 1996, M. Hommersand	KM254469	AY172572
O. spectabilis	Mexico, Baja California, Punta Santo Thomas, 2 Jul. 1996, M. Hommersand	-	AY172574
Osmundea splendens (Hollenb.) K.W.Nam	United States, California, Santa Cruz, Four Mile, 19 May 2010, B. Clarkston, K. Hind & S. Toews (GWS021992, -998)	HQ919290	KU564461
Osmundea sp.	Portugal, Madeira, Madeira, Reis Magos, 08 July 2011, M.T. Fujii, A. Neto, H. Encarnacao & M. Machín-Sánchez	KU566541	-
Osmundea sp.	Spain, Canary Islands, Gran Canarias, Punta Caldar, 2008	-	JF781519
Osmundea truncata (Kutz.) K.W.Nam & Maggs	Spain, Canary Islands, Tenerife, El Pris, 07 May 2012, M. Machín-Sánchez Spain, Canary Islands, Tenerife, La Barranquera, 5 May 2008, M. Gil-Rodríguez, M. Fujii, V. Cassano & J. Díaz-Larrea	KU566544	JF781523

Samples	Collection data	GenBank accession numbers	
		COI-5P	*rbc*L
Palisada cervicornis (Harv.) Collado-Vides, Cassano & M.T.Fujii	Bermuda, Brackish Pond Flats, inner reef, north shore (3-4 m), 17 Jan. 2012, C. Schneider, C. Lane & T. Popolizio	OK209888	OK209863
P. cervicornis (Harvey) Collado-Vides, Cassano & M.T. Fujii	United States, Florida, Key Largo, Pickles Reef, 14. Aug. 2013, A. Duran	-	MG030375
Palisada corallopsis (Mont.) Sentíes, M.T. Fujii & Díaz-Larrea	Bermuda, north shore Bermuda I., Bailey's Reef, 17 Aug. 2012, C. Schneider, C. Lane & T. Popolizio	OK209890	OK209865
P. corallopsis	Mexico, Yucatán, Cancún, Chaac Mool Beach, 2005, J. Díaz-Larrea, A. Sentíes	-	EF061646
P. corallopsis	United States, 12 Aug. 2013, L. Collado-Vides, A. Duran, M.T. Fujii, V. Cassano & J. Díaz-Larrea	-	MG020477
***P. corallopsis* Havana, Cuba**	Cuba, La Habana, Rincón de Guanabo, 20 Feb. 2020, A. Areces, D. Reyes	XXX	XXX
Palisada crustiformans	USA, Hawaii, Oahu, Makapuu, 26 May 2007, A. Kurihara	--	KX146196
Palisada flagellifera (J.Agardh) K.W.Nam	Spain, Canary Islands, Tenerife, Punta del Hidalgo, 13 Jan. 2012, M.C. Gil-Rodríguez, M. Machín-Sánchez Spain, Canary Islands, Tenerife, Playa Paraiso, 12 Jul. 2006, M. Gil Rodríguez, M. Fujii & A. Sentíes	KF492772	EF685998
P. flagellifera	Brazil, Rio de Janeiro, Rio das Ostras, Praia do Cemitério, 03 Aug. 2005, V. Cassano, M.B.B. Barreto	-	GU330227
P. flagellifera	Bermuda, SW of North Rock BAMZ "pink sand" collecting site, 16 Nov. 2012, T. Popolizio	OK209892	OK209866
P. flagellifera (17)	Venezuela, Estado Falcón, Puerto Escondido, 20 May 2015, S. Ardito et al.	XXX	
P. flagellifera (18)	Venezuela, Estado Falcón, Tumatey, 20 May 2015, S. Ardito et al.	XXX	
***P. furcata* Ceará, Brazil**	Brazil, Ceará, município de Trairi, Praia de Emboaca, 30 Mar. 2018, L. Soares	XXX	XXX
Palisada papillosa (C.Agardh) K.W.Nam	Spain, Canary Islands, Tenerife, Playa Paraiso, 14 Jul. 2006, M.C. Gil-Rodríguez, A. Sentíes, G. & M.T.Fujii	-	EU256325
Palisada parvipapillata (C.K.Tseng) K.W.Nam 2007	New Caledonia, Ilot Bayes, 01 Jan. 2001, C. Payri	KX258839	KX146194
P. parvipapillata	United States, Hawaii, Oahu, Hauula Beach Park, 18 Sep. 2007, A. Kurihara	GU223895	-
Palisada patentiramea (Mont.) Cassano, Sentíes, Gil-Rodríguez & M.T.Fujii	Philippines, A. Lluisma	-	AF489862
Palisada perforata (Bory) K.W. Nam	Spain, Canary Islands, Tenerife, Punta del Hidalgo, 13 Jan. 2012, M.C. Gil-Rodríguez, M. Machín-Sánchez Spain, Canary Islands, Tenerife, Punta del Hidalgo, Faro, Bahia Izquierda, 06 Oct. 2005, M. Gil-Rodríguez	KF492773	EU256327
P. perforata	United States, Florida, Key West, Low Key Channel, 27 May 2013, C. Schneider, C. Lane, D. McDevit & T. Popolizio	OK209883	OK209867
P. perforata	Mexico, Quintana Roo, Isla Mujeres, 2007, A. Sentíes, M.C. Gil-Rodríguez	-	EF658641
P. perforata	Bermuda, Bermuda I., Gravelly Bay, 22 Aug. 2012, C. Schneider, C. Lane & T. Popolizio	OK209891	OK209868

Samples	Collection data	GenBank accession numbers	
		COI-5P	*rbc*L
P. perforata	Brazil, Rio de Janeiro, Parati, Praia Vermelha, 30 Dec. 2005, V. Cassano	-	EU256331
P. perforata	Dominica, Portsmouth Harbor. Rocks E of Anchorage, 12 Mar. 2016, BLB	OM460643	-
P. perforata	Venezuela, Falcon, Playa Buchuacos, 6 Oct. 2012, G. García-Soto Venezuela, Falcon, Cabo San Román, 06 Oct. 2012, G. García-Soto	MH388710	MH388525
P. cf. *perforata*	New Caledonia	-	FJ785320
P. aff. *perforata* 1 (AG1)	Venezuela, Estado Falcón, Animas de Guazare, 29 Jan. 2011, B. Vera, S. Ardito	XXX	-
P. aff. *perforata* 1 (T14, T18, T22)	Venezuela, Estado Vargas (La Guaira), Taguao, 11 Aug. 2015, A. Brito, B. Vera.	XXX	XXX
P. aff. *perforata* 1 (16)	Venezuela, Estado Falcón, Puerto Escondido, 20 May 2015, S. Ardito et al.		
P. aff. *perforata* 1 (20)	Venezuela, Estado Falcón, Tumatey, 20 May 2015, S. Ardito et al.		
P. aff. *perforata* 1 (21)	Venezuela, Estado Falcón, Puerto Cumarebo, 20 May 2015, S. Ardito et al.		
P. aff. *perforata* 1 (SR2)	Venezuela, Estado Falcón, Santa Rosa, 26 Jul. 2011, B. Vera, S. Ardito.	XXX	XXX
P. aff. *perforata* 1 (CV4, CV5)	Venezuela, Estado Miranda, 25 Jun. 2015, S. Ardito et al.	XXX	-
P. aff. *perforata* 2 (4, 5)	Venezuela, Estado Falcón, Punta Brava, 18 May 2015 B. Vera, S. Ardito.	XXX	XXX
P. aff. *perforata* 2 (12)	Venezuela, Estado Falcón, Cayo Sal, 19 May 2015, S. Ardito *et al.*		
P. aff. *perforata* 2 (13)	Venezuela, Estado Falcón, Cayo Muerto, 19 May 2015 S. Ardito *et al.*		
P. aff. *perforata* (RL0)	Cuba, Cienfuegos, Rancho Luna, Jul. 2018, A. Moreira	XXX	-
P. aff. *perforata* (GH1)	Cuba, Holguín, Gíbara, 19 May 2019, P. González-Sánchez, E. Reynaldo de la Cruz	XXX	-
P. aff. *perforata* (SO2, SO3)	Cuba, Santiago de Cuba, La Socapa, 20 May 2019, P. González-Sánchez, E. Reynaldo de la Cruz	XXX	-
P. aff. *perforata* (FSC3)	Cuba, Santiago de Cuba, Playa El Francés, 21 May 2019, P. González-Sánchez, E. Reynaldo de la Cruz, A. Jover	XXX	-
P. cf. *robusta*	New Caledonia, Isle of Pines, 02 Dec. 2005, Payri, C. New Caledonia, Lifu, 23 Mar. 2005, C. Payri	KX258838	FJ785321
Palisada sp. 1	United States, Florida, Cudjoe Key, Summerland Bridge, 31 May 2013, C. Schneider, C. Lane, D. McDevit & T. Popolizio	OK209889	OK209864

Samples	Collection data	GenBank accession numbers	
		COI-5P	*rbc*L
Palisada sp.	United States, Hawaii, Oahu, Makapuu, 26 Dec. 2009, Kurihara, A.	KX258834	KX146190
Palisada sp.	United States, Florida, Fort Pierce, 10 Aug. 2013, G. Garcia-Soto	MH388699	-
Palisada sp.	New Caledonia, Lifou, 23 Mar. 2005, C. Payri	KX258835	-
Palisada sp.	New Caledonia, Nouméa, 4 Apr. 2008, J. Martin-Lescanne	KX258837	-
Palisada sp.	India, 12 Jan. 2016, Felix Bast	MT996228	-
Palisada sp.	Sri Lanka, Polhena Beach, 16 Aug. 2006, Coppejans, Eric	KX258836	-
Palisada sp.	South Africa, KwaZulu-Natal, Sodwana Bay, Jesser Point, 9 Sep. 2010, J. Bolton, R. Anderson & C. Francis	-	KY927798
Palisada sp.	Venezuela, Falcon, Playa Buchuacos, 06 Oct. 2012, G. Garcia-Soto	MH388716	-
Palisada sp. (CSC1)	Cuba, Santiago de Cuba, Cazonal, 21 May 2019, P. González-Sánchez, E. Reynaldo de la Cruz, A. Jover	XXX	-
Palisada sp.	Cuba, Holguín, Playa Cayuelo, Guardalavaca, 15 Aug. 2019, E. Reynaldo de la Cruz	-	XXX
P. yamadana (M.A. Howe) K.W. Nam	United States, Hawaii	HQ422794	-
Laurencia tenerrima J.Cremades	Italy, Torre Faro, Messina, 08 Oct. 2009, V. Fiore	MF544099	-
Yuzurua iridescens (M.J.Wynne & D.L.Ballant.) Sentíes, M.J.Wynne, Cassano, Gil-Rodríguez & M.T.Fujii	Bermuda, Bermuda I., Southampton, West Whale Bay boiler reefs, 20 Jan. 2012, C. Schneider, C. Lane & T. Popolizio	OK209902	OK209858
	Bermuda, Bermuda I., Southampton, West Whale Bay boiler reefs, 18 Jun. 2006, C. Schneider & C. Lane		
Yuzurua poiteaui (J.V. Lamouroux) Martin-Lescanne var. *gemmifera* (Harvey) M.J. Wynne	Mexico, Quintana Roo, Puerto Morelos, Ojo de Agua, 2004, J. Díaz-Larrrea, A. Sentíes	-	EF061648
Yuzurua poiteaui var. *gemmifera*	Cuba, Havana, Rincón de Guanabo, 2005, J. Díaz-Larrea, A. Areces	-	EF061650
Yuzurua poiteaui (J.V.Lamouroux) Martin-Lescanne	Bermuda, St. George's I., Ferry Reach, BIOS dock, 19 Aug. 2010, C. Schneider, C. Lane & T. Popolizio	OK209894	OK209860
Y. poiteaui var. *poiteaui*	Mexico, Quitana Roo, Cancún, Playa del Carmen, 2005, J. Díaz-Larrea, A. Sentíes	-	EF061653
Y. poiteaui var. *poiteaui*	United States, Florida, Long Key, Ovan Side, 1998, S. Fredericq	-	EF061652
Y. poiteaui	United States, Florida, Key West, White Street Pier, 29 May 2013, C. Schneider, C. Lane, D. McDevit & T. Popolizio	OK209895	OK209861
Y. poiteaui (G7)	Cuba, Pinar del Río, Guanahacabibes, Mar. 2018, Y. Alfonso	XXX	-
Yuzurua sp. 1	Bermuda, Bermuda I., Southampton, West Whale Bay boiler reefs, 30 May 2012, T. Popolizio	OK209893	OK209862
Yuzurua sp.	West Indies, Guadeloupe, Grand Cul-de-Sac Marin, Chenal ilet Colas, 03 May 2012, F. Rousseau, Y. Buske, J. Espinosa, M. Snyder, G. Dirberg	KX258843	KX146198

		GenBank accession numbers	
Samples	**Collection data**	**COI-5P**	***rbc*L**
Yuzurua sp. (BPC6)	Cuba, Ciego de Ávila, Playa Flamenco, Cayo Coco, 27 Feb. 2008, D. de la Nuez	XXX	
Yuzurua sp. (RGbo3)	Cuba, Havana, Rincón de Guanabo, 24 Apr. 2019	XXX	XXX

MATERIAL SUPLEMENTAR

Table S3. Intra- and interspecific divergence (%) of the COI-5P and *rbc*L genes for the *Laurencia* complex of this study and according to different authors. Based on Medeiros (2022).

		INTRAESPECIFIC		INTERSPECIFIC		
Genus	**Marker**	**This study**	**Literature**	**This study**	**Literature**	**Reference**
Laurencia	COI-5P	0-2.93 (4.58)	0-1.3	3.46-11.79	2.6-10.2	Cassano et al. 2019
			0-2.4		2.8-9.3	Popolizio et al. 2022
			0-4.58*		1.74-13.87	Medeiros 2022
	*rbc*L	0-1.90	0-0.62	0.82-9.32	1.0-6.8	Cassano et al. 2012a
			0-1.35		-	Metti et al. 2013
			0.06-0.5		1.2-7.3	Popolizio et al. 2022
			0-1.18		1.23-10.26	Medeiros 2022
Palisada	COI-5P	0-2.96	0-1.3	3.05-10.04	3.9-6.7	Popolizio et al. 2022
			0-3.29*[1]		3.16-9.27	Medeiros 2022
	*rbc*L	0-3.27	0-0.2	1.33-8.18	1.4-6.4	Cassano et al. 2012a
			0-0.7		2.0-3.0	Popolizio et al. 2022
			0-0.76		1.88-7.50	Medeiros 2022
Yuzurua	COI-5P	0.15-0.87	0-0.8	5.24-8.07	5.7-6.9	Popolizio et al. 2022
			-		-	Medeiros 2022
	*rbc*L	0-0.57	0.01-0.02	3.53-4.98	1.2-1.3	Sentíes et al. 2015
			-		4.7	Rousseau et al. 2017
			-		-	Díaz-Larrea et al. 2007
			0-0.2		3.7-5.3	Popolizio et al. 2022
			0-0.14		-	Medeiros 2022
Laurenciella	COI-5P	0.17-1.75	-	2.84-9.83	7.4-9.2	Collado-Vides et al. 2018
			0-0.7		-	Machín-Sánchez et al. 2014
			0-5.99		5.21-9.33	Serio et al. 2020
			0.4-1.1		6-9.4	Popolizio et al. 2022
			0.17-1.74		2.10-9.18	Medeiros 2022

	*rbc*L	0-0.62	0-0.2	1.31-3.94	0.7-1.5	Cassano et al. 2012a
			-		3.1-3.9	Collado Vides et al. 2018
			0-1.51		3.38-4.50	Serio et al. 2020
			0.2-0.4		0.9-3.9	Popolizio et al. 2022
			0-0.48		3.18-3.67	Medeiros 2022
Chondrophycus	COI-5P	0.3	0-0.3	0.97-9.83	5.4-8.76	Cassano et al. 2020
			0.4		8.8-9.3	Popolizio et al. 2022
			0-0.48		2.27-12.34	Medeiros 2022
	*rbc*L	1.35	0-1.34	1.87-9.28	1.86-7.8	Cassano et al. 2020
			-		2.2-9.5	Popolizio et al. 2022
			0-1.35		1.86-10.02	Medeiros 2022
Osmundea	COI-5P	0.17	0.17-2.15	6.08-13.48	2.4-10.96	Machín-Sánchez et al. 2016
			0.5-0.8		5.7-12.1	Popolizio et al. 2022
			1.65		6.62-13.10	Medeiros 2022
					-	
	*rbc*L	0.08-0.17	0.4-1.0	1.32-10.63	1.9-10	Cassano et al. 2012a
			0.05-0.6		1.7-5.1	Popolizio et al. 2022
			-		1.97-9.80	Medeiros 2022
					-	
Corynecladia	*rbc*L	-	0.3	2.48-6.89	2.5-6.1	Cassano et al. 2019
			-		2.5	Popolizio et al. 2022
			-		2.48-6.89	Medeiros 2022

* *Laurencia dendroidea* complex

*[1] *Palisada flagellifera*

Table S4. Intra- and interspecific genetic variations (%) for COI-5P sequences between the subclades of *Palisada perforata* and *P.* aff. *perforata*. Species from the type locality in bold.

		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
1	***P. perforata* Spain (KF492773)**																								
2	*P. perforata* Bermuda (OK209891)	4.14																							
3	*P. perforata* USA (OK209883)	4.14	0																						
4	*P.* sp. USA (MH388699)	5.06	1.09	1.09																					
5	*P. perforata* Dominica (OM460643)	4.59	0.21	21	1.93																				
6	*P. perforata* Venezuela (MH388710)	3.83	3.49	3.49	3.64	4.53																			
7	*P. perforata* Venezuela (SR2)	3.83	3.49	3.49	3.64	4.53	0.31																		
8	*P. perforata* Venezuela (5)	4.01	0.43	0.43	1.38	1.22	3.46	3.4																	
9	*P. perforata* Venezuela (AG1)	3.83	3.49	3.49	3.64	4.53	0	0.31	3.42																
10	*P. perforata* Venezuela (T14)	3.83	3.49	3.49	3.64	4.53	0	0.31	3.43	0															

11	*P. perforata* Venezuela (T18)	3.83	3.49	3.49	3.64	4.53	0	0.32	3.56	0	0															
12	*P. perforata* Venezuela (CV4)	4.18	3.49	3.49	4.34	4.87	1.35	1.7	4.06	1.4	1.4	1.45														
13	*P. perforata* Venezuela (CV5)	4.01	3.49	3.49	3.99	4.53	0.9	1.23	3.46	0.93	0.93	0.97	0.6													
14	*P. perforata* Venezuela (4)	3.83	0.21	0.21	1.21	1.04	3.31	3.24	0.15	3.26	3.27	3.4	3.91	3.31												
15	*P. perforata* Venezuela (12)	4.01	0.21	0.21	1.38	1.22	3.46	3.4	0.3	3.42	3.43	3.56	4.06	3.46	0.15											
16	*P. perforata* Venezuela (13)	4.01	0.21	0.21	1.38	1.22	3.46	3.4	0.3	3.42	3.43	3.56	4.06	3.46	0.15	0										
17	*P. perforata* Venezuela (16)	3.66	3.27	3.27	3.47	4.35	0.15	0.15	3.31	0.15	0.15	0.16	1.5	1.05	3.16	3.31	3.31									
18	*P. perforata* Venezuela (20)	3.66	3.27	3.27	3.47	4.35	0.15	0.15	3.31	0.15	0.15	0.16	1.5	1.05	3.16	3.31	3.31	0								
19	*P. perforata* Venezuela (21)	3.83	3.49	3.49	3.64	4.53	0.3	0	3.46	0.31	0.31	3.24	1.65	1.2	3.31	3.46	3.46	1.51	0.15							
20	*P. perforata* Cuba (RL0)	4.53	5.24	5.24	5.9	6.44	4.22	4.21	4.84	4.21	4.23	4.21	5.31	4.84	5	5.15	5.15	4.37	4.37	4.21						
21	*P. perforata* Cuba (GH1)	4.53	5.24	5.24	5.9	6.27	4.22	4.32	4.66	4.35	4.36	4.37	5.27	4.81	4.81	4.97	4.97	4.36	4.36	4.21	0.46					

22	*P. perforata* Cuba (SO2)	5.23	5.24	5.24	6.59	6.27	4.52	4.63	5.57	4.66	4.68	4.7	5.57	5.12	5.42	5.57	5.57	4.66	4.66	4.51	1.4	1.5			
23	*P. perforata* Cuba (SO3)	5.23	5.24	5.24	6.59	6.27	4.54	4.66	5.6	4.69	4.69	4.7	5.6	5.15	5.45	5.6	5.6	4.69	4.69	4.54	1.41	1.51	0		
24	*P. perforata* Cuba (FSC3)	5.23	5.24	5.24	6.59	6.27	4.58	4.63	5.48	4.66	4.68	4.7	5.64	5.18	5.33	5.48	5.48	4.72	4.72	4.57	1.4	1.52	0	0	

Table S5. Intra- and interspecific genetic variations (%) for COI-5P sequences between the subclades of *Palisada*. Species from the type locality in bold.

		1	2	3	4	5	6
1	***P. corallopsis* Cuba**						
2	*P. corallopsis* Bermuda (OK209890)	2.18					
3	***P. furcata* Brazil (CE34)**	1.65	1.09				
4	*P. cervicornis* Bermuda (OK209888)	3.71	4.14	3.05			
5	*Palisada* sp. Cuba (CSC1)	3.31	3.27	3.16	2.83		
6	*Palisada* sp. 1 Florida, USA (OK209889)	3.49	3.71	3.49	4.36	3.49	

Table S6. Intra- and interspecific genetic variations (%) for COI-5P sequences between the subclades of *Yuzurua*.

		1	2	3	4	5	6	7	8
1	*Y. iridescens* Bermuda (OK209902)								
2	*Y. poiteaui* Bermuda (OK209894)	6.33							
3	*Y. poiteaui* USA (OK209895)	6.55	0.87						
4	*Y. poiteaui* Cuba (G7)	6.76	0.43	0.87					
5	*Yuzurua* sp. Guadeloupe (KX258843)	6.33	7.64	7.86	7.71				
6	*Yuzurua* sp. Cuba (RGbo3)	6.11	7.42	7.64	7.62	0.15			
7	*Yuzurua* sp. Cuba (BPC6)	7.86	7.42	6.76	7.68	8.16	7.92		

8	*Yuzurua* sp. 1 Bermuda (OK209893)	7.42	5.45	5.24	5.89	7.2	6.98	5.67																												

Table S7. Intra- and interspecific genetic variations (%) for COI-5P sequences between the subclades of *Laurencia dendroidea* and *L.* aff. *dendroidea*. Species from the type locality in bold.

		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**
1	***L. dendroidea* Brazil (LfilAngra)**																																			
2	***L. dendroidea* Brazil (LVParati)**	0.15																																		
3	***L. dendroidea* Brazil (LobtRasa)**	0.6	0.75																																	
4	*L. dendroidea* Bermuda (OK209900)	3.05	3.27	3.71																																
5	*L. dendroidea* USA (OK209901)	3.05	3.27	3.71	0																															
6	*L. dendroidea* USAUSVI (OK209899)	3.05	3.27	3.71	0	0																														
7	*L. dendroidea* Cuba (AB)	2.86	3.01	3.46	0	0	0																													

8	*L. dendroidea* Cuba (CG2)	2.56	2.71	3.16	0.21	0.21	0.21	0.3																													
9	*L. dendroidea* Cuba (CG5)	2.56	2.71	3.16	0.21	0.21	0.21	0.3	0																													
10	*L. dendroidea* Cuba (CG6)	2.56	2.71	3.16	0.21	0.21	0.21	0.3	0	0																												
11	*L. dendroidea* Venezuela (MH388711)	2.75	2.91	3.07	3.49	3.49	3.49	3.39	3.07	3.07	3.07																										
12	*L. dendroidea* Venezuela (1)	2.86	3.01	3.46	0.21	0.21	0.21	0.3	0.3	0.3	0.3	3.39																									
13	*L. dendroidea* Venezuela (2)	2.86	3.01	3.46	0.21	0.21	0.21	0.3	0.3	0.3	0.3	3.39	0																								
14	*L. dendroidea* Venezuela (3)	2.86	3.01	3.16	0.21	0.21	0.21	0.3	0.3	0.3	0.3	3.39	0.3	0.3																							
15	*L. dendroidea* Venezuela (9)	2.71	2.86	3.31	0	0	0	0.15	0.15	0.15	0.15	3.23	0.15	0.15	0.15																						
16	*L. dendroidea* Venezuela (10)	2.71	2.86	3.31	0	0	0	0.15	0.15	0.15	0.15	3.23	0.15	0.15	0.15	0																					
17	*L. dendroidea*	2.56	2.71	2.86	3.27	3.27	3.27	3.01	2.71	2.71	2.71	0.16	3.01	3.01	3.01	2.86	2.86																				

	Venezuela (14)																																				
18	*L. dendroidea* Venezuela (15)	2.46	2.62	2.77	3.27	3.27	3.27	3.08	2.77	2.77	2.77	0.16	3.08	3.08	3.08	2.93	2.93	0																			
19	*L. dendroidea* Venezuela (CH12)	2.56	2.71	2.86	3.27	3.27	3.27	3.01	2.71	2.71	2.71	0.16	3.01	3.01	3.01	2.86	2.86	0	0																		
20	*L. dendroidea* Venezuela (CH13)	2.52	2.68	2.83	3.27	3.27	3.27	3.15	2.83	2.83	2.83	0.16	3.15	3.15	3.15	2.99	2.99	0	0	0																	
21	*L. dendroidea* Venezuela (CH14)	2.56	2.71	2.86	3.27	3.27	3.27	3.01	2.71	2.71	2.71	0.16	3.01	3.01	3.01	2.86	2.86	0	0	0	0																
22	*L. dendroidea* Venezuela (CH15)	2.51	2.66	2.82	3.27	3.27	3.27	3.14	2.82	2.82	2.82	0.16	3.14	3.14	3.14	2.98	2.98	0	0	0	0	0															
23	*L. dendroidea* Venezuela (CH16)	2.56	2.71	2.86	3.27	3.27	3.27	3.01	2.71	2.71	2.71	0.16	3.02	3.01	3.01	2.86	2.86	0	0	0	0	0	0														
24	*L. dendroidea* Venezuela (CH17)	2.56	2.71	2.86	3.27	3.27	3.27	3.01	2.71	2.71	2.71	0.16	3.02	3.01	3.01	2.86	2.86	0	0	0	0	0	0	0													
25	*L. dendroidea* Venezuela (CH18)	2.56	2.71	2.86	3.27	3.27	3.27	3.01	2.71	2.71	2.71	0.16	3.02	3.01	3.01	2.86	2.86	0	0	0	0	0	0	0	0												
26	*L. dendroidea*	2.56	2.71	2.86	3.27	3.27	3.27	3.01	2.71	2.71	2.71	0.16	3.02	3.01	3.01	2.86	2.86	0	0	0	0	0	0	0	0	0											

	Venezuela (T1)																																				
27	*L. dendroidea* Venezuela (CM1905)	2.55	2.71	3.19	0	0	0	0.16	0.16	0.16	0.16	3.33	0.16	0.16	0.16	0	0	3.03	3.03	3.03	3.05	3.05	3.08	3.03	3.03	3.03	3.03									
28	*L. dendroidea* Spain (KF492728)	1.92	2.09	1.92	4.58	4.58	4.58	4.18	3.83	3.83	3.83	1.39	4.18	4.18	4.18	4.01	4.01	1.22	1.22	1.22	1.22	1.22	1.22	1.22	1.22	1.22	1.22	4.01								
29	*L. dendroidea* Spain (KF492725)	1.74	1.92	2.09	4.36	4.36	4.36	4.01	3.66	3.66	3.66	1.57	4.01	4.01	4.01	3.83	3.83	1.39	1.39	1.39	1.39	1.39	1.39	1.39	1.39	1.39	1.39	3.83	0.17							
30	*L. dendroidea* Molokai, USA (GU223887)	5.42	5.27	5.72	5.67	5.67	5.67	5.87	5.57	5.57	5.57	4.69	5.87	5.87	5.87	5.72	5.72	4.81	4.78	4.81	4.73	4.81	4.86	4.81	4.81	4.81	4.81	5.75	4.71	4.53						
31	*L. dendroidea*Australia (MT822859)	5.45	5.28	6.16	5.67	5.67	5.67	6.16	5.81	5.81	5.81	5.1	6.16	6.16	6.16	5.98	5.98	5.28	5.28	5.28	5.28	5.28	5.28	5.28	5.28	5.28	5.28	5.98	5.28	5.1	3.34					
32	*L. dendroidea* Australia (MT822855)	5.98	5.81	5.98	5.24	5.24	5.24	5.81	5.45	5.45	5.45	5.98	5.81	5.81	5.81	5.63	5.63	6.16	6.16	6.16	6.16	6.16	6.16	6.16	6.16	6.16	6.16	5.63	6.16	6.33	4.04	3.52				
33	*L. dendroidea* Australia (MT822858)	6.16	5.98	6.51	5.24	5.24	5.24	6.16	5.81	5.81	5.81	6.51	6.16	6.16	6.16	5.98	5.98	6.69	6.69	6.69	6.69	6.69	6.69	6.69	6.69	6.69	6.69	5.98	6.69	6.51	4.04	3.34	1.05			
34	*L. dendroidea* Australia (MT822857)	6.69	6.51	7.04	5.45	5.45	5.45	6.33	5.98	5.98	5.98	7.04	6.33	6.33	6.33	6.16	6.16	7.21	7.21	7.21	7.21	7.21	7.21	7.21	7.21	7.21	7.21	6.16	7.21	7.04	4.57	3.87	1.58	0.52		
35	*L. dendroidea* Australia	6.51	6.33	6.86	5.24	5.24	5.24	6.16	5.81	5.81	5.81	6.86	6.16	6.16	6.16	5.98	5.98	7.04	7.04	7.04	7.04	7.04	7.04	7.04	7.04	7.04	7.04	5.98	7.04	6.86	4.4	3.69	1.4	0.35	0.17	

	(MT822856)																															

Table S8. Intra- and interspecific genetic variations (%) for COI-5P sequences between the subclades of *Laurencia microcladia*, *L.* aff. *microcladia* and *L. caduciramulosa*. Species from the type locality in bold.

		1	2	3	4	5	6	7	8	9	10
1	***L. microcladia* USAUSVI (OK209896)**										
2	*L. microcladia* Bermuda (OK209905)	1.52									
3	*L. microcladia* Cuba (PCSC1)	0.43	1.52								
4	*L. microcladia* Cuba (PCSC2)	0.43	1.52	0							
5	*L.* aff. *microcladia* Venezuela (PA8)	1.74	1.96	2.62	2.62						
6	*L.* aff. *microcladia* Venezuela (LC3)	1.74	1.96	2.71	2.71	0					
7	*L.* aff. *microcladia* Venezuela (PA20)	1.74	1.96	2.71	2.71	0	0				
8	*L. caduciramulosa* Cuba (B1)	1.52	2.18	2.41	2.41	2.25	2.25	2.25			
9	*L. caduciramulosa* Brazil (LCPV)	2.18	2.4	3.01	3.01	2.77	2.71	2.71	0.8		
10	*L. caduciramulosa* Spain (LCTE)	2.18	2.83	3.16	3.16	2.77	2.71	2.71	0.64	1.05	

Table S9. Intraespecific genetic variations (%) for COI-5P sequences between the subclades of *Laurencia catarinensis*. Species from the type locality in bold.

		1	2	3	4	5	6	7	8	9
1	***L. catarinensis* Brazil (VC)**									
2	*L. catarinensis* Bermuda (OK209904)	0.43								
3	*L. catarinensis* USA (OK209903)	0.43	0.43							
4	*L. catarinensis* USAUSVI (OK209898)	0.21	0.21	0.21						
5	*L. catarinensis* Cuba (CH3)	0.15	0.21	0.21	0					
6	*L. catarinensis* Venezuela (N2)	0.45	0.65	0.65	0.43	0.3				
7	*L. catarinensis* Venezuela (LC2)	0.47	0.65	0.65	0.43	0.31	0			
8	*L. catarinensis* Venezuela (PA1)	0.47	0.65	0.65	0.43	0.31	0	0		
9	*L. catarinensis* Spain (KF492718)	0.17	0.21	0.21	0	0	0.34	0.34	0.34	

Table S10. Intra- and interspecific genetic variations (%) for *rbc*L sequences between the subclades of *Laurencia caduciramulosa, L. laurahuertana, L. venusta, L. microcladia* and *L.* aff. *microcladia*. Species from the type locality in bold.

		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	*L. caduciramulosa* Spain (JF781525)													
2	*L. caduciramulosa* USA (KJ700867)	0.76												
3	*L. caduciramulosa* Cuba (FJ904933)	0.13	0.62											
4	*L. caduciramulosa* Cuba (B1)	0.76	0	0.62										
5	*L. caduciramulosa* Brazil (KJ700865)	0.13	0.62	0	0.62									
6	*L. laurahuertana* Mexico (KF279401)	1.47	0.79	1.24	0.79	1.24								
7	*L. venusta* Mexico (EF061655)	1.1	0.48	0.96	0.48	0.96	0.45							
8	***L. microcladia* USAUSVI (OK209881)**	1.23	0.49	1.06	0.49	1.06	0.48	0.16						
9	*L. microcladia* Bermuda (OK209880)	1.15	0.57	1.15	0.57	1.15	0.72	0.41	0.41					
10	*L. microcladia* Cuba (PCSC1)	0.99	0.36	0.81	0.36	0.81	0.37	0.18	0.1	0.43				
11	*L.* aff. *microcladia* Venezuela (LC3)	0.69	0.34	0.69	0.34	0.69	0.79	0.69	0.74	0.65	0.63			
12	*L.* aff. *microcladia* Venezuela (LPA8PAEV)	0.7	0.21	0.56	0.21	0.56	0.67	0.49	0.57	0.65	0.46	0.14		
13	*L.* aff. *microcladia* Venezuela (PA20)	0.69	0.34	0.69	0.34	0.69	0.79	0.69	0.74	0.65	0.63	0	0.14	

Table S11. Intra- and interspecific genetic variations (%) for *rbc*L sequences between the subclades of *Yuzurua*.

		1	**2**	**3**	**4**
1	*Y. iridescens* Bermuda (OK209858)				
2	*Yuzurua* sp. 1 Bermuda (OK209862)	4.27			
3	*Yuzurua* sp. Guadeloupe (KX146198)	3.55	3.78		
4	*Yuzurua* sp. Cuba (RGbo3)	3.55	3.53	0.089	

Table S12. Intra- and interspecific genetic variations (%) for *rbc*L sequences between the subclades of *Palisada*. Species from the type locality in bold.

		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	*P. cervicornis* Bermuda (OK209863)										
2	*P. cervicornis* USA (MG030375)	0.9									
3	*P. corallopsis* Bermuda (OK209865)	2.21	2.3								
4	*P. corallopsis* Mexico (EF061646)	2.54	2.76	0.57							
5	*P. corallopsis* USA (MG020477)	2.95	3.17	0.98	0.41						
6	***P. corallopsis* Cuba (B)**	2.3	2.41	0.82	1.02	1.46					
7	*P. furcata* Brazil (GU330226)	2.99	3.34	1.33	1.88	2.3	2.22				
8	***P. furcata* 1 Ceará, Brazil**	2.46	2.52	0.49	0.21	0.64	0.95	1.38			
9	*Palisada* sp. 1 Florida, USA	2.38	2.38	2.13	2.22	2.62	1.97	2.66	2.13		
10	*Palisada* sp. Cuba (R)	2.71	2.79	2.59	2.56	3.03	2.24	2.44	2.44	0	

MATERIAL SUPLEMENTAR

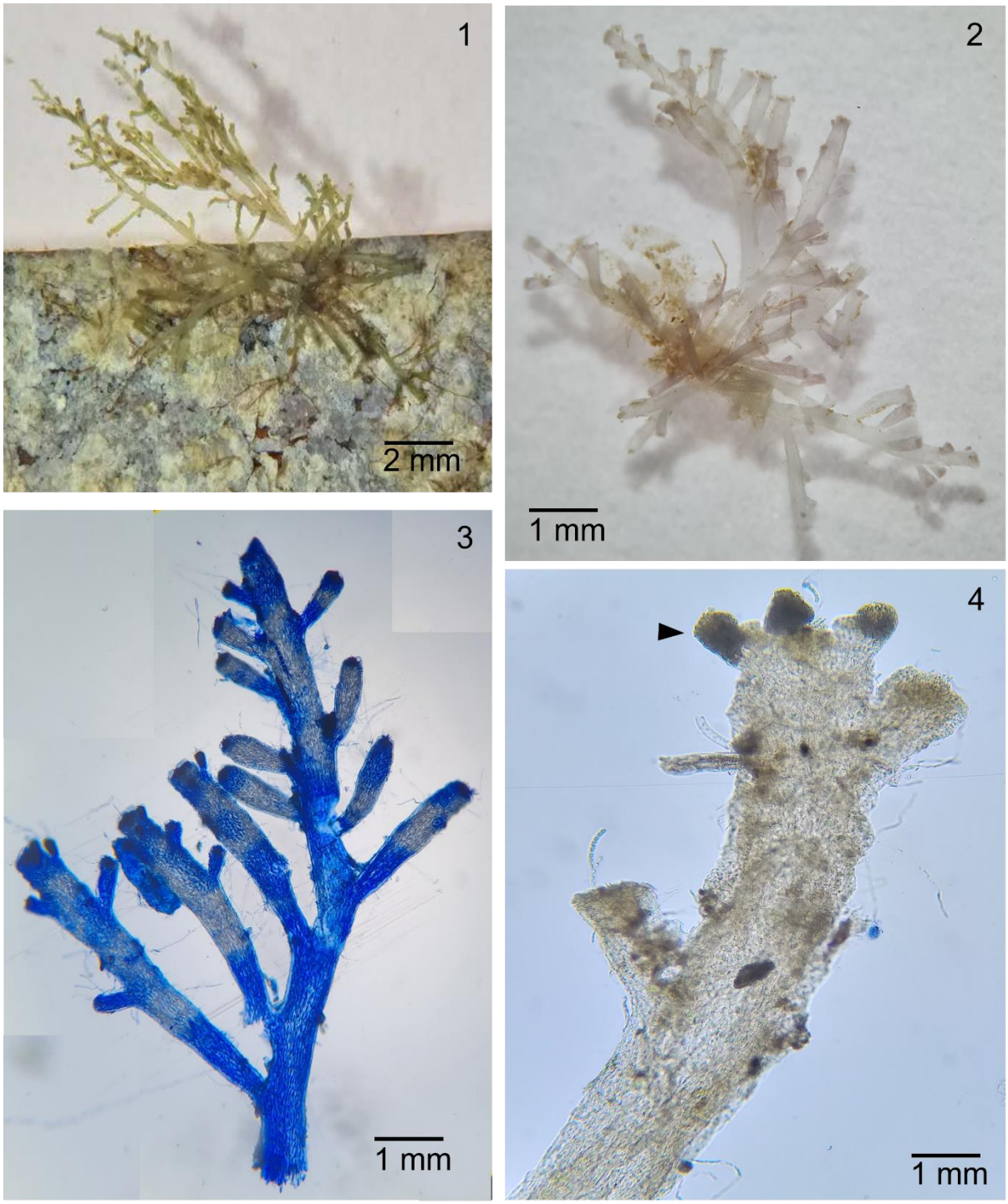


Figura 1-4. *Laurencia caduciramulosa* Masuda & S.Kawaguchi.
Figura 1. Aspecto geral do talo epífita em *Thalassia testudinum*.
Figura 2, 3. Detalhe dos ramos.
Figura 4. Porção superior dos ramos mostrando coroa de râmulos decíduos nos ápices dos ramos (ponta de seta).

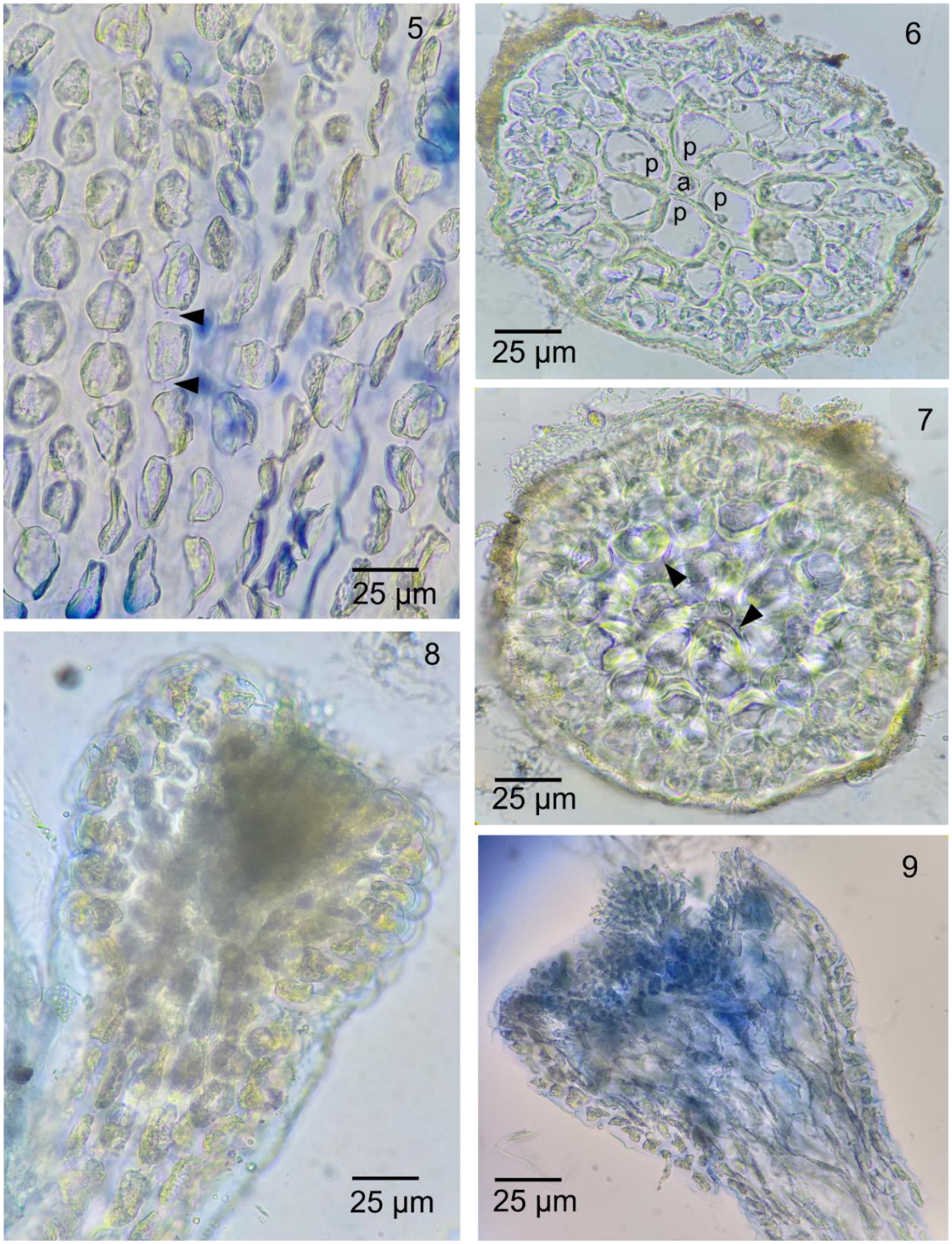
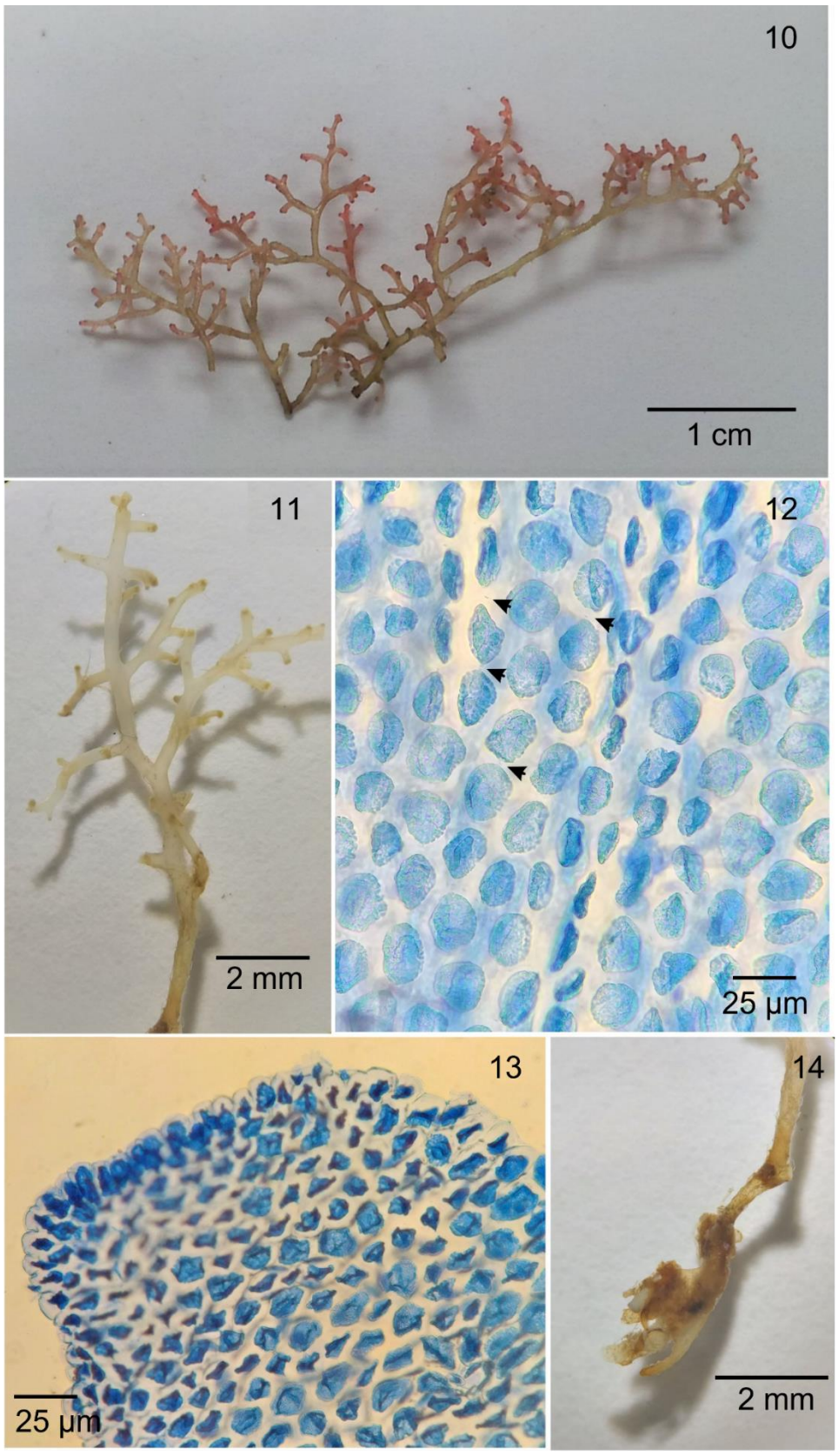


Figura 5-9. *Laurencia caduciramulosa* Masuda & S.Kawaguchi.
Figura 5. Vista superficial do talo com células corticais mostrando ligações secundárias (seta).
Figura 6. Corte transversal da porção apical de um ramo mostrando célula axial (a) com quatro células pericentrais (p).
Figura 7. Corte transversal do talo mostrando espessamentos lenticulares nas paredes das células pericentrais.
Figura 8. Corte longitudinal de um ramo com células corticais projetadas próximas ao ápice.
Figura 9. Corte longitudinal de um râmulo espermatangial.



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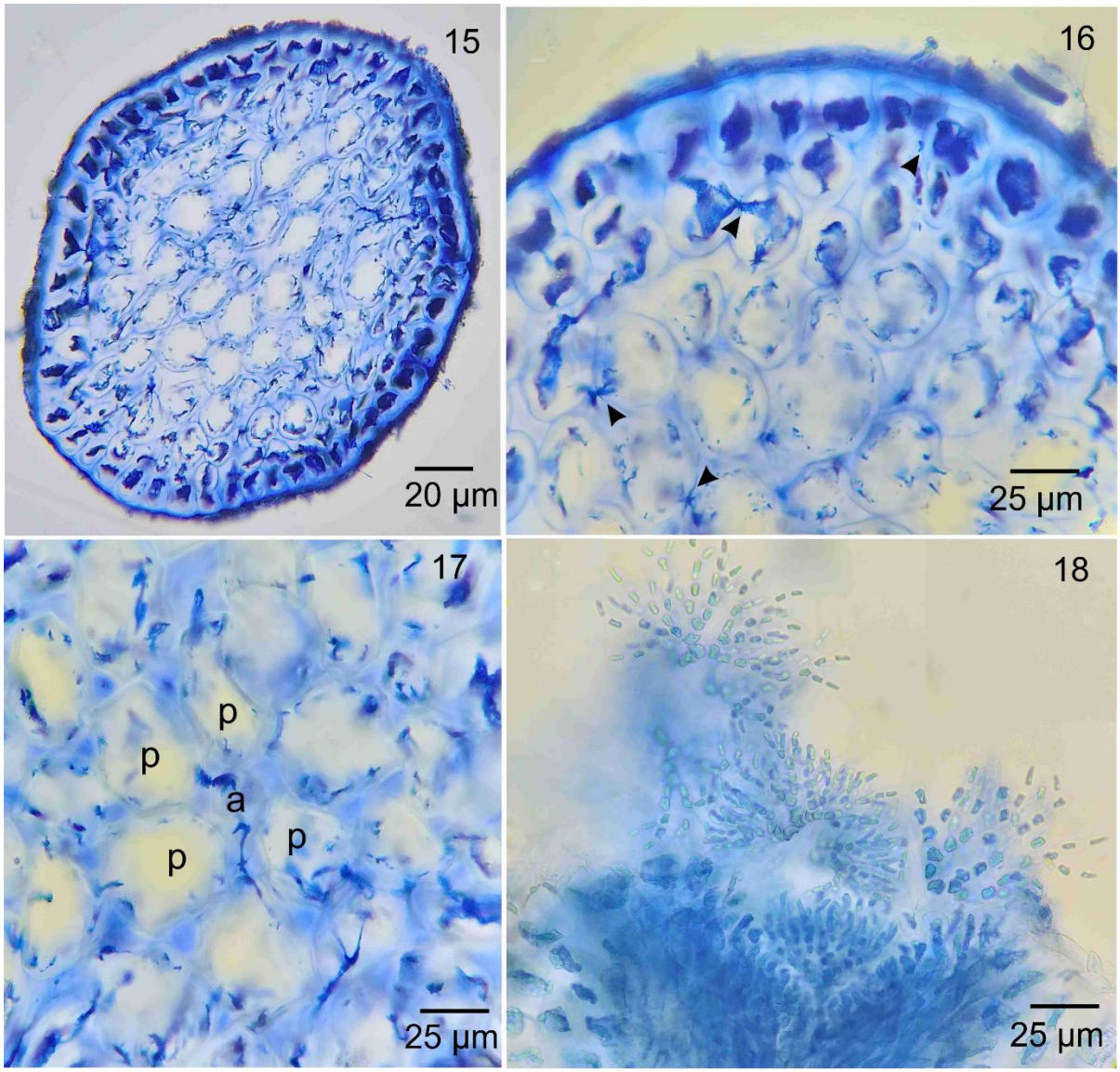

Figura 15-18. *Laurencia catarinensis* Cordeiro-Marino & Fujii.
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Figura 18. Corte longitudinal de um râmulo espermatangial.

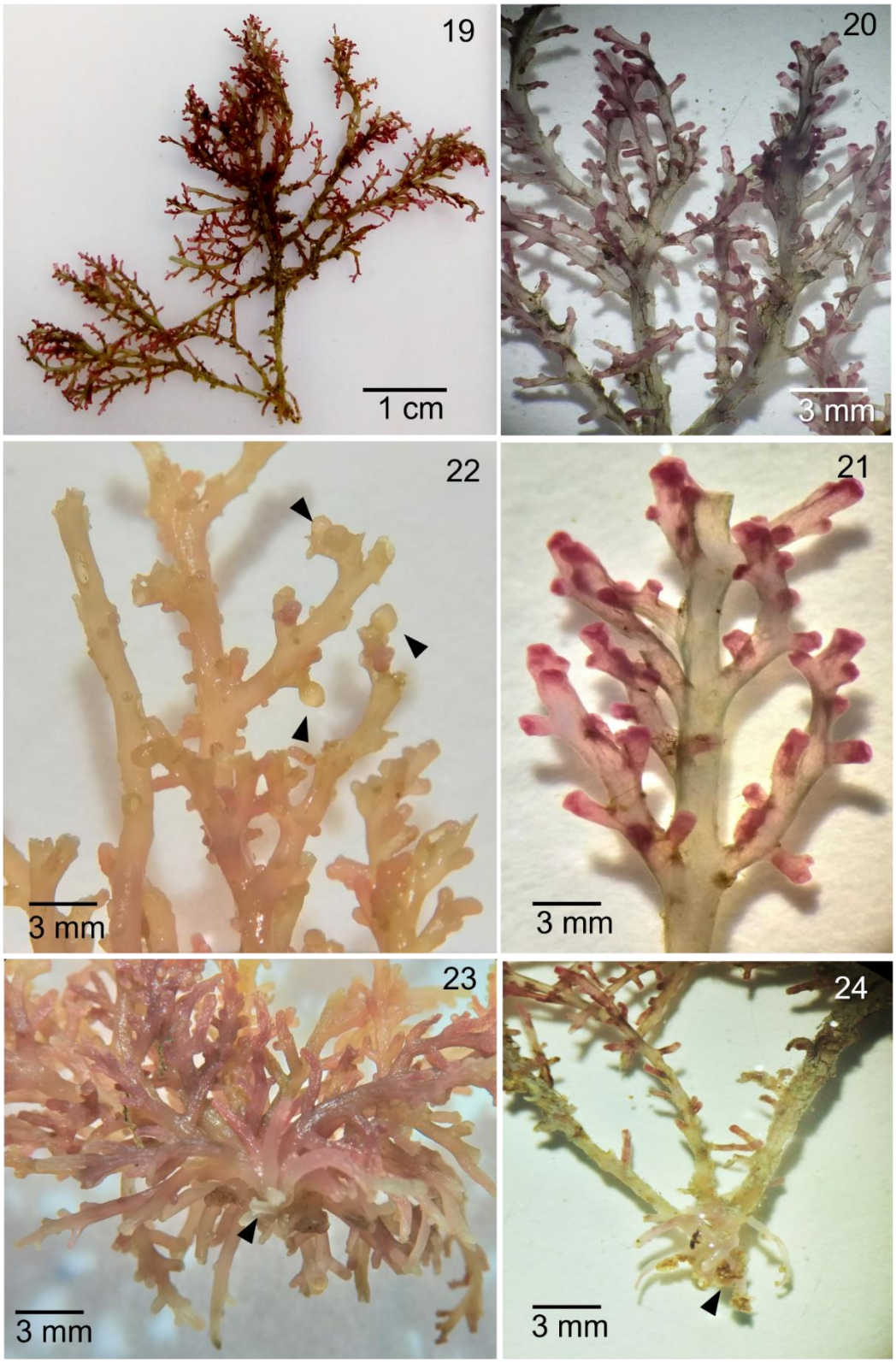

Figura 19-24. *Laurencia dendroidea* J.Agardh.
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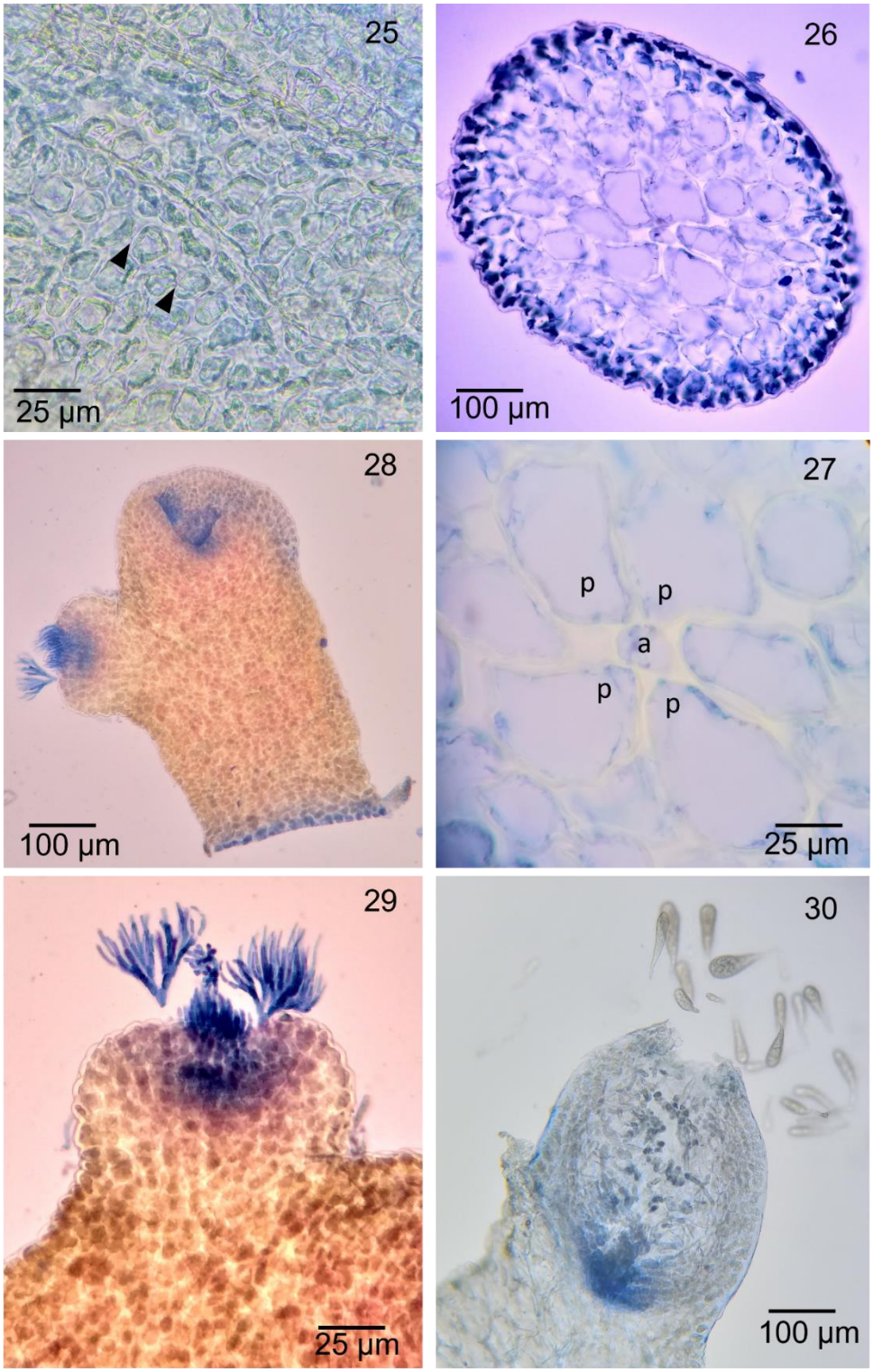

Figura 25-30. *Laurencia dendroidea* J.Agardh.
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Figuras 28, 29. Detalhe do râmulo espermatangial.
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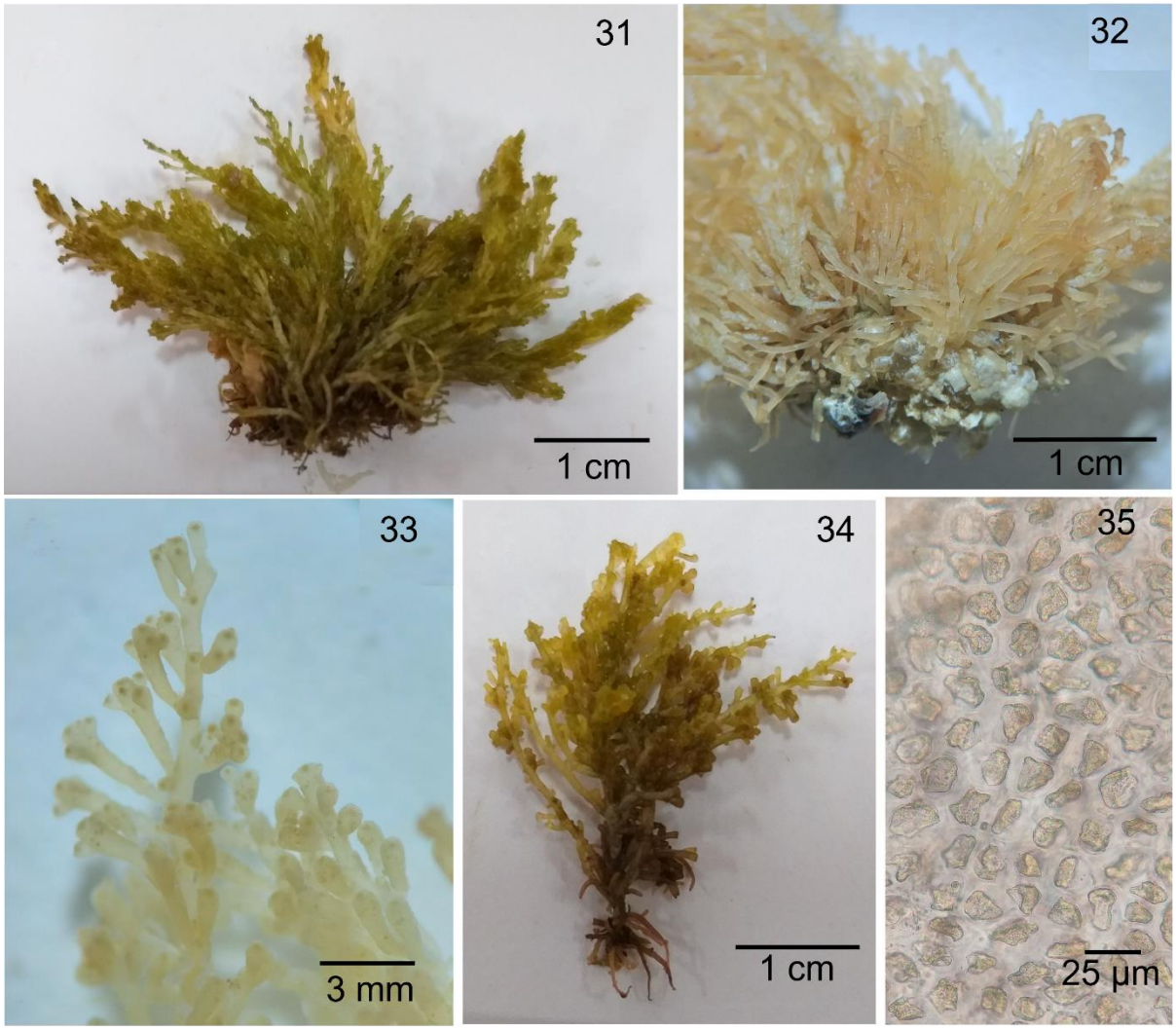

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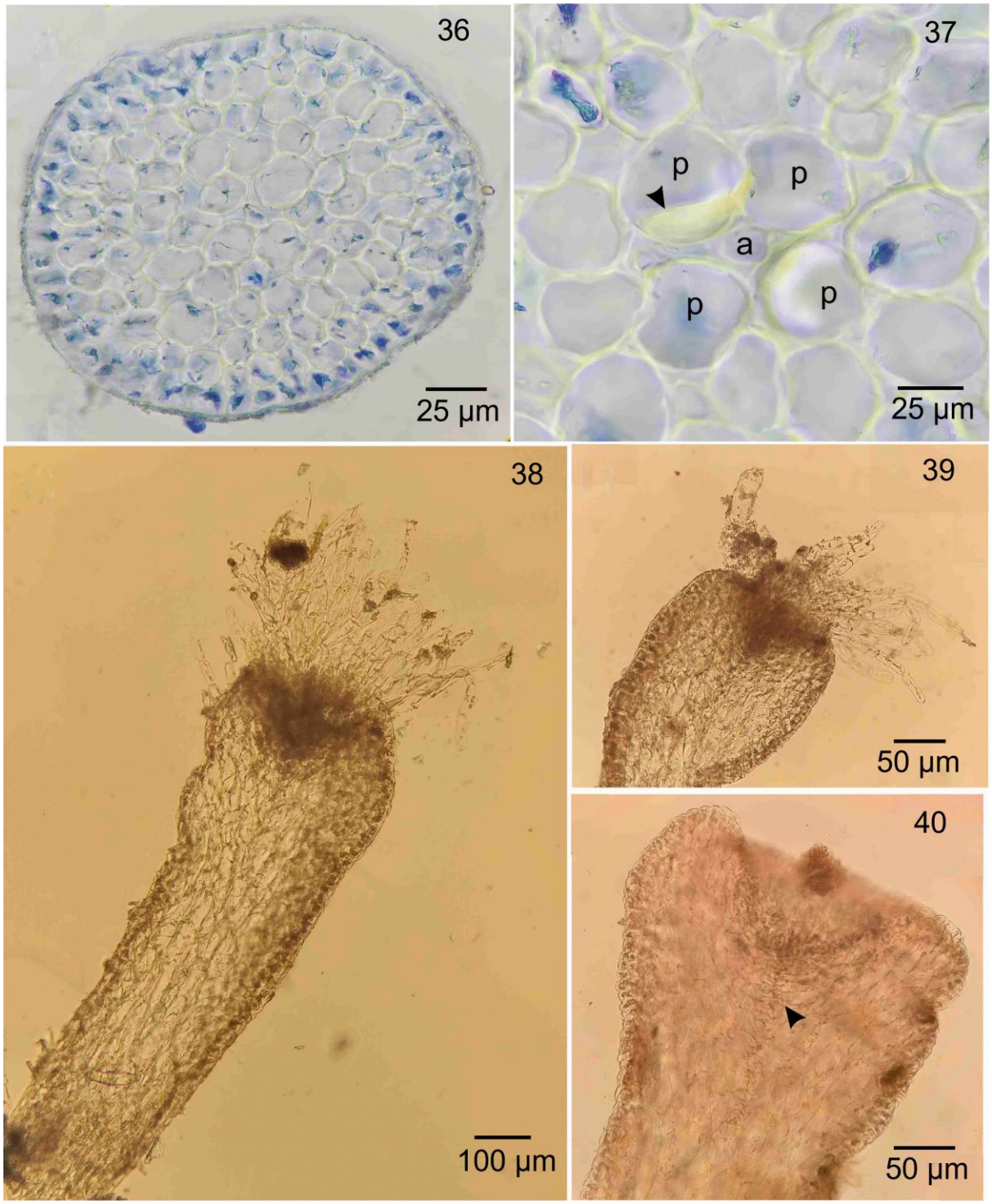

Figura 36-40. *Laurencia microcladia* Kützing.
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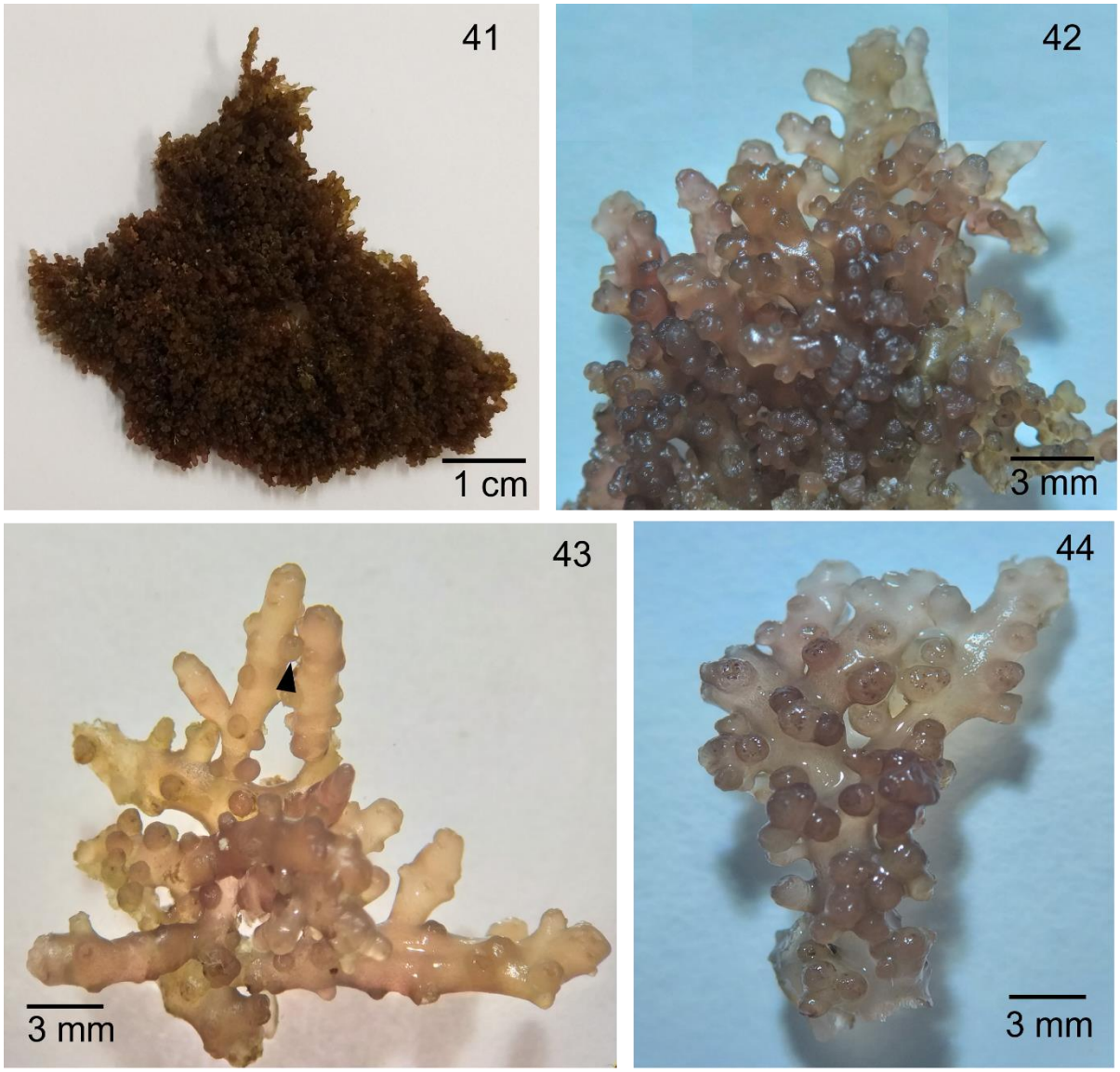

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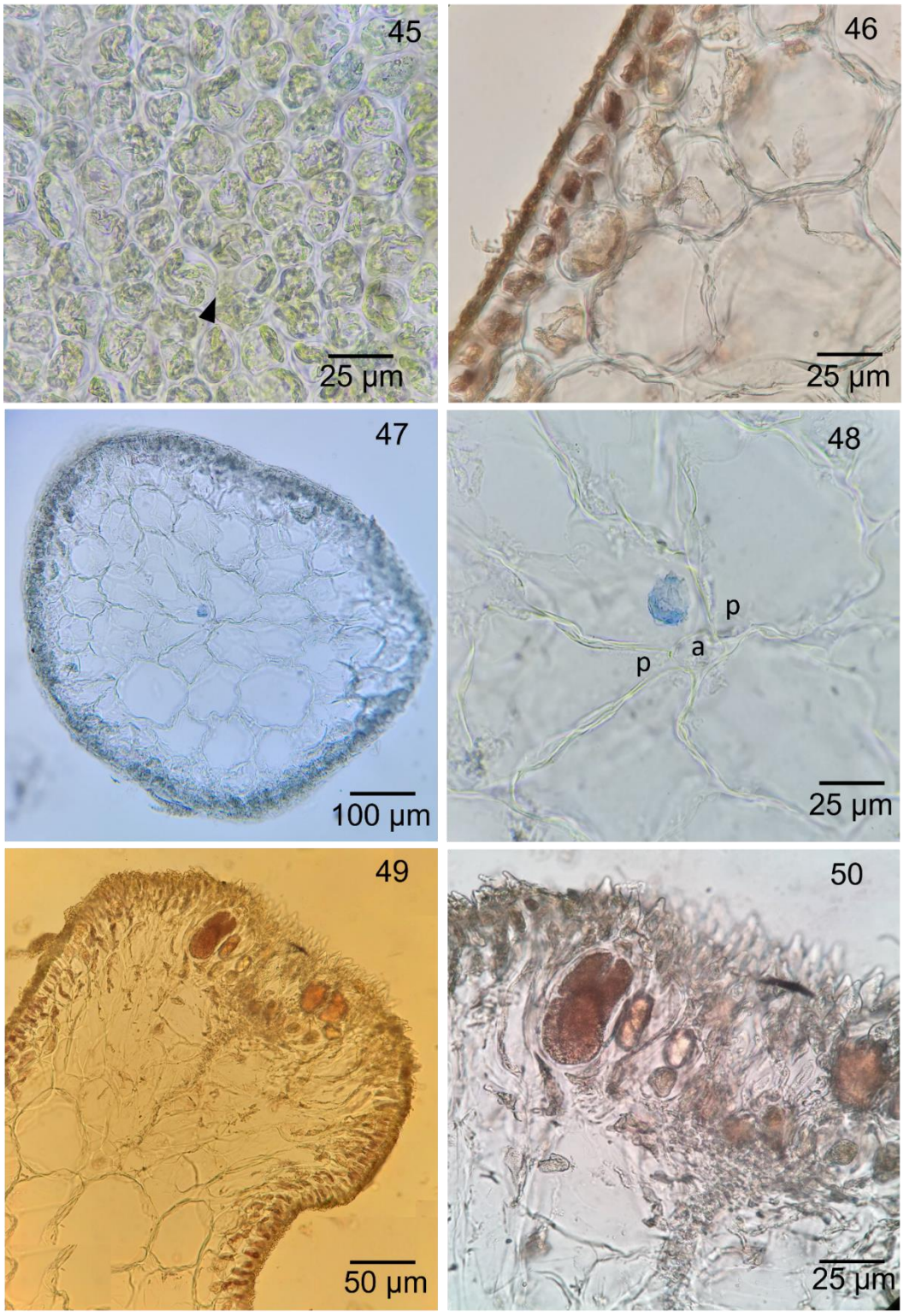

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CAPÍTULO 3

PALISADA CORALLOPSIS AND *P. FURCATA* (CERAMIALES, RHODOPHYTA) ARE CONSPECIFIC

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CAPÍTULO 3

***Palisada corallopsis* and *P. furcata* (Ceramiales, Rhodophyta) are conspecific**

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Abstract: For the first time, *Palisada corallopsis* was collected in the type locality (Havana, Cuba) and used for molecular analysis. *Palisada corallopsis* has a wide distribution in the western tropical Atlantic and some areas of the Pacific Ocean whereas *P. furcata* has a more restricted distribution, reported only for the northeast coast of Brazil and Cuba. We carried out morphological and molecular analyses using COI-5P and *rbc*L genes of *P. corallopsis* and *P. furcata* from their type localities, Havana, Cuba, and Ceará state, Brazil, respectively. The *rbc*L phylogeny placed the topotype sequences of the two species in the same clade, with low genetic divergence, 0.95%, and 1.6% for COI-5P. Morphologically the species share similar habit with branchlets bi- to trifurcated, cortical cells that may be arranged as a palisade and connected each other by secondary pit-connections. Our detailed morphological observations, including examination of the type specimens, as well molecular analyses demonstrate that *P. corallopsis* and *P. furcata* are conspecific being *P. furcata* a later synonym of *P. corallopsis*. This study confirms the occurrence of *P. corallopsis* in Brazil for the first time using molecular data and its wide distribution in the western Atlantic Ocean.

Keywords: COI-5P; morphology; *Palisada*; *rbc*L; taxonomy

1 Introduction

The taxonomy of the *Laurencia* J.V. Lamouroux complex has undergone several changes since the early 1990 based on morpho-anatomy and cladistic analyses of vegetative and reproductive characters (Nam et al. 1994; Nam and Saito 1995; Nam 1999, 2006, 2007; Garbary and Harper 1998). Afterwards, phylogenetic studies, mainly using *rbc*L gene, led to proposition of new genera, re-circumscription of old genera, and corroborated current classification system (Nam et al. 2000; Martin-Lescanne et al. 2010; Cassano et al. 2012a, 2019; Rousseau et al. 2017). Thus, the *Laurencia* complex currently comprises eight genera: *Laurencia sensu stricto*, *Osmundea* Stackhouse, *Chondrophycus* (Tokida *et* Y. Saito) Garbary *et* J.T. Harper, *Palisada*

K.W. Nam, *Yuzurua* (K.W. Nam) Martin-Lescanne, *Laurenciella* Cassano, Gil-Rodríguez, Sentíes, Díaz-Larrea, M.C. Oliveira *et* M.T. Fujii, *Ohelopapa* F. Rousseau, Martin-Lescanne, Payri *et* L. Le Gall, and *Corynecladia* J. Agardh (as *Coronaphycus* Metti in Metti et al. 2015).

Palisada is distributed from tropical to subtropical regions of the Atlantic and Indo-Pacific Oceans and is the second largest genus of the *Laurencia* complex with 26 accepted species names (Guiry and Guiry 2022). Based on the generitype, *Palisada robusta* K.W. Nam, the genus is characterized by a combination of vegetative and reproductive features such as: vegetative axial segments with two pericentral cells, first pericentral cell with underneath position of trichoblast, a palisade arrangement of cortical layer in some species, trichoblast type spermatangial development with spermatangial branches produced from one of two laterals on suprabasal cell of trichoblasts, procarp-bearing segments with four or five pericentral cells, auxiliary cells normally formed after fertilization, tetrasporangia arranged at right angles to the main axis, originated from particular pericentral cells, the second pericentral cell is always fertile, the first one remaining sterile, additional fertile pericentral cells may be produced in the tetrasporangial axis (Nam et al. 1994; Nam 2006, 2007).

Molecular studies carried out on *Palisada* species from the western and eastern Atlantic inferred phylogenetic affinities, proposed conspecificity of species and new combinations (Fujii et al. 2006, as *Chondrophycus*; Sentíes and Díaz-Larrea 2008; Cassano et al. 2009, 2012b; Collado-Vides et al. 2017; Popolizio et al. 2022). Of the 26 species described for the genus, six are cited for the western Atlantic: *Palisada cervicornis* (Harvey) Collado-Vides, Cassano *et* M.T. Fujii, *P. corallopsis* (Montagne) Sentíes, M.T. Fujii *et* Díaz in Sentíes and Díaz-Larrea, *P. flagellifera* (J. Agardh) K.W. Nam, *P. furcata* (Cordeiro-Marino *et* M.T. Fujii) Cassano *et* M.T. Fujii, *P. intermedia* (Yamada) K.W. Nam and *P. perforata* (Bory) K.W. Nam (Wynne 2022). Among these, *P. corallopsis* and *P. furcata* are the most closely related in terms of gross morphology and anatomic characteristics of the thallus (Montagne1842, Cordeiro-Marino et al. 1994).

Palisada corallopsis is widely distributed in the western Atlantic Ocean from North Carolina, USA to Brazil, including several Caribbean islands and Bermuda, whereas in the eastern Atlantic it is reported only for insular waters, in the Canary Islands, Madeira, Salvage Islands and Cape Verde Islands (Guiry and Guiry 2022). Its distribution also extends to the Indo-Pacific Ocean but with a more restricted occurrence, reported only for Indonesia, the Philippines, and the South China Sea (Guiry and Guiry 2022). On the other hand, *P. furcata* has a much more restricted distribution in the western Atlantic, occurring specifically in Brazil (Cordeiro-Marino et al. 1994; Fujii and Sentíes 2005; Cassano et al. 2012b) and Cuba (Areces et al. 2003; Suárez et al. 2015).

Molecular study of type specimens or topotype material is important in order to correctly apply specific names (Soares et al. 2019), which can lead to the correction of misapplied names and synonymizations, representing a huge advance in solving taxonomic problems. *Palisada corallopsis* and *P. furcata* are morphologically closely related, but until now, molecular data on *P. corallopsis* was lacking, making genetic comparison impossible.

During this study we collected samples of *P. corallopsis* from Havana, Cuba, and *P. furcata* from Ceará, Brazil, corresponding to their type localities, and performed molecular analyses using *rbc*L and COI-5P genes. We also compared these newly collected material with the holotypes of *P. corallopsis* and *P. furcata*, and provided for the first time a detailed and comparative morphological study of both species. Updated images illustrating the characteristics of *P. corallopsis* from the type locality are also provided.

2 Materials and methods

2.1 Morphology

Samples of *Palisada corallopsis* and *P. furcata* from Havana, Cuba and Ceará state, Brazil were collected between 2018 and 2020. Samples for morphological studies were collected and frozen, kept in this way until the moment of study or dried to allow transport to Brazil, where it was analyzed as a postgraduate objective, whereas material used in the molecular studies was dehydrated on silica gel. After completing the morphological analyses, the specimens were properly prepared, pressed and deposited in the herbaria (SP) of the Instituto de Pesquisas Ambientais, São Paulo, Brazil, and in the National Aquarium of Cuba (HANC) collections from Havana, Cuba.

Transverse and longitudinal hand sections were made with a stainless-steel razor blade and stained with 0.5% aqueous aniline blue solution, acidified with 1N HCl (Tsuda and Abbott 1985). Photographs were taken with a Panasonic Lumix DMC-FH4 camera (Panasonic Corporation, Osaka, Japan) coupled to a Zeiss Stemi 2000-C stereomicroscope and Carl Zeiss Axio Scope A1 microscopes (Germany).

2.2 Molecular analysis

Total genomic DNA was extracted from silica gel-dried material after automated grinding in a Precellys®24 tissue homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France) using the NucleoSpin® Plant II Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. DNA sequences were generated for the 5’ region of the mitochondrial cytochrome c oxidase 1 gene (COI-5P), and the plastid-encoded large subunit of the ribulose-1,5-bisphosphate carboxylase/oxygenase gene (*rbc*L) for *P. corallopsis* and *P. furcata*. Polymerase

chain reactions (PCRs) were run in 25 µl volumes using GoTaq® Green Master Mix (Promega, Madison, Wisconsin, USA) in a Techne TC-512 thermocycler (Bibby Scientific Ltd, Staffordshire, UK). The COI-5P region was amplified using the primers GazF1 and GazR1 (Saunders 2005), and PCR conditions described in Cassano et al. (2019) were used. The *rbc*L gene was amplified according to Geraldino et al. (2009) in three fragments, using specific primer pairs for *P. corallopsis* Fstart and R492, F492 and R1150, F993 and *rbc*LrevNew, and for *P. furcata* F57 and R753, F577 and R1150, F993 and *rbc*S as described in Freshwater and Rueness (1994) and Saunders and Moore (2013). Amplified products were checked for length, purity and yield in agarose gels and purified with the Illustra GFX PCR DNA and Gel Band Purification kit (GE Healthcare, Buckinghamshire, UK) following the manufacturer's recommendations. Successfully purified PCR products were sequenced using the Big Dye Terminator v.3.1 reaction cycle sequencing kit (Applied Biosystems, Foster City, California, USA) on a 3730 Genetic Analyzer (Applied Biosystems) by the sequencing service of the Human Genome Research Center (Biosciences Institute, University of São Paulo, Brazil).

2.3 Phylogenetic analysis

Forward and reverse reads were manually assembled to generate consensus sequences for each marker using BioEdit 7.2.6.1 (Hall 1999). A total of four sequences were newly generated of COI-5P and *rbc*L for *P. corallopsis* and *P. furcata*. Additional sequences were retrieved from GenBank (Table S1) and added to each dataset to construct the final alignments, which included a total of 85 *rbc*L and 71 COI-5P of sequences.

Phylogenetic trees were obtained using Maximum Likelihood (ML) and Bayesian inference (BI), and the evolution model was selected using jModeltest on CIPRES Science Gateway v3.3 (Miller et al. 2010) under the Akaike information criterion (AIC). ML trees were inferred in IQ-TREE (Nguyen et al. 2015); nodal support was assessed with 2,000 ultrafast bootstrap replicates (Minh et al. 2013). For Bayesian analysis, trees were built using BEAST v1.8.4 (Suchard et al. 2018), with four MCMC chains with 100 million generations and sampling every 1,000 generations. We discard the first 100,000 generations in both runs as burn-in to build the consensus tree. Pairwise distances were calculated using the uncorrected "p" distances in PAUP. *Chondria collinsiana* M.Howe (GU330225) and *Chondria dasyphylla* (Woodward) C.Agardh (U04021) were used as outgroups for *rbc*L and *Chondria baileyana* (Montagne) Harvey (KU564345) for COI-5P.

3 Results

3.1 Molecular analysis

We sequenced topotypes of *Palisada corallopsis* and *P. furcata* for both markers, *rbc*L and COI-5P. For *rbc*L analysis was built a matrix with 85 sequences with 1448 bp length (Figure 1), and 71 sequences with 664 bp length for COI-5P (Figure S1). The *rbc*L sequence of *P. furcata* from the type locality (GU330226) previously obtained by Cassano et al. (2012a) was not included in the analysis due to its poor-quality of sequence.

The *rbc*L phylogeny placed *P. furcata* from the type locality in the same clade with sequences of *P. corallopsis* from Cuba (type locality), Bermuda, Mexico and USA (Figure 1) with moderate to high support. The *rbc*L sequences of *P. corallopsis* presented 0.41-1.46% of intraspecific divergence, whereas *P. furcata* diverged from *P. corallopsis* in a lower range, 0.21-0.95%, with the highest value observed between their topotype sequences (Table 1). Sequences of *P. corallopsis* and *P. furcata* were placed in a well-supported sister relationship with *P. cervicornis* from Bermuda and the USA, along with an unidentified species of *Palisada* (*P*. sp. 1) from Florida (Figure 1). These sequences showed an interspecific divergence of 2.30-2.41% and 2.46-2.52% between the sequences of *P. corallopsis* and *P. furcata* with those of *P. cervicornis* from Bermuda and the USA, respectively.

Chondria dasyphylla USA (U04021)
Chondria collinsiana Brazil (GU330225)
Ch. anabeliae Mexico (KX815262)
Ch. anabeliae Venezuela (MN597441)
Ch. dotyi Hawaii (KX815263)
Ch. cf. *undulatus* New Caledonia (FJ785307)
Ch. tronoi Philippines (AF489864)
Chondrophycus
89/0.96
O. hybrida France (FJ785317)
O. osmunda France (FJ785318)
O. pinnatifida Ireland (JX828140)
83/1
O. silvae Portugal (KU566561)
90/1
99/1
O. oederi Ireland (AF281880)
O. truncata Spain (JF781523)
O. blinksii USA (AY172575)
O. splendens USA (KU564461)
76/-
97/1
O. spectabilis USA (AY172572)
O. sinicola USA (AY588407)
O. sanctarum Brazil (KC012601)
O. caspica Azerbaijan (KX146188)
Osmundea
C. clavata Australia (MF094079)
86/1
C. elatus Australia (KY120339)
C. novus Australia (KY120340)
Corynecladia
Y. iridescens Bermuda (OK209858)
99/1
Y. poiteaui var. *gemmiferus* Cuba (EF061650)
99/1
Y. poiteaui var. *poiteaui* Mexico (EF061653)
Y. poiteaui var. *poiteaui* USA (EF061652)
Y. poiteaui var. *gemmiferus* Mexico (EF061648)
Y. poiteaui Bermuda (OK209860)
Yuzurua
L. alfredensis South Africa (KY927749)
L. venusta Australia (KY120345)
98/1
L. brongniartii Australia (KY120341)
L. complanata South Africa (KY927738)
-/0.95
L. dendroidea Brazil (AF465810)
L. dendroidea Venezuela (MH388528)
L. obtusa Venezuela (AF465812)
L. caraibica Mexico (EF658642)
90/1
L. corymbosa South Africa (KY927728)
L. stegengae South Africa (KY927743)
100/-
L. intricata Cuba (GU330238)
L. longiramea Brazil (MH704451)
-/0.99
98/1
L. obtusa Ireland (AF281881)
L. viridis Spain (EF685999)
L. pyramidalis France (FJ785316)
L. tasmanica Australia (MF094081)
L. caduciramulosa Spain (JF781525)
L. caduciramulosa Cuba (FJ904933)
99/1
L. laurahuertana Mexico (KF279401)
79/0.99
L. microcladia Virgin Islands (OK209881)
84/0.99
L. dichotoma South Africa (KY927786)
L. glomerata South Africa (KY927763)
98/1
L. natalensis South Africa (AF465816)
L. decussata Australia (KY120344)
94/1
L. oliveirana Brazil (JF810352)
L. flexuosa South Africa (AF465815)
L. catarinensis Brazil (VC)
96/1
L. catarinensis USA (OK209873)
L. multiclavata South Africa (KY927789)
-/0.96
87/1
L. digitata South Africa (KY927748)
L. karachiana Pakistan (MK796228)
L. pumila South Africa (KY927765)
Laurencia
La. marilzae Spain (EF686001)
-/0.97
La. marilzae Brazil (GU938189)
La. namii Bermuda (OK209870)
La. namii USA (MG004184)
La. mayaimii USA (MG004182)
Laurenciella
Oh. flexilis Tahiti (KX146187)
-/0.99
Ohelopapa
89/1
P. cervicornis Bermuda (OK209863)
-/0.96
P. cervicornis USA (MG030375)
P. sp. 1 Florida, USA (OK209864)
99/1
99/0.99
P. corallopsis Bermuda (OK209865)
91/0.99
P. corallopsis Mexico (EF061646)
P. corallopsis USA (MG020477)
90/1
***P. furcata* Ceará, Brazil**
***P. corallopsis* Havana, Cuba**
Palisada corallopsis
Palisada furcata
P. flagellifera Spain (EF685998)
-/0.95
P. flagellifera Brazil (GU330227)
P. papillosa Spain (EU256325)
99/1
P. perforata Spain (EU256327)
98/1
P. perforata USA (OK209867)
P. perforata Bermuda (OK209868)
P. perforata Brazil (EU256331)
P. perforata Venezuela (MH388525)
P. patentiramea Philippines (AF489862)
P. parvipapillata New Caledonia (KX146194)
P. crustiformans Hawaii (KX146196)
Palisada
0.03

Figure 1. Phylogenetic tree based on maximum likelihood analysis of *rbc*L sequences. Value above branches indicate maximum likelihood Bootstrap (BS) values ≥70% and Bayesian posterior probabilities (BPP) ≥0.95. (-) indicates lack of bootstrap support or values under 70; (*) indicates BS 100% or BPP 1.00. Sequence generated in this study in bold.

The COI-5P tree is shown in figure S1. The results were similar to the *rbc*L gene with *P. furcata* from Brazil grouping with *P. corallopsis* from Cuba and Bermuda. The intraspecific divergence between sequences of *P. corallopsis* ranged 1.09-2.18%, and the topotype sequences of *P. furcata* and *P. corallopsis* diverged by 1.6% (Table 1). *Palisada corallopsis*/*P. furcata* was closest to *P. cervicornis* and also *P.* sp, 1 as in the *rbc*L analyses (Figure S1). *Palisada corallopsis*/*P. furcata* diverged from *P. cervicornis* by 3.05–3.71%.

Table 1. Comparison of the genetic divergence (%) for the molecular markers *rbc*L (above the diagonal) and COI-5P (under the diagonal) between *Palisada corallopsis* and *P. furcata*.

	P. corallopsis **Bermuda**	***P. corallopsis*** **Mexico**	***P. corallopsis*** **Florida, USA**	***P. corallopsis*** **Cuba**	***P. furcata*** **Ceará, Brazil**
P. corallopsis **Bermuda**	-	0.57	0.98	0.82	0.49
P. corallopsis **Mexico**		-	0.41	1.02	0.21
P. corallopsis **Florida, USA**			-	1.46	0.6
P. corallopsis **Cuba**	2.18			-	0.95
P. furcata **Ceará, Brazil**	1.09			1.6	-

3.2 Morphology

Palisada corallopsis (Montagne) Sentíes, Fujii & Díaz-Larrea (2008: 69)

Figures 2 A-G, 3 A-G

Basionym. *Sphaerococcus corallopsis* Montagne (1842, Histoire, physique, politique *et* naturelle de l'ile de Cuba: 49, pl. 3, fig. 1).

Type. PC 0062369 (Herbier Muséum Paris Cryptogamie) Herb. Montagne in Paris (Yamada 1931, p. 198) (Figure 4).

Type Locality. La Habana, Cuba.

Homotypic synonyms. *Laurencia corallopsis* (Montagne) M.Howe (1918: 519), *Chondrophycus corallopsis* (Montagne) K.W.Nam (1999: 463).

Heterotypic synonym. *Palisada furcata* (Cordeiro-Marino & M.T. Fujii) Cassano & M.T. Fujii (2012b: 79).

Habit. Thalli are erect, forming large tufts, up to 8 cm high, wine-red to brownish in color, fleshy to cartilaginous in texture adhering slightly to herbarium paper when dried (Figures 2 A, B) and attached to the substratum by a large and firm discoid hold fast (Figure 2 G). Thalli are cylindrical at the base, up to 3 mm in diameter and slightly flattened in the rest of the plant. Main axes are long, subdichotomous to dichotomous branched below, measuring 0.75-2 mm in diameter, irregular, opposite to alternate above becoming subcorymbose. Branchlets numerous, alternate or irregular; club-shaped, short, simple, upcurved, 1 - 4 mm long and 1 - 1.5 mm in diameter, usually swollen, often bifurcated, when trifurcated the branch between the bifurcations is smaller, and with slightly constricted bases (Figures 2 C, D).

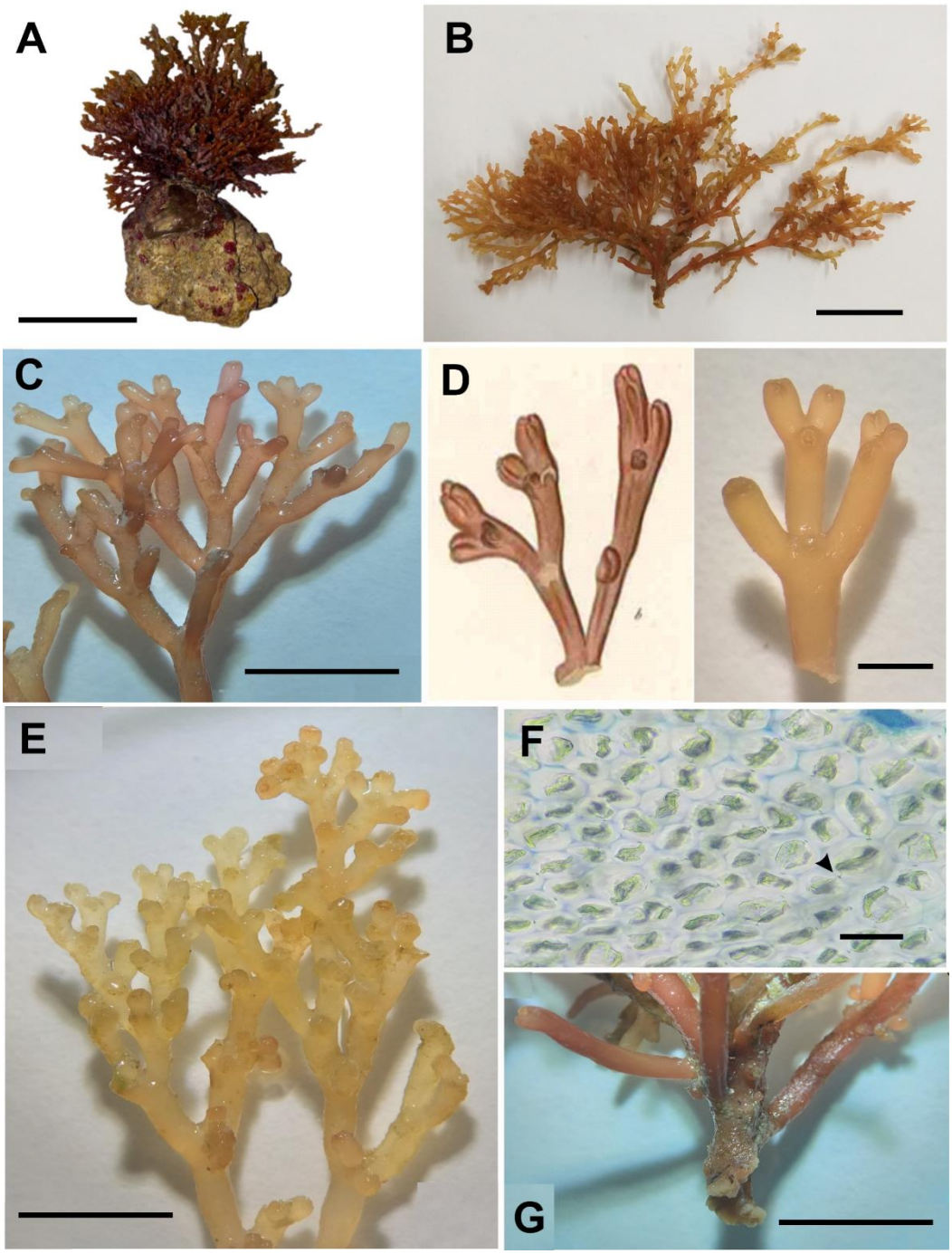

Figure 2. *Palisada corallopsis*. (A, B) Habit of the thalli. (C) Detail of branches. (D left) Illustration of the apical branches of *P. corallopsis* by Montagne (1842, as *Sphaerococcus corallopsis*) from Havana, Cuba. (D right) Detail of the apical branches of a freshly collected specimen of *P. corallopsis* from Havana, Cuba. (E) Tetrasporangial branches. (F) Surface view of the cortical cells showing secondary pit-connections between the cells (arrowhead). (G) Basal portion of thallus showing a discoid holdfast. Scale bar: A, 2 cm; B, C, E, G, 1 cm; D, 5 mm; F, 25 µm.

Vegetative structures. In surface view, cells are polygonal, isodiametric, sometimes hexagonal, presenting secondary pit-connections between the cells, 23-48 µm in diameter (Figure 2 F). In transverse section of median region of the thallus, cortical cells in one or two layers, pigmented, quadratic to subquadratic or radially elongated in palisade arranged, 30-50 x 20-35 µm, with secondary pit-connection between adjacent cells (Figure 3 B, D, E), cells of the inner layer rounded. Colorless medullary cells rounded, gradually decreasing in size towards the center, thin-walled with secondary pit-connection between them, 50-145 x 45-130 µm (Figure 3 A); pericentral cells, rounded, smaller than those of the intermediate layer. Each vegetative axial segment cuts off two pericentral cells (Figure 3 C). Cell-walls of the outer cortical cells at the apex not projecting above the surface of the thallus (Figures 3 E, G). *Corps en cerise* and lenticular thickening absent.

Reproductive structures. In longitudinal section through a fertile male branchlet, the spermatangial pits are cup-shaped and an axial cell row is discernible at the base (Figures 3F, G). Tetrasporangial branches are swollen (Figure 2E). Tetrasporangia oval to spherical, 20-48 µm in diameter, tetrahedrally divided and arranged at right-angle in relation to the longitudinal axis of the branchlets (Figures 3F, G). No female plants were observed.

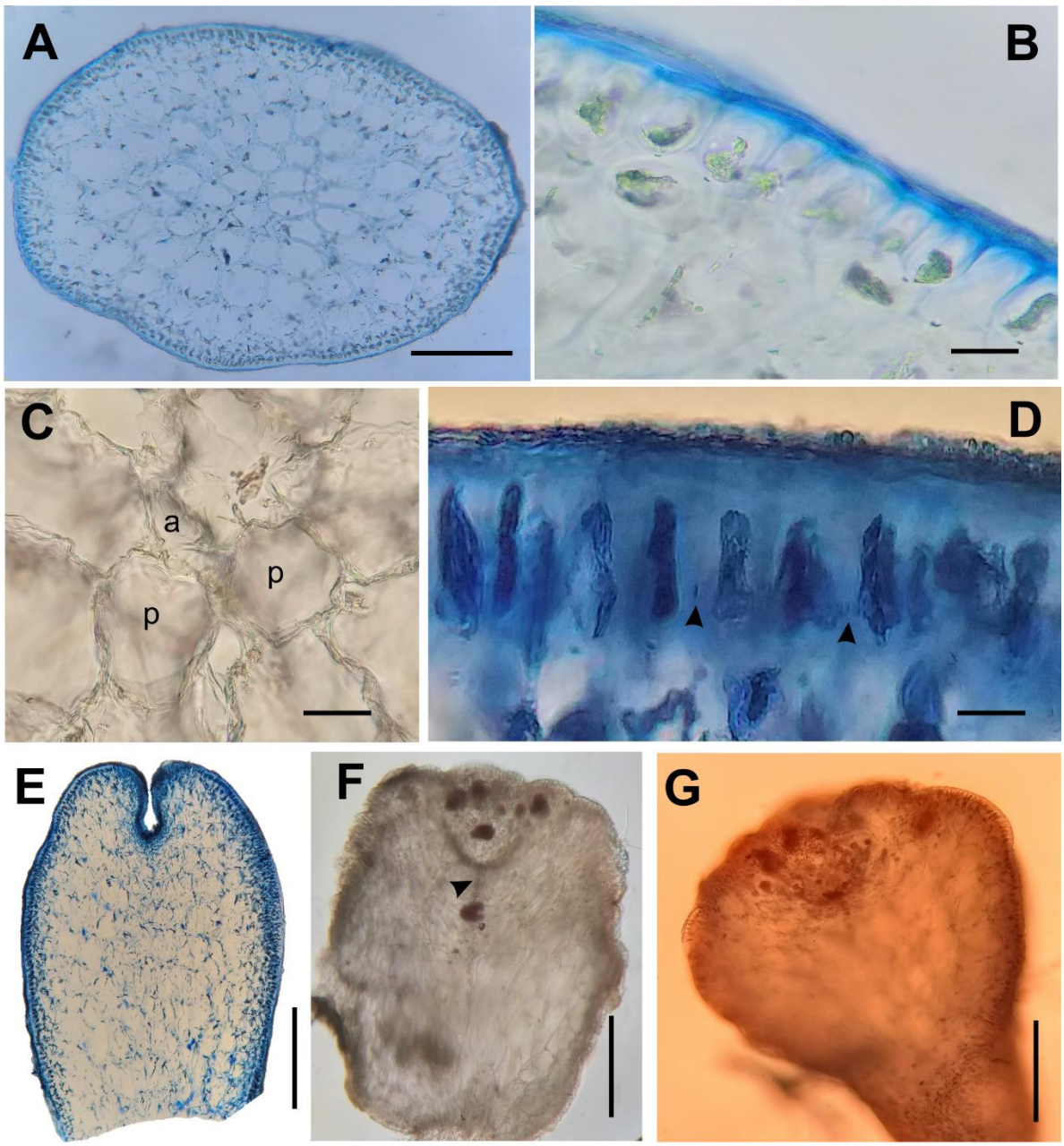

Figure 3. *Palisada corallopsis*. (A) Transverse section showing an elliptical shape of the thallus. (B) Transverse section of a branch showing quadratic to subquadratic cortical cells. (C) Transverse section of upper portion of branch showing an axial cell (a) with two pericentral cells (p). (D) Longitudinal section showing cortical cells with secondary pit-connections (arrowhead). E. Longitudinal section of a branchlet. F. Longitudinal section through tetrasporangial branchlet showing right-angle arrangement of the tetrasporangia and an axial cell row is discernible at the base (arrowhead). G. Longitudinal section through male branchlet showing spermatangial branches in cup-shaped tips. Scale bar: A, E, F, G 100 µm; B, C, D, 25 µm.

Habitat. Plants are found from the northwestern to the southeastern coast of Cuba, on rocky substrate exposed to waves, in the subtidal zone, up to 7 m deep or even in deeper waters, up to 60 m. Also found in shallow calm waters. Very abundant in coral reefs.

Specimens Examined Morphologically. ***Palisada corallopsis*** (Montagne) Sentíes, Fujii *et* Díaz-Larrea – **Cuba. Havana.** Rincón de Guanabo (leg. A.J. Areces, 23 Feb. 2001, HANC 184), (leg. A.J. Areces and D. Reyes, 20 Feb. 2020, SP 514123). **Ciego de Ávila.** Playa Flamenco, cayo Coco (leg. D. de la Nuez, 27 Feb. 2008, HANC 137). ***Palisada furcata*** (Cordeiro-Marino *et* M.T. Fujii) Cassano *et* M.T. Fujii – **Brazil. Bahia.** Salvador, Villas do Atlântico (leg. M.T.Fujii, 24 Aug. 2002, SP 400806). **Paraíba.** Jacuma, praia Carapibus (leg. M.T.Fujii, 01 Apr. 2014, SP 401518). **Pernambuco.** Jaboatão dos Guararapes, praia Candeias (leg. M.I.L.G. Cavalcanti, 25 Feb. 2018, SP 513854). **Espírito Santo.** Marataízes, praia do Pontal (leg. M.I.L.G. Cavalcanti, 09 Jun. 2017, SP 470199).

Additional Material Examined: Type: ***Sphaerococcus corallopsis*** Montagne (PC 0062369, Figure 4). Holotype: ***Laurencia furcata*** Cordeiro-Marino *et* M.T. Fujii (SP 239661); Isotype (SP 239661, Figure 5).

4 Discussion

Palisada corallopsis was originally described by Montagne (1842) as *Sphaerococcus corallopsis* from Havana, Cuba, and later transferred to the genus *Laurencia* by Howe (1918). Nam (1999) transferred *Laurencia corallopsis* (Montagne) M. Howe to the subgenus *Palisada* (Yamada) K.W. Nam, section *Palisadae*, within the genus *Chondrophycus*, based on the presence of radially elongated cortical cells arranged in a palisade, with no secondary pit-connections between adjacent cells, a single sterile pericentral cell in tetrasporangial axial segments, and a right-angle arrangement of tetrasporangia. Finally, based on molecular analyses, Sentíes and Díaz-Larrea (2008) verified that *Chondrophycus corallopsis* (Montagne) K.W. Nam positioned taxonomically close to its congeners previously transferred to *Palisada* and proposed the new combination *Palisada corallopsis* (Montagne) Sentíes, Fujii *et* Díaz-Larrea.

Palisada furcata was originally proposed as *Laurencia furcata* Cordeiro-Marino *et* M.T.Fujii in Cordeiro-Marino et al. (1994) from Brazil. However, the authors pointed out the presence of intermediate characteristics between the subgenera *Laurencia* and *Chondrophycus* for this species, as defined by Saito (1967), such as the presence of secondary pit-connections between adjacent cortical cells and cortical cells not radially elongated as palisade (*Laurencia*), and presence of two pericentral cells per vegetative axial segment, absence of *corps en cerise* and lenticular thickenings, and tetrasporangia arranged at right-angles in relation to the

longitudinal axis of the branches (*Chondrophycus*). Subsequently, Fujii and Sentíes (2005) transferred *L. furcata* to *Chondrophycus furcatus* (Cordeiro-Marino & M.T. Fujii) M.T. Fujii & Sentíes, primarily due to the presence of two pericentral cells per vegetative axial segment. Cassano et al. (2012b) based on phylogenetic analyses proposed the nomenclatural transfer of *Chondrophycus furcatus* to *Palisada furcata* (Cordeiro-Marino & M.T. Fujii) Cassano & M.T. Fujii.

Our material from Cuba, collected at Rincón de Guanabo, Havana, corresponds to *Palisada corallopsis* as defined by Montagne (1842), being similar to his original description (Figure 4, Table 2). The examination of the morpho-anatomical characteristics of the holotype (Figure 5) and isotype of *P. furcata*, as well as other materials of the species deposited in the SP herbarium and material of *P. corallopsis* from Cuba, showed that both species are very similar (Table 2). They share the presence of outermost cortical cell layer with secondary pit-connections between cortical cells, radially elongated in palisade arragement or not, absence of *corps en cerise* and lenticular thickenings, and the often presence of a third branch (trifurcation) at the apices of branches and branchlets. Morphological differences pointed out by Popolizio et al (2022) to separate *P. corallopsis* and *P. furcata* such as larger thallus, shape of branchlet tips (cylindrical to slightly flattened vs. swollen), and smaller size of the outermost cortical cells for *P. furcata*, can be considered intraspecific variations. Furthermore, the distribution range for *P. furcata* coincides with that of *P. corallopsis*, being the former mentioned only for the northeastern Cuba (Playa Guardalavaca at 3 m depth in a *Thalassia* biotope, Areces et al. 2003), apart from its type locality.

Figure 4. Photograph image of the type specimen of *Sphaerococcus corallopsis* (= *Palisada corallopsis* PC 0062369).

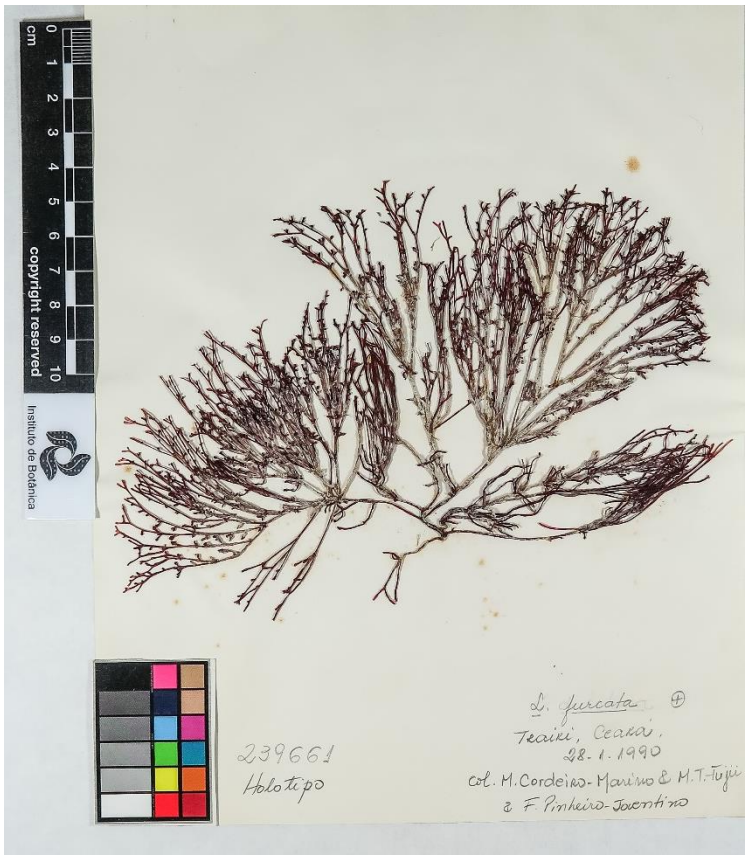

Figure 5. Photograph image of the holotype of *Laurencia furcata* (= *Palisada furcata* SP 239661).

Table 2. Comparison of morphological features between *Palisada corallopsis* and *P. furcata* from the western Atlantic Ocean. Based on Popolizio et al. (2022).

Characteristics	***Sphaerococcus corallopsis***	***Palisada corallopsis***	***Palisada corallopsis***	***Palisada corallopsis***	***Palisada corallopsis***	***Palisada furcata***
Sample collection site	Havana, Cuba (type locality)	Havana, Cuba (type locality)	Mexican Caribbean	Florida, USA	Bermuda	Ceará, Brazil (type locality)
Height plant (cm)	7.62 (= three inches)	to 8	7.5-12	4-16	to 8	to 15
Thallus shape (cross section)	-	Not cylindrical	-	-	-	Not cylindrical
Main axis basal diam. (mm)	-	3	-	-	1.5-2.0	1050-1560 μm
Branching pattern	Irregularly dichotomous or whorled; dichotomous above with short nearly dichtomous branchlets solitary or in clusters	Subdichotomous to dichotomous below; irregular, opposite to alternate above becoming subcorymbose	Spiral branching below, irregular above, up to 4 orders of branching.	Subdichotomously to dichotomously branched below, irregularly alternate above	Irregularly cervicorn; apices often appearing dichotomous; some adventitious branching	Di-totrichotomous
Branchlet diam. (mm)	1-2	1-1.5	1-2	0.75-2	1-2	1-1.5
Branchlet tips	Slightly swollen with apical pit; trichoblasts present	Swollen; trichoblasts present	Rarely swollen	Tips are obtuse, usually swollen, with an apical cell in a terminal pit; trichoblasts present	Swollen; broad in proportion to length; trichoblasts present	-
Outer cortex cell in surface view	Hexagonal	Polygonal and isodiametric	Polygonal or circular	Compact, angular to rectangular	Polygonal (hexagonal rectangular)	Polygonal and isodiametric

Characteristics	***Sphaerococcus corallopsis***	***Palisada corallopsis***	***Palisada corallopsis***	***Palisada corallopsis***	***Palisada corallopsis***	***Palisada furcata***
		(sometimes hexagonal)				
Arrangement of cortical cells (cross section)	-	Quadratic to subquadratic and palisade	Palisade	-	-	Not palisade
Outer cortex cell diam. (µm), surface view	-	23-48	8-52 x 20-34	to 50	23-45	7-33 x 21-33
Secondary pit-connections	-	Present	Absent	-	Present	Present
Medullary cells (µm)	-	50-145 x 45-130	6.0-12.0	-	-	Rounded, 50-150 x 45-135
Lenticular thickenings	-	Absent	Absent	-	Absent	Absent
Corps en cerise	-	-	Absent	-	-	Absent
Outer cortex cell projections	-	Absent	Absent	-	Absent	Absent
Tetrasporangium diam. (µm)	-	20-48 long	28-57	60-100 long	-	80-110 X 60-75
Arrangement of tetrasporangia	-	right-angle	right-angle	right-angle	-	right-angle
Shape of spermatangial branches tips	-	cup-shaped		-	-	cup-shaped
Shape of cystocarp	-	-	-	oval, subglobose to urn-shaped, and with obvious necks	-	Partly immersed
References	Montagne (1842)	This study	Sentíes and Fujii (2002)	Dawes and Mathieson (2008)	Popolizio et al. (2022)	Cordeiro-Marino et al. (1994), Fujii

Characteristics	***Sphaerococcus corallopsis***	***Palisada corallopsis***	***Palisada corallopsis***	***Palisada corallopsis***	***Palisada corallopsis***	***Palisada furcata***
						and Sentíes (2005)

Molecular data strongly support the conclusion that *P. corallopsis* and *P. furcata* correspond to the same taxonomic entity. *Rbc*L phylogeny shows low genetic divergence of the only 0.95% for *rbc*L and 1.6% for COI-5P (Table 1). Popolizio et al. (2022) in their *rbc*L analyses verified that *P. furcata* from Brazil (GU330226) also grouped in the *P. corallopsis* clade, but showed higher divergence (1.8%) between it and *P. corallopsis* from Mexico. The sequence of *P. furcata* (GU330226) has 14 ambiguities, then Popolizio et al. (2022) raised the possibility that the distance estimate could change with better quality sequence data. Our genetic divergence results for the entire *P. corallopsis*/*P. furcata* clade from new sequences generated with good quality was, in fact, slightly lower reaching to 1.46% for *rbc*L. The maximum of *rbc*L intraspecific divergence observed in our study (1.46%) is above that found for the genus *Palisada* by Cassano et al. (2009, 0.4%), and by Popolizio et al. (2022, 0.7%), however it is below the range of interspecific variation observed between *P. corallopsis*/*P. furcata* and its species closest phylogenetically *P. cervicornis* (2.30-2.41% from Bermuda and 2.46-2.52% from USA, this study; 2.7-3.5%, Collado-Vides et al. (2017), and between *Palisada* species (2.0-3.0%, Popolizio et al. 2022).

For COI-5P, our intraspecific divergence for *P. corallopsis*/*P. furcata* (1.09-2.18%) is higher than that found by Popolizio et al. (2022, 0-1.3%), but there was no overlap with the interspecific divergence values between *Palisada* species (Popolizio et al. 2022, 3.9-6.7%).

5 Conclusions

In conclusion, our study provides the first DNA sequences of *P. corallopsis* and *P. furcata* from their type localities, Havana, Cuba and Ceará state, Brazil, and detailed description and illustrations of the morpho-anatomical characters of the *P. corallopsis* topotype. Based on our morphological comparisons and molecular results, there is no evidence to consider *P. corallopsis* and *P. furcata* as different entities. Thus, *P. furcata* should be synonymized with *P. corallopsis* on the basis of the principle of priority (ICN, Principle III, Turland et al. 2018).

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Conflict of interest statement: The authors declare that they have no conflicts of interest regarding this article.

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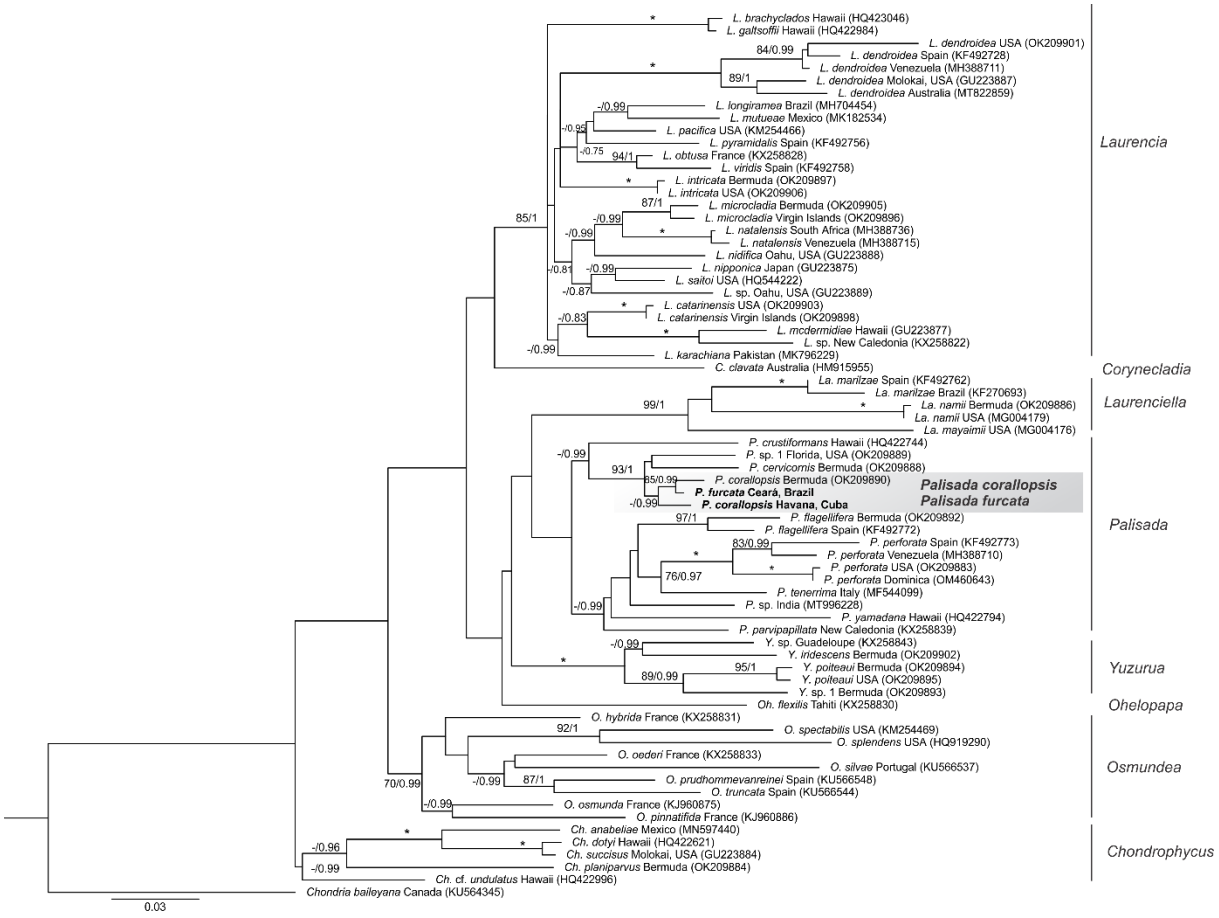
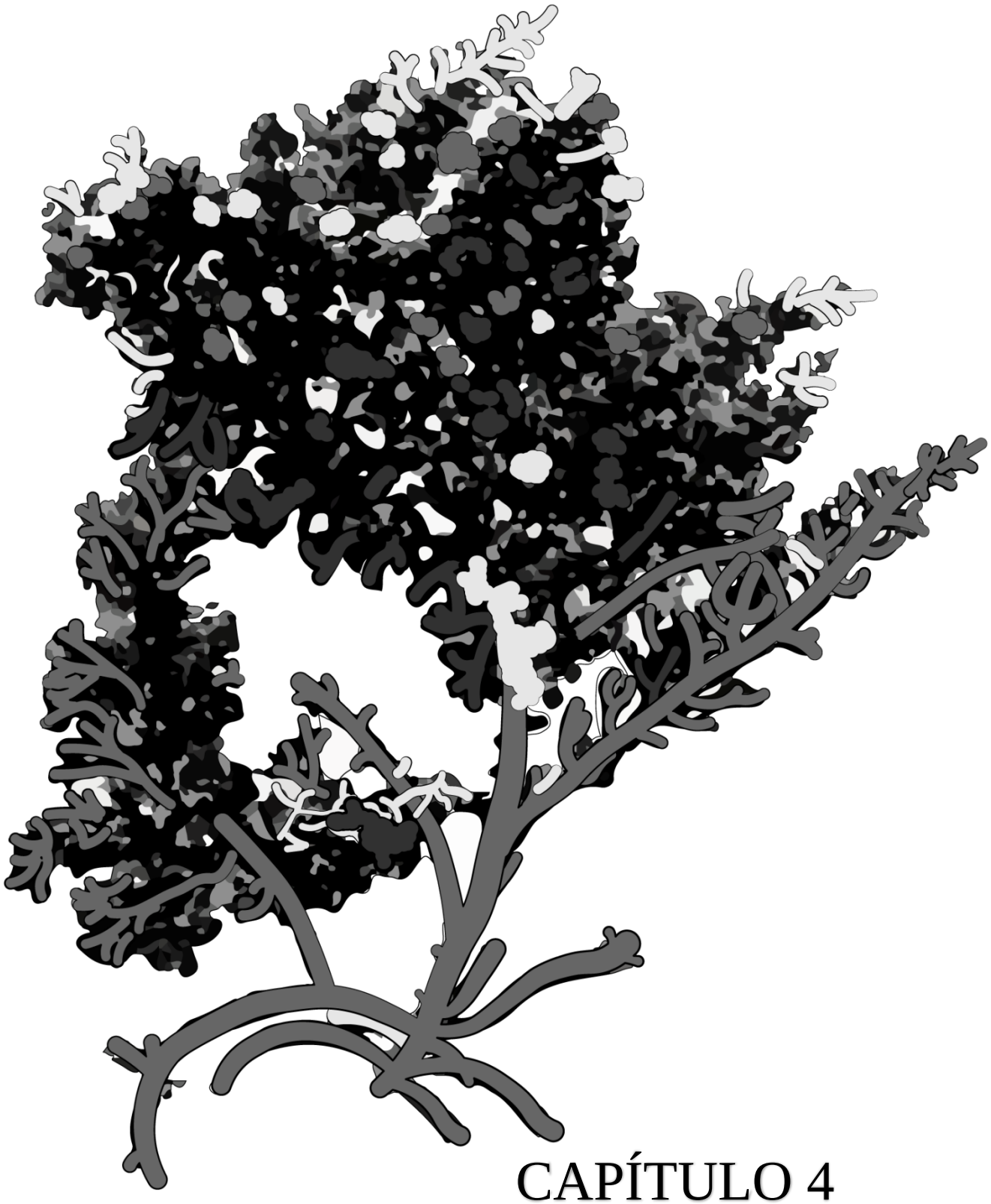


Figure S1: Maximum likelihood tree of COI-5P of the *Laurencia* complex. Value above branches indicate maximum likelihood Bootstrap (BS) values ≥70% and Bayesian posterior probabilities (BPP) ≥0.95. (-) indicates lack of bootstrap support or values under 70; (*) indicates BS 100% or BPP 1.00. Sequence generated in this study in bold.



CAPÍTULO 4

SIMILARITY AND AREAS OF ENDEMISM OF THE *LAURENCIA* COMPLEX (CERAMIALES, RHODOMELACEAE) IN CUBA

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ARTICLE

Similarity and areas of endemism of the *Laurencia* complex (Ceramiales, Rhodomelaceae) in Cuba

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Resumen

Similitud y áreas de endemismo del complejo Laurencia *(Ceramiales, Rhodomelaceae) en Cuba*

Las especies del complejo *Laurencia* son elementos importantes en la estructura y diversidad de los ecosistemas costeros. Por primera vez, se analiza la distribución geográfica del complejo dentro de la plataforma cubana, sus áreas marinas protegidas y se identifican áreas potenciales endémicas, considerando las ecozonas en que se dividió la plataforma. A la fecha, se han inventariado 19 especies, de estas 10 pertenecen a *Laurencia*, una a *Osmundea*, cinco a *Palisada*, dos a *Yuzurua*, y se incorporó *Laurenciella* como nuevo género para Cuba. La distribución fue desigual, con un alto número de especies y puntos de ocurrencia, especialmente en las regiones Occidental y Central de la costa norte. El análisis de parsimonia de endemicidad indicó dos ecozonas, una en Habana-Matanzas y otra en la Costa Nordeste, sustentadas por tres y dos especies endémicas, respectivamente.

Palabras clave: Biogeografía marina; Conservación; Análisis de parsimonia de endemicidad; Algas rojas

Abstract

Species of the *Laurencia* complex are important elements in the structure and diversity of coastal ecosystems. For the first time, we analyzed the geographical distribution of the complex within the Cuban shelf, its marine protected areas and identified potential endemic areas, considering the ecozones into which the shelf was divided. To date, 19 species have been inventoried, of which 10 belong to *Laurencia*, one to *Osmundea*, five to *Palisada*, two to *Yuzurua*, and *Laurenciella* a new record of genus for Cuba. The distribution was uneven, with a high number of species and occurrence points, especially in the Western and Central regions of the northern coast. The parsimony analysis of endemicity indicated two ecozones, one in Habana-Matanzas and the other in the Northeast Coast, supported by three and two endemic species, respectively.

Key words: Marine biogeography; Conservation; Parsimony analysis of endemicity; Red seaweeds.

Introduction

Cuba is the largest archipelago in the Caribbean Sea. It includes the main island of Cuba, Isla de la Juventud, and about 4000 islands and keys, which host the highest marine biodiversity in the region (Miloslavich *et al.* 2010, Roman 2018). The Cuban archipelago is surrounded by the deep basins and trenches of the Caribbean Sea, the Gulf of Mexico, the Straits of Florida, and the Bahamas. Extensive reefs border the shelf for most of its length before steeply descending to a depth of 400 m or more (Claro 2006, García-Machado *et al.* 2018). This configuration promotes divergent ecosystems that support many species throughout their different life stages. Among the main biotopes present on the Cuban shelf are non-reef rocky bottoms, coral reefs, seagrass meadows, estuaries, beaches, mangroves, and rocky intertidal zones (García-Machado *et al.* 2018).

The patterns of species composition and richness of the algal assemblages in the Cuban archipelago result mainly from the coastal proximity of the islands and archipelagos that make up the Caribbean, the southern Gulf of Mexico, and Florida (Suárez & Martínez-Daranas 2020), combined with large and mesoscale oceanographic patterns (Claro 2006, Suárez *et al.* 2015).

The marine algal flora of Cuba has been extensively studied over the past centuries, receiving more attention in the last decades in the areas of systematics and ecology (Montagne 1842, Farlow 1871, Howe 1917, Suárez *et al.* 2015). However, phytogeographic studies are still scarce (Suárez 1984, 1989a, Suárez *et al.* 2008, Suárez & Martínez-Daranas 2020), and are lacking particularly for the algae of the *Laurencia* complex.

Within the order Ceramiales (Rhodophyta), the *Laurencia* complex is the best represented, with 396 species of red macroalgae. However, currently, only 213 are accepted taxonomically (Guiry & Guiry 2022), which are distributed in the genera *Laurencia* J.V. Lamouroux *sensu stricto* (*s.s.*), *Osmundea* Stackhouse, *Chondrophycus* (Tokida & Y. Saito) Garbary & J.T. Harper, *Palisada* K.W. Nam, *Yuzurua* (K.W. Nam) Martin-Lescanne, *Laurenciella* Cassano, Gil-Rodríguez, Sentíes, Díaz-Larrea, M.C. Oliveira and M.T. Fujii, *Corynecladia* J. Agardh, and *Ohelopapa* F. Rousseau, Martin-Lescanne, Payri, and L. Le Gall.

For the Caribbean and the Gulf of Mexico, 41 species and two varieties are currently recognized, distributed in seven genera within the complex. The genus *Corynecladia* has not yet been recorded for this region. To date, *Laurencia*, *Osmundea*, *Palisada* and *Yuzurua* have been reported in Cuba based on morphological and molecular data (Cassano *et al.* 2012, Sentíes *et al.* 2015, Suárez *et al.* 2015, Fujii *et al.* 2016). Recently, González-Sánchez *et al.* (unpublished) also found the genus *Laurenciella* in Cuba. A list of records of the *Laurencia* complex for the Cuban archipelago is presented in Supplementary Material 1, Table S1).

Cuba shows the highest richness within the large islands of the Caribbean region, with 19 species and two varieties. All members of the *Laurencia* complex found along the Cuban coasts have also been reported in the Atlantic coasts of Central America, Florida, Bermuda and the Gulf of Mexico, except for *Palisada furcata*, which was recorded only in Brazil. However, these values are still low since they represent less than 50% of the taxa reported for this region.

At present, knowing the species richness and distribution patterns in an area is a priority for science, especially when the ecosystem functions decline due to increased anthropogenic threats and climate change (Hall *et al.* 2013). Estimating species richness and distribution changes would provide critical ecological perspectives for conservation and understanding of how they react to environmental perturbations (Primack *et al.* 2018). Furthermore, estimating the areas of endemism for a particular taxon using tools such as the parsimony analysis of endemism (PAE; Morrone 2014), is useful to identify priority areas for conservation (Posadas & Miranda-Esquivel 1999).

Suárez *et al.* (2015) mentioned the areas of the Cuban shelf where species of the *Laurencia* complex have been recorded. However, the richness of these algae and their distribution patterns in shelf areas remain to be characterized. This work aimed to describe the distribution patterns of the species composition of the *Laurencia* complex in Cuba.

Materials and methods

Sampling sites

Samples were collected between 2017 and 2019 in the intertidal or subtidal zone (0-2 m) at 21 sites of the Cuban archipelago, representing the nine

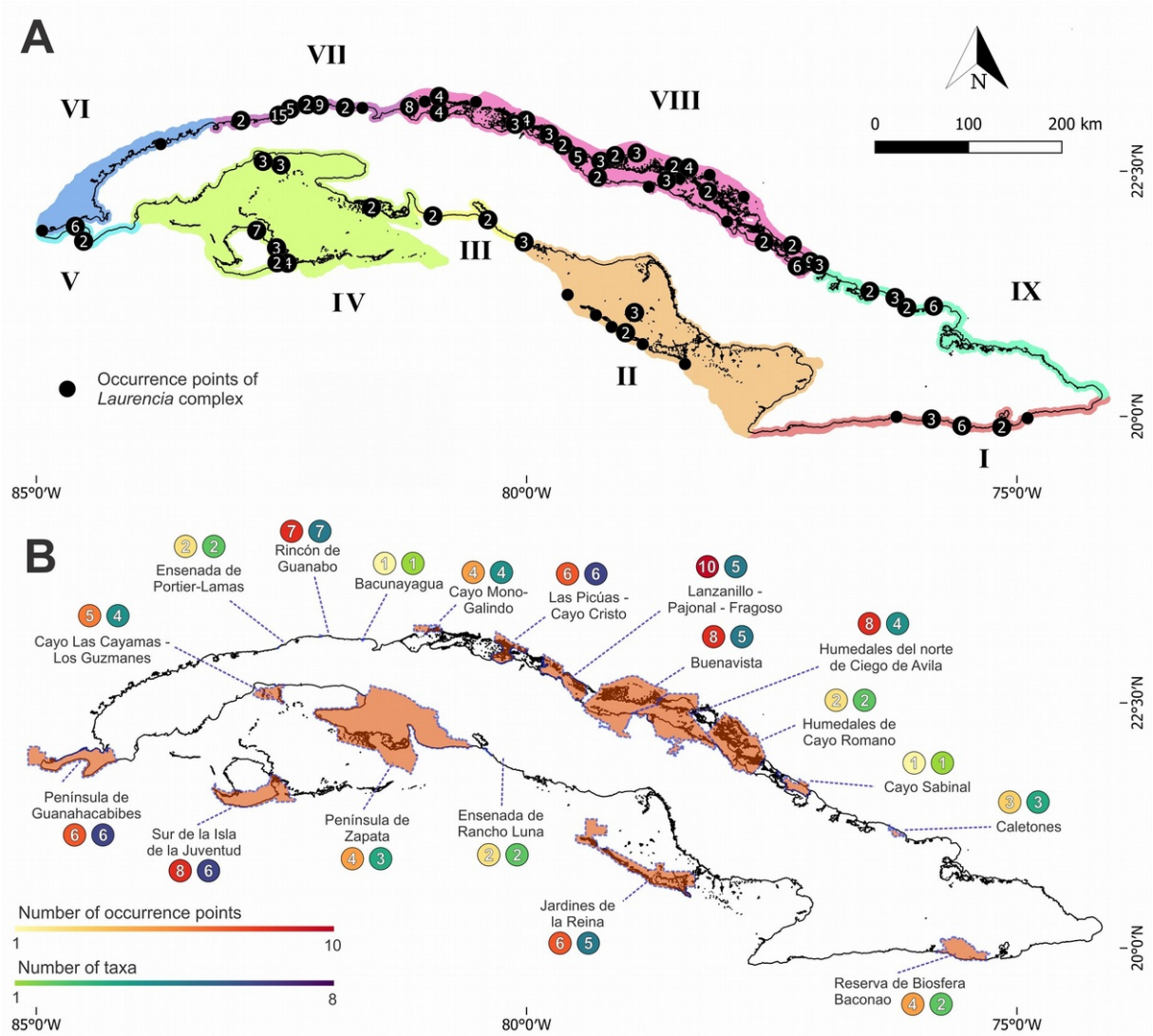

Figura 1. Distribución del complejo *Laurencia* dentro del archipiélago cubano. **A:** Puntos de ocurrencia recogidos por las zonas ecológicas propuestas por Areces (2002). Cuando se registró más de un punto de ocurrencia en la misma localidad, el número total de puntos se muestra en puntos negros. **B:** Áreas marinas protegidas (AMP: polígonos rojos) con registros del complejo *Laurencia*. Los números correspondientes de puntos de ocurrencia (izquierda) y taxones (derecha) registrados dentro de cada área se muestran junto a sus nombres.

Figure 1. Distribution of the *Laurencia* complex within the Cuban archipelago. **A:** Occurrence points gathered by the ecological zones proposed by Areces (2002). When more than a single occurrence point was recorded in the same locality, the total number of points is shown in black dots. **B:** Marine protected areas (MPAs: red polygons) with records of the *Laurencia* complex. The corresponding numbers of occurrence points (left) and taxa (right) recorded within each area are shown next to their names.

ecological zones (ecozones hereafter) established during a 2001 workshop (Areces 2002, Fig. 1A).

Morphological observations and data collection

For molecular analysis, small thallus fragments from each sample were dried on silica gel. The remaining material was preserved in 4% formalin-seawater or as herbarium vouchers for morphological studies. Transverse and longitudinal hand sections were obtained with a razor blade, stained with 0.5% aqueous aniline blue, and acidified with 1N HCl (Tsuda & Abbott 1985). Habitat images and photomicrographs were taken using a Panasonic Lumix DMC-FH4 camera (Panasonic Corporation, Osaka, Japan) coupled to a Zeiss Stemi 2000-C stereomicroscope and a Zeiss Primo Star microscope (Carl Zeiss Microscopy, Göttingen, Germany).

Voucher specimens were deposited in the herbarium of the Botanical Institute, São Paulo (SP), and in the National Aquarium of Cuba collections. Additionally, we examined specimens of the *Laurencia* complex deposited in the following herbaria: a closed collection of "Instituto de Oceanología" (IDO); the herbarium of "Acuario

Nacional de Cuba" (HANC); Universidad Autónoma Metropolitana-Iztapalapa (Metropolitan Herbarium-UAMIZ); the herbarium of "Instituto de Botanica de São Paulo" (SP); the University of Michigan Herbarium (MICH); the US National Herbarium, Department of Botany, Smithsonian Institution (NMNH); the New York Botanical Garden Herbarium (NY); Bernice Pauahi Bishop Museum (BPBM; current code: BISH); and the Museum National d'Histoire Naturelle (MNHN). Herbarium abbreviations follow the online Index Herbariorum (http://sciweb.nybg.org/science2/IndexHerbariorum.asp) (Thiers 2021, continuously updated).

Infrageneric taxa of the *Laurencia* complex for Cuba from the online database AlgaeBase (Guiry & Guiry 2022) were used as complementary data. Distribution maps were prepared using QuantumGIS 3.10 software, considering the nine ecozones (Fig. 1A).

Ecozone delimitation within the Cuban insular shelf was made from an *a priori* definition according to the observation scale. However, some biogeographic aspects were considered, such as the habitat geographic "place" or "location" in the coastal zoning of the Cuban insular shelf and its nature. The habitat nature was derived from the substrate type and degree of bottom coverage (Areces 2002, Areces & Salinas-Chávez 2020).

Characteristics of the nine ecozones (Fig. 1A)

I-Southeast: this coastline is high and steep, with a predominance of rocky substrates and a very narrow shelf. It has no keys or low areas, and the beaches are made of pebbles.

II-South Central (Jardines de la Reina): includes Guacanayabo and Ana María gulfs, and the Jardines de la Reina archipelago. Mangroves, coastal lagoons, muddy and sandy-muddy bottoms predominate in the gulfs, where the coral patches are commonly known as "heads". Keys and reef lagoons have well-developed seagrass meadows. The archipelago is to the South, bordering the gulfs, and has a great development of coral reefs.

III-South of the Guamuhaya Massif: is one of the smallest areas (Fig. 1A). It has a narrow shelf, with a rocky coast and estuarine characteristics in Cienfuegos Bay, such as muddy and sandy-muddy bottoms and mangroves. These estuarine characteristics are the result of the contribution of the rivers and the low tidal energy; it receives the drainage of urban and industrial waste from the city.

IV-Batabanó-Canarreos: it includes the Gulf of Batabanó and the Canarreos Archipelago. This area is dominated by seagrasses, with some rocky areas to the southwest, and coral reefs bordering the outer shelf edge.

V-Southwest of Guanahacabibes: it is an abrasive karst coastline, with some sandy beaches and a narrow shelf in its entirety, 200 to 300 m wide. Rocky substrates, coral reefs, and sandy bottoms predominate in this area.

VI-Northwest (Los Colorados): this archipelago includes keys and banks with well-developed coral reefs. The interior has muddy and sandy-muddy bottoms, with a great development of seagrasses and coastal mangroves, and some beaches.

VII-Habana-Matanzas: it is an area without keys. It has a narrow shelf, with sections of cliffs, important bays with mangrove, coastal reefs dominated by rocky ledges, rocky intertidal, and sandy beaches with seagrasses in some reef lagoons. Most research has been directed to studying coral reefs, the most abundant ecosystems in this area. The largest territory is part of the highly anthropized coastal area of Havana, the capital of the country.

VIII-North Central (Sabana-Camagüey): it is an extensive area with a wide shelf, keys, and islets; between these and the main island´s coast are extensive water bodies with soft bottoms and seagrasses. North of the keys, the area is bordered by coral reefs with reef lagoons.

IX-Northeast: as in the Southeast, the shelf is narrow but has several important bays and all kinds of substrates and biotopes, including coral reefs and rocky intertidal zones.

Currently, Cuba has 211 conservation areas for protection, including 105 Marine Protected Areas (MPAs) (CNAP 2013, Perera Valderrama *et al.* 2018). Fifty-seven of the proposed areas have been approved, and seven are currently in approval. Approved MPAs protect more than 2.5 million hectares of marine and coastal territories in the 15 provinces of Cuba and the special municipality of Isla de la Juventud. Individual MPAs and their locations are available at http://www.snap.cu/index.php/ct-menu-item-15.

Marine protected areas are considered of inter-

national, national, or regional importance. They constitute the core of the National System of Protected Areas (SNAP) due to their conservation value, representativeness, degree of conservation, uniqueness, and size (Perera Valderrama *et al.* 2018). Cuban MPAs are also classified according to national (n=46) and local (n=59) significance. These protected areas occupy a large part of the marine-coastal territory of the archipelago. For this reason, we consider it important to include the distribution of the species of the *Laurencia* complex recorded within the Cuban MPAs in our analysis (Fig. 1B, Table 1).

Analysis of data

The species composition of the *Laurencia* complex was compared between ecozones using Sørensen´s similarity index (Sørensen 1948). This index relates the number of species in common with the arithmetic mean of the species in the sites where they coincide (Magurran 1988). A clustering analysis (CLUSTER) was performed with this matrix, using the linkage method by group average (UPGMA) and 10,000 replacements. According to the highest percentage of replacement supported by each node within the cluster, a cut off line was established in the dendrogram (Hammer *et al.* 2001).

ANOSIM analyses were also employed to test for differences between similarity groups, following the criterion of the cut off line and using 10 000 permutations and Sørensen´s similarity index (Clarke & Warwick 2001). Similarity percentage (SIMPER) analysis was used to calculate the contribution of the species that most influenced the dissimilarity between groups (Clarke 1993).

Additionally, a distance-based test for homogeneity of multivariate dispersion (PERMDISP, Anderson 2006) and a non-metric multidimensional scaling (nMDS) analysis (Clarke & Warwick 2001) were performed to interpret and visualize data patterns. These analyses were based on 10 000 permutations and Sørensen´s similarity index (Clarke & Gorley 2006). All statistical analyses were performed with Past 4.07b (Hammer *et al.* 2001).

To identify endemism hotspots for the *Laurencia* species complex, we ran a parsimony analysis of endemicity (PAE) (Morrone 1994, 2014) using Nona 2.0 (Goloboff 1999) and Winclada v. 1.00.08 as a graphic interface (Nixon 2000). PAE uses a grid cell species matrix to identify endemism hotspots for a group. We adopted a 1.5° grid size for the analysis, with all cells encoded with an alphanumeric pattern. A presence/absence matrix was constructed, including a hypothetical taxon absent in all cells to root the resulting tree (Morrone 1994, 2014, Morrone & Crisci 1995, Posadas & Miranda-Esquivel 1999). For this analysis, 100 replicates were performed using TBR ("tree bisection and reconnection") as the adopted heuristic search algorithm and retaining a maximum of 1000 trees in each replicate. Most parsimonious trees were used to produce a strict consensus tree. On this tree, endemic areas were identified when at least two exclusive species supported them (Morrone 1994).

Results

Nineteen species of the *Laurencia* complex were recorded for the Cuban archipelago (Supplementary material 1). Of them, 10 belonged to *Laurencia*, one to *Laurenciella*, one to *Osmundea*, five to *Palisada*, and two to *Yuzurua* (one of them with two varieties). Only three species were found in at least 78% of the ecozones (six and seven ecozones), while five were recorded in just one.

Ecozones VII and VIII showed the highest number of taxa, with 12 species each (Fig. 1A). *Laurencia* was the richest genus, in all cases, while *Laurenciella* and *Osmundea* were the poorest (Supplementary material 2, Figs. S1-S4). The studied areas showed a similarity between 14.3 and 73%.

The similarity analysis based on the presence and absence of species of the *Laurencia* complex in the ecozones showed two large groups, A and B (Fig. 2). Both groups were established at 30% similarity due to the 100% replacement supported for the first node, inferring greater robustness and reliability in the dendrogram separation. The cophenetic correlation (82%) indicated a high correlation between the distances of the simulated matrix and the real matrix.

Group B included ecozones II and VI with 36% similarity, while the other ecozones were found in group A (Fig. 2). Within this last group, ecozones IV and VIII showed the greatest similarity between them (80%) and with ecozone VII (73%). Ecozones III and V also showed greater than 50% similarity. Cluster analysis, revealed

Name	Province	Ecozones	Significance	Management category	Occurrence points	(Number of) Taxa
Península de Guanahacabibes	Pinar del Río	V, VI	National	Protected Area with Managed Resources	6	(6) *Laurencia caraibica, L. intricata, L. microcladia, Palisada cervicornis, P. perforata, Yuzurua iridescens*
Cayos Las Cayamas - Los Guzmanes	Artemisa	IV	Local	Faunal Refuge	5	(4) *Laurencia intricata, L. microcladia, Palisada corallopsis, Yuzurua poiteaui* var. *poiteaui*
Ensenada de Portier-Lamas	La Habana	VII	Local	Protected Natural Landscape or Seascape	2	(2) *Laurencia dendroidea, Palisada perforata*
Rincón de Guanabo	La Habana	VII	Local	Protected Natural Landscape or Seascape	7	(7) *Laurencia caduciramulosa, L. dendroidea, L. minuscula, Palisada corallopsis, P. perforata, Yuzurua iridescens, Y. poiteaui* var. *gemmifera*
Bacunayagua	Matanzas - Mayabeque	VII	Local	Ecological Reserve	1	(1) *Laurencia caraibica*
Cayo Mono-Galindo	Matanzas	VIII	Local	Ecological Reserve	4	(4) *Laurencia intricata, L. obtusa, Yuzurua poiteaui* var. *poiteaui, Y. poiteaui* var. *gemmifera*
Península de Zapata	Matanzas	III, IV	National	Protected Area with Managed Resources	4	(3) *Laurencia dendroidea, Palisada perforata, Yuzurua poiteaui* var. *gemmifera*
Ensenada de Rancho Luna	Cienfuegos	III	Local	Natural Outstanding Landscape	2	(2) *Laurencia intricata, Palisada perforata*
Las Picúas-Cayo Cristo	Villa Clara	VIII	National	Faunal Refuge	6	(6) *Laurencia intricata, L. microcladia, L. obtusa, Palisada perforata, Yuzurua poiteaui* var. *poiteaui, Y. poiteaui* var. *gemmifera*
Lanzanillo-Pajonal-Fragoso	Villa Clara	VIII	National	Faunal Refuge	10	(5) *Laurencia intricata, L. obtusa, P. perforata, Yuzurua poiteaui* var. *poiteaui, Y. poiteaui* var. *gemmifera*
Buenavista	Sancti Spíritus - Villa Clara	VIII	National	Protected Area with Managed Resources	8	(5) *Laurencia intricata, L. obtusa, Palisada corallopsis, P. perforata, Yuzurua poiteaui* var. *poiteaui*
Humedales del Norte de Ciego de Ávila	Ciego de Ávila	VIII	National	Protected Area with Managed Resources	8	(4) *Laurencia dendroidea, L. intricata, Palisada perforata, Yuzurua poiteaui* var. *poiteaui*
Jardines de la Reina	Camagüey - Ciego de Ávila	II	National	National Park	6	(5) *Laurencia filiformis, L. intricata, L. obtusa, Laurenciella* sp., *Yuzurua iridescens*
Humedales de Cayo Romano	Camagüey	VIII	National	Protected Area with Managed Resources	2	(2) *Laurencia intricata, Yuzurua poiteaui* var. *poiteaui*
Cayo Sabinal	Camagüey	VIII	Local	Protected Area with Managed Resources	1	(1) *Palisada perforata*
Caletones	Holguín	IX	National	Ecological Reserve	3	(3) *Laurencia dendroidea, Palisada flagellifera, P. perforata*
Reserva de Biosfera Baconao	Santiago de Cuba - Guantánamo	I	National	Protected Area with Managed Resources	4	(2) *Osmundea coelenterata, Palisada perforata*
Sur de la Isla de la Juventud	Isla de la Juventud	IV	National	Protected Area with Managed Resources	8	(6) *Laurencia chondrioides, L. dendroidea, L. intricata, L. microcladia, Yuzurua poiteaui* var. *poiteaui, Y. poiteaui* var. *gemmifera*

Tabla 1. Áreas marinas protegidas dentro de la plataforma cubana con ocurrencia de taxones del complejo *Laurencia*. Ecozonas: I: Sureste, II: Centro Sur (Jardines de la Reina), III: Sur del Macizo Guamuhaya, IV: Batabanó-Canarreos, V: Suroeste de Guanahacabibes, VI: Noroeste (Los Colorados), VII: Habana - Matanzas, VIII: Centro Norte (Sabana-Camagüey), y IX: Noreste.

Table 1. Marine protected areas within the Cuban shelf with the occurrence of taxa of the *Laurencia* complex. Ecozones: I: Southeast, II: South Central (Jardines de la Reina), III: South of the Guamuhaya Massif, IV: Batabanó-Canarreos, V: Southwest of Guanahacabibes, VI: Northwest (Los Colorados), VII: Habana - Matanzas, VIII: North Central (Sabana-Camagüey), and IX: Northeast.

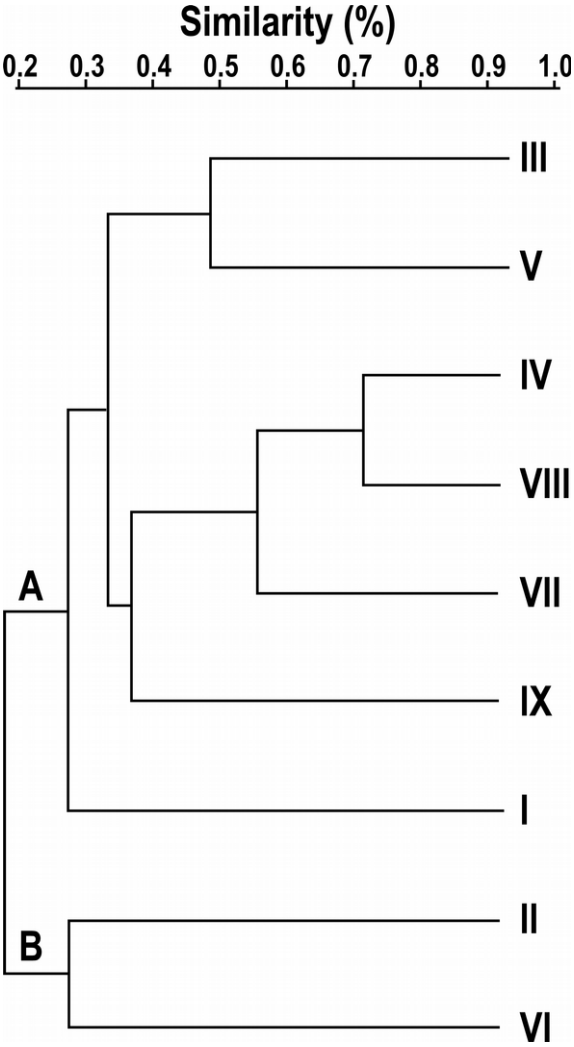

Figura 2. Dendrograma de similitud resultante del análisis de conglomerado de las ecozonas en las que se dividió la plataforma cubana, con registros de especies del complejo *Laurencia*. I: Sureste; II: Centro Sur (Jardines de la Reina); III: Sur del Macizo Guamuhaya; IV: Batabanó-Canarreos; V: Suroeste de Guanahacabibes; VI: Noroeste (Los Colorados); VII: Habana - Matanzas; VIII: Centro Norte (Sabana-Camagüey); IX: Noreste.

Figure 2. Similarity dendrogram resulting from the conglomerate analysis of the ecozones into which the Cuban shelf was divided, with records of species of the *Laurencia* complex. I: Southeast; II: South Central (Jardines de la Reina); III: South of the Guamuhaya Massif; IV: Batabanó-Canarreos; V: Southwest of Guanahacabibes; VI: Northwest (Los Colorados); VII: Habana - Matanzas; VIII: North Central (Sabana-Camagüey); IX: Northeast.

that ecozones I and IX had the least similarity with the rest.

ANOSIM analysis did not reveal any significant difference between the two groups established in the similarity analysis (R=0.03; p=0.20), suggesting that the species composition was not an excluding factor between both groups.

SIMPER results indicated that the maximum average dissimilarity between the groups was 62.18%. CLUSTER analysis showed that *Laurencia dendroidea* J. Agardh and *Laurencia microcladia* Kützing (both with 7.21%), *Yuzurua poiteaui* var. *poiteaui* (J.V. Lamouroux) Martin-Lescanne (7.13%), and *Laurencia caraibica* P.C. Silva (7.07%) were the species that most contributed to the dissimilarity between Groups A and B.

PERMDISP test for the structure and species composition of the *Laurencia* complex showed significant differences ($F=7.44$; $p<0.01$) in the dispersion between ecozones. In this sense, pairwise comparisons indicated significant differences between ecozones IV and VIII with I, III, and V ($p<0.0001$) and II, VI, and VII ($p<0.01$). In addition, ecozones VII and IX differed significantly from the rest of the ecozones ($p<0.01$). Non-metric multidimensional scaling confirmed the separation of ecozones II and VI from the other ecozones (Fig. 3). The ecological distance between ecozones IV and VIII was smaller than the other ecozones.

Of the 196 occurrence points for the *Laurencia* complex on the Cuban shelf, 87 were found within MPAs, mostly (73%) in those of national significance (Table 1, Fig. 1B). Lanzanillo-Pajonal-Fragoso refuge, in ecozone VIII and the area of resources, managed south of Isla de la Juventud, in ecozone IV, had the highest number of occurrence points and taxa (10, 5 and 8, 6, respectively). In contrast, smaller protected areas (Bacunayagua and Cayo Sabinal) usually had a few occurrence points and taxa. However, Rincón de Guanabo in ecozone VII was an exception. Despite its small area, it accounted for seven occurrence points from seven taxa (Fig. 1B). This ecozone also showed the single records of *Laurencia caduciramulosa* Masuda & S. Kawaguchi and *Laurencia minuscula* Schnetter within protected areas. Furthermore, *L. dendroidea*, *Laurencia intricata* J.V. Lamouroux, *Palisada perforata* (Bory) K.W. Nam, *Yuzurua poiteaui* var. *poiteaui*, and *Yuzurua poiteaui* var. *gemmifera* (Harvey) M.J. Wynne were recorded in at least five protected areas. In contrast, *Laurencia brongniartii* J. Agardh and *Palisada furcata* (Cordeiro-Marino & M.T. Fujii) Cassano & M.T. Fujii showed no records within protected areas.

The PAE tree, resulting from 31 equally parsimonious trees (CI=0.50 and RI=0.66), identified a large area of endemism that encompasses two smaller areas: A2 and B6 (Fig. 4). This large area of endemism mainly covered the Western and Central regions of the Cuban archipelago on both

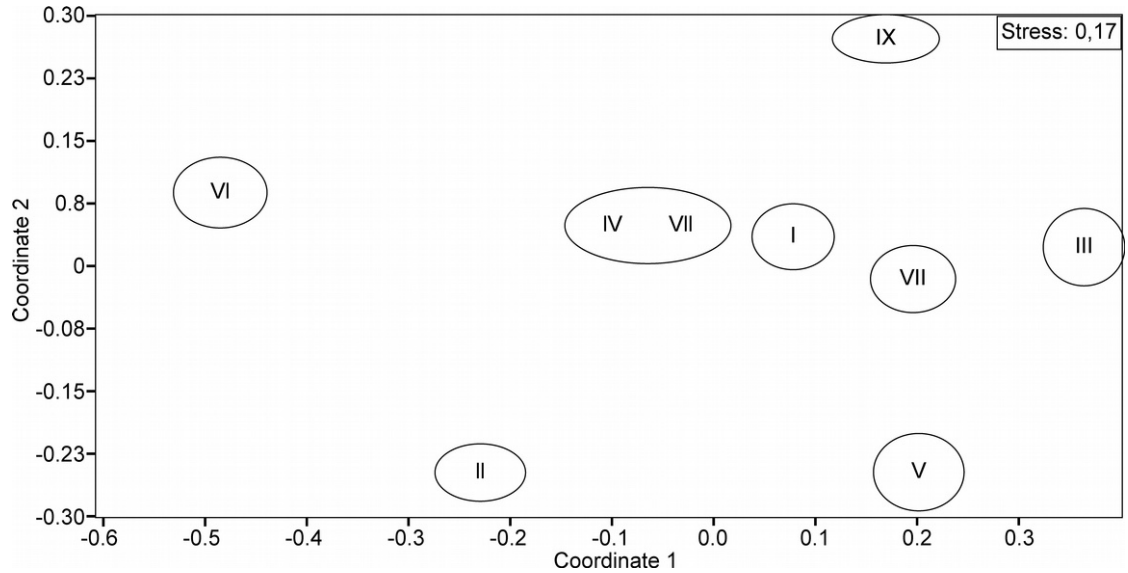

Figura 3. Escalado multidimensional no métrico (nMDS) de las ecozonas en las que se dividió la plataforma cubana y las especies del complejo *Laurencia*. I: Sureste; II: Centro Sur (Jardines de la Reina); III: Sur del Macizo Guamuhaya; IV: Batabanó-Canarreos; V: Suroeste de Guanahacabibes; VI: Noroeste (Los Colorados); VII: Habana-Matanzas; VIII: Centro Norte (Sabana-Camagüey); IX: Noreste.

Figure 3. nMDS of the ecozones in which the Cuban shelf was divided and the species of the *Laurencia* complex. I: Southeast; II: South Central (Jardines de la Reina); III: South of the Guamuhaya Massif; IV: Batabanó-Canarreos; V: Southwest of Guanahacabibes; VI: Northwest (Los Colorados); VII: Habana-Matanzas; VIII: North Central (Sabana-Camagüey); IX: Northeast.

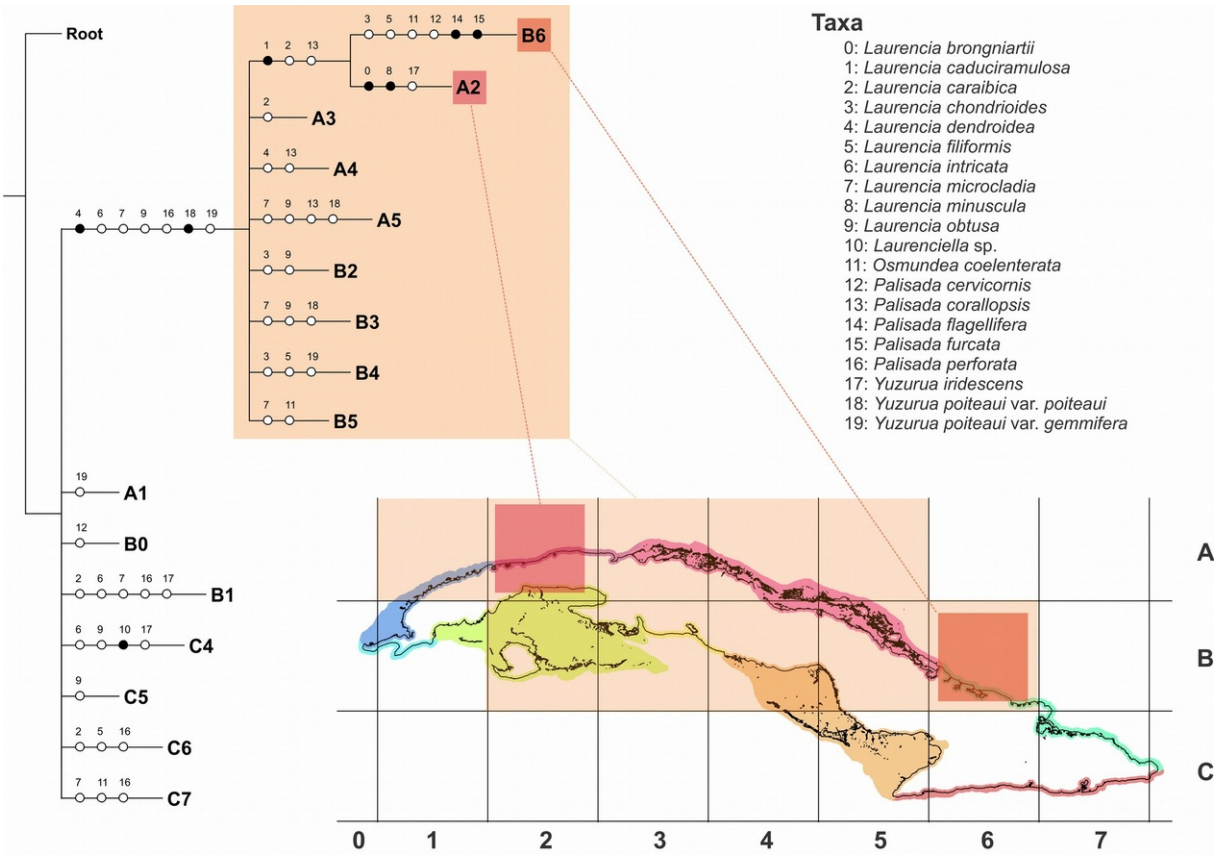

Figura 4. Árbol de consenso estricto resultante de 31 árboles igualmente parsimoniosos (IC=0,50; I0,66) en el análisis PAE. Los cuadrantes apoyados por al menos dos especies endémicas están resaltados en negro. Estas áreas están indicadas en el mapa, mostrando las zonas ecológicas reconocidas por Areces (2002). Los números sobre los círculos a lo largo de las ramas corresponden a las especies codificadas en la lista en la esquina superior derecha.

Figure 4. Strict consensus tree resulting from 31 equally parsimonious trees (CI=0.50; RI=0.66) in the PAE analysis. Quadrants supported by at least two endemic species are highlighted in black. These areas are indicated on the map, showing the ecological zones recognized by Areces (2002). Numbers above circles along the branches correspond to the species encoded in the list in the upper right corner.

shelf coasts and was supported by *L. dendroidea*, *Y. poiteaui* var. *poiteaui*, and *L. caduciramulosa*. Area A2 corresponded mainly to ecozone VII and was supported by *L. brongniartii*, *L. caduciramulosa*, and *L. minuscula*. Rincón de Guanabo was the protected area where almost all the species supporting A2 were found, except *L. brongniartii*. On the other hand, area B6, corresponded largely to ecozone IX and was supported by *Palisada*: *Palisada flagellifera* (J. Agardh) K.W. Nam, and *P. furcata*. The Eastern zones (I and IX) had the lowest number of protected areas with records of the *Laurencia* complex. Most *Palisada* species supporting B6 as an area of endemism were not recorded in any of the protected areas of these ecozones, except for *P. flagellifera* in Caletones Ecological Reserve.

Discussion

CLUSTER results indicated that the species composition had a low similarity between ecozones, showing two main groups with 30% similarity. ANOSIM test did not find any significant difference between the groups, reflecting that the species composition was not an excluding factor between them.

Despite this, the distribution of taxa within the ecozones of the Cuban marine shelf was not homogeneous, as shown by PERMDISP results and figure 3. The differences in the species number between the ecozones could be due to the variety of environments found in large areas such as ecozones II, IV, and VIII.

Similarity indices are used to identify bioregions and patterns of beta biodiversity. They are based on the number of species shared between localities but do not consider spatial or environmental proximity. Insufficient sampling due to species with limited distributions, difficult to observe or identify, would lead to false absences (Barbosa 2015). Therefore, we selected the Sørensen or Dice index, which doubles the importance of shared attributes and is helpful for more effective comparisons between rich and poor collections under conditions of great heterogeneity in the qualitative data matrix (Herrera 2000).

Another problem is the correct species identification. Recent phylogenetic studies have been useful to detect misidentifications of species records in the Atlantic Ocean, especially Florida, Venezuela, Brazil, and the Mexican Caribbean (Cassano *et al.* 2012, 2020, Sentíes *et al.* 2015, 2016, Collado-Vides *et al.* 2018). The molecular phylogenetic analysis of these species would allow us to identify new lineages in a wide distribution range, as happened with other red algal groups (Núñez-Reséndiz *et al.* 2015, Hernández *et al.* 2017, Popolizio *et al.* 2022), and better understand the historical biogeography of the *Laurencia* complex in the Atlantic Ocean.

Despite the above, ecozones IV and VIII and these with VII showed similarity greater than 70%. In addition to a spatial ecological approach between ecozones IV and VIII observed in the nMDS. Ecozones II and VI were separated with less than 40% similarity. These results partially coincide with those found by the PAE.

The greater diversity of representatives of the *Laurencia* complex in ecozones IV, VII, and VIII could be due to the variety of substrates, temperature, salinity, or other factors that regulate the development of marine flora, as well as geographical barriers or the establishment of artificial shores (Wiencke & Bischof 2012). Results of similarity in the species composition of the *Laurencia* complex between ecozones could be explained by the fact that Cuba is geographically located in a tropical region with formations of coral origin, where temperature, habitats, and substrates favor the development of many species of Ceramiales. In the Caribbean, members of the *Laurencia* complex are very abundant. They, can be found in soft and hard bottoms, and as epiphytes on other algae, seagrasses, and animals.

The north and south coasts of the Cuban shelf differ considerably (Suárez 1989b, Areces 2002, Areces & Salinas-Chávez 2020). The South coast has two large regions with a wide shelf (Batabanó-Canarreos Southwestern shelf - IV and the South Central Jardines de la Reina - II), shallow waters with reefs, mangrove keys, sand, and rocks. The northern shelf is much narrower and only at the north of Pinar del Río (Northwest Los Colorados shelf- ecozone VI) and Villa Clara-Camagüey (North Central Sabana-Camagüey shelf-VIII), it reaches about 10 miles. On the other hand, the south coast has a predominantly sandy-muddy substrate, while in the north, it is sandy and sandy-rocky of coral origin. For the entire shelf, the depth is seldom more than 25 m (Areces 2002, Areces & Salinas-Chávez 2020).

Cuba is considered a tropical island; however, its climate is not typically oceanic due to the con-

tinental influence of North America. Its temperature, light, and rainfall patterns vary depending on the time of year or the influence of climatological phenomena such as tropical storms and hurricanes, whose frequency and intensity have been modified in recent years due to climate change (Planos *et al.* 2012).

Ecozones IV, VII and VIII are large areas with diverse habitats, with IV and VIII being the widest. Ecozones IV and VIII are characterized by having all the substrates and biotopes recognized for Cuba (Jiménez & Alcolado 1990, Suárez *et al.* 2015, Cabrera *et al.* 2019). In them, the seagrasses predominate, but there are also keys and islets, coastal and of keys mangroves, and rocky coasts; coral reefs with lagoons usually bordered these ecozones. The ecozones have been one of the most studied within the marine shelf, and in the last two decades, there has been a great sampling intensity, mainly in ecozone VIII (Alcolado *et al.* 2007, Martínez-Daranas *et al.* 2007, 2021). For its part, ecozone VII has a narrow shelf without keys, with dominant intertidal and rocky subtidal biotopes. Coastal mangroves and sandy beaches with seagrasses in some reef lagoons, among other habitats, give the coastline a high algal wealth. It has also been extensively studied since the country's leading research centers are located in this ecozone, concentrating most phycologists (Suárez *et al.* 2015).

The variety of substrates is one of the factors that favored the high species richness of the *Laurencia* complex in the Cuban archipelago. Ninety-five percent of the *Laurencia* complex taxa found in Cuba have been collected from rocky or hard substrates. Taylor (1960) and Lüning (1990) pointed out that these substrates exposed to the waves host the greatest diversity of marine algae since they offer less competition with sessile invertebrates than coral reefs. Rock texture, degree of hardness, and color also influence algal communities (Areces *et al.* 2015).

Some red algae have effective adaptation mechanisms that allow them to occupy exposed habitats and avoid being uprooted by the influence of physical factors such as wave action. Some *Laurencia* species, such as *L. dendroidea* and *L. microcladia*, are fixed to the substrate through rhizoids and basal discs, which emerge from stoloniferous branches (Littler & Littler 2000, Gil-Rodríguez *et al.* 2012). Both species together with *P. perforata* were abundant and commonly observed on this type of substrate.

Meadows of *Thalassia testudinum* K.D. Koenig were common and abundant in ecozones IV and VIII. These meadows are generally found around river mouths, bays, estuaries, and coastal lagoons. Seagrasses play an essential role in stabilizing the sandy and silty substrate where algae can develop adequately, taking advantage of the spaces left free by seagrasses (Santelices 1977, Huerta 1978). Within the Cuban archipelago, these habitats are characterized by being shallow and in protected environments, with little water movement. *L. intricata* and the two varieties of *Yuzurua poiteaui* were recorded for this biotope.

In benthic marine communities, epiphytism leads to a greater number of strata and heterogeneity of habitats (Hicks 1980, Stewart 1982), potentially increasing the species diversity in these communities (Menge & Sutherland 1976, Krebs 1986, Jover *et al.* 2020). Epiphytism represents an option among colonization strategies. Epiphytic macroalgae might have advantages in the competition for light and nutrients (Wiencke & Bischof 2012), especially in the case of simpler morphofunctional types (Steneck & Dethier 1994). Mature leaves of *T. testudinum* constitute a substrate strongly colonized by algae, on which we recorded *L. caduciramulosa*, and Sentíes *et al.* (2010) found *L. minuscula*.

Suárez (1989b) carried out an ecological analysis of the macrophytobenthos of the Cuban shelf, and Suárez and Pérez (1989) analyzed the algae associated with *Rhizophora mangle* roots in the keys of Isla de la Juventud. These authors found that the order Ceramiales dominates in red mangrove roots, intertidal and rocky subtidal biotopes, coastal and of keys mangroves. Particularly referring to the epiphytism on phytobenthos in the Cuban shelf, Suárez (1989b) also found that the Ceramiales order dominates along with Bangiales. This author recorded *P. perforata* and *Laurencia obtusa* (Hudson) J.V. Lamouroux as epiphytes on the red alga *Digenea simplex* (Wulfen) C. Agardh and *L. caraibica* on *Sargassum filiforme* Montagne.

Specific richness, in general, is similar to the records made by Suárez *et al.* (2015) for Cuba and García-García *et al.* (2020) for red algae in the Mexican Atlantic. These results support the correlation that the greater the variety of habitats, the higher the richness of recorded species.

Ecozones II and VI with the lowest percentage

of similarity, constitute areas of greater extension and present a wide shelf with diverse habitats, which favor the growth of marine flora, mainly in ecozone II. Despite the low species richness and the limited abundance of *Laurencia* in these ecozones, it is possible that in these areas there are a greater number of species and new records for science in Cuba. This fact was verified with the new record of the genus *Laurenciella* within the protected marine area of Cayo Anclitas in Jardines de la Reina (ecozone II).

An area of endemism is defined as the sympatric congruence between two or more endemic species, based on the fact that these species share a common spatial history (Morrone 1994, 2007). Other areas that can be documented are the secondary areas, which have only one endemic species, or the so-called relic species that are important because these areas may be later isolations where species radiation has not yet occurred (Ippi & Flores 2001, Vargas *et al.* 2008). The PAE method allows us to identify areas of endemism from area cladograms. As in phylogenetic systematics, at least two synapomorphies (restricted species) are necessary to define an area of endemism (Morrone 2014, Hernández *et al.* 2017).

The species found by the PAE supporting areas of endemism (A2 and B6) are considered rare or infrequent for Cuba (Suárez *et al.*, 2015). Greater sampling efforts are required in other protected areas of the Northwest Coast (VII) since there is a discrepancy in the number of species/occurrence points recorded in Ensenada de Portier-Lamas (2/2) and Bacunayagua (1/1) compared to Rincón de Guanabo (7/7). Considering they all belong to the same area of endemism indicated by PAE (A2), future sampling efforts could reveal richer diversity of the *Laurencia* complex in the Cuban shelf.

Eastern zones had a low number of species/occurrence points and were represented mainly by the common species *P. perforata* (Table 1). Only one species, *P. flagellifera*, which supports B6 as an area of endemism, was recorded within a protected area Caletones. Therefore, for the area of endemism B6, both sampling and conservation efforts are necessary.

Despite being close to the capital city of Cuba, the MPA of Rincón de Guanabo has diverse ecosystems (rocky bottoms, sandy bottoms with seagrass patches, and coral reefs) where multiple communities live or seek refuge, giving it a high equity value. Based on our results, we propose to change the significance level of Rincón de Guanabo from Local to National, since it has a high diversity not only of species of the *Laurencia* complex but also of other important biological groups in the ecosystem (Alcolado 1989, Castellanos *et al.* 2004, Caballero *et al.* 2009, Hernández-Delgado *et al.* 2017, Gómez *et al.* 2019). In the case of Caletones Ecological Reserve, we propose that its status as MPA be analyzed and finally approved for its administration and management by the Integral Forestry Company (EFI) (see Table 1 in Perera Valderrama *et al.* 2018).

Suárez & Martínez-Daranas (2020) analyzed the marine phycoflora from tropical and subtropical western Atlantic areas. These authors found that the Cuban phycoflora has greater than 70% similarity with some of the ecoregions defined by Spalding *et al.* (2007) for the Tropical Northwestern Atlantic provinces, such as Florida, the southern Gulf of Mexico, the Western Caribbean, and the Southwestern Caribbean. This high similarity could be due to the location of Cuba in the center of the analyzed region, the increase in information on the macroalgae species in the different areas, changes in the regional phycoflora resulting from the dispersion of diasporas within the same phytogeographic province, the connectivity of oceanic waters or anthropic causes such as naval traffic and aquariums.

The Caribbean Sea is the Atlantic area with the highest species richness of *Laurencia* (22 spp, Guiry & Guiry 2022). Hernández *et al.* (2017) referred to some of these endemic species that inhabit this region, such as *Laurencia laurahuertana* Mateo-Cid, Mendoza-González, Sentíes & Díaz-Larrea in the western Caribbean, *Laurencia foldatsii* N. Rodríguez Ríos in the southern Caribbean, *Laurencia chondrioides* Børgesen, and *L. minuscula* in the Caribbean Sea. Recently, Popolizio *et al.* (2022) recorded the new species *Chondrophycus planiparvus* Popolizio, C.W. Schneider & C.E. Lane as endemic to Bermuda. This biogeographic pattern is also shared by the diversity distribution of several unrelated taxa, such as coastal fishes, mangroves, coral reefs, and seagrasses in the Atlantic Ocean (Tittensor *et al.* 2010).

The Northwestern Atlantic, Europe, and the Caribbean Sea share species of the *Laurencia* complex with other regions such as the Indo-Pacific. For example, *P. flagellifera*, whose type

locality is in the Indian Ocean, can be considered as a pantropical species with amphi-Atlantic distribution having been recorded from Cuba (Areces *et al.* 2003), Brazil (Fujii *et al.* 2006) and the Canary Islands (Gil-Rodríguez *et al.* 2010).

In addition, Brown & Lomolino (1998) found other areas of endemism in the south Atlantic, such as in Brazil, the Gulf of Guinea, and South Africa. The Gondwana breakup is the geological process that explains this endemism (Hernández *et al.* 2017).

Sentíes & Fujii (2002), Fujii & Sentíes (2005), Gil-Rodríguez *et al.* (2012), Machín-Sánchez *et al.* (2014, 2018) and Hernández *et al.* (2017) documented the spatial distribution patterns of the *Laurencia* complex in the Atlantic region. Although *Laurencia*, *Palisada*, *Osmundea*, and *Yuzurua* have a wide distribution in the different regions and corresponding oceans, other genera such as *Chondrophycus*, *Laurenciella*, *Ohelopapa*, and *Corynecladia* are restricted to a particular region (Guiry & Guiry 2022). The *Laurenciella* species have so far been recorded for the Atlantic Ocean and the Mediterranean Sea (Gil-Rodríguez *et al.* 2009, Cassano *et al.* 2012, Collado-Vides *et al.* 2018, Israel *et al.* 2020, Serio *et al.* 2020). On the other hand, *Chondrophycus*, *Corynecladia*, and *Ohelopapa* were found predominantly in the Indo-Pacific region, with few records in the Atlantic (Guiry & Guiry 2022). Of these genera, only *Ohelopapa flexilis* (Setchell) F. Rousseau, Martin-Lescanne, Payri & L. Le Gall, and *Chondrophycus anabeliae* Sentíes, M.T. Fujii, Cassano & Dreckmann have been reported for the Mexican Atlantic (Sentíes *et al.* 2016, Pedroche & Sentíes 2020). More recently, Cassano *et al.* (2020) found the latter species in Venezuela and Popolizio *et al.* (2022) recorded a new species of *Chondrophycus* (*C. planiparvus*) in Bermuda, which represents an expansion of the distribution of the genus toward northern Atlantic subtropical areas. *O. flexilis* was also quoted from Spain and the Canary Islands within the Atlantic (Guiry & Guiry 2022).

Conclusions

Our knowledge about the *Laurencia* complex in the Cuban archipelago is still insufficient, reflected in the differences in the species richness and distribution found within the shelf. The macroalgae of this complex differ between environments of the Cuban marine shelf. The characteristics of the substrate and associated benthos combined with the circulation patterns of ocean currents are probably some of the factors that determine the composition and distribution patterns of the *Laurencia* complex in Cuba. All these factors reinforce the need for a more exhaustive and comprehensive review of the *Laurencia* complex in the Cuban archipelago, carried out by a greater number of specialists, mainly in those less known areas such as the southern coast and the eastern region. Consequently, the incorporation of molecular characters to diversity studies considering a greater number of genera within the complex will increase the alpha diversity. MPAs are excellent refuges to preserve many species from extinction, including the macroalgae of the *Laurencia* complex. Given the above, the need for constant floristic monitoring along the coasts is imperative, particularly due to the large number of protected areas found in the Cuban archipelago.

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Appendix

This article includes the following electronic supplementary information:

Table S1: https://www.um.es/analesdebiologia/numeros/44/PDF/44_2022_06_SM1.pdf

Figures S1-S4: https://www.um.es/analesdebiologia/numeros/44/PDF/44_2022_06_SM2.pdf

Tabla S1. Especies de *Laurencia sensu stricto*, *Osmundea*, *Palisada* y *Yuzurua*, citadas para Cuba antes de 2022. Ecozonas donde se encontraron las especies. I: Sureste, II: Centro Sur (Jardines de la Reina), III: Sur del Macizo Guamuhaya, IV: Batabanó-Canarreos, V: Suroeste de Guanahacabibes, VI: Noroeste (Los Colorados), VII: Habana - Matanzas, VIII: Centro Norte (Sabana-Camagüey) y IX: Noreste. (*) representa las especies encontradas en los muestreos.
Códigos de herbario: colección cerrada del Instituto de Oceanología (IDO); el herbario del Acuario Nacional de Cuba (HANC); Universidad Autónoma Metropolitana-Iztapalapa (Herbario Metropolitano-UAMIZ); el herbario del Instituto de Botánica de São Paulo (SP); el herbario de la Universidad de Michigan (MICH); el Herbario Nacional de los Estados Unidos, Departamento de Botánica, Institución de la Smithsonian (NMNH); el Herbario del Jardín Botánico de Nueva York (NY); el Museo del Obispo Bernice Pauahi (BPBM; código actual: BISH); y el Museo Nacional de Historia Natural (MNHN). Las abreviaturas de herbario siguen el Index Herbariorum en línea: http://sciweb.nybg.org/ science2 / IndexHerbariorum.asp (Thiers 2021, actualizado continuamente).

Table S1. *Laurencia sensu stricto*, *Osmundea*, *Palisada* and *Yuzurua* species, cited for Cuba, prior to 2022. Ecozones where the species were found. I: Southeast, II: South Central (Jardines de la Reina), III: South of the Guamuhaya Massif, IV: Batabanó-Canarreos, V: Southwest of Guanahacabibes, VI: Northwest (Los Colorados), VII: Habana – Matanzas, VIII: North Central (Sabana-Camagüey), and IX: Northeast. (*) represents the species found in the samplings.
Herbarium codes: a closed collection of the Instituto de Oceanología (IDO); the herbarium of Acuario Nacional de Cuba (HANC); Universidad Autónoma Metropolitana-Iztapalapa (Metropolitan Herbarium-UAMIZ); the herbarium of Instituto de Botanica de São Paulo (SP); the University of Michigan Herbarium (MICH); the US National Herbarium, Department of Botany, Smithsonian Institution (NMNH); the New York Botanical Garden Herbarium (NY); the Bernice Pauahi Bishop Museum (BPBM; current code: BISH); and Museum National d'Histoire Naturelle (MNHN). Herbarium abbreviations follow the on-line Index Herbariorum: http://sciweb.nybg.org/ science2/IndexHerbariorum.asp (Thiers 2021, continuously updated).

Species	Type locality	Distribution in Cuba and ecozones	References	Examined material from Cuba	Examined material from other countries
Laurencia brongniartii J.Agardh	Martinique, West Indies	Jibacoa, Mayabeque (VII)	Areces 1999, Suárez *et al*. 2015	IDO158, collected 03 October 1991, *leg*. A. Areces, Jibaoca, Mayabeque, Cuba (23°08'57"N, 81°51'14"W)	SP317381, collected 15 December 1993, *leg*. M.C. Gil-Rodríguez, Punta Hidalgo, Canary Islands, Spain; MICH 701918, collected 10 March 2011, *leg*. R. Cabrera, playa Punta Uva, Caribe Sur, Costa Rica (9° 63' 95"N, 89° 69' 66" W).
*Laurencia caduciramulosa** Masuda & S.Kawaguchi	Hon Tre Island, Tien Hai Islands, Hatien, Kien Giang Province, Vietnam	Playa Baracoa, Artemisa (VII); La Habana (VII); Rincón de Guanabo, Mayabeque (VII)	Sentíes *et al*. 2010, Suárez *et al*. 2015	UAMIZ1017, collected 19 April 2006, *leg*. A. Areces, A. Sentíes, litoral este de la provincia de La Habana, Cuba (23°11'56.30"N, 82°25'44.98"W); HANC523, HANC524, collected 12 November 2013, *leg*. R. Cabrera, A. Areces, Júcaro, Bahía de Nuevitas, Camagüey (21°32'44"N, 77°08'47"W); HANC0959, collected 06 May 2019, *leg*. P.M. González-Sánchez, Baracoa, Artemisa (23°03'16"N, 82°33'06"W).	SP399809, collected 25 February 2007, *leg*. V. Cassano, M.T. Fujii, Ilha Comprida, Parati, Rio de Janeiro, Brazil; SP356403, collected 04 February 2003, *leg*. M.T. Fujii, Ilha do Brandão, Angra dos Reis (23°00'21"S, 44°28'43"W); SP365238, collected 22 October 2004, *leg*. B.L. Ignacio, V. Cassano, M.T. Fujii, Ilha de Itanhangá, Angra dos Reis, Rio de Janeiro; SP399922, collected 25 February 2005, *leg*. V. Cassano, M.T. Fujii, Praia Feiticeira, Angra dos Reis, Rio de Janeiro; SP356400, collected 21 March 2005, *leg*. M.T.M. de Széchy, V. Cassano, M.T. Fujii Praia do Velho, Saco de Piraquara de Fora, Angra dos Reis, Rio de Janeiro.

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Species	Type locality	Distribution in Cuba and ecozones	References	Examined material from Cuba	Examined material from other countries
Laurencia caraibica P.C.Silva	Abraham Bay, Mariguana, Bahamas	Barco Hundido, Guanahacabibes (V); Playa La Francesita, La Habana (VII); Tarará, La Habana; Bacunayagua, Matanzas (VII); Santa Lucía, Camagüey (VIII)	Areces *et al*. 2003, Suárez *et al*. 2015	HANC186, collected 11 November 2008, *leg*. A. Areces, Este de Socapa, Santiago de Cuba; IDO229, collected 24 August 1981, Bacunayagua, Matanzas (23°08'49"N, 81°40'31"W); IDO230, collected 21 June 1998, *leg*. A. Areces, Tarará, La Habana (23°11'07"N, 82°12'22"W); IDO231, collected 26 August 1999, *leg*. A. Areces, Jaimanitas, La Habana (23°05'47"N, 82°29'16"W); SP401495, collected 25 August 1999, *leg*. A. Areces, Playa Francés, Jaimanitas, Cuba (23°05'34"N, 82°28'27"W).	SP355383, collected 21 July 1999, leg. R. Villaça, M.T. Fujii, Plato central, Atol das Rocas, Brazil; SP355384, tetrasporophyte, collected 13 June 2000, leg. R. Villaça, M.T. Fujii, Barretão, Atol das Rocas, Brazil; SP399940, collected 07 July 2002, leg. R. Villaça, M.T. Fujii, Atol das Rocas, Brazil; SP391082, collected 21 March 2007, leg. R. Rocha-Jorge, Parque Estadual Marinho da Laje de Santos, Laje Principal, município Santos, São Paulo, Brazil (24°08'25"S, 46°17'36"W); SP400821, collected 20 September 2009, leg. M.T. Fujii, Praia de Suape, município de Cabo e Santo Agostinho, Pernambuco, Brazil (08°23'87"S, 35°57'15"W); SP401289, collected 25 February 2008, *leg*. I.B. Silva, E.O.V. Martins, recifes *offshore*, Praia de Maracajaú, município de Maxaranguape, Rio Grande do Norte, Brazil (5°25'38"S, 35°17'55"W).
Laurencia chondrioides Børgesen	Saint John Island, U.S. Virgin Islands	Alcatraz, Jardines de la Reina, Ciego de Ávila (II); Cayo Alonso, Isla de la Juventud (IV); Archipiélago de los Canarreos, Isla de la Juventud (IV); Santa Lucía, Camagüey (VIII)	Brito & Suárez 1994, Prado & Suárez 1997, Martínez-Daranas *et al*. 2008, Sentíes *et al*. 2010, Suárez *et al*. 2015		SP255307, collected 15 December 1992, *leg*. L. Collado-Villes, M. Cordeiro-Marino, M.T. Fujii, Sistema Lagunar de Nichupte, Quintana Roo, Mexico; SP336482, SP336483, collected 30 October 1993, leg. M.T. Fujii, Cancún, Quintana Roo, Mexico.

Species	Type locality	Distribution in Cuba and ecozones	References	Examined material from Cuba	Examined material from other countries
*Laurencia dendroidea** J.Agardh	Brazil [unspecified locality]	Caleta Buena, Playa Girón, Matanzas (III); Playa La Boca and Playa Ancón, Trinidad, Sancti Spíritus (III); Cayo San Juan, Isla de la Juventud (IV); Playa Baracoa, Artemisa (VII); Bajo de Santa Ana, Santa Fé, La Habana (VII); Rincón de Guanabo, Mayabeque (VII); West of Cayo Coco, Wetlands of North of Ciego de Ávila, Ciego de Ávila (VIII); Caletones, Gíbara, Holguín (IX)	Díaz-Piferrer 1957, Suárez *et al*. 2015	HANC0960, collected 18 May 2019, *leg*. P.M. González-Sánchez, E. Reynaldo de la Cruz, Caletones, Gíbara, Holguín (21°12'38"N, 76°14'39"W); HANC0961, collected 30 May 2019, *leg*. B. Martínez-Daranas, P.M. González-Sánchez, Playa Ancón, Trinidad, Sancti Spíritus (21°44'15"N, 80°00'43"W).	SP295097, collected 13 March 1986, *leg*. M.T. Fujii, Praia de Itaipava, município de Itapemirim, Espírito Santo, Brazil (20°54'08"S, 40°46'38"W); SP399945, collected 01 July 2007, *leg*. M.T. Fujii, Ponta dos Castelhanos, munícipio de Anchieta, Espírito Santo, Brazil (19°55'56"S, 40°07'24"W); SP400766, collected 23 June 2009, *leg*. M.T. Fujii, Arquipélago de Currais, Paraná, Brazil (22°44'1,0"S, 48°22'47"W); SP401496, collected 15 February 2003, *leg*. A. Areces, Hotel Américas, Varadero, Matanzas, Cuba; SP401492, collected 12 January 2009, *leg*. A. Areces Hotel Américas, Varadero, Matanzas, Cuba.
Laurencia caraibica P.C.Silva	Abraham Bay, Mariguana, Bahamas	Barco Hundido, Guanahacabibes (V); Playa La Francesita, La Habana (VII); Tarará, La Habana; Bacunayagua, Matanzas (VII); Santa Lucía, Camagüey (VIII)	Areces *et al*. 2003, Suárez *et al*. 2015	HANC186, collected 11 November 2008, *leg*. A. Areces, Este de Socapa, Santiago de Cuba; IDO229, collected 24 August 1981, Bacunayagua, Matanzas (23°08'49"N, 81°40'31"W); IDO230, collected 21 June 1998, *leg*. A. Areces, Tarará, La Habana (23°11'07"N, 82°12'22"W); IDO231, collected 26 August 1999, *leg*. A. Areces, Jaimanitas, La Habana (23°05'47"N, 82°29'16"W); SP401495, collected 25 August 1999, *leg*. A. Areces, Playa Francés, Jaimanitas, Cuba (23°05'34"N, 82°28'27"W).	SP355383, collected 21 July 1999, leg. R. Villaça, M.T. Fujii, Plato central, Atol das Rocas, Brazil; SP355384, tetrasporophyte, collected 13 June 2000, leg. R. Villaça, M.T. Fujii, Barretão, Atol das Rocas, Brazil; SP399940, collected 07 July 2002, leg. R. Villaça, M.T. Fujii, Atol das Rocas, Brazil; SP391082, collected 21 March 2007, leg. R. Rocha-Jorge, Parque Estadual Marinho da Laje de Santos, Laje Principal, município Santos, São Paulo, Brazil (24°08'25"S, 46°17'36"W); SP400821, collected 20 September 2009, leg. M.T. Fujii, Praia de Suape, município de Cabo e Santo Agostinho, Pernambuco, Brazil (08°23'87"S, 35°57'15"W); SP401289, collected 25 February 2008, *leg*. I.B. Silva, E.O.V. Martins, recifes *offshore*, Praia de Maracajaú, município de Maxaranguape, Rio Grande do Norte, Brazil (5°25'38"S, 35°17'55"W).

Species	Type locality	Distribution in Cuba and ecozones	References	Examined material from Cuba	Examined material from other countries
Laurencia chondrioides Børgesen	Saint John Island, U.S. Virgin Islands	Alcatraz, Jardines de la Reina, Ciego de Ávila (II); Cayo Alonso, Isla de la Juventud (IV); Archipiélago de los Canarreos, Isla de la Juventud (IV); Santa Lucía, Camagüey (VIII)	Brito & Suárez 1994, Prado & Suárez 1997, Martínez-Daranas *et al*. 2008, Sentíes *et al*. 2010, Suárez *et al*. 2015		SP255307, collected 15 December 1992, *leg*. L. Collado-Villes, M. Cordeiro-Marino, M.T. Fujii, Sistema Lagunar de Nichupte, Quintana Roo, Mexico; SP336482, SP336483, collected 30 October 1993, leg. M.T. Fujii, Cancún, Quintana Roo, Mexico.
*Laurencia dendroidea** J.Agardh	Brazil [unspecified locality]	Caleta Buena, Playa Girón, Matanzas (III); Playa La Boca and Playa Ancón, Trinidad, Sancti Spíritus (III); Cayo San Juan, Isla de la Juventud (IV); Playa Baracoa, Artemisa (VII); Bajo de Santa Ana, Santa Fé, La Habana (VII); Rincón de Guanabo, Mayabeque (VII); West of Cayo Coco, Wetlands of North of Ciego de Ávila, Ciego de Ávila (VIII); Caletones, Gíbara, Holguín (IX)	Díaz-Piferrer 1957, Suárez *et al*. 2015	HANC0960, collected 18 May 2019, *leg*. P.M. González-Sánchez, E. Reynaldo de la Cruz, Caletones, Gíbara, Holguín (21°12'38"N, 76°14'39"W); HANC0961, collected 30 May 2019, *leg*. B. Martínez-Daranas, P.M. González-Sánchez, Playa Ancón, Trinidad, Sancti Spíritus (21°44'15"N, 80°00'43"W).	SP295097, collected 13 March 1986, *leg*. M.T. Fujii, Praia de Itaipava, município de Itapemirim, Espírito Santo, Brazil (20°54'08"S, 40°46'38"W); SP399945, collected 01 July 2007, *leg*. M.T. Fujii, Ponta dos Castelhanos, munícipio de Anchieta, Espírito Santo, Brazil (19°55'56"S, 40°07'24"W); SP400766, collected 23 June 2009, *leg*. M.T. Fujii, Arquipélago de Currais, Paraná, Brazil (22°44'1,0"S, 48°22'47"W); SP401496, collected 15 February 2003, *leg*. A. Areces, Hotel Américas, Varadero, Matanzas, Cuba; SP401492, collected 12 January 2009, *leg*. A. Areces Hotel Américas, Varadero, Matanzas, Cuba.
Laurencia filiformis (C.Agardh) Montagne	Western Australia	La Puntita, Jardines de la Reina, Ciego de Ávila (II); Las Caletas, Holguín (IX)	Martínez-Daranas 2006, Suárez *et al*. 2015	HANC187, collected 11 November 2008, *leg*. A. Areces, La Socapa, Santiago de Cuba (19°58'25"N, 75°52'21"W); IDO153, collected 01 January 1995, *leg*. A. Sentíes Puerto Morelos, Quintana Roo, México.	

Species	Type locality	Distribution in Cuba and ecozones	References	Examined material from Cuba	Examined material from other countries
Laurencia intricata J.V.Lamouroux	Antilles	Cueva de Pipo, Cayo Los Guzmanes, Golfo de Batabanó, Artemisa (IV), Golfo de Batabanó, Artemisa (IV); Cayo San Juan, Isla de la Juventud (IV); Guanahacabibes, Pinar del Río (V); Bajo de Santa Ana, La Habana (VII); Cayo Mono-Galindo, Cayos de las Cinco Leguas, Cayo Buba, Cayo Cruz del Padre, Parte inferior Bahía de Cárdenas, Matanzas (VII); Bahía de Santa Clara, Bahía de Buena Vista, San Juan de los Remedios, Cayo Lanzanillo, Las Picúas-Cayo Cristo, Cayo Caimán Grande, Villa Clara (VIII); Cayo Coco, Ciego de Ávila (VIII); Las Loras, Sancti Spíritus; Bahía de la Gloria, Playa Varaderito, Nuevitas, Cayos Cuervo, Golfo de Ana María, Santa Lucía, Playa Cocal, Cachiboca, Jardines de la Reina, Río Máximo, Camagüey (VIII); Guardalavaca, Holguín (IX)	Montagne 1842, Howe 1918, Taylor 1960, Díaz-Piferrer *et al*. 1961, Suárez *et al*. 2015	HANC526, collected 30 July 2008, *leg*. Y. Alfonso, A. Areces, Litoral frente a calle 70, municipio Playa, La Habana; IDO187, IDO188, collected 23 February 2001, *leg*. A. Areces, Bajo de Santa Ana, Santa Fé, La Habana (23°03'47"N, 82°32'05"W); IDO197, collected 16 March 2001, tetrasporophyte, *leg*. J. Díaz-Larrea, D.M. Pérez, Cayo Cruz del Padre, Matanzas (23°15'49"N, 80°56'01"W); HANC176, collected 18 June 2008, *leg*. C. Varela, A. Areces, Cayería Los Caimanes, Norte de Caibarién, Villa Clara (22°40'59"N, 78°51'57"W); HANC525, collected 19 June 2008, *leg*. C. Varela, A. Areces, Bahía de Periquillo, Cayo Santa María, Villa Clara; HANC471, collected 21 July 2011, *leg*. M. Gómez, A. Moreira, Arrecifes del litoral Oriental, Cienfuegos; HANC177, collected 27 February 2008, D. de la Nuez, A. Areces, Playa Flamenco, Cayo Coco, Ciego de Ávila (22°31'52"N, 78°28'37"W); HANC531, collected 01 August 2002, *leg*. R. Cabrera, A. Areces, Júcaro, Bahía de Nuevitas, Camagüey; IDO324, collected 01 October 2002, *leg*. R. Cabrera, A. Moreira, Playa Varaderito, Nuevitas (21°40'04"N, 77°19'40"W).	MICH660984, collected 02 January 1952, *leg*. E.P. Killip, Playa Colombo, Isla de la Juventud, Cuba (21°53'51"N, 82°45'53"W); MICH660956, collected 30 April 1967, *leg*. O. Santana, Varadero, Matanzas, Cuba; SP401493, collected 12 January 2009, *leg*. A. Areces, Varadero, a la altura de calle 34, Matanzas, Cuba; SP401501, collected 23 February 2001, *leg*. A. Areces, Playa La Francesita, Jaimanitas, La Habana, Cuba; SP400754, collected 01 September 2007, *leg*. R. Cabrera, M.T. Fujii, Cayo Coco, Ciego de Ávila, Cuba (22°32'32"N, 78°21'41"W); IDO132, collected 01 January 1995, *leg*. A. Sentíes, Puerto Morelo, Quintana Roo, México; SP318028, collected 13 March 1986, *leg*. M.T. Fujii, costões entre Itaoca-Itaipava, municipio de Itapemirim, Espírito Santo, Brazil.

Species	Type locality	Distribution in Cuba and ecozones	References	Examined material from Cuba	Examined material from other countries
Laurencia microcladia Kützing	West Indies	Cayos Cuervos, Golfo de Ana María (II), Golfo de Batabanó, Artemisa (IV); Golfo de Guanahacabibes, Pinar del Río (V); Cayo Cristo, Villa Clara (VIII); Wetlands of Cayo Romano, Ciego de Ávila (VIII); Santa Lucía, Camagüey (VIII)	Montagne 1842, Díaz-Piferrer & López 1959, Taylor 1960, Suárez *et al*. 2015	IDO194, IDO195, IDO196, collected 29 October 1999, *leg*. A. Areces, Golfo de Guanahacabibes, Pinar del Río, Cuba (22°01'24"N, 84°33'06"W); HANC267, collected 09 January 2001, *leg*. A. Areces, Las Américas, Varadero, Matanzas, Cuba.	MICH661041, collected 30 September 1950, *leg*. R.N. Jervis, Playa Cable, Base Naval de Guantánamo, Guantánamo, Cuba (19°53'32"N, 75°09'20"W); MICH661059, collected 25 March 1967, *leg*. O. Santana, Acantilado de Punta del Este, Isla de la Juventud, Cuba (21°34'39"N, 82°32'45"W); MICH661058, collected 28 March 1955, *leg*. M.R. Diaz-Piferrer, Playa Larga Verraco, East of Santiago de Cuba, Cuba (19°53'28"N, 75°34'08"W); SP401494, collected 06 March 1998, *leg*. A. Areces, Varadero, Matanzas, Cuba; SP401497, collected 28.10.1999, *leg*. A. Areces, solapa intermareal, segundo farallón, Ensenada de Guanahacabibes, Pinar del Río, Cuba; IDO156, collected 01 January 1995, *leg*. A. Sentíes, Puerto Morelos, Quintana Roo, México.
Laurencia minuscula Schnetter	Puerto López (Alta Guajira), Departamento de la Guajira, Colombia	La Habana (VII); Rincón de Guanabo, Mayabeque (VII)	Sentíes *et al*. 2010, Suárez *et al*. 2015	UAMIZ1018, collected 13 June 2007, *leg*. A. Areces, R. Cabrera, Rincón de Guanabo, Mayabeque, Cuba (23°10'22.60"N, 82°05'47.17"W)	HOLOTYPE: COL126175, collected 22 February 1972, *leg*. R. Schnetter, deposited in the National Colombian Herbarium, Bogotá, on the leaves of *Thalassia testudinum* at Puerto López, Alta Guajira, Colombia.

Species	Type locality	Distribution in Cuba and ecozones	References	Examined material from Cuba	Examined material from other countries
Laurencia obtusa (Hudson) J.V.Lamouroux	Southern England (Hastings, Sussex; Devon	Cayo Ballenatos and El Júcaro, Cabeza del Este, Jardines de la Reina, Jardines de la Reina, Playa Santa Rita, Cayo Algodón Grande, Golfo de Ana María, Camagüey (II); La Herradura, Cabañas, Artemisa (VII); Jaimanitas, Alamar - Escuela Superior de Guerra, La Habana (VII); Jibacoa, Mayabeque (VII); Cayos Blancos, Matanzas (VII); Cayo Boca Chica, Cayo las Picúas, Cayo Pajonal, Bahía de Buena Vista, Villa Clara (VIII); Bahía de Nuevitas, Camagüey (VIII)	Montagne 1842, Howe 1918, Díaz-Piferrer 1957, Taylor 1960, Suárez *et al*. 2015	HANC026, collected 14 December 2007, *leg*. Y. Alfonso, litoral frente al Acuario, La Habana, Cuba (23°07'03"N, 82°26'13"W); HANC175, collected 18 June 2008, *leg*. C. Varela, A. Areces, Cayería Los Caimanes, Caibarién, Villa Clara, Cuba (22°40'59"N, 78°51'57"W); HANC193, collected 15 July 2008, *leg*. Y. Alfonso, Litoral frente Acuario Nacional, La Habana, Cuba (23°07'20"N, 82°26'14"W); IDO131, collected 01 January 1996, *leg*. A. Areces, Escuela Superior de Guerra, Alamar, La Habana, Cuba; IDO233, collected 25 May 1999, *leg*. A. Areces, Jardines de la Reina, Ciego de Ávila, Cuba (20°54'42"N, 79°08'00"W); IDO232, IDO234, tetrasporophyte, collected 14 May 1999, *leg*. A. Areces, Jibacoa, Mayabeque, Cuba (23°10'06"N, 81°50'34"W); IDO235, tetrasporophyte, collected 11 May 1999, *leg*. A. Areces, La Herradura, Cabañas, Artemisa, Cuba (23°01'20"N, 82°54'46"W); IDO327, collected 01 October 2002, *leg*. R. Cabreras, A. Moreira, Playa Santa Rita, Nuevitas, Camagüey, Cuba (21°33'36"N, 77°37'12"W).	MICH621541, collected 02 January 2006, *leg*. M.J. Wynne, ruinas de Tulum, Quintana Roo, México; MICH661254, collected 30 March 2006, *leg*. T. Sanderson, north side of San Salvador Island, Dump Beach, Bahamas (24°07'36"N, 74°28'18"W).

Species	Type locality	Distribution in Cuba and ecozones	References	Examined material from Cuba	Examined material from other countries
Laurenciella sp.* V.Cassano, Gil-Rodríguez, Sentíes, Díaz-Larrea, M.C.Oliveira & M.T.Fujii		Cayo Anclitas, Punta Practicas, Jardines de la Reina, Camagüey (II)	Popolizio 2015, Collado-Vides *et al*. 2018	HANC0962, collected 01 June 2017, *leg*. P.M. González-Sánchez, Dennis Hanisak, Jardines de la Reina, Punta Practicas, Cayo Anclitas, Ciego de Ávila, Cuba (20°48'44"N, 78°57'57"W).	-
*Osmundea coelenterata** (D.L.Ballantine & Aponte) M.T.Fujii, Sentíes & Areces	Pulaski Shoals, Dry Tortugas, Florida	Cazonal, Santiago de Cuba (I); Cayo Paredón Grande, Archipiélago Jardines del Rey, Ciego de Ávila (VIII); Gibara, Playa Guardalavaca, Holguín (IX)	Areces 1999 (as *Laurencia coelenterata*), Suárez *et al*. 2015	IDO161, IDO192, collected 21 October 1992, *leg*. A. Areces, Cayo Paredón Grande, Archipiélago Jardines del Rey, Ciego de Ávila, Cuba (22°27'43"N, 78°08'04"W); IDO162, collected 13 September 2007, *leg*. A. Areces, Playa Guardalavaca, Holguín, Cuba (21°07'46"N, 75°51'04"W); IDO193, collected 10 September 1980, *leg*. A. Areces, Gibara, Holguín, Cuba (21°06'07"N, 76°07'05"W); HANC266, collected 12 November 2008, *leg*. A. Areces, Cazonal, Santiago de Cuba, Cuba; HANC0963, collected 21 May 2019, *leg*. P.M. González-Sánchez, E. Reynaldo de la Cruz, A. Jover, Cazonal, Santiago de Cuba, Cuba (19°53'11"N, 75°28'32"W).	SP401499, collected 21 October 1992, *leg*. A. Areces, M.T. Fujii, Cayo Paredón Grande, Archipiélago Jardines del Rey, Ciego de Ávila. HOLOTYPE: NMNH Extant Biology 162713, collected 17 September 1991, *leg*. B. Kojis, deposited in the herbarium of the National Museum of Natural History, Smithsonian Institution, United States of America, Pulaski Shoals, Dry Tortugas, Monroe County, Florida, United States of America, U.S. (24°41'40"N, 82°42'18"W); NMNH Extant Biology 164181, collected 17 September 1991, *leg*. D.L. Ballantine, Pulaski Shoals, Dry Tortugas, Monroe County, Florida, United States of America, U.S.

Species	Type locality	Distribution in Cuba and ecozones	References	Examined material from Cuba	Examined material from other countries
Palisada cervicornis (Harvey) Collado-Vides, Cassano & M.T.Fujii	USA: Florida: Key West	Playa Guardalavaca, Holguín (IX)	Zayas *et al*. 2002, Suárez *et al*. 2015	IDO218, IDO219, tetrasporophyte, collected 27 October 2001, *leg*. P. García, A. Areces, Playa Guardalavaca, Holguín, Cuba (21°07'46"N, 75°51'04"W).	NY02216021, collected 24 May 1914, *leg*. J.B. Henderson, P. Bartsch, Cabo de San Antonio y Punta del Cajón, Cuba (21°53'36"N, 84°56'26"W); SP401500, collected 27 October 2001, *leg*. P. González, Playa Guardalavaca, Holguín; NMNH Extant Biology 204580, collected 26 June 1998, *leg*. D.S. Littler, M.M. Littler, B.L. Brooks, White Horse Key, Exuma Cays, Exuma District, Bahamas (23°48'09"N, 76°07'51"W); NMNH Extant Biology 13597787, collected 24 June 1998, *leg*. D.S. Littler, M.M. Littler, Normans Pond Cay, Exuma Cays, Exuma, Bahamas (23°47'25"N, 76°08'12"W); MICH660879, collected 23 February 1987, *leg*. M.J. Wynne, southeast of Moule, Porte d'Enfer de Moule, Guadeloupe (16°29'34"N, 61°26'30"W); MICH660882, collected 21 April 1944, leg. A. Questel, Le Moule, Guadeloupe (16°20'01"N, 61°20'40"W); MICH660868, collected 28 March 1960, *leg*. J.J. Frederick, Saint Georges, Fort St. Catherine beach, Bermuda (32°23'23"N, 64°40'28"W).
Palisada corallopsis (Montagne) Sentíes, Fujii & Díaz-Larrea	La Habana, Cuba	Cayo Las Cayamas, Golfo de Batabanó, Artemisa (IV); La Habana; Rincón de Guanabo, Mayabeque (VII); Cayo Cobos, Cayo Ensenachos, Sancti Spíritus (VII); Santa Lucía, Camagüey (VII)	Montagne 1842 (as *Sphaerococcus corallopsis*), Taylor 1941, Humm & Jackson 1955, Díaz-Piferrer 1957, Taylor 1960, Suárez *et al*. 2015	IDO184, collected 03 March 2007, *leg*. A. Areces, Rincón de Guanabo, Mayabeque, Cuba (23°11'18"N, 82°08'14"W); HANC137, HANC185, collected 27 February 2008, *leg*. D. de la Nuez, Y. Alfonso, A. Areces, Playa Flamenco, Cayo Coco, Ciego de Ávila, Cuba (22°31'52"N, 78°28'37"W).	SP317380, collected 19 September 1990, *leg*. M.C. Gil-Rodríguez, P. Floral, Canarias Island, Spain; SP317372, collected 29 October 1991, *leg*. M.C. Gil-Rodríguez, El Médano, Canarias Island, Spain.

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Palisada flagellifera (J.Agardh) K.W.Nam	India	Playa Caletones, Gibara, Holguín (IX)	Areces *et al*. 2003, Sentíes *et al*. 2010, Suárez *et al*. 2015	IDO154, collected 07 April 1979, *leg*. A. Areces, Gibara, Holguín, Cuba (21°06'07"N, 76°07'05"W).	IDO152, collected 01 January 1995, *leg*. A. Sentíes, Puerto Morelo, Quintana Roo, México; SP335932, collected 04 October 1985, *leg*. M.T. Fujii, Praia dos Padres, município de Aracruz, Espírito Santo, Brazil; SP335903, collected 05 July 1985, *leg*. S.M.P.B. Guimarães, Ponta da Fruta, município de Vila Velha, Espírito Santo, Brazil; SP335904, collected 03 July 1985, *leg*. S.M.P.B. Guimarães, Praia de Meaipe, município de Guarapari, Espírito Santo, Brazil; SP335920, collected 04 July 1981, *leg*. M.T. Fujii, Praia dos Padres, município de Santa Cruz, Espírito Santo, Brazil; SP468924, collected 29 November 2007, *leg*. M.T. Fujii, Praia Brava, município de Ubatuba, São Paulo, Brazil (23°27'40"S, 45°22'10"W).
Palisada furcata (Cordeiro-Marino & M.T.Fujii) Cassano & M.T.Fujii	Brazil: Ceará State; Praia de Guajiru, Trairi Municipality	Playa Guardalavaca, Holguín (IX)	Areces *et al*. 2003, Suárez *et al*. 2015		HOLOTYPE: SP239661, tetrasporophyte, collected 28 January 1990, *leg*. M.C. Marino, M.T. Fujii, F. Pinheiro-Joventino, deposited in the herbarium of the Botanical Institute, São Paulo, SP, Praia de Guajiru, Trairi, Ceará, Brazil; SP399928, collected 24 February 2004, *leg*. M.T. Fujii, Praia Tambari, Paraiba, Brazil; SP365658, collected 26 December 2005, *leg*. J.M.C. Nunes, M.T. Fujii, Praia de Catussaba, município de Salvador, Bahía, Brazil; SP239651, collected 29 June 1985, *leg*. S.M.P.B. Guimarães, M.C. Marino, Y. Tomita, M.P.R. Pique, E. Paula, Praia de Marataizes, Itapemirim, Espírito Santo, Brazil; SP254960, collected 27 April 1991, *leg*. M.T. Fujii, Arribada do Agazinho, Espírito Santo, Brazil.

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Species	Type locality	Distribution in Cuba and ecozones	References	Examined material from Cuba	Examined material from other countries
*Palisada perforata** (Bory) K.W.Nam	Santz Cruz de Tenerife, Islas Canarias	La Socapa, Cazonal, Pedro el cojo, Playa de Juraguá, Playa El Francés, Santiago de Cuba (I); Caleta Buena, Playa Girón, Matanzas (III); Rancho Luna, Cienfuegos (III); Cayo Aguado, Playa La Boca, Trinidad, Sancti Spíritus (III); Tetas de María la Gorda and Playa Antonio, Guanahacabibes, Pinar del Río (V); El Salado, Bauta, Playa Baracoa, Artemisa (VII); Bajo de Santa Ana, Playa La Puntilla and Marina Hemingway, Santa Fé, Jaimanitas, Celimar, La Habana (VII); Rincón de Guanabo, Mayabeque (VII); Bahía de Buena Vista, Cayo Cristo, Villa Clara (VIII); Caguanes, Centro Cayo Coco, Wetlands of the North of Ciego de Ávila, Ciego de Ávila (VIII); Punta Maternillos, Cayo Sabinal, Santa Lucía, Playa Varaderito, Nuevitas, Camagüey (VIII); Las Caletas, Caletones, Gibara, Holguín (IX)	Sánchez Alfonso 1930, Díaz-Piferrer 1957, Taylor 1960, Suárez *et al*. 2015	HANC170, collected 18 June 2008, C. Varela, *leg*. A. Areces, Cayería Los Caimanes, Caibarien, Villa Clara, Cuba (22°40'59"N, 78°51'57"W); HANC172, collected 15 June 2008, *leg*. D. de la Nuez, A. Areces, Las Guasas, Villa Clara, Cuba (22°40'06"N, 79°05'50"W); HANC171, collected 01 July 2008, *leg*. Y. Alfonso, A. Areces, Playa Baracoa, Artemisa, Cuba (23°02'49"N, 82°34'22"W); HANC173, collected 27 February 2008, *leg*. D. de la Nuez, A. Areces, Playa Flamenco, Cayo Coco, Ciego Ávila, Cuba (22°31'52"N, 78°28'37"W); HANC216, collected 24 August 2009, *leg*. C. Varela, Y. Alfonso, Punta Uvero, Puerto Padre, Las Tunas, Cuba; HANC268, collected 12 November 2008, *leg*. A. Areces, Cazonal, Santiago de Cuba, Cuba; HANC636, collected 09 March 1981, *leg*. A. Areces, Bahía de Corrientes, Península de Guanahacabibes, Pinar del Río, Cuba; IDO226, collected 29 August 2001, *leg*. A. Areces, Playa Antonio, Guanahacabibes, Pinar del Río, Cuba (21°58'34"N, 84°44'04"W); IDO228, collected 26 August 1999, *leg*. M. Cano, Jaimanitas, La Habana, Cuba (23°05'47"N, 82°29'16"W); IDO306, collected 01 January 2003, *leg*. R. Cabrera, A. Moreira, Playa Varaderito, Nuevitas,	SP317378, SP317397, collected 18 September 1992, *leg*. M.C. Gil-Rodríguez, Ponto Cruz, Islas Canarias, Spain; SP428793, collected 25 October 2000, *leg*. M.T. Fujii, Praia Mansa, município de Ilhabela, São Paulo, Brazil (23°51'11"S, 45°17'14"W); SP400166, collected 29 July 2002, *leg*. G. Amado Filho, M.T. Fujii, no médio litoral, Praia Rasa, município de Rio de Janeiro, Búzios, Rio de Janeiro, Brazil (22°45'06"S, 41°56'35"W); SP400572, collected 13 February 1978, *leg*. T.D. Beltrame, M.R.A. Braga, S.M.P.B. Guimarães, Praia do Farol, municipio de Fortaleza, Ceará, Brazil (03°43'45"S, 38°27'47"W); SP400573, collected 18 February 1978, *leg*. T.D. Beltrame, M.R.A. Braga, S.M.P.B. Guimarães, Canoa Quebrada, municipio de Aracati, Ceará, Brazil (04°31'45"S, 37°41'34"W); NMNH Extant Biology 210738, collected 07 October 1997, *leg*. G. Rosenberg, B. Martínez-Daranas, M. Hernandez, A. Fernandez, Playa la Herradura, Artemisa, Cuba (23°01'04"N, 82°54'42"W); MICH665370, collected 30 April 1967, *leg*. O. Santana, Varadero, Matanzas, Cuba (23°10'39"N, 81°12'37"W); MICH665307, collected 14 March 1954, *leg*. E.P. Killip, Columbo Beach, Isla de la Juventud, Cuba (21°53'51"N, 82°45'53"W); MICH665304, collected 03 April 1954, *leg*. E.P. Killip, Playa Bibijagua, Isla de la Juventud, Cuba (21°53'22"N, 82°43'37"W); MICH665308, collected 07 November 1954, *leg*. M.R. Diaz-Piferrer, Playa Arroyo la Costa, Santiago de Cuba, Cuba (19°56'14"N, 75°40'25"W); NY03531668, collected 08 February 1953, E.P. Killip, Playa

Species	Type locality	Distribution in Cuba and ecozones	References	Examined material from Cuba	Examined material from other countries
*Yuzurua iridescens** (M.J.Wynne & D.L.Ballantine) Sentíes & M.J.Wynne	Les Alizes, southeast of Moule, Grande-Terre, Guadeloupe, French West Indies	Cayo Caballones, Archipiélago Jardines de la Reina, Camagüey (II); Playa Antonio, Guanahacabibes, Golfo de Guanahacabibes, Pinar del Río (V, VI); Rincón de Guanabo, Mayabeque (VII)	Areces 1999 (as *Laurencia iridescens*), Suárez *et al*. 2015	IDO159, collected 10 September 1980, *leg*. A. Areces, Cayo Caballones, Jardines de la Reina, Camagüey, Cuba (20°53'41"N, 79°00'57"W); IDO160, collected 29 October 1999, *leg*. A. Areces, Playa Antonio, Guanahacabibes, Pinar del Río, Cuba (21°58'34"N, 84°44'04"W); IDO164, collected 20 October 1999, *leg*. A. Areces, Guanahacabibes, Pinar del Río, Cuba (22°01'24"N, 84°33'06"W); HANC0968, collected 24 April 2019, *leg*. P.M. González-Sánchez, A. Areces, Rincón de Guanabo Mayabeque, Cuba (23°10'20"N, 82°05'52"W).	ISOTYPE: BPBM 598479, collected 23 February 1987, *leg*. M. Wynne, M. Ballantine, SE of Moule, Grande-Terre, French West Indies, Guadeloupe; NMNH198001, collected 23 February 1987, *leg*. M. Wynne, Les Alizes, southeast of Le Moule, Grande Terre, Lesser Antilles, Guadeloupe, West Indies – Neotropics. MNHN PC0550111, collected 23 February 1987, *leg*. M. Wynne, M. Ballantine, Les Alizes, southeast of Moule, Grande-Terre, French West Indies, Guadeloupe (16°20'03"N, 61°19'43"W);
Yuzurua poiteaui var. *poiteaui* (J.V.Lamouroux) Martin-Lescanne	Santo Domingo, Dominican Republic	Las Cayamas, Mal País, Golfo de Batabanó, Artemisa (IV); Cayo Matías, Isla de la Juventud (IV); Zona pre-arrecifal, Bahía de Santa Clara, Bahía de Carahatas, Bahía de Buena Vista, San Juan de los Remedios, Las Picúas, Cayo Fragoso, Villa Clara (VIII); Cayo Lucas, Sancti Spíritus (VIII); Cayo Coco, Wetlands of Cayo Romano, Wetlands of the North of Ciego de Ávila, Ciego de Ávila (VIII); Río Máximo, Santa Lucía, Cayo Cruz, Camagüey (VIII)	Taylor 1941, Castellanos 1945, Díaz-Piferrer 1957, Taylor 1960, Suárez *et al*. 2015	HANC436, collected 13 October 1989, *leg*. M. Esquivel, B. Martínez-Daranas, Archipiélago Sabana-Camagüey, Cuba; IDO224, collected 17 August 1986, *leg*. M. Esquivel, A. Areces, Cayo Matías, Archipiélago Los Canarreos, Golfo de Batabanó, Isla de la Juventud, Cuba (21°33'33"N, 82°26'47"W).	MNHN PC0494324, NY03531700, MICH674840, MICH674833, collected 14 March 1953, *leg*. E.P. Killip, Playa de Bibijagua, Isla de la Juventud, Cuba (21°53'20"N, 82°43'40"W).

Species	Type locality	Distribution in Cuba and ecozones	References	Examined material from Cuba	Examined material from other countries
Yuzurua poiteaui var. *gemmifera* (Harvey) M.J. Wynne	Key West, Florida, USA	Punta Arenas, Cayos Buenavista, Golfo de Batabanó (IV); Punta del Este, Cayo Matías, Archipiélago de Los Canarreos, Isla de la Juventud (IV); Bajo de Santa Ana, Santa Fé, La Habana (VII); Rincón de Guanabo, Mayabeque (VII); Cayo Galindo, Cayos de las Cinco Leguas, Matanzas (VIII); Cayo Cristo, Bahía de Buena Vista, Isabela de Sagua, Villa Clara (VIII); Cayo Sabinal, Bahía de Nuevitas, Camagüey (VIII)	Díaz-Piferrer & López 1959, Taylor 1960, Suárez *et al*. 2015	IDO189, IDO190, IDO191, male plant, collected 23 February 2001, *leg*. A. Areces, Bajo Santa Ana, Santa Fé, La Habana, Cuba (23°03'47"N, 82°32'05"W); IDO220, collected 21 August 1986, *leg*. M. Esquivel, A. Areces, Punta Arenas, Golfo de Batabanó, Cuba (22°10'00"N, 81°33'36"W); IDO221, collected 27 February 1989, *leg*. A. Areces, Isabela de Sagua, Villa Clara, Cuba (22°55'00"N, 80°01'00"W); IDO222, collected 16 January 1995, *leg*. A. Areces, Punta del Este, Golfo de Batabanó, Isla de la Juventud, Cuba (21°33'22"N, 82°32'48"W); IDO223, collected 07 August 1986, *leg*. M. Esquivel, A. Areces, Cayo Matías, Archipiélago Los Canarreos, Cuba (21°33'33"N, 82°26'47"W).	SP401498, collected 12 January 2009, *leg*. A. Areces, M.T. Fujii, Varadero, Matanzas, Cuba; SP400753, collected 25 September 2005, *leg*. M.T. Fujii, Cayo Coco, Ciego Ávila, Cuba (22°32'25"N, 78°22'16"W); MICH674894, collected 27 December 1966, *leg*. O. Santana, Playa Guanabo, La Habana, Cuba (23°10'30"N, 82°07'47"W); MICH674902, collected 11 April 1955, *leg*. M.R. Diaz-Piferrer, Playa La Boca, Puerto Padre, Las Tunas, Cuba (21°16'00"N, 76°31'59"W); MICH674895, MICH674891, collected 26 March 1954, *leg*. E.P. Killip, Bibijagua beach, Isla de la Juventud, Cuba (21°53'24"N, 82°43'35"W); MNHN PC0550131, MICH674892, MICH674890, collected 03 April 1954, *leg*. E.P. Killip, Bibijagua beach, Isla de la Juventud, Cuba (21°53'36"N, 82°43'24"W); MICH674893, collected 23 April 1954, *leg*. E.P. Killip, Punta Piedra Beach, Isla de la Juventud, Cuba (21°54'15"N, 82°47'09"W); MICH674912, collected 10 May 1949, *leg*. E. Yale Dawson, Puerto Esperanza, Pinar del Rio, Cuba (22°46'36"N, 83°43'55"W).

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Note: Suárez et al. (2015) summarizes the records by areas. For more details on references go to this text.

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SUPPLEMENTARY MATERIAL 1 of Similarity and areas of endemism of the *Laurencia* complex (Ceramiales, Rhodomelaceae) in Cuba

Patricia María González-Sánchez, Otávio Luis Marques da Silva, Rubén Cabrera, Beatriz Martínez-Daranas, Enrique Reynaldo de la Cruz, Valéria Cassano & Mutue Toyota Fujii

http://dx.doi.org/10.6018/analesbio.44.0x

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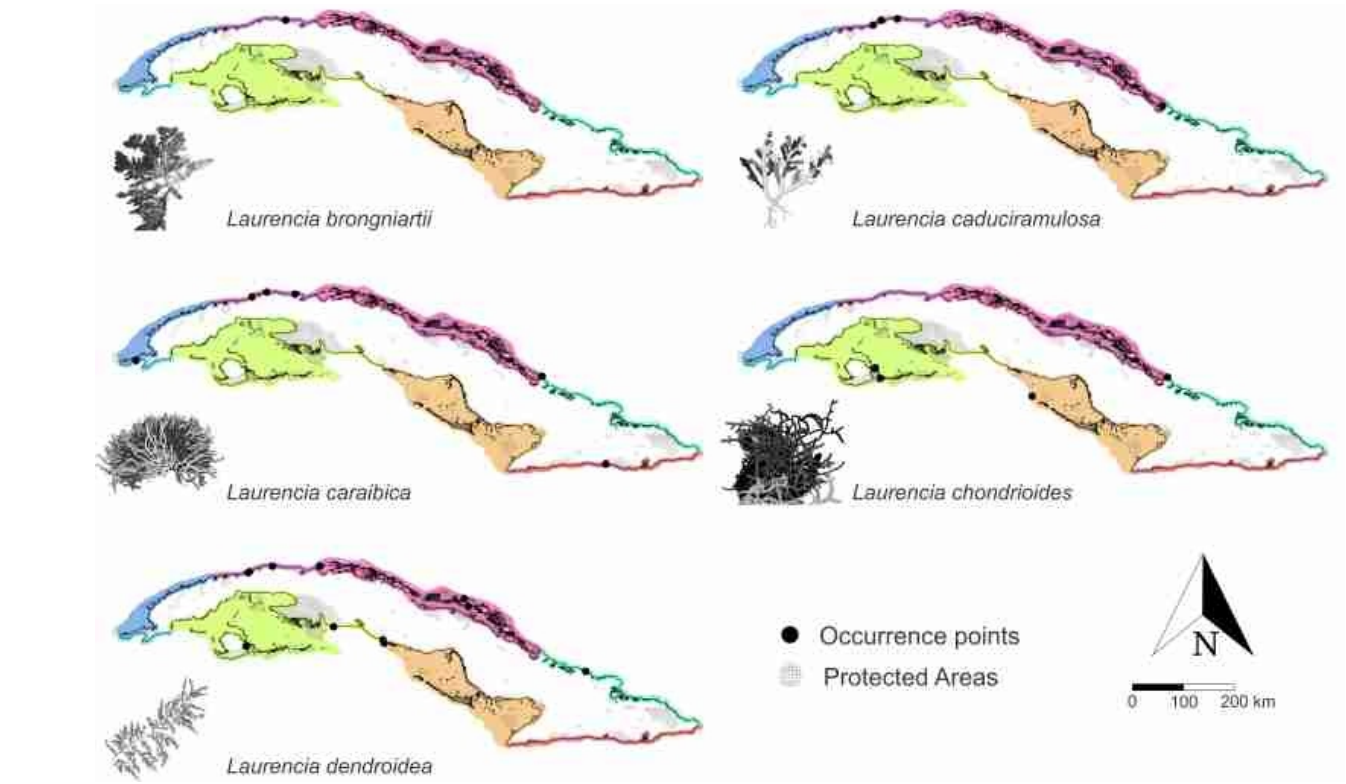
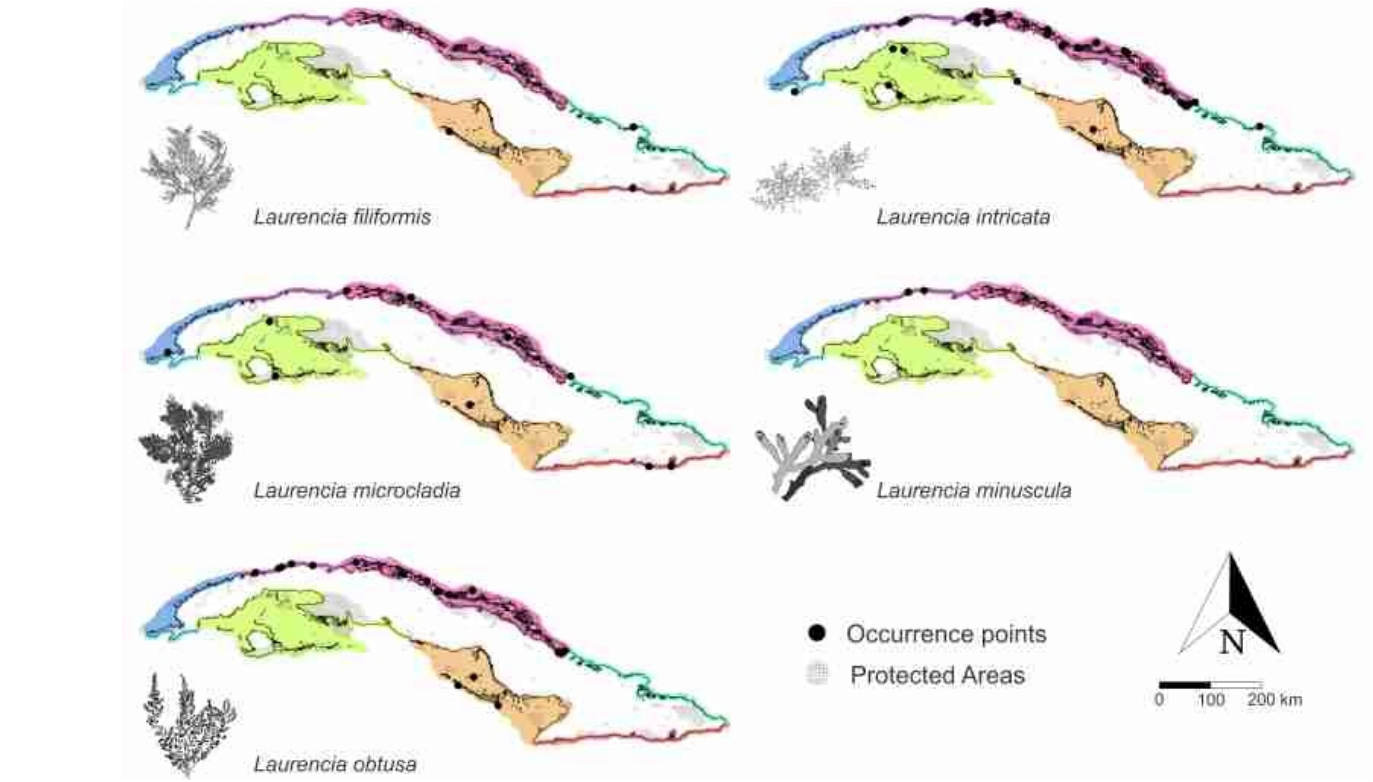


Supplementary Figure S1. Geographical distribution (black dots) of *Laurencia brongniartii*, *L. caduciramulosa*, *L. caraibica*, *L. chondrioides*, and *L. dendroidea* in the Cuban archipelago.



Supplementary Figure S2. Geographical distribution (black dots) of *Laurencia filiformis*, *L. intricata*, *L. microcladia*, *L. minuscula*, and *L. obtusa* in the Cuban archipelago.

SUPPLEMENTARY MATERIAL 1 of Similarity and areas of endemism of the *Laurencia* complex (Ceramiales, Rhodomelaceae) in Cuba

Patricia María González-Sánchez, Otávio Luis Marques da Silva, Rubén Cabrera, Beatriz Martínez-Daranas, Enrique Reynaldo de la Cruz, Valéria Cassano & Mutue Toyota Fujii

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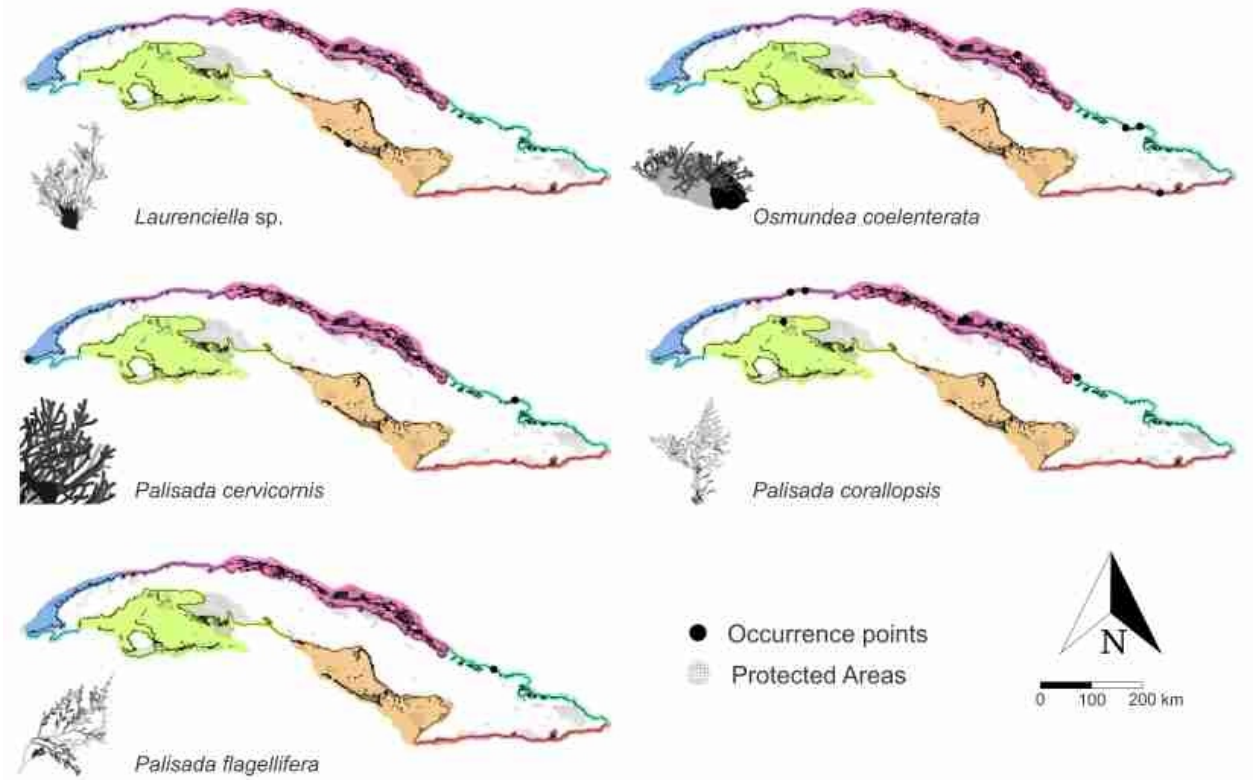
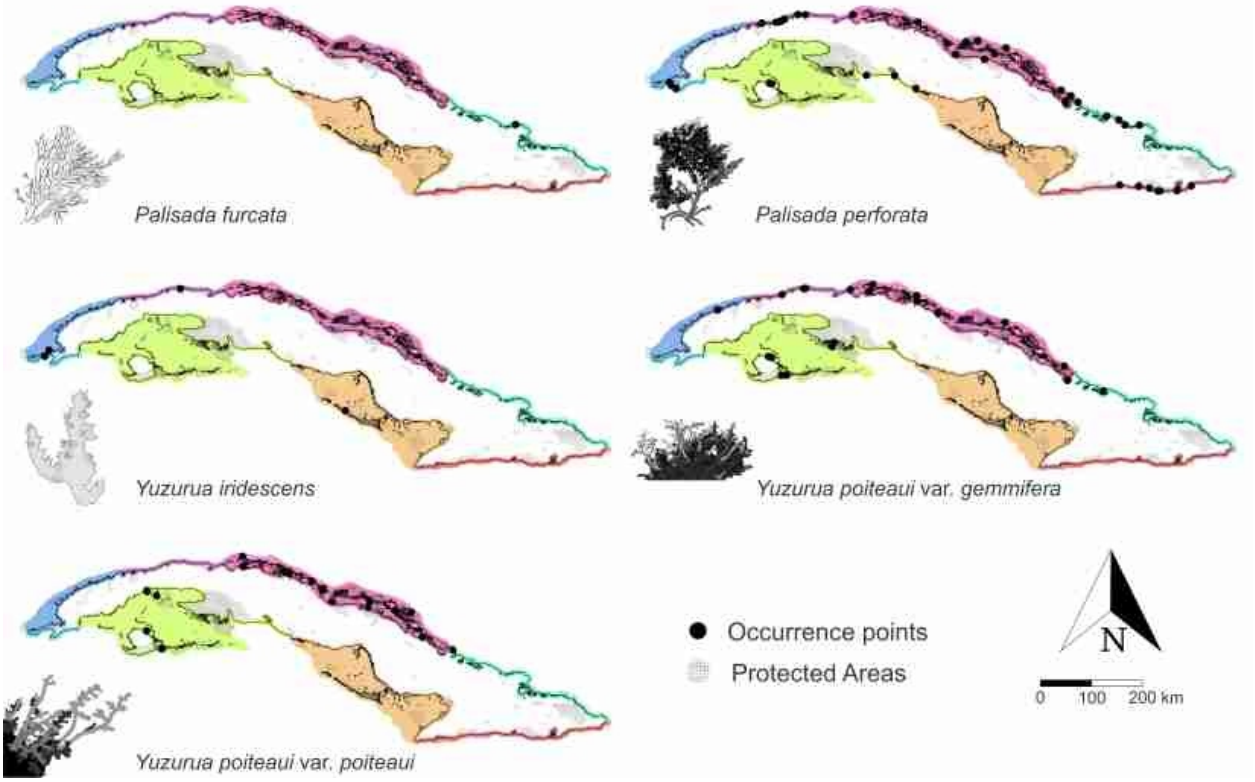


Supplementary Figure S3. Geographical distribution (black dots) of *Laurenciella* sp., *Osmundea coelenterata*, *Palisada cervicornis*, *P. corallopsis*, and *P. flagellifera* in the Cuban archipelago.



Supplementary Figure S4. Geographical distribution (black dots) of *Palisada furcata*, *P. perforata*, *Yuzurua iridescens*, *Y. poiteaui* var. *gemmifera*, and *Y. poiteaui* var. *poiteaui* in the Cuban archipelago.

CONSIDERAÇÕES FINAIS

CONSIDERAÇÕES FINAIS

Este estudo é o primeiro a reavaliar a diversidade de espécies do complexo *Laurencia* no arquipélago cubano usando marcadores moleculares e analisando sua distribuição a nível de país. Também constitui o primeiro banco de dados de código de barras de macroalgas para Cuba, fornecendo as bases para futuros estudos ficológicos na região. Nosso estudo gerou um total de 24 sequências para o GenBank, das quais 18 são do COI-5P e 6 do *rbc*L.

A literatura sobre o complexo *Laurencia* antes deste estudo relatou 19 espécies de *Laurencia sensu stricto* ao longo da costa de Cuba (Areces & Sentíes 2002, Areces et al. 2003, Sentíes et al. 2010, Suárez et al. 2015), das quais apenas três (*L. caduciramulosa*, *L. intricata* e *Yuzurua poiteuai* var. *gemmifera*) foram sequenciadas (sequências de DNA disponíveis no Genbank). No decorrer deste estudo, as análises morfológicas e moleculares das espécies do complexo *Laurencia* permitiram a confirmação de 13 táxons para a plataforma cubana: *Laurencia caduciramulosa, L. catarinensis, L. dendroidea, L. microcladia¸ Laurenciella namii, Palisada corallopsis, P.* aff. *perforata* 3, *Palisada* sp. 1, *Palisada* sp. 2, *Yuzurua iridescens, Y. poiteaui, Yuzurua* sp. 1 e *Yuzurua* sp. 2.

Apresentamos o primeiro relato da espécie *L. catarinensis* do Atlântico ocidental subtropical (localidade tipo Brasil) confirmado por análises moleculares, cuja distribuição se estende até o Caribe e áreas adjacentes (Venezuela). Outro novo registro foi o da espécie recentemente descrita *Laurenciella namii* das Bermudas e também presente na Flórida. *Laurenciella* é incorporada como um novo gênero ao grupo do complexo para Cuba.

Análises moleculares com o COI-5P e os resultados dos dois métodos de delimitação de espécies indicam que *Laurencia dendroidea* e *Palisada perforata* como atualmente reconhecidas formam um complexo de espécies. As espécies de *L. dendroidea* originalmente descritas para a Austrália como *L. majuscula* representam uma ou mais linhagens independentes das espécies do Atlântico. Portanto, se recomenda a reavaliação da coespecificidade de *L. majuscula* e *L. dendroidea* propostas por Metti et al. (2013). *Palisada peforata* foi subdividida em quatro linhagens distintas. O primeiro corresponde ao autêntico *P. peforata* da Espanha (topótipo), o segundo (*P.* aff. *perforata* 1) corresponde a espécimes da Venezuela, Dominica, EUA e Bermuda e é semelhante ao *P. intermedia* sensu Rodríguez de Ríos (1979), o terceiro (*P.* aff. *perforata* 2) a *P.* "*papillosa*" e corresponde a amostras da Venezuela e o quarto (*P.* aff. *perforata* 3) também é morfologicamente semelhante ao original *P.* "*papillosa*" e inclui o sequências de Cuba, que formaram um clado separado do resto do agrupamento deste complexo.

A comparação dos caracteres morfo-anatômicos e análises moleculares de espécimes coletados de *Palisada corallopsis* e *P. furcata* de suas localidades tipo com seus holótipos confirmou que ambas espécies são coespecíficas, sendo *P. furcata* sinônimo de *P. corallopsis*.

As espécies não identificadas de *Palisada* e *Yuzurua* requerem estudos futuros para sua determinação e descrição.

Os táxons *Laurencia brongniartii* J. Agardh, *Laurencia caraibica* P.C. Silva, *Laurencia chondrioides* Børgesen, *Laurencia intricata* J.V. Lamouroux, *Laurencia minuscula* Schnetter, *Laurencia obtusa* (Hudson) J.V.Lamouroux, *Palisada cervicornis* (Harvey) Collado-Vides, Cassano & M.T. Fujii, *Osmundea coelenterata* (D.L. Ballantine & Aponte) M.T. Fujii, Sentíes & Areces, *Palisada flagellifera* (J. Agardh) K.W. Nam e *Yuzurua poiteaui* var. *gemmifera* (Harvey) M. J. Wynne referidos para Cuba não foram confirmados neste estudo ou por não terem sido amostrados ou por representarem possíveis nomes mal aplicados para o país, como *Laurencia obtusa* e *Osmundea coelenterata*. Particularmente, estas duas espécies precisam de uma revisão com obtenção de sequências de *rbc*L para um melhor esclarecimento e confirmação de sua presencia no arquipélago cubano.

O marcador do tipo Código de Barras COI-5P mostrou-se eficaz na delimitação das espécies, e de fácil obtenção das sequências. Este marcador registrou um alto nível de divergência interespecífica, o que permitiu a resolução de espécies intimamente relacionadas. Como foi o caso de *Laurencia dendroidea* e *Palisada perforata* que formaram um complexo de especies. Por sua vez, *rbc*L nao se mostrou um marcador eficiente na separação de espécies próximas ou no reconhecimento dos complexos encontrados, evidenciando que análises utilizando outros marcadores ou filogenômica são necessárias para esclarecer as relações evolutivas do complexo *Laurencia*.

Métodos de delimitação de espécies combinados com análises morfológicas e de divergência genética dos marcadores provaram ser ferramentas úteis nas decisões taxonômicas.

O padrão de distribuição das espécies do complexo *Laurencia* no arquipélago cubano é heterogêneo. O litoral norte apresentou maior número de espécies e indivíduos do que o litoral sul. A baixa presença de centros científicos em áreas do litoral sul e especialistas do grupo, o pouco esforço amostral, as diferenças geomorfológicas do substrato e habitats marinhos, e também no padrão de circulação das correntes marinhas da plataforma cubana foram os principais variáveis responsáveis pelas marcantes diferenças observadas na distribuição das espécies do complexo *Laurencia* em Cuba

As áreas endêmicas obtidas pela Análise de Parcimônia de Endemicidade foram determinadas pela presença de espécies raras ou incomuns do complexo para o arquipélago dentro e fora das Áreas Marinhas Protegidas.

Nesse trabalho, foi possível atingir os objetivos previamente propostos. Contudo, ainda há lacunas no conhecimento do complexo *Laurencia* no arquipélago cubano e no Oceano Atlântico, uma vez que não foi possível obter amostras de todas as espécies previamente citadas e que as árvores filogenéticas apontam para um número maior de táxons do que o atualmente reconhecido. Uma amostragem mais completa no arquipélago cubano é necessária para estudos futuros com a inclusão de espécimes de áreas como Isla de la Juventud e Las Tunas, bem como coleções mais detalhadas de espécimes de países do Caribe que provavelmente revelarão maior diversidade a atualmente conhecida.

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