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## Diversity of *Hypnea* (Rhodophyta) in South Florida, with description of *H. spiniformis* sp. nov.

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

### Abstract

The genus *Hypnea* is well known to be rich in cryptic species, with many new species being recognized recently based on molecular data. Therefore, we used mitochondrial (COI-5P) and plastid (*rbcL*) molecular markers, in addition to aspects of morphology, to investigate the diversity of *Hypnea* on the coast of South Florida. Molecular divergences and phylogenetic inferences indicated that four species were present: *H. caribica* and *H. cryptica*, both new occurrences on the Florida coast; *H. musciformis*, already previously reported and *H. spiniformis* sp. nov. described in this work. Our data reinforce the importance of studies with an integrative taxonomy approach to delimit species and correctly identify them.

**Key words:** COI-5P; *rbcL*; systematics; taxonomy

### Introduction

The red algal genus *Hypnea* J.V. Lamouroux (1813: 131) is best known for the economic importance of some species used to produce carrageenan (Saito & Oliveira 1990). The genus is also known for its problematic taxonomy and difficulty in recognizing species based on morphological characters (Masuda *et al.* 1997, Yokoya *et al.* 2020). *Hypnea* has a wide geographical distribution and is present across both tropical and subtropical regions around the globe (Yokoya *et al.* 2020, Guiry & Guiry 2023). Like most red algae, *Hypnea* species have a simple and plastic morphology, often influenced by environmental factors, such as temperature and light (Masuda *et al.* 1997, Nauer *et al.* 2015). For this reason, molecular surveys often reveal new species, new cryptic species complexes, and new synonyms (Jesus *et al.* 2016, Nauer *et al.* 2015, 2016).

*Hypnea* molecular studies began in the Pacific Ocean (Yamagishi & Masuda 2000, Yamagishi *et al.* 2003, Geraldino *et al.* 2009, Geraldino *et al.* 2010), expanding to the South Atlantic, mainly on the Brazilian coast (Jesus *et al.* 2016, 2019a,b, Nauer *et al.* 2014, 2015, 2016), and in recent years, the Caribbean Sea (Nauer *et al.* 2019b, Cabrera *et al.* 2020) and the North Atlantic (Campbell *et al.* 2021). The molecular markers most used for species delimitation

were the plastid *rbcL*, encoding the large subunit of the enzyme ribulose-1,5-bisphosphate carboxylase-oxygenase, and the mitochondrial COI-5P, encoding the 5' end of the cytochrome c oxidase subunit 1, proposed for Rhodophyta barcoding by Saunders (2005).

Two important species complexes in the Atlantic region have been uncovered with molecular markers, the *Hypnea musciformis* (Wulfen) J.V.Lamouroux (1813: 43) and *H. cornuta* (Kützinger) J.Agardh (1851: 449) complexes. Nauer *et al.* (2015, 2019a, b) found molecular divergences among morphologically identified *H. musciformis* specimens collected throughout the western Atlantic, dividing them into four species clusters. Specimens from the coast of the United States were grouped with topotype specimens from the Italian Adriatic coast, considered the true *H. musciformis*. The other three clusters were considered distinct species with specific geographic distributions. *Hypnea pseudomusciformis* Nauer, Cassano & M.C. Oliveira (2015: 2407) was described for the Brazilian coast (Nauer *et al.* 2015), *H. caraibica* Nauer, Cassano & M.C. Oliveira (2019: 88) for the Caribbean Sea (Nauer *et al.* 2019b), and *H. schneideri* (2019: 88) Nauer, Cassano & M.C. Oliveira for the Dominican Republic (Nauer *et al.* 2019b).

Jesus *et al.* (2019a) used the COI-5P and *rbcL* markers to demonstrate that *Hypnea cornuta* and its related species, *H. stellulifera* (J.Agardh) Yamagishi & Masuda (2003: 535), consisted of five different cryptic species. *Hypnea cornuta* was revealed to be a polyphyletic taxon comprising three well-separated lineages. However, the lineage representing *H. cornuta sensu stricto* is yet to be determined because there are no sequences for type or topotype specimens (Jesus *et al.* 2019a). *Hypnea stellulifera* was revealed to be limited to Asia, and because of high molecular divergences, the Brazilian specimens originally identified as *H. stellulifera* were described as an endemic species, *H. cryptica* P.B.Jesus & J.M.C.Nunes (2019a: 142). Later, based on molecular markers, the species *H. cryptica* had its geographical distribution expanded to include the coasts of Cuba (Cabrera *et al.* 2020) and North Carolina in the USA (Campbell *et al.* 2021).

More recently, Jesus *et al.* (2023), analyzing modern and historical herbarium specimens previously identified as *H. nidulans* from Japan, Philippines, and Korea, described a new species, *H. djamilae* P.B. Jesus, Lyra, J.M.C. Nunes & M.C. Oliveira (2023: 9), as distinct from the *H. nidulans* Setchell specimens from French Polynesian. The authors also described specimens previously identified as *H. nidifica* from French Polynesia and the Indian Ocean as the new species, *H. davisiana* P.B. Jesus, Lyra, J.M.C. Nunes & M.C. Oliveira (2023: 9) and *H. evaristoae* P.B. Jesus, Lyra, J.M.C. Nunes & M.C. Oliveira (2023: 9), respectively (Jesus *et al.* 2023).

Concerning the biodiversity of *Hypnea* in Florida, initial collections were gathered and dispatched to phycologists such as J. Agardh and W.H. Harvey. Later, in his illustrated monograph on North American algae, Harvey (1853) documented the presence of *H. cornuta* and *H. musciformis* from the Florida Keys. Taylor (1928) reported five species for the Florida Keys and Dry Tortugas: *H. alopecuroides* Kützinger, *H. cervicornis* J. Agardh, *H. cornuta*, *H. musciformis* and *H. pannosa* J. Agardh. The same author suggested that the name *H. robusta*, previously assigned to the Florida coast by Hooper (1850), should be considered invalid based on morphological observations (Taylor 1929). Later, Taylor (1960) questioned the previous Florida reports of *H. pannosa* and did not include *H. alopecuroides* in the tropical Western Atlantic or *H. musciformis* in Florida. The latter was likely a misprint as his previous Florida listing of the species (Taylor 1928) included *H. musciformis*. Dawes (1974) reported *H. cervicornis*, *H. cornuta*, *H. musciformis* and *H. spinella* (C. Agardh) Kützinger for the west coast of Florida. The most recent marine algal flora for the state listed *H. cornuta*, *H. musciformis*, *H. spinella*, and *H. volubilis* Searles (Dawes & Mathieson 2008). *Hypnea valentiae* (Turner) Montagne was since reported for the Indian River Lagoon (Littler *et al.* 2008), and *H. volubilis* was transferred to *Calliblepharis* (Campbell *et al.* 2022), so the current list of Florida *Hypnea* species includes only *H. cornuta*, *H. musciformis*, *H. spinella*, and *H. valentiae*.

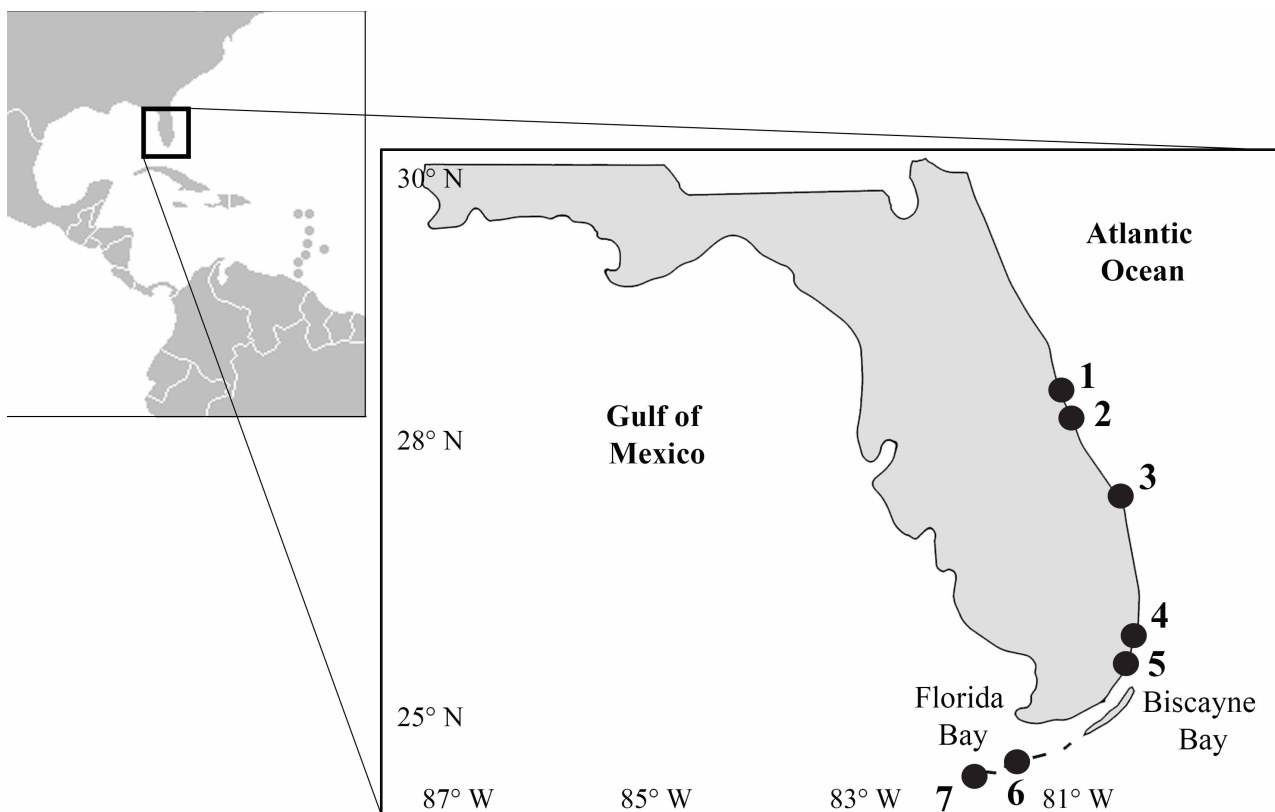
Despite significant progress in taxonomic studies using molecular markers, no molecular investigations of *Hypnea* along the Florida coast have been conducted. However, Cabrera *et al.* (2020) conducted a molecular phylogenetic analysis of the genus in the nearby island of Cuba, identifying only three species, *H. cryptica*, *H. musciformis*, and *H. spinella*. More recently, Campbell *et al.* (2022) conducted a comprehensive study that included both molecular and morphological analyses and identified only two species, *H. cryptica* and *H. musciformis*, in North Carolina, southeastern USA coast.

Based on this scenario, the objective of this study was to reassess the taxonomic classification of *Hypnea* species along South Florida using molecular markers together with morphological observations. We also make phylogenetic inferences and explore the relationships between South Florida species and those within the Western Atlantic Ocean.

## Materials and methods

### *Collection site and environment*

A total of 70 *Hypnea* specimens were collected from seven locations in South Florida (Figure 1, Table 1): (1) Sebastian Inlet State Park, (2) Humiston Beach Park, (3) John D. MacArthur Beach State Park, (4) Sandspur Island, (5) Crandon Park, (6) Bahia Honda State Park and (7) Higgs Memorial Beach Park. The specimens were collected at low tide in the intertidal zone (0–1 m depth). All specimens were epiphytic on other macroalgae or seagrass, except for specimens from Sebastian Inlet, which were attached to hard substrates. Apical regions were separated from the thalli, cleaned, and stored in silica gel. The remaining thalli were preserved in 4% formalin in seawater for morphological study. Representative specimens were deposited in the Herbarium of the University of São Paulo (SPF), Brazil. Herbarium abbreviations follow the online *Index Herbariorum* (Thiers, 2023, continuously updated).



**FIGURE 1.** Map of Florida region showing sample collection sites for this study: 1. Sebastian Inlet State Park. 2. Humiston Beach Park. 3. John D. MacArthur Beach State Park. 4. Sandspur Island. 5. Crandon Park. 6. Bahia Honda State Park. 7. Higgs Memorial Beach Park.

The South Florida region experiences a maritime climate that falls into the subtropical category, featuring distinct wet (May–October) and dry (November–April) seasons. The coastline is dominated by mangroves, seagrasses, coral reefs, and hard bottom ecosystems. These features are intricately connected through the Gulf Stream conveyor system, originating from various locations in the broader Caribbean region (Lirman *et al.* 2019). Two significant oceanic currents influence South Florida’s coastal dynamics. The Loop current that flows southward along the western coast of Florida in the Gulf of Mexico and extends through the Lower Florida Keys, while the Florida current moves northward along the southeastern Florida Shelf (Lirman *et al.* 2019).

### *Morphological approach*

Samples preserved in 4% formalin for morphological study were sectioned by hand with a razor blade and stained with 1% aniline blue acidified with 1N HCl. Habit and diagnostic characters of each species were obtained with digital capture and image analysis using a digital camera.

**TABLE 1.** Collection information of specimens analyzed in this work. \*GB—GenBank accession numbers. \*\*\*numbers will be added before publication.

Species	Collection site, Latitude, Longitude	Vouchers	COI-5P*	<i>rbcL</i> *
<i>Hypnea caraibica</i>	Bahia Honda State Park, Loggerhead Beach, Big Pine Key, Florida, USA. 24°39'23"N, 81°16'37"W. coll. F. Nauer, D. Hatt, 20 February 2022	SPF58796	OR589204 to OR589213	OR588072
	Higgs Memorial Beach Park, Key West, Florida, USA. 24°32'49"N, 81°47'14"W. coll. F. Nauer, D. Hatt. 20 February 2022	SPF58795	OR589214 to OR589223	OR588077
<i>Hypnea cryptica</i>	Sebastian Inlet State Park, Melbourne Beach, Florida, USA. 27°51'38"N, 80°26'57"W. coll. F. Nauer, D. Hatt. 27 February 2022	SPF58797	OR589244 to OR589253	OR588080
<i>Hypnea musciformis</i>	Humiston Beach Park, Vero Beach, Florida, USA. 27°38'58"N, 80°21'13"W. coll. F. Nauer, D. Hatt, 03 March 2022	SPF58794	OR589224 to OR589233	OR588078
	John D. MacArthur Beach State Park, Palm Beach County, Florida, USA. 26°49'28"N, 80°02'17"W. coll. F. Nauer, L. Collado-Vides, 12 February 2022	SPF58798	OR589234 to OR589243	OR588079
	Crandon Park, Key Biscayne, Miami, Florida, USA. 25°47'27"N, 80°08'51"W. coll. F. Nauer, D. Hatt, A. Durain, L. Collado-Vides, 26 February 2022	SPF58799	OR589254, OR589255	OR588073, OR588075
<i>Hypnea spiniformis</i> sp. nov.	Crandon Park, Key Biscayne, Miami, Florida, USA. 25°47'27"N, 80°08'51"W. coll. F. Nauer, D. Hatt, A. Durain, L. Collado-Vides, 26 February 2022	SPF58793	OR589265 to OR589273	OR588076
	Sandspur Island, Miami, Florida, USA. 25°53'56"N, 80°08'13"W. coll. F. Nauer, A. Durain. 02 July 2022	SPF58792	OR589256 to OR589264	OR588074

### Molecular procedures

DNA sequences were generated at the University of São Paulo and the University of North Carolina at Wilmington. Methods used to generate sequence data followed the procedures of Taylor *et al.* (2017), Nauer *et al.* (2019b), and Campbell *et al.* (2022). Primers used for PCR and sequencing of COI-5P included GAZF1-GAZR1 or GWSFn-GWSRx (Saunders 2005, Saunders and McDevit 2012), and for *rbcL* they included F57-R1144 and F753-RrbcSstart (Freshwater & Rueness 1994, Iha *et al.* 2016). Individual sequence reactions were edited and assembled using Sequencher 5.4.6 (GeneCodes Corp., Ann Arbor, MI).

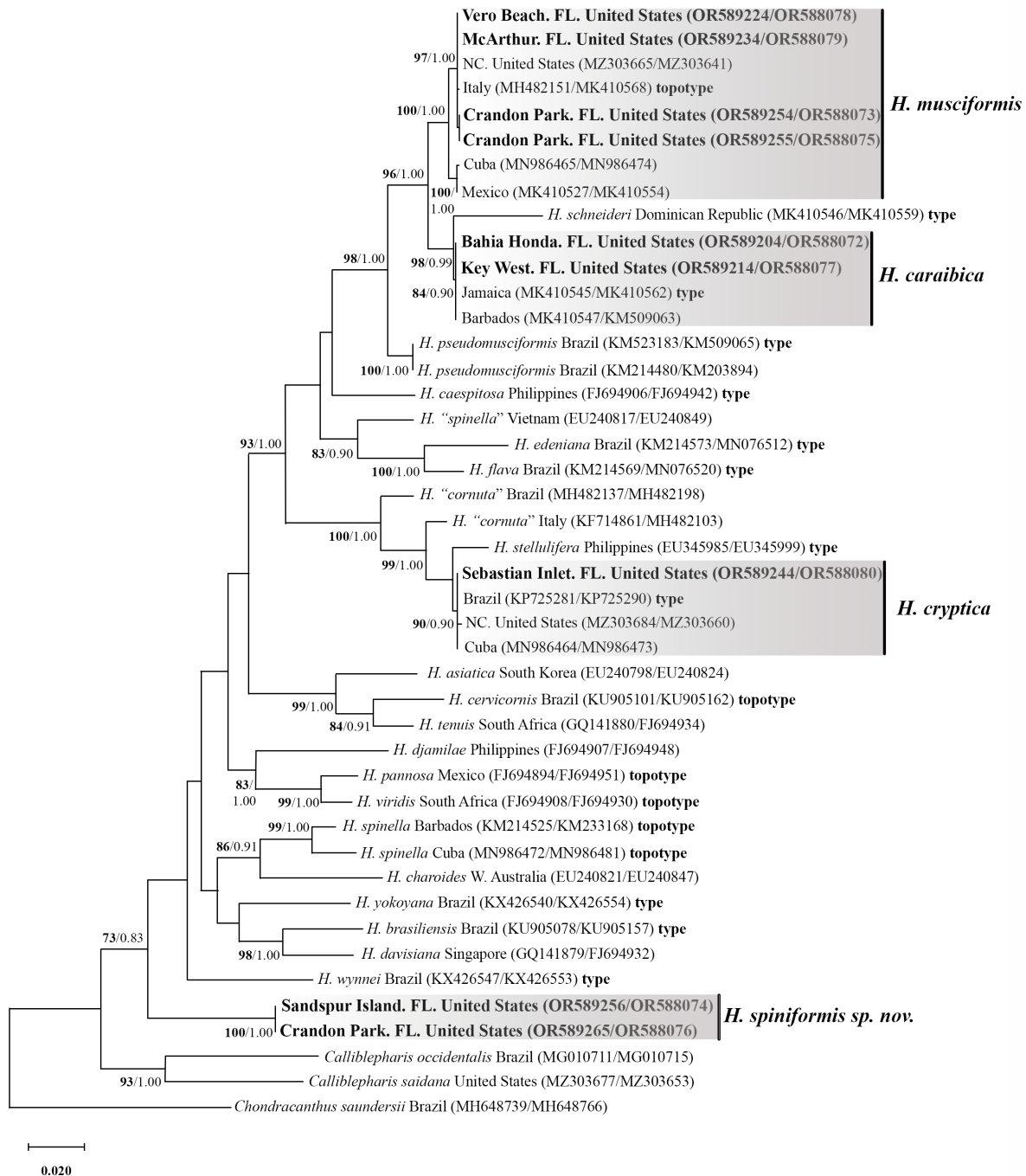
The COI-5P DNA alignment had 109 *Hypnea* sequences (70 new and 39 from GenBank) and 466 nucleotides (nt). The *rbcL* DNA alignment was comprised of 44 sequences and 825 nt (nine new and 35 from GenBank). The concatenated alignment (COI-5P + *rbcL*) was composed of 44 sequences (nine new and 35 from GenBank) with 1300 nucleotides (nt). For COI-5P, only Neighbor-Joining (NJ) analysis using a Maximum Likelihood (ML) model was performed with 2000 bootstrap replicates (Felsenstein 1985) using PAUP 4.0b10 (Swofford 2002). *Calliblepharis occidentalis* Joly & Yamaguishi-Tomita (MG010711), *Calliblepharis saidana* (Holmes) M. Y. Yang & M. S. Kim (MZ303677), and *Chondracanthus saundersii* C.W.Schneider & C.E.Lane (MH648739) were used as outgroups.

Phylogenetic relationships were estimated for the concatenated matrix (COI-5P + *rbcL*) using Maximum likelihood (ML) and Bayesian Inference (BI) analyses. Both analyses were performed on the CIPRES web portal (<http://www.phylo.org/index.php/>, Miller *et al.* 2010) using the GTR+I+G model. ML analysis was conducted using RAxML (Stamatakis *et al.* 2005) and a rapid hill-climbing algorithm (Stamatakis *et al.* 2008) for the best-scoring ML tree and 500 bootstrap trees. Bayesian analyses were performed using MrBayes 3.2.2 (Ronquist *et al.* 2012) using the same GTR model and two independent runs of four Monte-Carlo Markov Chains (MCMC) for 7,000,000 generations, sampling every 1000 generations and with a burn-in value of the first 25% of trees. Clades were considered supported when non-parametric bootstrap support was  $\geq 75\%$  in ML and Bayesian posterior probability (PP)  $\geq 0.9$ . *Calliblepharis occidentalis* (COI-5P: MG010711, *rbcL*: MG010715), *Calliblepharis saidana* (COI-5P: MZ303677, *rbcL*: MZ303653), and *Chondracanthus saundersii* (COI-5P: MH648739, *rbcL*: MH648766) were used as outgroups. Estimates of divergence values within and among species were computed using MEGA 11.0 (Tamura *et al.* 2021) to assess the level of variation in datasets, based on raw differences in similarity percentages between sequences.

## Results

### Molecular data

Concatenated analysis of COI-5P and *rbcL* DNA sequences (Figure 2) showed the presence of four *Hypnea* species among the sequenced specimens collected in Florida. Three of them grouped with type or toptype specimens of previously known species: *Hypnea caraiibica*, *H. cryptica*, and *H. musciformis*. A fourth clade, sister to all other *Hypnea* sequences analyzed here, was observed for samples from Crandon Park and Sandspur Island.



**FIGURE 2.** Maximum likelihood (ML) tree depicting phylogenetic relationships inferred among *Hypnea* species based on combined analyses of plastid *rbcL* and mitochondrial COI-5P sequences. ML bootstrap values (BS) are shown in bold; Bayesian posterior probabilities (BPP) are shown as regular text. Sequences generated in this work are also in bold. Accession numbers and country of origin, when available, are included for sequences obtained from GenBank. ‘Type’ indicates DNA sequences derived from type material, whereas ‘Topotype’ denotes DNA data from specimens from the type locality.

The *Hypnea musciformis* complex was recovered in four subclades with high bootstrap and posterior probability support in all analyses. Sequences from four South Florida locations grouped with the sequence of a *H. musciformis* topotype specimen from the Adriatic Sea (Italy). Interestingly, sequences from Florida Keys specimens clustered with the type sequence of *H. caraibica* from Jamaica. The latter is a new occurrence in the Florida coast. Sequences from *H. pseudomusciformis*, which occurs in the South Atlantic (Nauer *et al.* 2019a), formed a separate subclade with high bootstrap support. The divergence between *H. musciformis* sequences from South Florida and *H. caraibica* from the Florida Keys was 3.1% for the COI-5P and 1.5% for the *rbcL* markers (Table 2). Intraspecific diversity in the *H. musciformis* clade ranged from 0.3 to 2% for COI-5P and 0 to 0.2% for *rbcL* (Table 2). The highest divergence observed was between sequences from Cuba and those from Crandon Park and Sandspur Island, in the Miami, Florida region. No intraspecific divergence was found between the *H. caraibica* specimens.

The sequences from Sebastian Inlet grouped with the sequence of the *Hypnea cryptica* type specimen from Brazil (Jesus *et al.* 2019a), a species also previously reported for the coasts of Cuba (Cabrera *et al.* 2020) and North Carolina (Campbell *et al.* 2022). This species was only found at this site and was the only species of *Hypnea* found growing on hard substrate, while the others grew epiphytically on other macroalgae and seagrasses. *Hypnea cryptica* is also a new record for the Florida coast. *Hypnea cryptica* belongs to the *H. cornuta* species complex, a clade with at least five cryptic species (Jesus *et al.* 2019a).

Intraspecific divergences the four *Hypnea* species from Florida varied from 0 to 2.0% for COI-5P and 0 to 0.2% for *rbcL*. The interspecific divergences varied from 3.1 to 12.1% for COI-5P and 1.5 to 6.2% for *rbcL*. Crandon Park and Sandspur Island specimens formed a clade sister to all other *Hypnea* species. The divergences between those sequences and other *Hypnea* species used in the analyses were 13.2 to 16.2% for the COI-5P marker and 8.5 to 9% for *rbcL* (Table 2). However, there was a 0.3% divergence with the *rbcL* sequence (AF3856351) from a Florida Keys specimen identified as “*H. spinella*” by Hommersand & Fredericq (2003), which has a 7.2% divergence with a *H. spinella* topotype sequence (KM233168). Therefore, based on the phylogenetic species concept (Mayden 1997; Leliaert *et al.* 2014), we describe below a new species, *Hypnea spiniformis*, to accommodate a molecularly distinct lineage.

### Morphological observations

*Hypnea spiniformis* Nauer, Freshwater & M.T. Fujii *sp. nov.*  
(Figs 3–10)

DESCRIPTION: Thalli epiphytic, yellow, measuring 3–5 cm in height with cartilaginous texture (Fig. 3). One or multiple main axes percurrent, terete, 330–600 µm in diameter at middle portions. Dichotomous to subdichotomous branching at right angles in the basal portion and acute near the apex (Fig. 4). Rare anastomoses between branches. Apices of the branches and branchlets are acute, straight (Fig. 5), rarely forked, ending in an apical cell. Spinellike branchlets irregularly scattered at basal and middle portions of the thalli and alternately to distichous near the apex, turned upwards, 120–540 µm long, and 87–105 µm in diameter (Fig. 6). Secondary discoid holdfast usually in the last order of branches (Fig. 7). In cross-section 1–2 layers of pigmented cortical cells, 5–12 µm in diameter, and 1–2 layers of hyaline medullary cells (Fig. 8). Axial cell rounded to elliptical, 30–80 µm in diameter, surrounded by 4–6 periaxial cells, 40–120 µm in diameter. Tetrasporangial branchlets disposed throughout the thallus, mainly in their upper third part (Fig. 9). Tetrasporangia in sori surrounding the basal, median, and apical swollen portions of branchlets, 190–357 µm in diameter, zonately divided, 30–40 µm long and 12–25 µm in diameter (Fig. 10). Gametophytes not found.

HOLOTYPE: SPF58793, collected 26 February 2022 by F. Nauer, deposited in the Herbarium Collection of the São Paulo University (SPF), São Paulo, Brazil. Genbank accession numbers: OR589265 to OR589273 (COI-5P) and OR588076 (*rbcL*).

TYPE LOCALITY: Crandon Park. Key Biscayne. Florida. USA. (25°47'27"N / 80°08'51"W).

HABITAT: This species grows as epiphytes in lower intertidal and shallow subtidal seagrass beds.

DISTRIBUTION: Currently known only from locations on the southeastern coast of Miami and the Florida Keys.

ETYMOLOGY: The epithet refers to the spine-like branchlets of this species.

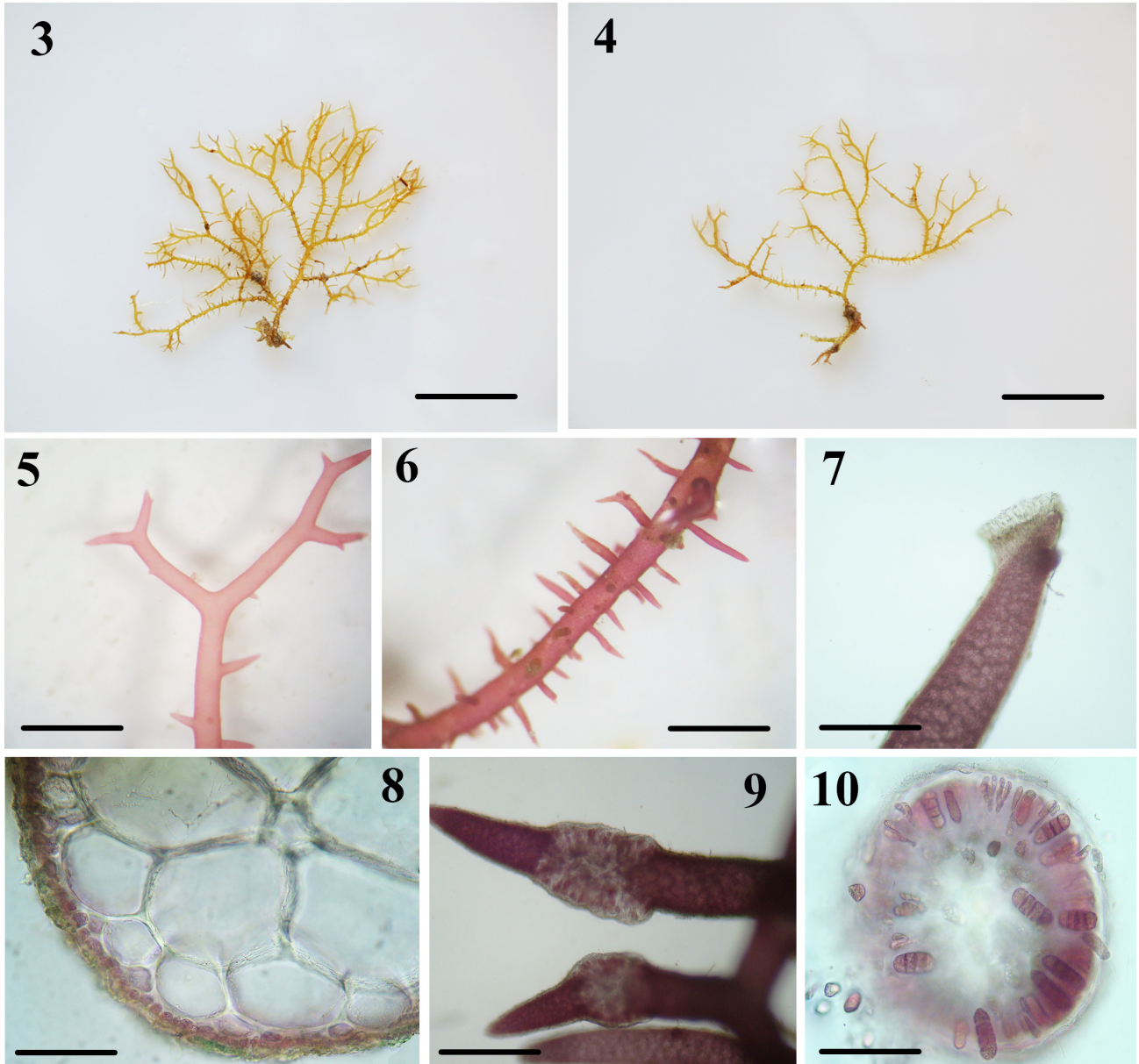
REPRESENTATIVE SPECIMENS EXAMINED: USA. FLORIDA: Crandon Park. Key Biscayne, Miami, 25°47'27"N / 80°08'51"W, coll. F.Nauer *et al.*, 26 February 2022 (SPF58793); Sandspur Island, Miami, 25°53'56"N / 80°08'13"W, coll. F.Nauer *et al.*, 02 July 2022 (SPF58792).

OBSERVATIONS: *Hypnea spiniformis* can be easily differentiated morphologically from *H. spinella* based on

**TABLE 2.** Divergence values for COI-5P (lower triangle, bold) and *rbcL* (upper triangle, italic) among some *Hypnea* specimens analyzed in this work.

<i>Hypnea caribbica</i>	Divergence (%)																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	<i>rbcL</i>
<b>Specimen</b>																				
1. US, FL, Bahia Honda (voucher)	-	0	0	0	6.2	6.2	6.2	6.2	6.2	1.5	1.5	1.5	1.5	1.6	1.6	9	9	11	8.9	
2. US, FL, Key West (voucher)	<b>0</b>	-	0	0	6.2	6.2	6.2	6.2	6.2	1.5	1.5	1.5	1.5	1.6	1.6	9	9	11	8.9	
3. Jamaica (MK410545/MK410562) <b>type</b>	<b>0</b>	<b>0</b>	-	0	6.2	6.2	6.2	6.2	6.2	1.5	1.5	1.5	1.5	1.6	1.6	9	9	11	8.9	
4. Barbados (MK410547/KM509063)	<b>0</b>	<b>0</b>	<b>0</b>	-	6.2	6.2	6.2	6.2	6.2	1.5	1.5	1.5	1.5	1.6	1.6	9	9	11	8.9	
5. US, FL, Sebastian Inlet (voucher)	<b>11.7</b>	<b>11.7</b>	<b>11.7</b>	<b>11.7</b>	-	0	0	0	0	5.6	5.6	5.6	5.6	5.8	5.8	8.5	8.5	9.9	8.2	
6. Brazil (KP725281/KP725290) <b>type</b>	<b>11.7</b>	<b>11.7</b>	<b>11.7</b>	<b>11.7</b>	<b>0</b>	-	0	0	0	5.6	5.6	5.6	5.6	5.8	5.8	8.5	8.5	9.9	8.2	
7. US, NC (MZ303684/MZ303660)	11.9	11.9	11.9	11.9	0.5	<b>0.5</b>	-	0	0	5.6	5.6	5.6	5.6	5.8	5.8	8.5	8.5	9.9	8.2	
8. Cuba (MN986464/MN986473)	11.7	11.7	11.7	11.7	<b>0</b>	<b>0</b>	<b>0.5</b>	-	5.6	5.6	5.6	5.6	5.6	5.8	5.8	8.5	8.5	9.9	8.2	
9. US, FL, Vero Beach (voucher)	3.1	3.1	<b>3.1</b>	<b>3.1</b>	<b>11</b>	<b>11</b>	<b>11</b>	<b>11</b>	-	0	0	0	0	0.2	0.2	8.5	8.5	10.7	8.7	
10. US, FL, McArthur (voucher)	3.1	<b>3.1</b>	<b>3.1</b>	<b>3.1</b>	<b>11</b>	<b>11</b>	<b>11</b>	<b>11</b>	<b>11</b>	<b>0</b>	-	0	0	0.2	0.2	8.5	8.5	10.7	8.7	
11. US, FL, Sandspur Island (voucher)	<b>3.3</b>	<b>3.3</b>	<b>3.3</b>	<b>3.3</b>	<b>11</b>	<b>11</b>	<b>12</b>	<b>11</b>	<b>0.3</b>	<b>0</b>	-	0	0	0.2	0.2	8.5	8.5	10.7	8.7	
12. US, FL, Crandon Park (voucher)	<b>3.3</b>	<b>3.3</b>	<b>3.3</b>	<b>3.3</b>	<b>11</b>	<b>11</b>	<b>12</b>	<b>11</b>	<b>0.3</b>	<b>0.3</b>	<b>0</b>	-	0	0.2	0.2	8.5	8.5	10.7	8.7	
13. US, NC (MZ303665/MZ303641)	<b>3.1</b>	<b>3.1</b>	<b>3.1</b>	<b>3.1</b>	<b>11</b>	<b>11</b>	<b>12</b>	<b>11</b>	<b>0</b>	<b>0</b>	<b>0.3</b>	<b>0.3</b>	-	0.2	0.2	8.5	8.5	10.7	8.7	
14. Cuba (MN986465/MN986474)	<b>3.1</b>	<b>3.1</b>	<b>3.1</b>	<b>3.1</b>	<b>11.9</b>	<b>11.9</b>	<b>12.1</b>	<b>11.9</b>	<b>1.8</b>	<b>1.8</b>	<b>2</b>	<b>2</b>	<b>1.8</b>	-	0	8.7	8.7	10.9	8.8	
15. Italy (MH482151/MK410568) <b>topotype</b>	<b>3.3</b>	<b>3.3</b>	<b>3.3</b>	<b>3.3</b>	<b>11.7</b>	<b>11.7</b>	<b>12.1</b>	<b>11.7</b>	<b>0</b>	<b>0</b>	<b>0.3</b>	<b>0.3</b>	<b>0</b>	<b>1.8</b>	-	8.7	8.7	10.9	8.8	
<i>Hypnea spiniformis</i> sp. nov.	13.2	13.2	13.2	13.2	16	16	16.2	16	13.6	13.6	13.6	13.6	13.6	14	<b>14.2</b>	-	0	8.8	8.2	
17. US, FL, Crandon Park (voucher)	13.2	13.2	13.2	13.2	16	16	16.2	16	13.6	13.6	13.6	13.6	13.6	<b>14</b>	<b>14.2</b>	<b>0</b>	-	8.8	8.2	
18. Brazil (MG010711/MG010715)	15.4	15.4	15.4	15.4	14.7	14.7	14.5	14.7	16.4	16.4	16.4	16.4	16.4	<b>16.4</b>	<b>16.4</b>	<b>16.4</b>	<b>16.4</b>	<b>16.4</b>	<b>16.4</b>	6.1
19. US (MZ303677/MZ303653)	<b>16.2</b>	<b>16.2</b>	<b>16.2</b>	<b>16.2</b>	<b>14.9</b>	<b>14.9</b>	<b>15.2</b>	<b>14.9</b>	<b>16.6</b>	<b>16.6</b>	<b>16.6</b>	<b>16.6</b>	<b>16.6</b>	<b>16.6</b>	<b>16.6</b>	<b>15.2</b>	<b>15.2</b>	<b>12.1</b>	-	
<b>COI-5P</b>																				

differences of the habit, thalli texture, anastomose frequency, and type of main axis. In *H. spinella*, the thalli exhibit a partially upright growth pattern, anchoring to rock surfaces by several disc-shaped holdfasts. *H. spinella* branches also possess a fleshy texture that form dense and intricately intertwined tufts, resembling cushion-like structures. Numerous anastomoses connect and interlace the thalli, allowing them to curve and twist in various directions, with the primary axes of the thalli often difficult to distinguish. However, *H. spinella* is morphologically similar to *H. cervicornis* and can only be correctly identified through molecular markers.



**FIGURES 3–10.** Holotype of *Hypnea spiniformis*. **3.** Habit of a tetrasporophytic thallus. Scale bar = 2 cm. **4.** Habit of a vegetative thallus. Scale bar = 2 cm. **5.** Detail of an apical portion of a branch. Scale bar = 200  $\mu\text{m}$ . **6.** Detail of spinelike branchlets. Scale bar = 200  $\mu\text{m}$ . **7.** Secondary fixation disc. Scale bar = 150  $\mu\text{m}$ . **8.** Transverse section of the thallus. Scale bar = 50  $\mu\text{m}$ . **9.** Tetrasporangial branchlets showing sori at the middle portions of branchlets. Scale bar = 100  $\mu\text{m}$ . **10.** Transverse section of a tetrasporangial branchlet showing zonately divided tetrasporangia. Scale bar = 30  $\mu\text{m}$ .

*Hypnea spiniformis* is also similar to *H. alopecuroides* and *H. valentiae*, two other species that have been reported for Florida. DNA comparisons are not possible because there is no type or topotype sequences available for these species, but they are morphologically distinct from *H. spiniformis*. Florida specimens identified as *H. alopecuroides* by Taylor (1928) are described as “intricate -caespitose, the larger branches alternately branched, spinulose except toward the apices,” while *H. spiniformis* does not form intricate tufts, and the branches can be easily separated by tweezers. *H. spiniformis* has also dichotomous to subdichotomous branching, and the spinelike branchlets are abundant throughout the thalli. Florida specimens described as *H. valentiae* by Littler *et al.* (2008) are brownish to red, with thalli exceeding



10 cm in height, and branches and branchlets at nearly right angles, creating a branching pattern that is more open and loosely arranged. Moreover, stellate branchlets could be found, in *H. valentiae* although it is an unreliable diagnostic feature. These characteristics differentiate it from *H. spiniformis*, in which the maximum observed height of the species was 5 cm, the color of the stalk of all specimens was yellow when alive and had the presence of secondary discoid holdfast in the last order of branches, that gave the thalli the tufted appearance.

*Hypnea caraibica* Nauer, Cassano & M.C. Oliveira  
(Figs. 11–12)

DESCRIPTION: Specimens were dark-red when alive, cartilaginous in texture, and 10–20 cm long (Fig. 11). Thalli fixed to the host plants by abundant tendrils at the ends of branches and branchlets. The branches are intricately interwoven, connecting through tendrils from either the same plant or different plants of the same species. Branches and branchlets also had straight or curved apices. Numerous spinescent branchlets irregularly arranged throughout the thalli with 200–425  $\mu\text{m}$  length (Fig. 12). In the subapical cross section, thalli had one layer of pigmented cortical cells and two to three layers of colorless medullary cells. The axial cell is smaller than or equal to the periaxial cells, although there are occasional instances where it may be larger. Axial cell with 28–150  $\mu\text{m}$  long and 40–112  $\mu\text{m}$  in diameter, surrounded by 5–7 periaxial cells with 40–190  $\mu\text{m}$  long and 52–148  $\mu\text{m}$  in diameter. Tetrasporangial sori completely or partially encircled the middle portions of branchlets. Tetrasporangia zonately divided, 25–50  $\mu\text{m}$  long and 15–27  $\mu\text{m}$  in diameter. Male and female plants were not observed.

OBSERVATIONS: *H. caraibica* was found on islands in the Florida Keys archipelago. All the collected plants were epiphytes of macroalgae growing in the shallow subtidal zone. No epilithic plants were found. Detailed descriptions and illustrations of this species are in Nauer *et al.* (2019b).

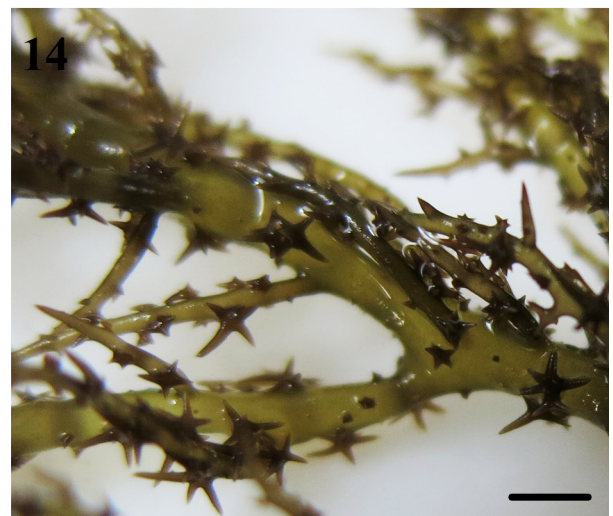
*Hypnea cryptica* P.B. Jesus & J.M.C. Nunes  
(Figs. 13–14)

DESCRIPTION: Thalli were erect, subcartilaginous, rosy to brownish red and measured 8–17 cm in height (Fig. 13). Star-shaped structures, known as stellate processes, possess 3 to 6 rays, exhibit a range of sizes, spanning from 300  $\mu\text{m}$  to 1536  $\mu\text{m}$ , commonly found through the thalli (Fig. 14). In cross section, the lower portion of axes showed a small, pigmented, circular axial cell, 29–73  $\mu\text{m}$  in diameter, surrounded by 4–6 oval periaxial cells, 47–264  $\mu\text{m}$  in diameter, 2–3 layers of hyaline medullary cells, 45–180  $\mu\text{m}$  in diameter that became gradually smaller towards the periphery, and 1–2 layers of pigmented cortical cells, 9–38  $\mu\text{m}$  in diameter. Tetrasporangial sori were found on the proximal portions of branchlets, containing zonate tetrasporangia, 24–53  $\mu\text{m}$  in length and 16–30  $\mu\text{m}$  in diameter. Cystocarps were globose, 647–1058  $\mu\text{m}$  in length and 672–1187  $\mu\text{m}$  in diameter, formed singly or aggregated. Carposporangia ovate, 24–34  $\mu\text{m}$  in diameter. Male gametophytes were not found.

OBSERVATIONS: *H. cryptica* was found at a single site, Sebastian Inlet State Park, and was the only species collected in this study that was not epiphytic. Plants occurred solitary or in aggregates, sometimes forming loose tufts. Easily identifiable from other species due to stellate processes on the branches. Detailed descriptions and illustrations of this species are in Jesus *et al.* (2019a).

*Hypnea musciformis* (Wulfen) J.V.Lamouroux  
(Figs. 15–16)

DESCRIPTION: Specimens were 5–15 cm long (Fig. 15), epiphytic, and attached to other macroalgae by tendrils. Thalli greenish to blackish-red when alive and cartilaginous in texture. The main axes are often undistinguishable, 525–925  $\mu\text{m}$  in diameter at middle portions. Branching irregular to alternate, with 3 or 4 orders of branches. Apices of the main axes and first- and second-order branches formed hooks or tendrils. Spine-like branchlets were straight or curved and irregularly scattered throughout the thalli; 300–900  $\mu\text{m}$  long and 130–240  $\mu\text{m}$  in diameter. In cross section, thalli with 1–2 layers of pigmented cortical cells and 3–4 layers of hyaline medullary cells. Axial cells rounded to elliptical, equal to or larger than periaxial cells, 32–150  $\mu\text{m}$  long and 40–137  $\mu\text{m}$  in diameter. Each axial cell surrounded by 5–8 rounded to elliptical periaxial cells, 45–137  $\mu\text{m}$  long and 42–150  $\mu\text{m}$  in diameter. Tetrasporangial branchlets disposed throughout the thalli, with tetrasporangial sori surrounding the swollen basal portions of branchlets or sometimes extending into the middle portions. Tetrasporangia zonately divided, cut off from the cortical cells, 37–65  $\mu\text{m}$  long and 15–27  $\mu\text{m}$  in diameter. Female and male gametophytes were not observed.



**FIGURES 11–16.** *Hypnea* species from South Florida. **11.** *H. caraibica*, habit of a tetrasporophytic thallus. Scale bar = 2 cm. **12.** *H. caraibica*, detail of hooks and tendrils in the apex of branches. Scale bar = 1 cm. **13.** *H. cryptica*, habit of a tetrasporophytic thallus. Scale bar = 2 cm. **14.** Detail of stellate processes on branches throughout the thallus of *H. cryptica*. Scale bar = 500  $\mu$ m. **15.** *H. musciformis*, habit of thallus from a Miami specimen. Scale bar = 1 cm. **16.** *H. musciformis*, detail of hooks, tendrils and spinescent branchlets from a Vero Beach specimen. Scale bar = 2 cm.

**OBSERVATIONS:** While *H. caraibica* inhabited islands of the Florida Keys, *H. musciformis* specimens were found from Miami northward. Morphologically, both species were very similar, but the spinescent branches of *H. musciformis* were generally shorter than those present in *H. caraibica*, and the abundance of apices ending in hooks

and tendrils was smaller. However, these characteristics can vary within and between species (Nauer *et al.* 2019b), and their correct identification can only be based on molecular markers. Compared to the specimens from the Gulf of Trieste, Italy (type locality), Florida specimens are similar, with long, flexible, and abundantly branched thalli that have fewer and shorter spiny branchlets scattered throughout the thallus and fewer tendrils present (Nauer *et al.* 2015). Detailed descriptions and illustrations of *H. musciformis* are in Nauer *et al.* (2015).

## Discussion

Since the advancement of molecular techniques, the systematics and taxonomy of red algae have been drastically modified, revealing a previously unknown diversity of cryptic species (Menezes *et al.*, 2015). This DNA survey, based on mitochondrial COI-5P and plastid *rbcL* markers, confirmed the occurrence of *Hypnea musciformis* plus three newly recorded species of *Hypnea* on the east coast of Florida, with *H. caraibica* and *H. cryptica* being the first reports for the state. Furthermore, the molecular data revealed that a fourth taxon presented high interspecific divergences, consistent with a distinct species described here as *H. spiniformis*.

*Hypnea cryptica*, despite being rarely found in this study, is the species most easily identified by morphology, distinct from the other *Hypnea* in the region because of its stellate processes. In the past, specimens with this characteristic were identified as *H. cornuta* (Dawes 1967, 1974, Dawes & Mathieson 2008, Littler *et al.* 2008, Taylor 1928). Only molecular data can confirm the correct identification of *H. cornuta*, *H. corona* Huisman & Petrocelli, *H. cryptica*, *H. stellulifera*, or other, yet undescribed, species in the complex (Jesus *et al.* 2019a, Huisman *et al.* 2021). Although *H. cryptica* was initially described for the coast of Brazil, later molecular studies revealed the presence of this species on the coast of Cuba (Cabrera *et al.* 2020) and North Carolina, USA (Campbell *et al.* 2021). Our data connect the distribution of this species between Cuba and the USA and suggest that this species has a continuous distribution along the Southeastern USA coast.

Another critical aspect to be considered is the breaks between the distributions of *Hypnea* species along the South Florida coast. Specimens of *H. caraibica* were only collected from the Florida Keys, while *H. musciformis* were sampled at sites from Biscayne Bay to Vero Beach. Based on molecular markers, Nauer *et al.* (2019) found that *H. musciformis* in the Atlantic Ocean represents a complex of four species with distinct geographic distributions. *Hypnea pseudomusciformis* occurred in the South Atlantic, *H. caraibica* and *H. schneideri* in the Caribbean Sea and *H. musciformis* in the North Atlantic. Further studies are needed to determine if geographical barriers separate their distributions along the Florida coast or if their distributions somehow overlap.

The combination of physiological and ecological data for *Hypnea* species and populations along the coast of Florida can help us explain which environmental factors are responsible for patterns of beta-diversity and intraspecific genetic breaks. Morphological differences in thallus architecture, size, and the number of branches were found between *H. pseudomusciformis* populations from Brazil's tropical and subtropical marine provinces (Nauer *et al.* 2015). Later, Nauer *et al.* (2019) found a genetic differences among populations from the same marine provinces.

*Hypnea spiniformis* is morphologically similar to *H. cervicornis* J. Agardh (1851: 451), which has already been reported from the Caribbean, Florida, and North Carolina (Børgesen 1920, Dawes 1974, Schneider & Searles 1976). Both species have multiple percurrent main axes, dichotomous to subdichotomous branching, and spinelike branchlets irregularly scattered on portions of the thalli turning upwards. Based on COI-5P and *rbcL* data, Jesus *et al.* (2016), following the International Code of Nomenclature for Algae, Fungi, and Plants, synonymized *H. aspera* Kützinger (1868) (as *H. boergesenii*) and *H. flexicaulis* Yamagishi and Masuda (2000) with *H. cervicornis* J. Agardh (1851), which has priority of publication. More recently, based on molecular markers, Jesus *et al.* (2023) also synonymized *H. marchantiae* Setchell & N.L.Gardner with *H. cervicornis*. The sequences used by the authors to represent *H. cervicornis* were collected in the type locality of that species, Bahia State, Brazil, "in litore maris prope Bahian." The interspecific divergence between *H. spiniformis* and *H. cervicornis* from the type locality was 13.4% for COI-5P and 8% for *rbcL*, values that corroborate them as distinct species.

In the past, *Hypnea cervicornis* was reduced to a synonym of *H. spinella* by Haroun & Prud'Homme van Reine (1993) based on ecological data. However, this synonymization was disputed by other studies (Geraldino *et al.* 2006) based on morphological and molecular data. Posteriorly, Nauer *et al.* (2014) provided molecular sequences of *H. spinella* specimens from the type locality in the Caribbean Sea (West Indies). Here, the interspecific divergence between *H. spiniformis* and *H. spinella* from the type locality was 14.2% for COI-5P and 8.1% for *rbcL*, confirming that they are different species. As with *H. cervicornis*, specimens morphologically identified as *H. spinella* have been

reported from both the east and west coasts of Florida (Dawes 1974, Dawes & Mathieson 2008, Littler *et al.* 2008), but these data have not been molecularly verified.

The phylogenetic reconstruction presented in this study recovers the same relationships between species presented in recent studies within the genus (Cabrera *et al.* 2020, Campbell *et al.* 2022, Jesus *et al.* 2019b, 2023). The novelty of the phylogeny presented here is that *H. spiniformis* is resolved as a sister clade to all other currently sequenced species of the genus *Hypnea*. A similar position was observed for a *rbcL* sequence (AF3856351) from a Florida Keys specimen identified as *H. spinella* by Hommersand & Fredericq (2003). After specimens of *H. spinella* from the type locality were sequenced by Nauer *et al.* (2014), this *rbcL* sequence (AF3856351) was labeled as *Hypnea* sp. by Jesus *et al.* (2019b). It is only 0.3% different from *H. spiniformis*, which is the correct identification of the specimen.

Other species previously cited for the Florida coast based on morphology were also not found and/or detected by molecular data. *Hypnea alopecuroides*, cited by Taylor (1929), has little information available in the literature. Still, it was recently mentioned in a marine algae checklist for the South China Sea (Phang *et al.* 2016) and Central and Southern Vietnam (Belous *et al.* 2020), although no morphological information is presented. The situation regarding *H. valentiae* is more obscure and taxonomically problematic. The species was first proposed as *Fucus valentiae* by Turner (1809) based on specimens from the Red Sea. Harvey (1834) reduced *F. valentiae* to a variety of *H. musciformis*. Later, Montagne (1841) restored the variety *valentiae* to a specific level, justifying the separation of *H. valentiae* from *H. musciformis* mainly by the presence of star-shaped stellate branchlets. In several subsequent studies in the literature, there was a confusion between *H. valentiae* and *H. cornuta*, another species that also has these stellate branchlets. That confusion led Price *et al.* (1992) to consider *H. cornuta* as a synonym of *H. valentiae*. Subsequently, Mshigeni and Chapman (1994) maintained the species as separate taxa and confirmed that both species have stellate branchlets. While molecular data showed that *H. cornuta* represents a complex of up to five species (Jesus *et al.* 2019a), including *H. cryptica*, Nauer *et al.* (2015), also based on molecular data, showed that the specimens described for the Brazilian coast as *H. valentiae* belong to *H. pseudomusciformis*. Although the species is widely cited in marine algae checklists around the globe (e.g. Greece, Tsiamis & Panayotidis 2019; Hawaiian Islands, Sherwood & Guiry 2023; México, Pedroche & Senties 2020; South China Sea, Phang *et al.* 2016), sequences of the type and/or type locality are necessary to clarify the validity of this species.

In conclusion, four species of *Hypnea* were confirmed from the coast of South Florida. Two of these (*H. caraibica* and *H. cryptica*) are new reports for the coast, and a new species, *H. spiniformis*, is described. *H. caraibica* and *H. musciformis*, both part of the *H. musciformis* species complex, have clear geographic distributions with a north-south separation at Biscayne Bay. Future phylogeography studies are needed to explore the genetic diversity of the populations of these two species and the possible marine biogeographical barriers responsible for isolating them.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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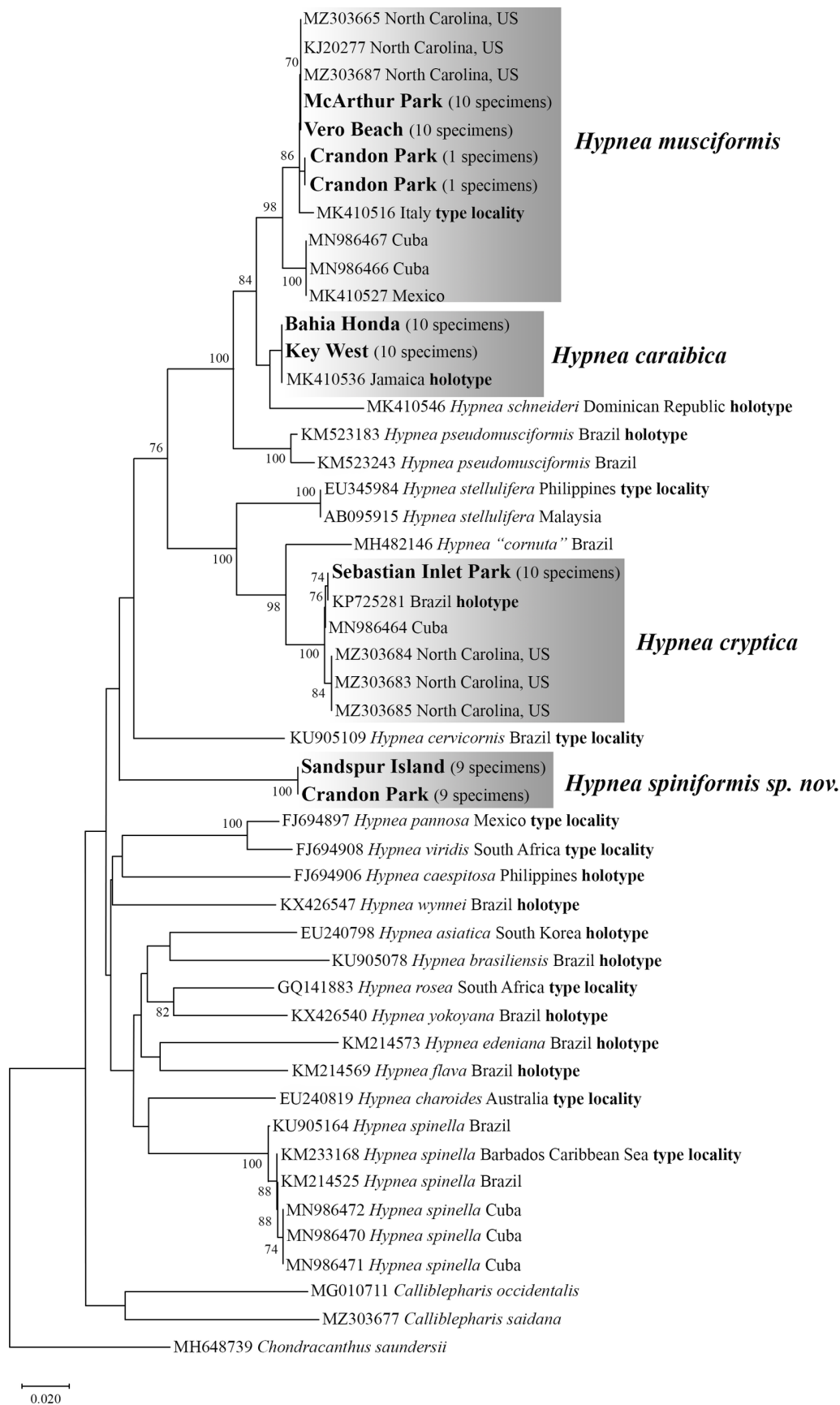
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**FIGURE S1.** Neighbor joining (NJ) tree depicting phylogenetic relationships inferred among *Hypnea* species based on mitochondrial COI-5P sequences. NJ bootstrap values (BS) are shown in bold. Sequences generated in this work are also in bold. When available, accession numbers and country of origin are included for sequences obtained from GenBank. ‘Type’ indicates DNA sequences derived from type material, whereas ‘Topotype’ denotes DNA data from specimens from the type locality.