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Two new flat species of *Gracilaria* (Gracilariales, Rhodophyta) from Brazil: *G. abyssalis* sp. nov. and *G. brasiliensis* sp. nov.

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A recent survey of the Brazilian Gracilariaceae (Rhodophyta, Gracilariales) flora aided by molecular-based phylogenetic analyses and comparative morphological data revealed two unidentified flat species of *Gracilaria* described here: *G. abyssalis* sp. nov. and *G. brasiliensis* sp. nov. *Gracilaria abyssalis* is a deep-water species characterized by a conspicuous holdfast; a long, branched, robust stipe; irregular to sympodial branching of the thallus; occasional presence of midrib; and an extensive range of broad blade morphologies. *Gracilaria brasiliensis* is a shallow intertidal species characterized by an inconspicuous stipe (< 1 cm), regular dichotomously branched thalli, and narrow blades and rounded apices. DNA sequence analyses of the chloroplast-encoded *rbc*L gene showed that flat species of *Gracilaria* in the western Atlantic belong to two distinct lineages. Maximum parsimony results placed *G. brasiliensis* as a member of the *G. mammillaris* clade with no support, while Bayesian results identified this species as a disntict new independent lineage. Furthermore, it was found that *G. cuneata* and *G. curtissiae* are recently diverged sister species and that *rbc*L phylogenetic distances among the morphologically distinct *G. intermedia* and *G. yoneshigueana* are quite small.

Key Words: Brazil, *Gracilaria*, EEZ, Marine algae, New species, Phylogeny, *rbc*L, Rhodophyta, Systematics, Western Atlantic

INTRODUCTION

The Gracilariaceae Nägeli 1847 (Gracilariales, Fredericq & Hommersand 1989) is composed of at least eight genera (Fredericq & Hommersand 1990; Zuccarello *et al.* 2004). Among them, *Gracilaria* Greville (1830) is a taxonomically challenging genus because of its structural simplicity, high morphological plasticity, and great species diversity. Since 1830 the genus *Gracilaria* has undergone a dynamic taxonomic history (e.g., Silva *et al.* 1996; Liao & Hommersand 2003; Gurgel *et al.* 2004a, b). This genus has received special research focus largely because of its economic importance as one of the main sources of agar in the world (Zemke-White & Ohno 1999). In this study we follow the global Gracilariaceae classification proposed by Gurgel & Fredericq (2004) and focus on the genus *Gracilaria sensu stricto*.

Chloroplast-encoded *rbc*L has been shown to be less conserved and more phylogenetically informative below the family level than the other two markers most used in the molecular systematics of red algae, namely, the nuclear 18S and the 28S rDNA genes (Freshwater *et al.* 1999). Therefore, the *rbc*L gene is well suited for phylogenetic reconstruction at and below the species level (McIvor *et al.* 2001; Gurgel *et al.* 2004b; Kim *et al.* 2006). Regarding the flat species of *Gracilaria*, *rbc*L-based phylogenies have helped to clarify phenotypic variation and elucidate species boundaries, such as confirming that the '*Gracilaria mammillaris* species-complex' was composed of several distinct species (Gurgel *et al.* 2004a).

The Brazilian benthic marine flora, with few exceptions (Yoneshigue & Valentin 1988; Yoneshigue-Valentin & Valentin 1992), has tropical and subtropical affinities and is rich in number of species of Gracilariaceae (Oliveira 1977). In Brazil, as in the rest of the world, flat species of *Gracilaria* are possibly the most morphologically variable and taxonomically difficult group of Gracilariaceae (e.g. Yamamoto 1984; Norris 1985; Millar 1997; Gurgel *et al.* 2004a; Skelton *et al.* 2004). During 2002 and 2003, collections were made between latitudes 10° and 21°S to reassess the systematics of the local Gracilariaceae flora. Here, we present results obtained from an *rbc*L sequence analysis and morphological comparisons of selected Brazilian members of the family based on these new collections. Two new species are described.

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MATERIAL AND METHODS

Collection and specimen preservation

Algal specimens were fixed in the field, in silica gel or 95% ethanol for the molecular study, and in 5% Formalin/seawater solution for morphological study. Whenever possible, voucher specimens were pressed on herbarium paper without being previously fixed in formalin solution and were deposited in SPF, UC, and Alg. Coll. US (herbarium abbreviations follow Holmgren *et al.* 1990).

Morphology

Cross sections were handmade using stainless-steel razor blades, stained with a modified iron-aceto-hematoxylin stain (Wittmann 1965) following the procedure of Hommersand & Fredericq (1988) or with a 3% aniline blue solution for 10–15 minutes, then fixed with 1 drop of 3% acetic acid and rinsed with distilled water (Tsuda & Abbott 1985) and mounted in a 50% clear Karo™ corn syrup/4% Formalin distilled water solution. Photomicrographs were taken with a Polaroid DMC Ie digital camera (Polaroid, Inc., Cambridge, MA) attached to an Olympus BX60 (Olympus, Melville, NY). Images were edited and assembled in plates using Photoshop 6.0 (Adobe Systems Inc., San Jose, CA).

Molecular analysis

DNA samples were prepared using the DNeasy Plant Mini Kit (QIAGEN, Valencia, CA). The *rbc*L amplification and sequencing primers were *Frbc*Lstart, F57, F577, F753, F993, R753, R1381, and *Rrbc*Sstart (as listed in Freshwater & Rueness 1994; Hommersand *et al.* 1994). Best PCR primer combinations used were FrbcL+R753, F557+R1381, and F993+RrbcS. Protocols for gene amplification, automated sequencing and alignment are identical to those given in Lin *et al.* (2001). Sample information and GenBank accession numbers are presented in Table 1.

DNA alignment was composed of one out-group, *Rhodymenia pseudopalmata*, and 45 in-group taxa. The first 100 nucleotides on the 5′ end of the alignment were removed from the analyses because some sequences were incomplete at this end. Maximum parsimony (MP) phylogenetic analysis was performed with PAUP* v. 4.0 beta 10 (Swofford 2002), and MP trees were inferred using unordered and unweighted characters in a heuristic search of 5000 random sequence addition replications (allowing only 25 trees to be held at each step) with the Tree Bisection Reconnection swapping algorithm (TBR) and saving multiple trees (MULTREES) options in effect. Support for nodes was assessed by calculating bootstrap proportions (BP) values (Felsenstein 1985) based on 1000 resamplings of heuristic searches with ‘simple’ sequence addition, TBR, and MULTREES options in effect. The ‘simple’ option was used during bootstrapping because in several different previous MP bootstrap proportion analyses, we used distinct sequence addition options (i.e. ‘simple’, ‘as is’, and ‘closest’) and no major differences were observed. The ‘random’ option using a robust number of replications was not computationally feasible with our computer facilities.

The optimal model of DNA sequence evolution to fit the alignment was estimated and chosen after comparing the results from four different analytical methods: the hierarchical likelihood ratio tests (hLRT), the standard Akaike information criterion (AIC), the corrected Akaike information criterion (AICc), and the Bayesian information criterion (BIC) performed by Modeltest version 3.7 (Posada & Crandall 1998; Posada & Buckley 2004). Alpha level of significance used in all model-selection tests was 0.01. Sample size function set for the AICc and the BIC tests was the total number of characters (i.e. 1367). HLRT and weighted and unweighted AIC tests selected the same model, the general time-reversible model with a portion of the data set composed of invariable sites and rate of variation among variable sites following a gamma distribution (GRT+I+G). Optimum model parameters obtained were as follows: base frequencies A = 0.3366, C = 0.1293, G = 0.1710, and T = 0.3632; substitution model rate matrix: A–C = 1.225, A–G = 6.9802, A–T = 0.8953, C–G = 2.0621, C–T = 11.7530, and G–T = 1.0; proportion of invariable sites = 0.5498; and the Gamma distribution shape parameter = 1.2927. Averaged or multimodal inferred parameters were not calculated because there were no uncertainties or incongruent results among different optimal model selection tests performed. The selected optimal model was applied in the Bayesian phylogenetic analysis using the software MrBayes 3.6 (Ronquist & Huelsenbeck 2003). The command line used in MrBayes that corresponds to the model chosen was *lset nst=6 rates =invgamma*. Starting parameter exact values (i.e. substitution matrix, alpha shape parameter for the gamma distribution, and the exact proportion of invariable sites) were automatically set arbitrarily for substitution model parameters (= default options) because it normally makes little practical sense to override this function by forcing specific values onto prior parameters in Bayesian analysis (Posada & Buckley 2004). The Bayesian analysis was conducted by running four chains of the Markov chain Monte Carlo (three hot, one cold), sampling one tree every 100 generations for 1,000,000 generations starting with a random tree. Reliability of the Bayesian consensus tree is given by the frequency at which each node appears among all saved trees after the moment when the maximum likelihood values from the trees sampled along the MCMC run reached stationary. Inferences about the phylogeny were based on a 50% majority rule consensus of those trees sampled after removal of the ‘burn-in’. This frequency corresponds to the true probability of the clades, the posterior probability (Hall 2001).

RESULTS

Gracilaria abyssalis Gurgel & Yonesh. *sp. nov.*

Figs 1–16

Algae 14–21 cm longae, pallidae rhodoroseae, complanatarum, cuneatarum, ramosarum laminarum conspicua stipite. Ramificatio

Table 1. Taxonomic identification and information about specimens included in the *rbc*L DNA alignment, including collection site, collector's name, date of collection, and GenBank accession number.

Species studied	Locality; collector and date	GenBank accession no.
Gracilaria apiculata J. Agardh	La Encrucijada, Peninsula Paraguaná, Falcón, Venezuela; coll. C.F. Gurgel, C.S. Carmona & J.E. Conde, 13 Jul. 1999	AY049333
G. arcuata Zanardini	Hilutangdu, Cebu, the Philippines; coll. S.M. Lin, 19 Apr. 1998	AY049383
G. beckeri (J. Agardh) Papenf.	Sharks Bay, Port Alfred, South Africa; coll. M.H. Hommersand, 19 Jul. 1993	AY049377
G. brasiliensis Gurgel & Yonesh.	Praia Rasa, Búzios City, Rio de Janeiro State, Brazil; coll. C.F. Gurgel & C. Simões, *FG#:249*, 5 Jan. 2002, voucher no. US Alg. Coll. 209094	EU380716
G. bursa-pastoris (S.G. Gmel.) P.C. Silva	Taranto, Mar Piccolo, Italy; coll. E. Cecere, 2 Apr. 1994	AY049373
G. canaliculata Sond.	Philippines; coll. S.M. Lin, Apr. 1998	AY049390
G. capensis F. Schmitz ex Mazza	Sharks Bay, Port Alfred, South Africa; coll. M.H. Hommersand, 19 Jul. 1993	AY049378
G. cervicornis (Turner) J. Agardh	Key West, FL, USA; coll. C.F. Gurgel, Jul. 1998	AY049365
G. chilensis Bird, McLachlan & Oliveira	Playa Charya, Coquimbo, Chile; coll. S. Fredericq, 19 Jan. 1995	AY049396
G. cuneata Aresch.	Itapoã Beach, Salvador City, Bahia State, Brazil; coll. C.F. Gurgel, 04 Nov. 2002	EU380717
G. curtissiae J. Agardh	Mangue Lloroso, Peninsula Paraguaná Peninsula, Venezuela; coll. C.F. Gurgel, J.E. Conde & C.S. Carmona, 13 Jul. 1999	AY049327
G. aff. damaecornis J. Agardh	Harbor Branch Oceanographic Institution jetty, Fort Pierce, FL, USA; coll. C.F. Gurgel, 13 Jul. 1998	AY049326
G. hayi Gurgel, Fredericq & J.N. Norris	Galeta Point, Panama; coll. B. Wysor, 21 Sep. 1999	AY049315
G. flabelliformis (P. & H. Crouan *in* Schramm & Maze) Fredericq & Gurgel in Gurgel & Fredericq	Buchuaco, Peninsula Paraguaná, Falcón, Venezuela; coll. C.F. Gurgel, C.S. Carmona & J.E. Conde, 13 Jul. 1999	AY049334
G. galetensis Gurgel, Fredericq & J.N. Norris	Galeta Point, Panama; coll. B. Wysor, 20 Apr. 1999	AY049320
G. gracilis (Stackh.) Steentoft, Irvine & Farnham	West Angle Bay, Wales, England; coll. M.H. Hommersand, 22 Jul. 1997	AY049399
G. intermedia J. Agardh subsp. *intermedia*	Buchuaco, Peninsula Paraguaná, Falcón, Venezuela; coll. C.F. Gurgel, C.S. Carmona & J.E. Conde, 13 Jul. 1999	AY049335
G. isabellana Gurgel, Fredericq & J.N. Norris	La Encrucijada, Peninsula Paraguaná, Falcón, Venezuela; coll. C.F. Gurgel, C.S. Carmona & J.E. Conde, 13 Jul. 1999	AY049332
G. mammillaris (Mont.) M. Howe	Offshore Louisiana, Gulf of Mexico, USA; coll. S. Fredericq, 26 May 2000	AY049323
G. multipartita (Clemente) Harv.	Carantec, Brittany, France; coll. J. Cabioch, 22 Jun. 1993	AY049317
G. occidentalis (Børgesen) Bodard	offshore Louisiana, Gulf of Mexico, USA; coll. S. Fredericq, 26 May 2000	AY049322
G. oliveirarum Gurgel, Fredericq & J.N. Norris	La Vela de Coro, Falcón State, Venezuela; coll. C.F. Gurgel, C.S. Carmona & J.E. Conde, 13 Jul. 1999	AY049330
G. ornata Aresch.	Fort Randolph, Panama; coll. B. Wysor, 26 Feb. 1999	AY049318
G. pacifica I.A. Abbott	Indian Inlet, WA, USA; coll. M.H. Hommersand	AY049397
G. salicornia (C. Agardh) E.Y. Dawson	Sulpa, Cebu, Philippines; coll. S.M. Lin, 19 Apr. 1998	AY049385
G. smithsoniensis Gurgel, Fredericq & J.N. Norris	Galeta Point, Panama; coll. B. Wysor, 20 Apr. 1999	AY049321
G. spinulosa (Okamura) C.F. Chang et B.M. Xia	Taiwan; coll. S.M. Lin, 11 May 1998	AY049395
G. tenuistipitata C.F. Chang & B.M. Xia	Sông Cái, Nha Trang, Vietnam; coll. Le Nhu Hau, 21 Mar. 2003	EU380718
G. textorii (Suringar) De Toni	Gobogahana, Japan; 10 Jul. 1994	AY049325
G. tikvahiae McLachlan	Morret pond, Pomquet Harbor, Antigonish Co., Nova Scotia, Canada; coll. C.J. Bird, 3 Jul. 1999	AY049434
G. venezuelensis W.R. Taylor	HBOI jetty, Indian River, Fort Pierce, FL, USA; coll. C.F. Gurgel, 20 Oct. 1998	AF539603
G. vermiculophylla (Ohmi) Papenf.	Hog Island Bay, Eastern Shore, VA, USA; coll. C. Tyler, 10 Feb. 1999	AY049312
G. vieillardii P.C Silva	Taiwan; coll. S.M. Lin, 22 Apr. 1998	AY049394
G. yoneshigueana Gurgel, Fredericq & J.N. Norris	Prainha Beach, Arraial do Cabo City, Rio de Janeiro State, Brazil; coll. A. Taouil, 13 Mar. 1998	AY049372
Hydropuntia caudata (J. Agardh) Gurgel & Fredericq	Walton Rocks, St. Lucie Co., FL, USA; coll. C.F. Gurgel, J.N. Norris & S. Fredericq, 11 Apr. 1998	AY049358
H. cornea (J. Agardh) M.J. Wynne	Venezuela, coll. C.F. Gurgel, J.E. Conde & C.S. Carmona, 13 Jul. 1998	AY049338
H. crassissima (Crouan & Crouan) M.J. Wynne	Fort Randolph, Colon, Panama; coll. B. Wysor, 6 Mar. 1999	AY049351
H. edulis (S.G. Gmel.) Gurgel & Fredericq	Little Santa Cruz, Philippines; coll. L.M. Liao, 28 Apr. 1998	AY049387
H. eucheumatoides (Harv.) Gurgel & Fredericq	Tambuli, Cebu, Philippines; coll.: S.M. Lin; 18 Apr. 1998	AY049389
H. preissiana (Sond.) Gurgel & Fredericq	Cervantes, Australia; coll. M.H. Hommersand, 20 Sep. 1995	AY049403
H. rangiferina (Kütz. Gurgel & Fredericq	Tema city, Ghana; coll. G. Ameka, leg. M.H. Hommersand, Mar. 2001	AY049379
H. secunda Gurgel & Fredericq	Tampa Bay, FL, USA; coll. C. Dawes, 26 Oct. 1999	AY049360
H. urvillei Mont.	Lee Point, Darwin, Australia; coll. M.H. Hommersand, 22 Nov. 1995	AY049402
H. usneoides (C. Agardh) Gurgel & Fredericq	Santa Rosalia bridge, Campeche Bay, Mexico; coll. C.F. Gurgel, 14 Feb. 1999	AY049346
Rhodymenia pseudopalmata (J.V. Lamour.) P.C. Silva (OUTGROUP)	Port Aransas Jetty, TX, USA; coll. C.F. Gurgel 17.v.98	AY1686565

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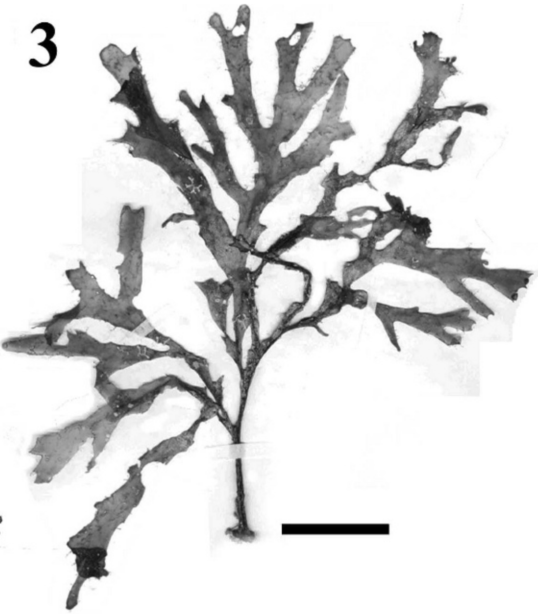

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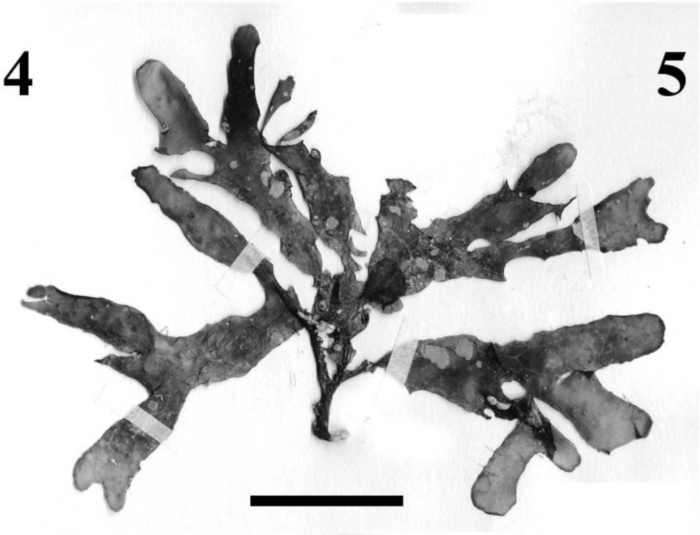

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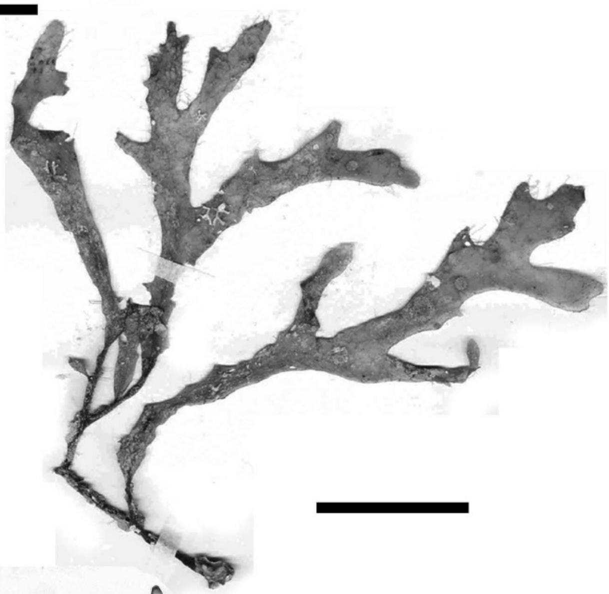

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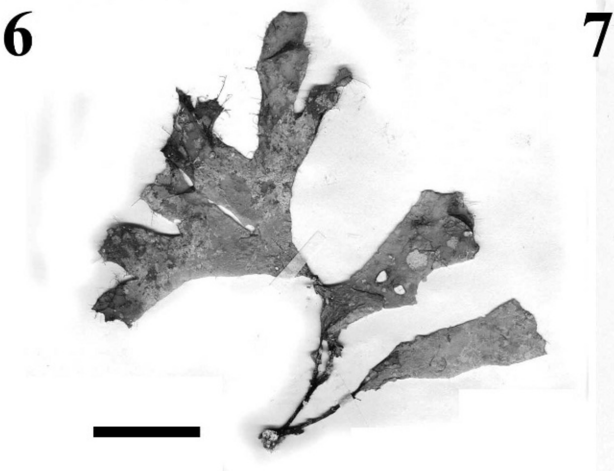

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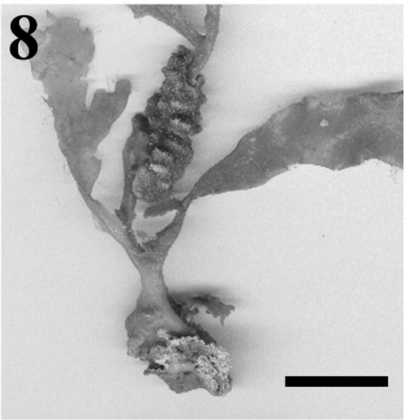

Figs 1–8. *Gracilaria abyssalis* sp. nov. phenotypic variation of the mature tetrasporic thalli (Figs 1–6, 8) and a sterile young thallus (Fig. 7).

Fig. 1. Holotype, tetrasporophyte, US Alg. Coll. 209083, scale bar = 4 cm.

Fig. 2. SPF *s.n.* 8/23, scale bar = 3 cm.

Fig. 3. US Alg. Coll. 209087, scale bar = 4 cm.

subdichotoma. Stipes cylindriceus usque ad compressus, (2) 4–5 (7) cm altus, 1–3 mm latus. In plantis juvenibus laminae sine costis marginesque simplices, angustae lanceolataeque aut latae flabellataeque. In maturis plantis, laminae coriaceae, 0.7–2.5 cm altae, 237–325 μm latae, basali costa, margines simplices irregulares aut saepe spinis latis broad marginalibus, interdum laceratae. Cellulae corticales formae variabilis, isodiametrae quadrataeque sed maximam paterm radialiter elongatae, 2.5 μm × 6.25 μm; cellular subcorticales compressae periclinaliter, 11.25 μm × 6.25 μm. Medulla 4–6 stratorum amplitudine cellularum variabili, transitio inter corticem medullamque abrupta. Cystocarpia brevia, 1.4–1.7 mm diam., subglobosa usque ad tholiformia, rostrata, variantibus constrictionis basalibus. Pericarpium 20–25 stratorum cellularum. Basis carposporophyti lata, conspicuis cellulis nutritriciis tubulosis. Cellulae fusionis genialis inconspicuae. Gonimoblasti placenta irregularis, lobata, ferens fasciculos parvorum, globosorum usque ad obovatorum carposporangiorum, 19–22.5 μm diam. Tetrasporangia decussata-cruciata, in non profundis soris.

Algae 14–21 cm long, pale pink to pale red, composed of flat, cuneate, branched blades supported by a conspicuous subdichotomously branched stipe. Stipe coriaceous to brittle, cylindrical to compressed, (2) 4–5 (7) cm tall, 1–3 mm thick. In young plants, blades lack midribs and margins are simple; blades narrow and lanceolate or broad and flabellate. In mature specimens, blades coriaceous, 0.7–2.5 cm wide, 237–325 μm thick, with basal midrib, simple margins; irregular or often with regular series of broad marginal spines, occasionally lacerate. Cortical cells of variable shape, isodiametric and quadrate but mostly radially elongated, 2.5 μm by 6.25; subcortical cells compressed periclinally, 11.25 μm by 6.25 μm. Medulla of 4–6 cell layers of variably sized cells, transition between cortex and medulla is abrupt. Cystocarps small, 1.4–1.7 mm diam. in traverse sections, subglobose to dome-shaped, rostrate, with variable levels of constrictions at base. Pericarp composed of 20–25 cell layers. Carposporophyte with broad cuneate based, with conspicuous tubular nutritive cells connecting gonimoblasts to cystocarp base. Fusion cells inconspicuous. Gonimoblast placenta irregular, lobed, bearing clusters of small, globose to obovate carposporangia, 19–22.5 μm in diameter. Tetrasporangia decussate-cruciate in shallow sori.

HOLOTYPE: US Alg. Coll. 209083, formalin-fixed (Fig. 1).

ISOTYPES: UC1819295 and UC1819296, US Alg. Coll. 209084 to 209087, SPF *s.n.*, four specimens on two sheets (8/23 and 9/23).

PARATYPES: US Alg. Coll. 209088 to 209094, UC1819297 to 1819299, SPF *s.n.* 35 specimens on four sheets (14/23 to16/23, and 23/23).

ETYMOLOGY: Species name was derived from *abyssus* (L.) referring to its deep-water habitat (Brown 1956).

TYPE LOCALITY: Revizee Central VI, station Y3, 21°09′55″S; 40°19′44″W, dredged, 47 m, 14 Jun. 2002.

DISTRIBUTION: Brazil, 21°00′00″S to 21°37′57″S, 40°19′00″W to 40°19′80″W.

COLLECTION SITES: Station Y3, 21°09′55″S, 40°19′44″W, 47 m, 14.vi.2002; Station Y4, 21°37′57″S; 40°01′27″W, 54 m, 14.vi.2002; Station Bio III, dredge II, 21°00′00″S, 40°19′00″W, 38 m, 25.vii.1999; Station 0481, 21°04′00″S; 40°48′00″W, 18 m, 25.vi.1999; and Station D19-C2/19, 21°10′59″S, 40°19′80″W, 50 m, 26.ii.1996.

HABITAT: *Gracilaria abyssalis* was collected between 18 and 54 m depth and is restricted to deep-water habitats of the SE Brazilian continental shelf on substrate of rhodoliths and other calcareous rocks of biogenic origin. This deep-water flora typically has temperate affinities caused by local upwelling (Yoneshigue-Valentin & Valentin 1992). Many of the specimens had large portions of their thalli covered by different species of crustose bryozoans. Although it was not possible to obtain a *rbc*L sequence from *G. abyssalis* fixed in ethanol, this species is morphologically distinct from any other described *Gracilaria* species (Table 2).

HABIT AND VEGETATIVE STRUCTURES: Algae 14–21 cm long, pale pink to pale red, composed of flat, cuneate, branched blades supported by a conspicuous subdichotomously branched stipe (Figs 1–8) borne on an incrusting discoid holdfast (Fig. 8). Stipe coriaceous to brittle, cylindrical to compressed, (2) 4–5 (7) cm tall, 1–3 mm thick, about 33 cell layers in diameter, composed of compact, roundish to compressed medullary cells decreasing in size towards the cortex; central medullary cells larger, 74–99 by 54–72 μm (Fig. 9). Stipe cortical cells radially elongated, 22.5–25 by 8.8–12.5 μm, occasionally isodiametric. In young plants, blades lack midribs and margins are simple; blades narrow and lanceolate or broad and flabellate (Fig. 7). In mature specimens, blades coriaceous, 0.7–2.5 cm wide, 237–325 μm thick, with basal midrib, simple margins; irregular or often with regular series of broad marginal spines, occasionally lacerate in habit (Figs 1–6). Blade cortex composed of 1–2 (3) cell layer(s), cortical cells of variable shape, isodiametric and quadrate but mostly radially elongated, 2.5 μm by 6.25; subcortical cells compressed periclinally, 11.25 μm by 6.25 μm (Figs 10, 11). In traverse blade sections, medulla of 4–6 cell layers of variably-sized medullary cells, 99–116 μm by 30–62 μm, decreasing in size towards the cortex, but transition between cortex and medulla is abrupt (Fig. 10).

REPRODUCTIVE STRUCTURES: Cystocarps small, 1.4–1.7 mm diam. in traverse sections, subglobose to dome-shaped, rostrate, with variable levels of constrictions at base, scattered on upper part of blade, isolated or in clusters. Pericarp composed of 20–25 cell layers; cells of innermost four pericarp layers spherical, anticlinally elongated or star-shaped, with numerous secondary pit connections, cells of outer layers becoming more compact and progressively radially elongated (Fig. 12). Carposporophyte with broad cuneate based, with conspicuous tubular nutritive cells connecting gonimoblasts to cystocarp base (Figs 12, 13). Floor of cystocarp composed of 3–5 layers of small, darkly staining cortical cells (Fig. 13). Fusion cells

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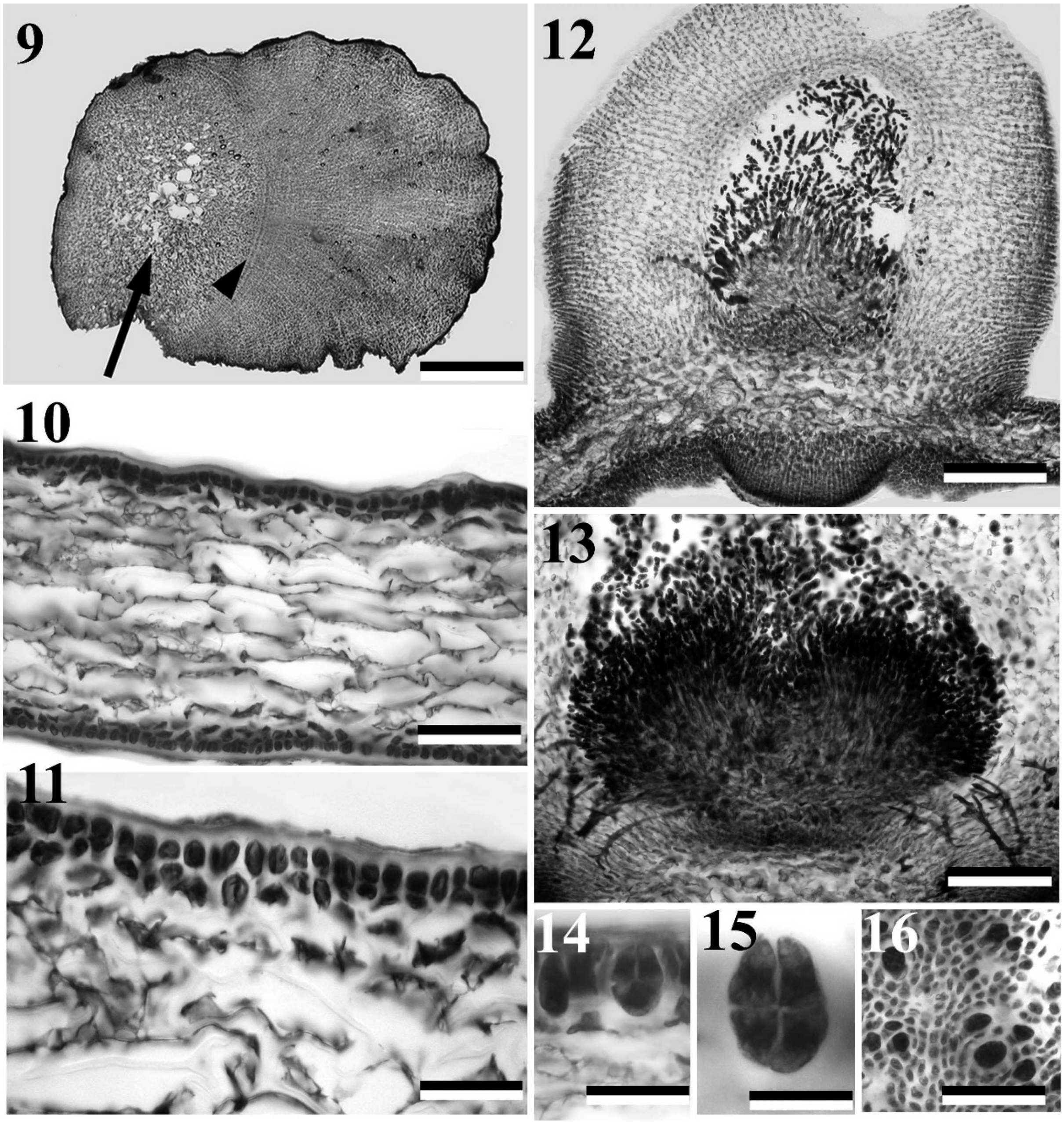
Fig. 4. UC 1819296, scale bar = 3 cm.
Fig. 5. US Alg. Coll. 209092, scale bar = 3 cm.
Fig. 6. US Alg. Coll. 209093, scale bar = 3 cm.
Fig. 7. US Alg. Coll. 209089, scale bar = 3 cm.
Fig. 8. SPF *s.n.* 8/23 (= Fig. 2). Detail of holdfast and small stipe, scale bar = 1 cm.



Figs 9–16. *Gracilaria abyssalis* sp. nov. (Figs 9–11, 14–16. US Alg. Coll. 209083; Figs. 12–13. US Alg. Coll. 209088).

Fig. 9. Traverse section through stipe, central compact medulla (arrow), separation between old cortex and new cortical growth (arrowhead), scale = 0.7 mm.

Fig. 10. Traverse section of blade, showing abrupt transition between cortex and medulla, scale bar = 100 μm.

Fig. 11. Detail of cortex composed of 1–2 cortical cell layers, scale bar = 40 μm.

Fig. 12. Longitudinal section of young cystocarp, showing layers of small cortical cells at cystocarp floor, lateral tubular nutritive cell connecting gonimoblast mass to pericarp (left side) and an aborted cystocarp on opposite side of blade, scale bar = 150 μm.

Fig. 13. Longitudinal section of cystocarp detailing carposporophyte; note darkly stained basal and lateral tubular nutritive cells and carpospores, scale bar = 200 μm.

Fig. 14. Traverse section through tetrasporic sorus, decussate tetrasporangium, scale bar = 50 μm.

Fig. 15. Cruciate tetrasporangium, scale bar = 36 μm.

Fig. 16. Surface view of tetrasporic blade, scale bar = 75 μm.

inconspicuous. Gonimoblast placenta irregular, lobed, bearing clusters of small, globose to obovate carposporangia, 19–22.5 μm in diameter (Figs 12, 13). Tetrasporangia decussate-cruciate (Figs 14, 15), scattered (Fig. 16), flanked by radially elongated cortical cells, 19 by 6 μm, in shallow sori on one or both sides of blade. Mature tetrasporangia 25–30 by 19–24 μm to 42–44 by 27–31 μm. Spermatangia not found.

Table 2. Comparative summary of main morphological characters of flat species of *Gracilaria* from the western Atlantic Ocean and other locations.

Characters	*G. abyssalis* (this study)	*G. brasiliensis* (this study)	*G. cearensis* (Joly *et al.* 1965)	*G. cuneata* (Ganesan 1989)
Plant length (cm)	14–21	11–14 (20)	15	3–12
Blade width (mm)	70–250	1–4 (5)	2–11	10–20
Thallus thickness (µm) (*1)	237–325	290–315	180	600–1000
Stipe length × thickness (mm)	20–70 × 1–3	4–12 × 0.4–1.0	30 in length	10 × 1
Thallus branching	pseudodichotomous/ variable	pseudodichotomous/ irregular	di-polychotomous	pseudodichotomous
Branching position	marginal, all in one plane	marginal, all in one plane	marginal only	marginal, all in one plane
Cortical cell layers (*1)	1–2 (3)	1 (2)	1–2	1 (2)
Cortical cell shape	isodiametric, variable (*3)	variable, mostly radially elongated	–	isodiametric, (quadrate)
Cortical cell size (µm) (*1)	2 × 6	(4) 6 (8) × (6) 9 (11)	7.4 × 9.2	–
Gland/hair cells	No	No	no	large
Cortex-medulla transition	sharp	gradual	sharp	sharp
Number of medullary cell rows	4–6	7–9	5–6	8–11
Shape of medullary cells in transverse sections	compressed periclinaly	compressed periclinaly	compressed	isodiametric
Medullary cell size (µm) (*1)	99–116 × 30–62	29 (75) 104 × 85 (121) 185	120 × 66	–
Distinct cystocarpic features	1.4–1.7 mm diam., thick and branched basal lateral tubular nutritive cells only	tubular nutritive cells evenly distributed, fusion cell not persisting	broad-based, tubular nutritive cells evenly distributed	up to 1 mm diam., base not constricted, basal and lateral tubular nutritive cells only
Habitat	deep water, on rodoliths, upwelling region	shallow subtidal in moderately exposed sites, partially buried by sand	in drift	always collected in drift
Remarks	*4	crystals inside medullary cells	types examined in SPF	*6

Gracilaria brasiliensis Gurgel & Yonesh. *sp. nov*

Figs 17–30

Algae complanatae omnino, 11–14 (20) cm longae, 1–4 (5) cm altae, 290–315 µm latae, erectae, carnosae, cartilagineae, ruber ribis saepe partibus superis luteis. Ramificationes partibus basalibus infernisque sparsae, subdichotomae, partibus superis abundae pseudodichotomae plana eadem. Stipes subcylindriceus, 0.4–1.2 cm long, 0.4–1 mm lat. Laminae magnarum centralium acoloratarum medullarium cellularum. Medulla 7–9 stromatica, amplitudinibus variabilibus, transitio inter corticem medullamque gradatim, cellulis centralibus medullosis leviter periclinaliter compressis. Crystallum in centralibus cellulis medullaribus solum. Cortex 1 (2) stromaticus cellularum formis variabilibus, cellulae subcorticales, rotundae usque ad ovales. Cystocarpia prominentia, subglobosae usque ad tholiformia, constrictae prope basim, rostratae, basi constricta. Pericarpium usque ad 19–22 strata, conspicuis cellulis nutritriciis tubulosis. Cellula fusionis interdum conspicua, 25 µm lata, 130 µm alta. Tetrasporangia cruciata aut decussata-cruciata. Cortex tetrasporangialis 3 (4) stratosa, cellulae corticales externae elongatae radialiter, 6–10 µm long., 3–6 µm lat. Spermatangia not visa.

Algae flat throughout, 11–14 (20) cm long, 1–4 (5) cm wide, 290–315 (mean = 305) µm thick; erect, fleshy, cartilaginous, dark red to vinaceous color throughout or often with yellow apical portions. Branching rarely in basal and lower portions of the blade, pseudodichotomous to irregular on the distal portions of the blade, always on the same plane. Stipe subcylindrical, 0.4–1.2 cm long, 0.4–1.0 mm diameter. Blades composed of large, colorless medullary cells. Medulla composed of 7–9 cell layers of variable size, transition between cortex and medulla gradual, central medullary cells slightly periclinally compressed. Crystals present in central medullary cells only. Cortex composed of 1 (2) cell layers, cortical cells of variable shape. Subcortical cells round to oval. Cystocarps prominent, subglobose to dome shaped, rostrate, constricted at base. Pericarp of up to 19–22 cell layers thick. Presence of regularly distributed tubular nutritive cells connecting the gonimoblasts to the pericarp. Fusion cell sometimes conspicuous, 25 µm wide by 130 µm tall. Tetrasporangia cruciately divided or decussate-cruciate. Tetrasporophyte cortex of 3 (4) cortical cell layers, outer cortical cells radially elongated, 6–10 µm long by 3–6 µm wide. Spermatangia not found.

HOLOTYPE: US Alg. Coll. 209095, formalin-free specimen (Fig. 17).

ISOTYPES: US Alg. Coll. 209096, SPF s.n. (three specimens on one sheet).

PARATYPES: UC 1819279–1819284, US Alg. Coll. 209097–209105, SPF s.n. (22 specimens in four sheets).

ETYMOLOGY: The epithet refers to the endemic nature of this species in Brazil.

TYPE LOCALITY: Brazil, Rasa Beach, Búzios City, Rio de Janeiro State (22°45′S, 41°52′W).

DISTRIBUTION AND SPECIMENS EXAMINED: Brazil, Rio de Janeiro State: Buzios City, Rasa Beach (as per type collection) and Caravelas Beach (SPF 55606, 27 Jan. 2000, *E.C. Oliveira*; 56115, 17 Jan. 2000, *E.C. Oliveira*). Espirito Santo State: Ponta da Fruta (SPF 53214, 19 Aug. 1978, *E.J. Paula*), Barra do Sai (SPF 26251, 20 Aug. 1979, *E.C. Oliveira*), Peracanga Beach (SPF 52824, 09 Jul. 1967, *E.C. Oliveira*) and Curva da Baleia Beach (US Alg. Coll. 209097–8, 3 Mar. 2002 *C.F. Gurgel & V.P. Oliveira*).

HABITAT: So far *Gracilaria brasiliensis* is known only from northern Rio de Janeiro and Espirito Santo States, Brazil. It is a perennial species, attached to rocky substratum but with basal

Table 2. Extended

G. curtissiae (this study)	*G. galetensis* (Gurgel *et al.* 2004a)	*G. halogenea* (Millar 1990)	*G. hayi* (Gurgel *et al.* 2004a)	*G. intermedia subsp. intermedia* (Gurgel *et al.* 2004a)
up to 40	1–16 (20)	40	5–10 (15)	18 (20)
up to 150	5–8	15–20	14–16 (25)	3–5
620–1000	254–275	2200	up to 100	685–884
absent	8–10 × 1–1.5	55 × 7.0	10–20 in length	absent
pseudodichotomous/ irregular	pseudodichotomous/ subdichotomous	irregular to alternate	pseudodichotomous/ subdichotomous	pseudodichotomous/ subdichotomous
marginal and in middle of frond	no marginal branchlets, branches sometimes constricted at base	marginal, and in middle of frond	marginal, all in one plane	marginal only, opposite to alternate, young branchlets constricted at base
1	1–2 (3)	1–3	1 (2)	2 (3)
isodiametric	isodiametric, mostly radially elongated	slightly radially elongated	radially compressed	variable, isodiametric to radially elongated
4–6 max. diam.	(4) 5 (10) × (5) 9 (13)	10 × 12	3–6 × 6–10	5–9 × 5–7
large but uncommon	yes	No	small	conspicuous, 13.8 × 6.3 (–8.8)
sharp	gradual	gradual	sharp	gradual
3–4	4–5	38,542	4–5	8–10
ovoid-inflated, thin cell walls	isodiametric to compressed	compressed periclinaly	compressed periclinaly	isodiametric to compressed
261–329 (398) max. diam.	(101) 133 (153) × (57) 81.5 (111)	450 × 250	50–65 (86) max. diam.	(225) 245 (275) × (110) 117 (145)
evenly scattered on both surfaces	wide base, usual absence of cortical cell layers at cystocarp base	tubular nutritive cells evenly distributed, fusion cell mostly persisting	1–2 mm diam., constricted base	750–1700 μm diam.; constricted base; conspicuous ostiole; large, rounded, thick-walled gonimoblasts
subtidal	shallow subtidal	subtidal, in heavily shaded environs	deep water, 50–90 m	shallow subtidal, protected sites
most cortical cells dome-shaped	outer medullary cells pigmented	thick stipe with 'growth rings'	blade margins smooth	plants with thick, mucilaginous cell walls, round cortical cells

portions of the thalli often buried in sand, in the shallow subtidal of areas with little to moderate wave action. The habitat of *G. brasiliensis* is not influenced by coastal upwelling and is characterized by inshore turbid waters resulting from the discharge of fine sediments into the ocean from nearby rivers.

HABIT AND VEGETATIVE STRUCTURES: Algae flat throughout, 11–14 (20) cm long, 1–4 (5) cm wide, 290–315 (mean = 305) μm thick; erect, fleshy, cartilaginous, dark red to vinaceous color throughout or often with yellow apical portions (Figs 17–19); attached by a small round to irregularly shaped holdfast. Branching rarely in basal and lower portions; and abundantly pseudodichotomous to irregular branched in a same plane on the distal portions. Stipe subcylindrical, 0.4–1.2 cm long, 0.4–1.0 mm diameter, composed of a small central medulla of compressed, 96–141 (mean = 119) by 56–90 (mean = 72) μm, thick-walled medullary cells 9.0–16 (mean = 12.5) μm thick; and a thick cortex (Fig. 20); pit connections conspicuous (Fig. 21). Stipe cortex of 6–14 cell layers of densely compacted, isodiametric to mostly radially elongated, small cortical cells, 6–13 μm long (Fig. 20); outer cortical cell walls 10–15 μm thick. Blades composed of large, colorless medullary cells and a cortex of 1–2 cells (Figs 22, 23). In traverse sections, medulla composed of 7–9 cell layers of variable size, larger cells in the center, decreasing in size outwards to the cortex; transition between cortex and medulla gradual (Figs 22, 23); central medullary cells slightly periclinally compressed, 71–77 (mean = 75) by 98–112 (mean = 110) μm, with cell walls 6.0–6.5 μm thick. In longitudinal sections, medullary cells elongated, 87–125 (mean = 108) by 50–54 μm. Crystals present in central medullary cells only, one crystal per cell, size proportional to cell size (Fig. 22). Proteinaceous material adjacent to cell wall connecting pit plugs. Outer cortical cells variable, most radially elongated, oval or isodiametric, 4–8 (mean = 6) by 6–11 (mean = 9) μm; subcortical cells slightly larger, round to oval, 9–11 μm diameter (Fig. 23).

REPRODUCTIVE STRUCTURES: Cystocarps prominent, subglobose to dome-shaped, rostrate, constricted at base, 0.8 (1.5) mm tall by 1.5 (2.0) mm wide, scattered over both sides of the blade and margins, singular or in clusters (Figs 24, 25). Pericarp of up to 19 (22) cell layers; internal cells of first four layers star-shaped, with numerous secondary pit connections; cells of outer layers becoming more compact and progressively quadrate to radially elongated. Mature carposporophyte 540–1000 μm diameter, with constricted base and regularly distributed tubular nutritive cells connecting the gonimoblasts to the pericarp (Figs 24–26). Carposporophyte base of 3–5 layers of small, darkly-staining cells of cortical origin (Figs 27, 28). Fusion cell sometimes conspicuous, centrally placed inside the gonimoblast mass, subclavate, 25 μm wide by 130 μm tall (Fig. 27). Gonimoblast placenta irregularly organized, lobed, bearing irregular clusters of carposporangia at different stages of maturity; terminal mature carposporangia 25–31 (mean = 36) μm diameter (Fig. 25). Tetrasporangia cruciately divided or decussate-cruciate, radially

Table 2. Extended

G. isabellana (Gurgel *et al.* 2004b)	*G. mammillaris* (Gurgel *et al.* 2004a)	*G. occidentalis* (Gurgel *et al.* 2004a)	*G. oliveirarum* (Gurgel *et al.* 2004a)	*G. smithsoniensis* (Gurgel *et al.* 2004a)
12	7–10	27–31	10–14	3.0–5.5
18	5–8	6	15	2–2.5
1400	250–330	810 (at the margins)	500–750	up to 300
up to 6 in length	40–70 in length	up to 15 in length	absent	10–35 in length
irregular and variable	irregular, pseudodichotomous	pseudodichotomous	irregular	pseudodichotomous, wide angle
marginal, all in one plane	marginal, all in one plane	marginal, all in one plane	marginal, all in one plane	branchlets not common
1 (2)	1 (2)	1 (2)	1 (2)	1 (2)
variable, mostly radially elongated	mostly isodiametric; sometimes anticlinally elongated	periclinaly compressed	variable; inconspicuous, mostly radially elongated	variable; mostly periclinally elongated
(12) 21 (31) × (8) 13 (19)	(4) 6 (8) × (6) 9 (12)	(5) 7.0–10 × (9) 14 (16)	3–5 (9) max. diam.	4–6 × (5) 6.5 (8)
Rare	no	small	rare	no
gradual	sharp	sharp	sharp	sharp
7–11	4 (–5); rounded to slightly compressed	5–7 (8)	4–5 with 2 distinct large central cells	5–6; smaller and isodiametric
compressed periclinaly	variable	variable, round to compressed	compressed periclinaly	isodiametric
198 (329) 516 × 101 (175) 247	100–120 (125)	103–120 (135)	150–190 × 304–400	(62) 66 (74) × (79) 106 (116), thick-walled
conspicuous central fusion cell	thick internal gonimoblasts	–	not seen yet	conspicuous, wide base, 3–4 layers of small cortical cells at base
intertidal, under strong wave action and sand scour	deep water, on calcareous substrata, 50–100 m	deep water, on rodoliths, 50–100 m	shallow subtidal, moderately exposed sites	shallow subtidal
formerly *G. lacinulata* Vahl	*5	*2	blades often elongated, even in young branchlets	medullary cells often pigmented

^{*1} = value range, exceptional maximum or minimum value observed in parentheses.
^{*2} = with pigmented outer medullary cells, with the largest and most spherical medullary cells among these species; gland cells more common at margins.
^{*3} = different thallus parts, with different cortical cell shapes as seen in transverse sections.
^{*4} = presence of midrib in well-developed specimens.
^{*5} = presence of distinct subcortex formed by single large strongly compressed cell layer.
^{*6} = lateral margins sometimes undulated. Tetrasporangia in sori, 15–20 µm broad, 30–40 µm long.

elongated, 31–16 µm long, 14–19 µm wide (Figs 29, 30); scattered on both blade surfaces, sorus formation not evident. Tetrasporophyte cortex of 3 (–4) cortical cell layers, outer cortical cells radially elongated, 6–10 µm long by 3–6 µm wide; small, thin, long, cortical filaments of 3–4 cells surround mature and immature tetrasporangia (Fig. 30). Spermatangia not found.

Morphological analyses. Comparison of morphological characters among flat *Gracilaria* species known from the western Atlantic plus other selected species were summarized in Table 2.

Phylogenetic analyses. MP produced a single MPT with the phylogenetic position of five species carrying very low support (i.e. ≤ 57): *G. brasiliensis*, *G.* sp. Philippines, *G. textorii*, *G. vermiculophylla*, and *G. spinulosa* (Fig. 31). After removing a 'burn-in' composed of 146 trees, the 50% majority-rule consensus tree was constructed based on the remaining 9854+1 trees. Bayesian consensus tree was fully resolved with the exception of two nodes (Fig. 32). Main topological differences between both trees were: (1) the two *Hydropuntia* lineages formed a monophyletic and well-supported clade in the Bayesian tree (PP = 0.86), but in the MP tree they were paraphyletic; (2) *G. brasiliensis* is a well-supported (PP = 1.0) and independent monotypic lineage in the Bayesian tree but part of the *G. mammillaris* clade in the MPT with very low support (BV = 55); (3) the *G. tikvahiae – G. isabellana – G. aff. damaecornis* clade is a well-supported (PP = 0.99) and an independent lineage in the Bayesian tree but sister to the *G. mammillaris* clade in the MPT with no bootstrap support; and (4) the *G. venezuelensis – G. yoneshigueana – G. intermedia* clade is well supported (BV = 99) and basal to all other derived clades in the MPT (which are characterized by *Textorii*-type of spermatangial conceptacles; Yamamoto 1984) but internal and well supported in the Bayesian tree (PP = 0.80). The 15 most derived species in both trees were resolved in a number of relatively well-supported smaller clades, with the exception of *G. spinulosa*, *G.* sp. from the Philippines and *G. textorii* (all three western Pacific species), and low to no support among some of the deeper nodes, the latter particularly pronounced in the MPT (Figs 31, 32).

Gracilaria cuneata Areschoug (1854) is sister to *G. curtissiae* J. Agardh (1885) with high phylogenetic support (BV = 100, PP = 1.0) and there is very low sequence divergence between the two taxa (0.8%, uncorrected

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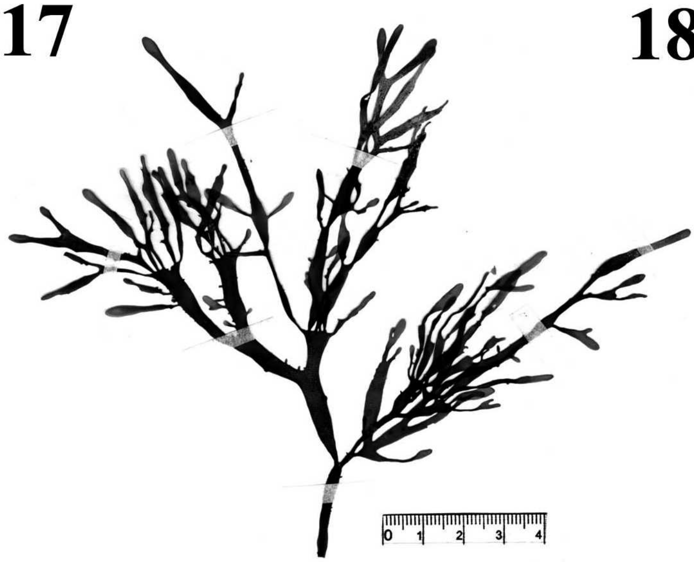

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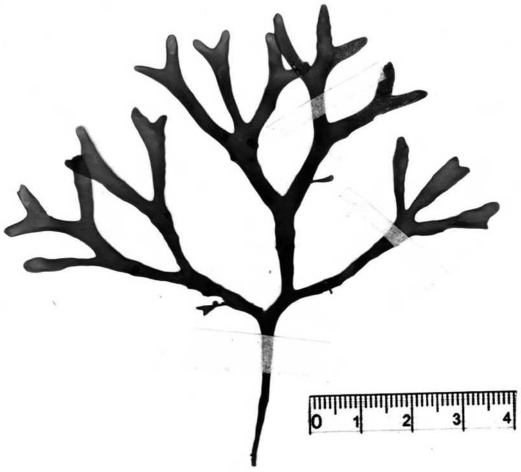

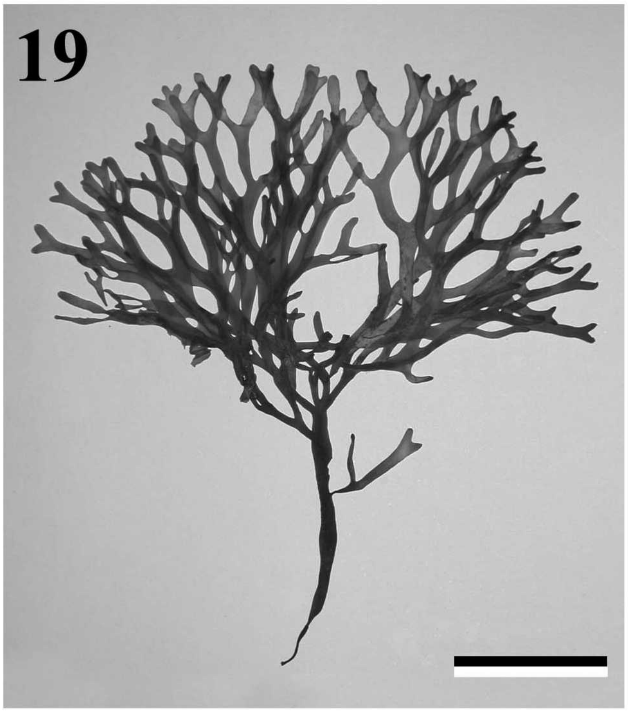

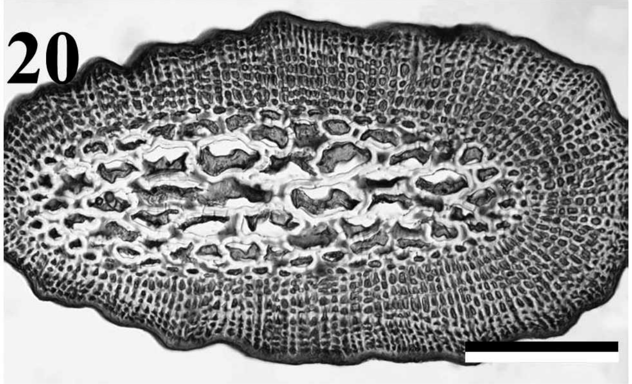

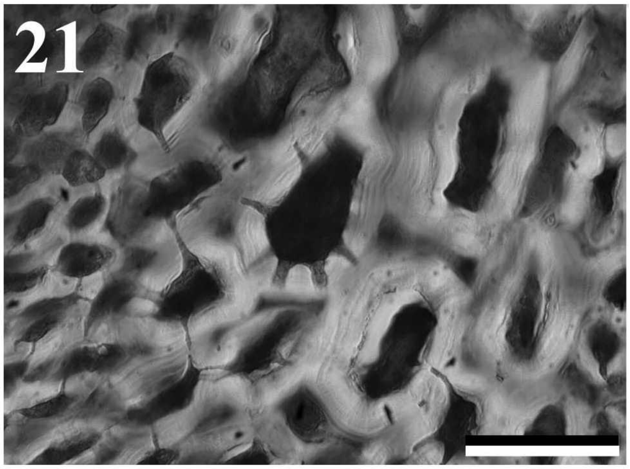

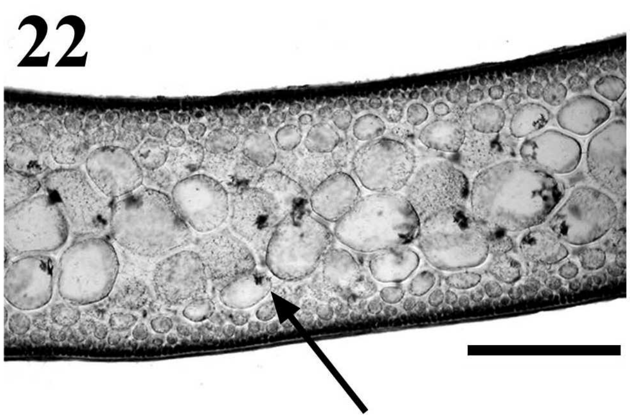

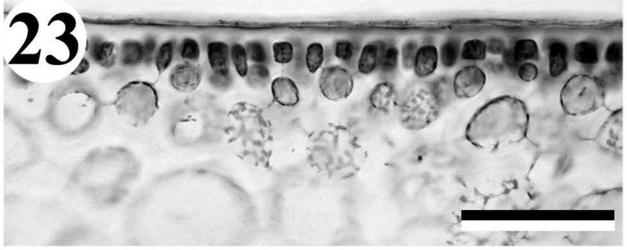

Figs 17–23. *Gracilaria brasiliensis* sp. nov. (Figs 17, 20–23: US Alg. Coll. 209095).

Fig. 17. Holotype, cystocarpic specimen, scale bar = 4 cm.

pairwise distance). These two species form a clade sister to a second clade that is also solely composed of western Atlantic *Gracilaria* species such as *G. cervicornis* (Oliveira *et al.* 1983) and *G. apiculata* (Gurgel *et al.* 2004a). The phylogenetic relationship of these two clades with yet another tropical-subtropical western Atlantic clade (*G. flabelliformis* – *G. occidentalis* – *G. ornata*) remained unresolved in both the Bayesian and MP trees (Figs 31, 32).

DISCUSSION

Gracilaria abyssalis was collected on hard rhodolith substratum during the Central REVIZEE cruise VI off the SE coast of Brazil. While *G. abyssalis* is morphologically very distinct from any other Gracilariaceae species in the western Atlantic, it resembles *G. halogenea* Millar (1990:337) from Australia (Table 2). Both share the presence of a robust stipe composed of several layers of cortical cells, and some *G. abyssalis* specimens display lacerations or 'margins with acute teeth' as described for *G. halogenea* (fig. 17B in Millar 1990). Despite similarities among the habit of some specimens, other morphological features distinguishing these two taxa include stipe length and thickness (far more robust in *G. halogenea*), the arrangement of tubular nutritive cells surrounding the carposporophyte (basal and lateral in *G. abyssalis* vs homogeneously distributed in *G. halogenea*), presence or absence of midrib (present in *G. abyssalis* vs absent in *G. halogenea*) and cystocarp size (larger and clearly constricted at the base in *G. halogenea* vs smaller and variably constricted in *G. abyssalis*). Another difference is that *G. abyssalis* does not emit the distinctive smell noted for *G. halogenea* (Millar 1990).

The second-closest species to *G. abyssalis* is *G. stipitata* Withell, Millar & Kraft (1994), which is also endemic to eastern Australia (i.e. Lord Howe Island). They share the presence of terete to slightly compressed, single to branched conspicuous stipes; transition from stipe to blade gradual (sometimes abrupt in *G. abyssalis*), stipe persisting as thickened midrib that gradually tapers into the blade (not always present in some delicate specimens of *G. abyssalis*); blade width varying from broad to narrow fronds; thallus texture tough to soft and pliable when fresh but brittle when dry; color pinkish-red to yellow-brown; and abrupt transition between cortical and medullary cells, presence of traversing filaments since early stages of cystocarp development and traversing filaments restricted to basal regions of the carposporophyte. Major morphological differences between these two species include maximum observed thallus height (8 cm in *G. stipitata* and 21 cm in *G. abyssalis*), the shape of stereotypic fronds (palmate in *G. stipitata* and lacerated in *G. abyssalis*), presence of marginal projections in surface view of the blade (absent in *G. abyssalis*) and thallus thickness (350–600 μm in *G. stipitata* and 237–325 μm in *G. abyssalis*).

Even though flat species of *Gracilaria* along the Brazilian coast display enormous morphological variation, recent abundant collections and the phylogenetic results herein clearly delineate *G. brasiliensis* as distinct from all other flat *Gracilaria* species. In the western Atlantic, similar-looking species to *G. brasiliensis* are *G. galetensis*, *G. smithsoniensis* and *G. oliveirarum* (see Gurgel *et al.* 2004a). In habit *G. brasiliensis* blades are always narrower than those of *G. galetensis* and *G. oliveirarum* but broader and longer than those of *G. smithsoniensis*. *Gracilaria brasiliensis* has two distinguishable morphotypes, one with simple dichotomous branching (Figs 18, 19) and another with a more proliferous and irregular branching pattern (Fig. 17). A unique feature observed in *G. brasiliensis* was the presence of crystals inside medullary cells (Fig. 22, arrows). Another species superficially resembling *G. brasiliensis* is *G. cearensis* (Joly & Pinheiro) Pinheiro & Joly (1966, p. 131; basionym: *Tylotus cearensis* Joly & Pinheiro in Joly *et al.* 1965, p. 81) from Ceará, Brazil, but it can be distinguished from *G. brasiliensis* by its sparser branching and wider blades (Table 2).

Gracilaria cuneata vs *G. curtissiae*. *Gracilaria cuneata* Areschoug (1854, p. 351) was described from Pernambuco State, NE Brazil, and the type locality is probably the city of Recife (state capital of Pernambuco and a major local coastal port). Taylor (1969) provided a revised description of *G. cuneata* and lectotypified the species. Later, Ganesan (1994) selected another lectoptype specimen since Taylor's lectotype was missing from *S*. The original description of *G. cuneata* (Areschoug 1854) strongly emphasizes the cuneate nature of its blades. However, cuneate blades are characteristic of several species of *Gracilaria*, such as *G. hayi* Gurgel, Fredericq & J. N. Norris, *G. galetensis* Gurgel, Fredericq & J.N. Norris and *G. smithsoniensis* Gurgel, Fredericq & J.N. Norris (see Gurgel *et al.* 2004a), as well as *G. curtissiae* and *G. abyssalis*. Specimens of *G. cuneata* used in this study came from Itapoã beach, Salvador city, Bahia State, NE Brazil, and are morphologically identical to the lectotype specimens from Recife city, Pernambuco State, Brazil (cf. Taylor 1969; Ganesan 1994). Our results showed that *G. cuneata* is a sister taxon to *G. curtissiae* (Agardh 1885; type locality: Florida) and does not belong in the *G. mammillaris* complex (Fig. 31). Both species display overlapping phenotypes, and species identifications can be made only for specimens with habits matching those of their

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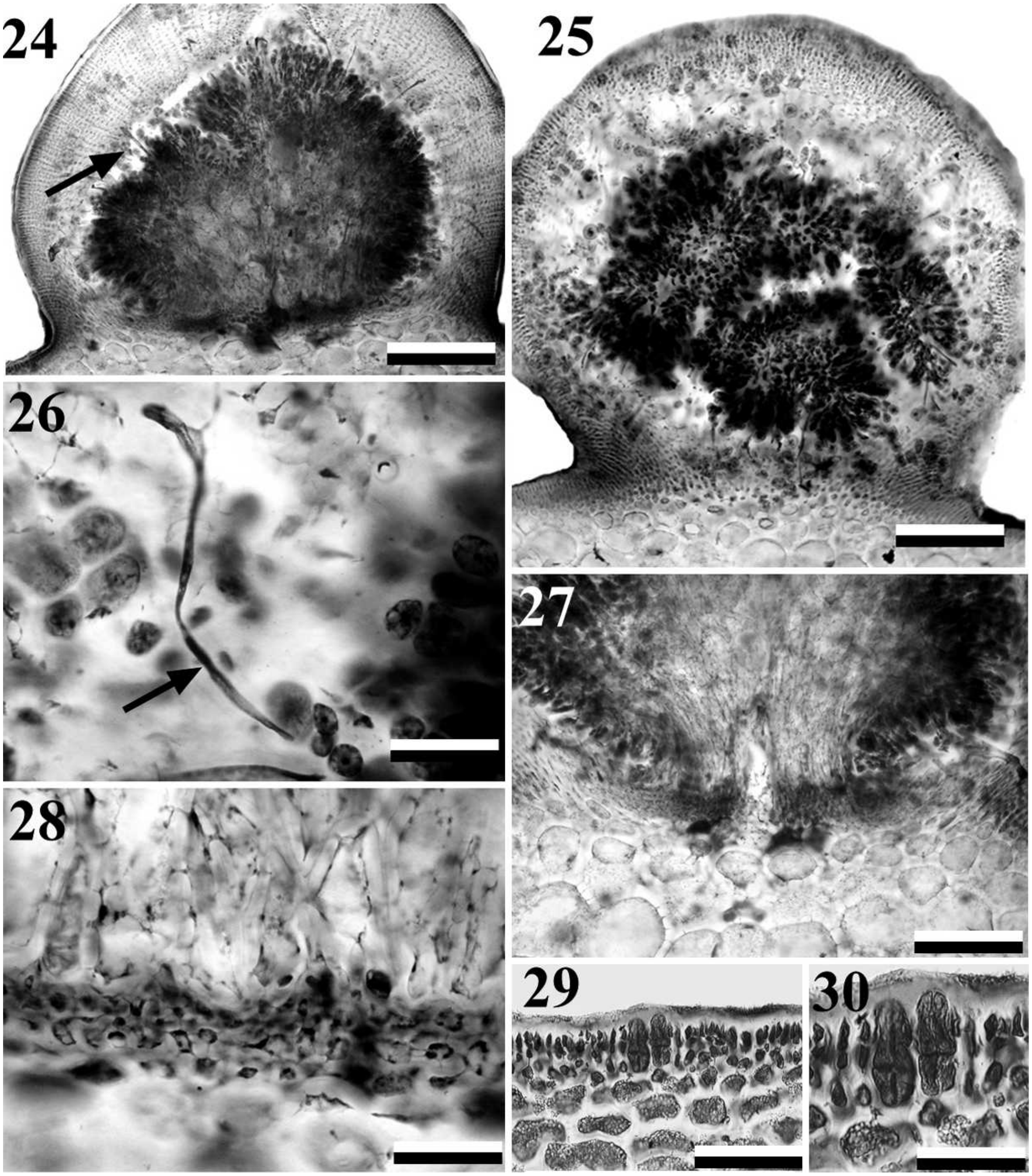
Fig. 18. Young cystocarpic specimen with regular pseudodichotomous branching pattern, scale bar = 4 cm (SPF#: *s.n.*).
Fig. 19. Older specimen with prolific branching; note simple unbranched basal portion subtending the branched part of the thallus, scale bar = 4 cm (UC# 1819279).
Fig. 20. Traverse section of stipe, showing medulla surrounded by thick multilayered cortex, scale bar = 100 μm.
Fig. 21. Medullary stipe cells, note thickened and striated cell walls and wide intercellular connections, scale bar = 40 μm.
Fig. 22. Traverse section through blade; some cylindrical medullary cells have internal crystals (arrow), scale bar = 100 μm.
Fig. 23. Cortical region, showing gradual transition between cortex and medulla, scale bar = 40 μm.



Figs 24–30. *Gracilaria brasiliensis* sp. nov. Figs 24–28: US Alg. Coll. 209094, Figs 29–30: US Alg. Coll. 209098, FG#251b).

Fig. 24. Longitudinal section of cystocarp, showing tubular nutritive cells evenly distributed (arrow is depicting one tubular cells), scale bar = 250 μm.

Fig. 25. Longitudinal section of cystocarp; note gonimoblasts organized in clusters and few tubular nutritive cells, scale bar = 250 μm.

Fig. 26. Tubular nutritive cell connecting pericarp to gonimoblasts (arrow), scale bar = 50 μm.

Fig. 27. Cystocarp with central fusion cell, scale bar = 200 μm.

Fig. 28. Layers of small cortical cells in cystocarp floor separating medullary cells (below) from gonimoblast cells (above), scale bar = 100 μm.

Fig. 29. Traverse section of tetrasporophyte cortex, scale bar = 100 μm.

Fig. 30. Cruciate tetrasporangia, scale bar = 40 μm.

respective types. Ganesan (1994) pointed out that based only on morphology, these two species could be considered synonymous or at least closely related. The most distinct morphological features that separate these two species are blade width and medullary cell size (both larger in *G. curtissiae* vs narrower and smaller in *G. cuneata*), branching pattern (abundant in *G. cuneata* vs often scarce in *G. curtissiae*), and apex shape (often lacerate, ragged or

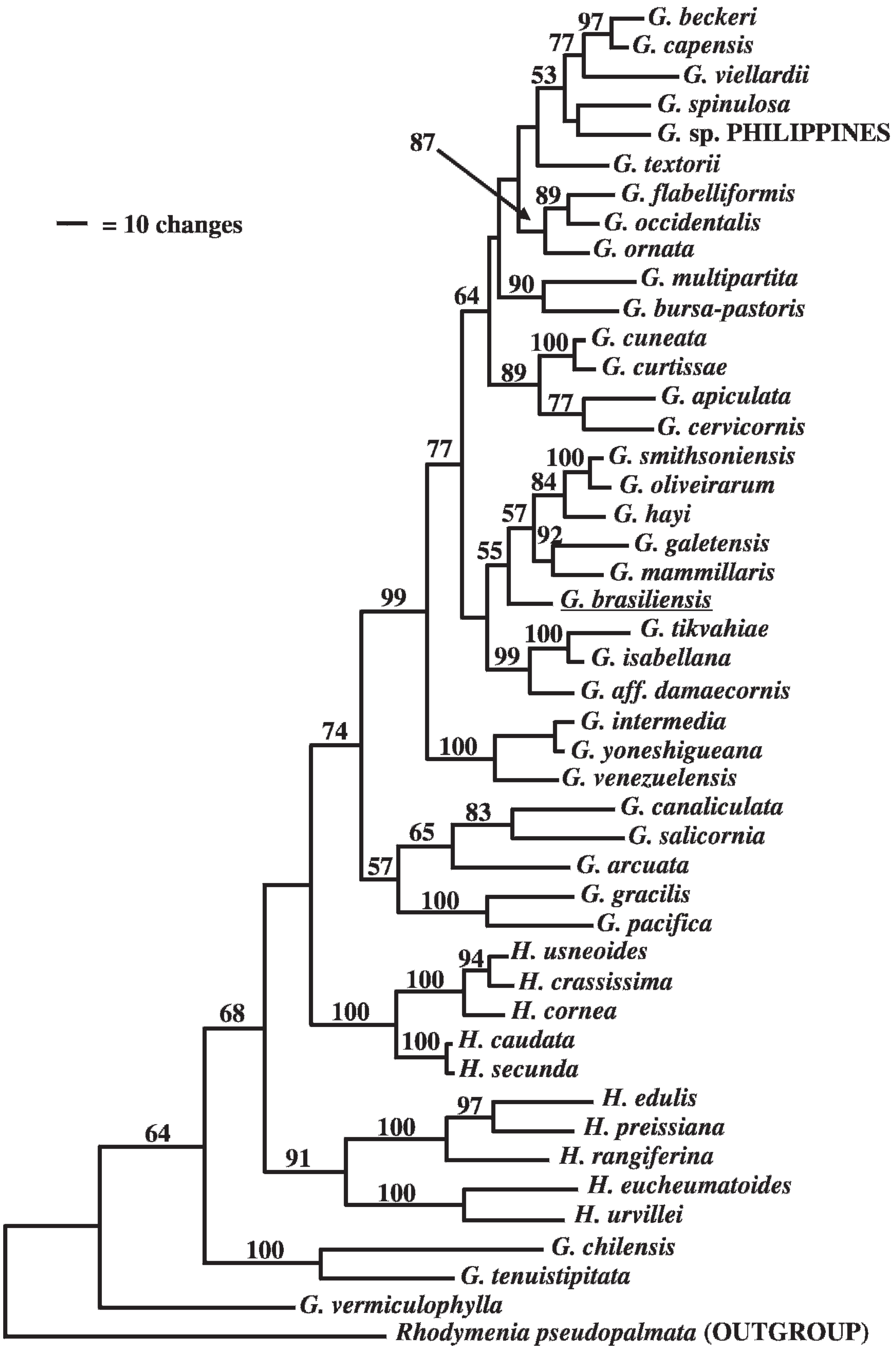


Fig. 31. Single most parsimonious tree of selected Gracilariaceae taxa based on *rbc*L DNA sequences showing the phylogenetic position of *Gracilaria brasiliensis* sp. nov. (tree length = 1748 steps, CI = 0.41, RI = 0.57). Numbers above branches correspond to MP bootstrap proportion values (1000 replicates).

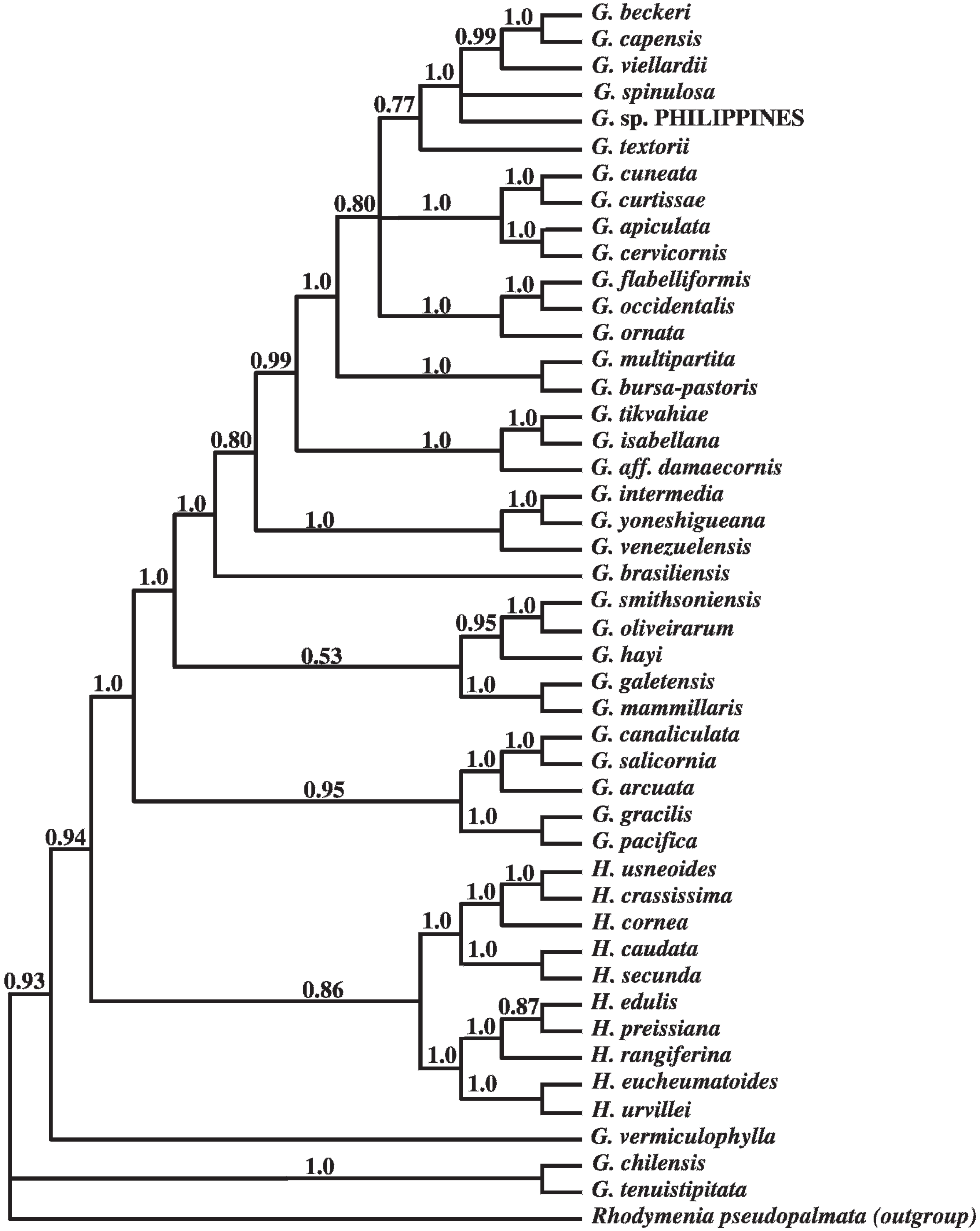


Fig. 32. The 50% majority-rule consensus tree resulting from Bayesian analysis of *rbc*L DNA sequences from selected Gracilariaceae taxa. Note the phylogenetic position of *Gracilaria brasiliensis* sp. nov. Numbers above branches denote support values from Bayesian posterior probabilities.

irregular in *G. cuneata* vs blunt or regular in *G. curtissiae*) (see Table 2). The genetic distance between *G. cuneata* and *G. curtissiae* is small, and their phylogenetic distances based on the parsimony criterion correspond to only 11 steps in the *rbc*L data set.

Gracilaria intermedia J. Agardh (1901) and *G. yoneshigueana* Gurgel, Fredericq & J. Norris (2004) are two taxa with very distinct habits but nearly identical *rbc*L sequences. New DNA sequences of other genetic markers (e.g. nuclear ITS rDNA, mitochondrial cox1, and cox2–cox3 spacer) are being generated to help resolve this lack of congruence between morphology and *rbc*L phylogeny. Because of its 'in concert' mode of evolution (Hillis & Dixon 1991), nuclear ITS rDNA sequences may prove to be a very important tool in helping delineate taxa in which morphological plasticity is problematic.

CONCLUSION

Morphological analyses of recently described species have revealed characters useful to distinguish *Gracilaria* species (Table 2). However, identification in this genus continues to be problematic especially when attempted on small, juvenile thalli or on specimens exhibiting altered phenotypes (due to sand scour, herbivory pressure, etc.). The continuing discovery of new *Gracilaria* species in the western Atlantic (e.g. Bellorin *et al.* 2002; Gurgel *et al.* 2004a, b) supports Hay & Norris's (1984) suggestion that this may be the macroalgal genus with the greatest number of sympatric species (i.e. 12).

This study described and delineated two new species of flat *Gracilaria* from the western Atlantic and showed that the Brazilian Gracilariaceae is far richer than previously recognized. With agar quantity and quality and other characters of economic interest varying between species (Araño *et al.* 2000), a better knowledge and elucidation of species is important and will lead to improved recognition of the diversity and species richness in this genus.

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