Crouania pumila sp. nov. (Callithamniaceae: Rhodophyta), a new species of marine red algae from the Seaflower International Biosphere Reserve, Caribbean Colombia

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Abstract: ***Crouania pumila* sp. nov. (Callithamniaceae: Rhodophyta), una nueva especie de alga roja marina de la Reserva Internacional de la Biosfera Seaflower, Caribe colombiano.** In the Colombian Caribbean, the marine macroalgal flora of the Seaflower International Biosphere Reserve has been little studied, despite its ecological importance. Historical records have reported only 201 macroalgae species within its area of almost 350 000km^2. However, recent surveys have shown a diversity of small algae previously overlooked. With the aim to determine the macroalgal diversity in the Reserve, we undertook field surveys in different ecosystems: coral reefs, seagrass beds, and rocky and sandy substrates, at different depths, from intertidal to 37m. During these field surveys, we collected a small described species belonging to the genus *Crouania* (Callithamniaceae, Rhodophyta), *Crouania pumila* sp. nov. that is decribed in this paper. This new species was distinguished from other species of the genus by a distinctive suite of traits including its diminutive size (to only 3.5mm in length), its decumbent, slightly calcified habit (epiphytic on other algae), its ramisympodial branching, the ecorticate main axes, and the elongate shape of the terminal cells of the cortical filaments. The observations were provided for both female (cystocarpic) and tetrasporangiate thalli; however, male thalli were not seen. Further studies have to be undertaken in this Reserve in order to carry out other macroalgal analysis and descriptions. Rev. Biol. Trop. 61 (3): 1015-1023. Epub 2013 September 01.

Key words: Caribbean, Colombia, *Crouania pumila*, new species, Old Providence Island, red algae.

Seaweeds are widely distributed and support most oceanic ecosystems, and generally have small sizes (Norton *et al.* 1996). They are at the base of the food web and, along with phytoplankton, constitute the primary producers in the ocean.

Crouania J. Agardh is a genus of filamentous terete red algae characterized by a uniaxial organization of its indeterminate axes, each axial cell bearing a whorl of three determinate branchlets, and new indeterminate axes (branches) arising from an axial cell or from the basal cell of a whorl-branchlet (Wollaston & Womersley 1998). The primordium of a new indeterminate branch appears as a chain of 10-20 cells before the formation of its own whorl-branchlets. Tetrasporangia are borne on the basal cells of whorl-branchlets, and gland cells are absent. Characters used to distinguish the species include habit type, thallus size, presence or absence of cortication on the main axis, the branching pattern and origin, shape of terminal cortical cells, length/diameter ratio of axial cells and position of the tetrasporangia

(Saenger & Wollaston 1982, Norris *et al.* 1984, Wollaston & Womersley 1998, Mateo-Cid *et al.* 2002, Schneider 2004).

With the re-organization of the family Ceramiaceae, as proposed by Choi *et al.* (2008) on the basis of gene-sequence data, *Crouania* is now assigned to the family Callithamniaceae. The genus is widely distributed in warm temperate and tropical seas (Norris *et al*. 1984), with 16 species currently recognized (Guiry & Guiry 2012), four of which are reported for the Western Atlantic (Wynne 2011): *C. attenuata* (C. Agardh) J. Agardh, *C. elisiae* C.W. Schneid., *C. mayae* Mateo-Cid, Mendoza-González & Searles and *C. pleonospora* W.R. Taylor. The status of a fifth species, *C. capricornica* Saenger & E.M. Woll., in the flora of the Western Atlantic, is uncertain. That species, which has a type locality of Port Curtis, Queensland, Australia (Saenger & Wollaston 1982), was reported to occur in Martinique, Lesser Antilles, by Bucher & Norris (1995), which was its first report from the Atlantic Ocean. Schneider (2004), however, pointed out that Bucher & Norris (1995) depicted elongated outer cortical cells and more than one sporangium per whorl-branchlet in their material, features that are in conflict with Saenger & Wollaston's (1982) original description of this species. This led Wynne (2005) to dismiss the record from his checklist. But a second record of this species was missed, namely, the report of *C. capricornica* by Díaz-Pulido & Díaz-Ruiz (2003) from Caribbean Colombia. In addition to their record of this Australian-based species for the Atlantic coast of Colombia, Díaz-Pulido & Díaz-Ruiz (2003) reported the occurrence of *C. attenuata* and *C. pleonospora*.

In the present paper we describe the new species *Crouania pumila*, collected subtidally from Old Providence Island, Seaflower Biosphere Reserve, Caribbean Colombia.

MATERIALS AND METHODS

The Archipelago of San Andres, Old Providence and Santa Catalina islands are located in the western portion of the Caribbean Sea, between 10-18° N - 78-82° W, with a marine extension of more than 250 000km^2, of which 5 000km^2 are shallow water. Three main islands (San Andres, Old Providence and Santa Catalina) and several cays, shoals and banks forming the archipelago, were declared as the Seaflower International Biosphere Reserve in 2000. Old Providence is a small (17km^2) volcanic island, 228km off the coast of Nicaragua (Fig. 1). The island harbors the second largest barrier reef of the Caribbean Sea (32km long), situated on the east side of the island. Among the southern cays of the Archipelago, Albuquerque lies about 137km southwest of Old Providence, 190km^2 off the Nicaraguan coast. The key is made up of two islands that were formed by the accumulation of sediments on the reef lagoon (Fig. 1).

During our field surveys, specimens were collected by SCUBA at a depth of 16-37m, in October 2009, in Old Providence, at two diving sites. The first one is El Planchón, a ship wreck lying at about 20m depth, covered by a rich coral community and associated macroalgae. The second site is Nick´s Place, a leeward cliff on the platform of the island, with a terrace at about 16m and a slope descending abruptly, where the specimen was collected at 37m.; the specimens were epiphytic on larger red algae (*Chondria* sp.) or as part of turf. Additional specimens were collected in Albuquerque Cay, by SCUBA, at a depth of 1-2m, in October 2010, also epiphytic on larger algae. The alga was fixed in 5% formalin in seawater and mounted on microscope slides, unstained, in 50% Karo corn syrup in a distilled water solution to which few phenol crystals were added

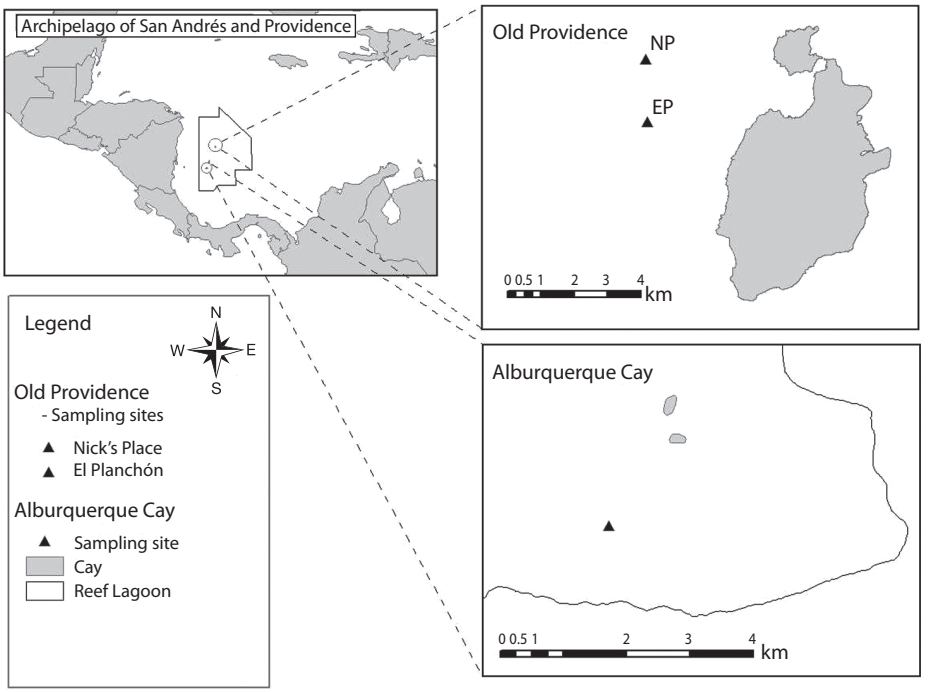


Fig. 1. Map of the sample sites, in Old Providence island and Albuquerque Cay.

to prevent fungal growth. The slides for herbarium specimens were sealed with nail polish. Cross-sections were made by hand with a razorblade. The specimens were observed and photographed with an Olympus BX52 light microscope, connected to a digital camera Moticam 2 300.

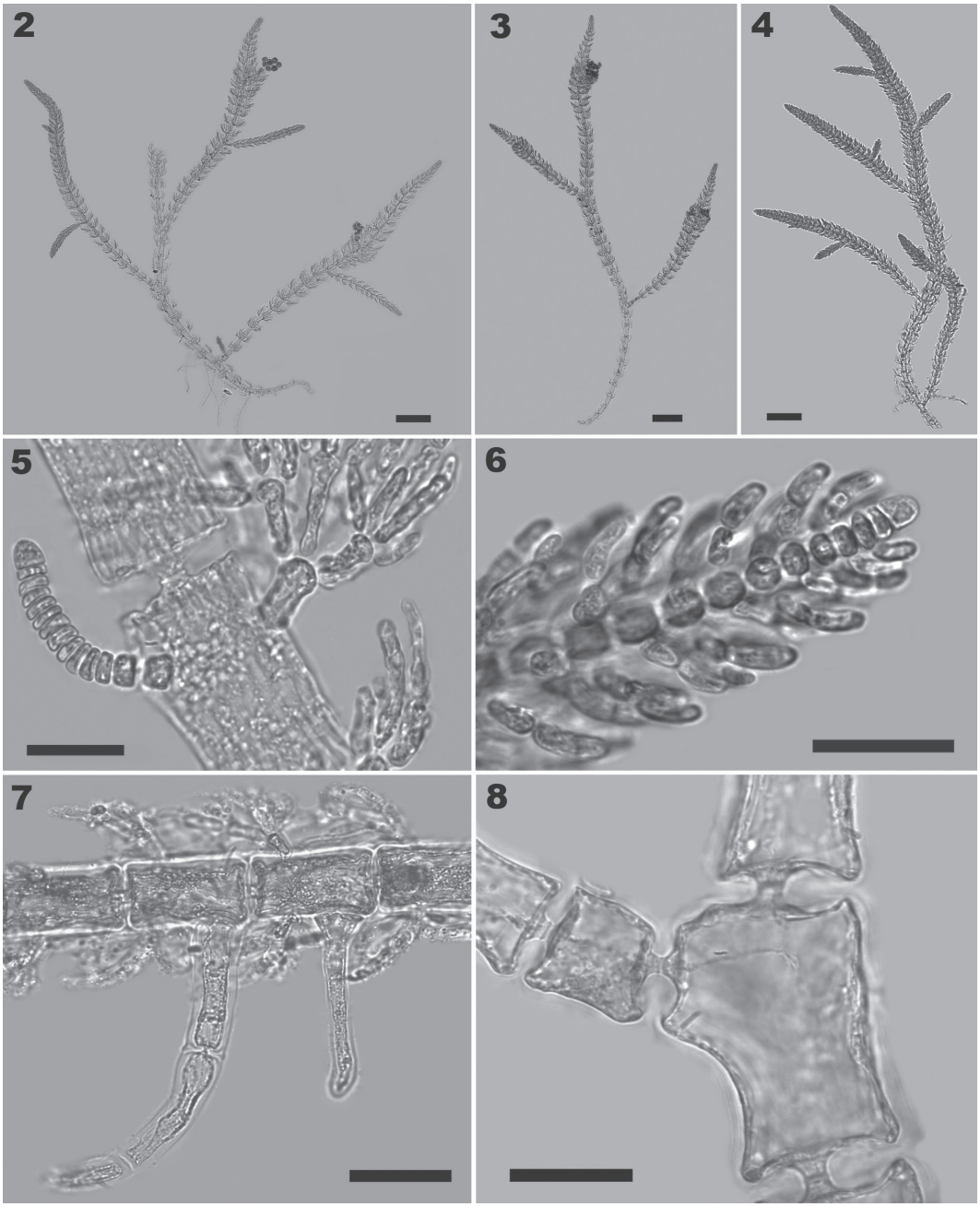
RESULTS

Crouania pumila B. Gavio, V. Reyes-Gómez et M.J. Wynne sp. nov.

Plantae epiphyticae, pusillae, usque 3.5mm altae, roseolae, leviter calcareae. Axis ramique cylindrici, articulati, gelatinosi, ecorticati. Plantae sistema ramisympodiale formantes. Cellulae axiales 30-50µm diametro et 70-100µm altae, gradatim decrescentes usque cellula apicalis tholiformis, 7.0-7.5µm diámetro et 7.0-10µm alta. Tres rami breves verticillati atque determinati in omni nodo, ramulis verticillorum di-trichotome ramosis. Prima cellula ramorum magis parva, isodiametra, 15-25µm diametro et 15-25µm alta. Secunda cellula ramorum diametro similare sed magis alta, 20-50µm longa. Cellulae terminales ramorum elongatae, 10-17.5µm altae, 5µm latae.

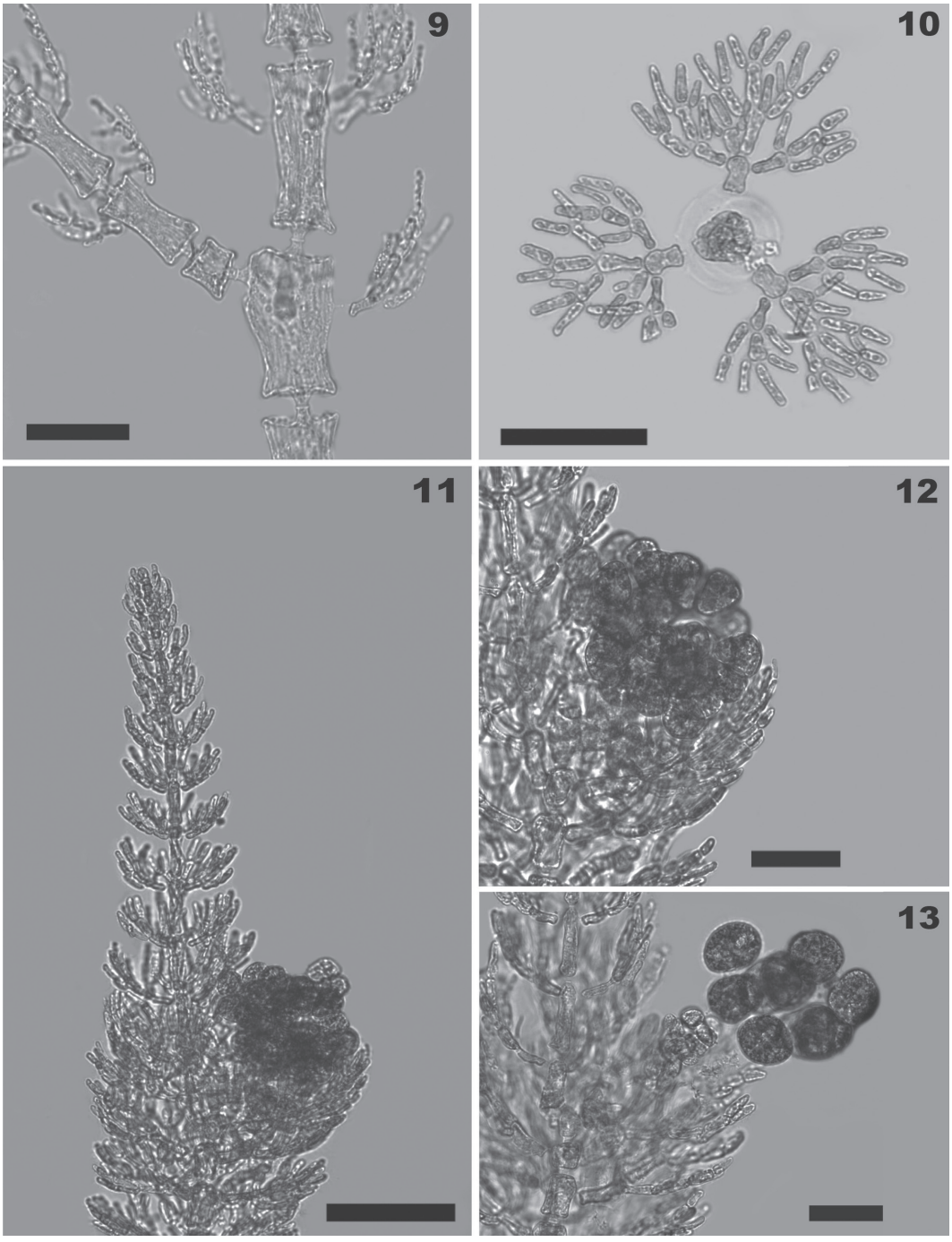
Rhizoidea multicellulares cum cellulis 10-23µm diametro et 75-120µm longis. Gonimoblasta cum carposporas isodiametras, 25-30µm diametro. Tetrasporangia ovales 37-50µm diámetro et 50-60µm longa, uno vel duo in quo verticillo. Plantae masculae ignotae.

Plants epiphytic, small, to 3.5mm long, light pink, lightly calcified. Thallus decumbent with prostrate main axes attached to other algae, ramisympodially branched (Figs. 2-4). Main axis completely ecorticate (Figs. 5-8), with primary axial cells 30-50µm in width and 70-100µm in length (length/width ratio: 1.5-2.5), gradually tapering distally to a dome-shaped apical cell 7-7.5µm wide and 7-10µm long (Fig. 6). Rhizoids multicellular, uniseriate, composed of cells 10-23µm wide and 75-120µm long, arising proximally from



Figs. 2-8. *Crouania pumila* sp. nov. Figs 2-4. Habit, scale bars= 200µm. Fig. 5. Main axis completely ecorticated, scale bar= 30µm. Fig 6. Apical cell in dome-shaped, scale bar= 30µm. Fig. 7. Rhizoids multicellular and uniseriate, scar bar= 50µm. Fig 8. First cell of indeterminate branches shorter than the others, scale bar 30µm.

axial cells (Fig. 7). First cell of indeterminate branches shorter than the others, almost isodiametric, 15-25µm wide (Figs. 5, 8-9). Second cell of indeterminate branches longer, up to 20-25µm long (Fig. 9). Indeterminate branches arising distally from axial cells (Figs. 5, 8-9). Determinate branchlets in whorls of three (Fig. 10), tri-tetrachotomously divided, with first cell



Figs. 9-13. *Crouania pumila* sp. nov. Fig. 9. Indeterminate branches, second cell of indeterminate branches longer, scale bar= 50µm. Fig. 10. Determinate branchlets in whorls of three, tri-tetrachotomously divided, with first cell of the branchlet elongate, scale bar= 30µm. Fig 11-13. Carposporophyte a compact rounded mass of carpospores.

of the branchlet elongate, 15-17.5µm long, 7.5-10µm wide, terminal cells elongate, 10-17.5µm long, 5µm wide. Carposporophyte a compact rounded mass of carpospores (Figs. 11-12); individual carpospores 25-30µm in diameter (Fig. 13). Tetrasporangia ovoid, 37-50µm in diameter, 50-60µm long, one or two per whorl (Figs 14-17, Table 1). Spermatangial thalli not seen.

HOLOTYPE: VR 191, collected by B. Gavio and J.F. Ortiz, 18 October, 2009, at a depth of 16m COL 550268 (Herbario Nacional de Colombia, Bogotá Colombia), a female plant.

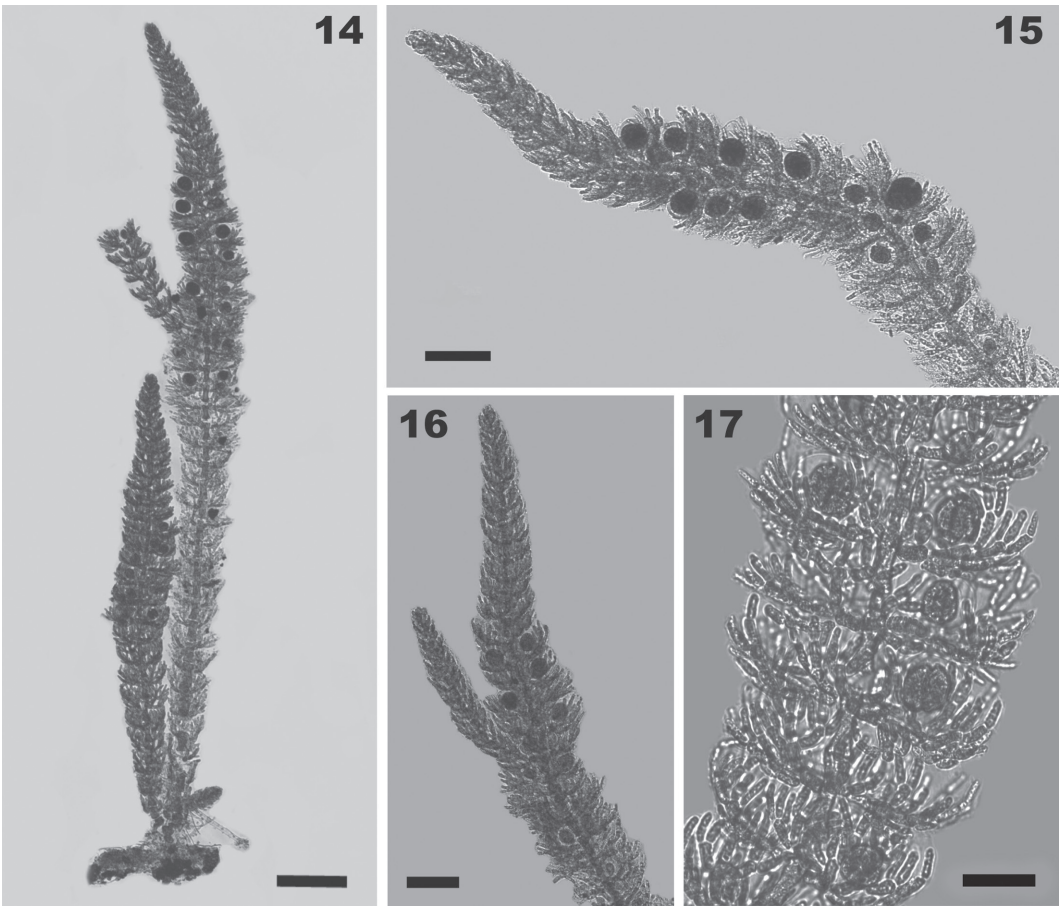
ISOTYPES: COL 550269, female; COL 550270 vegetative plant.

TYPE LOCALITY: The type specimens were epiphytic on *Chondria* sp., at El Planchón, Old Providence Island, Colombia. 13° 22'17" N, 81° 25'05" W

ADDITIONAL COLLECTIONS EXAMINED [PARATYPES]:

LRP 78, collected by M. Albis in Albuquerque Cay, 22 September, 2010, 1-2m depth. COL 553429, tetrasporophyte, epiphyte on *Chondria* sp.

VR 185, collected by V. Reyes and H. Velasquez, 18 October, 2009, at Nick´s Place,



Figs. 14-17. *Crouania pumila* sp. nov. Fig. 14. Tetrasporic specimen, scale bar = 100µm. Figs. 15-16. Branch with tetrasporangia, scale bar= 100µm. Fig. 17. Close-up on tetrasporangia, scale bar= 50µm.

TABLE 1
Comparison among *Crouania* species in the Western Atlantic

	C. attenuata	*C. elisiae*	*C. mayae*	*C. pleonospora*	*C. pumila*
Habit	erect	erect	erect	erect	decumbent
Height	to 5 cm	2-3 cm	2.5-4 cm	to 6.5 cm	to 3.5 mm
Cortication	ecorticate	ecorticate	rhizoidal	basal	ecorticate
Branching	monopodial	sympodial	irregular	monopodial	sympodial
Terminal cells	elongate	spherical	subspherical/ conical	elongate	elongate
⊕ per lateral	1-2	1-2	1 (2)	1-several	1-2
⊕ diameter (µm)	45-64	35-67	40-68	60-90	37-50x50-60
Axial cell diameter (µm)	to 170; 24-56[1]	70-180	to 185	250-400	30-50
Axial cell length (µm)	300; 72-224[1]	130-275	80-530	250-800	70-100
Origin indeterminate branches	axial cell	axial cell	basal cell det lat & axial cell	axial cell	axial cell
References	Taylor 1960, Maggs & Hommersand 1993	Schneider 2004	Mateo-Cid et al. 2002	Taylor 1960	This work

Old Providence Island, Colombia, 13° 23' 18.5" N-81° 25' 07.3" W, at a depth of 37m, turf with *Centroceras* sp.

DISTRIBUTION: Old Providence Island and Albuquerque Cay, Caribbean Colombia.

DISCUSSION

In all of its characters, *C. pumila* conforms well to the genus. Its most distinctive character is the diminutive size of the thalli, which, in all the specimens examined, do not exceed 0.5cm in length. Considering that we found both cystocarpic and tetrasporic thali, we can state that the alga reaches full maturity at this size, and probably does not exceed the size that we observed. Of the 16 currently accepted (Guiry & Guiry 2012) only three other species of *Crouania* have a comparable size: *C. destriana* Wollaston from D'Estree Bay, Kangaroo Island, South Australia (Wollaston 1968), *C. ischiana* (Funk) Boudouresque & M. Perret-Boudouresque from Isla Ischia, Italy, Mediterranean Sea (Funk 1955), and *C. minutissima* Yamada from the Atoll of Ant, Caroline Islands, South Pacific (Yamada 1944). However, it is easily distinguishable from *C. destriana* by the complete absence of cortication and the elongate terminal cells of the determinate branches. Such terminal cells in *C. destriana* are ovoid (Wollaston & Womersley 1998). *C. minutissima* has relatively large axial cells, 200-250µm long and 64µm wide, with a L/W ratio of 3-4: 1 (Yamada 1944), whereas in our species the axial cells are 70-100µm long and 30-50µm wide (L/W ratio: 1.5-2.5:1). *C. ischiana* has a known distribution limited to the Mediterranean (Gómez-Garreta *et al.* 2001), ranging from the Andalucian coast of Spain in the West (Conde *et al*. 1996) to the Gulf of Saronikos of Greece in the East (Diapoulis & Verlaque 1981). Its size is more similar to our specimens; however, it has a monopodial branching pattern in contrast to the ramisympodial branching present in the new species from Colombia (Funk 1955). Furthermore, in our species the main axes are widest in their middle part and taper towards the apex, while in *C. ischiana* the diameter of the axes is more even (Coppejans 1983). The presence of hyaline hairs at the apex, reported in *C. ischiana* (Coppejans 1983), has not been observed in the new species.

In the Western Atlantic, the four other species of *Crouania* reported to date can be distinguished from our specimens because these other species are much larger in size.

It seems prudent to include *C. capricornica* in the discussion on the basis that there have been reports of that species from the Western Atlantic. Similar to the new species, *C. capricornica* has a prostrate habit, and the axial cells in that species have a size range closer to *C. pumila*. However, *C. capricornica* has sub-spherical terminal cells of the determinate branchlets, whereas in *C. pumila* they are very elongate. The Atlantic occurrence of *C. capricornica* remains uncertain. Material of *C. capricornica* from Martinique, described by Bucher & Norris (1995), should be examined carefully to determine its taxonomic status. Schneider (2004) questioned the identity of the Martinique material based on the presence of more than one sporangium per whorl-branch and the elongated outer cortical cells, which, according to him, are not in agreement with the original description of *C. capricornica*. Saenger & Wollaston (1982), however, found slightly elongate terminal cells in the type specimen of the taxon and stated that "*in the limited material available, no more than one tetrasporangium has been observed on each fertile whorl-branchlet*" (page 81) suggesting that they had limited access to tetrasporic material of the species, and they could not dismiss *a priori* the presence of several tetrasporangia per whorl. We consider that the shape of the terminal cells of the determinate branches is not a good taxonomic character because in the same plant, spherical, slightly elongate to elongate cells may be observed (pers. obs.). Thus, species differentiation based solely or mainly on this morphological character should be reconsidered.

We are able to dismiss the record of *C. capricornica* for the Atlantic coast of Colombia, reported by Díaz-Pulido & Díaz-Ruiz (2003). We analyzed the specimen (INV-RDH 398) collected by G. Díaz-Pulido, which is deposited in the Museum of Marine Natural History of Colombia MHNMC, Santa Marta, and could determine that the material does not correspond to the genus *Crouania* because the axial cells bear a whorl of four determinate branchlets, not three as in the genus *Crouania*. The identity of the specimen could not yet be resolved, but at this time we can exclude *C. capricornica* from the Colombian Caribbean marine flora.

There are many examples where size has been used as one of the characters to distinguish species of red algae from their congeners, for example: *Jania pumila* J.V. Lamouroux, *Amphiroa minutissima* W.R. Taylor, *Predaea pusilla* (Berthold) Feldmann, *Grateloupia minima* P.L. Crouan & H.M. Crouan, *Symphiocladia pumila* (Yendo) Uwai & Masuda, and *Scagelothamnion pusillum* (Ruprecht) Athanasiadis. These species are currently recognized (Guiry & Guiry 2012).

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RESUMEN

Crouania pumila sp. nov. (Ceramiaceae, Rhodophyta) es descrita para la isla de Providencia, en el Caribe colombiano. La nueva especie se destaca de las otras especies del género por características como su tamaño diminuto (hasta 3.5mm de largo), su hábito decumbente y poco calcificado (epífita sobre otras algas), su ramificación ramisimpodial, la ausencia de corticación y la forma alargada de las células terminales de los filamentos corticales.

Se proveen observaciones sobre talos femeninos (cistocárpicos) y tetraspóricos. No se observaron talos masculinos.

Palabras clave: Caribe, Colombia, *Crouania pumila*, nueva especie, isla de Providencia.

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