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Crouania mageshimensis Itono, 1977 (Ceramiales, Rhodophyta) and three other species new to the Eastern Tropical Pacific

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Abstract
We report new records of four macroalgae species in the Eastern Tropical Pacific, specifically from the rocky reefs of northern Chocó, Colombian Pacific. Among them, three species of Rhodophyta are included—*Crouania mageshimensis* Itono, 1977; *Monosporus indicus* Børgesen, 1931; *Jania articulata* N'Yeurt & Payri, 2009—and one species of Chlorophyta—*Ulothrix subflaccida* Wille, 1901. The new records increase the knowledge of tropical marine algae in the Pacific, open the discussion about possible dispersal mechanisms, and recall the importance of conducting molecular studies to define phylogenetic and biogeographic aspects of macroalgae.

Keywords
Benthic flora, Chlorophyta, Colombia, Panama Bight, rocky reefs.

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Introduction

The Eastern Tropical Pacific (ETP) is an isolated region of great value in terms of biodiversity due to its high endemism in several biological groups (Cortés et al. 2017; Miloslavich et al. 2011). The region is continually subjected to extreme oceanographic conditions, including low pH, high variation in temperature, low salinity, and seasonal upwelling (Cortés et al. 2017). This area has a high climatic variation with very dry regions as Baja California, coastal Mexico, northern Central America, southern Ecuador, and most oceanic islands, to very humid from central Costa Rica to northern Ecuador (Amador et al. 2006; Cortés et al. 2017). The study on marine biodiversity in this region has shown that although there are areas in the ETP that are currently considered more diverse than others, more studies are required to corroborate this hypothesis because the richness of local species is probably correlated with sampling effort (Cruz et al. 2003; Wehrtmann et al. 2009; Fernández-García et al. 2011).

The diversity of macroalgae in the region is considered rather low, due to several factors that include the appearance of the Isthmus of Panama (Lessios et al. 2001; Wysor 2004) and the influence of cold water due to a geological event associated with seasonal upwelling (Glynn 1972; Cortés 2011). This cold-water condition has caused local species extinctions that had been part of a homogeneous regional floral community (Wysor 2004). Another factor that may be affecting the distribution of macroalgae is the low prevalence of suitable habitats, which reduces the establishment of certain species (Cortés et al. 2017).

The studies on the macroalgae in this region over the last 70 years have resulted in a total of 300 recognized species, the red algae being the most diverse group, followed by green algae and finally brown algae (Cortés et al. 2017). It is estimated that around 70% of these macroalgae belong to coral or rocky reef environments (Alvarado et al. 2011, 2015; Murillo-Muñoz and Peña-Salamanca 2014). To date, in the Colombian Pacific, there is a record of 172 species, the great majority recorded in Gorgona Island. It is clear that the Pacific coast of Colombia has not been thoroughly studied from a phycological point of view, and several portions of the coast remain unexplored. Here, we report new records for four species of macroalgae from the Eastern Tropical Pacific, discovered during underwater exploration in the rocky reef ecosystem of the North Pacific of Colombia.

Methods

An exploration of rocky reefs was carried out in August 2015 at four localities at northern Chocó, between Cabo Corrientes (05°29′N, 077°32′W) and Cabo Marzo (06°49′N, 077°41′W; Fig. 1), an area inside the Panama Bight ecoregion (Spalding et al. 2007). The seafloor of the study area is predominantly rugged, and a large portion consists of mafic and ultramafic basaltic igneous

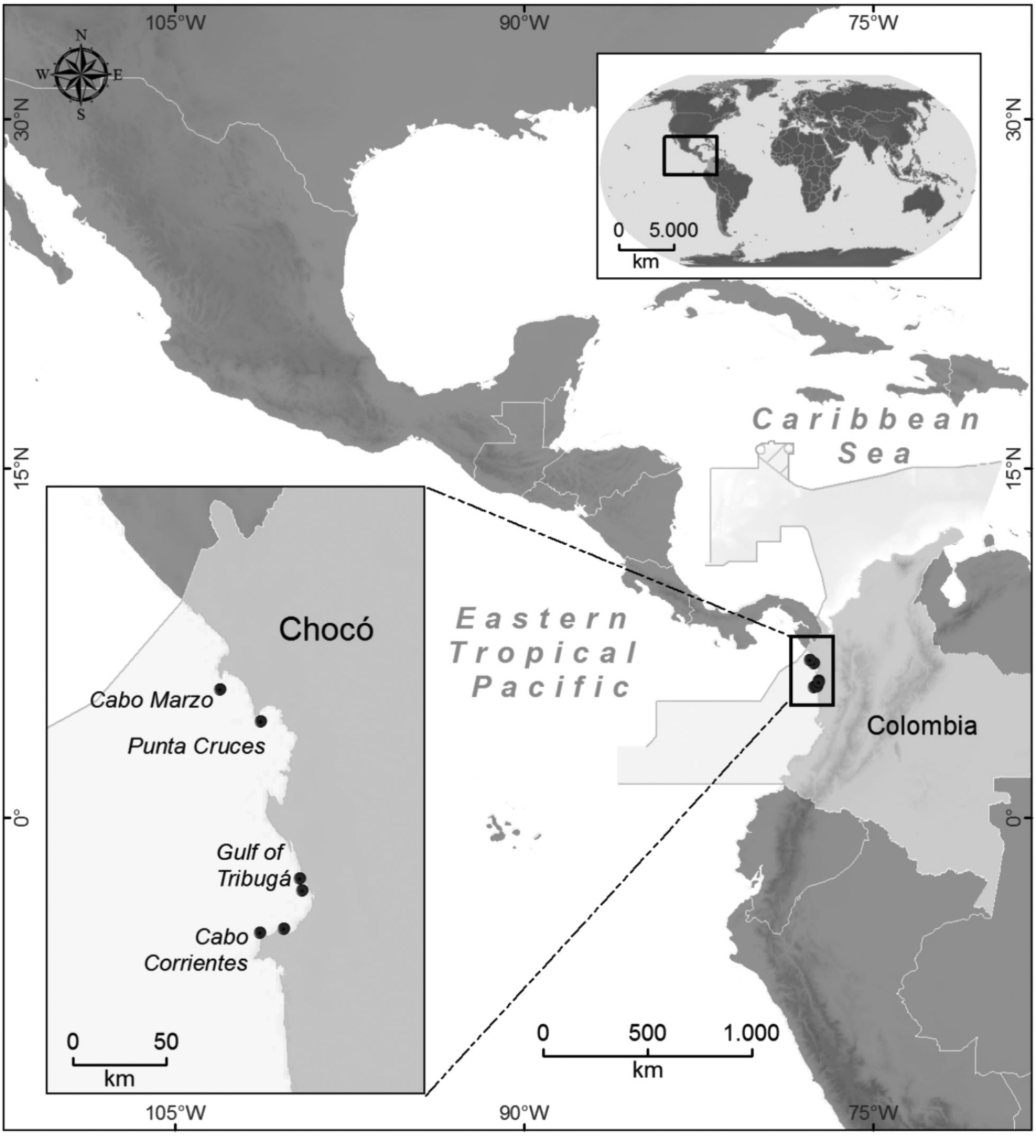

Figure 1. Sampling sites and localities on the ETP rocky reefs in the northern Chocó, Colombian Pacific.

rocks that make up the rocky reefs. Some of these are permanently submerged or emerge briefly during low tide and are local knows as "riscales", while others form small islets that remain emerged and are called "morros" (Posada et al. 2009; Velandia and Díaz et al. 2016). These rocky structures offer settlement substrate for various species of benthic flora and fauna such as corals, sponges, and macroalgae, while at the same time providing refuge for species of motile invertebrates and fish. These marine habitats are considered important artisanal fishing grounds (Díaz et al. 2016).

All the specimens were collected randomly by SCUBA diving over the rocky reef formations between 7 and 20 m depth (Fig. 1). The algae were collected by hand and preserved in 96% ethanol, and a replica of each one was also fixed in 4% formalin diluted in seawater.

The specimens were identified using an optical microscope and stereoscope (ZEISS, Axio Lab.A1). Morphological and anatomical characteristics were used to identify the algae based on Abbott and Hollenberg (1976), Schnetter and Bula-Meyer (1982), Bula-Meyer (1995), Abbott (1999), Littler and Littler (2010), Murillo-Muñoz and Peña-Salamanca (2014), Norris (2010, 2014), and N'Yeurt and Payri (2009). All specimens are deposited in the biological collection of the Museo de Historia Natural Marina de Colombia (MHNMC) of the Instituto de Investigaciones Marinas y Costeras José Benito Vives de Andréis.

Results

Here we report new records for three species of Rhodophyta and one species of Chlorophyta for the Eastern

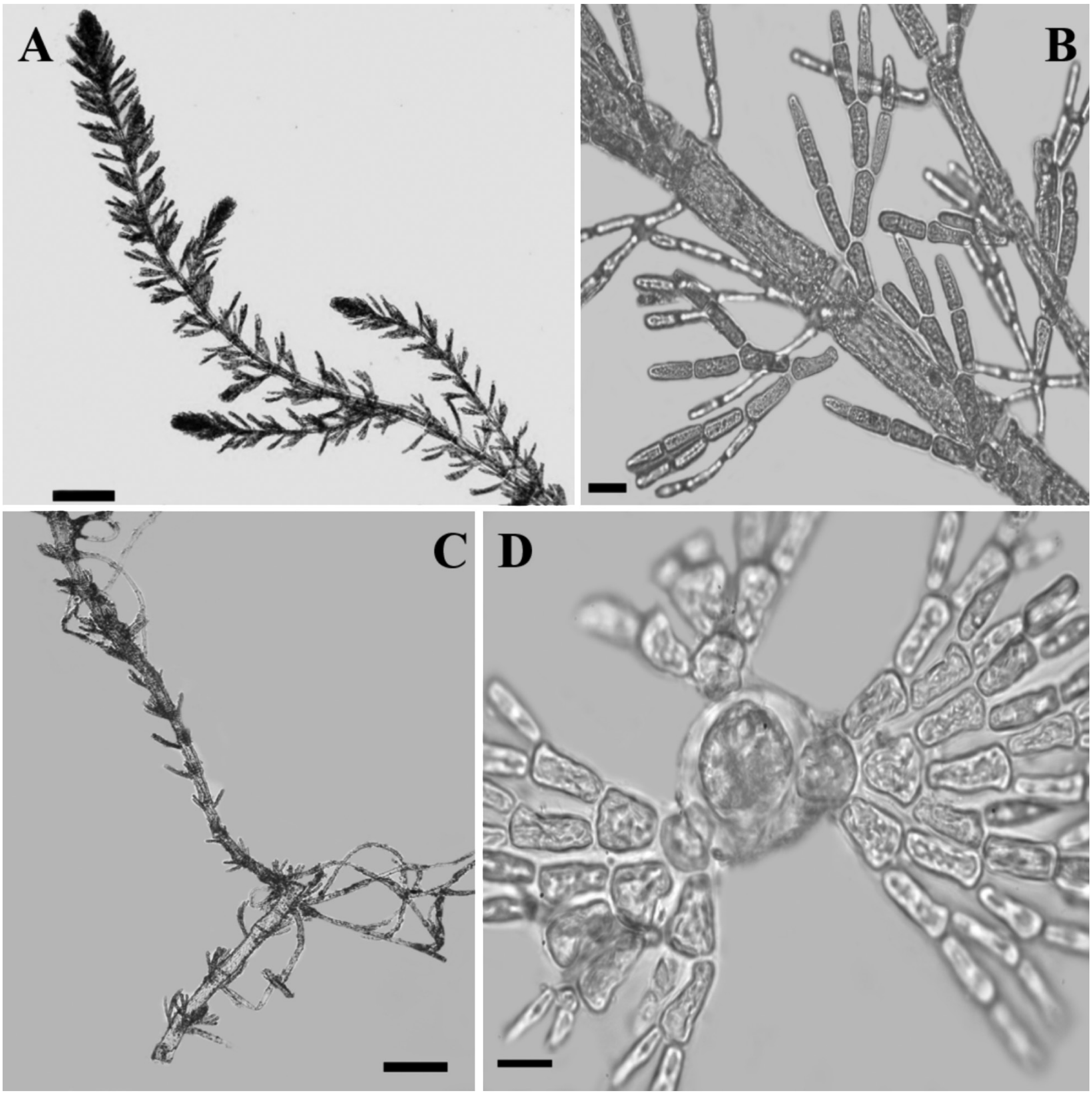

Figure 2. *Crouania mageshimensis*. **A.** Habit of vegetative thalli. **B.** Detail of axis showing wider spacing of whorl branchlets. **C**. Randomly rhizoids bearing from whorl branchlet, elongate and with digitate ends. **D.** Determinate branchlets in whorls of three. Scale bars: A, C = 200 µm; B = 20 µm; D = 10 µm.

Tropical Pacific (ETP), with descriptions of the morphology and anatomy of the species.

***Crouania mageshimensis* Itono, 1977**
Figure 2

New record. COLOMBIA • Vegetative specimen; Chocó, Gulf of Tribugá; Morros de Jurubidá; 05°48′47″N, 077° 17′49″W; 7 m depth; 20 Aug. 2015; M. Natalia Rincón-Díaz leg; INV RHD555. • Vegetative specimen; Chocó, Gulf of Tribugá, Punta Orión; 05°37′50″N, 077°23′18″W; 7 m depth; 20 Aug. 2015; M. Natalia Rincón-Díaz leg; INV RHD590.

Type locality. Mageshima, southern Japan.

Pacific distribution. American Samoa (Tsuda et al. 2011), Central Polynesia (Tsuda and Walsh 2013), Federated States of Micronesia (Lobban and Tsuda 2003; Tsuda 2006), Hawaiian Islands (Abbott 1999; Vroom and Timmers 2009), Jarvis Island and Kingman Reef (Tsuda and Fisher 2012), Northwestern Hawaiian Islands (Tsuda 2014).

Description. Epiphytic alga, prostrate, or partially erect, up to 1 cm high (Fig. 2a). Thallus is decumbent with prostrate main axes attached to other algae. Cylindrical mucilaginous axes ecorticated, cells 20–50 μm diameter, and up to 110 μm long (Fig. 2b). Elongated and multicellular rhizoids with digitate ends, arising randomly from the prostrate axis (Fig. 2c). Determinate branchlets in whorls of three, arising from each axial cell (Fig. 2d). Primary dichotomous or trichotomous ramifications, with the first cell of branchlet fan-shaped, 9.5-10 μm wide, 11.5–12 μm long, apical cells acute, up to 4 μm in diameter and approximately 16 μm long. The specimens were found as epiphytes on *Pterocladia sonorensis* (E.Y.Dawson) J.G.Stewart & J.N.Norris.

Remarks. This species was described by Itono (1977). There are currently nineteen species of *Crouania* J.Agardh, and most taxa are distributed in Australia and within the geographic region of Oceania (Guiry and Guiry 2020). Characteristics used to distinguish the species include thallus size, habit type, presence or absence of cortication on the main axis, the branching pattern and origin, the shape of terminal cortical cells, length/diameter ratio of axial cells, and position of the tetrasporangia (Saenger and Wollaston 1982; Norris et al. 1984; Wollaston and Womersley 1998; Mateo-Cid et al. 2002; Schneider 2004; Gavio et al. 2013).

Among the distinctive characteristics of *Crouania mageshimensis* is the arrangement of the branchlets in that each whorl separates widely from the next, especially towards older portion*s* (Abbott 1999), giving a ring-shape appearance to the segments. Both the size and the branchlet appearance of the observed specimens agree with the description of *C. mageshimensis*. To date, the only other species of the genus reported for ETP is *Crouania attenuata* (C.Agardh) J.Agardh recorded for the Revillagigedo Archipelago (Dawson 1962). Our specimen is easily distinguishable from *C. attenuata* because this latter species is greater in size, exhibits a bushy branching habit and may be lightly calcified; furthermore, thalli of *C. attenuata* are erect, to 5 cm high and are attached by digitate discs at the ends of multicellular rhizoids (Maggs and Hommersand 1993; Schneider 2004; Gavio et al. 2013). Although no reproductive structures were observed in the present study, the alga fits well the description of the species.

***Monosporus indicus* Børgesen, 1931**
Figure 3

New record. COLOMBIA • Monosporangia; Chocó, Punta Cruces; La Viuda; 06°37′58″N, 077°29′58″W; 15 m depth; 14 Aug. 2015; M. Natalia Rincón-Díaz leg; INV RHD580. • Monosporangia; Chocó, Gulf of Tribugá, Punta Orión; 05°37′50″N, 077°23′18″W; 7 m depth; 20 Aug. 2015; M. Natalia Rincón-Díaz leg; INV RHD589. • Monosporangia; Chocó, Gulf of Tribugá, Morromico; 05° 52′19″N, 077°18′38″W; 15 m depth; 20 Aug. 2015; M. Natalia Rincón-Díaz leg; INV RHD623, INV RHD658.

Type locality. Bombay, India.

Pacific islands distribution. Australia and New Zealand (e.g., Lewis 1984; Millar and Kraft 1993; Huisman 1997), central Polynesia (Tsuda and Walsh 2013), Hawaiian Islands (Abbott 1999), Jarvis Island and Kingman Reef (Tsuda and Fisher 2012).

Description. Thallus filamentous, uniseriate, formed by a prostrate axis to 45–70 μm in diameter, and 304–350 μm in length. Erect axes arising from prostrate joints to 30 μm in diameter, and 170 μm in length in basal portion (Fig. 3a), with subdichotomous branchlets and rounded apices (Fig. 3b). Unicellular rhizoids up to 2 mm long and 40–50 μm in diameter which arise one per each cell of the prostrate axis, claw-like ends (Fig. 3c). Oval one-celled propagules to 80–100 μm wide (Fig. 3d). The specimens were found as epiphytes on *Dictyota* sp., *Amphiroa crosslandii* M. Lemoine*,* and *Aglaothamnion cordatum* (Børgesen) Feldmann-Mazoyer.

Remarks. The species was originally described from the shores of India in 1931 by Børgesen and since then it has been reported at several localities in the Indo-Pacific basin: Korea, Australia, central Polynesia and the Hawaiian Islands (Lewis 1984; Abbott 1999; Kim and Lee 2012). In the Atlantic Ocean, it has been reported from Florida, Martinique, and Puerto Rico in the 1990s (Ballantine 1996; Ballantine and Aponte 1997). More recently, it has been reported for the southern Caribbean Sea: Venezuela (García et al. 2011) and the Archipelago of San Andrés, Colombia (Rincón-Díaz et al. 2018). In the Mediterranean basin, *M. indicus* is considered an introduced species of Lessepsian origin (Hoffman and Wynne 2016). Due to its small size, it is difficult to determine whether its wide distribution has been overlooked in the past or if the species has been introduced in the Caribbean and perhaps at other localities in the Indo-Pacific, being possibly an example of a cryptogenic species.

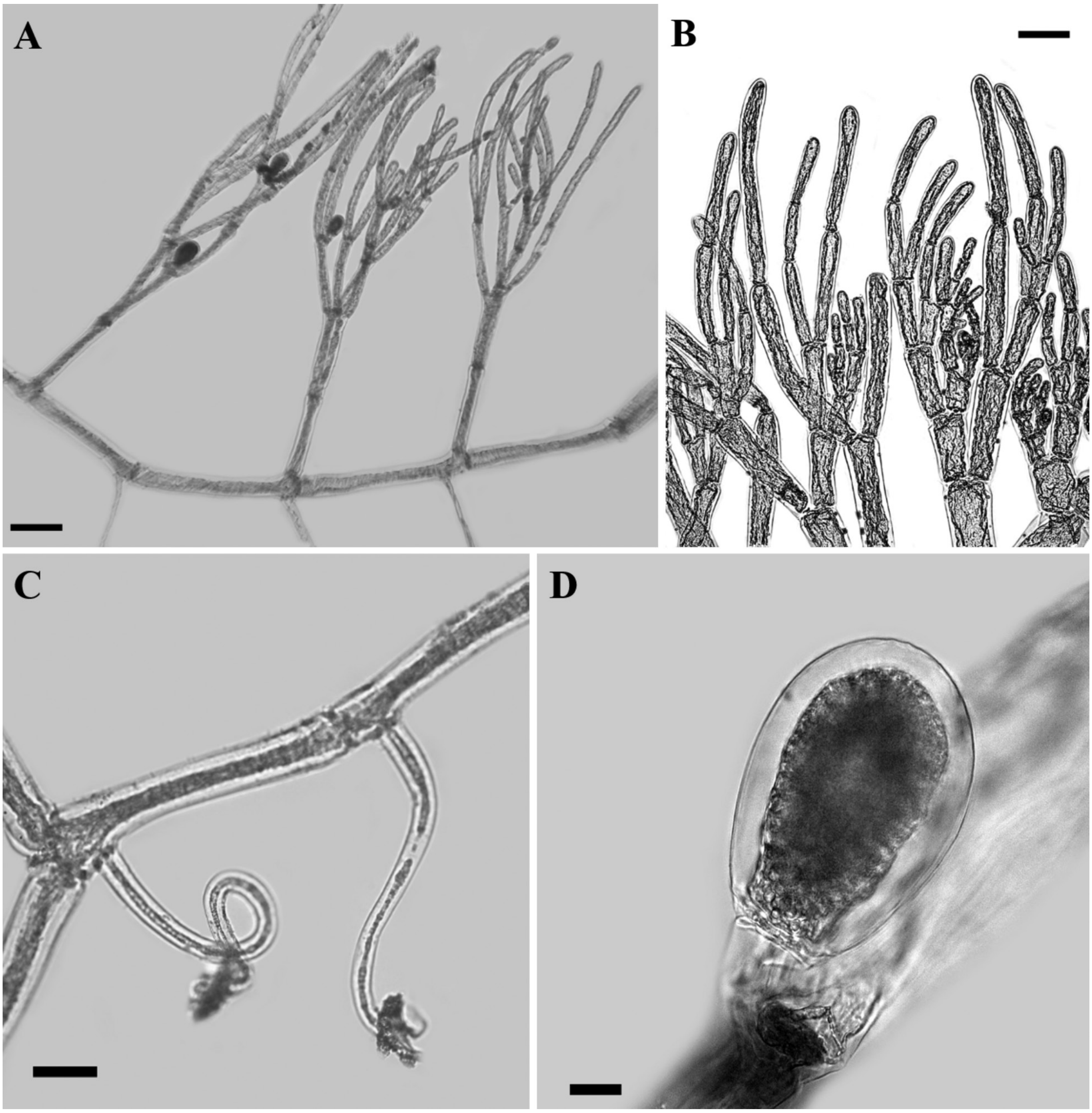

Figure 3. *Monosporus indicus*. **A.** Habit. **B.** Detail of distal branchlets portion. **C.** Detail of rhizoids. **D.** Oval one-celled propagules. Scale bars: A–C = 100 μm; D = 10 μm.

Jania articulata **N'Yeurt & Payri, 2009**
Figure 4

New record. COLOMBIA • Vegetative specimen; Chocó, Cabo Marzo, La Foca; 06°47′10″N, 077°41′32″W; 17 m depth; 18 Aug. 2015; M. Natalia Rincón-Díaz leg; INV RHD569. • Vegetative specimen; Chocó, Gulf of Tribugá, Punta Orión; 05°37′50″N, 077°23′18″W; 7 m depth; 20 Aug. 2015; M. Natalia Rincón-Díaz leg; INV RHD592.

Type locality. Fangatau Atoll, Tuamotu, French Polynesia.

Pacific distribution. Archipelago of Tuamotu, French Polynesia and Manihiki, Cook Islands of the North (N'Yeurt and Payri 2009, 2010); Central Polynesia (Tsuda and Walsh 2013).

Description. Thallus erect, heavily calcified (Fig. 4a), emerging from a well-defined calcareous base and with a growth pattern resembling plumes (Fig. 4b); dichotomies uncommon but when present, they are formed every four joints (Fig. 4c). Segments are of similar size reaching up to 313 μm long and to 240 μm in diameter in the basal portion. Intergenicular length: width ratio small, between 1.2 and 1.4. Joint composed of a row of elongated cells of 80–100 μm long and 5–6 μm in diameter (Fig. 4d). Surface cells 4–7 μm wide (Fig. 4e). The collected specimens were growing directly on rocks and as epiphytes on *Aglaothamnion cordatum, Monosporus indicus,* and *Dasya sinicola* (Setchell & N.L. Gardner) E.Y. Dawson.

Remarks. The genus *Jania* J.V. Lamouroux currently includes 48 species and is widely distributed in tropical and subtropical waters worldwide (Guiry and Guiry 2020); it

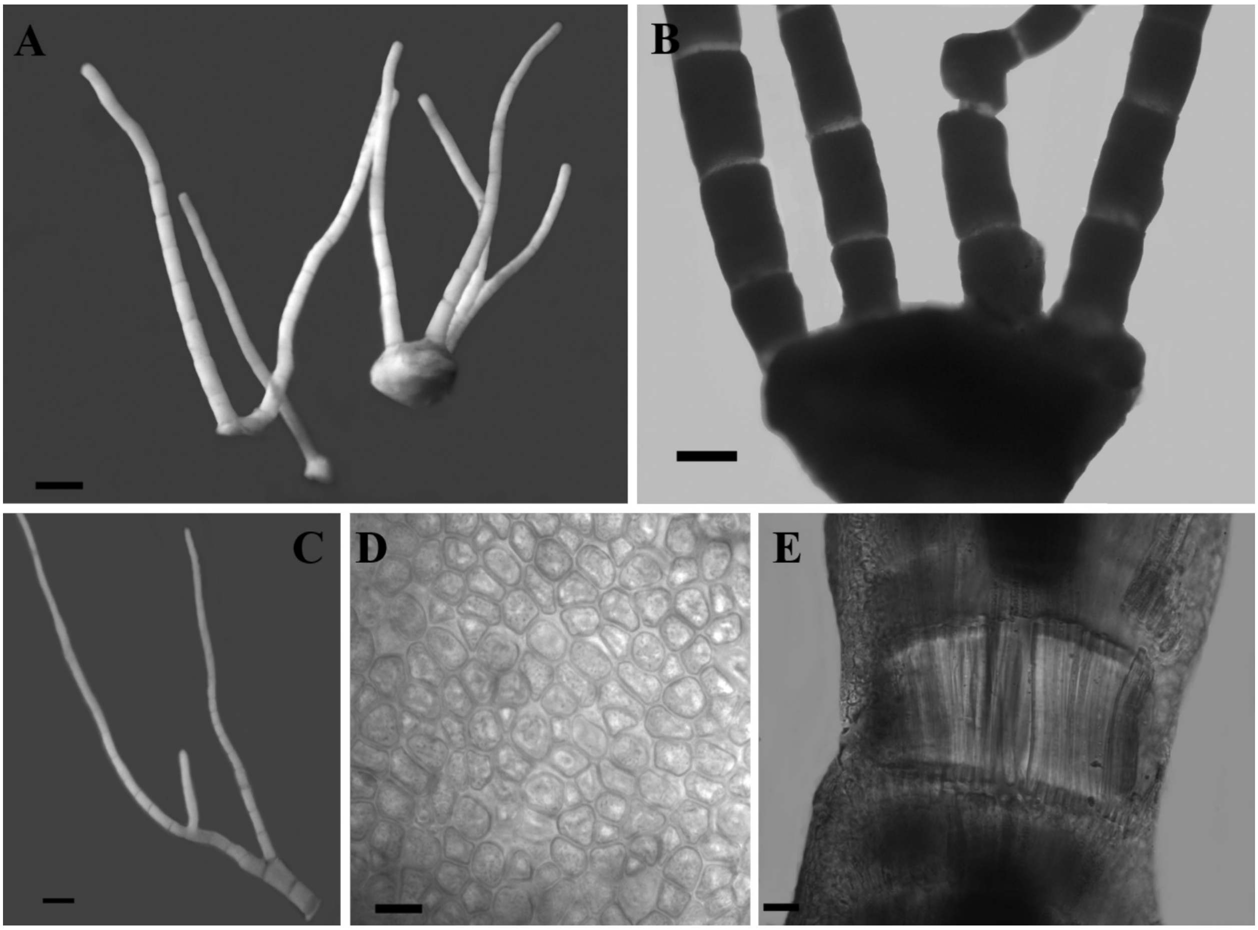

Figure 4. *Jania articulata.* **A.** Habit. **B.** Calcified base. **C.** Detail of branches; **D.** Surface cells. **E.** Genicula and intergenicula. Scale bars: A = 500 µm; B = 200 µm; C = 500 µm; D = 10 µm; E = 20 µm.

is characterized by its branching pattern that varies from dichotomous to pinnate or alternate to irregular. Among the species of *Jania*, the large branch diameter and the numerous interdichotomal genicula are distinctive characteristics of *J. articulata.* The intergenicular length to width ratio of *J. articulata* is also by far the smallest among tropical reported species (N'Yeurt and Payri 2009). *Jania articulata* may superficially resemble some species of *Amphiroa* J.V. Lamouroux; however, the latter genus has irregularly dichotomous branching, and axes are more than 1000 µm wide (N'Yeurt and Payri 2009).

Ulothrix subflaccida **Wille, 1901**
Figure 5

New record. COLOMBIA • Vegetative specimen; Chocó, Cabo Corrientes, Piedra de Jairo; 05°36′36″N, 077°30′02″W; 19 m depth; 18 Aug. 2015; M. Natalia Rincón-Díaz leg; INV CHL214, INV CHL217. • Vegetative specimen; Chocó, Punta Cruces, La Viuda; 06°37′58″N, 077°29′58″W; 15 m depth; 14 Aug. 2015; M. Natalia Rincón-Díaz leg; INV CHL216.

Lectotype locality. Dröbak Norway.

Pacific island distribution. Australia and New Zealand (e.g., Chapman 1956; Silva et al. 1996; Broady et al. 2012); Hawaiian Islands (Abbott and Huisman, 2004); Northwestern Hawaiian Islands (Tsuda 2014).

Description. Solitary filament 370–750 µm long, simple, and unbranched (Fig. 5a), attached to host alga by a single elongated cell 10–12.5 µm in diameter and 12–14 µm long (Fig. 5b). Small apical cells 7.5 µm in diameter and 10 µm long with a single plastid (Fig. 5c), and a single pyrenoid per cell (Fig. 5d). The specimens were found as epiphytes on *Rhodymenia dawsonii* W.R.Taylor, *Dictyota stolonifera* E.Y. Dawson, and *Dictyota* sp.

Remarks. To date, the genus *Ulothrix* Kützing has been reported for the ETP only in El Salvador, with the species *Ulothrix flacca* (Dillwyn) Thuret (Gutiérrez 1985; Tejada 2003). *Ulothrix subflaccida* has a basal cell, which is elongated and tapering (John 2007; Albis-Salas and Gavio 2015); while *U. flacca* is characterized by having the basal portion attached by a basal cell and rhizoids formed as downward-growing extensions from a few intercalary cells above. Furthermore, *U. flacca* has cells always wider than long; its cell walls are thicker and mucilaginous and each cell can present up to eight pyrenoids per chloroplast, while *Ulothrix implexa* (Kützing) Kützing presents cells of variable length, 1–1.5 (–2) diameters long, and up to four pyrenoids per cell; these characters differentiate these two species from *U. subflaccida.* Our specimens coincide with the description by Cormaci et al. (2014), who also agreed with Lokhorst (1978, 1985) and John (2007) in considering *U.*

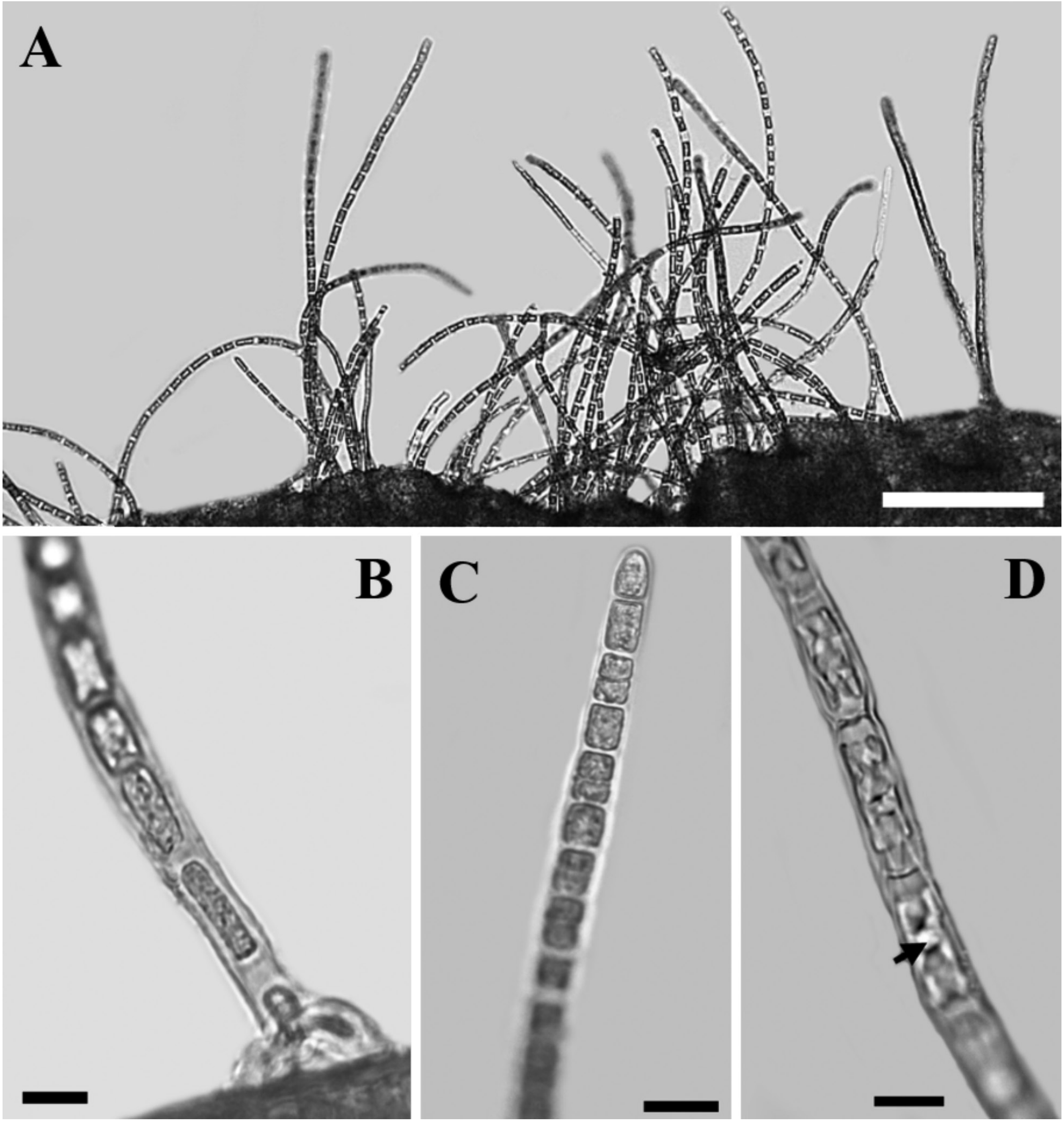

Figure 5. *Ulothrix subflaccida.* **A.** Filaments epiphytic on *Rhodymenia dawsonii.* **B.** View of single elongated basal cell. **C.** Filament and apical cell. **D.** Detail of the cells showing the pyrenoid (arrow). Scale bars: A = 200 µm; B, D = 10 µm; C = 20 µm.

subflaccida distinct from *U. implexa,* while Setchell and Gardner (1920), Hamel (1931) and Burrows (1991) considered *U. subflaccida* as a taxonomic synonym of *U. implexa.* Based on this, the samples described by Sfriso (2010) as *U. implexa* may correspond to *U. subflaccida* considering the diameter of the filaments, the shape and size of the cells, and the presence of a single pyrenoid per chloroplast (Cormaci et al. 2014). Despite being considered a freshwater species, *U. subflaccida* has been reported for coastal marine environments.

Discussion

The seaweed flora of the Eastern Tropical Pacific has not been thoroughly studied. Recent research on the marine flora of Pacific Panama (Wysor 2004) and Costa Rica (Fernández-García et al. 2011) has highlighted the paucity of studies, at least in some portions of ETP. In Colombia, the marine algae of the Pacific coast have received little attention, in part because of difficulties to access, due to lack of roads and public security issues.

The presence of these four taxa may be the result of unnoticed introductions into the region, from ballast water or hull fouling associated with intense shipping by the closeness of the Panama Canal, the latter having been considered one of the most ancient ways for the introduction of alien species in the marine system (Boudouresque and Verlaque 2010). Global ocean warming and increasing connectivity through human activities are currently causing rapid changes in the biogeography of seaweeds (Straub et al. 2016), promoting expansions in the distribution ranges of some species. In that sense, the study area has two possible sources of propagule pressure, the Buenaventura harbor in the south and the Panama Canal in the north, both of them located around 200 km from the sites of collection.

The species *Monospurus indicus* has been reported in the Caribbean Sea, including the coast of Colombia (San Andrés Archipelago, Rincón-Díaz et al. 2018), and a possible route of introduction for this species may be through the Panama Canal from the Caribbean Sea, rather than the Western Pacific Ocean.

The possibility cannot be discarded, however, that these species have been part of the ETP marine flora, but due their small sizes, epiphyte habit, and the lack of surveys, they have not been detected until now. Because it is not possible to determine the exact origin of these species in the study area, they should be considered cryptogenic species (Carlton 1996). Determining the origin of the introduction as well as the colonization route for small species such as the ones found in this study is a challenging task, which in general requires an approach from molecular genetics (e.g., Holland 2000; Saltonstall 2002; Estoup and Guillemaud 2010; Rius et al. 2015; Yang et al. 2015; Geoffroy et al. 2016).

It is well known that morphology-based research for several groups, including macroalgae, should be supported with molecular methods to assert the proposed taxonomic information. However, this is a preliminary study based on morpho-anatomic characters of macroalgae, which aims to contribute to the baseline of Colombian Pacific rocky reefs biodiversity, an ecosystem and area for which biological information is still very scarce.

The present report contributes to the knowledge of the marine macroalgal biodiversity in the Eastern Tropical Pacific, specifically for the Panama Bight ecoregion (Spalding et al. 2007), an area that has been grossly overlooked from a scientific point of view. These new records from the ETP show a plausible scenario of recent introduction events, possibly through biofouling on ship hulls or ballast water, emphasizing the importance of shipping as a primary vector for alien marine algae and the susceptibility of the area to bioinvasions. However, in the absence of molecular evidence, the native or exotic character of these newly reported species cannot be established in the study area; therefore, they should be considered cryptogenic species. The inclusion of molecular analysis to encompass phylogeographic aspects of the macroalgae species could define the non-native or native status in the ETP.

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Authors' Contributions

NRD collected, prepared, and identified specimens; also wrote the manuscript; BG confirmed the identity of the specimens and wrote and revised the manuscript; JVSM identified and photo-documented the specimens; LC wrote and revised the manuscript. All authors agree with the final version of the manuscript.

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