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# Some rarely reported deep-water macroalgal species from Bonaire, Caribbean Sea, including *Verdigellas discoidea* sp. nov. (Palmophyllaceae, Chlorophyta) based on submersible collections

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**Abstract:** Two rarely reported and one newly described species of benthic marine algae are herein recognized from deep-water habitats at Bonaire, representing the first Caribbean reports of each. *Archestenogramma profundum* is previously known only from its type collection in Bermuda at 17 m depth and the rarely reported *Halymenia integra* is known originally from its type locality at Cabo Frio, Brazil. *Verdigellas discoidea* is newly described on the basis of morphological and molecular evidence. It forms flat circular thalli to 6.5 cm in diameter, measuring to 390 µm thick. The disc-like algae are attached by several small holdfasts on the ventral surface, but the margins are mostly free from their substrata.

**Keywords:** *Archestenogramma*; Caribbean; *Halymenia*; macroalgae; *Verdigellas*.

## Introduction

There is a limited literature dealing specifically with the algal flora of the Islands Aruba, Bonaire and Curaçao comprising the former political entity Netherlands Antilles, located in the southern Caribbean Sea. Most of these accounts dealt with shallow-water algae (incl. Hoek 1969, Hoek et al. 1972, 1975, 1978, Wanders and Wanders-Faber 1974, Wanders 1976a,b, 1977, Fricke et al. 2018). Taylor's (1960) flora of the region also includes a number of records from the Netherlands Antilles. Hoek (1978) reported on the occurrence of four algal species from deep reef habitats, to 65 m and is the sole published account of deep-water algae from Curaçao.

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The genus *Verdigellas* currently comprises three species, *V. peltata* D.L. Ballant. et J.N. Norris (1994), *V. nektongamma* D.L. Ballant. et N. Aponte (1996) and the generitype, *V. fimbriata* D.L. Ballant. et J.N. Norris (1994). *Verdigellas* species share a soft gelatinous habit and are composed of small spherical cells arranged in a gelatinous matrix. Reports of the genus are relatively few, due to its mostly deep-water distribution. *Verdigellas fimbriata* is known only from Puerto Rico (Ballantine and Norris 1994); *V. peltata*, also from Puerto Rico (Ballantine and Norris 1994, Ballantine et al. 2016), is additionally known from the Bahamas (Ballantine and Aponte 1996), Florida (Dawes and Mathieson 2008), Bermuda (Schneider et al. 2010), Brazil (Bravin et al. 1999) and the northwestern Gulf of Mexico (Leliaert et al. 2016); and *V. nektongamma* is known only from the Bahamas archipelago (Ballantine and Aponte 1996). *Verdigellas*, a member of the Palmophyllales, is a source of recent interest due to its distinct and early diverging position in the phylogeny of green algae (Zechman et al. 2010, Leliaert et al. 2011, 2016).

Beginning in 2011, a multi-disciplinary group of scientists from the Smithsonian Institution, began an examination of deep-water fauna and flora in an ongoing Deep Reef Observation Project (DROP) in the southern Caribbean using the deep research submersible, *Curasub* (<http://www.substation-curaçao.com>). Collections from the January 2017 expedition to Bonaire, led to this contribution.

## Materials and methods

All collections were made by the manned submersible, *DSVCurasub*. Voucher specimens were fixed in a 4% formaldehyde and seawater solution. Microscopic preparations were stained with 1% acidified aniline blue and mounted in 60% Karo® (ACH Food Company, Inc., Memphis, TN, USA). Photomicrographs were taken utilizing an Olympus BX63 microscope with Olympus CellSens software (Olympus Optical Co., Tokyo, Japan) for Extended

Focal Imaging. The plates were assembled from digital photographs utilizing Adobe Photoshop CC (ver. 20.0.4, Adobe Systems, San Jose, CA, USA). Type and voucher specimens of the reported species are deposited in the US Algal Collection of the National Herbarium, Smithsonian Institution. Authority designations are according to Brummitt and Powell (1992).

Portions of fresh algal tissue for DNA analysis were dried in silica gel and kept in individual polyethylene bags at  $\sim 2^{\circ}\text{C}$  until accessed for DNA isolation, at which time a 0.5-cm<sup>2</sup> fragment of dried thallus was pulverized in a zirconium bead and garnet sand mixture and then processed utilizing a modified phenol: chloroform extraction (Sambrook and Russell 2006). The *rbcL* plastid gene was amplified using either the 6F or 7F forward primer from Lam and Zechman (2006) in combination with the 1391R primer developed by Verbruggen et al. (2009). The 5' half of the *rbcL* sequence was also amplified and sequenced using the 6F-791R, 6F-893R, 6F-904R primer pairs. The 3' half of *rbcL* was both amplified and sequenced with 712F-1391R (Verbruggen et al. 2009). Polymerase Chain Reactions were prepared in 25- $\mu\text{l}$  volumes with 2.5  $\mu\text{l}$  Bionline NH<sub>4</sub> reaction buffer (10 $\times$ ), 2.0  $\mu\text{l}$  dNTP mix (10 mM), 1.25  $\mu\text{l}$  MgCl<sub>2</sub> (50 mM), 1.0  $\mu\text{l}$  of each primer (10 nM), 0.5  $\mu\text{l}$  bovine serum albumin (10 mg  $\cdot$  ml<sup>-1</sup>), 14.05  $\mu\text{l}$  water, 0.2  $\mu\text{l}$  Bionline DNA polymerase (5 U  $\cdot$   $\mu\text{l}$ <sup>-1</sup>); (Bionline USA, Taunton, MA, USA) and 2.5  $\mu\text{l}$  DNA template solution ( $\sim 10$  ng  $\cdot$   $\mu\text{l}$ <sup>-1</sup>). Amplification conditions were 95 $^{\circ}\text{C}$  for 3 min, followed by 35 cycles of 94 $^{\circ}\text{C}$  for 45 s, 55 $^{\circ}\text{C}$  for 60 s and 72 $^{\circ}\text{C}$  for 90 s, with a 5 min final extension at 72 $^{\circ}\text{C}$ . The products were treated with ExoSAP-IT (Affymetrix-USB, Santa Clara, CA, USA) prior to sequencing using BigDye Terminator v3.1 [Applied Biosystems, Foster City, CA, USA], where 0.8  $\mu\text{l}$  BigDye mix was combined with 1.0  $\mu\text{l}$  of 1  $\mu\text{M}$  primer, 2.0  $\mu\text{l}$  of 5 $\times$  buffer, 4.2  $\mu\text{l}$  water, and 4  $\mu\text{l}$  of DNA template. The Sanger reaction was conducted for 30 cycles of 95 $^{\circ}\text{C}$  for

30 s, 50 $^{\circ}\text{C}$  for 30 s and 60 $^{\circ}\text{C}$  for 4 min. The resulting fragments were filtered through Sephadex G-50 (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA), heat-denatured in formamide and then read on an ABI 3730 $\times$ 1 DNA Analyzer (Applied Biosystems). Geneious Prime<sup>®</sup> 2019.0.3 (Biomatters Ltd., Newark, NJ, USA) was used to edit and assemble the chromatograms to consensus sequences that were aligned with other taxa of Palmophyllales found in GenBank (Table 1). The *rbcL*, nuclear 18S and internal transcribed spacer 2 region were sequenced for the new *Verdigellas* species [BLB1233 (BONB 17073)] and were issued GenBank accession numbers MK583199, MK726277 and MK726276, respectively.

Phylogenetic relationships between the newly proposed *Verdigellas* species and those found in GenBank were estimated using Bayesian Inference with MrBayes 3.2.6 (Ronquist et al. 2012), separately. Two separate Markov chains were calculated for 3,001,000 generations and stopped when the average standard deviation was  $< 0.01$ . The first 25% of resulting topologies were discarded as burn-in before calculating the posterior probabilities on each node of the majority-rule consensus tree. The resulting phylogram images (not shown) were produced using FigTree 1.4.3 (Rambaut 2009). ITS2 secondary structures were predicted using mFold (Zuker 2003); the structures best approximating the conserved domains described by Mai and Coleman (1997) were chosen to identify possible compensatory base changes.

## Results

**Rhodophyta F. Wettst**  
**Florideophyceae Cronquist**  
**Gigartinales F. Schmitz**  
**Phylloporaceae Willkomm**

**Table 1:** Species used for 18S and *rbcL* phylogenetic analysis.

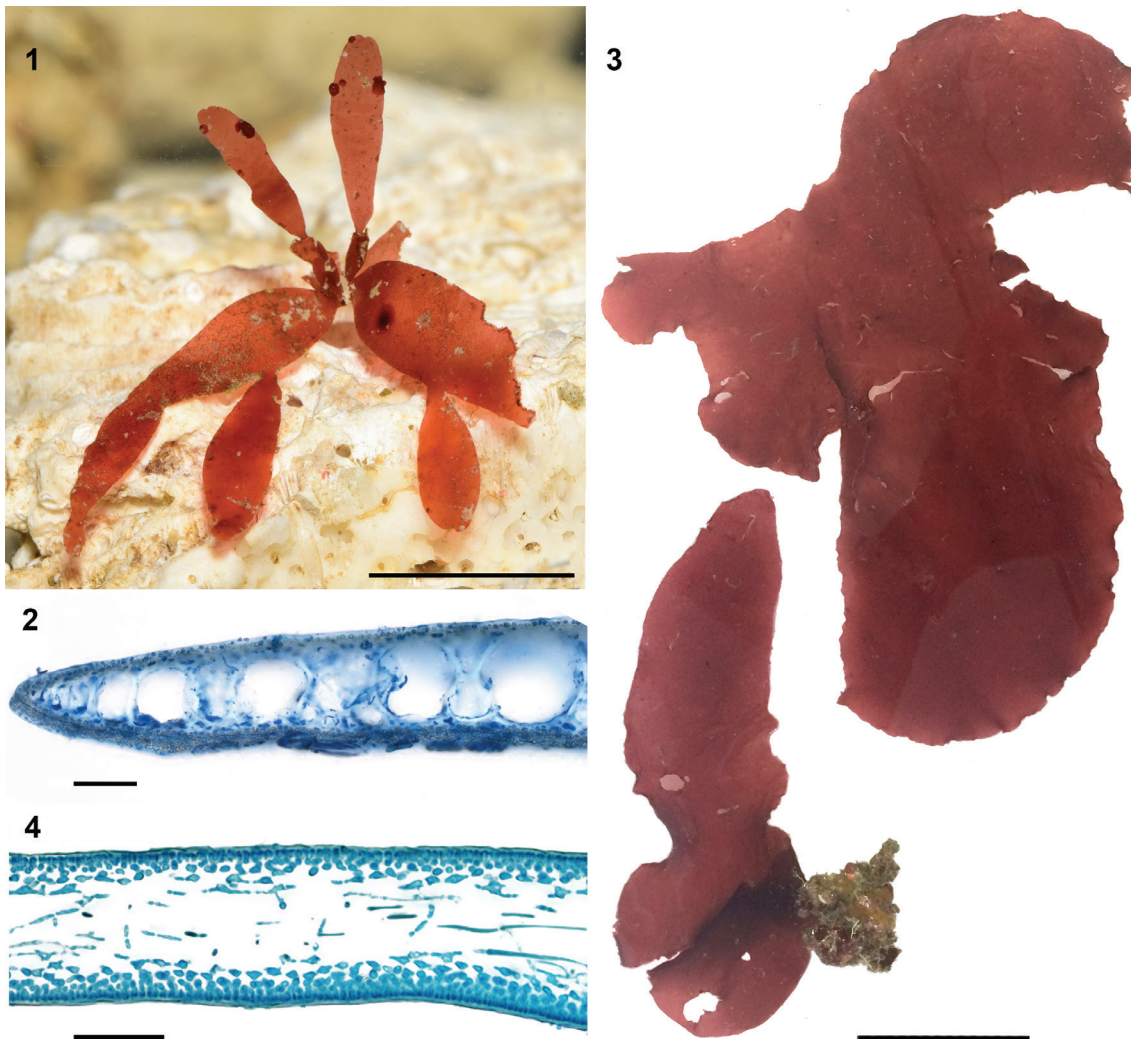
Species	GenBank 18S accession no.	GenBank <i>rbcL</i> accession no.	Locality	Reference
<i>Palmophyllum crassum</i>	MF769361	MF769368	South Korea	Lee and Kim 2017
<i>P. crassum</i>	MF769362	MF769367	South Korea	Lee and Kim 2017
<i>P. umbricola</i>	FJ619275	EU586180	California, USA	Zechman et al. 2010
<i>P. umbricola</i>	FJ619276	EU586182	New Zealand	Zechman et al. 2010
<i>Verdigellas discoidea</i>	MK726277	MK583199	Bonaire	This contribution
<i>V. peltata</i>	FJ619277	EU586183	Guadaloupe	Zechman et al. 2010
<i>V. peltata</i>	LT174528	LT174527	NW Gulf of Mexico	Leliaert et al. 2016

***Archestenogramma profundum* C.W. Schneider, Chengsupanimit et G. Saunders (Figures 1, 2)**

Specimens examined: Bonaire, *BLB1238* (BONB 17078), US Alg. Coll. 226937, south of Punt Vierkant, 12.097° N, 68.289° W, 116 m, 19.i.2017, Coll: L. Weigt, B. Thoma, I. Silver-Gorges, B. van Bebbber; *BLB1239* (BONB17079) US Alg. Coll. 226938, *ibid.*, 119 m.

Schneider et al. (2011) described *Archestenogramma profundum* based on collections made in Bermuda. Their specimens were reported to be ligulate branched blades to 3.0 cm tall, to 4.0 mm wide and 260  $\mu\text{m}$  thick.

Cortical cells measured to 8  $\mu\text{m}$  diam. and medullary cells to 160  $\mu\text{m}$  diam. The Bonaire specimens (Figure 1) produced up to 4 erect ligulate blades from a common base. The blades measured to 2.0 cm long and to 5.0 mm wide and are sparsely branched from the margins. Trans sections revealed a medulla of 1–2 (–3) cell layers (Figure 2) and a blade thickness of 200  $\mu\text{m}$ . The medullary cells are thick-walled and reach 110  $\mu\text{m}$  diam. Cortical cells measured 4.0–6.5  $\mu\text{m}$  diam. All specimens examined were sterile. The dimensions of the Bonaire specimens are similar although somewhat less than reported for original Bermuda collections. Despite the specific epithet, the original species collection was only from water of moderate depth, 17 m. The Bonaire specimens



**Figures 1–4:** (1–2) *Archestenogramma profundum* (*BLB1238*).

(1) Habit of living specimen (in aquarium), scale bar = 1.0 cm. (2) Trans-section, showing single and multiple layers of medullary cells, scale bar = 100  $\mu\text{m}$ . (3–4) *Halymenia integra* (*BLB1161*). (3) Habit, scale bar = 2.5 cm. (4) Trans-section showing cortex and medullary filaments, scale bar = 100  $\mu\text{m}$ .



are more appropriate to the epithet, having been collected to 119 m. We consider the discrepancy in size of our measurements not to be at serious variance and may easily represent the substantially deeper habitat of the Bonaire specimens.

**Halymeniales G.W. Saunders et Kraft**  
**Halymeniaceae Bory**

***Halymenia integra* M. Howe et W.R. Taylor**  
**(Figures 3, 4)**

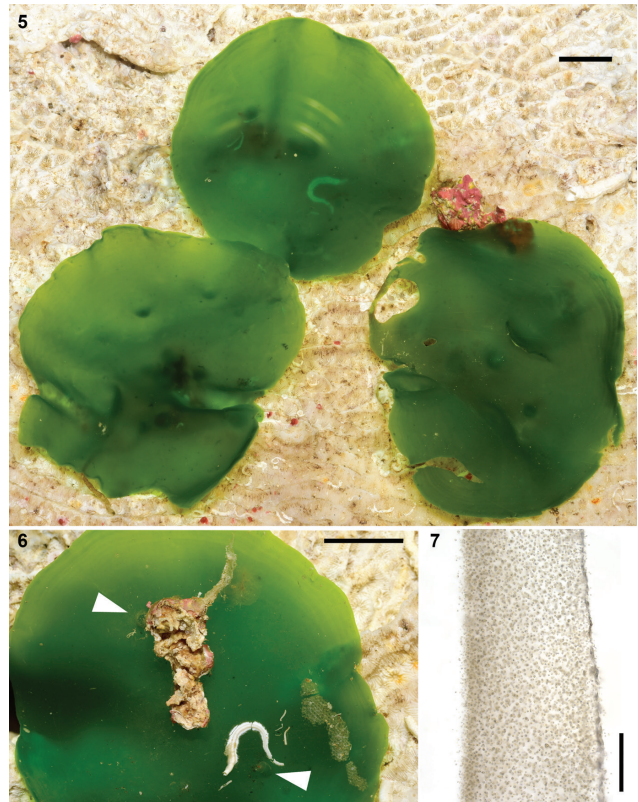
Specimen examined: *BLB1161* (BONB 17001) US Alg. Coll. 226932; between Harbor Village and Klein Bonaire, 12.159° N, 68.290° W, 91 m, Coll: C.C. Baldwin, A. Schrier, B. Brandt and M. Patterson, 19.i.2017.

The species was originally described by Howe and Taylor (1931) based on a dredged collection (no depth provided) from Cabo Frio, Brazil during the Hassler Expedition of 1871 and 1872. Utilizing the U.S. Coast Surveyor Hassler, the expedition under the direction of ichthyologist Louis Agassiz, spent time dredging coastal areas of eastern Brazil. Subsequent collections include those of Hanisak and Blair (1988) from the east Florida continental shelf at a depth of 44 m. *Halymenia integra* is also listed by Yoneshigue-Valentin et al. (1995) as a member of the deep (45–90 m) continental shelf algal community of Brazil.

Two individual specimens attached to the same rock have now been collected from Bonaire. One specimen was strap-shaped, tapering distally to an acute apex with a lobed branch and the other was lobed. Both possessed entire margins and cuneate bases (Figure 3). Dimensions of the larger specimen were 14 cm long, 5.0 cm wide and 175  $\mu\text{m}$  thick. Medullary filaments were mostly parallel to the surface with fewer filaments traversing the medulla (Figure 4). Medullary filaments measured 3.0–5.0  $\mu\text{m}$  diam. and reduced stellate ganglia were rarely observed.

Taylor (1960) referred to *Halymenia integra* as an apparently deep-water species. The type collection was dredged and both the Hanisak and Blair (1988) and Yoneshigue-Valentin et al. (1995) collections were at 44 m or greater. This latest report at 91 m further supports this characterization.

**Chlorophyta, Reichenbach**  
**Palmophyllophyceae. Leliaert et al.**  
**Palmophyllales Zechman et al.**  
**Palmophyllaceae Zechman et al.**



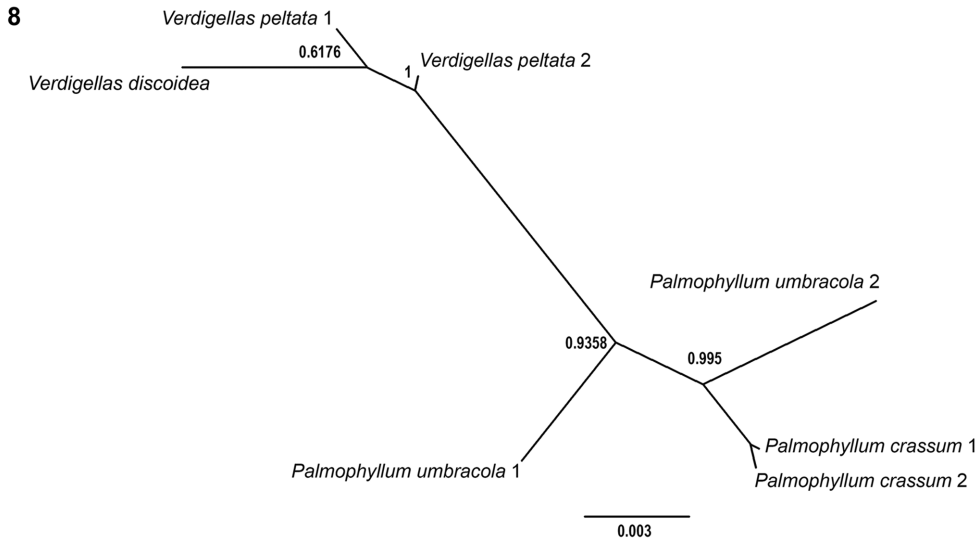
**Figures 5–7: *Verdigellas discoidea* (BLB1233).**  
 (5) Habit of the holotype (in aquarium), scale bar=1.0 cm. (6) Ventral view (in aquarium) of the top central specimen (Figure 5) showing small attachment stipes (arrowheads), scale bar=1.0 cm. (7) Trans-section showing dense placement of unicells, scale bar=200  $\mu\text{m}$ .

***Verdigellas discoidea* sp. nov. (Figures 5–7)**

Specimens examined: *BLB1233* (BONB 17073) Holotype US Alg. Coll. 226933, south of Punt Vierkant, 12.090° N, 68.289° W, 117 m, Coll: B.L. Brooks, C.C. Baldwin, L. Tornabene and B. Brandt, 19.i.2017.  
 Isotypes: US Alg. Coll. 226934, 226935, and 226936;

Lubricous thalli are disk-like, perfectly round or with slightly eroded margins (Figure 5) and bright green. Observable when living, algae are concentrically zonate towards the margins. Thalli are anchored by occasional very short stipes, apparently originating at random (Figure 6), and the margins are largely free from the substratum. The disks are 3.3–6.5 cm diam. and 325–390  $\mu\text{m}$  in thickness. Margins of the disk may be slightly thickened. Dorsal surfaces are smooth (Figure 5) and internally made up of densely placed coccoid cells, 5.2–9.1  $\mu\text{m}$  diam. (Figure 7).

*Verdigellas discoidea* is easily distinguished from its congeners on the basis of gross morphology. *Verdigellas*



**Figure 8:** Bayesian inference of phylogenetic relationships among concatenated SSU and 5' *rbcL* accessions of *Verdigellas* and *Palmophyllum* available on GenBank and sequenced in this study.

The scale bar shows genetic distance and node labels indicate the posterior probability of the relationship. [*V. peltata* 1 is from Guadeloupe, W.I.; *V. peltata* 2 is from Ewing Bank, NW Gulf of Mexico (USA); *P. crassum* 1 is from Udo, Jeju, Korea; *P. crassum* 2 is from Chujado, Jeju, Korea].

*discoidea* is the only species of the genus demonstrating zonation and a flat discoid habit. The new species and *Verdigellas peltata* share the character of possessing a dorsi-ventral organization. The new species is disk-like in shape, differing from *V. peltata* that is peltate and somewhat globose above multiple short stipes, to 1.0 mm long. *Verdigellas peltata* is substantially thicker than the new species (to 2.0 mm vs. to <0.4 mm). In *V. peltata*, vegetative cells are more densely placed at the surface while they are densely distributed throughout in the new species. The two remaining *Verdigellas* species, *V. fimbriata* and *V. nektongamea* both differ in having an erect habit. *Verdigellas fimbriata* reaches 9.0 mm in height and to 11 mm in width, is flat and possesses irregular marginal branching. *Verdigellas nektongamea* reaches 2.0 cm in height and 4.0 cm in width; it also differs from the new species in possessing vegetative cells arranged in reticulate strands internally.

Prior to this study, *Verdigellas peltata* was the only species of the genus for which DNA sequence data was available (Table 1). Aligning the 5' half of *rbcL* of the new species against that of a *V. peltata* specimen from NW Gulf of Mexico, revealed 1.1% divergence with seven single nucleotide polymorphisms, resulting in three amino acid differences (Leucine vs. Serine, Proline vs. Glutamic Acid, and Isoleucine vs. Leucine). Moreover, there were 8 non-compensatory base changes in the nuclear ribosomal internal transcribed spacer 2 (ITS2) between the new species and a *V. peltata* sample from NW Gulf of Mexico. There was

only one base difference between the 18S sequence for *Verdigellas discoidea* and that of *V. peltata*. Nevertheless, the phylogenetic relationships among *Verdigellas* species and their sister taxa, *Palmophyllum*, were reconstructed using Bayesian inference of concatenated nuclear 18S and plastid *rbcL* sequences. Importantly, this unrooted tree (Figure 8) depicts how the genetic distance between *V. peltata* and *V. discoidea* is comparable to the distances between *Palmophyllum* species. Lee and Kim (2017) reported that *Palmophyllum crassum* (Naccari) Rabenh. from Korean and Japanese collections showed 0.3% SSU and 18S rRNA genetic divergences with *P. umbracola* W.A. Nelson et K.G. Ryan and *V. peltata*, respectively. Further, Leliaert et al. (2016, p. 9) state “The small, compact and intronless cpDNA of *V. peltata* shows remarkable similarities in gene content and organization with the cpDNAs of Prasinococcales and the streptophyte *Mesostigma viride*, indicating that cpDNA architecture has been extremely well conserved in the early-branching lineages of green plants.” Thus, while the genetic difference between *V. discoidea* and *V. peltata* is not great, species level genetic divergence is apparently low in the Palmophyllaceae for the genes analyzed. We therefore conclude that the combination of genetic distance in addition to substantial morphological differences support recognition of *V. discoidea* as a new species.

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## Bionotes



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## Graphical abstract

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David L. Ballantine, Barrett L. Brooks and Gabe P. Johnson

**Some rarely reported deep-water macroalgal species from Bonaire, Caribbean Sea, including *Verdigellas discoidea* sp. nov. (Palmophyllaceae, Chlorophyta) based on submersible collections**

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**Research article:** Two rarely reported marine rhodophytes (*Archestenogramma profundum* and *Halymenia integra*) in addition to *Verdigellas discoidea* sp. nov., all from deep-water habitats are reported from Bonaire Caribbean Sea.

**Keywords:** *Archestenogramma*; Caribbean; *Halymenia*; macroalgae; *Verdigellas*.

