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A new encrusting deep-water coral reef alga, *Peyssonnelia incomposita* (Peyssonneliaceae, Rhodophyta), from Puerto Rico, Caribbean Sea

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Abstract – A new *Peyssonnelia* species is described from deep-water habitats in Puerto Rico as well as the U.S. Virgin Islands. *Peyssonnelia incomposita* is distinctive when living due its background orange coloration with highly contrasting bright yellow highlights. Internally the thallus is distinguished by abundant cell fusions that occur both laterally and longitudinally among cell rows of the perithallus as well as between cells of adjacent perithallial filaments at different tier levels. Tetrasporangia are cut off distally and possess an enlarged pedicel. They measure to 30 µm wide and 75 µm long. The crusts are closely appressed to the substratum and while variable, normally measure approximately 75 µm in thickness.

Caribbean / deep-water algae / *Peyssonnelia incomposita* / Peyssonneliaceae / Puerto Rico / Rhodophyta / western Atlantic

Résumé – Une nouvelle algue encroûtante des récifs coralliens profonds, *Peyssonnelia incomposita* (Peyssonneliaceae, Rhodophyta), de Puerto Rico, Mer des Caraïbes. Une nouvelle espèce de *Peyssonnelia* est décrite à partir habitats d'eau profonde à Puerto Rico, ainsi que des îles Vierges américaines. *Peyssonnelia incomposita* se distingue à l'état vivant par sa coloration de fond orange avec des contrastes jaunes très intenses. De façon interne, le thalle se distingue par les fusions cellulaires abondantes qui se produisent à la fois latéralement et longitudinalement entre les rangées de cellules du périthalle, ainsi qu'entre les cellules appartenant à des filaments périthalliques adjacents à différents niveaux. Les tétrasporanges sont coupés distalement et possèdent un pédoncule élargi. Ils mesurent 30 µm de large et 75 µm de long. Les croûtes sont étroitement accolées au substrat et, bien que variables, elles mesurent généralement 75 µm d'épaisseur environ.

Algues des eaux profondes / Atlantique occidental / Mer des Caraïbes / *Peyssonnelia incomposita* / Peyssonneliaceae / Puerto Rico / Rhodophyta

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INTRODUCTION

A quantitative and qualitative examination of the benthic marine algae of deep-water coral reef habitats in southwestern Puerto Rico was begun in 2007 as part of a NOAA-sponsored "mesophotic" coral reef ecosystem study. Among the numerous crustose algal species observed, a distinctive undescribed *Peyssonnelia* species was regularly encountered at depths greater than 50 m.

Peyssonnelia is a large genus presently credited with 70 species (Guiry & Guiry, 2010; Ballantine & Ruiz, 2010) and with a principally warm temperate to tropical distribution (Denizot, 1968; Kato & Masuda, 2003). The already speciose genus is growing steadily as there has been an increased interest in the group. A number of new species mostly from deep-water habitats in Puerto Rico and the Bahamas have recently been described, including: *P. abyssica* D.L.Ballant. *et* Aponte (2005), *P. imbricans* D.L.Ballant. *et* H.Ruiz (2006, 2007), *P. flavescens* D.L.Ballant. *et* H.Ruiz (2005), *P. iridescens* D.L.Ballant. *et* H.Ruiz (2010) and *P. gigaspora* D.L.Ballant. *et* H.Ruiz (2010). The latter two brings to 16 the total number of *Peyssonnelia* species currently recognized from the greater Caribbean (Wynne, 2005) of which eight are known from Puerto Rico (Ballantine & Aponte, 2002; Ballantine & Ruiz, 2010).

Species of *Peyssonnelia* are distinguished from one another by a variety of morphological and reproductive characters including whether rhizoids are unicellular or multicellular, the degree of crust adherence to the substratum, disposition of the hypothallial filaments (whether they are in straight and parallel or fan-shaped arrangements), perithallus anatomy (angle of perithallial filaments, whether or not perithallial coxal cells are cut off from the entire dorsal surface of hypothallial cells, number of erect perithallial filaments from the perithallial coxal cell, degree of lateral adherence of perithallial filaments), presence or absence of cystoliths, presence or absence of hair cells, presence or absence of cell fusions, whether nemathecia are immersed or superficial, position and size of the tetrasporangia within the nemathecia, and development of spermatangia (Boudouresque & Denizot, 1975; Maggs & Irvine, 1983; Schneider & Reading, 1987; Guimarães & Fujii, 1999; Dixon, 2010).

MATERIALS AND METHODS

Plants were collected utilizing trimix rebreather diving with mixed gases and were preserved in 10% formalin-seawater. Prior to sectioning, pieces of *Peyssonnelia* were decalcified in 5% HCl and hardened for 1 minute in N-Methylmorpholine. Transections (30-40 μm thick) were made with a Leica model CM1850 freezing microtome. Microscopic preparations were stained in acidified 1% aniline blue and mounted in 60% Karo® corn syrup on glass slides. Photomicrographs were taken with an Olympus DP720 digital camera through an Olympus BMAX light microscope (Olympus Optical Co., Tokyo, Japan). The plates were assembled from digital photographs utilizing Adobe Photoshop® CS2 (Adobe Systems, Inc., San Jose, USA). Voucher specimens were deposited in MICH, MSM and US. Herbarium abbreviations follow Holmgren *et al.* (1990), and authority designations are in accordance with Brummitt & Powell (1992).

RESULTS

Peyssonnelia incomposita D.L. Ballant. *et* H. Ruiz **sp. nov.** **Figs 1-10**

Diagnosis

Plantae encrustationes aurantiacas notis aureis formantes; crustae confertim ad substratum appressae; crustae rhizoideis unicellularibus ca 100 µm longae; cellulae hypothallinae in ordinibus parallelis depositae 12.5-17.5 µm longae et 15 µm altae; cellulae proximales perithallinaeque, e centro cellulae hypothallinae qui fert, abscissa, et filamenta 1 aut 2 assurgentia producentes quae sunt 12.5 µm lata infra et usque ad 7.5 µm distaliter; crassities crustae varians, ab 60 usque usque ad 175 µm sed plerumque ca 75 µm et 8 cellulas crassa; coniunctiones cellularum inter cellulas perithallinas valde pervulgatae; tetrasporangia in pedicellis magnis profunde tingentibus quae a cellulis proximalibus paraphysalibusque abscissa; gametophyta non visa.

Plants form reddish orange encrustations with bright yellow markings; crusts are closely appressed to the substratum; crusts with unicellular rhizoids averaging approximately 100 µm in length; hypothallial cells are arranged in parallel files, these measure 12.5 to 17.5 µm long and to 15 µm high; perithallial coxal cells cut off from the center of the bearing hypothallial cell and give rise to one or two assurgent filaments, these measuring to 12.5 µm in breadth below, decreasing to 7.5 µm distally; crust thickness varies from 60 to 175 µm, normally averaging 75 µm and 8 cells in thickness; cell fusions among perithallial cells very common; tetrasporangia borne on large deeply staining pedicels which themselves are cut off from proximal paraphysal cells, gametophytes not observed.

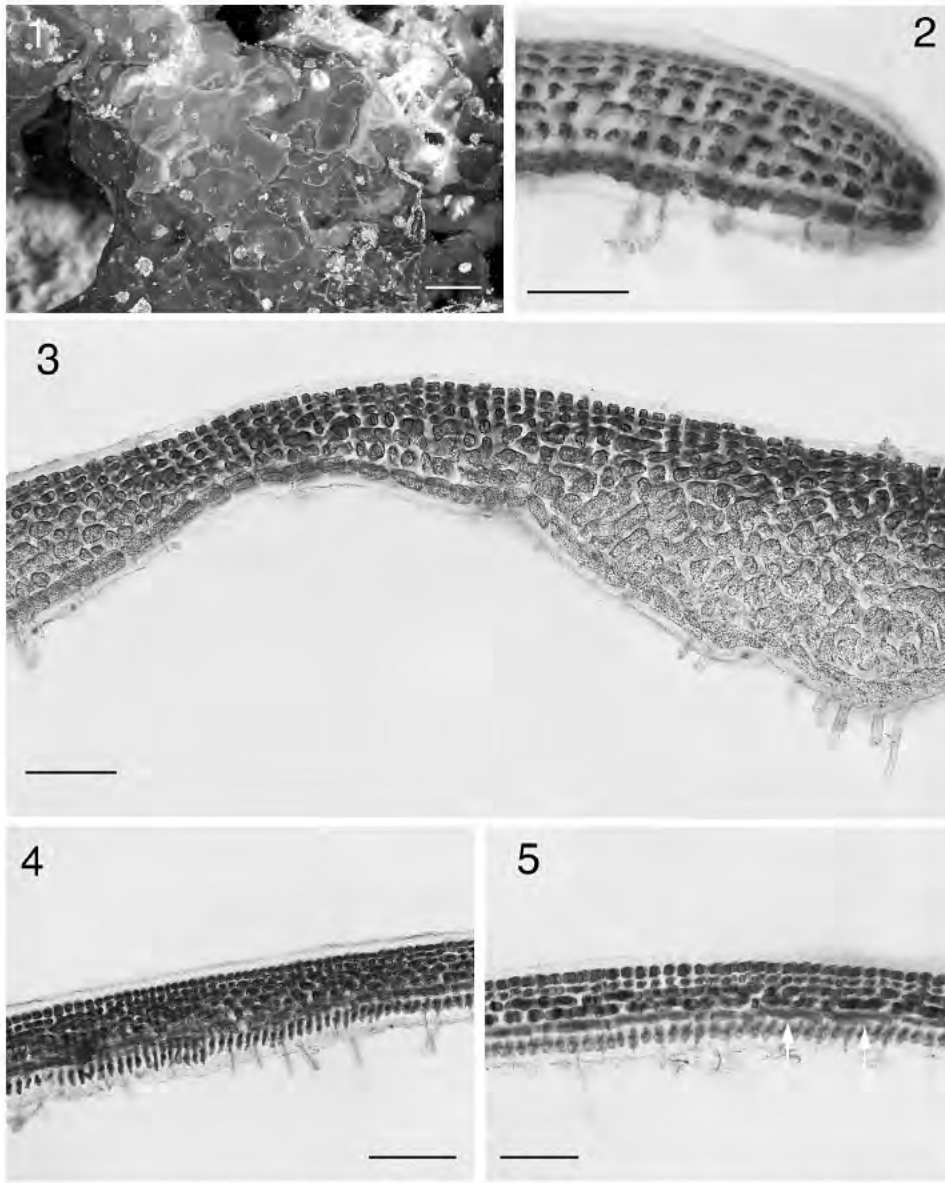
Holotype: *David L. Ballantine7777*, Edge of Insular Shelf ("Weinberg") (17°53.423' N, 66°59.320' W), La Parguera, Puerto Rico, 50 m, Coll. Hector Ruiz, 3.iii.2008 (#*US Alg. Coll. 211257*).

Isotypes: MICH, MSM, US.

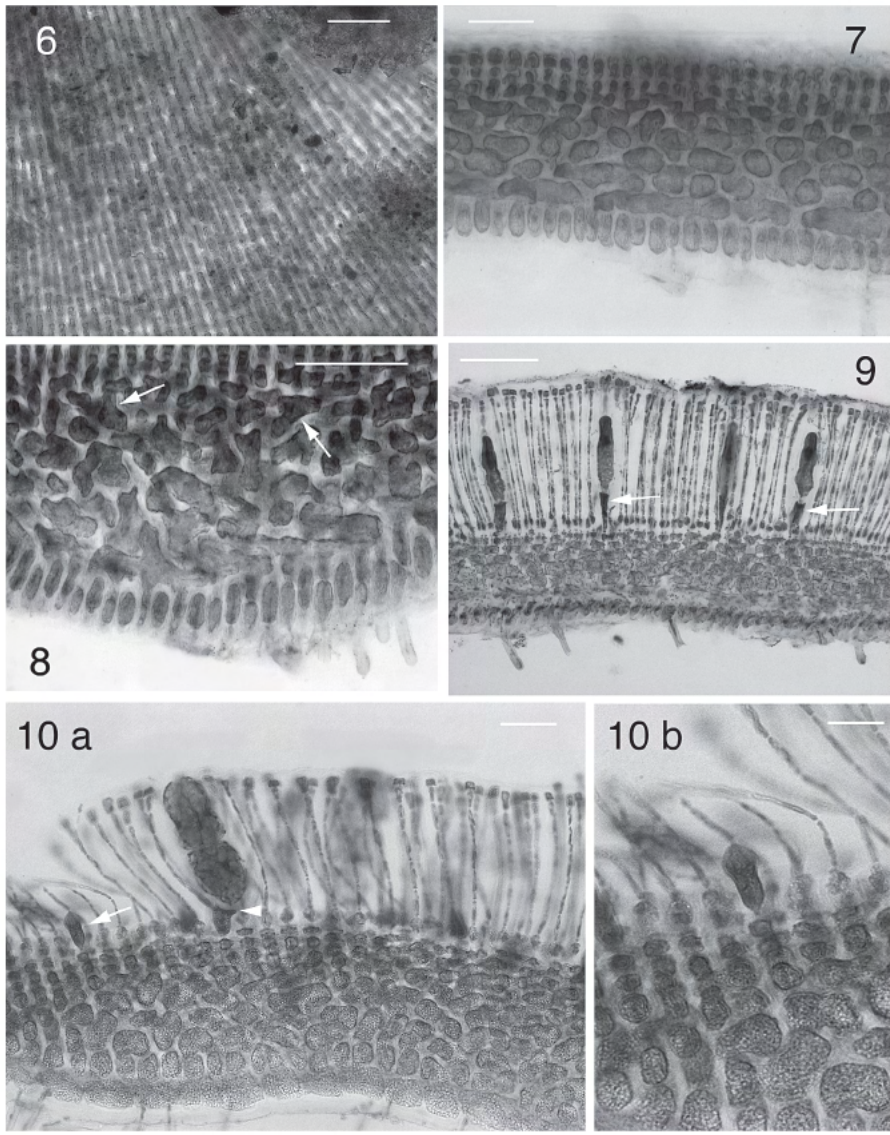
Paratypes (all in MSM): *DLB7818,* Edge of Insular Shelf ("Hole in the Wall"), La Parguera, Puerto Rico, 73 m, Coll. H.R., 11.vi.2009; *DLB7945*, *ibid.*, 67 m, 18.xi.2009; *DLB7830*, Edge of Insular Shelf ("El Hoyo"), La Parguera, Puerto Rico, 52 m, Coll. H.R., 9.vi.2009; *DLB7859*, *ibid.* 82 m, 1.ix.2009; *DLB7907, ibid.*, 70 m, 15.ix.2009; *DLB7987*, south Mona Island, Puerto Rico, 62 m, Coll. Michael Nemeth, Ivonne Bejarrano, Clark Sherman, 12.i.2010; *DLB8021*, *ibid.*, 70 m, Coll. H.R., 10.i.2010; *DLB8039*, Vieques Island ("El Seco"), Puerto Rico, 70 m, Coll. M.N., I.B., C.S., 17.i.2010; *DLB8127*, St. Thomas ("Grammanik Bank"), U.S. Virgin Islands, 70 m, Coll. M.N., I.B., C.S., 21.i.2010

Etymology: The specific epithet refers to the confused or chaotic pattern of cells of the perithallus caused by the abundant cell fusions.

Peyssonnelia incomposita is an exclusively deep-water species, having been collected from 50 to 73 m in depth. Plants typically form reddish orange encrustations that are highlighted with bright yellow markings. Concentric and longitudinal striations or patterns are absent. The blades are closely appressed to the substratum with entire colonies (Fig. 1) measuring up to 30 cm across. Calcification is strictly hypobasal. Hypothallial filaments are arranged in more or less parallel files (Fig. 6). In tangential section (Figs 4, 5), hypothallial cells



Figs 1-5. *Peyssonnelia incomposita* sp. nov. (All figures *DLB7777*). **1.** Habit of the holotype (Scale bar = 1 cm). **2.** Radial transverse section through growing apex. Note cell fusions occurring almost immediately behind the apex (scale bar = 50 µm). **3.** Radial transverse section showing irregular thickness of crusts ranging from 60 to 180 µm and 6 to approximately 15 cells in thickness (scale bar = 100 µm). **4.** Tangential transverse section showing abundant lateral fusions at all perithallial filament tier levels except the distal most (scale bar = 100 µm). **5.** Tangential transverse section showing abundant lateral fusions (arrows). Note that fusions among coxal cells encompass up to eight different cells (scale bar = 100 µm).



Figs 6-10. *Peyssonnelia incomposita* sp. nov. (All figures *DLB7777*). **6.** Ventral portion of alga showing parallel arrangement of hypothallial cells (scale bar = 50 µm). **7.** Transverse section through the crust showing numerous mostly lateral fusions, disrupting an organized appearance of the thallus (scale bar = 50 µm). **8.** Transverse section through the crust showing lateral, vertical fusions as well as fusions between cells perithallial files at a different tier level (arrows), disrupting an organized appearance of the thallus (scale bar = 10 µm). **9.** Young developing tetrasporocytes in superficial nemathecium; arrows denote large deeply stained pedicels (scale bar = 100 µm). **10a.** Mature tetrasporangium in superficial nemathecium; note recently cut off tetrasporocyte (arrow) at left which is enlarged in 10b. Arrowhead denotes pedicel supporting mature tetrasporangia (scale bar = 50 µm). **10b.** Enlarged portion (at left) of Fig. 10a showing recently cut off tetrasporocyte (arrow) directly from apical perithallial cell. Note absence of a pedicel (scale bar = 25 µm).

measure up to 16 μm wide and up to 15 μm high. The hypothallial cells in radial section (Figs 2, 3) are seen to be square to rectangular in shape, measuring 15 to 22.5 μm long by 11 to 15 μm high. The perithallial coxal cells, to 8-10 μm high X 10-13 μm long, are cut off from the center of the bearing hypothallial cell; however, they only cover approximately half the length of the bearing cell (Figs 2, 3). The coxal cell generally gives rise to a single, less commonly two, assurgent filament(s) (Figs 2, 3). The perithallial cells measure to 12.5 μm in breadth below, decreasing to 7.5 μm distally. The crust is typically 8 cells in thickness and according to microtopography of the substratum, measures from 60 to 175 μm although generally approximately 75 μm in thickness. The unicellular rhizoids average approximately 100 μm in length and are produced from the proximal end of hypothallial cells. Hair cells are not produced. *Peyssonnelia incomposita* is highly distinctive in section due to the abundant cell fusions between adjacent perithallial cells as well as between cells of adjacent perithallial filaments at different tier levels (Figs 7, 8). The fusions apparently occur in all directions. Particularly among the coxal cells, fusions may include up to 8 adjacent cells, with fusions measuring to 70 μm across (Figs 4, 5). With age, the perithallus appears to be extremely unorganized due to the fusions. Fusions were not observed among hypothallial cells.

Tetrasporangia are produced in very large nemathecia that extend up to 125 μm above the level of surrounding vegetative cells (Figs 9, 10a). Nemathecial filaments are multicellular and slender, to 2 μm in diameter (Fig. 9), and terminate in enlarged squarish cells, to 10 μm in diameter, or rectangular cells to 12 μm broad. Tetrasporangia (Fig. 10a) are cruciately divided, elongate, oval, measuring up to 30 X 80 μm (width X length). They are cut off directly from proximal nemathecial cells (Fig. 10b). The young tetrasporocyte presumably then divides to produce a tetrasporangium and a pedicel (Fig. 10a). The pedicel enlarges with maturation and is deeply staining (Figs 9, 10a). Virtually every apical vegetative cell within the nemathecium produces a tetrasporangium or a paraphysal filament. Gametophytes were not observed.

DISCUSSION

The principal diagnostic feature of *Peyssonnelia incomposita* is the presence of abundant fusions among perithallial cells. This character may be used to differentiate the new species from all Atlantic, including Caribbean, *Peyssonnelia* species. In the western Atlantic, the new species could only possibly be mistaken for the recently described *Ramicrusta textilis* Pueschel *et* G.W. Saunders (2009). *Ramicrusta textilis* and the generitype, *R. nanhaiensis* D.R. Zhang *et* J.H. Zhou (1981), are recognized as possessing both cell fusions and secondary pit connections; however, at least for *R. textilis*, the perithallus is characterized by a "highly patterned fabric of secondary pit connections" (Pueschel & Saunders, 2009, p. 481). They (*op. cit.*) indicated that the abundant lateral secondary pit connections produced by adjacent perithallial cells could be falsely interpreted as fusions; however, the dominant pit connections give the perithallus a decidedly different appearance than that of *P. incomposita* which lacks secondary pit connections. *Ramicrusta textilis* further differs from the new species in its growth habit, coloration and depth range (Pueschel & Saunders, 2009).

Denizot (1968) indicated that cell fusions were present in *Cruoriopsis reinboldia* Weber Bosse (1921). This genus, whose taxonomic status within the Peyssonneliaceae remains uncertain, is characterized by a mucilaginous perithallus. The mucilaginous perithallus serves to differentiate *C. reinboldia* from the new species. Questionable presence of cell fusions and perithallial morphology in *Peyssonnelia calcea* Heydrich was explained by Pueschel and Saunders (2009). Denizot (1968) transferred *Peyssonnelia frutescens* Me. Lemoine (1960) to a variety of *P. calcea*. Lemoine (*op. cit.*) indicated that her species possessed cell fusions and lacked secondary pit connections, while Weber-van Bosse (1921) in her account of *P. calcea*, reported presence of secondary pit connections. Dixon's (2010) examination of a piece of the surviving holotype specimen of *P. calcea* showed that it exhibited features of *Ramicrusta* (*i.e.* presence of pit connections). There remains the possibility that the reports of *P. calcea* from different geographic areas and with conflicting morphological attributes may represent different taxonomic entities.

Deep-water ("mesophotic") coral reef habitats in Puerto Rico have already yielded several new species to science. The *Peyssonnelia* species newly described herein undoubtedly has a broader distribution than only Puerto Rico and the U.S. Virgin Island. Other Peyssonneliaceae crusts including *Peyssonnelia* and *Polystrata* species have also been recognized; however, remain undescribed until reproductive plants are collected. Further deep-water collecting activities in other regions will undoubtedly result in extension of geographic ranges of these recently described species and will yield further undescribed diversity.

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