

## ***Peyssonnelia stratos* sp. nov. (Peyssonneliaceae, Rhodophyta), a new shallow-water species from Puerto Rico, Caribbean Sea**

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**Abstract** – A new *Peyssonnelia* species from shallow water habitats in southwest Puerto Rico is described. *Peyssonnelia stratos* is yellowish beige or reddish in color and is composed of small overlapping blades 2-3 cm in diameter and forms extensive colonies. *Peyssonnelia stratos* exhibits small cells at mid level in the perithallus, both immediate distal and proximal cells being much longer, giving the appearance of stratification within the perithallus. Tetrasporangia, cut off laterally from an enlarged proximal nemathecium cell, reach 190 µm in length.

**Caribbean / *Peyssonnelia* / Peyssonneliaceae / Puerto Rico / Rhodophyta / western Atlantic**

**Résumé** – Une nouvelle espèce se trouvant dans les eaux peu profondes du sud ouest de Porto Rico et appartenant au genre *Peyssonnelia* est décrite. *Peyssonnelia stratos* est d'un beige jaunâtre ou rougeâtre et est constitué de petites lames de 2-3 cm qui se recouvrent les unes les autres et forment des colonies étendues. *Peyssonnelia stratos* a de petites cellules à mi-niveau du périthalle, les cellules adjacentes distales et proximales sont beaucoup plus longues ce qui donne l'impression que le périthalle est stratifié. Les tétrasporocystes émergent latéralement à partir d'une cellule némathecium proximale et atteignent 190 µm de long.

**Caribes / *Peyssonnelia* / Peyssonneliaceae / Puerto Rico / Rhodophyta / Atlantique ouest**

### **INTRODUCTION**

*Peyssonnelia* is a large genus with a principally warm-temperate to tropical distribution (Denizot, 1968; Kato & Masuda, 2003). Presently 71 species are recognized (Guiry, 2011; Ballantine & Ruiz, 2011). In the tropical western Atlantic, Wynne (2011) listed 19 *Peyssonnelia* species, including *P. polymorpha* (Zanardini) F.Schmitz cited as a questionable record. In addition to the recent documentation of cryptic species within the Peyssonneliaceae (Dixon, 2010), recent research has resulted in the description of a number of new species. In

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Puerto Rico alone, new *Peyssonnelia* species have recently been described based on collections from both deep- and shallow-water habitats, including *Peyssonnelia flavescens* D.L. Ballant. et H. Ruiz (2005), *P. imbricans* D.L. Ballant. et H. Ruiz (2006, 2007), *Peyssonnelia iridescens* D.L. Ballant. et H. Ruiz (2010), *P. gigaspora* D.L. Ballant. et H. Ruiz (2010) and *P. incomposita* D.L. Ballant. et H. Ruiz (2011). Despite the apparently simple thallus plan of a horizontal hypothallus that gives rise dorsally to a layer of vertically assurgent perithallial filaments, a number of morphological and reproductive characters have been recognized that vary between species (Denizot, 1968; Boudouresque & Denizot, 1975; Marcot *et al.*, 1977; Guimaraes & Fujii, 1999; Ballantine & Ruiz, 2006; Dixon, 2010).

## MATERIALS AND METHODS

Plants were collected by snorkeling and specimens preserved in 10% formalin-seawater. Prior to sectioning, fragments of thallus were decalcified in 5% HCl and subsequently hardened in N-Methylmorphiline for approximately one minute. Transections (40  $\mu\text{m}$  thick) were made with a Leica<sup>®</sup> model CM1850 freezing microtome. Microscopic preparations were stained in acidified 1% aniline blue and mounted in 60% Karo<sup>®</sup> corn syrup on glass slides. Photomicrographs were taken with an Olympus<sup>®</sup> DP2 digital camera through an Olympus<sup>®</sup> BX60 light microscope utilizing a PRIOR motorized focusing drive and Olympus<sup>®</sup> Microsuite Five imaging software for extended focal imaging. The plates were assembled from digital photographs utilizing Adobe Photoshop<sup>®</sup> CS2. The holotype is deposited in US and isotypes in MICH and MSM. Herbarium abbreviations follow Holmgren *et al.* (1990) and initials for given names of authorities are according to Brummitt & Powell (1992).

## RESULTS

*Peyssonnelia stratos* sp. nov. D.L. Ballant. et H. Ruiz

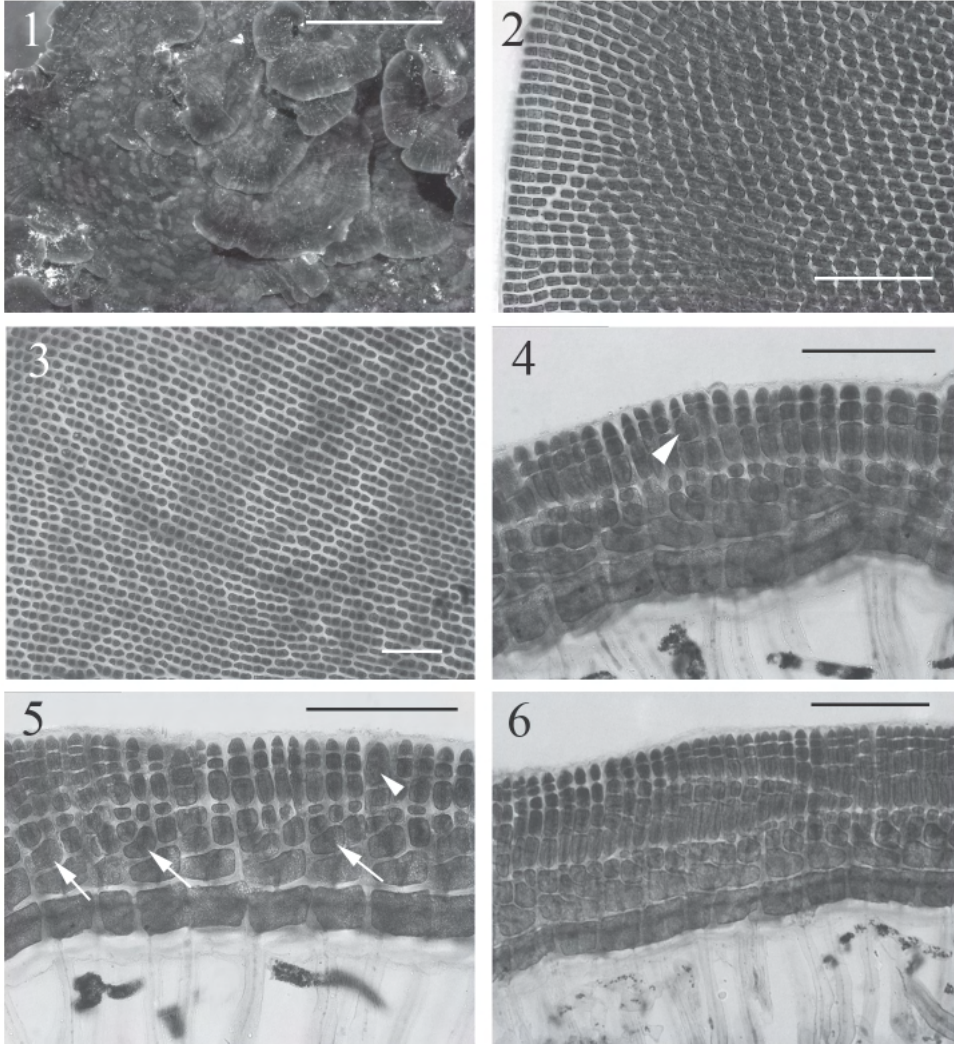
**Figs 1-10**

**Diagnosis.** Crusts are yellowish to beige with prominent radial striations, the extensive colonies are made up of small overlapping blades, 2-3 cm across; hypothallial cells are arranged in straight parallel files, the cells mostly squarish, 30-35  $\mu\text{m}$  in length and height when viewed in radial transverse section. Hypothallial cells produce elongate unicellular rhizoids, that measure to 400  $\mu\text{m}$  in length, at their anterior ends. Calcification is hypobasal, to 50  $\mu\text{m}$  in thickness immediately behind the growing edge and increasing to approximately 150  $\mu\text{m}$  at a distance of 1.0 cm from the growing edge. Perithallial filaments possess a single short cell 5-10  $\mu\text{m}$  long, generally at the same height within the perithallus; the adjacent cells above and below in the perithallial filament are ca. 30  $\mu\text{m}$  long and ca. 12  $\mu\text{m}$  broad. Perithallial filaments are 6 to 10 cells long, the entire crust 100-230  $\mu\text{m}$  in thickness; occasional hair cells measure to 16  $\mu\text{m}$  in width by 35  $\mu\text{m}$  in length. Tetrasporangial nemathecium are in discrete patches and measure to 220  $\mu\text{m}$  in height; typically paraphysal filaments are 7 cells long; enlarged proximal paraphysal cells, to 10  $\mu\text{m}$  in width by 30  $\mu\text{m}$  in length, divide laterally to give rise to cruciately divided tetrasporangia; tetrasporangia are 160-190  $\mu\text{m}$  long  $\times$  45-70  $\mu\text{m}$  broad. Gametophytic plants not observed.

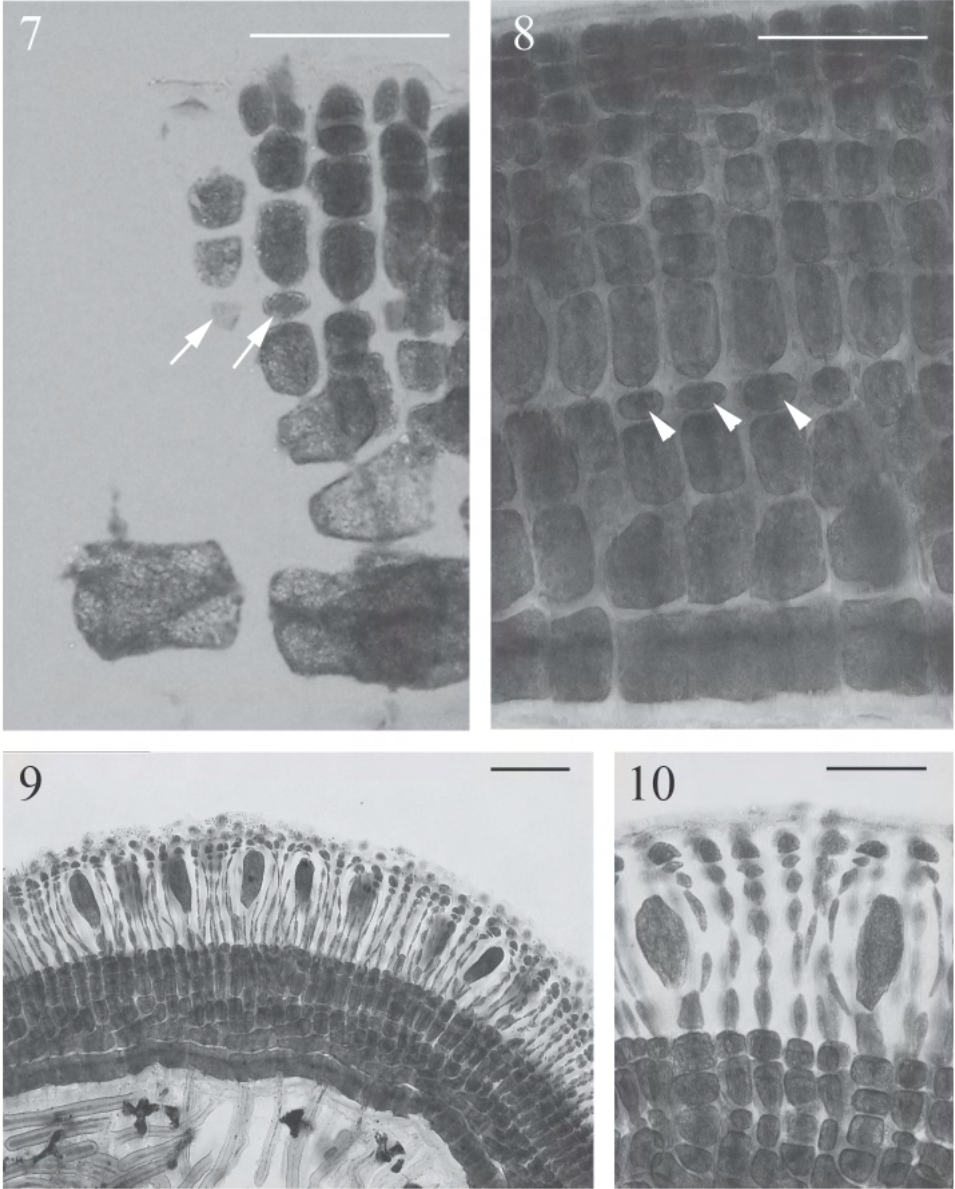
**Holotype:** DLB 8400, West side of Cabo Rojo Lighthouse point (17°56.029' N; 67°11.759' W), 0.5-1.0 m, Coll. D.L.B. and H.R., 28.ix.2011 (#US Alg. Coll.-217936).

**Paratypes** (in MSM and US): *DLB 8353*, West side of Cabo Rojo Lighthouse point ( $17^{\circ}55.865' N$ ;  $67^{\circ}11.347' W$ ), 0.5-1.0 m, Coll. H.R., 20.iv.2011; *DLB 8397*, East side of Cabo Rojo Lighthouse point, 0.5-1.0 m, Coll. D.L.B. and H.R., 28.ix.2011

**Etymology:** The specific epithet refers to the layered nature of the perithallus.



Figs 1-6. *Peyssonnelia stratosa* sp. nov. **1.** The living holotype (*DLB 8400*) (Scale bar = 2.0 cm). **2.** Hypothallial cells in straight files (*DLB 8400*) (Scale bar = 250  $\mu\text{m}$ ). **3.** Dorsal view of crust showing apical perithallial cells arranged directly over the hypothallial cells (*DLB 8353*) (Scale bar = 100  $\mu\text{m}$ ). **4.** Radial vertical section through crust showing tiered aspect and presence of small perithallial cells. A hair mother cell is indicated by an arrowhead. (*DLB 8353*) (Scale bar = 100  $\mu\text{m}$ ). **5.** Radial vertical section through crust showing unicellular rhizoids from the anterior end of hypothallial cells (*DLB 8353*), proximal perithallial cells giving rise to two cells, the anterior of which (arrows) also gives rise to two files of cells. A hair mother cell is indicated by an arrowhead. (Scale bar = 100  $\mu\text{m}$ ). **6.** Appearance of two to three layers within the perithallus (*DLB 8353*) (Scale bar = 100  $\mu\text{m}$ ).



Figs 7-10. *Peyssonnelia stratososa* sp. nov. **7.** Radial vertical section through a young thallus portion showing short perithallial cells (arrows) in a perithallial file (*DLB 8353*) (Scale bar = 50  $\mu$ m). **8.** Perithallial files of cells (tangential section) with a single short cell (arrowheads) in each file (*DLB 8397*) (Scale bar = 50  $\mu$ m). **9.** Tetrasporangial nemathecium (*DLB 8353*) (Scale bar = 100  $\mu$ m). **10.** Tetrasporocytes cut off laterally from enlarged proximal nemathecial cells (*DLB 8353*) (Scale bar = 50  $\mu$ m).

*Peyssonnelia stratosa* was collected on the walls of a shallow-cave at 0.5 m depth, from a nearby shallow, shaded vertical rock wall and a wave exposed rock outcrop. Crusts are yellowish to beige with prominent radial striations and are largely free at their margins (Fig. 1). The hypothallus cells are arranged in slightly curved parallel files (Fig. 2) and these are mostly squarish, 30-35  $\mu\text{m}$  in length and height, when viewed in radial vertical section (RVS), producing unicellular rhizoids below at their anterior ends (Fig. 4). The rhizoids are elongate measuring to 400  $\mu\text{m}$  in length. Calcification is hypobasal and uneven in thickness, to 50  $\mu\text{m}$  immediately behind the growing edge and increasing to approximately 150  $\mu\text{m}$  at a distance of 1.0 cm from the growing edge. Calcification incorporates the hypothallium as well the proximal-most perithallial cells in mature plant portions. Rhizoids penetrate the hypobasal calcification. Proximal perithallial cells are cut off from the center of the hypothallial cells and are nearly the same length as their bearing cells when viewed in RVS. The proximal perithallial cells normally cut off two cells, one of these (to the anterior, Figs 4, 5) cuts off two files of cells, and the other is undivided until near the dorsal surface where it commonly divides dichotomously. Perithallial cells immediately arising from coxal cells measure to 15  $\mu\text{m}$  broad and to 35-40  $\mu\text{m}$  high. At approximately the lower third of the perithallus, each of the regular files of perithallial filaments is typically interrupted by the presence of a single short cell, 5-10  $\mu\text{m}$  long. Cells immediately above and below the small cells are to approx. 30  $\mu\text{m}$  long and 12  $\mu\text{m}$  broad (Figs 4, 5, 7, 8). This gives the perithallus (when viewed in section), the appearance of possessing two layers (Figs 4, 5). Occasionally production of short perithallial cells more distally gives rise to the appearance of three tiers (Fig. 6). The perithallial cells become shorter and narrower distally. Apical perithallial cells are dome-shaped and measure 10  $\mu\text{m}$  in diameter. The perithallus is comprised of 6 to 10 cells and the entire crust ranges from 100-230  $\mu\text{m}$  in thickness. Occasional hair mother cells (Figs 4, 5) are observed in the upper perithallus, these typically measure 16  $\mu\text{m}$  in width by 35  $\mu\text{m}$  in length. When viewed from above the perithallial apical cells closely mirror the parallel arrangement of hypothallial cells (Fig. 3). Tetrasporangia are in discrete nemathecia that measure to 220  $\mu\text{m}$  in height (Fig. 9). Tetrasporangia derive laterally from the proximal nemathecial cell that becomes enlarged and ultimately measures to 10  $\mu\text{m}$  in width by 30  $\mu\text{m}$  in length (Fig. 10). The cruciately divided tetrasporangia measure 160-190  $\mu\text{m}$  long  $\times$  65  $\mu\text{m}$  broad. Nemathecial filaments are typically comprised of seven very slender cells, 2.5-5  $\mu\text{m}$  in diameter, the apical and subapical nemathecial cells being shorter than proximal cells; however are substantially enlarged to 10-15  $\mu\text{m}$  broad (Fig. 10). Gametophytic plants were not observed.

## DISCUSSION

*Peyssonnelia stratosa* was initially collected on walls of a partially enclosed cave and subsequently on vertical walls in a protected cove and on wave-exposed rocks, both located within several tens of meters of each other. It was also subsequently collected on a nearby exposed rocky outcrop. *Peyssonnelia* species are virtually all separated from one another by a combination of differing characters as is the case for *P. stratosa*. Morphological features for tropical west Atlantic *Peyssonnelia* species are shown in Table 1. Nevertheless, the presence of small perithallial cells at the same height within the perithallus of *P. stratosa* is, to our knowledge, unique in the genus. Otherwise *P. stratosa* shares with west Atlantic

Table 1. Comparison of western Atlantic *Peyssonnelia* species

<i>Species</i>	<i>Color</i>	<i>Calcification</i>	<i>Concentric Rings/ Radial Lines</i>	<i>Crust Margin</i>	<i>Perithallial cells rigid</i>	<i>Vegetative Crust Thickness (µm)</i>	<i>Anatomy Type</i>	<i>No. Uprights From 1<sup>st</sup> Perithallial Cell</i>
<i>P. abyssica</i>		Hypobasal	Lacking/ Lacking	Appressed	+	70-75	<i>P. rubra</i> -type	2
<i>P. armorica</i>		Hypobasal	Lacking/ Lacking	Appressed		60-100	<i>P. rubra</i> -type	1-2
<i>P. boergesenii</i>	Yellow-green with red markings	Hypobasal	Lacking/Present	Appressed	+	100-500	<i>P. rubra</i> -type	1-2
<i>P. boudouresquei</i>	Pink to dark red	Hypobasal	Lacking/ Lacking	Appressed	-?	80-220	<i>P. rubra</i> -type	2-3
<i>P. capensis</i>	Rose red to brownish	Hypobasal	Conspicuous/ Lacking	Free		140-310	<i>P. atropurpurea</i> -type	1
<i>P. conchicola</i>			?Lacking/ ?Lacking	Appressed	+	220	<i>P. rubra</i> -type	(2-)3
<i>P. flavescens</i>	Yellow or brown, red spots		Lacking/Faint to Conspicuous	Appressed	+	310-640	<i>P. rubra</i> -type	2(-3)
<i>P. gasporea</i>	Tan to reddish	Hypobasal		Appressed	+	130	<i>P. rubra</i> -type	2
<i>P. inamoena</i>	Pink to deep red	Hypobasal	Faint/Faint	Free	+	57-215	<i>P. rubra</i> -type	2
<i>P. imbricans</i>	Brown to orange	Complete	Lacking/ Conspicuous	Variably adherent	+	110-180	<i>P. atropurpurea</i> -type	2
<i>P. incomposita</i>	Orange with bright yellow margins	Hypobasal		Appressed	+	To 75	<i>P. rubra</i> -type	1 or 2
<i>P. iridescens</i>	Orange, purple margin	Hypobasal	Lacking/ Faint	Mostly free	+	To 100	<i>P. atropurpurea</i> -type	2
<i>P. nordstedtii</i>	Red-purple	Hypobasal	Faint/?	Appressed	+	?	<i>P. rubra</i> -type	3
<i>P. polymorpha</i>	Dark reddish purple	Almost complete	?	Free	+	300	<i>P. rubra</i> -type	1 or 2
<i>P. rosamarina</i>		Hypobasal	Conspicuous/ Conspicuous	Free	+	To 650	<i>P. rubra</i> -type	1-2
<i>P. rosenvingii</i>	Brownish purple		Faint/?	Appressed	+	To 500 or more	<i>P. atropurpurea</i> -type	1?
<i>P. simulans</i>	Bright pink		Lacking/ Lacking	Loose	+	200	<i>P. rubra</i> -type	2
<i>P. stoechas</i>	Rosy red	Hypobasal	Conspicuous/ Lacking	Free	+	150	<i>P. rubra</i> -type	2
<i>P. valentini</i>			Lacking/ Lacking	Free	+	50-150	<i>P. rubra</i> -type	2-3
<i>P. stratosa</i>	Yellowish beige to red	Hypobasal	Lacking/ Conspicuous	Free	+	100-230	<i>P. rubra</i> -type	2

<i>Hypothallus Orientation</i>	<i>Hair mother cells</i>	<i>Rhizoids, (µm)</i>	<i>Nematocia</i>	<i>Tetra-sporangial paraphysal width (µm)</i>	<i>Tetrasporangia Diameter X Length (µm)</i>	<i>Spermatangial Sorus Type</i>	<i>Particular features</i>	<i>References</i>
Polyflabellate		Unicellular to 100	Superficial		To 90 × to 150	?		1
Polyflabellate		Unicellular to 20	Superficial		18-20 × 60-70	?	Perithallial filaments not laterally coherent	2
Polyflabellate		Multicellular to 1000	Superficial		30-52 × 110-170	?		2, 3
Parallel		Unicellular to 43	Immersed		12-25 × 40-70	<i>P. dubyi</i> -type		2, 4 (as <i>P. atlantica</i> ), 5
Parallel		Multicellular to 1000	Superficial		14-24 × 60-70	<i>P. dubyi</i> -type		2
Parallel		Unicellular to 60	Immersed		15-25 × 32-53	<i>P. dubyi</i> -type		4, 6
Parallel		Unicellular to 450	Superficial		To 80 × to 170	<i>P. flavescens</i> -type	Large colony size	7
Parallel		Unicellular to 300	Superficial	1.5-2.5			Intercalary tetrasporangia	8
Parallel		Unicellular to 160	Superficial		27-57 × 70-100	<i>P. harveyana</i> -type		2, 4, 9
Polyflabellate		Unicellular, spherical	Superficial		To 45 × 110	Simple chains	Calcified throughout	10
Parallel		Unicellular to 175	Superficial	1.5	To 30 × 80	?	Lateral cell fusions	11
Parallel			Superficial	To 15	To 55 × 135	?	Iridescent margin	8
Polyflabellate		Unicellular to 96	Superficial		? X × 80	?		2, 6
Parallel		Multicellular, to 260			?	?	Swollen excrescens on surface	9, 12, 13, 14
Parallel		Unicellular & Multicellular, to 330	Superficial		35-90 × 80-135	?	Only monosporangia	2, 13, 15
Polyflabellate/ Parallel		Unicellular to 66	Superficial		50-55 × 88-110	?		16, 17, 18
Parallel		Unicellular to 65	Superficial		12-30 × 40-80	?		4, 6
Parallel		Unicellular to 230	Unknown		Unknown	?		4, 9
Parallel		Unicellular to 200	Superficial		21-43 × 66-85	<i>P. harveyana</i> -type		2, 19
Parallel	+	Unicellular, to 400	Superficial	To 5.0	To 65 × 190	?	Stratified perithallus	3

References: 1) Ballantine & Aponte 2005; 2) Guimarães & Fujii 1999; 3) This paper; 4) Schneider & Reading 1987; 5) Yoneshigue 1984; 6) Taylor 1960; 7) Ballantine & Ruiz 2005; 8) Ballantine & Ruiz 2010; 9) Boudouresque & Denizot 1975; 10) Ballantine & Ruiz 2006; 11) Ballantine & Ruiz 2011; 12) Lawson & John 1982; 13) Feldmann 1939; 14) Athanasiadis 1987; 15) Boudouresque & Denizot 1973; 16) Denizot 1968; 17) Rosenvinge 1893; 18) Kato and Masuda 2000; 19) Yoneshigue 1985.

species *P. inamoena* Pilg., *P. polymorpha*, *P. rosa-marina* Boudour. et Denizot, *P. simulans* Weber Bosse, *P. stoechas* Boudour. et Denizot, *P. valentini* Yonesh. et Boudour. parallel hypothallial filaments, a *P. rubra*-type of perithallial development and blades that become free at their margins. *Peyssonnelia polymorpha* differs from the new species in possessing a nearly completely calcified perithallus (Lawson & John, 1982) and in possessing multicellular rhizoids (Boudouresque & Denizot, 1975). Among the remaining species for which tetrasporangia size is known, *P. inamoena* (Schneider & Reading, 1987; Guimarães & Fujii, 1999), *P. simulans* (Schneider & Reading, 1987), and *P. valentii* (Schneider & Searles, 1998; Guimarães & Fujii, 1999) all have much smaller tetrasporangia. The thallus of *P. rosa-marina* lacks the pronounced radial striations in the new species, is thicker, possesses a nearly completely calcified perithallus and has multicellular rhizoids (Boudouresque & Denizot, 1973, 1975). Guimarães & Fujii (1999) reported unicellular rhizoids for eastern Atlantic *P. rosa-marina* possibly indicating that more than a single entity has been referred to this species. *Peyssonnelia stoechas* is relatively poorly known in the western Atlantic (Schneider & Reading, 1987). While Schneider and Reading (1987, Fig. 52) illustrate inconsistently-placed small cells among the perithallial filaments of *P. stoechas* (not illustrated by Boudouresque & Denizot, 1975), the resulting appearance of the perithallus is not that of being layered. *Peyssonnelia stratosa* also lacks the branching pattern of perithallial filaments, the extensive extracellular calcification and the conspicuous concentric rings found in *P. stoechas* (Boudouresque & Denizot, 1975; Schneider & Reading, 1987).

*Peyssonnelia harveyana* P.L. Crouan et H.M. Crouan ex J.Ag. is widely distributed from the eastern Atlantic and Mediterranean and Pacific (Guiry, 2011) bears some morphological similarities to the new species. Both have margins that become free, possess unicellular rhizoids, hypothallial cells arranged in parallel files, are prominently radially striate and possess similar hypobasal calcification (Boudouresque & Denizot, 1975; Irvine & Maggs, 1983). They differ, however, with respect to size and production of tetrasporangia. In *P. harveyana*, tetrasporangia are cut off terminally from an inconspicuous stalk cell and measure to a length of 100-130  $\mu\text{m}$  (Irvine & Maggs, 1983). Tetrasporangia in the new species are cut off laterally from enlarged basal cells within nemathecium and measure to 190  $\mu\text{m}$  in length.

A layered appearance of the perithallus is not unique within the *Peyssonneliales*. A tiered perithallus was figured for *Peyssonnelia obbessii* (Weber Bosse) Denizot by Dixon (2010, Fig. 5.54B) with the layered appearance being due to basal constrictions of perithallial cells at the same level in adjacent filaments. A layered appearance may also be due to superposed thalli as in *Polystrata dura* Heydrich figured by Dixon (2010, Fig. 5.71D) as well as in *Peyssonnelia imbricans* (Ballantine & Ruiz, 2006). In some *Peyssonnelia* species a layered appearance is due to secondary growth of the perithallus, i.e. perithallial growth stops and regrowth occurs at a later time. This explanation was invoked for the tiered appearance in *Peyssonnelia harveyana* by Boudouresque and Denizot (1975: Fig. 133) as well as for a species designated as Vanuatu 2 *Peyssonnelia inamoena* by Dixon (2010, Fig. 5.39E). We feel that the small cells cut off in the perithallus of *P. stratosa* are produced during normal perithallial development and are not indicative of growth inhibition and regrowth. In other words, the intercalary small cell seen within a perithallial file is more than likely to be a normal division product (see Fig. 7 of early perithallus development). Typically in a *Peyssonnelia* thallus, the distal two or three cells of perithallial files are shortened and therefore a series of several short cells would be seen beneath the tier in which regrowth has



occurred. Such an arrangement of perithallial cells is seen in section of a sympatrically growing (and currently non-described) Peyssonneliaceae (Fig. 11) leading to a tiered appearance which is fundamentally different from that seen in the new species.

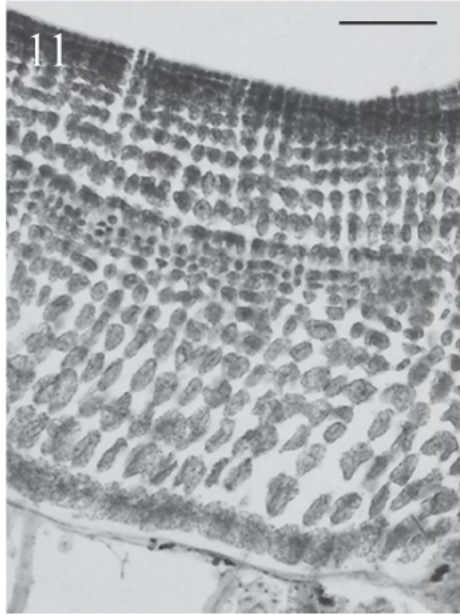


Fig. 11. Radial vertical section of *Peyssonnelia* sp. illustrating apparent secondary growth and consequent tiered appearance (*DLB 8391*) (Scale bar = 100  $\mu$ m).

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