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Research article

Two new species of *Crassitegula* (Sebdeniaceae, Sebdeniales, Rhodophyta) extend the diversity and biogeographic range of the genus to the Caribbean and Mediterranean seas

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Abstract. *Crassitegula* (Sebdeniaceae, Sebdeniales) is a relatively new genus of red algae, currently consisting of three species, that was first described based on a western Atlantic species in 2006. In the present paper, two species of *Crassitegula* are newly described from the Caribbean and Mediterranean seas, both being first detected using COI-5P barcoding, then followed by phylogenetic analysis. *Crassitegula bouchetii* L.Le Gall & C.W.Schneid. sp. nov. and *C. garyi* L.Le Gall & C.W.Schneid. sp. nov. represent the fourth and fifth species in the genus, which, prior to this study, has been confined to Bermuda (western Atlantic) and Lord Howe Island (Australia). *Crassitegula garyi*, from France, Corsica and Italy in the north-central Mediterranean, has a strap-shaped habit reminiscent in part of *C. walsinghamii* C.W.Schneid., C.E.Lane & G.W.Saunders and *C. laciniata* C.W.Schneid., Popolizio & C.E.Lane, whereas *C. bouchettii* is presently known only as small, peltate, kidney-shaped blades that possibly represent immature specimens.

Keywords. Caribbean Sea, COI-5P, *Crassitegula*, Mediterranean Sea, *rbc*L, Rhodophyta, Sebdeniales.

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Introduction

The family Sebdeniaceae Kylin was elevated to the order Sebdeniales Withall & G.W.Saunders based upon molecular analyses, as well as vegetative and reproductive differences from members of the Halymeniales G.W.Saunders & Kraft s. lat. (Withall & Saunders 2007). Withall & Saunders (2007) found the Sebdeniales weakly linked to the Rhodymeniales Nägeli and more distant from the Halymeniales in their Bayesian analyses of SSU rDNA sequences. At the time, the order held only a single family and genus, *Sebdenia* (J.Agardh) Berthold, and an undescribed generic grouping. *Sebdenia* presently comprises 13 species (Guiry & Guiry 2024) that display a wide range of habits, from dichotomously divided, swollen, terete axes (Huisman 2019) to strongly compressed and blade-like (Gavio *et al.* 2005). When

Crassitegula C.W.Schneid., C.E.Lane & G.W.Saunders was first described based upon material collected from Bermuda in the western Atlantic, it became the second genus in the Sebdeniaceae and the first with a dorsiventral habit (Schneider *et al.* 2006). The generitype, *C. walsinghamii* C.W.Schneid., C.E.Lane & G.W.Saunders, a subtidal mat-forming species on vertical walls, produces thick, overlapping, dorsiventral blades reminiscent of roof tiles, giving the genus its name. Secondary holdfasts produced from the ventral surface and margins of blades firmly affix the plants to the rock substrate. Also new for the Sebdeniaceae, the generitype of *Crassitegula* was found to produce tetrasporangia in nemathecia, a feature not known in *Sebdenia* (Schneider *et al.* 2006). Since the description of *Crassitegula*, two additional species have been described in the genus, *C. imitans* G.W.Saunders & Kraft from Lord Howe Island (Australia) in the Pacific Ocean (Kraft & Saunders 2011) and *C. laciniata* C.W.Schneid., Popolizio & C.E.Lane from Bermuda (Schneider *et al.* 2014a). Both of these species are likewise thick-bladed and dorsiventrally organized, producing overlapping plants in localized mats. Although tetrasporangia have yet to be found in specimens of *C. laciniata*, Kraft & Saunders (2011) did report them forming within discrete nemathecia on *C. imitans*. Tetrasporangia of *C. walsinghamii* and *C. imitans* are divided in an irregularly cruciate pattern, at times appearing zonate, and this seems to be typical for the genus (Schneider *et al.* 2006; Kraft & Saunders 2011).

Since the protologue of *Crassitegula* was published, the genus *Lesleigha* Kraft & G.W.Saunders from Australia, Hawaii and Japan was added to the Sebdeniaceae based upon molecular sequencing as well as the lack of a fusion cell subtending the gonimoblast filaments in carposporophytes (Kraft & Saunders 2011). Similar to *Crassitegula*, *Lesleigha* produces tetrasporangia in nemathecia and bears 3-celled carpogonial branches on female gametophytes. However, based on morphology and anatomy, Kraft & Saunders (2011) found it impossible to separate non-cystocarpic plants of *L. howensis* Kraft & G.W.Saunders from the sympatric *C. imitans*. They noted that only gene sequences could separate vegetative specimens of the two species from Lord Howe Island. Huisman & Saunders (2018) added a fourth genus to the Sebdeniaceae, *Cryptocallis* Huisman & G.W.Saunders from north-western Australia and the Philippines. This genus shares a vegetative habit with both *Crassitegula* and *Lesleigha* and is best distinguished by molecular sequencing. Vegetatively, *Cryptocallis dixoniorum* Huisman & G.W.Saunders appears very similar to the broad blades of *C. walsinghamii*. However, on female gametophytes, carpogonia of *C. dixoniorum* are formed directly on intercalary supporting cells in the outer cortex, and multiple carposporophytes share a single nemathecial pericarp (Huisman & Saunders 2018).

In the course of the Paris Museum's expedition program "Our Planet Reviewed" that surveyed the benthic fauna and flora of Corsica, as well as surveys conducted along the Mediterranean coasts of France and Italy, and off the island of Guadeloupe in the Caribbean Sea, two unique genetic species groups that nested with the genus *Crassitegula* were uncovered. In the present paper, we morphologically compare these new genetic groups with the three known species of the genus.

Material and methods

Collections and morphological methods

Collections from Guadeloupe and the northern Mediterranean were sampled using Open Circuit or Close Circuit rebreather SCUBA diving. Voucher specimens are deposited in PC (abbreviations follow the online *Index Herbariorum* (http://sweetgum.nybg.org/science/ih/); Thiers 2024 [continuously updated]). Fragments of individuals were dried in silica gel for DNA extraction, and the remainder of the specimens were pressed fresh on herbarium paper and dried as permanent vouchers and for anatomical study. Cross-sections were cut with a single-edged razor and mounted in 30% corn syrup with acidified 1% aniline blue in a ratio of 20:1. Dried specimens were scanned on an Epson ET-2650 scanner (Seiko Epson Corporation, Suwa, Nagano, Japan), and photomicrographs were taken using a Zeiss Axioskop

40 microscope (Oberkochen, Germany) equipped with a Spot Idea 28.2–5MP digital camera (Diagnostic Instruments, Sterling Heights, Michigan, USA).

Molecular methods

Genomic DNA was extracted from the silica-dried fragments using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol except for the extraction buffer which was prepared in the lab as follows: 1M Tris-base, 1M Tris-HCl, 0.05M Na2EDTA, 0.2M NaCl, 2.5M potassium acetate, 10% Tween 20 and 0.2 mg ml–1 Pro K. The quality and concentration of the extracted DNA were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The target gene regions, including the mitochondrial CO1-5P' marker (known as DNA barcode) as well as the large subunit (LSU) of the nuclear ribosomal DNA, were amplified using specific primers. COI-5P was amplified using the forward GWSFn (Le Gall & Saunders 2010) and reverse primers GWSRx (Saunders & McDevit 2012), and LSU (28S) was amplified as three overlapping fragments using primers T01N/T20, T04/T08 and T05/T15, and using the PCR primers and the internal primers T10, T16N, T19N, T22, T24, T25, T30, T33, following protocols of Harper & Saunders (2001) and Le Gall & Saunders (2010). The PCR reactions were performed in a 25 µL volume containing 10 ng of genomic DNA, 1x PCR buffer, 2.5 mM MgCl2, 0.2 mM dNTPs, 0.2 µm of each primer, and 0.5 units of Taq DNA polymerase (Qiagen, Hilden, Germany). The thermal cycling conditions were as follows: an initial denaturation at 95°C for 5 mins, followed by 35 cycles of denaturation at 95°C for 30 secs, annealing at 55°C for 30 secs and extension at 72°C for 1 min, with a final extension at 72°C for 10 mins. The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced in both directions using the Sanger method at a commercial sequencing facility (Eurofins Genomics, Ebersberg, Germany). The raw sequence data were edited and assembled using MUSCLE ver. 3.8.31 (Robert C. Edgar, https://drive5.com/muscle/downloads_v3.htm) implemented in SeaView ver. 4 (Gouy *et al.* 2010). The LSU alignment was manually inspected to optimize the alignment of the loop regions. Phylogenetic analyses were performed using IQ-TREE ver. 2.1.3 (Nguyen *et al.* 2015). The best-fit substitution model for each dataset was determined using the ModelFinder option in IQ-TREE, which implements the Bayesian Information Criterion for model selection. Maximum likelihood trees were inferred for each gene region separately, and a concatenated dataset was also analyzed. The IQ-TREE analysis was executed with 1000 ultrafast bootstrap (UFBoot) replicates to assess node support (Hoang *et al.* 2018). The resulting phylogenetic trees were visualized and edited using SeaView ver. 4.

All sequence data generated in this study have been deposited in GenBank as accession numbers PQ213414–PQ213425, and all other sequence data used in this study are presented in Table 1. The alignment files and resulting phylogenetic trees are available in the Dryad Digital Repository (https://datadryad.org/stash).

Table 1 (continued on next two pages). Collection details and GenBank accession numbers for specimens included in analyses. Specimens sequenced for this study are shown in boldface type.

Species	Voucher	Collector(s) / Collection date	Collection site	COI-5P	LSU rDNA
Asteromenia bermudensis G.W.Saunders, C.E.Lane, C.W.Schneid. & Kraft	*GWS001252*	G. Saunders / 13 Nov. 2001	West Whale Bay, Southampton, Bermuda Islands, Bermuda	AY970560	DQ068297
Crassitegula bouchetii L.Le Gall & C.W.Schneid. **sp. nov.**	*FRA1944* **(Holotypus)**	L. Le Gall, Y. Turpin, R. Moolenbeek, K. Netchy, E. Vassard, J. Espinosa, A. Antonioli / 21 May 2012	Marina de Rivière Sens, Basse-Terre, Guadeloupe, West Indies, Caribbean	**PQ213414**	**PQ213421**
Crassitegula garyi L.Le Gall & C.W.Schneid. **sp. nov.**	*LLG3706* (**Holotypus**)	L. Le Gall, J. Utge / 13 Apr. 2011	Capo dell'Armi, Reggio Calabria, Italy	**PQ213415**	**PQ213422**
	LLG2562	L. Le Gall, J. Utge / 7 Jun. 2009	Montremian, Port-Cros, Hyères, France, Mediterranean Sea	**PQ213416**	**PQ213423**
	LLG5005	L. Le Gall / 7 Jun. 2013	Agay-Saint Raphaël, Var, French Riviera, Mediterranean Sea	**PQ213419**	**PQ213424**
	LLG6821	B. Gouillieux, L. Le Gall, A. Le Viavant, W. Bay-Nouailhat, E. Vassard, F. Otero Ferrer / 16 Oct. 2020	Deep reef, southeast of the Cerbicale Islands, Corsica, France, Mediterranean Sea	**PQ213417**	–
	LLG6822	B. Gouillieux, L. Le Gall, A. Le Viavant, W. Bay-Nouailhat, E. Vassard, F. Otero Ferrer / 16 Oct. 2020	Deep reef, southeast of the Cerbicale Islands, Corsica, France, Mediterranean Sea	**PQ213418**	–
Crassitegula imitans G.W.Saunders & Kraft	*GWS002089* (**Holotypus**)	G. Saunders / 3 Feb. 2004	Malabar Reef, Lord Howe Island, New South Wales, Australia	JN641182	–
	GWS001076	G. Kraft, G. Saunders / 16 Mar. 2001	Noddy Island, Lord Howe Island, New South Wales, Australia	JN641180	DQ343700
	GWS002026	R. Withall / 30 Jan. 2004	Yellow Rock, Lord Howe Island, Australia	HM915970	–
	GWS002071	G. Saunders / 2 Feb. 2004	Ned's Beach, Lord Howe Island, Australia	JN641181	–
	GWS023521	G. Saunders, K. Dixon, R. Withall / 24 Nov. 2010	Algae Hole North, Lord Howe Island, Australia	JN641792	–
Crassitegula laciniata C.W.Schneid., Popolizio & C.E.Lane	*BDA0134*	C. Schneider, C. Lane, D. McDevit, T. Popolizio / 20 Aug. 2010	Middle Buoy, Eastern Blue Cut Channel, Bermuda	KF561827	–
	BDA1132	T. Popolizio / 28 May 2012	Hog Reef, north of Bermuda Islands, Bermuda	KF561829	–
	BDA1134	T. Popolizio / 28 May 2012	Hog Reef, north of Bermuda Islands, Bermuda	KF561831	–
	BDA1305 **(Holotypus)**	T. Popolizio / 11 Aug. 2012	Hog Breaker, north of Bermuda Islands, Bermuda	KF561832	–
	BDA1306 (**Isotypus**)	T. Popolizio / 11 Aug. 2012	Hog Breaker, north of Bermuda Islands, Bermuda	KF561833	KF761654

Table 1 (continued). Collection details and GenBank accession numbers for specimens included in analyses. Specimens sequenced for this study are shown in boldface type.

Species	Voucher	Collector(s) / Collection date	Collection site	COI-5P	LSU rDNA
Crassitegula walsinghamii C.W.Schneid., C.E.Lane & G.W.Saunders	*GWS001245* (**Isotypus**)	G. Saunders / 12 Nov. 2001	Walsingham Pond, Bermuda Islands, Bermuda	JN641185	EF033623
	GWS001260	G. Saunders / 16 Nov. 2001	Walsingham Pond, Bermuda Islands, Bermuda	JN641184	–
Crassitegula sp. 1REU	*JPQ1062*	T. Rauby / 30 Oct. 2019	Lapérouse Bank, Réunion Island, Indian Ocean	PQ733018	–
Crassitegula sp. 1WA	*GWS025666*	K. Dixon, R. Dixon, G. Belton / 18 Nov. 2010	The Basin, Rottnest Island, Western Australia, Australia	JN641183	JN602192
Cryptocallis dixoniorum Huisman & G.W.Saunders	*GWS020687*	J. Huisman / 20 Oct. 2011	Brue Reef, Kimberley, Western Australia, Australia	MG894157	–
	GWS020689	J. Huisman / 22 Oct. 2011	Mavis Reef, Kimberley, Western Australia, Australia	MG894138	–
	GWS020688	J. Huisman / 23 Oct. 2011	Brue Reef, Kimberley, Western Australia, Australia	MG894137	–
	GWS025841	K. Dixon / 11 Dec. 2010	South Balatasan, Oriental Mindoro, Philippines	JN641191	JN602193
Dictyothamnion saltatum A.Millar	*G0231*	A. Millar, A. Wright / 12 Aug. 1994	Split Solitary Island, New South Wales, Australia	JN641186	EU624161
Fryeella gardneri (Setchell) Kylin	*GWS004232*	G. Saunders, B. Clarckston, D. McDevit / 21 Jun. 2006	Saxe Point, Victoria, British Columbia, Canada	GQ497306	–
	G0335	T. Schaeffer / 8 Jul. 1995	San Juan Islands, north side Turn Island, Washington USA	–	EF033622
Halopeltis sp. 1WA	*G0402*	G. Kraft, G. Saunders / 10 Nov. 1995	Northeast of White Island, Easter Group, Abrolhos Islands, Australia	EF101939	DQ343679
Hymenocladiopsis prolifera (Reinsch) M.J.Wynne	*CCMP455*	M. Hoban	Antarctica, Center for Culture of Marine Phytoplankton	HQ919249	EU624144
Lesleigha hawaiiensis Kraft & G.W.Saunders	*G0436*	G. Kraft / 20 Jun. 1996	Maunalua By, Oahu, Hawaii, USA	JN641187	DQ343699
Lesleigha howensis Kraft & G.W.Saunders	*GWS002087*	G. Saunders / 3 Feb. 2004	Malabar Reef, Lord Howe Island, New South Wales, Australia	HM915995	–
	GWS002088 **(Holotypus)**	G. Saunders / 3 Feb. 2004	Malabar Reef, Lord Howe Island, New South Wales, Australia	JN641188	JN602198
	GWS022878	G. Saunders, K. Dixon, R. Withall / 22 Nov. 2010	Malabar Reef, Lord Howe Island, New South Wales, Australia	JN641190	–
	GWS022923	G. Saunders, K. Dixon, R. Withall / 22 Nov. 2010	Malabar Reef, Lord Howe Island, New South Wales, Australia	JN641189	–

Table 1 (continued). Collection details and GenBank accession numbers for specimens included in analyses. Specimens sequenced for this study are shown in boldface type.

Species	Voucher	Collector(s) / Collection date	Collection site	COI-5P	LSU rDNA
Lesleigha yamadae (Okamura & Segawa) G.W.Saunders & Kraft	*GWS018503*	G. Saunders, H.-G. Choi / 19 May 2010	Big Munseom Island, Jeju, South Korea	JN641192	JN602195
	GWS018683	G. Saunders, H.-G. Choi / 19 May 2010	Big Munseom Island, Jeju, South Korea	HQ544145	–
	GWS018686	G. Saunders, H.-G. Choi / 19 May 2010	Big Munseom Island, Jeju, South Korea	HQ544148	–
Perbella minuta (Kylin) Filloramo & G.W.Saunders	*GWS015600*	F. Scott / 16 Mar. 2010	Deep reef west of Verona Sands, mouth of Huon River, Tasmania, Australia	HQ919321	–
	G0273	G. Kraft / 6 Jan. 1995	Warrnambool, Victoria, Australia	–	DQ068295
Rhodymenia wilsonis (Sonder) G.W.Saunders	*GWS002530*	G. Saunders / 17 Jan. 2005	Warrnambool, Victoria, Australia	HM033153	HM033182
Sebdenia flabellata (J.Agardh) P.G.Parkinson	*CL031201*	C. Schneider, C. Lane / 3 Apr. 2003	Green Bay, Harrington Sound, Bermuda	JN641197	JN602196
	GWS011903	C. Schneider / 3 Apr. 2003	Green Bay, Harrington Sound, Bermuda	JN641196	–
Sebdenia monardiana (Mont.) Berthold	*LLG3983*	J. Utge, L. Le Gall/ 21 Apr. 2011	Santa Maria la Scala, Acireale, Sicily, Italy	**PQ213420**	**PQ213425**
Sebdenia cf. ***polydactyla*** (Børgesen) M.Balakrishnan	*GWS002027*	R. Withall / 30 Jan. 2004	Yellow Rock, Lord Howe Island, Australia	HM915971	–
	GWS023452	G. Saunders, K. Dixon, R. Withall / 24 Nov. 2010	Algae Hole North, Lord Howe Island, Australia	JN641198	–
	GWS023509	G. Saunders, K. Dixon, R. Withall / 24 Nov. 2010	Algae Hole North, Lord Howe Island, Australia	JN641199	–
Sebdenia sp. 1LH	*GWS002074*	G. Saunders / 2 Feb. 2004	Middle Beach, Lord Howe Island, New South Wales, Australia	JN641194	JN602197
	GWS023442	G. Saunders, K. Dixon, R. Withall / 24 Nov. 2010	Algae Hole North, Lord Howe Island, Australia	JN641193	–
	G0262	A. Millar, P. Richards / 11 Dec. 1994	Clayton Reef, Lord Howe Island, Australia	HM916189	–
Sebdenia sp. 2WA	*G0192*	G. Saunders, G. Kraft / 2 Feb. 1994	Roe Reef, Rottnest Island, Western Australia, Australia	JN641195	–

Results

Phylogenetic analysis

Phylogenetic analyses inferred from COI-5P (Supp. file 1), LSU (Supp. file 2) and both genes combined (Fig. 1) revealed that specimens collected in Guadeloupe and along the coast of France and Italy in the western basin of the Mediterranean Sea are resolved with full support in the lineage encompassing all the described species of the genus *Crassitegula* and a species from western Australia pending formal description. The specimen from Guadeloupe joined the generitype of *Crassitegula*, *C. walsinghamii*, with full support in all the analyses. The five specimens from the Mediterranean Sea that were barcoded showed no difference at all in their COI-5P sequences suggesting that they are conspecific. These sequences were resolved in all analyses as the sister taxa to the yet undescribed Australian species suggesting that they are likely to be a relic from the Mesozoic Tethyan Sea.

Unfortunately, a small specimen of *Crassitegula* (*C.* sp. 1REU, *JPQ1062*; Supp. file 1) from Réunion Island that grouped with *C.* sp. 1WA (*GWS025666*, Western Australia) was entirely sacrificed for DNA sequencing. Therefore, we will have to wait until additional material is collected to determine if this pair represents additional species in the genus, or might represent a new undescribed genus.

We propose the following taxonomic treatment for two new species of *Crassitegula* from the Caribbean and Mediterranean seas.

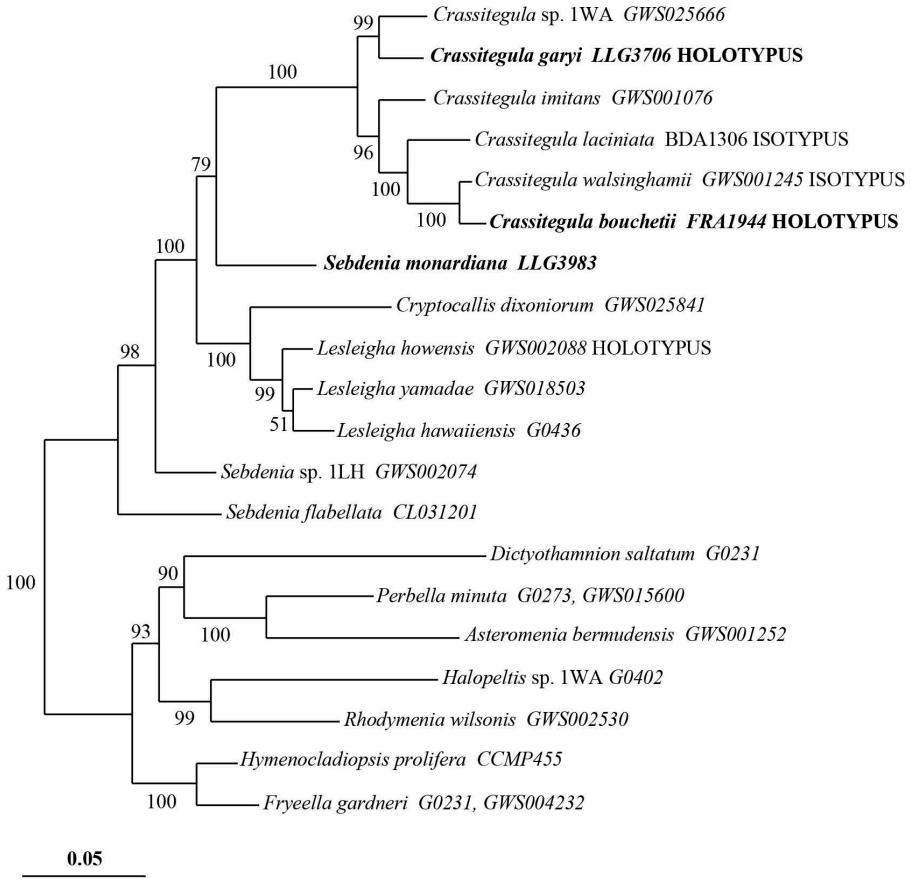

Fig. 1. Concatenated maximum likelihood tree inferred from combined COI-5P + LSU markers of members of the Sebdeniales Withall & G.W.Saunders (outgroup Rhodymeniales Nägeli). Numbers on branches show the bootstrap support calculated with ultrafast bootstrap (1000 replicates) in IQ-TREE. Scale = substitutions per site. Specimens newly sequenced for this study are shown in **boldface** type.

Taxonomic treatment

Phylum Rhodophyta Wettst.
Subphylum Eurhodophytina G.W.Saunders & Hommers.
Class Florideophyceae Cronquist
Subclass Rhodymeniophycidae G.W.Saunders & Hommers.
Order Sebdeniales Withall & G.W.Saunders
Family Sebdeniaceae Kylin
Genus *Crassitegula* C.W.Schneid., C.E.Lane & G.W.Saunders

Crassitegula bouchetii L.Le Gall & C.W.Schneid. sp. nov.
Registration: http://phycobank.org/105326
Fig. 2

Diagnosis

The new species is genetically distinct from all of the other known species in the genus (Fig. 1), and has characteristics reminiscent of immature blades of the generitype, *C. walsinghamii*.

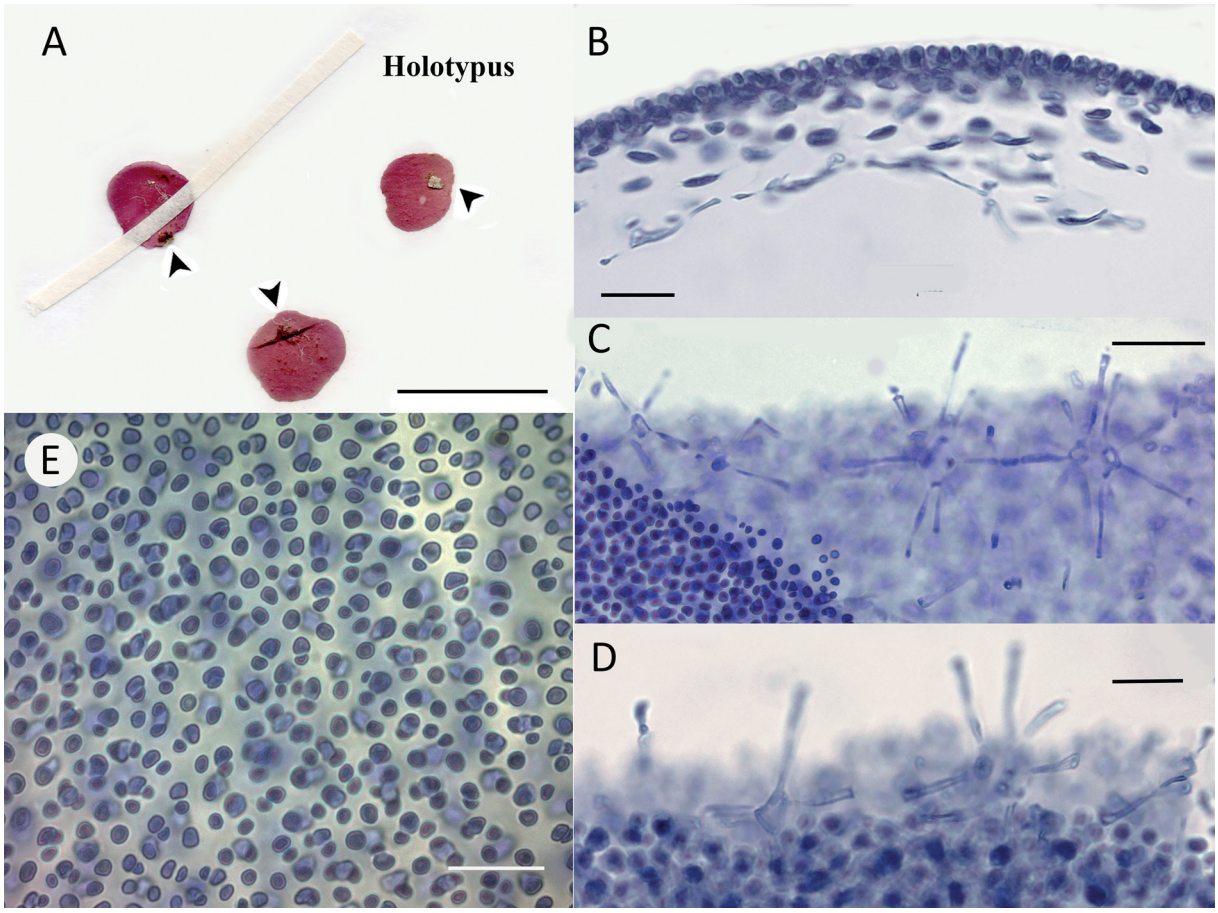

Fig. 2. *Crassitegula bouchetii* L.Le Gall & C.W.Schneid. sp. nov., *FRA1944* [PC0143905]. **A**. Holotype, a gathering of peltate specimens with ventral surfaces and single thick holdfasts (arrowheads) showing. **B**. Transverse section through blade and margin showing layers of cortical cells with inner margin of stellate ganglia. **C–D**. Stellate ganglial cell network under the inner cortex in surface view with opposite cortex pulled away. **E**. Surface view of outer cortex. Scale bars: A = 1 cm; B, D–E = 25 µm; C = 50 µm.

Etymology

The species is named for Prof. Emeritus Philippe Bouchet of the Muséum national d'Histoire naturelle, who initiated the expedition program "Our Planet Reviewed" and coordinated the 2012 karubenthos expedition to Guadeloupe, where these collections were made.

Type material

GUADELOUPE • Deep reef off Marina de Rivière Sens, Basse-Terre, Guadeloupe, West Indies, Caribbean Sea; 15°58′59.988″ N, 61°43′5.016″ W; depth 28 m; 21 May 2012; *L. Le Gall FRA1944*; GenBank nos PQ213414 (COI-5P), PQ213421 (LSU); holotype: PC [PC0143905].

Description

Plants saxicolous, prostrate, dorsiventrally organized, firm and smoothly textured, vermilion-red (Graf1x 2023); blades irregularly orbicular to ovate and up to 6.4 mm wide (Fig. 2A), attached by a short, thick, submarginal to more central holdfast (Fig. 2A); blades 350–380 µm thick with crenate margins, margins rounded in section; axes multiaxial, medulla loosely filamentous with long, thin, mostly anticlinal filaments 2–3 µm in diam., connected to inner cortex by regular network of stellate ganglia with spherical to rounded angular bodies 6–12 µm diam., each pit-connected to multiple radiating medullary filaments (Fig. 2B–D); cortex 3–4 layered with periclinally flattened larger inner cortical cells 9–15 µm diam., grading to smaller outer cortical cell layers (Fig. 2B), surface cells irregularly rounded to spherical and small, 3–6 µm in diam., loosely spaced, often with paired larger and smaller cells (Fig. 2E); tetrasporangia and gametangia unknown.

Distribution and habitat

Presently known only from a deep reef off Marina de Rivière Sens, Guadeloupe, in the Caribbean Sea.

Crassitegula garyi L.Le Gall & C.W.Schneid. sp. nov.
Registration: http://phycobank.org/105327
Fig. 3

Diagnosis

Crassitegula garyi sp. nov. produces branched ligulate blades from marginal proliferations that are similar to the finger-like branches of mature *C. laciniata*, but those of the latter are directly produced from blade margins and not from marginal proliferations. The apices of the new species are rounded, not acute like those of *C. laciniata*.

Etymology

The species is named for the Canadian macroalgal phylogeneticist, Prof. Gary W. Saunders of the University of New Brunswick, who sorted out the Sebdeniales and co-authored three of the five genera in the order including *Crassitegula*.

Type material

ITALY • Mesophotic reef off Capo dell'Armi, Reggio Calabria, Calabria, Mediterranean Sea; 37°57′13.932″ N, 15°40′37.200″ E; depth 50 m; 13 Apr. 2011; *L. Le Gall and J. Utge LLG3706*; GenBank nos PQ213415 (COI-5P), PQ213422 (LSU); holotype: PC [PC0164776].

Additional material examined

FRANCE • Deep reef, Montremian, Port-Cros, Hyères; 43°01′07.26″ N, 06°21′50.09″ E; depth 30 m; 7 Jun. 2009; *L. Le Gall and J. Utge LLG2562*; GenBank nos PQ213416 (COI-5P), PQ213423 (LSU);

PC [PC0162308] • Deep reef, Agay-Saint Raphael, south of Cap Dramont, Var, French Riviera; 43°24′27.41″ N, 06°51′19.44″ E; depth 40 m; 7 Jun. 2013; *L. Le Gall LLG5005*; GenBank nos PQ213419 (COI-5P), PQ213424 (LSU); PC [PC0144755] • ⊕; Deep reef, southeast of the Cerbicale Islands, Corsica; 41°31′43.85″ N, 09°24′07.96″ E; depth 32–42 m; 16 Oct. 2020; *B. Gouillieux, L. Le Gall, A. Le Viavant, W. Bay-Nouailhat, E. Vassard and F. Otero Ferrer LLG6821*; GenBank no. PQ213417 (COI-5P); PC [PC0626136] • same collection data as for preceding; *B. Gouillieux, L. Le Gall, A. Le Viavant, W. Bay-Nouailhat, E. Vassard and F. Otero Ferrer LLG6822*; GenBank COI-5P no. PQ213418 (COI-5P); [PC0626138].

Description

Plants saxicolous, prostrate, dorsiventrally organized, firm and smoothly textured, scarlet to fire brick-red (Graf1x 2023); blades originally reniform to cuneate and irregular, 1–2 cm wide, initially attached by short, submarginal holdfasts, later developing secondary holdfasts from ventral surfaces of spreading blades, blades also affixed to substrate directly by a cuticle-like surface covering ventral surface cells (Fig. 3A); primary blade later proliferating multiple new small circular to spathulate blades from margins,

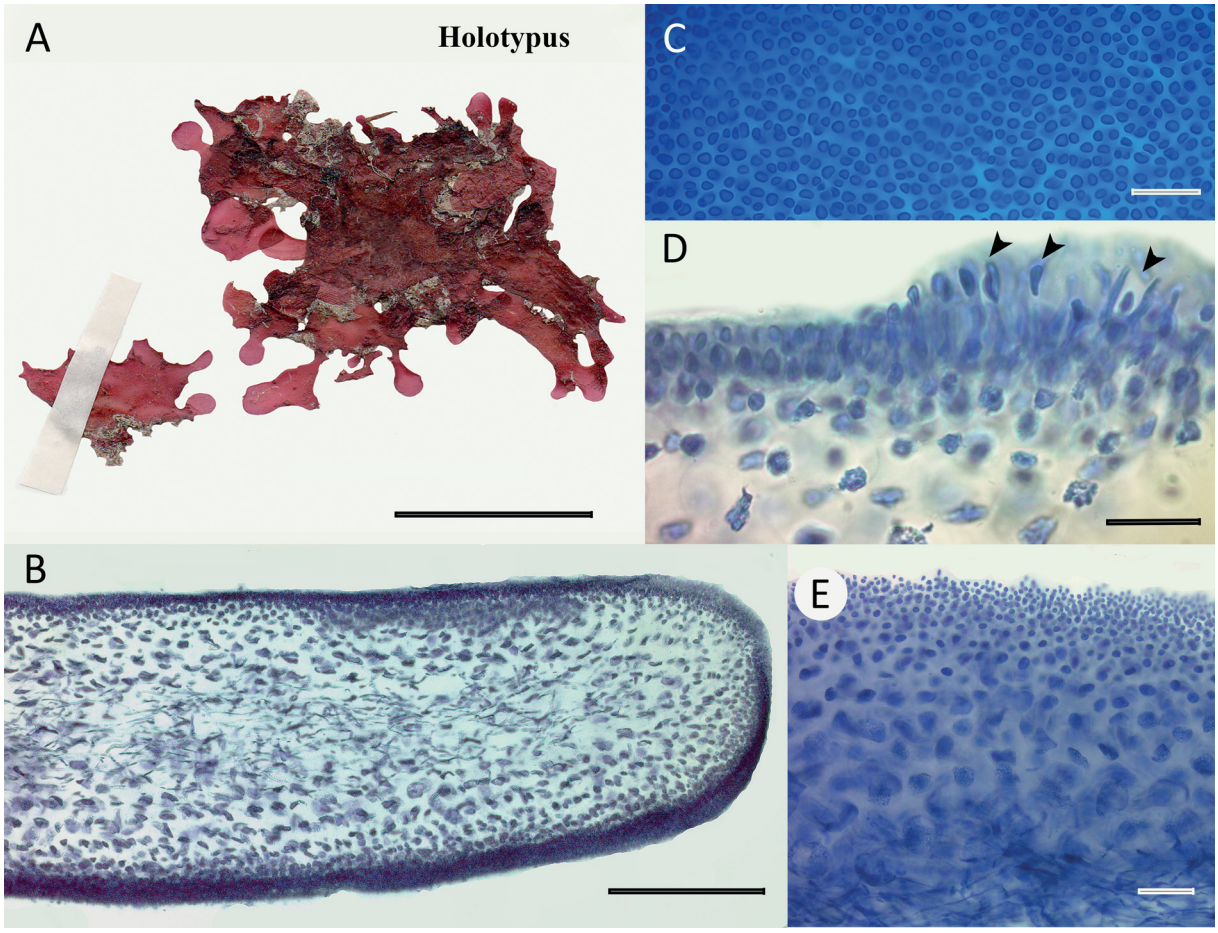

Fig. 3. *Crassitegula garyi* L.Le Gall & C.W.Schneid. sp. nov. **A**. Holotype, *LLG3706* [PC0164776]. **B**. Transverse section through blade and margin, *LLG3706* [PC164776]. **C**. Surface view of outer cortex, *LLG3706* [PC164776]. **D**. Section through raised tetrasporangial nemathecium, with immature tetrasporangia (arrowheads), *LLG6822* [PC0626138]. **E**. Thick section through focusing on gradation from inner to outer cortex in surface view, *LLG3706* [PC0164776]. Scale bars: A = 3 cm; B = 200 µm; C = 20 µm; D = 25 µm; E = 50 µm.

these eventually developing into long ligulate dorsiventral blades in the same plane; ligulate blades overlapping and affixed to one another by occasional secondary holdfasts issued from margins (Fig. 3A); blades 340–630 µm thick with rounded to nearly squared-off margins (Fig. 3B); axes multiaxial, medulla densely filamentous with long, thin, interwoven filaments 3–6 µm in diam., mostly periclinal to blade axis (Fig. 3B), and secondarily pit-connected to other medullary filaments and inner cortical layer by stellate ganglia with bodies 6–12 µm diam. with multiple radiating extensions; cortex 6–9 layered with broad transversely ellipsoidal to subglobose large inner cortical cells 36–60 µm in longest diam., each layer grading to the smallest outer cortical cell layer (Fig. 3E), cells irregularly ellipsoidal perpendicular to surface, 3.5–4.5 µm diam. and 6.0–7.5 µm tall, rounded angular and loosely to closely spaced in surface view, at times in arching lines (Fig. 3C); tetrasporangia elongated obovate, irregularly cruciately divided, 7.5–? µm diam., 15–? µm long, on attenuated outer cortical cells in slightly raised nemathecia on the ventral surface (Fig. 3D); gametangia and carposporophytes unknown.

Remarks

The only sporangia of *Crassitegula garyi* sp. nov. that were discovered were undivided or just once-divided in their nemathecia, but the oblique first meiotic division showed that this species was likely going to have similar irregularly cruciate tetrasporangia to others in the genus.

Distribution and habitat

At present, known from 30–50 m reefs off France, including Corsica, and Italy in the northern Mediterranean Sea.

Discussion

The collection of *Crassitegula bouchetii* sp. nov. from the Caribbean is made up of several small, smooth, suborbicular plants taken offshore of Guadeloupe during the spring growing season. Given their size and lack of reproduction, as noted above, they are likely juveniles having not yet achieved fully mature size and form. As they are genetically distinct (Fig. 1), and from a location where the genus is previously unknown, it is worthwhile to add them to the literature with hopes that they may be discovered later in a more mature state, or if they remain diminutive, reproductive. The type material does exhibit vegetative characteristics of the genus with its dorsiventral habit, submarginal holdfasts and pattern of the outer cortical cells in surface view.

Young broad blades of *Crassitegula garyi* sp. nov. could easily be confused with young blades of *C. walsinghamii* and *C. imitans*, as well as *Lesleigha howensis* and *Cryptocallis dixoniorum*, all characterized by thick dorsiventral blades with submarginal holdfasts. When mature, however, *C. garyi* produces branched ligulate blades from marginal proliferations similar to the finger-like branches of mature *C. laciniata*, but with more rounded blades and lacking acute apices. The blades of *C. laciniata* are directly produced from blade margins and not from marginal proliferations as in *C. garyi*.

The tetrasporangia of *Crassitegula garyi* sp. nov. are formed in nemathecia on attenuated cortical cells on the ventral surface as was the case in *C. walsinghamii.* Kraft & Saunders (2011) demonstrated nemathecia on *C. imitans* developed on the dorsal surface. Thus far, the remaining two species in the genus, *C. bouchetii* sp. nov. and *C. laciniata*, have not been found bearing sporangia.

It is noteworthy that a genus first described with only the generitype in 2006 now includes five species nearly two decades later. Prior to their descriptions, none of the known species of *Crassitegula* had ever been collected previously in their native habitats, and none were previously known species that were moved to the genus based upon genetic analyses. Most of the known species are from deep water (*C. bouchetii* sp. nov., 13–28 m; *C. garyi* sp. nov., 30–50 m; *C. imitans*, 6–22 m; *C. laciniata*, 7–12 m),

thus often living in habitats that minimally required Scuba diving. They join other species found at depth that escaped detection until the 21st century, species that after genetic analysis could be placed in their rightful genera even if known only as vegetative specimens (Schneider *et al.* 2014b, 2020). *Crassitegula walsinghamii*, however, is found in more shallow inland saltwater ponds and sink holes from 1.5–4 m depths in Bermuda, these fed by subterranean caves connected to the sea. These well-known inland habitats were often visited by previous workers who failed to collect *C. walsinghamii*, e.g., Collins, Hervey, Howe, Taylor and Bernatowitz (Schneider 2003). Of note, this species is also found on offshore Bermuda reefs to at least 18 m depths. As more and more collections are made globally from deeper subtidal habitats, the geographic ranges of *Crassitegula* and other elusive genera may well follow the trend of more species in more places than is currently known.

Sister to the *Crassitegula* clade in the COI-5P barcode analysis (Supp. file 1), the generitype of *Sebdenia*, *S. monardiana* (Mont.) Berthold, is shown to be separated in the tree from other members of the genus, *S. flabellata* (J.Agardh) P.G.Parkinson [type locality Guadeloupe, West Indies], *S. polydactyla* (Borgesen) M.S.Balakr. [type locality Gujarat, India] (see Huisman & Saunders 2018), *S.* sp. 1LH and *S.* sp. 2WA. *Sebdenia monardiana* is morphologically as well as genetically distinct from the soft, terete, flabellate species included in our analyses. Clearly, a phylogenetic analysis of the genus is needed to sort out undescribed species as well as many other known species of *Sebdenia* lacking molecular data at present to see where they fall. It seems obvious that the two flabellate species included here, *S. flabellata* and *S.* cf. *polydactyla* will need a new generic placement after additional future study.

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Supplementary files

Supp. file 1. Maximum likelihood tree inferred from the COI-5P marker of members of the Sebdeniales Withall & G.W.Saunders (outgroup Rhodymeniales Nägeli). Labels on branches show the bootstrap support calculated with ultrafast bootstrap (1000 replicates) in IQ-TREE. Scale = substitutions per site. Specimens newly sequenced for this study are shown in **boldface** type.
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Supp. file 2. Maximum likelihood tree inferred from the LSU marker of members of the Sebdeniales Withall & G.W.Saunders (outgroup Rhodymeniales Nägeli). Labels on branches show the bootstrap support calculated with ultrafast bootstrap (1000 replicates) in IQ-TREE. Scale = substitutions per site. Specimens newly sequenced for this study are shown in **boldface** type.
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