A molecular study of *Mazzaella* (Gigartinaceae, Rhodophyta) and morphological investigation of the *splendens* clade from Pacific North America

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HUGHEY J.R. AND HOMMMERSAND M.H. 2010. A molecular study of *Mazzaella* (Gigartineaceae, Rhodophyta) and morphological investigation of the *splendens* clade from Pacific North America. *Phycologia* 49: 113–135. DOI: 10.2216/08-68.1

The delineation of species of Mazzaella remains problematic because of similarities in thallus shape and color. To better define species boundaries, molecular phylogenetic analyses and developmental studies of Mazzaella were performed on one of the more taxonomically challenging clades, the splendens group (Mazzaella flaccida, Mazzaella linearis, Mazzaella sanguinea and Mazzaella splendens). Using an expanded rbcL data set, internal transcribed spacer sequences, and a combined analysis, six clades were identified: splendens, californica, rosea, cobinae, oregona, and South American/ African. Morphological examination of members of the splendens clade identified differences in their cystocarpic and tetrasporangial development and supported the recognition of two subspecies, M. splendens subsp. splendens and M. splendens subsp. fulgens Hughey & Hommersand stat. nov. The gonimoblasts of M. flaccida, M. linearis and M. sanguinea produced numerous terminal tubular cells that radiated from the gonimoblasts and fused to gametophytic cells; whereas, tubular gonimoblast cells were rare in *M. splendens* subsp. splendens and absent in *M. splendens* subsp. fulgens. The development of gonimoblasts was associated with the production of secondary gametophytic filaments derived from inner cortical and medullary cells. Their formation was progressive, continuing beyond the edges of the radiating gonimoblasts and giving rise to cystocarps of indefinite dimensions (M. flaccida and M. splendens subsp. splendens), or they developed primarily before the production of gonimoblasts from an auxiliary cell resulting in cystocarps of more or less definite size (M. linearis, M. sanguinea and M. splendens subsp. fulgens). The secondary filaments persisted, leaving a well-defined involucre (M. splendens subsp. fulgens), a poorly defined involucre (M. linearis) or were completely digested at maturity (M. splendens subsp. splendens), sometimes leaving a halo (M. sanguinea). Tetrasporangia were produced in secondary medullary filaments that formed lateral bands on both sides of the blade (most species) or extended across the center of the medulla (M. splendens subsp. fulgens). The tetrasporangia differentiated in sori within these bands and consisted of short filaments that reconnect by secondary pit connections forming loops.

KEY WORDS: Gigartinaceae, ITS, Mazzaella, Pacific North America, Phylogenetic analysis, rbcL

INTRODUCTION

Mazzaella G. De Toni (1936) currently contains 24 species distributed in the northeast and west Pacific, Pacific South America, Gough Island and South Africa (Hommersand et al. 1993). Of relevance to Mazzaella was the major reorganization of the Gigartinaceae by Hommersand et al. (1993) who resurrected the genera Chondracanthus Kützing (1843) and Mazzaella in addition to recognizing Chondrus Stackhouse (1797), Gigartina Stackhouse (1809), Iridaea Bory, nom. cons. (1826) and Rhodoglossum J. Agardh (1876) on the basis of a new interpretation of cystocarp and tetrasporangial morphology. Seventeen species were transferred to Mazzaella, of which 12 were recorded from the North Pacific, including two taxa previously assigned to Gigartina, five to Rhodoglossum and five to Iridaea. On the basis of phylogenetic analyses of rbcL gene sequences, Hommersand et al. (1994, 1999) later transferred three additional species to Mazzaella from Pacific North America: Mazzaella sanguinea (Setchell & N.L. Gardner) Hommersand, Mazzaella splendens (Setchell & N.L. Gardner) Fredericq and M. coriacea (E.Y. Dawson)

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Hughey. In addition, Hommersand et al. (1999) outlined an evolutionary history for Mazzaella that identified five groups: californica, heterocarpa, rosea, splendens and South American/African. Using nuclear and chloroplast DNA, Hughey et al. (2001) definitively linked the current application of species names with their type specimens for many Gigartinaceae from Pacific North America. They proposed three new combinations [Mazzaella oregona (Doty) Hughey, P.C. Silva & Hommersand, Mazzaella parksii (Setchell & N.L. Gardner) Hughey, P.C. Silva & Hommersand, Mazzaella parva (G.M. Smith & Hollenberg) Hughey, P.C. Silva & Hommersand] and established the legitimacy of the new combination, Mazzaella phyllocarpa (Postels & Ruprecht) Perestenko. A molecular investigation of six Mazzaella species (Ross et al. 2003) using sequences of the internal transcribed spacer (ITS) regions 1, 2, and the 5.8S gene (nuclear ribosomal DNA) supported the earlier conclusions reached by Shaughnessy (1995) and Hughey et al. (2001). A detailed summary of the nomenclatural history of the genus and its species was given by Hommersand et al. (1993, 1994, 1999), Hughey et al. (2001) and Ross et al. (2003), and a review of the species currently treated as Mazzaella from Pacific North America is provided in Table 1.

Postels & Ruprecht 1840	Setchell & Gardner 1933, 1937	Dawson 1961	Abbott & Hollenberg 1976	Kim 1976	Hommersand <i>et a</i> 1993, 1994	<i>al.</i> Hughey <i>et al.</i> 2001
Ird. heterocarpa ¹ Ird. cornucopiae ² Ird. lilacina Ird. punicea ³ Ird. phyllocarpa	Irs. heterocarpum Irs. cornucopiae NT NT NT Irs. agardhianum Irs. coriaceum Irs. flaccidum Irs. flaccidum Irs. fulgens Irs. furcatum Irs. ineare Irs. parksii Irs. parvulum Irs. reediae Irs. sanguineum Irs. sinicola Irs. splendens Irs. whidbeyanum ⁵ G. leptorynchos G. volans	NT NT NT NT NT NT NT NT NT NT NT NT NT N	Ird. heterocarpa Ird. cornucopiae Ird. cordata G. punicea NT Ird. cordata Ird. cordata Ird. cordata Ird. flaccida Ird. flaccida Ird. flaccida Ird. cordata Ird. heterocarpa Ird. lineare Ird. cordata Ird. cordata Ird. cordata Ird. cordata Ird. cordata Ird. punicea G. leptorynchos G. volans R. affine R. roseum R. californicum G. leptorynchos NT Ird. cordata P. ovodata	G. heterocarpa G. cornucopiae NT Ird. punicea G. phyllocarpa NT G. cordata NT G. flaccida NT G. lineare NT NT NT G. punicea NT G. punicea NT G. cordata NT G. cordata NT G. leptorynchos G. volans G. colifornica NT G. cobiniae G. coriacea	M. heterocarpa M. cornucopiae M. lilacina NT NT NT Ird. cordata ⁴ NT M. flaccida NT M. flaccida NT M. linearis NT M. linearis NT M. sanguinea NT M. splendens NT M. splendens NT M. leptorynchos M. volans M. affinis M. rosea M. californica NT M. cobinae NT	M. oregona M. parksii M. phyllocarpa NT M. phyllocarpa NT M. splendens M. flaccida M. splendens M. flaccida M. splendens M. linearis M. parksii NT M. splendens NT M. splendens NT M. splendens NT NT M. splendens NT NT NT NT NT NT M. affinis M. rosea NT NT NT NT NT NT NT
			R. parvum	NT	NT	M. parva

Table 1. Nomenclatural history of currently accepted names of Mazzaella from Pacific North America. G., Gigartina; Ird., Iridaea; Irs., Iridophycus; M., Mazzaella; R., Rhodoglossum; NT, not treated.

¹ Iridaea heterocarpa is a heterotypic synonym of Chondrus crispus (Hughey et al. 2001).

² Iridaea cornucopiae is a heterotypic synonym of Mazzaella laminarioides (Hughey et al. 2001).

³ Iridaea punicea is a heterotypic synonym of Turnerella mertensiana (Gabrielson 1989).

⁴ Leister (1977) concluded that the type specimen of *Fucus cordatus* was collected from the Southern Hemisphere (probably Isla de los Estados, Argentina).

⁵ Iridophycus whidbeyanum was placed in synonymy with Iridaea sanguinea (Gabrielson 1989).

Although recent analyses (Hommersand et al. 1994, 1999; Hughey et al. 2001; Ross et al. 2003; Saunders 2005) have helped to clarify the species boundaries at the molecular level, the absence of 75% of the species from the ITS phylogenetic analysis (Ross et al. 2003), the lack of duplicate sequences for Mazzaella species from the rbcL study (Hommersand et al. 1999), together with a need for detailed morphological studies has left these works incomplete. A group of species for which the latter is abundantly apparent is the splendens clade from Pacific North America: Mazzaella flaccida (Setchell & N.L. Gardner) Fredericq, Mazzaella linearis (Setchell & N.L. Gardner) Fredericq, M. sanguinea and M. splendens. Traditionally these species have been distinguished on the basis of thallus shape, size, color and intertidal position (Smith 1944; Abbott 1972a; Abbott & Hollenberg 1976). Variability in these characters, however, continues to lead to erroneous identifications. To date, several descriptions of medullary filament diameters and cortical thickness have been published for members of the group (Setchell & Gardner 1937; Abbott 1972a) and some useful reproductive features illustrated by Kim (1976), but a thorough documentation accurately delineating these species on the basis of anatomy and reproduction is still lacking. The purpose of this investigation is to (1) expand on the phylogenetic analyses inferred for species of *Mazzaella* from *rbc*L (Hommersand *et al.* 1994, 1999) and ITS sequence data (Ross *et al.* 2003), (2) perform a combined phylogenetic analysis of *Mazzaella* on the basis of *rbc*L and ITS DNA sequences and (3) clarify species boundaries of these four *Mazzaella* species from Pacific North America by describing and illustrating their reproductive morphology.

MATERIAL AND METHODS

Species investigated in this study, authorities, collection information and GenBank accession numbers are listed in Table 2. DNA extraction, amplification and sequencing followed the methods described by Hughey & Hommersand (2008).

Alignment and choice of outgroup

The boundaries of the ITS regions were determined from published sequences (Hughey *et al.* 2001). Alignment of *rbcL* sequences was performed visually with PAUP 4.0.0b10, [©]Florida State University (Swofford 2002). The ITS 1, 2, and 5.8S regions were preliminarily aligned with

~ .			GenBank accession number	
Species name	Authority	Collection information	rbcL	ITS
Iridaea cordata	(Turner) Bory 1826	Punta Daniel Este, Magellanes, Chile, coll. S. Fredericq and M.F. Ramírez, 19 February 1994	AF146207	EU090992
Mazzaella affinis	(Harvey) Hommersand in Hommersand <i>et al.</i> 1993	Pacific Grove, Monterey County, California, coll. M.H. Hommersand, 2 January 1993	U03081	AF401064
Mazzaella	(J. Agardh) G.B. DeToni f.	Jalama Beach State Park, Santa Barbara County, California,	U03082	EU090990
Mazzaella californica	(J. Agardh) G.B. DeToni f. 1936	Buhne Pt., Humboldt County, California, coll. F. Shaughnessy, 15 July 1997	—	EU090988
Mazzaella capensis	(J. Agardh) Fredericq in Hommersand <i>et al.</i> 1993	Kommetjie, Cape Peninsula, South Africa, coll. J. Bolton, 23 February 1993	U03083	
Mazzaella	(Bory) Fredericq in Hommersand <i>et al.</i> 1993	Quintay, Valparaiso Province, central Chile, coll. M.E. Ramírez, 07 April 1993	U03380	EU090981
Mazzaella	(Dawson) Fredericq in	Punta María, Baja California, Mexico, coll. J.R. Hughey,	AF146210	EU090980
cobinae Mazzaella	(Areschoug ex J. Agardh)	04 Jun 1996 Kommetjie, Cape Peninsula, South Africa, coll. J. Bolton,	U03084	
convoluta	Hommersand in Hommersand <i>et al.</i> 1994	23 February 1993		
Mazzaella	(Dawson) Hughey in Hommersand <i>et al</i> 1999	Cypress Pt., Monterey #2, Monterey County, California,	—	EU090987
Mazzaella	(Dawson) Hughey in	La Bufadora, Ensenada, Baja California, Mexico,	_	AY048051
coriacea Mazzaella	Hommersand <i>et al.</i> 1999 (Dawson) Hughey in	coll. M.H. Hommersand, 06 July 1996 N. reef, Punta Baja, Baja California, Mexico,	EU082425	EU090986
coriacea	Hommersand <i>et al.</i> 1999	coll. J.R. Hughey, 03 July 1996	A E14(211	A X/049050
coriacea	(Dawson) Hugney in Hommersand <i>et al.</i> 1999	coll. J.R., P.A., and D.R. Hughey, 14 July 1996	AF146211	AY048050
Mazzaella	(Setchell & Gardner)	Pigeon Point, San Mateo County, California, coll. M.H.	U03378	EU090971
Jlacciaa	Hommersand <i>et al.</i> 1993	Hommersand, 21 December 1992		
Mazzaella flaccida	(Setchell & Gardner) Fredericq in	Horseshoe Cove, Bodega, Sonoma County, California, coll. J.R. Hughey, 1995		EU090972
Mazzaella	Hommersand <i>et al.</i> 1993 (Setchell & Gardner)	Carmel Pt., Carmel, Monterey County, California, coll. J.R.		EU090970
flaccida	Hommersand <i>et al.</i> 1993	Hughey and M.F. Perez, 03 November 1994		
Mazzaella flaccida	(Setchell & Gardner) Frederica in	Otter Crest, Newport Co., Oregon, coll. G. Hansen, 04 June 1996	EU082417	EU090973
Juccuu	Hommersand <i>et al.</i> 1993	of Julie 1990		
Mazzaella	(Setchell & Gardner) Frederica in	N. end of Carmel Beach, Carmel, Monterey County,		AY048045
Juccuu	Hommersand <i>et al.</i> 1993	Camorina, con. J.K., 1.A., and D.K. Hughey, 15 July 1990		
Mazzaella	(Mikami) Hommersand in Hommersand at al. 1993	Cape Todogasaki, Miyako-wan, Iwate Prefecture, Japan,	AF146213	EU090982
Mazzaella	(Bory) Fredericq in	Punta Horcón, Chile, coll. M.H. Hommersand, 29 July 1994	EU082420	AF400054
laminarioides	Hommersand <i>et al.</i> 1993	El Desembra dans Chile cell M II Hammanard 02 Jamman	EL 1092421	A E 400055
laminarioides	Hommersand <i>et al.</i> 1993	1995	EU082421	AF400055
Mazzaella	(J. Agardh) Leister in	Jalama Beach State Park, Santa Barbara County, California, coll	U03381	EU090989
Mazzaella	(Setchell & Gardner)	Reef at Botany Beach, Port Renfrew, Vancouver Island #2,		EU090961
linearis	Fredericq in	Canada, coll. M.H. Hommersand, 10 July 1980		
Mazzaella	(Setchell & Gardner)	Barkley Sound, Vancouver Island, Canada,		AY048048
linearis	Fredericq in	coll. F. Shaughnessy, 24 June 1994		
Mazzaella	(Setchell & Gardner)	Drift, Malpaso Creek, Carmel, Monterey County, California,		EU090964
linearis	Fredericq in	coll. J.R. Hughey and R. Stabler, 31 May 1995		
Mazzaella	Hommersand <i>et al.</i> 1993 (Setchell & Gardner)	Drift, Cypress Pt., Monterey, Monterey County, California.	EU082426	EU090963
linearis	Fredericq in	coll. J.R., P.A. and D.R. Hughey, 12 July 1996	10001120	20070700
Mazzaella	Hommersand <i>et al.</i> 1993 (Setchell & Gardner)	Drift Moss Beach Monterey County California coll F	AF148520	AV048047
linearis	Fredericq in	Shaughnessy, 23 July 1996	111 140320	/1 I UHOUH/
Mazzaella	Hommersand <i>et al.</i> 1993 (Setchell & Cordner)	Mission Dt. Carmal Montaray County California call LD		E11000042
linearis	Fredericq in Hommersand <i>et al.</i> 1993	Hughey and M.F. Perez, 03 November 1994		E-0090902

Table 2. Species names, authorities, collection information, and GenBank accession numbers for Mazzaella analyzed in this study.

Table	2.	Continue	ed
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			GenBank accession number	
Species name	Authority	Collection information	rbcL	ITS
Mazzaella membranacea	(J. Agardh) Fredericq in Hommersand <i>et al.</i> 1993	Punta Daniel Este, Magellanes, S Chile, coll. S. Fredericq and M.E. Ramírez,	AF146214	EU090993
Mazzaella	(Doty) Hughey, Silva,	Seal Rock, Lincoln County, Oregon, coll. E. Henry,	U03379	AF398530
Mazzaella	(Doty) Hughey, Silva,	Cypress Pt., Monterey County, California, coll. J.R., P.A.,	_	AF398531
oregona Mazzaella	(Doty) Hughey, Silva,	and D.K. Hughey, 12 July 1996 Chetzomoka State Park, Port Townsend, Washington,		EU090974
oregona Mazzaella	& Hommersand 2001 (Doty) Hughey, Silva,	coll. J.R., P.A. and D.R. Hughey, 04 May 1997 Gravina Island, Ketchikan area, Alaska,		AF398532
oregona Mazzaella parksii	& Hommersand 2001 (Setchell & Gardner) Hughey Silva &	coll. S.C. Lindstrom 8662, 30 June 2000 Offshore rock near Trinidad, Humboldt County, California, coll. F.V. Dawson, 25 July 1965	—	AF400059
Mazzaella	Hommersand 2001 (Setchell & Gardner)	Attu Island, Aleutian Islands, Alaska, coll. K.A. Miller,		AF400061
parksii	Hughey, Silva, & Hommersand 2001	19 June 1987		
Mazzaella parksii	(Setchell & Gardner) Hughey, Silva, & Hommersand 2001	S. side, Horseshoe Cove, Bodega Head, Sonoma County, California, coll. M.H. Hommersand, 22 December 1992	U03377	EU090983
Mazzaella parksii	(Setchell & Gardner) Hughey, Silva, &	Perevalnie Passage, Kodiak Island, Alaska, coll. G. Hansen, 20 August 1993	AF146212	EU090984
Mazzaella parksii	(Setchell & Gardner) Hughey, Silva, &	Cape Arago, Coos County, Oregon, coll. J.R. Hughey, 04 September 1998	—	AF400060
Mazzaella parva	Hommersand 2001 (Smith & Hollenberg) Hughey, Silva, & Hommersand 2001	Cypress Pt., Monterey County, California, coll. G.M. Smith, 28 November 40, holotype collection, cystocarpic specimen on upper sheet beneath the plant illustrated in publication, G.M. Smith 40.178	_	AF401060
Mazzaella parva	(Smith & Hollenberg) Hughey, Silva, & Hommersand 2001	Puerto San Tomás, Baja California, Mexico, coll. J. R. Hughey, 02 July 1996	_	AF401063
Mazzaella phyllocarpa	(Postels & Ruprecht) Perestenko 1994	Perevalnie Pass, Kodiak Island, Alaska, coll. G. Hansen,	EU082418	AF398536
Mazzaella	(Postels & Ruprecht)	Snug Harbor, Alaska, coll. S.C. Lindstrom, 24 June 1994	EU082419	AF398535
Mazzaella	(Kylin) Fredericq in	Drift, Chetzomoka State Park, Port Townsend, Washington,		EU090978
rosea Mazzaella	(Kylin) Fredericq in	Pacific Grove, Monterey County, California, coll. J.R. Hughey,	AF146215	EU090979
rosea Mazzaella sanguinea	Hommersand <i>et al.</i> 1993 (Setchell & Gardner) Hommersand in	14 July 1996 Drift, Horseshoe Cove, Bodega Head, Sonoma County, California, coll. M.H. Hommersand, 22 December 1992	U03384	EU090967
Mazzaella sanguinea	Hommersand <i>et al.</i> 1994 (Setchell & Gardner) Hommersand in	Partridge Pt., Washington, coll. S.C. Lindstrom, 12.iv.1994	EU082422	EU090968
Mazzaella sanguinea	(Setchell & Gardner) Hommersand <i>et al.</i> 1994	Drift, Cambria, California, coll. M.H. Hommersand, 10 July 1996	—	EU090966
Mazzaella sp.1	N/A ¹	Punta María, Baja California, Mexico, coll. J.R. Hughey, 04 December 1996	EU082423	EU090976
Mazzaella sp.1	N/A	Reef below Nazarene College, San Diego County, California, coll. J.R. Hughey, 07 December 1996		EU090975
Mazzaella sp.1	N/A	China Pt., San Clemente Island, Los Angeles County, California, coll. S. Murray, 23 February 1997	EU082424	EU090977
Mazzaella	N/A	Cape Douglas, Alaskan Peninsula, Alaska, coll. P.W. Gabrielson, 15 August 1989	EU930197	EU930198
Mazzaella sp.3	N/A	Caleta Errazuriz, Antofagasta, Chile, coll. M.E. Ramírez, 27 November 1997	AF146209	
Mazzaella splendens	(Setchell & Gardner) Fredericq in Hommersand et al. 1004	Reef S. Jalama Beach County Park, Santa Barbara County, California, coll. M.H. Hommersand, 21 July 1966	_	AF401067
Mazzaella splendens	(Setchell & Gardner) Fredericq in Hommersand <i>et al.</i> 1994	Pigeon Point, San Mateo County, California, coll. M.H. Hommersand, 21 December 1992	U03385	

			GenBank nur	accession nber
Species name	Authority	Collection information	rbcL	ITS
Mazzaella splendens	(Setchell & Gardner) Fredericq in Hommersand <i>et al.</i> 1994	S. side of Horseshoe Cove, Bodega Head, Sonoma County, California, coll. M.H. Hommersand, 22 December 1992, published as <i>M. linearis</i> by Hommersand <i>et al.</i> 1994	U03383	EU090965
Mazzaella splendens	(Setchell & Gardner) Fredericq in Hommersand <i>et al.</i> 1994	Shannon Point, Skagit County, Washington, coll. S.C. Lindstrom, 11 May 1993	U03382	EU090958
Mazzaella splendens	(Setchell & Gardner) Fredericq in Hommersand <i>et al.</i> 1994	Taigud Island, site 2, Alaska, coll. S.C. Lindstrom, 29 April 1994		AF401066
Mazzaella splendens	(Setchell & Gardner) Fredericq in Hommersand <i>et al.</i> 1994	Otter Crest, Newport County, Oregon, coll. G. Hansen, 04 June 1996	—	EU090960
Mazzaella splendens	(Setchell & Gardner) Fredericq in Hommersand <i>et al.</i> 1994	N. end of Carmel Beach #1, Carmel, Monterey County, California, coll. J.R., P.A. and D.R. Hughey, 13 July 1996	EU082427	AF398529
Mazzaella volans	(C. Agardh) Fredericq in Hommersand <i>et al.</i> 1993	Pigeon Point, San Mateo County, California, coll. M.H. Hommersand, 21 December 1992	U03386	EU090991

the Clustal W 1.61 alignment program using the settings reported previously by Hughey *et al.* (2001), then optimized manually in PAUP. On the basis of previous phylogenetic relationships, *Iridaea cordata* (Turner) Bory was selected to root the tree (Hommersand *et al.* 1994, 1999).

Phylogenetic analysis of *rbcL* and ITS data sets

Maximum-parsimony and maximum-likelihood analyses were performed using PAUP 4.0.0b10 following the parameters described by Hughey and Hommersand (2008). For maximum-likelihood analyses, best-fit models were determined using Modeltest 3.8 (Posada & Crandall 1998). The g1 statistic was used to measure skewness (Sokal & Rohlf 1981; Hillis & Huelsenbeck 1992; Hughey & Hommersand 2008). Support for nodes was assessed by calculating bootstrap proportion (BP) values (Felsenstein 1985) on the basis of 1000 resamplings using maximum parsimony. Genetic distances were calculated using PAUP 4.0.0b10 and represent uncorrected ("p") distances for *rbcL* and ITS data.

Phylogenetic analysis combining data sets

The combined data were analyzed using maximumlikelihood methods with a substitution model selected using Modeltest 3.8. The level of incongruence between partitions was tested using partition homogeneity tests (PHT) with 100 replicates. A probability of 0.05 was taken as the threshold for significance.

Anatomical examination

Material was preserved in 10% formalin/seawater and then maintained in 5% formalin/seawater or mounted on herbarium paper. Algal fragments were hydrated in seawater for 1–5 min and then frozen in a 1% gum arabic solution on the stage of a freezing microtome (American Optical Corp., acquired by Sola International Inc., Menlo Park, CA, USA). Sections were made at 10 µm and stained for 2-10 min with a solution of 0.125% aniline blue, 1% HCl, 20% Karo[®] corn syrup (Englewood Cliffs, NJ, USA), and 79% seawater. Sections were destained with a 50% glycerine/50% seawater solution, sorted and transferred to a slide with 25 µl of a mixture containing 1 g of aniline blue, 70 ml of Karo, 30 ml of distilled water and 5 ml of acetic acid (Wynne 2001). Slides with sections were allowed to set for 24 h, then photographed with a Zeiss (Montpelier, MD, USA) photomicroscope III, using Kodak[®] T-Max 100 film (Rochester, NY, USA). Herbarium thalli were photographed with a Minolta (Konica Minolta, Tokyo, Japan) Maxxum 400si camera and T-Max film. Negatives were scanned with a Polaroid Sprint Scan 35 (Waltham, MA, USA) and adjusted using Adobe Photoshop 5.0 (Adobe Systems Incorporated, San Jose, CA, USA). Herbarium abbreviations are from Holmgren et al. (1990).

Typification

We followed Division II, Section 2, Article 8.2, which states that "For the purpose of typification a specimen is a gathering, or part of a gathering, of a single species" (McNeill *et al.* 2006).

RESULTS AND DISCUSSION

Molecular analyses

Nucleotide sequence data for the *rbc*L gene were generated for 34 specimens. The length of the *rbc*L gene analyzed was 1321 base pairs. The number of variable sites was 291, of which 213 (73%) were phylogenetically informative. Base frequencies showed an A+T bias: A = 0.3052, C = 0.1652, G = 0.2061, T = 0.3234. Maximum-parsimony and -likelihood analyses yielded trees that were in topological agreement. The transition model plus invariable sites plus γ (TIM+I+G) model, where Nst = 6, R(AC) = 1.0, R(AG) = 4.310, R(AT) = 1.5646, R(CG) = 1.5646, R(CT) = 10.8088, R(GT) = 1.0, Rates = gamma, pinvar = 0.5916, and γ distribution = 1.1420, was selected as the best fit for the *rbcL* data. Maximum-likelihood analysis of *rbcL* sequences yielded a single tree with an ln likelihood score of 5138.7506 (Fig. 1). The *rbcL* data set provided strong phylogenetic signal according to the skewness of the random tree-length distribution (mean = 1085.2, g1 = -0.70).

Sequence data for the ITS regions were generated for 51 specimens. Nucleotide lengths varied in ITS 1 from 146 to 159, ITS 2 from 335 to 436 and 5.8S from 151 to 153. The number of variable sites was 314, of which 242 (77%) were phylogenetically informative. Base frequencies showed an A+T bias: A = 0.3001, C = 0.1933, G =0.1992, T = 0.3073. Maximum-parsimony and -likelihood analyses generated trees that were in topological agreement. The Hasegawa–Kishino–Yano plus γ (HKY+G) model, where Nst = 2, Tratio = 1.4408, Rates = gamma, pinvar = 0.0, and γ distribution = 0.2742, was identified as the best fit for the ITS data. Maximum-likelihood analysis of the ITS sequences yielded a single tree with an In likelihood score of 4507.9245 (Fig. 2). The ITS data set showed convincing phylogenetic signal according to the random tree-length distribution hypothesis (mean = 1864.5, g1 = -0.47).

The first clade includes M. splendens, M. linearis, M. flaccida and M. sanguinea and is strongly supported in all three analyses [BP rbcL = 98%, ITS = 89%, combined (comb.) = 100% (Figs 1–3). These data support the recognition of four closely related, fleshy-bladed species, previously referred to as the splendens group (Hommersand et al. 1999). A sister group relationship between M. splendens and M. linearis is strongly supported (BP rbcL = 94%, ITS = 99%, comb. = 100%) (Figs 1–3), as previously shown by Hughey et al. (2001) and Ross et al. (2003). The other two sister species, M. flaccida and M. sanguinea, form a grade as demonstrated by Hommersand et al. (1994, 1999). Hughey et al. (2001) and Ross et al. (2003) showed previously that M. splendens and M. linearis are the most closely related taxa in this group. At the species level, M. splendens (BP rbcL = 84%, ITS = 56%, comb. = 96%) and M. linearis (BP rbcL = 100%, ITS = 90%, comb. = 100%) have mostly strong support. Pairwise sequence divergences are low for these two species (rbcL =1.4%, ITS = 0.6-0.8%) compared with that of *M. splendens* vs *M. flaccida* (*rbc*L = 2.1-2.6%, ITS = 3.1-3.2%) and *M.* splendens vs M. sanguinea (rbcL = 2.5-2.7%, ITS = 2.8-2.9%). With the exception of M. splendens, intraspecific variation within species is negligible. Specimens of M. splendens, representing a range of morphologies, segregate into two lineages in all three analyses. One lineage corresponds to I. cordata var. cordata sensu I.A. Abbott and the other to I. cordata var. splendens (Setchell & N.L. Gardner) I.A. Abbott (1972a). Mazzaella flaccida (BP rbcL = 100%, ITS = 94%, comb. = 100%) and *M. sanguinea* (BP rbcL = 100%, ITS = 100\%, comb. = 100\%) are wellsupported species that show low sequence divergence (rbcL = 2.9 - 3.2%, ITS = 4.6 - 4.9%).

A second clade of species, previously referred to as the *californica* group (Hommersand *et al.* 1999), contains

Mazzaella affinis (Harvey) Fredericq, Mazzaella californica (J. Agardh) G. De Toni, Mazzaella leptorynchos (J. Agardh) Leister, M. parva and Mazzaella volans (C. Agardh) Fredericq and is strongly supported in rbcL, ITS and combined analyses (BP rbcL = 95%, ITS = 84%, comb. = 100%) (Figs 1–3). These data support the recognition of a morphologically heterogeneous assemblage of five species that is sister to the splendens clade. The position of this clade is supported in all three analyses (BP rbcL = 71%, ITS = 68%, comb. = 99%) and is consistent with previous phylogenetic hypotheses (Hommersand et al. 1994, 1999). Within the clade, M. affinis, M. californica, M. leptorynchos form a moderately supported lineage (BP rbcL = 73%, ITS = 58%, comb. = 80%) in which M. affinis and *M. leptorynchos* share a branch (BP rbcL = 93%, ITS = 73%, comb. = 60%). Mazzaella volans and M. parva form the other lineage, but this relationship is poorly supported (BP ITS = 61%). Mazzaella californica differs measurably in pairwise sequence distance from *M. affinis* (rbcL = 2.3%, ITS = 5.7%), *M. leptorynchos* (*rbc*L = 2.3%, ITS = 13.8%), M. volans (rbcL = 3.1%, ITS = 8.3%) and M. parva (ITS = 5.9%). The ITS data suggest that M. volans and M. parva are closely allied; however, the two differ by 7.5% in pairwise sequence distance (ITS).

A third clade includes M. coriacea and Mazzaella rosea (Kylin) Fredericq and is strongly supported in all three analyses (BP rbcL = 100%, ITS = 100\%, comb. = 100\%) (Figs 1-3). In the case of the rbcL analysis (Fig. 1), these two species occupy an unsupported (BP rbcL = < 50%) paraphyletic position to a fourth clade containing two species, Mazzaella cobinae (E.Y. Dawson) Fredericq and an undescribed species from northern Mexico and California that resembles *M. affinis* in habit, *Mazzaella* sp. 1. In the case of ITS and combined analyses (Figs 2, 3), a monophyletic relationship between M. coriacea and M. rosea and the fourth clade is observed, but not supported (BP ITS = 58%, comb. = <50%). These findings are similar to those of Hommersand et al. (1999), who found very weak support (BP rbcL = 51%) for a lineage containing M. coriacea, M. rosea, and M. cobinae. The lineage was designated the rosea group. Hommersand et al. (1999) characterized the group as having "large cystocarps of indefinite size in which new carposporangia differentiate at the periphery of the cystocarp cavity." These molecular data support the splitting of rosea into rosea and cobinae groups; however, anatomical evidence validating this is lacking. Sequence divergences between M. coriacea and M. rosea are low (rbcL = 2.5%, ITS = 5.7–5.8%), as are those between *M. cobinae* and *Mazzaella* sp. 1 (rbcL = 2.3%, ITS = 6.9%). Comparison of sequence divergences between clades three and four show little to nearly double the variation (rbcL = 2.7-4.8%, ITS = 8.7-11.3\%).

A fifth clade includes *M. oregona*, *M. parksii*, *M. phyllocarpa* and *Mazzaella* sp. 2 from Cape Douglas, Alaska, that is representative of *Iridaea laminarioides* var. *parvula* Kjellman (1889) [Homotypic synonyms: *Iridophycus parvulum* (Kjellman) Setchell & N.L. Gardner (1937) and *Iridaea parvula* (Kjellman) Papenfuss (1958)], and is strongly supported in ITS and combined analyses (BP ITS = 94%, comb. = 98%) (Figs 2, 3), but unsupported by *rbcL* data (BP *rbcL* = < 50%) (Fig. 1). In the case of the *rbcL*



0.005 substitutions/site

Fig. 1. Maximum-likelihood phylogeny of species of *Mazzaella* on the basis of rbcL gene sequences. Numbers along branches represent maximum parsimony bootstrap values (> 50%).

analysis, the clade is paraphyletic with respect to *Mazzaella* sp. 2, but monophyletic for ITS and combined analyses. Hommersand *et al.* (1999) previously named this cluster of species (as *Mazzaella heterocarpa* and *Mazzaella cornucopiae*) the *heterocarpa* group. The name *M. heterocarpa*, however, is referable to *Chondrus crispus* Stackhouse (Hughey *et al.* 2001). Therefore, a suitable name for the members of this clade is the *oregona* group. In all three analyses, *Mazzaella japonica* (Mikami) Hommersand is positioned as the closest relative to the fifth clade (Figs 1–3) and is excluded from all clades (BP rbcL = < 50%, ITS = < 50%, comb. = < 50%). This topology is consistent with the phylogram published by Hommersand *et al.* (1999). Within the clade, rbcL fails to resolve interspecific



— 0.01 substitutions/site

Fig. 2. Maximum-likelihood phylogeny of species of *Mazzaella* on the basis of internal transcribed spacer regions 1, 2, and 5.8S gene sequences. Numbers along branches represent maximum parsimony bootstrap values (> 50%).

relationships (Fig. 1). ITS and combined analyses, however, suggest an affinity between *M. oregona* and *Mazzaella* sp. 2 (BP ITS = 78%, comb. = 92%). On the basis of genetic distances, *M. japonica* is the most distantly related to species in the fifth clade, differing moderately from *M. phyllocarpa* (*rbcL* = 1.5–2.0%, ITS = 5.6%), *M. oregona* (*rbcL* = 2.4%, ITS = 5.6–6.0%), *M. parksii* (*rbcL* = 2.2– 3.5%, ITS = 7.4–8.7%) and *Mazzaella* sp. 2 (*rbcL* = 2.0%, ITS = 6.0%). *Mazzaella phyllocarpa* is approximately equally distant in genetic sequence from *M. oregona* (*rbcL* = 1.2–1.3%, ITS = 5.2–5.8%), *M. parksii* (*rbcL* = 1.4– 2.4%, ITS = 6.7–6.9%) and *Mazzaella* sp. 2 (*rbcL* = 0.9– 1.2%, ITS = 8.7%). Among these species, *Mazzaella* sp. 2 is most closely related in genetic distance to *M. phyllocarpa* (*rbcL* = 0.9–1.2%, ITS = 8.7%) and *M. oregona* (*rbcL* = 1.5%, ITS = 4.5–5.2%). Morphological and molecular studies of *Mazzaella* sp. 2 and its conspecificity to *I. laminarioides* var. *parvula* are ongoing. Significant intraspecific variation is observed in *M. parksii. Mazzaella parksii* from Bodega, CA, USA differs from a specimen of *M. parksii* from Attu Island by 1.1% (ITS) and from a specimen from Perevalnie Passage, AK, USA by 1.9% (*rbcL*) and by 0.7% (ITS). In comparison, the two Alaskan specimens differ by 0.5% (ITS) from each other. Distances between the closely related *M. splendens* and *M. linearis* are nearly equivalent (*rbcL* = 1.4%, ITS = 0.6–0.8%). A more thorough sampling of *M. parksii* is needed to understand the genetic relationship and taxonomic status of this species.

A sixth clade includes species from the Southern Hemisphere: *Mazzaella capensis* (J. Agardh) Fredericq, *M*.



Fig. 3. Maximum-likelihood phylogeny of species of *Mazzaella* on the basis of a combined analysis of the *rbc*L gene and internal transcribed spacer regions 1, 2, and 5.8S gene sequences. Numbers along branches represent maximum-parsimony bootstrap values (> 50%). Congruence tests showed no serious differences between the two partitions (PHT = 0.02).

laminarioides (Bory) Fredericq, *Mazzaella membranacea* (J. Agardh) Fredericq, *Mazzaella convoluta* (Areschoug ex J. Agardh) Hommersand and an undescribed species *Mazzaella* sp. 3 from Chile that resembles *M. affinis* from the northeastern Pacific (the last two are not represented in the ITS data set), and receives strong bootstrap support (BP rbcL = 91%, ITS = 94%, comb. = 100%) (Figs 1–3). Its topological position as a basal lineage, however, is only moderately supported by ITS (BP ITS = 78%), unsupported as a sister group to clade three by rbcL data (BP rbcL = < 50%), and unsupported as a basal group from the combined analysis (BP comb. = < 50%). On the basis of rbcL sequence analysis, Hommersand *et al.* (1994, 1999) reported similar support for the clade and inferred for it a basal position. Within the clade, interspecific resolution is poor for rbcL,

ITS and combined analyses (Figs 1–3). Mazzaella capensis and M. membranacea represent the only observable affinity (BP ITS = 75%, comb. = < 94%). Results from Hommersand et al. (1994) suggested that M. capensis and M. laminarioides were identical in the rbcL tree within the limits of sequence resolution. An ITS sequence obtained from the specimen of M. laminarioides analyzed from the Hommersand et al. (1994) study differs significantly from two identical sequences of M. laminarioides. It appears that the sequence of M. laminarioides in Hommersand et al. (1994) (accession U03380) may be based on a misidentification or the result of contamination. On the basis of genetic distances, M. laminarioides is closely related to M. membranacea (rbcL = 3.1%, ITS = 8.2-8.7%), M. convoluta (rbcL = 3.1%), and Mazzaella sp. 3 (rbcL = 3.3%), and distantly to *M. capensis* (rbcL = 4.7-6.5%, ITS = 10.8–11.4%). *Mazzaella capensis* shows similar divergences to *M. membranacea* (rbcL = 4.3-4.9%, ITS = 6.6%), *M. convoluta* (rbcL = 4.3-5.0%), and *Mazzaella* sp. 3 (rbcL = 4.6-5.6%). The closest genetic similarity is observed between *M. membranacea* and *M. convoluta* (rbcL = 2.5%).

Combined analysis of the rbcL and ITS data sets includes sequences from 29 ingroup and one outgroup taxon (I. cordata). The combined data was analyzed using maximum-likelihood methods with the best-fit model Tamura-Nei plus invariable sites plus γ (TrN+I+G), where Nst = 6, R(AC) = 1.0, R(AG) = 2.0851, R(AT) = 1.0, R(CG) =1.0, R(CT) = 3.6602, R(GT) = 1.0, base frequencies A = 0.3117, C = 0.1796, G = 0.2061, T = 0.3026, Rates =gamma, pinvar = 0.4079 and γ distribution = 0.6174. The analysis resulted in a single tree with an ln likelihood score of 10927.7215 (Fig. 3). Visual comparison with the individual analyses suggests that simultaneous analysis largely expressed the ITS topology. Resampling support is similar for all nodes in comparison with the trees generated from rbcL and ITS data sets. Bootstrap support for the splendens clade is 100%, californica clade 100%, rosea clade 100%, cobinae clade 100%, oregona clade 98%, and the South American/African clade 100%. Congruence tests show minor differences between the two partitions (PHT = 0.02).

Preliminary phylogenetic analysis of the *rbcL* gene from 45 Gigartinaceae supported a paraphyletic Mazzaella with respect to Chondrus (Hommersand et al. 1994). A second analysis on the basis of 65 specimens identified six clades for Mazzaella, five of which were designated groups by Hommersand et al. (1999): californica, heterocarpa (oregona), rosea, splendens and South American/African. The analyses herein strongly support the splendens, californica, rosea, cobinae and South American/African groups. The oregona group, however, received no support for the rbcL data set, yet was well resolved in ITS and combined analyses. The phylogram published by Hommersand et al. (1999) arranged Southern Hemisphere taxa in a basal position with respect to other Mazzaella. Analysis of ITS and combined character sets inferred this relationship (Figs 2, 3); however, the rbcL data set failed to show this polarity. Above the group level, the analysis of Hommersand et al. (1999) and the data presented here, both were unable to adequately provide nodal support for the position of the groups within Mazzaella.

Morphological observations

For a generic diagnosis of *Mazzaella* refer to Hommersand *et al.* (1993).

Mazzaella flaccida (Setchell & N.L. Gardner) Fredericq in Hommersand et al. (1993, p. 110)

Figs 4–12

BASIONYM: *Iridophycus flaccidum* Setchell & N.L. Gardner 1937, p. 171.

HOLOTYPE: Figs 4, 5. A collection by A.V. Manza, 18 July 1935, from the first point north of Carmel Beach, Carmel, Monterey County, California. UC 539561! (cystocarpic),

547655! (male), 547654! (tetrasporic designated as lectotype by I.A. Abbott 1972a, p. 64).

HOMOTYPIC SYNONYMS: *Iridaea flaccida* (Setchell & N.L. Gardner) P.C. Silva in Stoloff & Silva 1957, p. 328; *Gigartina flaccida* (Setchell & N.L. Gardner) D.H. Kim 1976, p. 41.

HETEROTYPIC SYNONYMS: *Iridophycus agardhianum* Setchell & N.L. Gardner 1937, p. 170; *Iridaea agardhiana* (Setchell & N.L. Gardner) Kylin 1941, p. 23, pl. 8, fig. 41; *Iridaea minor* J. Agardh 1849, p. 86.

TYPE: Collector and date unknown, Hab. ad Californiam. LD 23248! (cystocarpic).

ILLUSTRATIONS: Smith 1944, p. 288, pl. 72, fig. 2 (as *Iridophycus flaccidum*); Abbott 1972a, p. 64, figs 9, 10 (as *Iridaea flaccida*); Abbott 1972b, p. 261, figs 10–13 (as *Iridaea flaccida*); Abbott & Hollenberg 1976, p. 533, fig. 477 (as *Iridaea flaccida*).

HABITAT: High to mid-intertidal, intermediate to low wave exposure.

DISTRIBUTION: Pine Island, British Columbia (UBC A49074); Vancouver Island, British Columbia (Abbott 1972a, p. 64); Oregon (Hansen 1997, p. 194); Monterey Peninsula, California (Smith 1944, p. 288); San Luis Obispo County, California (Sparling 1977, p. 70); Government Pt., Santa Barbara County, California (Dawson 1959, p. 187).

REPRESENTATIVE SPECIMENS: British Columbia: Pine Island, 15 September 1970, UBC A49074, tetrasporic; Oregon: Otter Crest, Newport County, 04 June 1996, GIH/HMSC 88, tetrasporic; California: N. Cuffey's Pt., Mendocino County, 17 August 1977, NCU 584078-79, cystocarpic; Stillwater Cove, Sonoma County, California, 12 August 1998, NCU 584310-11, cystocarpic & tetrasporic; Horseshoe Cove Bodega Head, Sonoma County, 23 May 1992, NCU 584080, male & tetrasporic; Horseshoe Cove Bodega Head, Sonoma County, 10 August 1979, NCU 584081-83, tetrasporic; Dillon Beach, Marin County, 13 August 1977, NCU 584084-85, cystocarpic, male, & tetrasporic; Pigeon Pt., San Mateo County, 21 July 1992, NCU 584086, cystocarpic; Davenport Landing, Santa Cruz County, 31 December 1979, NCU 584087-88, 584092, tetrasporic & juveniles; Davenport Landing, Santa Cruz County, 09 August 1979, NCU 564089-91, cystocarpic & tetrasporic; N. end Carmel Beach, Carmel, Monterey County, 13 July 1996, NCU 584243-52, cystocarpic & tetrasporic; Mission Pt., Monterey County, 19 August 1977, NCU 584093-98, tetrasporic; Carmel Bay, Monterey County, 18 July 1935, UC 547655, male (holotype); Montaña de Oro State Park, San Luis Obispo County, 24 August 1977, NCU 584099-100, male & tetrasporic; S. of Jalama County Park, Santa Barbara County, 21 July 1966, NCU 584101, cystocarpic; Government Pt., Santa Barbara County, 16 May 1957, E.Y.D. 16586, UC 1853883, tetrasporic.

DESCRIPTION: Thalli 30–60 cm tall with several blades arising from a discoid holdfast, fleshy and flaccid, green to purple, sometimes green above and purple below; blades 8–20 cm wide, ovate-lanceolate with a gradually tapering apex, margins ruffled, usually entire, approximately 400 µm thick, stipe short and apophysis canaliculate (Figs 4, 5), variable in thickness: cystocarpic (0.6-1.5 mm), male (430-779 µm), tetrasporic (615-820 µm). Cortex composed of six to seven layers of isodiametric cells that become stellate in inner cortical region. Medullary filaments narrow, loosely interwoven, 1.5-3.5 µm in diameter. Auxiliary cell initially bearing a few gonimoblast cells surrounded by a cluster of short-celled secondary gametophytic filaments (Fig. 6). Gonimoblast filaments growing through the short-celled filaments and linking to vegetative filaments by means of terminal tubular cells throughout their development (Fig. 7). Carposporangia differentiating progressively, while gonimoblasts continue to produce long terminal tubular cells that radiate into the medulla on all sides (Fig. 8). Cystocarp bulging on both sides of the thallus at maturity, 0.8–1.4 mm in width and releasing spherical to ovoid carpospores, 8-23 µm in diameter. Initially carpospores released through a pore in the outer surface and later the cystocarp eroding away (Fig. 9), leaving a hole. Spermatangia scattered over surfaces of smooth blades that are uniform in color. Tetrasporangial chains transformed from secondary filaments produced in the outer medulla in narrow bands on opposite sides of the thallus (Fig. 10). Chains forming loops of three to five ellipsoidal tetrasporocytes that link together to produce an interconnecting network (Fig. 11). Tetrasporangial sori forming dense, irregularly shaped packets, 512-615 µm in diameter and 190-220 µm thick that elevate the outer wall slightly (Fig. 12). Mature tetraspores released through a small, elevated pore in the blade surface.

Mazzaella linearis (Setchell & N.L. Gardner) Fredericq in Hommersand *et al.* (1993, p. 110)

Figs 13-21

BASIONYM: *Iridophycus lineare* Setchell & N.L. Gardner 1937, p. 171.

HOLOTYPE: Fig. 13. A collection by N.L. Gardner, May 1916, from the rocks in the lower littoral, Carmel, Monterey County, California. UC, 507637! (cystocarpic), 507582! (male; designated as lectotype by I.A. Abbott 1972a, p. 67), & 266510! (tetrasporic).

HOMOTYPIC SYNONYMS: *Iridaea lineare* (Setchell & N.L. Gardner) Kylin 1941, p. 23; *Gigartina lineare* (Setchell & N.L. Gardner) D.H. Kim 1976, p. 42.

ILLUSTRATIONS: Smith 1944, p. 290, pl. 72, fig. 3 (as *Iridophycus lineare*); Scagel 1967, p. 256, fig. 111 (as *Iridaea lineare*); Abbott 1972a, p. 68, figs 20, 21 (as *Iridaea lineare*); Abbott & Hollenberg 1976, p. 533, fig. 479 after Smith (as *Iridaea lineare*).

HABITAT: Low intertidal, exposed habitats.

DISTRIBUTION: Near Sitka, Alaska (Abbott 1972a, p. 68); Bamfield, Vancouver Island, British Columbia (Scagel 1973, p. 140); Oregon (Hansen 1997, p. 194); Monterey Peninsula, California (Smith 1944, p. 290); San Luis Obispo County, California (Sparling 1977, p. 70); Arroyo Honda, Santa Barbara County (AHF 64497 in UC).

REPRESENTATIVE SPECIMENS: Southeast Alaska: Cape Ommaney, Baranof Island, Sitka Borough, 15 June 1965, UBC A20673, tetrasporic & sterile; Cape Bartolome, Prince of Wales-Outer Ketchikan Borough, 02 July 1965, UBC A22094, tetrasporic; Cape Chacon, Prince of Wales Island, Prince of Wales-Outer Ketchikan Borough, 12 June 1965, UBC A20183, tetrasporic & sterile; California: Pacific Grove, Monterey County, June 1953, NCU 584102-03, tetrasporic; Drift, Moss Beach, Monterey County, 23 July 1996, NCU 584308, sterile; Mission Pt., Monterey County, 17 July 1974, NCU 584104-05, male; Mission Pt., Monterey County, 19 August 1977, NCU 584106-08, cystocarpic & male; Drift, Cypress Pt., Monterey, Monterey County, 12 July 1996, NCU 584253, tetrasporic; N. end of Carmel Beach, Carmel, Monterey County, 13 July 1996, NCU 584263-67, cystocarpic & tetrasporic; Carmel Pt., Monterey County, 03 November 1994, NCU 584109, male; Mission Pt., Carmel, Monterey County, 12 July 1996, NCU 584309, tetrasporic; Malpaso Creek, Carmel, Monterey County, 31 May 1995, NCU 584110, tetrasporic; Beach at Cayucos, San Luis Obispo Coun ty, 03 February 1954, UC M160860, tetrasporic; Pecho Beach, San Luis Obispo County, October 1954, UC 392782, reproductive state undetermined.

DESCRIPTION: Thalli 0.25-1.0 m long, in dense clumps up to 0.5 m in diameter from a large discoid holdfast, rubbery and slick to the touch with crisped margins, purplish brown to dark purple, drying with a brown undertone, usually iridescent; stipe distinct, 4-7 cm long, 2.6-3.5 mm in diameter, cylindrical, occasionally with fine lateral papillae and with a well-defined arc-shaped apophysis in tetrasporic plants; blades 3-7 cm broad, linear-lanceolate, planar or mostly spiraled, with attenuate apices, these sometimes divided, entire (Fig. 13) or the divisions with two or three lobes (Fig. 14), bases cohering laterally, sometimes furrowed, variable in thickness: cystocarpic (0.8-1.4 mm), male (574-615 µm), tetrasporic (656-779 µm). Cortex composed of six to seven layers of spherical cells. Medullary filaments narrow and loosely interwoven, 1.5-3.5 µm in diameter. Auxiliary cell initially bearing a few gonimoblast cells surrounded by a cluster of short-celled secondary gametophytic filaments (Fig. 15). Gonimoblasts irregularly shaped, consisting of short-celled filaments that connect to the gametophytic cells by means of numerous long terminal tubular cells beginning early in development (Figs 16, 17); carposporangia differentiating progressively as the cystocarp matures (Fig. 16). Terminal tubular cells abundant and exceedingly long throughout gonimoblast development, radiating in all directions and attaching to medullary and cortical cells (Fig. 17). Mature cystocarps 1.1-1.5 mm in width, not bulging, releasing spherical carpospores 13-20 µm in diameter through a single pore in the thallus surface (Fig. 18). Spermatangia scattered over surfaces of smooth blades, which are paler and thinner than cystocarpic and tetrasporic plants. Tetrasporangial chains formed from secondary filaments in the outer medulla (Fig. 19). Chains consisting of three to five ellipsoidal tetrasporocytes arranged loosely in a looping pattern (Fig. 20), forming lateral bands on both sides of the blade. Tetrasporangial sori



 $550-650 \,\mu\text{m}$ in diameter and $200-230 \,\mu\text{m}$ thick, closely set on both sides of the blade (Fig. 21). At maturity, tetrasporangia $35-44 \,\mu\text{m}$ in diameter, released through a small porelike structure in an elevated region of the blade surface.

Mazzaella sanguinea (Setchell & N.L. Gardner) Hommersand in Hommersand et al. (1994, p. 198)

Figs 22–30

BASIONYM: *Iridophycus sanguineum* Setchell & N.L. Gardner 1937, p. 172.

HOLOTYPE: Fig. 22. A collection by N.L. Gardner, June 1933, drift, Duxbury Reef, Marin County, California. UC, 507503! (cystocarpic; designated as lectotype by I.A. Abbott 1972a, p. 69) & 507504! (male).

HOMOTYPIC SYNONYM: *Iridaea sanguinea* (Setchell & N.L. Gardner) Hollenberg & I.A. Abbott 1965, p. 1184.

HETEROTYPIC SYNONYMS: *Iridophycus whidbeyanum* Setchell & N.L. Gardner 1937, p. 172.

TYPE: Collections by N.L. Gardner, date unknown, west coast of Whidbey Island, Washington. UC 132749! (cystocarpic), 94378! (tetrasporic), & 94380! (male); *Iridaea laminarioides* f. *punicea* Setchell & N.L. Gardner 1903, p. 300; *Iridaea whidbeyana* (Setchell & N.L. Gardner) Scagel 1957, p. 191.

ILLUSTRATIONS: Abbott 1972a, p. 69, fig. 23 (as *Iridaea sanguinea*) & fig. 24 (as *Iridaea whidbeyana*); Abbott & Hollenberg 1976, p. 538, fig. 480 (as *Iridaea punicea*) & fig. 481 (as *Iridaea sanguinea*).

HABITAT: Subtidal.

DISTRIBUTION: Wingham & Kayak islands, Alaska & Amphitrite Pt., Vancouver Island, British Columbia (Abbott 1972a, p. 68); West Beach, Washington (Scagel 1957, p. 191); Bald Pt. & Jockey Cap Rock, Oregon (Markham & Celestino 1976, p. 262); Duxbury Reef, Bolinas, California (Setchell & N.L. Gardner 1937, p. 172); Carmel Submarine Canyon, Monterey & Shell Beach, San Luis Obispo County, California (Abbott 1972a, p. 68). REPRESENTATIVE SPECIMENS: Alaska: Wingham Island, Valdez-Cordova Borough, 04 July 1966, UBC A25484 & A25670, cystocarpic, male, & tetrasporic; Kayak Island, Valdez-Cordova Borough, 25 June 1965, UBC A20424-27, A20437, A22661-62, A22568-72, A22578, cystocarpic, male, tetrasporic, & sterile; Washington: West coast of Whidbey Island, Island County, 1908, UC 132749 (cystocarpic), unknown dates, UC 94380 (male), & unknown date, UC 94378 (tetrasporic) (type collections of *Iridophycus whidbeyanum*); Oregon: Strawberry Hill, Lane County, 15 May 1999, NCU 584111, tetrasporic; California: Crescent City, Del Norte County, 7 September 1998, NCU 584268-69, cystocarpic; Bodega Head, Sonoma County, 24 July 1966, NCU 584112, sterile; Drift, Cambria, 10 July 1996, NCU 584113-15, sterile.

DESCRIPTION: Thalli 20-40 cm tall, from a discoid holdfast and consisting of a stipe approximately 1 cm long, a short apophysis and a single, broadly lanceolate to cordate blade, rubbery, pliable, pink to deep red or purplish iridescent; blades up to 20 cm wide, entire or with the apices sometimes cleft or lobed (Figs 22, 23), variable in thickness: cystocarpic (900–1100 µm) and tetrasporic (820–902 µm). Cortex five to six cell layers thick, composed of isodiametric cells. Medullary cells bulbous, spherical to ellipsoidal, 5-20 µm in diameter. Gonimoblast filaments short-celled (Figs 24-27), connecting at an early stage to gametophytic medullary cells by means of long terminal tubular cells; carposporangia differentiating progressively as the cystocarp matures (Figs 24-27). Gonimoblasts surrounded by secondary gametophytic filaments beginning along their upper edges at an intermediate stage of development (Fig. 25). When mature cystocarps 697–756 µm in diameter and surrounded by a hemispherical to umbonate halo (Figs 26, 27) that is formed as secondary gametophytic filaments break down; medullary and secondary gametophytic filaments traversed on all sides by numerous terminal tubular gonimoblast cells 200 µm in length that connect to surrounding medullary and cortical cells; cystocarps not bulging on either side of the thallus (Fig. 26); carpospores spherical to ovoid, 13-22 µm in diameter, the carposporophyte disintegrating after carpospore release, leaving holes.

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Figs 4–12. Mazzaella flaccida.

Fig. 5. Holotype of Iridophycus flaccidum S. & G., UC 547654. Scale bar, 2 cm.

Figs 6, 7. NCU 584310.

Fig. 7. Close-up of long terminal tubular gonimoblast cells (arrows) on side away from auxiliary cell of mature cystocarp. Scale bar, 100 µm.

Fig. 8. Section through cystocarp with partly eroded surfaces bulging from both sides of blade. Scale bar, 200 µm.

Fig. 9. Section through eroded cystocarp that has ruptured on only one side of blade. Scale bar, 200 µm.

Figs 10-12. Tetrasporangial development. NCU 584311.

Fig. 10. Young, secondarily produced tetrasporangial chains (arrows) forming more or less continuous bands on both sides of outer medulla. Scale bar, 100 μm.

Fig. 11. Close-up of young, secondarily produced tetrasporangial chains, showing network of interconnecting ellipsoidal tetrasporocytes (arrows). Scale bar, 50 μm.

Fig. 4. Holotype of Iridophycus flaccidum S. & G., UC 539561. Scale bar, 2 cm.

Figs 6–9. Cystocarpic development.

Fig. 6. Section through young cystocarp showing auxiliary cell (ac) bearing a few gonimoblast cells (g) surrounded by a cluster of short-celled secondary gametophytic filaments (arrows). Scale bar, $100 \ \mu m$.

Figs 8, 9. NCU 584243-52.

Fig. 12. Mature tetrasporangial sorus with cleaved tetrasporangia being released through small elevated pore in blade surface. Scale bar, $100 \ \mu m$.



Spermatangia scattered over the surface of the blade, formed in the outermost layer of the cortex with each spermatangial mother cell producing one spermatium. Tetrasporangial chains forming in the outer medulla (Fig. 28), composed of numerous, spherical to cuboidal tetrasporocytes, three to four per chain (Fig. 29). Tetrasporangial sori 450–615 μ m wide and 240–290 μ m thick, uniformly distributed in bands on opposite sides of the thallus (Fig. 30). Tetraspores, 22–37 μ m in diameter at maturity, released through slightly elevated regions in the outer wall followed by repair of the exposed region by the inward anticlinal growth of secondary filaments.

Mazzaella splendens (Setchell & N.L. Gardner) Fredericq in Hommersand et al. (1994, p. 198) subsp. splendens

Figs 31-39

BASIONYM: *Iridophycus splendens* Setchell & N.L. Gardner 1937, p. 170.

HOLOTYPE: Fig. 31. A collection by A.V. Manza, 19 July 1935, north end of Carmel Beach, Carmel, Monterey County, California. UC, 539564! (cystocarpic & tetrasporic generations), 539565! (cystocarpic & tetrasporic generations, tetrasporic designated as lectotype by I.A. Abbott 1972a, p. 58), 539566! (cystocarpic & male generations).

HOMOTYPIC SYNONYMS: *Iridaea splendens* (Setchell & N.L. Gardner) Papenfuss 1958, p. 106; *Iridaea cordata* var. *splendens* (Setchell & N.L. Gardner) Abbott 1972a, p. 55; *Gigartina cordata* var. *splendens* (Setchell & N.L. Gardner) D.H. Kim 1976, p. 40; *Mazzaella splendens* (Setchell & N.L. Gardner) Fredericq in Hommersand *et al.* 1994, p. 198.

HETEROTYPIC SYNONYMS: *Iridophycus coriaceum* Setchell & N.L. Gardner 1937, p. 170.

TYPE: Collections by N.L. Gardner, July 1917, Cypress Pt. near Pacific Grove, Monterey County, California. UC, 236589! (cystocarpic), 296600! (tetrasporic), & from Pebble Beach, Carmel, date unknown, 276269! (male); *Iridophycus reediae* Setchell & N.L. Gardner 1937, p. 172.

TYPE: Collections by R.S. Reed, July 1917, Bushnell's Beach, San Luis Obispo County, California. UC, 510685!

(cystocarpic), 510684! (tetrasporic); *Iridophycus sinicola* Setchell & N.L. Gardner 1937, p. 172.

TYPE: Collections by N.L. Gardner, 16 November 1933, near Pt. Cavallo, San Francisco Bay, Marin County, California. UC, 392651! (cystocarpic), 392669! (male); *Iridaea coriacea* (Setchell & N.L. Gardner) Papenfuss 1958, p. 106; *Iridaea reediae* (Setchell & N.L. Gardner) Papenfuss 1958, p. 106; *Iridaea sinicola* (Setchell & N.L. Gardner) Papenfuss 1958, p. 106.

ILLUSTRATIONS: Smith 1944, p. 289, pl. 72, fig. 1 (as *Iridophycus splendens*); Abbott 1972a, p. 55, figs 1, 3, 7 (as *Iridaea cordata* var. *splendens*), fig. 4 (as *Iridophycus splendens*), fig. 8 (as *Iridophycus sinicola*), fig. 12 (as *Iridophycus reediae*), figs 13, 14 (as *Iridophycus coriaceum*); Abbott 1972b, p. 260, figs 1–3, 5, 6, 8, 9 (as *Iridaea cordata* var. *splendens*); Abbott & Hollenberg 1976, p. 530, fig. 475 (as *Iridaea cordata* var. *splendens*).

HABITAT: Low intertidal, sheltered to exposed localities.

DISTRIBUTION: Shell Beach, Sonoma County, California (NCU 584116-17); San Francisco Bay, California (Silva 1979, p. 327); Monterey Peninsula, California (Smith 1944, p. 289); Montaña de Oro St. Park, San Luis Obispo County, California (Sparling 1977, p. 71); Southern California (Murray 1974, p. 51); S. side of Punta Baja, Baja California, Mexico (NCU 584133-34, 34A).

REPRESENTATIVE SPECIMENS: California: Shell Beach, Sonoma Co., 6 July 1966, NCU 584116-17, tetrasporic; Horseshoe Cove, Bodega Head, Sonoma County, 10 August 1979, NCU 584270-73, tetrasporic; Dillon Beach, Marin County, 13 August 1977, NCU 584118-19, cystocarpic; Pigeon Pt., San Mateo County, 12 December 1992, NCU 584274, cystocarpic & tetrasporic; Davenport Landing, Santa Cruz County, 09 August 1979, NCU 584120-24, cystocarpic & tetrasporic; Moss Beach, Monterey County, 19 August 1977, NCU 584125, male; Reef at Islay Montaña de Oro State Park, San Luis Obispo County, 19 July 1966, NCU 584126-27, cystocarpic; Jalama Beach County Park, Santa Barbara County, 21 July 1966, NCU 584128-30, tetrasporic; Port Hueneme, Ventura County, 30 December 1956, E.Y.D. 15869, UC 1823140, tetrasporic & sterile;

Figs 15, 16, 18. NCU 584263-67.

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Figs 13–21. Mazzaella linearis.

Fig. 13. Holotype of Iridophycus lineare S. & G., UC 507637. Scale bar, 2 cm.

Fig. 14. Tetrasporic specimen, NCU 584253. Scale bar, 2 cm.

Figs 15–18. Cystocarp development.

Fig. 15. Auxiliary cell (ac) and young gonimoblasts (g) surrounded by short-celled secondary filaments (arrows) and unmodified medullary filaments. Scale bar, 100 μm.

Fig. 16. Young cystocarp showing developing gonimoblasts (arrowheads) surrounded by sphere of secondary vegetative filaments (arrows). Gonimoblast filaments and differentiating carposporangia grow and mature progressively, beginning at an early stage. Scale bar, 100 µm.

Fig. 17. Numerous long terminal tubular gonimoblast cells (arrows) on far side of medium-sized cystocarp. NCU 584106-08. Scale bar, 100 μm.

Fig. 18. Centrally located mature cystocarp that has not raised surface layer of thallus. Scale bar, 200 μm.

Figs 19–21. Tetrasporangial development. NCU 584253. Scale bars, 100 µm.

Fig. 19. Broad band of secondarily produced tetasporangial chains (arrows).

Fig. 20. Close-up of young ellipsoidal tetrasporocytes (arrows) in looping chains three to five cells long.

Fig. 21. Two mature tetrasporangial sori.



Baja California: Punta Santo Tomás, 08 November 1969, NCU 584131-32, cystocarpic; S. side of Punta Baja, 21 June 1974, NCU 584133-34, 34A, tetrasporic.

DESCRIPTION: Thalli 20-40 cm tall, rubbery and slick, liverbrown, purple, or violet, but usually iridescent, consisting of one to several lanceolate to broadly ovate blades from a fleshy discoid holdfast and stipes 2-6 cm long, with short, cuneate apophyses; blades, 12-40 cm wide, 0.5 to 1 mm thick, with margins entire and ruffled and typically with a tapering apex, or terminally cleft or divided (Fig. 31), variable in thickness: cystocarpic (0.4-1.2 mm) and tetrasporic (615–700 µm). Cortex five to seven cell layers thick composed of spherical cells. Medullary cells slender, 1.5-3.5 μ m in diameter in sterile plants, and bulbous, 10–20 μ m in fertile plants. Gonimoblasts composed of short-celled filaments (Fig. 32). Secondary gametophytic filaments initiated from medullary cells in the vicinity of a functional auxiliary cell and continuing to be produced progressively forming a bounding outline as gonimoblast filaments advance (Figs 33-35) and virtually disappearing at maturity (Fig. 36). Gonimoblasts composed of loosely arranged, short-celled filaments with carposporangia differentiating progressively as the carposporophyte expands (Fig. 36), probably linking to secondary gametophytic cells, but typically not producing terminal tubular cells; gonimoblasts occasionally connecting to medullary cells by a few sinuous-shaped terminal tubular gonimoblast cells; cystocarps 800-1000 µm in diameter, moderately emergent on both sides of the blade. Carposporangia spherical to ovoid, 10–19 µm in diameter, released through a cup-shaped pore (Fig. 36). Spermatangial plants smooth, spermatangial sori indefinite and superficial. Tetrasporangial chains initiated as continuous networks from outer and inner medullary filaments on both sides of the thallus (Fig. 37). Defined regions transformed periodically into chains of spherical tetasporocytes, three to four per chain (Fig. 38), which mature into tetrasporangia 23-50 µm in diameter that are released through a crack or gelatinous pore from an elevated region of the outer cortex (Fig. 39); tetrasporangial sori 460-550 µm broad and 170-200 µm high, tending to bulge the outer cortical layer slightly (Fig. 39). In some material (e.g. specimens from Santa Barbara County) the sorus does not raise the outer blade surface.

Mazzaella splendens (Setchell & N.L. Gardner) Fredericq in Hommersand *et al.* (1994, p. 198) subsp. *fulgens* (Setchell & N.L. Gardner) Hughey & Hommersand *stat. nov.*

Figs 40-45

BASIONYM: *Iridophycus fulgens* Setchell & N.L. Gardner 1937, p. 173.

HOLOTYPE: Fig. 40. A collection by H.E. Parks, July 1934, near Trinidad, Humboldt County, California. UC 543970! (cystocarpic & tetrasporic generations, "stipitate specimen on right of the sheet" designated as lectotype by I.A. Abbott 1972a, p. 58).

HOMOTYPIC SYNONYM: *Iridaea fulgens* (Setchell & N.L. Gardner) Papenfuss 1958, p. 106.

ILLUSTRATIONS: Scagel 1967, p. 254, fig. 110 (as *Iridaea cordata*); Abbott 1972a, p. 54, figs 2, 5 (as *I. cordata* var. *cordata*), fig. 11 (as *Iridophycus fulgens*); Abbott 1972b, p. 260, fig. 4 (as *Iridaea cordata* var. *cordata*); Abbott & Hollenberg 1976, p. 530, fig. 474 (as *I. cordata* var. *cordata*).

HABITAT: Low intertidal, sheltered to exposed localities.

DISTRIBUTION: Harbor Pt., Lituya Bay, Skagway-Hoonah-Angoon Area, Alaska (UBC A23240); Loran Station, Biorka Island, Sitka Borough Alaska (UBC A13011-12, A13014); Banks & Calvert islands to Port Renfrew & Victoria, Vancouver Island, British Columbia (Scagel 1957, p. 190; Abbott 1972a, p. 54); Richmond Beach, Puget Sound, Washington (Thom *et al.* 1976, p. 272); S. Jetty of the Columbia River & Arch Cape, Clatsop County, Oregon (Markham & Celestino 1976, p. 262); Trinidad, Humboldt County, California (Setchell & N.L. Gardner 1937, p. 173); Bodega Head, Sonoma County, California (NCU 585808-10).

REPRESENTATIVE SPECIMENS: Alaska: Harbor Pt., Lituya Bay, Skagway-Hoonah-Angoon Area, 29 June 1965, UBC A23240, tetrasporic; Taigud Island, Sitka Borough, Site 2, 29 April 1994, NCU; Loran Station, Biorka Island, Sitka Borough, 11 July 1960, UBC A13011-12, A13014, cystocarpic & sterile; British Columbia: Brockton Pt. Stanley Park, Vancouver, 14 July 1980, NCU 584136-37, cystocarpic; Sheringham Pt., Vancouver Island, 11 July 1980, NCU 584139-40, cystocarpic; Tofino, Esowista Peninsula,

Fig. 22. Holotype of Iridophycus sanguineum S. & G., UC 507503. Scale bar, 2 cm.

Fig. 27. Close-up of terminal tubular cells (arrows) seen in Fig. 26 radiating from mature cystocarp on side opposite auxiliary cell. **Figs 28–30.** Tetrasporangial development. NCU 584111. Scale bars, 100 μm.

Fig. 28. Section through fertile blade showing a continuous band of secondary filaments (arrows) on both sides of thallus that will become tetrasporangia.

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Figs 22–30. Mazzaella sanguinea.

Fig. 23. Holotype of Iridophycus whidbeyanum S. & G., UC 94378. Scale bar, 2 cm.

Figs 24–27. Cystocarp development. NCU 584268-69. Scale bars, 100 µm.

Fig. 24. Young cystocarp showing auxiliary cell (ac) and radiating gonimoblasts that penetrate and also compress secondary and medullary filaments (coarse arrows). Note terminal tubular gonimoblast cells fusing with gametophytic cells in medulla (fine arrows) at this early stage.

Fig. 25. Gonimoblasts and secondary gametophytic filaments forming involucre (arrows) around upper edges of cystocarp.

Fig. 26. Mature cystocarp not raising surface cells of thallus. At this stage, numerous, long, terminal tubular gonimoblast cells (arrows) radiating from edge of cystocarp. Secondary gametophytic filaments absent and appear to have been digested, leaving halo around entire periphery of cystocarp.

Fig. 29. Soral area with tetrasporocytes (arrows) bulbous and cuboidal or spherical. Cortex of files of six to seven spherical cells. Fig. 30. Sorus with mature tetrasporangia.



Vancouver Island, 09 July 1980, NCU 584275-77, tetrasporic; Execution Rock, Bamfield, Vancouver Island, 04 June 1985, UBC A68132, tetrasporic; Botany Beach, Vancouver Island, 02 June 1985, UBC A68063, tetrasporic; Washington: Indian Island, Puget Sound, 10 June 1994, NCU 584135, cystocarpic; California: Trinidad Bay, Humboldt County, 01 July 1965, E.Y.D. 25243a in NCU 584146-47, tetrasporic & sterile; Buhne Pt., Humboldt Bay, 16 August 1977, NCU 548138, 584148-50, tetrasporic; Caspar Anchorage, Mendocino County, 16 August 1977, NCU 584151-54, tetrasporic; N. of Cuffey's Pt., Mendocino County, 17 August 1977, NCU 584155-59, tetrasporic.

DESCRIPTION: Thalli similar in habit to M. splendens subsp. splendens, but developmentally different. Shortcelled secondary cortical and medullary filaments formed around a functional auxiliary cell before the initiation of the gonimoblasts. These occupy nearly the same volume as the mature carposporophyte and effectively limit its growth (Fig. 41); a few additional secondary medullary filaments are formed as the gonimoblast matures, but their development is not progressive, as in subsp. splendens (Fig. 42). Gonimoblast cells probably link up with cells of the secondary medulla; however, the two are virtually indistinguishable at maturity. Most of the gonimoblast cells are transformed into carposporangia when the cystocarp is medium to large in size; however, the process of their formation was not resolved. The mature cystocarp is similar in size to subsp. splendens and does not possess any terminal tubular cells at its edge; however, it is bounded by a well-defined, thick involucre, composed of compressed medullary cells and secondary medullary filaments at maturity (Fig. 43). Similar to M. splendens subsp. splendens, tetrasporangia form in chains throughout the entire blade as continuous networks in the outer and inner medulla (Figs 44, 45). In general, the tetrasporangial chains tend to form deeper inside the medulla than in M. splendens subsp. splendens. Tetrasporangial sori 380-460 µm broad and 220-300 µm high. In some regions of the blade the sori extend across the medulla, forming a compound sorus that is greater than 450 µm high.

Although the names of each of the species belonging to the *splendens* clade have been recognized in the literature for a

long time, each of them has proved to be difficult to distinguish, and species identities have often been confused. Mazzaella flaccida has sometimes been distinguished from M. splendens by its yellowish-green tint compared with the blue-iridescent sheen or liver-brown color that characterizes forms of *M. splendens*, and by its tendency to be found in habitats higher in the littoral. The extent of the confusion was illustrated by Foster (1982) who recognized the two as ecological variants of a single species reflecting environmental factors that were correlated with thallus position on the reef. We find that the color differences are a good first indicator of species identity, but any identification on the basis of color needs to be confirmed with anatomical observations. Female plants are common in both species and the variation in cystocarp size provides a second character useful for distinguishing between them. Cystocarps are relatively constant in size and are uniformly spread over the thallus in M. splendens, whereas cystocarp size tends to vary from proximal to distal as well as central to marginal areas of the blade and is not constant in M. flaccida. This difference is related partly to the way in which the thallus grows in the two species and partly to the features of cystocarp development itself. Mazzaella flaccida grows primarily at its apex and from its margins, often exhibiting a sterile strip along the margin in tetrasporophytic plants. Cystocarps vary in diameter, becoming larger as the gonimoblasts grow led by the sterile tubular cells that continue to penetrate vegetative cells at the periphery of the expanding cystocarp. In contrast, growth and thallus expansion is primarily intercalary rather than marginal in M. splendens (Norris & Kim 1972), and new cystocarps tend to be constant in size and are interpolated amongst existing cystocarps as the thallus increases in length and width. Secondary cortical and medullary filaments develop around the auxiliary cell in response to its diploidization before any gonimoblast filaments are formed, and these serve as the main nutritive tissue that is consumed as gonimoblast filaments grow. Few or no tubular filaments are produced, and gonimoblasts are limited in diameter by the amount of nutritive tissue formed initially or later on from surrounding gametophytic tissues.

Mazzaella flaccida overlaps a large part of the geographical range of *M. splendens*; however, *M. splendens* consists

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Figs 31-39. Mazzaella splendens subsp. splendens.

Fig. 31. Holotype of Iridophycus splendens S. & G., UC 539566. Scale bar, 2 cm.

Figs 32–36. Cystocarpic development.

Figs 32-34, 36. NCU 584270-73.

Fig. 32. Close-up of auxiliary cell (ac), gonimoblast cells (coarse arrows) and carposporangia (fine arrows). Scale bar, 50 µm.

Fig. 33. Young cystocarp showing gonimoblast filaments (coarse arrow) together with differentiated carposporangia (fine arrows). Scale bar, 100 μm.

Fig. 34. Edge of maturing cystocarp (center right) showing short-celled secondary filaments at periphery (arrows). Scale bar, 50 μm. **Fig. 35.** Upper left edge of mature cystocarp showing loose network of irregularly shaped cells (arrows) resembling involuce, NCU 584118-19. Scale bar, 100 μm.

Fig. 36. Cystocarp with carposporangia at margins that has released carpospores from center through pore in blade surface. Scale bar, 100 µm.

Figs 37–39. Tetrasporangial development. Scale bars, 100 µm.

Fig. 37. Bands of secondary filaments (arrows) on both sides of blade where tetrasporangial sori are developing, NCU 584128-30. Figs 38, 39. NCU 584118-19.

Fig. 38. Partly cleaved tetrasporangia.

Fig. 39. Mature tetrasporangial sorus with elevated pore.



Figs 40-45. Mazzaella splendens subsp. fulgens.

Fig. 40. Holotype of *Iridophycus fulgens* S. & G., UC 453970. Scale bar, 2 cm. **Figs 41–43.** Cystocarp development. NCU 584139-40.

Fig. 41. Young cystocarp showing an auxiliary cell (ac) bearing few gonimoblast cells (g) surrounded by irregularly branched secondary filaments (arrows) interconnected to form network. Scale bar, 100 µm.

Fig. 42. Cystocarp showing auxiliary cell and a dense mass of gonimoblast filaments. Scale bar, 100 µm.

Fig. 43. Upper right edge of mature cystocarp showing carposporangia on right surrounded by compacted secondary filaments (arrows) forming involucre. Scale bar, 50 µm.

Figs 44, 45. Tetrasporangial development. NCU 584275-77.

Fig. 44. Section through blade showing young tetrasporangial sorus differentiating from secondary medullary filaments in center of medulla. Scale bar, 100 µm.

Fig. 45. Tetrasporocytes (arrows) formed in short chains from secondary filaments in central medulla. Scale bar, 50 µm.

of two subspecies that overlap in distribution in Sonoma County, California, M. splendens subsp. splendens extending to the south and M. splendens subsp. fulgens to the north. Mazzaella splendens subsp. splendens tends to be narrower and more linear and the latter broader and more orbicular with a cordate base; however, these differences are not definitive species characters. In fact, the two subspecies can be told apart with certainty only upon examining the anatomy of cystocarpic and tetrasporangial plants. Cystocarps of M. splendens subsp. splendens increase slightly in diameter as the preformed secondary filaments are consumed and some additional secondary filaments are produced, but those of M. splendens subsp. fulgens are entirely restricted in growth to the dimensions of the initial secondary nutritive filaments formed around the auxiliary cell immediately after fertilization. In fact, not all of these are consumed, and those that remain form a conspicuous involucre surrounding the mature cystocarp. Tetrasporangial plants are even more distinct. Whereas tetrasporangia form in two bands on opposite sides of the medulla in M. splendens subsp. splendens, as in other members of the splendens clade, tetrasporangial development continues across the medulla to produce a single central band in M. splendens subsp. fulgens.

Mazzaella sanguinea is a subtidal species that is easily recognized when it is brick-red, as in its northern distribution; however, color can be misleading as plants found at Bodega Head, California and further south are often purple and rubbery in texture. They can be told from specimens of *M. splendens* by their vegetative anatomy, which is distinguished by the presence bulbous secondary filaments in the medulla of both sterile and tetrasporangial plants that resemble those found only in fertilized female plants and tetrasporic plants of *M. splendens*. Cystocarps are of definite diameter and size, as in *M. splendens*, but terminal tubular cells are present and the preformed nutritive tissue is so completely consumed as to leave a distinct halo around the periphery of the cystocarp.

Mazzaella linearis is frequently confused with narrow forms of *M. splendens*. It is most readily distinguished by the presence of a prominent cylindrical stipe at the base of the blade. The identity of *M. linearis* and the utility of this character was tested by Shaughnessy (1995, 2004), who investigated the two species in transplant studies in Barkley Sound on Vancouver Island in Canada and concluded that they were environmentally and genetically distinct. The detailed molecular work by Ross et al. (2003) confirmed Shaughnessy's findings, that thalli intermediate in habitat and morphology to classic M. splendens and M. linearis are assignable to M. splendens. Anatomically, M. linearis is more like M. splendens subsp. fulgens in that the gonimoblasts are limited in size with part of the secondary nutritive filaments retained around the mature cystocarp as a poorly defined involucre. The species is also characterized by the presence of terminal tubular cells that extend into the surrounding medulla.

Our molecular and anatomical results confirm the conventional treatment of the *splendens* clade in *Mazzaella* with the recognition of four species that are morphologically distinct and are well separated in phylogenetic trees. Our treatment differs only with the addition of two

subspecies under *M. splendens*, *M. splendens* subsp. *splendens* and *M. splendens* subsp. *fulgens*, which are morphologically distinct but closely related in molecular analyses, suggesting that they are incipient rather than fully differentiated species.

Dichotomous key to the *splendens* clade from Pacific North America

- 1. Blades deep red in color (sometimes purplish iridescent when fresh), uniform throughout in color; stipe less than 0.5 cm in length; medullary filaments bulbous in sterile and fertile plants, 5–20 μ m in diameter; cystocarps surrounded by a hemispherical to umbonate halo; subtidal M. sanguinea

- 3. Thalli generally green in color, sometimes green above and purple below; apophysis canaliculate; tetrasporangial plants typically with a sterile margin measuring 1–3 mm in width; medullary filaments narrow in sterile and fertile thalli, 1.5–3.5 μ m in diameter; cystocarp connecting to gametophytic cells by means of numerous long terminal tubular cells; high to mid-intertidal M. flaccida
- Thalli brown, purple, or black in color; apophysis flattened; tetrasporangial plants producing sporangia that extend to the margins of the blade; medullary filaments narrow in sterile plants, bulbous and tightly packed in fertile plants, 10–20 μm in diameter; cystocarp lacking terminal tubular cells or rare when present; low intertidal. 4
- 4. Short-celled secondary medullary filaments formed before the initiation of the gonimoblasts, filaments compact; mature cystocarp with a well-defined involucre; tetrasporangial development taking place in the outer medulla as well as continuing across the central medulla; southeast Alaska to Sonoma County, California *M. splendens* subsp. *fulgens*

ACKNOWLEDGEMENTS

We thank Fran Hommersand for assembling specimens for accessioning. We also thank Linda Jennings at UBC for sending loan material. We are indebted to Paul Gabrielson for his critical review of this manuscript, retrieving some of the vouchers cited in this study and generously providing the complete sequences for Mazzaella sp. 3. We are grateful as always for Susan Whitfield's preparation of the figures. We thank Suzanne Fredericq for graciously supplying the DNA from a previous rbcL study. We express our gratitude to Geoff Leister for sharing kleptotype material of Iridaea minor and for lab assistance. Special appreciation to the following for collecting samples used in this work: Sandra Lindstrom, Steven Murray, Pat and Dave Hughey and to Franchesca Perez and Miguel Volovsek who helped with DNA sequencing. Rangasamy Elumalai, Len Krall and Punita Nagpal provided technical assistance, and Jason Reed and Clifford Parks offered work space in their laboratories. This project was supported in part by a Cooley Trust Scholarship and Coker Fellowship from the Department of Biology at the University of North Carolina, Chapel Hill and the Hans & Margaret Doe Charitable Trust Scholarship Foundation.

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Received 20 August 2008; accepted 19 August 2009 Associate editor: Paul Gabrielson