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New, resurrected and redefined species of *Mastocarpus* (Phyllophoraceae, Rhodophyta) from the northeast Pacific

Sandra C. Lindstrom[1]*, Jeffery R. Hughey[2] and Patrick T. Martone[1]

[1]*Department of Botany, 3529-6270 University Blvd, University of British Columbia, Vancouver V6T 1Z4, Canada*
[2]*Division of Science and Mathematics, Hartnell College, Salinas, California 93901, USA*

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Recent molecular phylogenetic investigations of the red algal genus *Mastocarpus* from the northeast Pacific resolved numerous cryptic species. Although species were clearly defined through genetic analyses, the correct names to apply to the species remained unclear due to both morphological variability within species and morphological similarity between species. To determine the appropriate name for each entity, we analyzed DNA from type material of taxa previously ascribed to *Mastocarpus*. In combination with this analysis, an updated phylogeny based on a broad range of geographical and morphological collections is presented that includes data from nuclear (ribosomal internal transcribed spacers [ITS]), chloroplast (*rbc*L) and mitochondrial [cytochrome oxidase I (COI)] genomes. By analyzing partial ITS region sequences of type specimens, we are able to match currently accepted names (*Mastocarpus papillatus*, *M. pacificus* and *M. jardinii*) to modern collections. We resurrect the following specific epithets and propose the new combinations *Mastocarpus cristatus*, *Mastocarpus latissimus* and *Mastocarpus agardhii*, and we create new species for which we were unable to verify an existing name: *Mastocarpus alaskensis*, *Mastocarpus intermedius*, *Mastocarpus vancouveriensis*, *Mastocarpus californianus* and *Mastocarpus rigidus*. The species formerly included in *M. papillatus* are now identified as *Mastocarpus alaskensis*, *M. papillatus*, *Mastocarpus intermedius*, *Mastocarpus cristatus*, *Mastocarpus vancouveriensis* and *Mastocarpus latissimus*. The name *M. jardinii* applies to a species thus far collected only from Moss Beach in San Mateo County and the Monterey Peninsula, both in California. Specimens other than the type previously assigned to *M. jardinii* are now separated into three species: *Mastocarpus rigidus*, *Mastocarpus californianus* and *Mastocarpus agardhii*. *Mastocarpus cristatus* represents a species closely allied to Clade 3 (*Mastocarpus intermedius*), and *M. pacificus* represents Clade 7. Morphological and anatomical diagnoses, along with vertical distributions and geographic ranges, are provided for each species.

Key Words: Biogeography, COI, ITS, *Mastocarpus*, Pacific North America, Phylogeny, *rbc*L, Rhodophyta, Species, Taxonomy, Type material

INTRODUCTION

The genus *Mastocarpus* Kützing 1843 was originally established to accommodate four cartilaginous 'Gigartineae' with stipitate, frequently divided thalli and protruding cystocarpic papillae. Kützing's proposal, however, was dismissed by J. Agardh (1851, 1876, 1899), who retained all of the *Mastocarpus* species in *Gigartina* Stackhouse 1809. Setchell & Gardner (1933) later resurrected the name but at the rank of subgenus, as *Mastocarpus* (Kützing) Setchell & N.L. Gardner, effectively lectotypifying the Kützing genus when they chose *Gigartina mamillosa* (Goodenough & Woodward) J. Agardh as the 'typical species'. *Mastocarpus* went without treatment until Guiry *et al.* (1984) reinstated the genus based on morphological, life history and biochemical features. These features included 'channelled' gametophytic thalli with female reproductive structures and carposporophytes borne in papillae and a heteromorphic (or direct) life history with the crustose *Petrocelis*-like tetrasporophyte phase producing tetrasporangia singly. The reinstatement has been supported by molecular analyses (Fredericq & Lopez-Bautista 2002; Lindstrom 2008a; LeGall & Saunders 2010).

* Corresponding author (Sandra.Lindstrom@botany.ubc.ca).

The number of *Mastocarpus* species recognized worldwide at the time of Setchell & Gardner (1933, as subgenus *Mastocarpus*) was 17. Of those, 12 were distributed in Pacific North America. Abbott (1972) reduced all but two names (*Gigartina agardhii* Setchell & N.L. Gardner, *Gigartina jardinii* J. Agardh) into synonymy under *Gigartina papillata* (C. Agardh) J. Agardh. In support of this reduction, she noted that intermediate characters between the different species were common, and sometimes characters attributed to more than one species appeared on a single thallus. West *et al.* (1978) recognized *Gigartina papillata* as a distinct entity and on the basis of priority placed *Gigartina agardhii* into synonymy under *Gigartina jardinii*.

Recent molecular studies on species of *Mastocarpus* from the northeast Pacific (Lindstrom 2008a) identified five distinct *Mastocarpus papillatus* clades (species) and a single, strongly supported clade containing *Mastocarpus jardinii*. Unfortunately, no reliable morphological distinctions were discernible among the species identified as *Mastocarpus papillatus*, and although a lengthy list of names extending back to *Sphaerococcus papillatus* C. Agardh (1821) was provided, it was unclear which names to apply to which clades (Lindstrom 2008a). The present study expands on the northeast Pacific data set of Lindstrom (2008a) by

including more samples from a wider geographic area and sequences of the chloroplast large subunit of the ribulose-1, 5-biphosphate carboxylase/oxygenase (*rbc*L) gene, the mitochondrial cytochrome oxidase I (COI) gene and the nuclear internal transcribed spacer region (ITS1, 5.8S rRNA gene and ITS2). Here we assign names to clades by performing genetic analyses on type material (Hughey *et al.* 2001, 2002). In addition, we present distributional, ecological and morphological data to aid in the delineation and identification of the northeast Pacific species of *Mastocarpus*.

MATERIAL AND METHODS

DNA analysis of newly collected material

DNA extraction, amplification and sequencing of ITS and *rbc*L genes followed the procedures in Lindstrom (2008a). The primer pair ITS1-JO6 was used to amplify and sequence the complete ITS region including the 5.8S rRNA gene (Lindstrom 2008a), and the primer pair F57-RrbcSst was used to produce 1356 bp of the *rbc*L gene (Freshwater & Rueness 1994). Amplification and sequencing of 665 bp at the 5′ end of the COI gene followed Saunders (2005) using primers GazF1 and GazR1. Polymerase chain reaction (PCR) products were sequenced using the ABI Applied Biosystems (Foster City, California, USA) Big Dye Terminator V.3.1 cycle sequencing kit by the Nucleic Acid Protein Service Unit (University of British Columbia, Vancouver, British Columbia, Canada).

DATA ANALYSIS: Fully alignable ITS, *rbc*L and COI sequences from the specimens listed in Table 1 were concatenated and then subjected to maximum parsimony (MP), neighbour-joining (NJ) and maximum likelihood (ML) analyses using PAUP* 4.0b10 (Swofford 2002) following Lindstrom & Fredericq (2003). The generalized time-reversible model + I + G was determined to be the appropriate model of evolution for the ML analysis using the Akaike Information Criterion in Modeltest 3.7 (Posada & Crandall 1998). Model parameters included nucleotide frequencies A = 0.2878, C = 0.1814, G = 0.2041; substitution rates A > C 1.7580, A > G 6.5293, A > T 0.6466, C > G 1.1034, C > T 12.9896; gamma shape parameter (G) = 0.6719 and proportion of invariable sites (I) = 0.5819. Bootstrap proportions were determined based on 10,000 replicates for MP, 1000 replicates for NJ and 100 replicates for ML. Because the specimens in the concatenated analysis represented a random assortment for which all three parts of the genome had been sequenced, we also analyzed all unique ITS genotypes (Table 1) using MP, NJ and ML analyses to determine whether the concatenated results were consistent with a much larger taxon sample. The transversion model + I + G was determined to be the appropriate model for this ML analysis with the following parameters: nucleotide frequencies A = 0.2688, C = 0.2195, G = 0.2179 and substitution rates A > C 1.7592, A > G 3.5976, A > T 0.8863, C > G 1.1063, C > T 3.5976, G = 0.7453 and I = 0.3408.

DNA analysis of type material

DNA EXTRACTION: All extractions and amplifications of type material (Table 2) were performed in a laboratory at Hartnell College, Salinas, California, in which modern DNA from *Mastocarpus* has not been analyzed. Standard negative controls were processed in parallel with type material to monitor for contamination from exogenous DNA. Extractions were carried out using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, California, USA). Approximately 15 mg of dried tissue (~5 mm^2 in size) was preincubated for 30 min at 56°C in a 1.7 ml microcentrifuge tube in a solution of 270 µl of Buffer ATL and 30 µl of Proteinase K. Partially digested samples were then ground with the wooden end of sterile swab and further incubated for 30 min to 2 h. Samples were centrifuged for 3 min at 8000 rpm to separate cellular debris from the supernatant. Two hundred microlitres of the supernatant (including any precipitate) was transferred to a new microcentrifuge tube containing 200 µl of ethanol (96–100%) and 200 µl Buffer AL. The resulting sample was mixed by pipetting, transferred to the DNeasy Mini spin column and then centrifuged for 90 s at 8000 rpm. For cases in which phycocolloids inhibited the flow-through of the supernatant, extended centrifugation at the same rate was carried out. The spin column was removed and placed in a new 2 ml collection tube and washed with 500 µl of Buffer AW1 by centrifugation for 1 min at 8000 rpm. The column was then placed in a new 2 ml collection tube and washed with 500 µl of Buffer AW2 by centrifugation for 3 min at 14,000 rpm. The DNA was eluted with 70 µl of Buffer AE for 5 min followed by centrifugation for 1 min at 8000 rpm.

DNA AMPLIFICATION PROTOCOL: For all PCR reactions, 6 µl of stock or 6 µl of diluted DNA (10-fold) served as template for 50 µl reactions following the protocol outlined in the TopTaq™ PCR Handbook using 10 µl of 5X Q-Solution (Qiagen). To eliminate competition from nonspecific fungal contaminants, a *Mastocarpus* specific forward ITS 1 primer 5′ GGACATTCGTAGTGGGATAGT 3′ was used in conjunction with the universal ITS 2 reverse primer (GCTGCGTTCTTCATCGATGC) designed by White *et al.* (1990). Reactions were run on a Perkin Elmer Cetus DNA Thermal Cycler (Waltham, Massachusetts, USA) with the following parameters: 94°C for 90 s; followed by 40 cycles of 94°C for 25 s, 53°C for 30 s and 72°C for 60 s and final extension of 72°C for 5 min. PCR products were purified using the QIAquick PCR Purification Kit following the manufacturer's instructions (Qiagen). The samples were sequenced using both forward and reverse primers by Functional Biosciences, Inc. (Madison, Wisconsin, USA) and edited using Chromas Lite Version 2.01 (Technelysium Pty Ltd, Queensland, Australia).

RESULTS

Phylogenetic analysis of concatenated ITS, COI and *rbc*L sequence data representing 43 ingroup and two outgroup taxa generated the ML phylogram in Fig. 1. With the

Table 1. Specimens of *Mastocarpus* sequenced, including extract number, collection data and GenBank and voucher accession numbers. Specimens for which all three genomes (COI, ITS and *rbc*L) were analyzed were used to construct Fig. 1. Sequences in brackets are from other studies.

Extract number	Collection site and habitat[1]	Date	Collector[2]	COI acc.	ITS acc.[3]	*rbc*L acc.	Voucher
Mastocarpus alaskensis (Clade 1)							
M5	Sunshine Cove, AK, high boulder	24 Jun. 2002	SCL		HQ437761		UBC A85244
M8	Birch Bay, WA, mid to high cobble	03 Nov. 2001	SCL		DQ872469		UBC A85238
M25	Rosario Beach, WA, mid	24 Jan. 2002	SCL		HQ437762		UBC A85253
M42	Sunshine Cove, AK, high	24 Jun. 2002	SCL		HQ437769		UBC A85244
M53	Moses Point, BC, high cobble	27 Apr. 2002	SCL		DQ872472		UBC A85239
M57	Glencoe Cove, BC, high	27 Apr. 2002	SCL		HQ437763		UBC A85246
M65	Port Hardy, BC, high intertidal	16 May 2003	SCL	HQ437708	DQ872470	DQ872500	UBC A85240
M68	Miracle Beach, BC, high rock in sand	16 May 2003	SCL		DQ872471		UBC A85355
M72	Sayward, BC, mid	18 May 2003	SCL		HQ437764		UBC A85245
M134	Sitka, AK, low cobble	27 Jul. 2003	SCL		HQ437751		UBC A85254
M136	Lantzville, BC, high	16 May 2003	SCL		HQ437765		UBC A85264
M137	Hornby Island, BC, mid to high	29 Feb. 2004	VR		HQ437770		UBC A85249
M161	Second Priest Rock, AK, high	05 Aug. 2004	SCL		EU186038		UBC A85817
M168	Triple Islets, BC, high	14 Sep. 2004	LG		HQ437752		UBC A88733
M180	Beaver Harbour, BC, high mid	18 May 2003	SCL		HQ437753		UBC A85290 UBC A85291
M181	Miracle Beach, BC, high rock in sand	16 May 2003	SCL		HQ437748		UBC A85355
M187	Glencoe Cove, BC, high	27 Apr. 2002	SCL		DQ872468		UBC A85317
M188	Birch Bay, WA, mid to high cobble	03 Nov. 2001	SCL		HQ437749		UBC A85238
M191	Dupont, AK, mid	23 Jun. 2002	SCL		EU186039		UBC A85818
M193	Chugach Bay, AK, high	09 Jul. 2002	SCL		HQ437771		UBC A88736
M200	Second Priest Rock, AK, high	05 Aug. 2004	SCL		HQ437772		UBC A85817
M201	Second Priest Rock, AK, high	05 Aug. 2004	SCL		HQ437758		UBC A85817
M255	Cape Ilktugitak, AK, mid	13 Jun. 2003	SCL		HQ437759		UBC A85284
M261	Passage Island, AK, high intertidal	30 Jun. 2003	MRL	HQ437709	HQ437768	EU186029	UBC A85281
M263	Judith Island, AK	28 Jun. 2003	MRL		HQ437773		UBC A88740
M292	NW Olympic Peninsula, WA, mid boulder	31 May 2003	SCL		EU 186040		UBC A85279
M298	Monashka Bay, AK, high	19 May 2005	SCL		HQ437774		UBC A88741
M299	Afognak Island, AK, high	25 May 2005	SCL		HQ437754		UBC A85350
M305	Near Island, AK, mid boulder	20 May 2005	SCL		HQ437775		UBC A85347
M317	Spruce Island, AK, high	29 May 2005	SCL		HQ437776		UBC A88742
M354	Tigalda Bay, AK	02 Jul. 2006	MRL		HQ437755		UBC A88743
M361	Peacock Point, AK	14 Jul. 2006	MRL		HQ437777		UBC A88533
M362	Tigalda Bay, AK	02 Jul. 2006	MRL		HQ437778		UBC A88536
M382	Butze Rapids, BC, low intertidal	17 Jun. 2007	SCL	HQ437710	HQ437750	HQ437877	UBC A88520
M394	Harpoon Point, AK, high	29 Jun. 2007	MRL		HQ437779		UBC A88538
M459	Akun Bay, AK, low boulder	13 Jun. 2008	SCL		HQ437760		UBC A88503
M462	Sunset Beach, OR, high	06 Apr. 2008	SCL		HQ437781		UBC A88493
M463	Sunset Beach, OR, mid	06 Apr. 2008	SCL		HQ437782		UBC A88498
M464	Sunset Beach, OR, high	06 Apr. 2008	SCL		HQ437783		UBC A88497
M468	Crescent City, CA, high	06 Apr. 2008	SCL		HQ437780		UBC A88490
M500	Pearse Island, BC, high	24 May 2009	SCL		HQ437766		UBC A87901
M511	Malcolm Island, BC, mid boulder	28 May 2009	SCL		HQ437767		UBC A88459
M516	Walter's Island, Kyuquot Sound, BC	26 May 2009	PTM		HQ437756		UBC A88756
M528	Trinidad, CA, mid boulder	14 Feb. 2010	SCL		HQ437757		UBC A88465
Mastocarpus papillatus (Clade 2)							
M1	Clover Point, BC, mid	27 Apr. 2002	SCL		EU186041		UBC A85272
M10	Baker Beach, Humboldt County, CA, high intertidal	25 May 2002	SCL	HQ437711	HQ437784	DQ872501	UBC A85271

Table 1. Continued

Extract number	Collection site and habitat[1]	Date	Collector[2]	COI acc.	ITS acc.[3]	*rbc*L acc.	Voucher
M104	Seppings Island, BC, mid	18 Apr. 2003	SCL		HQ437786		UBC A85270
M156	Low Arch, SW Farallon Island, CA	14 Aug. 2004	KD	HQ437712	EU186044	HQ437878	UBC A85820
M210	Agate Beach, CA	13 Jan. 1973	ARP		HQ437787		UC 1471472
M238	Baker Beach, CA, mid	25 May 2002	SCL		DQ872478		UBC A85318
M284	Golden Gardens, Seattle, WA, high riprap	30 May 2003	SCL		DQ872466		UBC A85276
M469	Crescent City, CA, high	06 Apr. 2008	SCL		HQ437788		UBC A88489
M471	Humboldt Bay jetty, CA, high	07 Apr. 2008	SCL		HQ437789		UBC A88487
M476	Cape Mendocino, CA, high	08 Apr. 2008	SCL		HQ437790		UBC A88495
M526	Moss Beach, San Mateo County, CA, high intertidal at rock/sand interface	16 Nov. 2009	KAM & PTM	HQ437713	HQ437785	HQ437879	UC 1944776
M527	Moss Beach, CA	16 Nov. 2009	KAM		HQ437791		UC 1944779
M529	Trinidad, CA, mid boulder	14 Feb. 2010	SCL		HQ437792		UBC A88466
M537	Fort Bragg, CA, mid rock in sand	15 Feb. 2010	SCL		HQ437793		UBC A88473
M538	Point Arenas, CA, mid	16 Feb. 2010	SCL		HQ437794		UBC A88474
M539	Bodega Head, CA, high	16 Feb. 2010	SCL		HQ437795		UBC A88475
M540	Bodega Head, CA, mid	16 Feb. 2010	SCL		HQ437796		UBC A88476
M541	Bodega Head, CA, mid	16 Feb. 2010	SCL		HQ437797		UBC A88477
M542	Bodega Head, CA, mid	16 Feb. 2010	SCL		HQ437798		UBC A88478
M544	Bodega Head, CA, mid	16 Feb. 2010	SCL		HQ437799		UBC A88480
M546	Cambria, CA, mid	18 Feb. 2010	SCL		HQ437800		UBC A88481
M548	Pescadero State Beach, CA, high rock in sand	17 Feb. 2010	SCL		HQ437801		UBC A88508
Mastocarpus intermedius (Clades 3A & 3B)							
M2	Barkley Sound, BC, mid to low	10 Aug. 2002	SCL		HQ437819		UBC A85324
M11	Baker Beach, Humboldt County, CA, low intertidal boulder	25 May 2002	SCL	HQ437723	DQ872481	DQ872502	UBC A85235
M174	Triple Island, BC, mid intertidal	01 Sep. 2004	LG	HQ437724	EU186047	HQ437886	UBC A85833
M234	Birch Bay, WA, low	04 Jul. 2004	SCL		HQ437820		UBC A88739
M245	Whiffin Spit, BC, low intertidal boulder	03 Feb. 2002	SCL	HQ437725	HQ437814	EU186031	UBC A85823
M288	NW Olympic Peninsula, WA, low	31 May 2003	SCL		DQ872482		UBC A85296
M344	Cape Kaguyak, AK, low	25 Jun. 2005	SCL		HQ437821		UBC A88513
M375	Ventura Beach, CA, low intertidal riprap	10 Nov. 2007	SCL	HQ437726	HQ437815	HQ437887	UBC A88510
M378	Ventura Beach, CA, low intertidal riprap	10 Nov. 2007	SCL	HQ437727	HQ437816	HQ437888	UBC A88510
M407	Hopkins Marine Station, Pacific Grove, CA, mid intertidal	07 Jan. 2008	PTM	HQ437729	HQ437817	HQ437890	UBC A88558
M440	Pacific Grove, CA, high	31 Dec. 2007	PWG		HQ437822		UBC A88548
M473	Cape Mendocino, CA, upper mid	08 Apr. 2008	SCL		HQ437823		UBC A88484
M474	Mussel Rock, Cape Mendocino, CA, low intertidal boulder	08 Apr. 2008	SCL	HQ437732	HQ437818	HQ437893	UBC A88485
M479	Van Damme State Park, CA, upper mid	09 Apr. 2008	SCL		HQ437824		UBC A88482
M550	Ensenada, Mexico	24 Feb. 2010	PWG		HQ437825		UBC A88550
Mastocarpus cristatus (Clade 3C)							
M406	Hopkins Marine Station, Pacific Grove, CA, high intertidal	07 Jan. 2008	PTM	HQ437728	HQ437804	HQ437889	UBC A88557
M408	Hopkins Marine Station, Pacific Grove, CA, low intertidal	07 Jan. 2008	PTM	HQ437730	HQ437805	HQ437891	UBC A88745
M410	Hopkins Marine Station, Pacific Grove, CA, low intertidal	07 Jan. 2008	PTM	HQ437731	HQ437806	HQ437892	UBC A88746
M416	Pacific Grove, CA, mid	07 Jan. 2008	PTM		HQ437807		UBC A88561
M417	Pacific Grove, CA, mid	07 Jan. 2008	PTM		HQ437808		UBC A88561
M421	Pacific Grove, CA, mid	07 Jan. 2008	PTM		HQ437809		UBC A88562
M426	Pacific Grove, CA, high	07 Jan. 2008	PTM		HQ437810		UBC A88557

Table 1. Continued

Extract number	Collection site and habitat[1]	Date	Collector[2]	COI acc.	ITS acc.[3]	*rbc*L acc.	Voucher
M427	Pacific Grove, CA, mid	07 Jan. 2008	PTM		HQ437811		UBC A88563
M429	Pacific Grove, CA, mid	07 Jan. 2008	PTM		HQ437812		UBC A88563
M431	Pacific Grove, CA, mid	07 Jan. 2008	PTM		HQ437813		UBC A88564
Mastocarpus vancouveriensis (Clade 4)							
M50	Fishboat Bay, BC, mid boulder	29 Apr. 2002	SCL		DQ872483		UBC A85234
M63	Sandy Beach, Sitka, AK, mid intertidal boulder/cobble	20 Apr. 2000	SCL	HQ437718	EU186048	HQ437882	UBC A85325 UBC A85391 UBC A85392
M224	China Beach, BC, low intertidal boulder	29 Apr. 2002	SCL	HQ437719	HQ437826	HQ437883	UBC A88738
M287	Tongue Point, WA, lower mid rock in sand	01 Jun. 2003	SCL		EU186050		UBC A85297
M477	Cape Mendocino, CA, low rock near sand	08 Apr. 2008	SCL		HQ437827		UBC A88486
Mastocarpus latissimus (Clade 5)							
M44	Chance Cove, AK	12 Jul. 2002	MRL		HQ437831		UBC A85338
M66	Lantzville, BC, mid	16 May 2003	SCL		DQ872492		UBC A85230
M106	Shakun Islets, AK	16 Jun. 2003	SCL		HQ437832		UBC A85331
M108	Kiukpalik Island, AK, low	16 Jun. 2003	SCL		DQ872491		UBC A85231
M109	Shakun Islets, AK	16 Jun. 2003	SCL		HQ437833		UBC A85329
M110	Shakun Islets, AK, low	16 Jun. 2003	SCL		HQ437834		UBC A85330
M129	Halibut Point, AK, low	02 Aug. 2003	SCL		HQ437835		UBC A85334
M185	Sombrio Beach, BC, low boulder	30 Apr. 2002	SCL		HQ437836		UBC A88734
M189	Brockton Point, BC	08 Feb. 2002	CB		HQ437837		UBC A88735
M235	Baker Beach, CA, mid	25 May 2002	SCL		HQ437838		UBC A85319
M241	Knoll Head, AK, lower mid	28 Aug. 2004	SCL		HQ437839		UBC A85308
M291	NW Olympic Peninsula, WA, low intertidal boulder	31 May 2003	SCL	HQ437720	HQ437828	EU186036	UBC A85298
M309	Cape Sitkinak, AK, low	24 Jun. 2005	SCL		EU186054		UBC A85352
M313	Akhiok Island, Kodiak Island, AK, low intertidal rock	21 Jun. 2005	SCL	HQ437721	HQ437829	HQ437884	UBC A85348
M386	Big Beach, Ucluelet, BC, lower mid intertidal	18 May 2007	SCL	HQ437722	HQ437830	HQ437885	UBC A88504
M490	La Desembocadora de Bío Bío, Chile, mid	09 Nov. 2008	PWG		HQ437840		NCU 588649
Mastocarpus rigidus (Clade 6A)							
M3	Botany Bay, BC, mid intertidal	30 Apr. 2002	SCL	HQ437735	DQ872496	DQ872504	UBC A85227
M7	Baker Beach, CA	25 May 2002	SCL		DQ872495		UBC A85385
M38	Fishboat Bay, BC, mid boulder	29 Apr. 2002	SCL		DQ872497		UBC A85228
M64	Halibut Point, AK, lower mid	20 Apr. 2000	SCL		HQ437847		UBC A85340
M73	Cape Palmerston, BC, mid intertidal boulder	17 May 2003	SCL	HQ437736	HQ437841	HQ437896	UBC A85341
M293	NW Olympic Peninsula, WA, mid intertidal boulder	31 May 2003	SCL	HQ437737	EU186057	HQ437897	UBC A85316
M307	Cape Kaguyak, AK, low	25 Jun. 2005	SCL		EU186059		UBC A85354
M379	Dundas Island, BC, mid intertidal	19 Apr. 2007	SCL	HQ437738	HQ437842	HQ437898	UBC A88744
M454	Outside Chernofski Harbor, Unalaska Island, AK, high intertidal	11 Jun. 2008	SCL	HQ437742	HQ437843	HQ437902	UBC A88463
M460	Clover Point, BC, mid intertidal	02 Mar. 2008	SCL	HQ437743	HQ437844	HQ437903	UBC A88517
M465	Sunset Beach, OR, mid intertidal rock	06 Apr. 2008	SCL	HQ437744	HQ437845	HQ437904	UBC A88494
M466	Sunset Beach, OR, mid intertidal rock	06 Apr. 2008	SCL	HQ437745	HQ437846	HQ437905	UBC A88492
M505	Plumper Island, BC, low	25 May 2009	SCL		HQ437848		UBC A88755
M531	Trinidad, CA, mid boulder	14 Feb. 2010	SCL		HQ437849		UBC A88468
M534	Humboldt Bay jetty, CA, mid	15 Feb. 2010	SCL		HQ437850		UBC A88506

Table 1. Continued

Extract number	Collection site and habitat[1]	Date	Collector[2]	COI acc.	ITS acc.[3]	*rbc*L acc.	Voucher
Mastocarpus californianus (Clade 6B)							
M475	Mussel Rock, Cape Mendocino, CA, low intertidal boulder	08 Apr. 2008	SCL	HQ437746	HQ437851	HQ437906	UBC A88483
M480	Van Damme State Park, CA, mid intertidal	09 Apr. 2008	SCL	HQ437747	HQ437852	HQ437907	UBC A88462
M543	Bodega Head, CA, mid	16 Feb. 2010	SCL		HQ437853		UBC A88479
Mastocarpus agardhii (Clade 6C)							
M436	Hopkins Marine Station, Pacific Grove, CA, mid intertidal	07 Jan. 2008	PTM	HQ437739	HQ437854	HQ437899	UBC A88565
M438	Hopkins Marine Station, Pacific Grove, CA, mid intertidal	07 Jan. 2008	PTM	HQ437740	HQ437855	HQ437900	UBC A88567
M439	Hopkins Marine Station, Pacific Grove, CA, high intertidal	07 Jan. 2008	PTM	HQ437741	HQ437856	HQ437901	UBC A88526
Mastocarpus pacificus (Clade 7)							
M35	Kurumoishi, Hokkaido, Japan	28 Jul. 2002	SCL		HQ437857		UBC A88732
M49	Kurumoishi, Hokkaido, Japan, mid	28 Jul. 2002	SCL		DQ872486		UBC A85274
M52	Kurumo Ishi, Hokkaido, Japan, mid intertidal	28 Jul. 2002	SCL	HQ437716	EU186060	DQ872506	UBC A88732
M96	Kukak Bay, AK, mid intertidal	14 Jun. 2003	SCL	HQ437717	DQ872487	DQ872507	UBC A85229
M312	Akhiok Island, AK, low	21 Jun. 2005	SCL		DQ872488		UBC A85359
M324	Shearwater Bay, AK, low pebble	26 Jun. 2005	SCL		HQ437858		UBC A88546
Mastocarpus stellatus (Clade 8)							
M154	Ormhilleren, Oygarden, Norway, mid intertidal	23 Jun. 2004	SCL	[DQ191350] [DQ191351]	DQ872485	DQ872508	UBC A85222
(Clade 9, included in *Mastocarpus papillatus*)							
M437	Hopkins Marine Station, Pacific Grove, CA, mid intertidal	07 Jan. 2008	PTM	HQ437714	HQ437802	HQ437880	UBC A88566
M443	Foot of 15th Street, Pacific Grove, CA, high intertidal	31 Dec. 2007	PWG & PTM	HQ437715	HQ437803	HQ437881	UBC A88525
Mastocarpus jardinii (Clade 10)							
M405	Hopkins Marine Station, Pacific Grove, CA, low intertidal	07 Jan. 2008	PTM	HQ437733	HQ437859	HQ437894	UBC A88556
M409	Pacific Grove, CA, low	07 Jan. 2008	PTM		HQ437860		UBC A88559
M411	Pacific Grove, CA, low	07 Jan. 2008	PTM		HQ437861		UBC A88559
M413	Hopkins Marine Station, Pacific Grove, CA, low intertidal	07 Jan. 2008	PTM	HQ437734	HQ437862	HQ437895	UBC A88747
M525	Moss Beach, San Mateo County, CA, low	16 Nov. 2009	KAM		HQ437863		UC 1944777
Mastocarpus pachenicus	Various			[GQ380177]	n/a[4]	Unpublished	
Mastocarpus yendoi	Japan	n/a[4]	n/a[4]		Unpublished		
Ahnfeltiopsis paradoxa	Various			n/a[4]	[AF388648]	[AF388568]	
Ahnfeltiopsis leptophylla	Various			[GQ380027]	HQ437864	HQ437908	

[1] Habitat is bedrock unless stated otherwise.
[2] Collectors: SCL = Sandra C. Lindstrom, VR = Vera Roningen, LG = Larry Golden, MRL = Mandy R. Lindeberg, KD = Kathleen Dickie, ARP = Alan R. Polanshek, KAM = Kathy Ann Miller, PTM = Patrick T. Martone, PWG = Paul W. Gabrielson, CB = Corban Bristow.
[3] Specimens with only ITS accession numbers represent unique genotypes that contributed to the construction of Fig. 2.
[4] n/a = not available.

Table 2. Type and other historic specimens of *Mastocarpus* analyzed.

Name of type	Collection site	Date	Collector	GenBank acc.	Type specimen
Sphaerococcus papillatus	Golden Gate, CA	1816	A. Chamisso	HQ437866	Hb. Ag. 23883 in LD
Petrocelis franciscana	Fort Point, CA			HQ437865	UC 188973
Gigartina obovata	Golden Gate, CA		S. Berggren	HQ437867	Hb. Ag. 23840 in LD
Gigartina jardinii	Monterey, CA	1855	E. Jardin	HQ437874	Hb. Ag. 23800 in LD
Gigartina pacifica	Bering Island, Russia	14–19 Aug. 1879	F.R. Kjellman	HQ437871	S syntype (upper left)
Chondrus mamillosus var. *unalaschcensis*	Unalaska, AK		I. Wosnessenski	HQ437872	S syntype (centre)
Gigartina mamillosa var. *latissima*	Esquimalt, BC	1859–1861	D. Lyall	HQ437868	Hb. Ag. 23855 in LD
				HQ437869	
Gigartina dichotoma	Duxbury Reef, CA	Nov.	N.L. Gardner	HQ437870	UC 296705
Gigartina agardhii	Pyramid Point, CA	18 May 1897	W.A. Setchell	HQ437873	UC 93733
Gigartina papillata f. *cristata*	Pyramid Point, CA	18 May 1897	W.A. Setchell	HQ437875	UC 93707
Other historic specimens					
Gigartina papillata obovata?				HQ437876	Hb. Ag. 23801 in LD

inclusion of additional taxa beyond those in Lindstrom (2008a), particularly specimens from California, and the addition of another gene (COI) to the analysis, 12 northeast Pacific species were resolved. *Mastocarpus* species received robust bootstrap support, ranging from 96% to 100% for all analyses. The only exception to this support was *Mastocarpus papillatus* (see below). *Mastocarpus alaskensis* and *M. papillatus* occurred on a strongly supported branch, as did *Mastocarpus intermedius* and *Mastocarpus cristatus. Mastocarpus rigidus* and *Mastocarpus californianus* also showed a strong sibling relationship, as did these two species to *Mastocarpus agardhii*; finally, these three species were clearly related to *Mastocarpus latissimus*. The remaining species showed no clear relationships with other species and occurred alone on their own robustly supported branches.

Sequence analysis of the ITS regions of 382 specimens of *Mastocarpus* resulted in the identification of 128 unique ribotypes. Maximum likelihood and maximum parsimony analyses of these unique ribotypes generated phylograms that were congruent with the concatenated tree, with the exception of *Mastocarpus cristatus*, which was nested within a branch containing *Mastocarpus intermedius* (Fig. 2). Separate analyses on 68 COI and 66 *rbc*L gene sequences, representing all known *Mastocarpus* clades, supported similar relationships as those found in Fig. 1 (data not shown).

Within species variation in *rbc*L ranged from 0% among many conspecifics to a maximum of 0.7% in *M. pacificus* (between Japanese and Alaskan specimens) and 1.3% among some specimens retained in *M. papillatus*. This latter figure was more than the level of difference between *Mastocarpus intermedius* and *Mastocarpus cristatus* (0.6–0.7%) and similar to the level of difference between *M. papillatus* and its sibling species *Mastocarpus alaskensis* (1.0–1.5%) and among the closely related *Mastocarpus rigidus*, *Mastocarpus californianus* and *Mastocarpus agardhii* (1.0–1.7%). For the COI gene, within species variation ranged from 0% among many conspecifics, to 1.7% for *M. pacificus* and up to 5.5% for specimens retained in *M. papillatus*. In contrast to the *rbc*L gene, which indicated little distance between *Mastocarpus intermedius* and *Mastocarpus cristatus*, there was significant distance in the COI gene (3.5–4.5%) between these species. *Mastocarpus rigidus* and *Mastocarpus californianus* were separated by a modest distance (2.2–2.3%), and both showed a similar and shorter distance to *Mastocarpus latissimus* (2.0–3.2%) than to *Mastocarpus agardhii* (8.6–9.1%).

Below we provide details of our identification of these clades with existing species names, and we create new species that cannot be matched with existing names. We follow Lindeberg & Lindstrom (2010, fig. 4) in the application of the habitat descriptors high, mid and low intertidal. In general, high intertidal is at the elevation of *Balanus glandula* Darwin and *Endocladia muricata* (Endlicher) J. Agardh and near the upper limit of *Fucus distichus* subsp. *evanescens* (C. Agardh) H.T. Powell, where these species occur. The mid intertidal zone is the elevation dominated by *Fucus distichus* and *Semibalanus cariosus* (Pallas). The low intertidal zone is often dominated by green or red algae at more sheltered sites and by kelps on

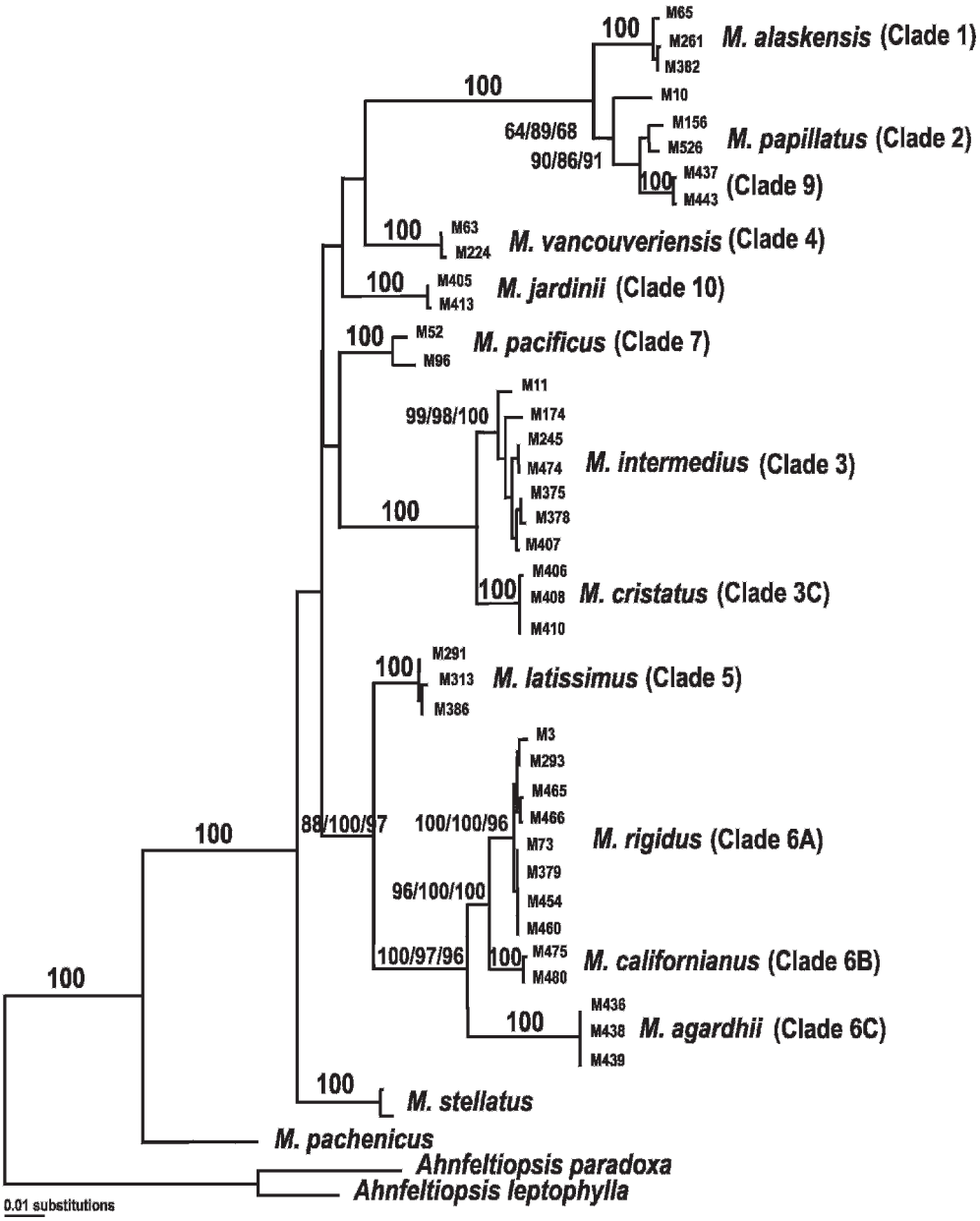


Fig. 1. Maximum likelihood tree (−ln L = 10226.8566) of concatenated ITS-*rbc*L-COI sequences of *Mastocarpus* specimens. Numbers above branches are bootstrap values for maximum parsimony/neighbour-joining/maximum likelihood, respectively. When the bootstrap value for all analyses was 100%, only that number is indicated. Individual specimens identified by their extract number (see Table 1), and clades (1–10) discussed in text indicated in parentheses.

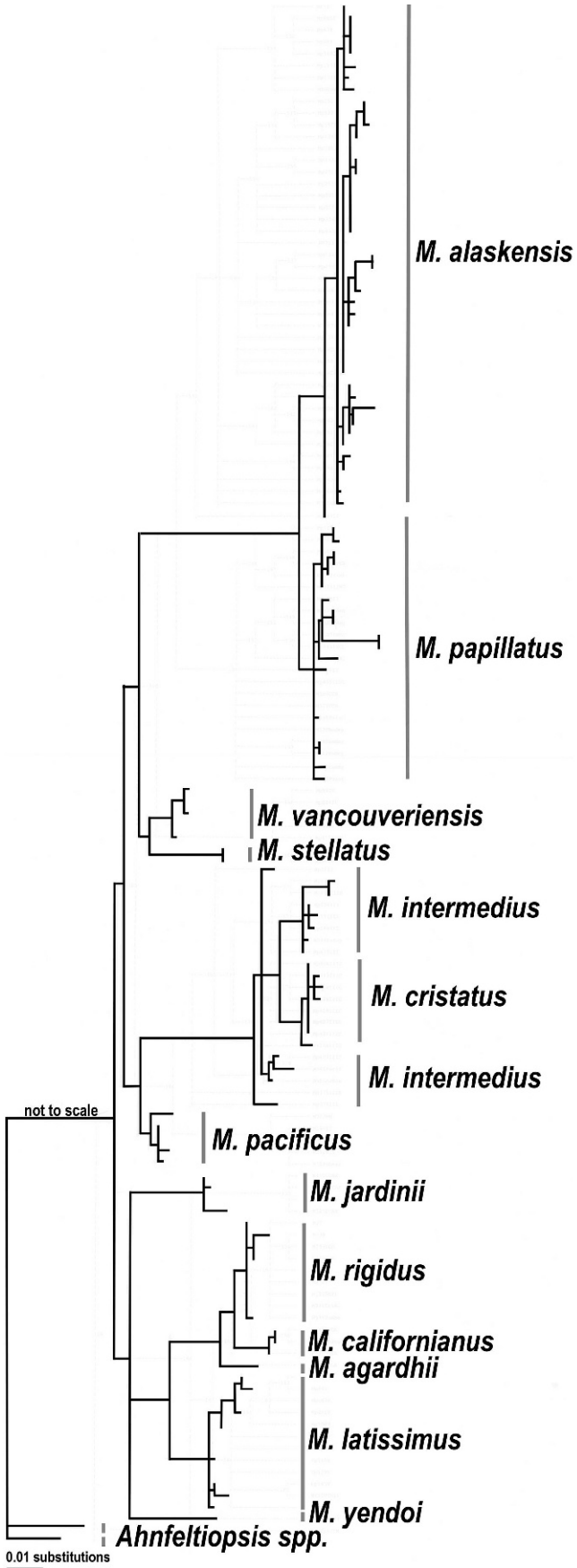
more exposed shores. Species descriptions are based on our collections.

Mastocarpus papillatus (C. Agardh) Kützing 1843: 398

Gametophytic thallus upright, crisp, up to 9 cm tall. Stipe terete, unbranched, 1–3 mm in length, expanding slightly into a flattened apophysis 6–15 mm long and 2–18 mm wide, then into blades 3–10 mm wide and 300–500 μm thick. Thallus uniformly reddish black. Margin of apophysis slightly swollen, giving thallus its canaliculate character; margin of blade slightly wavy, rarely proliferous. Branching highly variable, generally subdichotomous, to three orders, occasionally unbranched. Apices rounded, crenate, or irregular. In section, cortex of 6–8 cell layers, occupying about half the width of the section. Male thalli smooth, without papillae. Female thalli with simple papillae, initially nipple-like, becoming subspherical at maturity; papillae restricted to blade surface (not along margins); larger papillae occurring on older (lower) parts of the thallus in discrete patches (Figs 24, 25). Distinguished from other *Mastocarpus* species by molecular sequence differences.

BASIONYM: *Sphaerococcus papillatus* C. Agardh 1821: pl. 19.

HOMOTYPIC SYNONYM: *Gigartina papillata* (C. Agardh) J. Agardh 1846: pl. 19.



HETEROTYPC SYNONYMS: *Petrocelis franciscana* Setchell & N.L. Gardner in N.L. Gardner 1917: 391, pl. 33, fig. 1; *Gigartina obovata* J. Agardh 1899: 25.

TYPE: Golden Gate, San Francisco, California, *Leg*. Chamisso, 1816, LD! [Herbarium Agardh (Hb. Ag.) 23883] (http://botany.ubc.ca/sandral/Sphae-pap.jpg). We select the specimen in the upper right as lectotype.

ITS sequence of Hb. Ag. 23883: Clade 2 (Lindstrom 2008a). This 220 bp fragment at the 5′ end of the nuclear small subunit rDNA (nSSU) ITS1 region of the type specimen differed from the 41 other sequenced specimens in this clade by 0–6 nucleotides and 0–12 indels. The type specimen was identical to a 120 bp fragment from Agate Beach, California, 13 January 1973 (UC 1471472), to a 218 bp fragment from the holotype of *Petrocelis franciscana* (UC 188973, Fort Point, San Francisco, California) and to a 203 bp fragment from the type of *Gigartina obovata* (Hb. Ag. 23840, Golden Gate, California, *Leg*. Berggren, see Setchell & Gardner 1933: 288, pl. 62). We designate the upper specimen, which is larger, as lectotype of *Gigartina obovata*.

Assignment of the type material of *Mastocarpus papillatus* to Clade 2 solves a long-standing uncertainty as to the provenance of this collection. The herbarium sheet bears the note: '*e mari Atlantico Chamisso*'. In his description, C. Agardh (1821) wrote: '*Ad insulam O-wai-hee* [Hawaii] *Chamisso, in cujus collectione specimina vidi*'. Setchell & Gardner (1933) concluded that the type locality was in error, stating 'both specimens and plate agree perfectly with specimens from the Pacific coast of North America'. It had even been surmised that the specimen might have come from Unalaska, Alaska, one of the stops on Chamisso's voyage (M.J. Wynne, personal communications).

The occurrence of this species on the Monterey Peninsula, California, is debatable. We provisionally include high intertidal specimens of *Mastocarpus*, represented by Clade 9 (this study), in *M. papillatus*. Clade 9 is thus far known only from the Monterey Peninsula. This clade is separated from other specimens of *M. papillatus sensu stricto* (Clade 2) on a branch with high bootstrap support in separate analyses of the individual genes as well as the concatenated data (Fig. 1). However, its relationship with Clade 2 is paraphyletic in these analyses. To our knowledge, there are no previously existing names for Clade 9. Further study of *M. papillatus* in this region is required to understand how many species of this high intertidal entity should be recognized. Verified distribution: Cambria, San Luis Obispo County, California, to Cape Palmerston, northern Vancouver Island, British Columbia (Fig. 42).

←

Fig. 2. Maximum likelihood tree (−ln L = 3499.2610) of all unique *Mastocarpus* ITS sequences. The size of the datafile precluded bootstrap analyses.

Mastocarpus jardinii (J. Agardh) J.A. West in Guiry, West, Kim & Masuda 1984: 57

Gametophytic thallus upright, crisp, up to 9 cm tall. Stipe terete, unbranched, 1–3 mm in length, expanding slightly into a flattened apophysis 8–10 mm long and 2–5 mm wide, then into blades 4–10 mm wide and 250 μm thick. Thallus uniformly dark reddish black. Margin of apophysis and blade slightly swollen, giving thallus its canaliculate character; margin of blade straight, not wavy. Branching divaricate, up to three orders. Apices tapered and crenate or irregular. In section, cortex of 6–8 cell layers, occupying about half the width of the section. Papillae simple or becoming branched, sessile or cylindrical in shape, centrally or terminally enlarged, occurring on surfaces of blades as well as along margins; some papillae becoming bladelike (Figs 40, 41). Distinguished from other *Mastocarpus* species by molecular sequence differences.

BASIONYM: *Gigartina jardinii* J. Agardh 1872: 9.

TYPE: Monterey, California, *Leg*. E. Jardin, 1855 LD! (Hb. Ag. 23800).

ITS sequence of LD Hb. Ag. 23800 (type specimen): Clade 10 (this study). The 193 bp fragment at the 5′ end of the nSSU ITS1 region of the type specimen was identical to three sequences from Hopkins Marine Station, Pacific Grove, Monterey Peninsula, California: M409, 411 and 413. It differed from another Pacific Grove specimen, M405, by a single indel.

Setchell & Gardner (1933: 291, pl. 65) indicated that Hb. Ag. 23800 was the specimen that J. Agardh used to describe this species. This makes sense because it was collected by Jardin, after whom it was named. Thus, we consider this material to be the holotype rather than a lectotype. Thus far, this species is known only from Moss Beach in San Mateo County and the Monterey Peninsula, California (Fig. 42). These results clearly indicate that the name *Mastocarpus jardinii* represents a different entity from the narrower thalli that are characteristic of *Gigartina agardhii* and other species (see below). *Mastocarpus jardinii* occupies the low intertidal (0 to −0.25 m level) and has blades that are intermediate in width between *Mastocarpus papillatus* and *Mastocarpus agardhii*.

A second specimen (LD Hb. Ag. 23801), mounted on the same composite sheet as the type LD Hb. Ag. 23800 and thus representing potential syntype material of *Gigartina jardinii,* was also analyzed. Its ITS sequence placed it into Clade 6B (see below). It was identical to sequence M475 from Cape Mendocino, California. To our knowledge LD Hb. Ag. 23801 has not been previously assessed and is not associated with a specific epithet. It should not be considered part of the type of *Mastocarpus jardinii*.

Mastocarpus pacificus (Kjellman) Perestenko 1980: 72

Gametophytic thallus upright, crisp or flaccid, up to 7 cm tall. Stipe terete, unbranched, 2–8 mm in length, expanding slightly into an apophysis 7–12 mm long and 2 mm wide, then into blades 1–15 mm wide and up to 400 μm thick. Thallus uniformly reddish black, fading to dusky rose. Margin of apophysis not swollen, thallus not canaliculate. Branching subdichotomous to flabellate, to three orders. Apices crenate, rounded or acuminate. In section, cortex of 9–10 cell layers, occupying more than half the width of the section; cuboidal cells like those of inner cortex extending across most of the width of the medulla, with narrow filaments intertwined among them. Male thalli smooth, without papillae. Female thalli with papillae on both surface and margins (*pacificus* form) or restricted to margins, including terminal segments (*ochotensis* form). Distinguished from other *Mastocarpus* species by molecular sequence differences.

BASIONYM: *Gigartina pacifica* Kjellman 1889: 31.

TYPE: Bering Island, Commander Islands, Russia, *Leg.* F.R. Kjellman, 14–19 August 1879, Vega Expedition, UPS! S! (http://botany.ubc.ca/sandral/Gigar-pac.jpg).

HETEROTYPIC SYNONYM: *Chondrus mamillosus* var. *unalaschcensis* Ruprecht 1850: 126 (type locality: Unalaska, Alaska, *Leg.* Wosnessenski) = *Gigartina unalaschcensis* (Ruprecht) J. Agardh 1899: 11 (the latter name was first used by Kjellman 1889: 31, but he neither indicated that it was a new combination nor cited the basionym) = *Mastocarpus unalaschcensis* (Ruprecht) Makienko in Klochkova 1996: 191 (http://botany.ubc.ca/sandral/Chond-mam-una.jpg).

ITS sequences of type specimens in S: *Mazzaella* sp. and Clade 7 (Lindstrom 2008a). These sequences come from the upper right and upper left specimens of the S syntype collection, respectively (http://botany.ubc.ca/sandral/Gigar-pac.jpg). (UPS, where additional type material is housed, would not provide type material for sequencing.) The 225 bp fragment at the 5′ end of the nSSU ITS1 region from the *Mastocarpus* specimen differed by up to 2 indels from the two specimens in this clade we have sequenced from northern Japan. It was identical to all specimens from Russia and Alaska and to syntype material of *Chondrus mamillosus* var. *unalaschcensis* in S. We designate the upper left specimen of the S syntype as lectotype of *Gigartina pacifica*, and we designate the central specimen of the S syntype (http://botany.ubc.ca/sandral/Chond-mam-una.jpg) as lectotype of *Chondrus mamillosus* var. *unalaschcensis* since these are the specimens we obtained sequences from.

The type material of *Mastocarpus pacificus* has channelled, spreading, dichotomous, papillate blades and looks like a typical species of *Mastocarpus,* whereas all Clade 7 specimens that we sequenced were flat rather than canaliculate and lacked papillae. Ohno *et al.* (1982) previously noted that broad thalli with both surface and marginal papillae dominated in summer and narrow thalli with only marginal papillae dominated in winter. Because of this alternation in forms, they dubbed this species '*Gigartina pacifica-ochotensis*'.

Kjellman (1889) reported this species, from Bering Island, Commander Islands, Russia, to be quite abundant and gregarious on upper subtidal rock. Based on our collections, this species occurs intertidally, not subtidally. We have collected it on both emergent rock and in tidepools. Most if not all of the sites where we collected our smooth, linear specimens experience some shore ice during the winter. Ours were all summer collections, however, suggesting that this species does not alternate forms between summer and winter, as it does in Japan

(Ohno *et al.* 1982). Verified distribution: Cook Inlet and Kodiak Island, Alaska, west to northern Japan (Fig. 42).

Mastocarpus latissimus* (Harvey) *comb. nov.

Gametophytic thallus upright, crisp, up to 40 cm tall but usually smaller. Stipe terete, unbranched, 4–12 mm in length, expanding slightly into an apophysis 20 mm long, then into blades up to 40 mm wide and 250 μm thick. Thallus uniformly red to reddish black. Margin of apophysis slightly swollen, giving thallus its canaliculate character; margin of blade slightly wavy. Branching dichotomous, subdichotomous, or arising as proliferations from the apophysis, with proliferations becoming subdichotomous. Apices rounded or crenate. In section, cortex of 6–7 cell layers, occupying about half the width of the section. Male thalli with few, small, nipple-like papillae. Female thalli with mostly simple papillae, initially nipple-like, becoming spherical with cystocarps growing out laterally. Some papillae proliferous and bladelike, occurring on surface and along margins of thallus (Figs 32, 33). Distinguished from other *Mastocarpus* species by molecular sequence differences.

BASIONYM: *Gigartina mamillosa* var. *latissima* Harvey 1862: 172 [=*G. latissima* (Harvey) Eaton in J. Agardh 1899: 32].

LECTOTYPE: Hb. Ag. 23855 (LD), Victoria Harbour (Esquimalt), Vancouver Island, British Columbia, February 1860, *Leg.* Lyall (left-hand specimen lectotypified by Setchell & Gardner 1933: 285, pl. 60).

HETEROTYPIC SYNONYM: *Gigartina dichotoma* N.L. Gardner 1927: 333, pl. 59 (type locality: Duxbury Reef, Marin County, California, *Leg.* N.L. Gardner 6212, UC 296705).

ITS sequence of lectotype of *G. latissima*: Clade 5 (Lindstrom 2008a). The 221 bp fragment at the 5′ end of the nSSU ITS region of this specimen was identical to 12 sequences from Alaska and northern California. The 221 bp fragment of the right-hand specimen of Hb. Ag. 23855 was identical to most of the remaining specimens from Alaska and British Columbia except for a single unique transition.

ITS sequence of the holotype of *G. dichotoma* also belonged to Clade 5. The 223 bp fragment at the 5′ end of the nSSU ITS1 region of the type specimen was identical to two specimens (from La Desembocadora de Bío Bío, near Concepción, Chile, *Leg.* Paul Gabrielson). These sequences differed from other Clade 5 sequences by a 1 bp indel. Although the Chilean sequences differed from other Clade 5 sequences at up to five other nucleotide positions, either indels or base pair substitutions, across the 728 bp ITS region, these differences were uncommon among the 63 sequences compared. Habitat: mid to low intertidal on bedrock and boulders. Verified distribution: Moss Beach, San Mateo County, California, north to Attu Island, Aleutian Islands, Alaska; Chile (Fig. 42).

Mastocarpus agardhii* (Setchell & N.L. Gardner) *comb. nov.

Gametophytic thallus upright, cartilaginous, up to 15 cm tall. Stipe and apophysis terete, branched or unbranched, 25–55 mm in length, constituting a third to half the height of the thallus, expanding into narrow blades of uniform width (~3 mm) and 450–500 μm thick. Thallus uniformly dark reddish black. Margin of apophysis and blade slightly swollen, giving thallus its canaliculate character. Branching more or less dichotomous or divaricate, up to six orders. Apices narrowly rounded on female thalli to slightly crenate on male thalli. In section, cortex of 10–11 cell layers, occupying about half the width of the section. Papillae vermiform (Fig. 39), on blade surface and submarginal. Cystocarpic papillae with long stalks (pedicellate), each with a swollen subterminal cystocarp (Fig. 38). Distinguished from other *Mastocarpus* species by molecular sequence differences.

BASIONYM: *Gigartina agardhii* Setchell & N.L. Gardner 1933: 290.

HOLOTYPE: Pyramid Point, Monterey County, California, *Leg.* W.A. Setchell, 1666, 18 May 1897, UC 93733. Isotypes distributed as Phycotheca Boreali-Americana (PB-A) 427 (Setchell & Gardner 1933: 290, as 'cotypes').

ITS sequence of UC 93733 (holotype): Clade 6C (Lindstrom 2008a; this study). The 218 bp fragment at the 5′ end of the nSSU ITS1 region from the type specimen is identical to extracts M78, 381, 503 and 513 from Vancouver Island, British Columbia, and M436, 438 and 439 from the Monterey Peninsula, California.

Mastocarpus agardhii (Clade 6C) is one of three species formerly and erroneously referred to as *M. jardinii*. In contrast to *M. jardinii*, which occurs in the low intertidal, *Mastocarpus agardhii* grows on the rocks in the high to mid intertidal 'between the 3.5- and 1.0-foot tide levels' on the Monterey Peninsula (Smith 1944: 284) and in the mid intertidal elsewhere.

This species was first described as *Gigartina papillata* f. *dissecta* Setchell on the label to PB-A 427. Later Setchell & Gardner (1933) elevated the taxon to specific rank, adopting the epithet *agardhii*. Setchell & Gardner noted that the species was incorrectly identified by J. Agardh (1899: 24) as *Gigartina batracopus* [*batrachopus*] Bory (1828: 153, pl. 19, fig. 2), a species now considered to be referable to *Laurencia* (www.algaebase.org, visited 15 January 2010). Rather than illustrating their type specimen, however, they provided photos of Agardh's material, identifying it as *Gigartina agardhii* but with the misleading label, 'Type sheet of *G. batrachopus*'. *Mastocarpus agardhii* was included as a synonym of *Mastocarpus jardinii* by Guiry *et al.* (1984). The present work clearly indicates that the two species are distinct.

The specimens identified as *Mastocarpus agardhii* from Vancouver Island, based on nSSU ITS sequences, show either evidence of hybridization with other, closely related species or evidence of chimeric thalli due to coalescence (Santelices *et al.* 1999). M78 has the ITS sequence of *Mastocarpus agardhii*, a *rbc*L sequence allying it to Clade 6B (*Mastocarpus californianus*; see below) and a COI sequence allying it to Clade 5 (*Mastocarpus latissimus*). In contrast, M381, which also has the ITS sequence of *Mastocarpus agardhii*, has the COI sequence of Clade 6A (*Mastocarpus rigidus*; see below) and the *rbc*L sequence of Clade 6B. Compared with Clade 6A, Clade 6C (*Mastocarpus agardhii*) occurs slightly higher in the intertidal zone where the two species co-occur on Vancouver Island.

Verified distribution: Monterey Peninsula, California; north and west Vancouver Island, British Columbia (Fig. 42).

Mastocarpus cristatus (Setchell) *comb. nov.*

Gametophytic thallus upright, crisp, flabellate, up to 8 cm tall, terete at base, stipe unbranched, 3–5 mm in length, expanding slightly into an apophysis 5–15 mm long, then into blades mostly less than 8 mm wide and 250–300 μm thick (but often appearing thicker due to dense covering of papillae). Thallus uniformly reddish black. Margin of apophysis swollen, giving it a distinctive canaliculate appearance. Branches subdichotomous, to five orders, with crenate apices. In section, cortex of 6–8 cell layers, occupying at least half the width of the section. Papillae occurring on both male and female thalli and on both surfaces and margins of blades, including near the base of the thallus, often very dense (Figs 30, 31). Female papillae compound, uniform in size and shape along the length of the thallus. Male papillae initially elongate and acute, becoming proliferous and elliptical in shape. Distinguished from other *Mastocarpus* species by molecular sequence differences.

BASIONYM: *Gigartina papillata* f. *cristata* Setchell in Collins, Holden & Setchell 1898: 426.

HOMOTYPIC SYNONYMS: *Gigartina mamillosa* f. *cristata* Setchell in Setchell & Gardner 1903: 301; *Gigartina cristata* (Setchell) Setchell & N.L. Gardner 1933: 289.

HOLOTYPE: Pyramid Point, Monterey County, California, *Leg.* W.A. Setchell, 1665, 18 May 1897, UC 93707. Isotypes distributed as PB-A No. 426.

ITS sequence of UC 93707 (holotype): Clade 3C (Lindstrom 2008a; this study). The 227 bp fragment at the 5′ end of the nSSU ITS1 region from this type specimen differed by 1 indel from some specimens of this species and by the indel and a single additional nucleotide difference from other specimens. All specimens were collected from the Monterey Peninsula, the only area where this species is currently known to occur (Fig. 42). Specimens grew on bedrock from −0.25 m to +1.5 m, with the majority coming from +1.0 m (mid intertidal).

New species of Northeast Pacific *Mastocarpus*

CLADE 1: Known to occur from Trinidad, Humboldt County, California, to Attu Island, Alaska, and possibly further west, this may be the most abundant species of *Mastocarpus* based on its broad geographic range and local dominance in the mid to high intertidal. A potentially available name for this species is *Gigartina sitchensis*. However, since we have been unable to obtain type material of *Gigartina sitchensis* from LE, we propose the name *Mastocarpus alaskensis* for this taxon because it is the common high intertidal species in Alaska.

Mastocarpus alaskensis sp. nov.

Figs 3–6, 22, 23

Thallus gametophyticus erectus crispus usque ad 15 (ad 21) cm altus (Fig. 3). Stipes teres eramosus 1–3 mm longus, leviter expansus in apophysim 6–14 mm longam 4–10 mm latam, deinde in laminas 4–35 mm latas usque ad 350 μm crassas. Thallus rubroniger brunneoruber vel rubrobrunneus, decoloratus fusce roseus. Apophysis margine leviter tumido, thallum canaliculatum faciens; lamina margine leviter undulato raro prolifero. Ramificatio subdichotoma vel flabellata, ad quatuor ordines, thallus interdum eramosus. Apices crenati dissecti vel irregulariter lobati. Cortex in sectione transversali e stratis cellularum sex ad octo (ad 12) constare visus, circiter dimidiam partem sectionis occupans (Figs 4, 5). Thalli masculi laeves sine papillis (Fig. 6). Thalli foemini papillis simplicibus, primum barbatuliformibus dein cylindricis postremo subsphaericis, ad superficiem laminae (non ad margines) restrictis (Figs 22, 23); papillis majoribus in partibus thalli senioribus (inferioribus). Ab aliis speciebus Mastocarpi ordinibus nucleotidorum distinguendus.

Gametophytic thallus upright, crisp, up to 15 (to 21) cm tall (Fig. 3). Stipe terete, unbranched, 1–3 mm in length, expanding slightly into an apophysis 6–14 mm long and 4–10 mm wide, then into blades 4–35 mm wide and up to 350 μm thick. Thallus uniformly reddish black, brownish red, reddish brown, fading to dusky rose. Margin of apophysis slightly swollen, giving thallus its canaliculate character; margin of blade slightly wavy, rarely proliferous. Branching subdichotomous to flabellate, to four orders, occasionally unbranched. Apices crenate, dissected, or irregularly lobed. In section, cortex of six to eight (to 12) cell layers, occupying about half the width of the section (Figs 4, 5). Male thalli smooth, without papillae (Fig. 6). Female thalli with simple papillae, stubble-like at first, becoming cylindrical and then subspherical; papillae restricted to blade surface (not along margins; Figs 22, 23); larger papillae occurring on older (lower) parts of the thallus. Distinguished from other *Mastocarpus* species by molecular sequence differences.

HOLOTYPE: Left specimen (Fig. 3), UBC A88537, high intertidal bedrock, north of Deep Bay, Atka Island, Aleutian Islands, Alaska, 15 July 2007, *Leg*. Mandy R. Lindeberg (ALEUT07_387). DNA extract M392.

HABITAT: Occurring on rock, usually in the mid to high intertidal zone. Where they co-occur, this species can be slightly lower than *M. papillatus*.

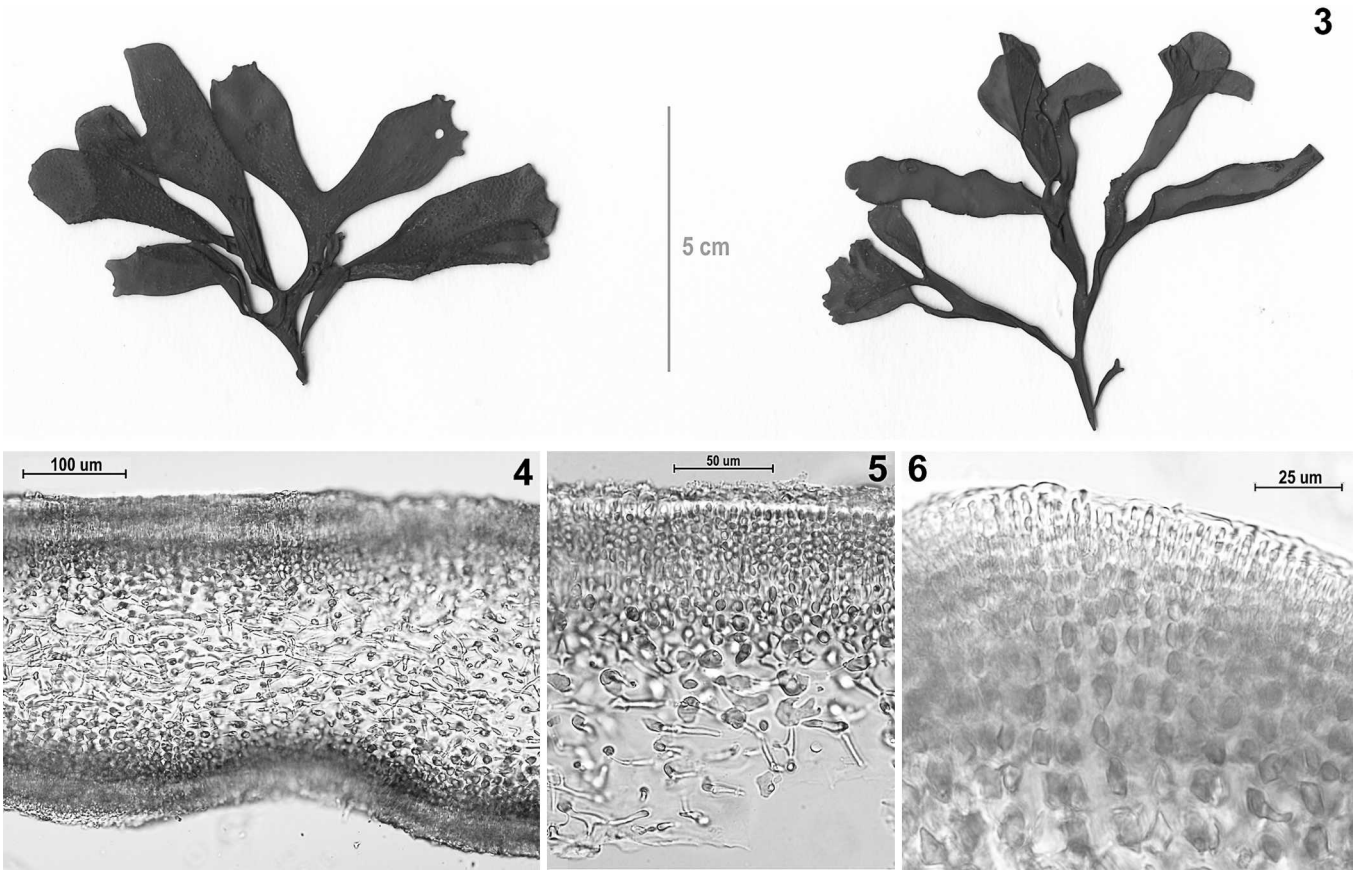
VERIFIED DISTRIBUTION: Trinidad, Humboldt County, California, north to Attu Island, Aleutian Islands, Alaska (Fig. 42).

CLADE 3: Is divided into two distinct, well supported clades (Fig. 1). As noted above, one of these is assignable to *Mastocarpus cristatus*, a species thus far known only from the Monterey Peninsula. We propose the name *Mastocarpus intermedius* for the other clade because it shares many characters with other species in the genus.

Mastocarpus intermedius sp. nov.

Figs 7–10, 28, 29

Thallus gametophyticus erectus crispus usque ad 9 cm altus (Fig. 7). Stipes teres eramosus 1–8 mm longus sensim expansus in apophysim 10–20 mm longam, deinde in laminas plerumque ad 20 mm latas 175–350 μm crassas. Thallus uniformiter rubroniger. Apophysis margine tumido, thallum



Figs 3–6. *Mastocarpus alaskensis* type material.
Fig. 3. Holotype (left) and isotype of *Mastocarpus alaskensis* (UBC A88537).
Fig. 4. Cross section of blade.
Fig. 5. Detail of cortex (and medulla) in cross section.
Fig. 6. Spermatangia in blade cross section.

distincte canaliculatum faciens. Ramificatio subdichotoma flabellata vel prolifera, usque ad quatuor ordines, vel thallus eramosus. Apices rotundati vel irregulares. Cortex in sectione transversali e stratis cellularum 8–15 constare visus, plus quam dimidiam partem sectionis occupans (Figs 8-10). Papillae in thallis maturis masculis foeminisque et in utraque superficie laminae, et prope basin frondis (Figs 7, 28, 29); papillae foeminae primum hemisphaericae, dein compositae interdum vermiformes (convolutissimae); papillae masculae ligulatae, dein magnificantes proliferantes. Ab aliis speciebus Mastocarpi ordinibus nucleotidorum distinguendus.

Gametophytic thallus upright, crisp, up to 9 cm tall (Fig. 7). Stipe terete, unbranched, 1–8 mm in length, expanding gradually into an apophysis 10–20 mm long, then into blades mostly to 20 mm wide and 175–350 μm thick. Thallus uniformly reddish black. Margin of apophysis swollen, giving it a distinctive canaliculate appearance. Branching subdichotomous, flabellate or proliferous, up to four orders, or unbranched. Apices rounded to irregular in shape. In section, cortex 8–15 layers, occupying more than half the section (Figs 8-10). Papillae occurring on both mature male and female thalli and on both surfaces and margins of blades, including near the base of the frond (Figs 7, 28, 29). Female papillae initially hemispheric, becoming compound and sometimes vermiform (very convoluted). Male papillae ligulate, becoming large and proliferous. Distinguished from other *Mastocarpus* species by molecular sequence differences.

HOLOTYPE: Leftmost specimen (Fig. 7), UBC A88558, epilithic, near coarse sand at base of boulders, +0.5 m above mean lower low water (MLLW), Hopkins Marine Station, Pacific Grove, Monterey County, California, 7 January 2008, *Leg*. Patrick T. Martone (Z). DNA extract M407.

HABITAT: Occurring on rock usually in the mid to low intertidal zone.

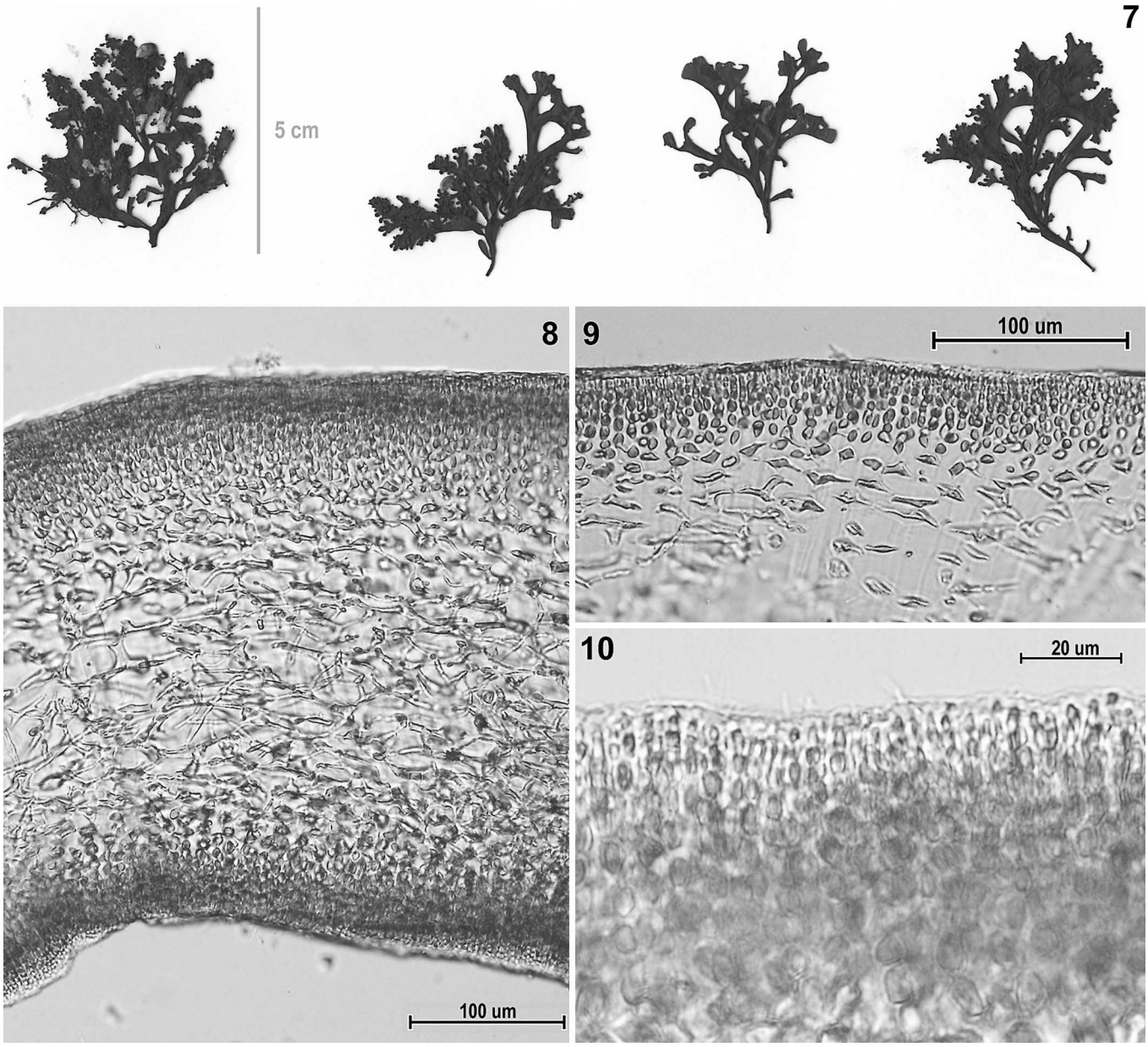
VERIFIED DISTRIBUTION: Ensenada, Baja California, Mexico, north to northern British Columbia; Cape Kaguyak, Kodiak Island, Alaska (Fig. 42).

CLADE 4: Is clearly distinguished in all analyses (Figs 1, 2). We propose the name *Mastocarpus vancouveriensis* for this species because its distribution is centred on Vancouver Island.

Mastocarpus vancouveriensis sp. nov.

Figs 11–14, 26, 27

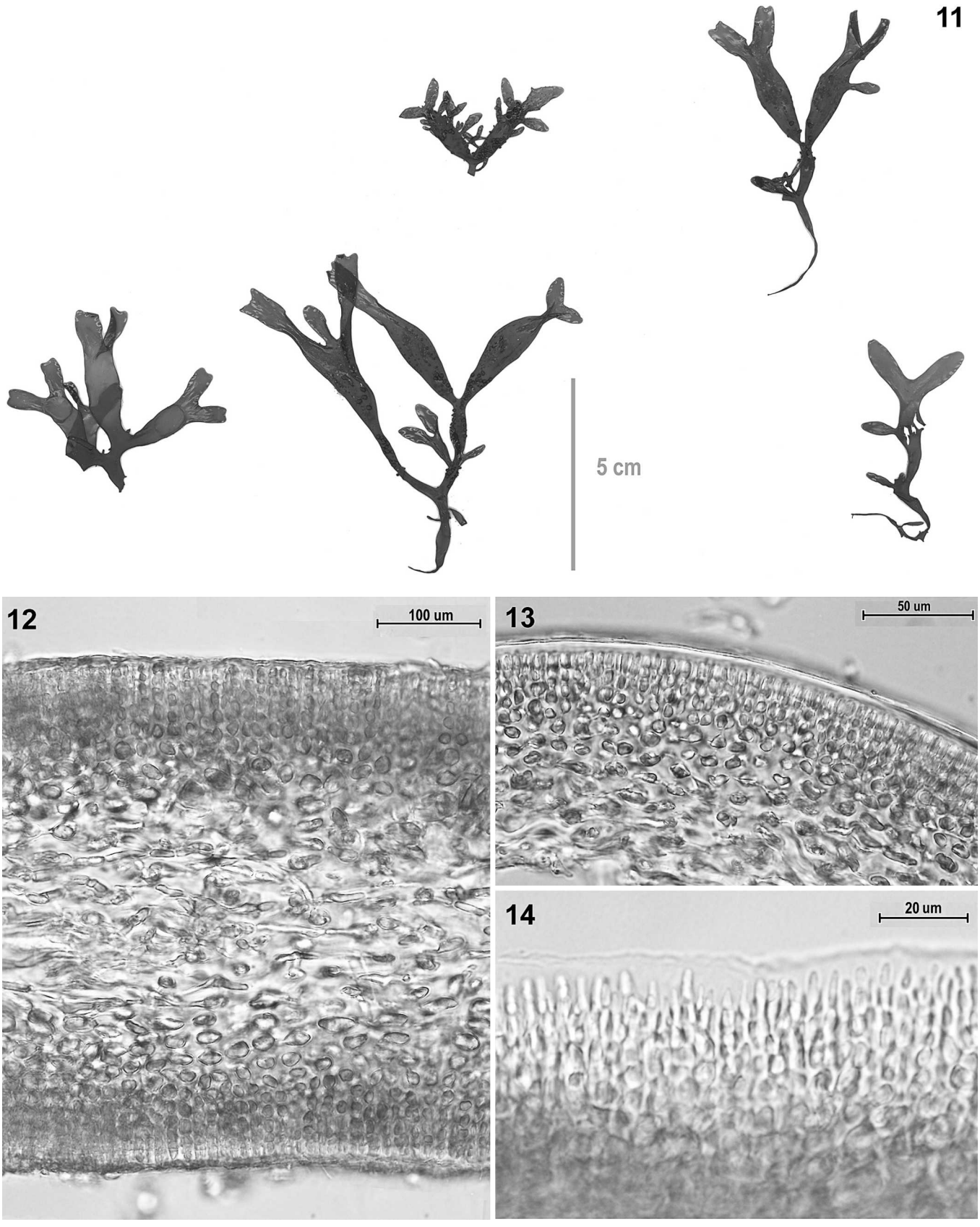
Thallus gametophyticus erectus aliquot flaccidus usque ad 8 cm altus, basi teres. Stipes eramosus 3–5 mm longus leviter



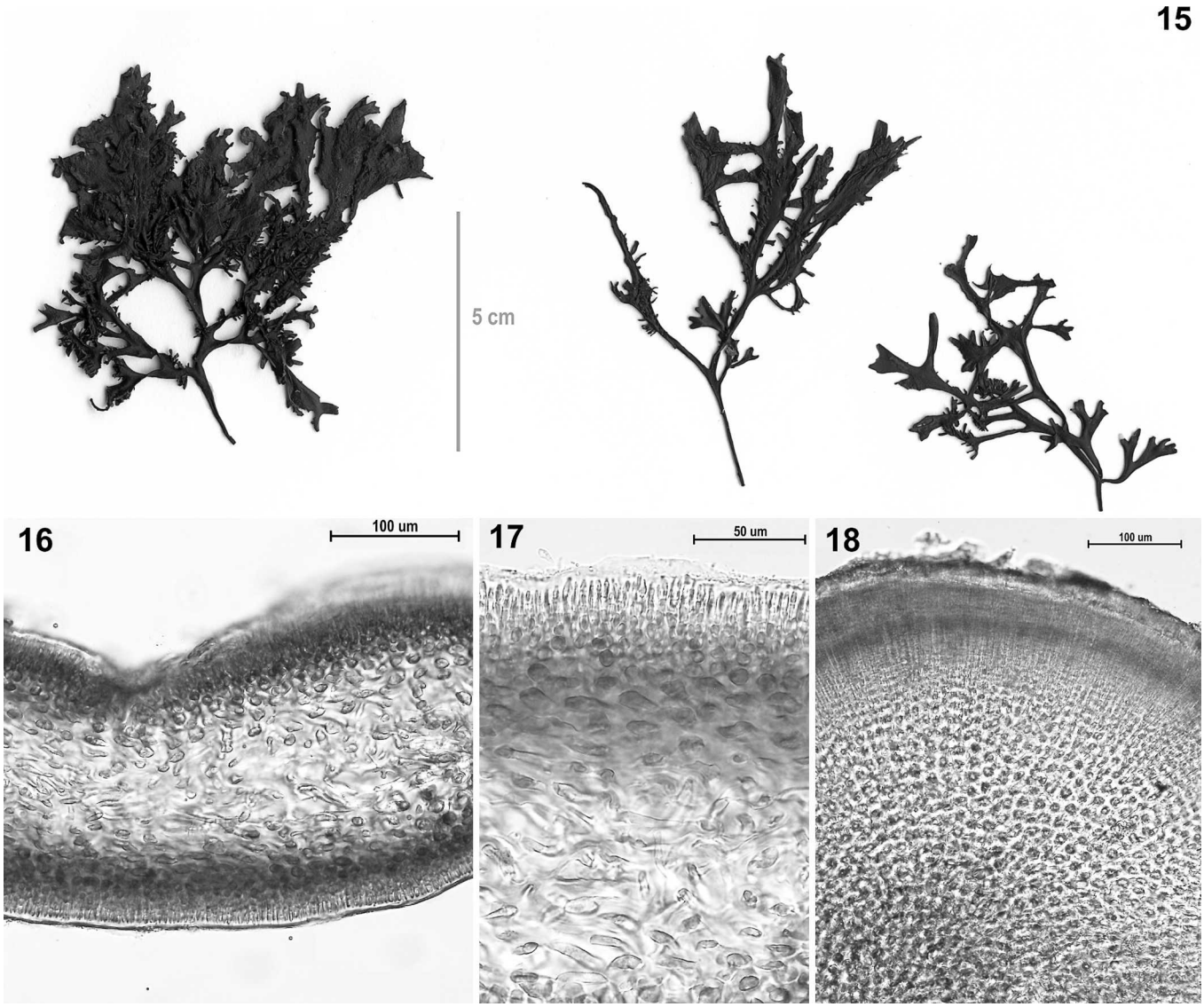
Figs 7–10. *Mastocarpus intermedius* type material.
Fig. 7. Holotype (left) and isotypes of *Mastocarpus intermedius* (UBC A88558).
Fig. 8. Cross section of blade.
Fig. 9. Detail of cortex (and medulla) in cross section.
Fig. 10. Spermatangia in blade cross section.

expansus in apophysim 10–15 mm longam 2–3 mm latam (Fig. 11). Thallus fusce ruber vel rubroniger. Apophysis margine leviter tumido, thallum canaliculatum faciens. Ramificatio prolifera ex apice apophysis, interdum subdichotoma, ad tres ordines. Laminae proliferae ad 1.5 cm latae 3 cm longae, primum orbiculares vel ellipticae, dein cordatae, stipite angustissimo affixae. Apices rotundati. Cortex in sectione transversali e stratis cellularum sex ad octo constare visus, plus quam dimidiam partem latitudinis sectionis (200 μm) vix occupans (Figs 12, 13). Spermatangia in superficie laminarum (Fig. 14). Thalli foeminae papillis plerumque simplicibus, subsphaericis, interdum ramis duo globularis instructis, sparsis, ad partes seniores thalli restrictis (Figs 26, 27). Ab aliis speciebus Mastocarpi ordinibus nucleotidorum distinguendus.

Gametophytic thallus upright, somewhat flaccid, up to 8 cm tall, terete at base, stipe unbranched, 3–5 mm in length, expanding slightly into an apophysis 10–15 mm long and 2–3 mm wide (Fig. 11). Thallus dark red to reddish black. Margin of apophysis slightly swollen, giving thallus its canaliculate character. Branching proliferous from apex of apophysis, occasionally subdichotomous, to three orders. Proliferous blades to 1.5 cm wide and 3 cm long, initially orbicular to elliptical, becoming cordate, attached by a very narrow stipe. Apices rounded. In section, cortex of six to eight cell layers, occupying slightly more than half the



Figs 11–14. *Mastocarpus vancouveriensis* type material.
Fig. 11. Holotype (lower centre) and isotypes of *Mastocarpus vancouveriensis* (UBC A85326).
Fig. 12. Cross section of blade.
Fig. 13. Detail of cortex (and medulla) in cross section.
Fig. 14. Spermatangia in blade cross section.



Figs 15–18. *Mastocarpus rigidus* type material.
Fig. 15. Holotype (left) and isotypes of *Mastocarpus rigidus* (UBC A88570).
Fig. 16. Cross section of blade.
Fig. 17. Spermatangia in blade cross section.
Fig. 18. Cross section of lower stipe.

200 μm width of the section (Figs 12, 13). Spermatangia on blade surface (Fig. 14). Female thalli with mostly simple papillae, subspherical, occasionally with two globular-shaped branches; sparse, restricted to older parts of thallus (Figs 26, 27). Distinguished from other *Mastocarpus* species by molecular sequence differences.

HOLOTYPE: Bottom centre specimen (Fig. 11), UBC A85326, high intertidal bedrock in sand, Brady's Beach, Barkley Sound, British Columbia, Canada, 17 April 2003, *Leg.* S.C. Lindstrom (SCL 10719). DNA extract M91.

HABITAT: Occurring on rock usually in the mid to high intertidal zone often surrounded by sand.

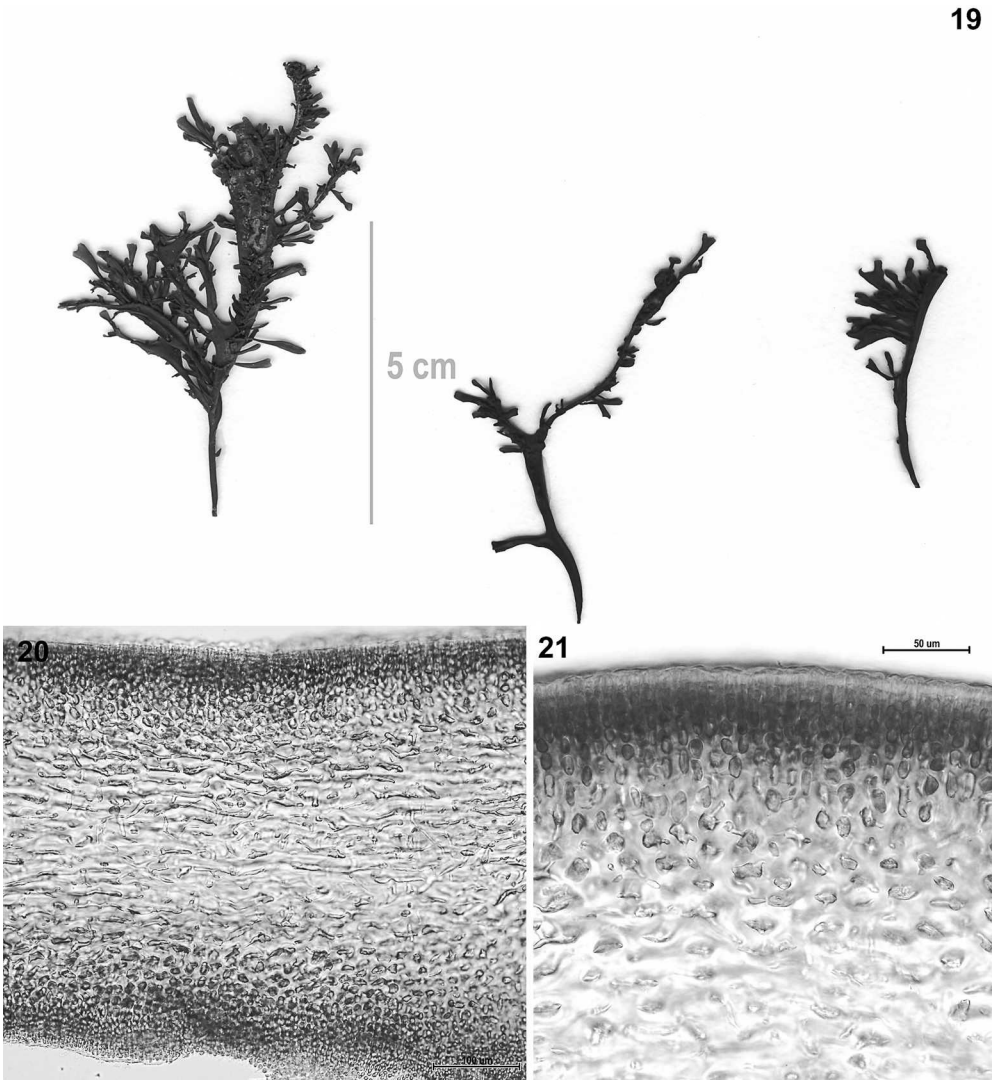
VERIFIED DISTRIBUTION: Cape Mendocino, California, north to Sitka, Alaska (Fig. 42).

CLADE 6A AND 6B: Share a habit of clustered, narrow, cartilaginous thalli with Clade 6C (*Mastocarpus agardhii*). These two species are more closely related to each other than either is to *Mastocarpus agardhii* as noted above. However, Clades 6A and 6B are sufficiently distinct to warrant recognition as separate species (Figs 1, 2), and we propose the names *Mastocarpus rigidus* for Clade 6A because of the upright, rigid character of this species and *Mastocarpus californianus* for Clade 6B because it is currently known only from northern California.

Mastocarpus rigidus sp. nov.

Figs 15–18, 34, 35

Thallus gametophyticus erectus cartilagineus usque ad 9 cm altus (Fig. 15). Stipes apophysisque teres (Fig. 18) eramosa

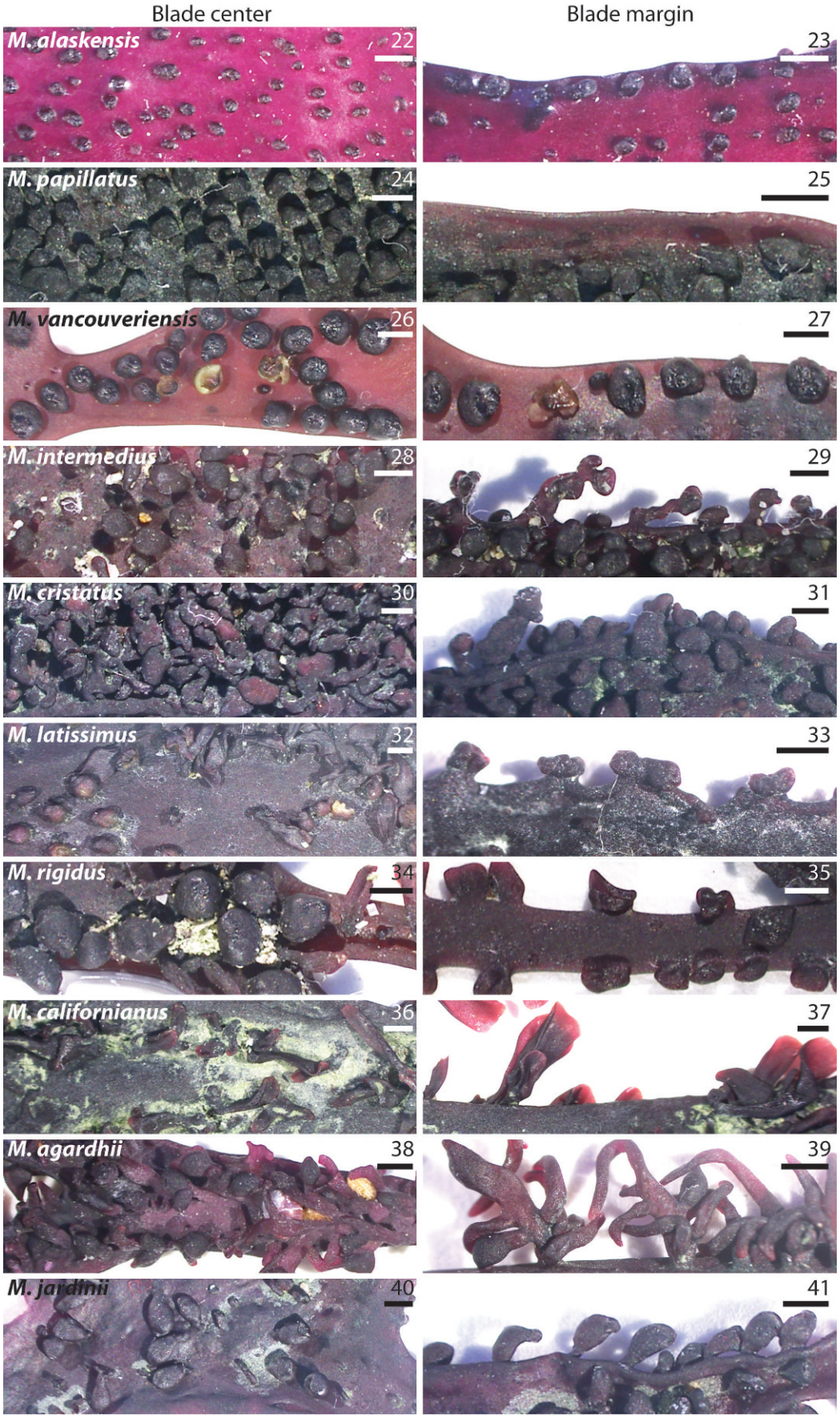


Figs 19–21. *Mastocarpus californianus* type material.
Fig. 19. Holotype (left) and isotypes of *Mastocarpus californianus* (UBC A88483).
Fig. 20. Cross section of blade.
Fig. 21. Detail of cortex (and medulla) in cross section.

20–35 mm longa, partem tertiam vel dimidiam altitudinis thalli attingens, expansa in laminas 5 mm latas in thallis foeminis, 12 mm latas in thallis masculis, 275 μm crassas. Thallus unicolor: rubroniger. Apophysis margine leviter tumido, thallum canaliculatum faciens. Ramificatio plus minusve dichotoma, usque ad quatuor ordines. Apices anguste rotundati vel crenato-fimbriati. Cortex in sectione transversali e stratis cellularum septem ad novem constare visus, dimidiam partem latitudinis sectionis occupans (Fig. 16). Papillae simplices in superficie marginibusque, hemisphaericae vel irregulares sed interdum prolificantes (laminiformes; Figs 34, 35), papillis senioribus majoribus basalibus, junioribus minoribus distalibus. Spermatangia et in papillis et in superficie laminae (Fig. 17). Ab aliis speciebus Mastocarpi ordinibus nucleotidorum distinguendus.

Gametophytic thallus upright, cartilaginous, up to 9 cm tall (Fig. 15). Stipe and apophysis terete (Fig. 18), unbranched, 20–35 mm in length, constituting a third to half the height of the thallus, expanding into blades 5 mm wide on female thalli, 12 mm wide on male thalli and 275 μm thick. Thallus uniformly reddish black. Margin of apophysis slightly swollen, giving thallus its canaliculate character. Branching more or less dichotomous, up to four orders. Apices narrowly rounded to crenate/fimbriate. In section, cortex of seven to nine cell layers, occupying half the width of the section (Fig. 16). Papillae simple, on surface and margins, hemispheric to irregular in shape but can become proliferous (bladelike; Figs 34, 35). Older, larger papillae basal; smaller, younger papillae distal. Spermatangia on both papillae and blade surface (Fig. 17). Distinguished from other *Mastocarpus* species by molecular sequence differences.

HOLOTYPE: Leftmost specimen (Fig. 15), UBC A88570, on mid intertidal bedrock with *Fucus distichus* subsp. *evanescens* and *Saccharina sessilis*, Botanical Beach, Vancouver Island, British Columbia, Canada, 11 July 2010, *Leg.* Patrick T. Martone (PTM 184). DNA extract M559.


Blade center
Blade margin
M. alaskensis
22
23
M. papillatus
24
25
M. vancouveriensis
26
27
M. intermedius
28
29
M. cristatus
30
31
M. latissimus
32
33
M. rigidus
34
35
M. californianus
36
37
M. agardhii
38
39
M. jardinii
40
41

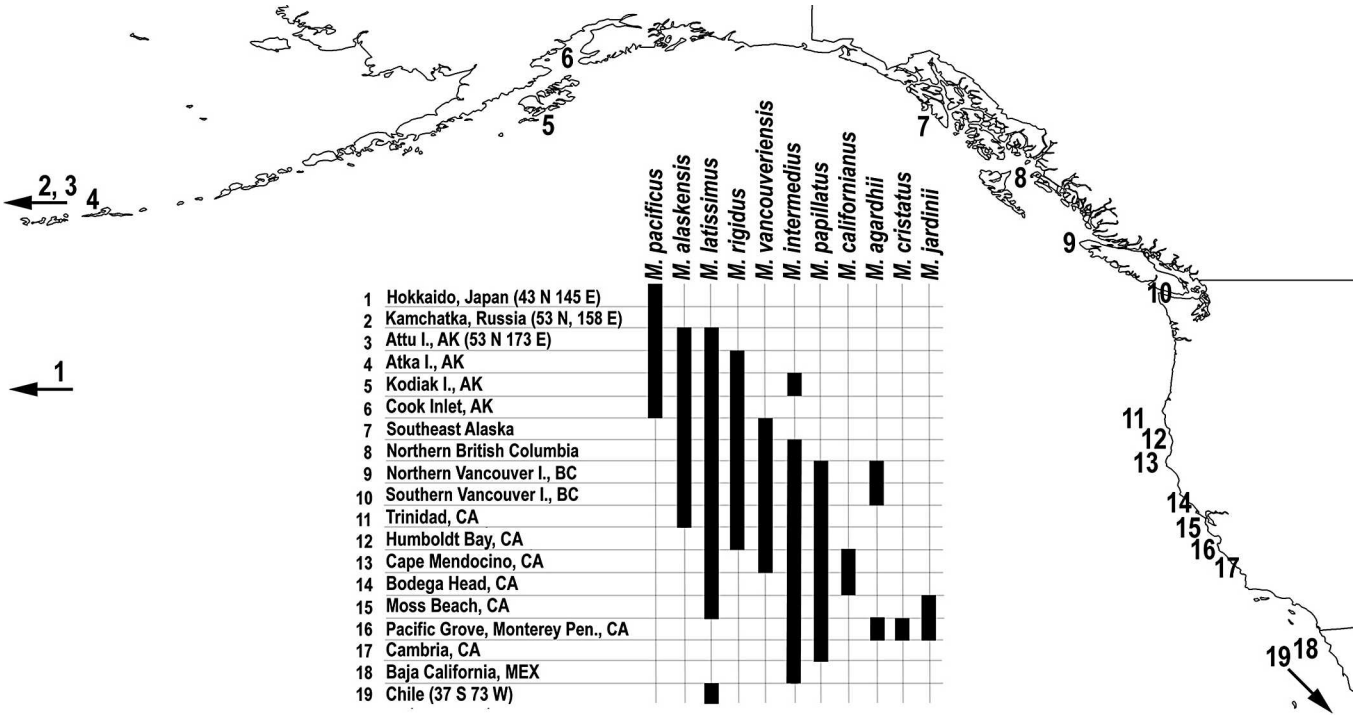


Fig. 42. Map of the northeast Pacific distribution of the species of *Mastocarpus* discussed in this paper.

HABITAT: Occurring on rock usually in the (lower) mid intertidal zone. Where they co-occur, this species can be slightly lower than *Mastocarpus agardhii*. Thalli often occur in clumps of 15–50 individuals.

VERIFIED DISTRIBUTION: North Jetty, Humboldt Bay, California, north to Atka Island, Aleutian Islands, Alaska (Fig. 42).

Mastocarpus californianus sp. nov.

Figs 19–21, 36, 37

Thallus gametophyticus erectus cartilagineus usque ad 8 cm altus (Fig. 19). Stipes apophysisque teres eramosa 15–35 mm longa, usque ad partem dimidiam altitudinis thalli attingens sed plerumque brevior, 2–3 mm lata, expansa in laminas proliferas 1–3 mm latas 350 μm crassas. Thallus unicolor: rubroniger. Apophysis margine leviter tumido, thallum canaliculatum faciens. Ramificatio irregularis, interdum prope basin thalli. Apices cordati vel crenati. Cortex in sectione transversali e stratis cellularum 10–11 constare visus, circiter dimidiam partem longitudinis sectionis occupans (Figs 20, 21). Papillae simplices in superficie marginibusque, interdum prolificantes (laminiformes; Figs 36, 37). Ab aliis speciebus Mastocarpi ordinibus nucleotidorum distinguendus.

Gametophytic thallus upright, cartilaginous, up to 8 cm tall (Fig. 19). Stipe and apophysis terete, unbranched, 15–35 mm in length, constituting up to half the height of the thallus but often considerably less, 2–3 mm wide, expanding into proliferous blades 1–3 mm wide and 350 μm thick. Thallus uniformly reddish black. Margin of thallus slightly swollen, giving it a canaliculate character. Branching irregular, can occur near base of thallus. Apices cordate to crenate. In section, cortex of 10–11 cell layers, occupying about half the width of the section (Figs 20, 21). Papillae simple, on surface and margins, some becoming proliferous (bladelike; Figs 36, 37). Distinguished from other *Mastocarpus* species by molecular sequence differences.

HOLOTYPE: Leftmost specimen (Fig. 19), UBC A88483, low intertidal boulder, ~0.3 m above MLLW, 'Mussel Rock', Cape Mendocino, Humboldt County, California, 8 April 2008, *Leg.* S.C. Lindstrom (SCL 13349). DNA extract M475.

HABITAT: Occurring on rock usually in the (lower) mid intertidal zone. Thalli often occur in clumps of 15–50 individuals.

←

Figs 22–41. Characteristic shapes of papillae near the centre of blades on their surfaces (left) and at their margins (right). Scale bars = 1 mm.
Figs 22, 23. *Mastocarpus alaskensis* (UBC A88520).
Figs 24, 25. *Mastocarpus papillatus* (UBC A88477).
Figs 26, 27. *Mastocarpus vancouveriensis* (UBC A85326).
Figs 28, 29. *Mastocarpus intermedius* (UBC A88510).
Figs 30, 31. *Mastocarpus cristatus* (UBC A88562).
Figs 32, 33. *Mastocarpus latissimus* (UBC A88534 left; UBC A88504 right).
Figs 34, 35. *Mastocarpus rigidus* (UBC A88494 left, UBC A88542 right).
Figs 36, 37. *Mastocarpus californianus* (UBC A88483).
Figs 38, 39. *Mastocarpus agardhii* (UBC A88526 left, UBC A88565 right).
Figs 40, 41. *Mastocarpus jardinii* (UBC A88747).

VERIFIED DISTRIBUTION: Bodega Head to Cape Mendocino, California (Fig. 42).

Names of North Pacific species of *Mastocarpus* that have not yet been assigned to contemporary specimens

Petrocelis middendorfii (Ruprecht) Kjellman 1883: 190

BASIONYM: *Cruoria middendorfii* Ruprecht 1850: 137.

TYPE: Asa Island, Sea of Okhotsk, Russia.

We have been unable to obtain type material of this species. If we are able to acquire this material, it is possible that this specific epithet will replace an existing or a proposed name, most likely *M. pacificus*, given its provenance.

Mastocarpus ochotensis (Ruprecht) Makienko in Klochkova 1996: 191

BASIONYM: *Chondrus mamillosus* var. *ochotensis* Ruprecht 1850: 126.

HOMOTYPIC SYNONYMS: *Gigartina ochotensis* (Ruprecht) Yendo 1916: 57. (The name was first used by Kjellman 1889: 31 but without indicating it was a new combination, nor citing the basionym.)

TYPE: Sea of Okhotsk.

We have been unable to obtain type material of this species. It is possible that this specific epithet will replace a proposed name but we expect that this species is a synonym of *M. pacificus*, which has nomenclatural priority.

Gigartina sitchensis (Ruprecht) Yendo 1916: 57

This name was first used by Kjellman 1889: 31 but without indicating it was a new combination, nor citing the basionym.

BASIONYM: *Chondrus mamillosus* var. *sitchensis* Ruprecht 1850: 126.

TYPE: Presumably Sitka, Alaska.

As noted above, we have been unable to obtain type material of this species. It is possible that this is an older name for *Mastocarpus alaskensis,* although other species of *Mastocarpus* are also known to occur in the Sitka area. Moreover, we have encountered a number of instances of misapplication of provenance to old Russian material, so we are reluctant to predict to which species this might belong.

DISCUSSION

These results underscore earlier observations that there are more species of *Mastocarpus* in the northeast Pacific than previously acknowledged (Lindstrom 2008a; Le Gall & Saunders 2010). Inclusion of mitochondrial COI sequence data supports earlier conclusions derived from nuclear ITS and chloroplast *rbc*L sequences (Lindstrom 2008a). Sequences from additional specimens from California indicate that even more species than those originally identified by Lindstrom (2008a) required names.

Small fragments of type material from public herbaria provided unambiguous ITS sequences that allowed us to match unequivocally these types with our own modern collections. These results highlight the importance of sequencing type material, where possible, to determine the correct application of existing names to cryptic species identified using molecular techniques. However, we acknowledge that this is often not as easy with other taxa as it has been with *Mastocarpus*. *Mastocarpus* is a genus for which many types are available in herbaria; many of the species are well-adapted to tough intertidal conditions (extended periods of desiccation, burial by sand), which may 'predispose' them to enhanced preservation compared with thalli from other environments.

Despite the success of this study, we were unable to find morphological features that would allow us to unequivocally identify many specimens. Nevertheless, *Mastocarpus* species show distinct geographic ranges and preferred habitats. However, even these observations require caveats since ranges may shift through time or by human-mediated activities. *Mastocarpus pacificus*, which is represented by the type of *M. unalaschcensis* from the Aleutian Islands from nearly 200 years ago, is much less common there now than *Mastocarpus alaskensis*, which appears to be absent from early Aleutian collections. Although *Mastocarpus alaskensis* is usually found in the mid to high intertidal zone, we have occasionally found it lower on the shore in areas with otherwise sparse algal cover. We were surprised to find specimens of *Mastocarpus agardhii* on Vancouver Island with sequences identical to those from the Monterey Peninsula more than 1000 km away and with no apparent geographically intermediate populations. Moreover, these Vancouver Island populations are odd because specimens with ITS of *Mastocarpus agardhii* have organelle sequences from other species as noted above. (Zuccarello *et al.* 2005 also observed mixed genotypes in European *Mastocarpus*, with asexual thalli having the plastid haplotype of the northern breeding group and the mitochondrial haplotype of the southern breeding group.) The occurrence of *Mastocarpus latissimus* in Chile, with little genetic differentiation from some California specimens, also raises the possibility of modern, anthropogenic dispersal.

These results support the hypothesis that biogeography is a major driver of speciation among seaweeds in the northeast Pacific (Lindstrom *et al.* 1996; Lindstrom 2006, 2008a, b). Sister species recognized in this paper show clear geographic differentiation, although sometimes with present-day overlap. *Mastocarpus alaskensis* is the northern counterpart of *M. papillatus,* although they overlap from northern Vancouver Island to northernmost California; where they co-occur, *M. papillatus* is slightly higher in the intertidal, as one might expect in a more southerly species. *Mastocarpus rigidus* and *Mastocarpus californianus* also show geographic differentiation, with *Mastocarpus rigidus* known to occur from Humboldt Bay to the Aleutian Islands, and *Mastocarpus californianus* thus far known only from Bodega Head to Cape Mendocino. These two species are in turn sister species to *Mastocarpus agardhii*, which occurs on the Monterey Peninsula, California.

This study does not address the fascinating question of the relationship between apomictic and sexual upright thalli in *Mastocarpus* spp., a phenomenon widely published in the genus (e.g. Polanshek & West 1977; Ohno *et al.* 1982; Fierst

et al. 2010) and discussed in Lindstrom (2008a). The sequence data from this study do provide the means for identifying individuals to determine whether they represent different species or even different genotypes of the same species in studies of apomixis and sexual reproduction in the genus. Nor do we address the relationship between the *Petrocelis* (sporophytic) phase and the gametophytic phase, such as whether all species have a *Petrocelis* phase and whether that phase occurs at the same elevation as its gametophyte. The type of *Petrocelis franciscana* had a sequence identical to the type of *Mastocarpus papillatus*. Field observations (Lindstrom, unpublished observations) confirmed the presence of a *Petrocelis* crust in the vicinity of at least some individuals of *Mastocarpus alaskensis*, *Mastocarpus latissimus* and *Mastocarpus pacificus*. We suspect that a *Petrocelis* phase occurs for most if not all the species.

Although we initially assumed that all *Mastocarpus* species were dioecious, we observed spermatangia on thalli of *Mastocarpus alaskensis*, *Mastocarpus intermedius*, *Mastocarpus vancouveriensis* and *Mastocarpus rigidus* that also bore what appeared to be cystocarpic papillae. This phenomenon requires further investigation. The frequency of spermatangia may suggest that individuals are protandrous. We often encountered distinct, pale, nonpapillate male thalli in populations of *Mastocarpus alaskensis* and *M. papillatus*. We don't know whether these thalli eventually produce cystocarpic papillae.

It is likely that at least some the species of *Mastocarpus* in the northeast Pacific are more widely distributed than our collections indicate. We hope this work provides the necessary framework for others to determine the range of morphological plasticity and details of their reproductive anatomy, ecology, physiology and geography.

We offer the following key to the gametophytic phase of species of *Mastocarpus* occurring in the northeast Pacific with a caveat: it will not work for all specimens. Most species of *Mastocarpus* are highly variable in their shape, particularly the widely distributed low intertidal species *Mastocarpus intermedius* and *Mastocarpus latissimus*, and it is impossible to account for that amount of polymorphism and still provide a useable key. To help cope with some of this variation, we refer the reader to Table S1 in Lindstrom (2008a) and to http://botany.ubc.ca/sandral/M[species epithet].jpg, where images of representatives of each species are posted. To help narrow the range of possible identifications, a map of the known distributions of northeast Pacific species of *Mastocarpus* discussed in this paper is presented in Fig. 42.

1. Thalli often of similar sizes and growing in clusters; stipe usually a third to three-quarters the height of the thallus; apophysis and most branches narrow, to ~2 mm wide, of more or less uniform width, except distal ends of branches, which may become bladelike 2
1. Thalli not clustered, or if clustered, of different sizes; stipe mostly one quarter or less the height of the thallus; apophysis and branches mostly >2 mm wide, broadening distally 6
2. Thalli to 6 cm tall; papillae usually absent on cystocarpic thalli (cystocarps at branch tips, in axes near branch tips or occasionally in papillae); habit similar to *Ahnfeltiopsis leptophylla* 3
2. Thalli to 15 cm tall; papillae present on cystocarpic thalli 4
3. Axes terete to compressed, cartilaginous; medullary cells large and isodiametric; occurring in mid intertidal pools or in the low intertidal or subtidally; known from west coast Vancouver Island, British Columbia, to central California . . . . . *M. pachenicus*[1]
3. Axes compressed to ribbonlike, flaccid; medullary cells including at least some narrow filaments; occurring in high intertidal pools or emergent on mid or low intertidal rock; known from northern Gulf of Alaska to northern Japan . . . . . *M. pacificus* (*ochotensis* form)
4. Branching irregular, thallus mostly proliferous, resembling in habit a high intertidal *Prionitis lanceolata* but itself occurring on low to upper mid intertidal bedrock; known only from northern California (Cape Mendocino to Bodega Head) . . . . . *Mastocarpus californianus*
4. Branching more or less dichotomous, with orderly, slender branches that are four or more times dichotomous; from central California to the Aleutian Islands, Alaska 5
5. Thalli up to 15 cm tall, divided up to six orders; papillae vermiform and pedicellate, generally not proliferous; occurring in the mid to high intertidal; known only from north and west Vancouver Island, British Columbia, and from the Monterey Peninsula, California . . . . . *Mastocarpus agardhii*
5. Thalli up to 9 cm tall, divided up to four orders; papillae simple and sessile, becoming proliferous; occurring in the low to mid intertidal; known from Humboldt Bay, California, north to the Aleutian Islands, Alaska . . . . . *Mastocarpus rigidus*
6. Branches or blades 4–10 mm wide, slender in appearance; papillae cylindrical, centrally or terminally enlarged; occurring in the low intertidal; known only from San Mateo to Monterey Counties, California . . . . . *M. jardinii*
6. Branches or blades greater than 10 mm wide, bladelike in appearance; papillae, habitats and distributions various 7
7. Vegetative blades stipitate, arising from the apex of the apophysis (occasionally subdichotomous); papillae sparse, mostly simple, restricted to older parts of the surface of female thalli; occurring on rock in the mid to high intertidal, often associated with sand; known from northern California to northern Southeast Alaska . . . . . *Mastocarpus vancouveriensis*
7. Vegetative blades part of a more or less dichotomous axis, proliferous branches when present not arising solely from the apophysis; papillae, habitats and distributions various 8

[1] This species was described by Le Gall and Saunders (2010) from the west coast of Vancouver Island; it is also known from central California (Hughey, personal observations). Its morphological similarity to *Ahnfeltiopsis leptophylla* suggests it is more widely distributed than just these two areas. Habitat information included here was provided by Saunders (personal communication, 10 August 2010).

8. Blades irregularly dichotomous or proliferous, sometimes constricted; papillae on both surfaces and margins of blades and on both female and male thalli, male papillae can become proliferous; usually occurring in the mid to low intertidal 9
8. Blades more or less regularly dichotomous, rarely proliferous or constricted; papillae only on female thalli, mostly solely on surface of blades, male papillae lacking; usually found in the mid to high intertidal 11
9. Vegetative blades mostly narrow (< 8 mm wide); papillae profuse on both female and male thalli, becoming branched or bladelike; known only from the Monterey Peninsula, California . . . . . *Mastocarpus cristatus*
9. Vegetative blades very narrow (< 2 mm wide) to 30 mm broad; widely distributed. 10
10. Female thalli with hemispheric to vermiform papillae, which can become very convoluted; known from Baja California, Mexico, to northern British Columbia and from a single site on Kodiak Island, Alaska . . . . . *Mastocarpus intermedius*
10. Female thalli with simple papillae; known from Moss Beach, San Mateo County, California, north to Attu Island, Aleutian Islands, Alaska; Chile . . . . . *Mastocarpus latissimus*
11. Papillae congested on margins of female plants, surfaces generally smooth; known from Kodiak Island, Alaska, to northern Japan . . . . . *M. pacificus* (*pacificus* form)
11. Papillae often stubble-like, restricted to older parts of the surface of the female thalli; known from California to the Aleutian Islands, Alaska 12
12. Blades up to 15 (to 21) cm tall and up to 350 μm thick; mid to high intertidal but lower than *M. papillatus* where they co-occur; known from northern California to the Aleutian Islands, Alaska . . . . . *Mastocarpus alaskensis*
12. Blades up to 9 cm tall and up to 500 μm thick; high intertidal, higher than *Mastocarpus alaskensis*, where they co-occur; known from central California to northern Vancouver Island, British Columbia . . . . . *M. papillatus*

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