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**Front cover:** Non-geniculate coralline red algae are widespread in all of the world's oceans, where they often form the dominant cover on primary rocky substrata. These are individuals of a new species *Heydrichia cerasina* from L'Agulhas, Cape Agulhas, Western Cape Province, growing on small pebbles in low shore surge channels, which is their usual habitat. The coin is 20 mm in diameter. (See Maneveldt and van der Merwe, pp. 11–21) Photo: Gavin Maneveldt.



## An examination of the red algal genus *Pugetia* (Kallymeniaceae, Gigartinales), with descriptions of *Salishia firma* gen. & comb. nov., *Pugetia cryptica* sp. nov. and *Beringia wynnei* sp. nov.

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CLARKSTON B.E. AND SAUNDERS G.W. 2012. An examination of the red algal genus *Pugetia* (Kallymeniaceae, Gigartinales), with descriptions of *Salishia firma* gen. & comb. nov., *Pugetia cryptica* sp. nov. and *Beringia wynnei* sp. nov. *Phycologia* 51: 33–61. DOI: 10.2216/11-01.1

The red algal family Kallymeniaceae was surveyed along the west coast of Canada using the DNA barcode (COI-5P – 5' region of the mitochondrial cytochrome c oxidase I gene) as a species identification tool. A total of 253 specimens field identified as *Pugetia* spp. subsequently resolved as five genetic species groups, although only two are reported in the flora. Additionally, COI-5P data were available for the Chilean *P. chilensis*, which resolved as a distinct species. Subsequent analysis of the internal transcribed spacer of the ribosomal cistron and the universal plastid amplicon (domain V of the 23S rRNA gene) resolved the same groups as COI-5P. Phylogenetic relationships of the Canadian groups were investigated using large-subunit ribosomal DNA (LSU) and a combined analysis of LSU and COI-5P data. One species was divergent from the *Pugetia* spp. in all analyses and grouped closely with the kallymeniacean genera *Erythrophyllum* (Kallymeniaceae, Gigartinales) and *Kallymeniopsis* (Kallymeniaceae, Gigartinales) – it is here described as *Beringia wynnei* sp. nov. The other '*Pugetia*' species fell into two divergent clusters in phylogenetic analyses, differing also in blade thickness, carpogonial branch morphology and the association of the auxiliary cell relative to the carpogonium (procarpic vs nonprocarpic). We retain the genus *Pugetia* for the type species *P. fragilissima* and *P. cryptica* sp. nov. and describe the new genus *Salishia* for *Salishia firma* (Kylin) comb. nov., *S. sanguinea* (Montagne) comb. nov. and *S. chilensis* (J. Agardh) comb. nov. Finally, we completed morphological and anatomical examinations of other *Pugetia* species to provide a comprehensive review of the genus.

KEY WORDS: Biodiversity, COI-5P, DNA barcode, Florideophyceae, Kallymeniaceae, *Pugetia*

### INTRODUCTION

The Kallymeniaceae is a large red algal family of c. 20 genera and 132 species with representatives in every ocean and its highest diversity in temperate waters. The genera possess similar female reproductive characters – namely, a carpogonial branch system composed of a clavate to lobed supporting cell with a variable number of three-celled carpogonial branches and subsidiary cells (Hansen & Lindstrom 1984) – and are delimited by vegetative (e.g. thallus branching and internal anatomy) and reproductive characters (e.g. morphology of the carpogonial branch and cystocarp, procarpy vs nonprocarpy; Kylin 1956; Hansen & Lindstrom 1984). Recently, genetic sequences have also been used in combination with morphological characters to delimit members of the family (Harper & Saunders 2002; Clarkston & Saunders 2010).

Along the coast of Canada, there are currently eight kallymeniacean genera (*Callocolax*, *Callophyllis*, *Erythrophyllum*, *Euthora*, *Hommersandia*, *Kallymenia*, *Kallymeniopsis* and *Pugetia*) and 18 species reported, most of which occur only in the Pacific. One species, *Euthora cristata* (C. Agardh) J. Agardh, is found on the Pacific, Atlantic and Arctic coasts of Canada, while another, *Kallymenia schmitzii* De Toni, is reported only from the

Arctic (Hooper & South 1974; Lee 1980). Two species of the genus *Pugetia* are found in Canada, *P. fragilissima* Kylin and *P. firma* Kylin, both in British Columbia.

Kylin (1925) erected the genus *Pugetia* based on *P. fragilissima* from Canoe Island, Washington, USA. He distinguished *Pugetia* from the related *Callophyllis* by the largely undivided or unevenly divided habit of the thallus (Kylin 1941) and by characters of the medullary filament cells – nearly isodiametric and pigmented in *Pugetia* and larger, elongate and unpigmented in *Callophyllis* (Kylin 1925). Since then, 12 additional species have been recognized: *Pugetia firma* Kylin (Kylin 1941) from Pacific Grove, California, USA; *Pugetia chilensis* (J. Agardh) Kylin (Kylin 1941) from the coast of Chile; *Pugetia sanguinea* (Montagne) Kylin (Kylin 1941) from the southern coast of Chile, all of which were transferred to the red algal genus *Callophyllis* (Norris 1957); *Pugetia japonica* Kylin (Kylin 1941) from Chiba Prefecture, Japan, which was also transferred to *Callophyllis* (Silva *et al.* 1987); *Pugetia kylinii* Baardseth (Baardseth 1941) from the Tristan da Cunha Islands off the west coast of Africa; *Pugetia palmatifolia* Tokida (Tokida 1948) from Higashisoya, southern Sakhalin, Japan (now part of Russia), which was recently transferred to *Hommersandia* (Selivanova & Zhigadlova 1997); *Pugetia delicatissima* R.E. Norris (Norris 1957), from Gore Bay, Canterbury, New Zealand; *Pugetia latiloba* (W.R. Taylor) R.E. Norris (Norris 1957) from Gardner Bay in the Galapagos

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Islands; *Pugetia porphyroidea* (F. Schmitz ex Holmes) R.E. Norris and *Pugetia harveyana* (J. Agardh) (Norris 1964), both from the Cape of Good Hope, South Africa; and, finally, *Pugetia mexicana* E.Y. Dawson (Dawson 1966) from Isla San Lorenzo del Sur, Mexico.

Since *Pugetia* was erected, it has been the subject of several detailed studies (e.g. Kylin 1941; Norris 1957, 1964). Despite these rigorous examinations, however, the taxonomic status of many *Pugetia* species remains uncertain. This is due, in part, to the fact that four of the nine currently recognized species of *Pugetia* are known virtually only from the type collections, and several others have been collected only rarely. In addition, the majority of taxonomic studies on *Pugetia* took place before the advent of molecular tools. To date, only *P. fragilissima*, *P. firma* and *P. chilensis* have been included in published molecular phylogenies (Harper & Saunders 2002; Le Gall & Saunders 2007; Clarkston & Saunders 2010), and only one of these studies examined the relationship of *Pugetia* within the Kallymeniaceae in any detail (Harper & Saunders 2002).

An increasingly common practice in algal systematics is to include multiple types of characters (e.g. morphological, anatomical, biogeographical, molecular) when describing species or completing monographs (e.g. Schneider & Lane 2008; Yamada *et al.* 2008; Le Gall & Saunders 2010; Saunders & McDonald 2010). Molecular tools are increasingly being utilized for assigning specimens to genetic species groups from which morphological and anatomical characters can be assessed and for assigning cryptic specimens to known species (e.g. Fox & Swanson 2007; Guillemin *et al.* 2008). Several such tools have been used in red algae for species assignment, notably, the DNA barcode (COI-5P, c. 664 base pairs from the 5' region of the mitochondrial cytochrome c oxidase I gene; Saunders 2005, 2008), the internal transcribed spacer of the ribosomal cistron (ITS; variable length; Ross *et al.* 2003; Saunders 2005), and the universal plastid amplicon (UPA; c. 400 base pairs, domain V of the 23S rRNA gene; Sherwood & Presting 2007; Sherwood *et al.* 2008; Clarkston & Saunders 2010). Of these tools, the COI-5P is currently the standard DNA barcode marker sanctioned by the Consortium for the Barcode of Life (<http://www.barcoding.si.edu>) – an international initiative dedicated to developing short, standardized DNA sequences as species-level identification tools – and has been the primary marker used during recent and ongoing surveys of Canadian Kallymeniaceae.

The objectives of this study were (1) to resolve the number of genetic species groups for *Pugetia* that occur along the coast of British Columbia using molecular tools, (2) determine whether previous groups matched recorded species from the region or were new records of *Pugetia* species from other geographic regions or were new species, (3) determine the phylogenetic affinities of the *Pugetia* species groups relative to other kallymeniacean genera using sequences of the large-subunit ribosomal DNA (LSU), and (4) conduct the first reappraisal of this taxonomically controversial genus in over 50 years (Norris 1957), including a re-examination of representative type specimens, in order to augment existing descriptions with an emphasis on incorporating molecular and morpholog-

ical characters to delimit species. During our work, we uncovered and subsequently characterized previously unrecognized kallymeniacean diversity in the North Pacific.

## MATERIAL AND METHODS

### Sample collection

All specimens were collected either in the subtidal by SCUBA or in the intertidal as attached individuals or drift. The date and collector(s) of each specimen are available online by searching the Barcode of Life Data Systems (BOLD) accession number at <http://www.barcodinglife.org>, and the location is reported in Table 1. Specimens were dried on herbarium paper with a subsample dried in a vial with silica gel for molecular analyses.

### DNA extraction, amplification and sequencing

Genomic DNA was isolated following a protocol modified from Saunders (1993; see Saunders 2008). The 5' end of the mitochondrial cytochrome c oxidase I gene (DNA barcode; COI-5P) region was amplified for all kallymeniacean samples except four (Table 1) using one of the following primer combinations: GazF1 and GazR1 (Saunders 2005), GazF1 and DumR1 (Saunders 2005), GazF1 and GazR4 (Saunders 2008), or GWSFn (Le Gall & Saunders 2010) and GWSRx (reverse; 5' ACTTCTGGRT-GICCRAARAAYCA 3'). For some of the COI-5P-amplified samples, there was either poor amplification using the above primers or contamination due to epi/endophytic biota; for these samples, the following primer combinations were used: GHaIF (Saunders 2008) and GazR1, GHaIF and DumR1, GHaIF and GHaIR (Clarkston & Saunders 2010), GHaIF and GWSR3 (Saunders 2009), ScyF1 (forward; 5' GGTACTCTRTATTTAATT 3') and GazR1, and GrF1 (forward; 5' ACTAATCATAARGATATYGG 3') and COX1R1 (Saunders 2008). The actual primer combination for each isolate is recorded on BOLD (<http://www.boldsystems.org>). The PCR amplification profile followed Hebert *et al.* (2003) but used an annealing temperature of 50°C. A portion of the plastid 23S rDNA (universal plastid amplicon; UPA) was PCR amplified and sequenced for select samples (Table 1), following the protocol outlined in Sherwood & Presting (2007), but used the modified reverse primer P23SnewR (Clarkston & Saunders 2010). All COI-5P and UPA PCR products were purified using ExoSAP-IT® (USB, Cleveland, OH, USA). The nuclear internal transcribed spacer region (ITS) was PCR amplified and sequenced for select samples (Table 1) following the procedure of Tai *et al.* (2001). Partial nuclear large-subunit ribosomal DNA (LSU) was also amplified and sequenced for a representative of each species (Table 1) following the protocol of Harper & Saunders (2001; but for modifications, see Le Gall & Saunders 2010). All ITS and LSU amplification products were cleaned using a glass wool column protocol (Saunders 1993).

Sequencing of all PCR products was carried out using the PE Applied Biosystems Big Dye (version 3.1) kit (following the manufacturer's instructions except only 1 µl of Big Dye



Table 1. Samples used in molecular analyses.

Species and voucher	Collection site	GenBank accession <sup>1,2</sup>			
		COI-5P	UPA	ITS	LSU
<i>Family Kallymeniaceae</i>					
<i>Beringia wynnei</i> Clarkston & G.W. Saunders					
GWS002222	Browning Wall, Port Hardy, BC, Canada	HM918467	N.D.	N.D.	N.D.
GWS003046	Seapool Rock, Bamfield, BC, Canada	JF903289	JF903448	N.D.	N.D.
GWS004487	Seapool Rock, Bamfield, BC, Canada	JF903291	JF903450	N.D.	N.D.
GWS004493	Seapool Rock, Bamfield, BC, Canada	JF903287	JF903446	JF903425	N.D.
GWS004495	Seapool Rock, Bamfield, BC, Canada	JF903290	JF903449	N.D.	N.D.
GWS004755	Browning Wall, Port Hardy, BC, Canada	JF903288	JF903447	N.D.	N.D.
GWS004783	Browning Wall, Port Hardy, BC, Canada	JF903293	JF903452	N.D.	N.D.
GWS004848	Tree Knob Islands, Prince Rupert, BC, Canada	HM916627	N.D.	N.D.	N.D.
GWS004863	Tree Knob Islands, Prince Rupert, BC, Canada	JF903286	JF903445	JF903424	JF833332
GWS004878	Tree Knob Islands Prince Rupert, BC, Canada	HM918715	N.D.	N.D.	N.D.
GWS008673	Seapool Rock, Bamfield, BC, Canada	HM917069	N.D.	N.D.	N.D.
GWS008928	Seapool Rock, Bamfield, BC, Canada	HM918847	N.D.	N.D.	N.D.
GWS008929	Seapool Rock, Bamfield, BC, Canada	HM918848	N.D.	N.D.	N.D.
GWS008930	Seapool Rock, Bamfield, BC, Canada	HM918849	N.D.	N.D.	N.D.
GWS008931	Seapool Rock, Bamfield, BC, Canada	HM918850	N.D.	N.D.	N.D.
GWS010421	Seapool Rock, Bamfield, BC, Canada	JF903292	JF903451	N.D.	N.D.
GWS010432	Seapool Rock, Bamfield, BC, Canada	HM917277	JF903444	N.D.	N.D.
<i>Callophyllis laciniata</i> (Hudson) Kützing					
GWS001792	Strangford Lough, Portaferry, N. Ireland, UK	JF903294	N.D.	N.D.	N.D.
GWS001795	Strangford Lough, Portaferry, N. Ireland, UK	N.D.	N.D.	JF903426	JF833333
<i>Erythrophyllum delesserioides</i> J. Agardh					
GWS000072	Piedras Blancas, California, USA	GU140104	GU140205	GU140205	<b>AF419123</b>
GWS001645	Seppings Is., Bamfield, BC, Canada	GU140109	N.D.	N.D.	N.D.
GWS001664	Cape Beale, Bamfield, BC, Canada	GU140100	GU140203	N.D.	N.D.
GWS001665	Cape Beale, Bamfield, BC, Canada	GU140103	N.D.	N.D.	N.D.
GWS001746	Cape Beale, Bamfield, BC, Canada	GU140099	GU140202	N.D.	N.D.
GWS001747	Cape Beale, Bamfield, BC, Canada	GU140113	GU140213	N.D.	N.D.
GWS001752	Cape Beale, Bamfield, BC, Canada	GU140110	GU140210	N.D.	N.D.
GWS002910	Blowhole at Bradys Beach, Bamfield, BC, Canada	GU140107	GU140208	GU140208	N.D.
GWS002911	Blowhole at Bradys Beach, Bamfield, BC, Canada	GU140105	GU140206	N.D.	N.D.
GWS002945	Seppings Is., Bamfield, BC, Canada	GU140106	GU140207	N.D.	N.D.
GWS003275	Seppings Is., Bamfield, BC, Canada	GU140101	N.D.	N.D.	N.D.
GWS003295	Cape Beale, Bamfield, BC, Canada	GU140102	GU140204	N.D.	N.D.
GWS004405	Botanical Beach, Port Renfrew, BC, Canada	GU140112	GU140212	N.D.	N.D.
GWS004688	Palmerston Recreation Reserve, Vancouver Is., BC, Canada	GU140111	GU140211	N.D.	N.D.
GWS006868	'Lands End', Pachena Bay, Bamfield, BC, Canada	GU140108	GU140209	N.D.	N.D.
<i>Kallymeniopsis oblongifruca</i> (Setchell) G.I. Hansen					
GWS001164	Seapool Rock, Bamfield, BC, Canada	GU140196	GU140238	N.D.	<b>AY171613</b>
GWS003003	Tapaltos Beach, Bamfield, BC, Canada	JF903298	N.D.	N.D.	N.D.
GWS003039	Seapool Rock, Bamfield, BC, Canada	GU140201	N.D.	N.D.	N.D.
GWS003040	Seapool Rock, Bamfield, BC, Canada	GU140186	GU140232	N.D.	N.D.
GWS003044	Seapool Rock, Bamfield, BC, Canada	GU140184	N.D.	N.D.	N.D.
GWS003055	Seapool Rock, Bamfield, BC, Canada	GU140200	N.D.	N.D.	N.D.
GWS003058	Seapool Rock, Bamfield, BC, Canada	GU140187	N.D.	N.D.	N.D.
GWS003065	Seapool Rock, Bamfield, BC, Canada	JF903299	N.D.	N.D.	N.D.
GWS003066	Seapool Rock, Bamfield, BC, Canada	GU140185	GU140231	GU140185	N.D.
GWS003143	Seapool Rock, Bamfield, BC, Canada	GU140197	N.D.	GU140197	N.D.
GWS004496	Seapool Rock, Bamfield, BC, Canada	GU140199	GU140240	N.D.	N.D.
GWS004530	Seapool Rock, Bamfield, BC, Canada	GU140194	GU140236	N.D.	N.D.
GWS004531	Seapool Rock, Bamfield, BC, Canada	GU140195	GU140237	N.D.	N.D.
GWS004532	Seapool Rock, Bamfield, BC, Canada	GU140192	N.D.	N.D.	N.D.
GWS004533	Seapool Rock, Bamfield, BC, Canada	GU140198	GU140239	N.D.	N.D.
GWS004534	Seapool Rock, Bamfield, BC, Canada	GU140191	N.D.	N.D.	N.D.
GWS004535	Seapool Rock, Bamfield, BC, Canada	GU140190	GU140235	N.D.	N.D.
GWS004909	Stenhouse Reef, Prince Rupert, BC, Canada	GU140189	GU140234	N.D.	N.D.
GWS004939	Stenhouse Reef, Prince Rupert, BC, Canada	GU140188	GU140233	N.D.	N.D.
GWS008676	Seapool Rock, Bamfield, BC, Canada	GU140193	N.D.	N.D.	N.D.
GWS008919	Seapool Rock, Bamfield, BC, Canada	JF903300	N.D.	N.D.	N.D.
GWS010428	Seapool Rock, Bamfield, BC, Canada	JF903301	N.D.	N.D.	N.D.
<i>Pugetia cryptica</i> Clarkston & G.W. Saunders					
B057	Diana Island, Bamfield, BC, Canada	N.D.	N.D.	N.D.	<b>AY171614</b>
GWS001737	Five Mile Reef, Haida Gwaii Islands, BC, Canada	JF903305	JF903457	N.D.	N.D.



Table 1. Continued

Species and voucher	Collection site	GenBank accession <sup>1,2</sup>			
		COI-5P	UPA	ITS	LSU
GWS003011	Black Fish Is., Bamfield, BC, Canada	JF903306	N.D.	JF903429	N.D.
GWS004125	Scotts Bay, Bamfield, BC, Canada	JF903308	JF903459	JF903430	JF833331
GWS004239	Saxe Pt., Victoria, BC, Canada	JF903304	JF903456	N.D.	N.D.
GWS008498	Backeddy Resort, Egmont, BC, Canada	JF903302	JF903454	JF903428	N.D.
GWS008499	Backeddy Resort, Egmont, BC, Canada	HM917043	N.D.	N.D.	N.D.
GWS008500	Backeddy Resort, Egmont, BC, Canada	JF903303	JF903455	N.D.	N.D.
GWS009089	Wizard Islet, Bamfield, BC, Canada	JF903307	JF903458	N.D.	N.D.
GWS009090	Wizard Islet, Bamfield, BC, Canada	HM918884	N.D.	N.D.	N.D.
GWS010373	Palliser Rock, Comox, BC, Canada	HM917257	N.D.	N.D.	N.D.
GWS010409	Savoie Rocks, Hornby Island, BC, Canada	HM917271	N.D.	N.D.	N.D.
GWS010412	Savoie Rocks, Hornby Island, BC, Canada	HM917273	N.D.	N.D.	N.D.
GWS012608	Mazarredo Islands, Haida Gwaii, BC, Canada	HM915302	JF903453	JF903427	N.D.
GWS012652	Mazarredo Islands, Haida Gwaii, BC, Canada	HM915331	N.D.	N.D.	N.D.
<i>Pugetia fragilissima</i> Kylin					
GWS001695	Shields Island Bay, Haida Gwaii Islands, BC, Canada	JF903316	N.D.	N.D.	N.D.
GWS001696	Shields Island Bay, Haida Gwaii Islands, BC, Canada	JF903343	N.D.	N.D.	N.D.
GWS001697	Shields Island Bay, Haida Gwaii Islands, BC, Canada	JF903320	N.D.	N.D.	N.D.
GWS002184	Bear Cove Park, Port Hardy, BC, Canada	JF903319	N.D.	N.D.	N.D.
GWS002205	Daphne Pt., Port Hardy, BC, Canada	JF903342	JF903469	N.D.	N.D.
GWS002207	Daphne Pt., Port Hardy, BC, Canada	JF903332	N.D.	N.D.	N.D.
GWS002209	Daphne Pt., Port Hardy, BC, Canada	HM918465	N.D.	N.D.	N.D.
GWS002870	Bradys Beach, Bamfield, BC, Canada	JF903338	N.D.	N.D.	N.D.
GWS002968	Scotts Bay, Bamfield, BC, Canada	JF903330	N.D.	N.D.	N.D.
GWS003267	Wizard Islet, Bamfield, BC, Canada	JF903339	N.D.	N.D.	N.D.
GWS004110	Scotts Bay, Bamfield, BC, Canada	JF903340	N.D.	N.D.	N.D.
GWS004111	Scotts Bay, Bamfield, BC, Canada	JF903335	N.D.	N.D.	N.D.
GWS004122	Scotts Bay, Bamfield, BC, Canada	JF903333	JF903466	N.D.	N.D.
GWS004127	Scotts Bay, Bamfield, BC, Canada	JF903329	N.D.	N.D.	N.D.
GWS004128	Scotts Bay, Bamfield, BC, Canada	JF903331	N.D.	N.D.	N.D.
GWS004335	Dixon Is., Bamfield, BC, Canada	JF903311	JF903461	N.D.	JF833330
GWS004551	Satellite Passage Reef, Bamfield, BC, Canada	JF903344	JF903470	N.D.	N.D.
GWS004553	Satellite Passage Reef, Bamfield, BC, Canada	JF903328	N.D.	N.D.	N.D.
GWS004554	Satellite Passage Reef, Bamfield, BC, Canada	JF903315	JF903462	JF903433	N.D.
GWS004555	Satellite Passage Reef, Bamfield, BC, Canada	JF903324	N.D.	N.D.	N.D.
GWS004556	Satellite Passage Reef, Bamfield, BC, Canada	HM918668	N.D.	N.D.	N.D.
GWS004557	Satellite Passage Reef, Bamfield, BC, Canada	JF903323	N.D.	N.D.	N.D.
GWS004558	Satellite Passage Reef, Bamfield, BC, Canada	JF903322	N.D.	N.D.	N.D.
GWS004559	Satellite Passage Reef, Bamfield, BC, Canada	JF903314	N.D.	N.D.	N.D.
GWS004560	Satellite Passage Reef, Bamfield, BC, Canada	JF903313	N.D.	N.D.	N.D.
GWS004561	Satellite Passage Reef, Bamfield, BC, Canada	JF903327	N.D.	JF903434	N.D.
GWS004562	Satellite Passage Reef, Bamfield, BC, Canada	JF903321	N.D.	N.D.	N.D.
GWS004563	Satellite Passage Reef, Bamfield, BC, Canada	JF903312	N.D.	JF903432	N.D.
GWS004564	Satellite Passage Reef, Bamfield, BC, Canada	HM918669	N.D.	N.D.	N.D.
GWS004565	Satellite Passage Reef, Bamfield, BC, Canada	JF903326	JF903465	N.D.	N.D.
GWS004777	Browning Wall, Port Hardy, BC, Canada	HM918697	N.D.	N.D.	N.D.
GWS004779	Browning Wall, Port Hardy, BC, Canada	JF903341	JF903468	N.D.	N.D.
GWS004792	Ruth Is., Port Hardy, BC, Canada	HM918701	N.D.	N.D.	N.D.
GWS004793	Ruth Is., Port Hardy, BC, Canada	JF903325	JF903464	N.D.	N.D.
GWS004851	Tree Knob Islands Prince Rupert, BC, Canada	HM918710	N.D.	N.D.	N.D.
GWS004855	Tree Knob Islands Prince Rupert, BC, Canada	HM918711	N.D.	N.D.	N.D.
GWS004870	Tree Knob Islands Prince Rupert, BC, Canada	HM918713	N.D.	N.D.	N.D.
GWS004864	Tree Knob Islands Prince Rupert, BC, Canada	JF903310	JF903310	N.D.	N.D.
GWS006581	Island #37, Tahsis, BC, Canada	JF903318	JF903463	N.D.	N.D.
GWS008178	Scotts Bay, Bamfield, BC, Canada	JF903336	N.D.	N.D.	N.D.
GWS008737	Wizard Islet, Bamfield, BC, Canada	JF903337	N.D.	N.D.	N.D.
GWS008972	Execution Rock, Bamfield, BC, Canada	JF903317	N.D.	N.D.	N.D.
GWS008992	Scotts Bay, Bamfield, BC, Canada	HM918865	N.D.	N.D.	N.D.
GWS008993	Scotts Bay, Bamfield, BC, Canada	HM918866	N.D.	N.D.	N.D.
GWS008994	Scotts Bay, Bamfield, BC, Canada	HM918867	N.D.	N.D.	N.D.
GWS008995	Scotts Bay, Bamfield, BC, Canada	HM918868	N.D.	N.D.	N.D.
GWS008996	Scotts Bay, Bamfield, BC, Canada	HM918869	N.D.	N.D.	N.D.
GWS009005	Scotts Bay, Bamfield, BC, Canada	HM918872	N.D.	N.D.	N.D.
GWS009017	Gilbert Island, Broken Group, Bamfield, BC, Canada	HM918873	N.D.	N.D.	N.D.
GWS009018	Gilbert Island, Broken Group, Bamfield, BC, Canada	HM918874	N.D.	N.D.	N.D.
GWS009021	Gilbert Island, Broken Group, Bamfield, BC, Canada	JF903309	N.D.	N.D.	N.D.
GWS009026	Gilbert Island, Broken Group, Bamfield, BC, Canada	HM915811	N.D.	N.D.	N.D.
GWS009045	Mears Bluff, Broken Group, Bamfield, BC, Canada	HM918876	N.D.	N.D.	N.D.



Table 1. Continued

Species and voucher	Collection site	GenBank accession <sup>1,2</sup>			
		COI-5P	UPA	ITS	LSU
GWS009056	Mears Bluff, Broken Group, Bamfield, BC, Canada	HM918880	N.D.	N.D.	N.D.
GWS010143	Flower Islet, Tahsis, BC, Canada	HM917194	N.D.	N.D.	N.D.
GWS010153	Flower Islet, Tahsis, BC, Canada	JF903334	JF903467	N.D.	N.D.
GWS010592	Wizard Islet, Bamfield, BC, Canada	HM917309	N.D.	N.D.	N.D.
GWS012589	Chaatl Island, Haida Gwaii, BC, Canada	HM915297	N.D.	N.D.	N.D.
GWS012590	Chaatl Island, Haida Gwaii, BC, Canada	HM915298	N.D.	N.D.	N.D.
GWS012596	Chaatl Island, Haida Gwaii, BC, Canada	HM915301	N.D.	N.D.	N.D.
GWS013398	Burnaby Narrows, Gwaii Haanas, Haida Gwaii, BC, Canada	HM915637	N.D.	N.D.	N.D.
GWS013401	Burnaby Narrows, Gwaii Haanas, Haida Gwaii, BC, Canada	HM915665	N.D.	N.D.	N.D.
GWS013402	Burnaby Narrows, Gwaii Haanas, Haida Gwaii, BC, Canada	HM915672	N.D.	N.D.	N.D.
GWS013403	Burnaby Narrows, Gwaii Haanas, Haida Gwaii, BC, Canada	HM915608	N.D.	N.D.	N.D.
GWS013404	Burnaby Narrows, Gwaii Haanas, Haida Gwaii, BC, Canada	HM915618	N.D.	N.D.	N.D.
GWS013405	Burnaby Narrows, Gwaii Haanas, Haida Gwaii, BC, Canada	HM915628	N.D.	JF903431	N.D.
GWS019665	Scotts Bay, Bamfield, BC, Canada	HQ919318	N.D.	N.D.	N.D.
GWS020711	Kwuna Island, Haida Gwaii, BC, Canada	HQ919378	N.D.	N.D.	N.D.
GWS020713	Kwuna Island, Haida Gwaii, BC, Canada	HQ919379	N.D.	N.D.	N.D.
GWS020766	Kwuna Island, Haida Gwaii, BC, Canada	HQ919381	N.D.	N.D.	N.D.
GWS020774	Kwuna Island, Haida Gwaii, BC, Canada	HQ919382	N.D.	N.D.	N.D.
GWS020806	Telephone Point, Haida Gwaii, BC, Canada	HQ919383	N.D.	N.D.	N.D.
GWS020821	Telephone Point, Haida Gwaii, BC, Canada	HQ919384	N.D.	N.D.	N.D.
GWS020826	Telephone Point, Haida Gwaii, BC, Canada	HQ919385	N.D.	N.D.	N.D.
GWS020833	Telephone Point, Haida Gwaii, BC, Canada	HQ919386	N.D.	N.D.	N.D.
GWS020844	Indian Head, Haida Gwaii, BC, Canada	HQ919387	N.D.	N.D.	N.D.
GWS020852	Indian Head, Haida Gwaii, BC, Canada	HQ919388	N.D.	N.D.	N.D.
GWS020856	Indian Head, Haida Gwaii, BC, Canada	HQ919389	N.D.	N.D.	N.D.
GWS020860	Indian Head, Haida Gwaii, BC, Canada	HQ919390	N.D.	N.D.	N.D.
GWS020922	Indian Head, Haida Gwaii, BC, Canada	HQ919391	N.D.	N.D.	N.D.
GWS021083	Masset Inlet, Haida Gwaii, BC, Canada	HQ919394	N.D.	N.D.	N.D.
GWS021086	Masset Inlet, Haida Gwaii, BC, Canada	HQ919395	N.D.	N.D.	N.D.
GWS021125	Masset Inlet, Haida Gwaii, BC, Canada	HQ919412	N.D.	N.D.	N.D.
<i>Salishia chilensis</i> (J. Agardh) Clarkston & G.W. Saunders					
GWS000501	Ancud Bay, Chiloe Is., Chile	JF903345	N.D.	N.D.	AY171602
<i>Salishia firma</i> (Kyllin) Clarkston & G.W. Saunders					
B063	Dixon Is., Bamfield, BC, Canada	JF903365	N.D.	N.D.	N.D.
GWS000625	Seppings Is., Bamfield, BC, Canada	JF903359	N.D.	N.D.	N.D.
GWS000825	Dixon Is., Bamfield, BC, Canada	JF903364	N.D.	N.D.	N.D.
GWS000826	Dixon Is., Bamfield, BC, Canada	JF903357	N.D.	N.D.	N.D.
GWS000850	Seppings Is., Bamfield, BC, Canada	JF903355	N.D.	N.D.	N.D.
GWS001094	Dixon Is., Bamfield, BC, Canada	JF903363	N.D.	N.D.	N.D.
GWS001105	Seppings Is., Bamfield, BC, Canada	JF903354	N.D.	N.D.	N.D.
GWS001335	Dixon Is., Bamfield, BC, Canada	JF903384	N.D.	N.D.	N.D.
GWS002747	Dixon Is., Bamfield, BC, Canada	JF903381	N.D.	N.D.	N.D.
GWS002748	Dixon Is., Bamfield, BC, Canada	JF903379	N.D.	N.D.	N.D.
GWS003457	Dixon Is., Bamfield, BC, Canada	JF903387	N.D.	N.D.	N.D.
GWS003458	Dixon Is., Bamfield, BC, Canada	JF903385	JF903481	N.D.	N.D.
GWS003459	Dixon Is., Bamfield, BC, Canada	JF903383	N.D.	N.D.	N.D.
GWS003463	Dixon Is., Bamfield, BC, Canada	JF903382	N.D.	N.D.	N.D.
GWS003464	Dixon Is., Bamfield, BC, Canada	HM918576	N.D.	N.D.	N.D.
GWS003479	Dixon Is., Bamfield, BC, Canada	JF903361	N.D.	N.D.	N.D.
GWS003903	Dixon Is., Bamfield, BC, Canada	JF903380	N.D.	N.D.	N.D.
GWS003969	Seppings Is., Bamfield, BC, Canada	JF903360	N.D.	N.D.	N.D.
GWS003970	Seppings Is., Bamfield, BC, Canada	JF903358	JF903473	N.D.	N.D.
GWS003971	Seppings Is., Bamfield, BC, Canada	JF903356	N.D.	N.D.	N.D.
GWS003972	Seppings Is., Bamfield, BC, Canada	JF903374	JF903476	N.D.	N.D.
GWS003973	Seppings Is., Bamfield, BC, Canada	JF903373	N.D.	N.D.	N.D.
GWS004154	Otter Point, BC, Canada	JF903377	JF903479	JF903437	JF833329
GWS004158	Otter Point, BC, Canada	HM918635	N.D.	N.D.	N.D.
GWS004159	Otter Point, BC, Canada	JF903376	JF903478	N.D.	N.D.
GWS004162	Otter Point, BC, Canada	JF903375	JF903477	JF903436	N.D.
GWS004169	Otter Point, BC, Canada	JF903370	N.D.	N.D.	N.D.
GWS004209	Whiffen Spit, Sooke, BC, Canada	JF903388	JF903482	N.D.	N.D.
GWS004250	Saxe Pt., Victoria, BC, Canada	N.D.	JF903471	N.D.	N.D.
GWS004918	Stenhouse Reef, Prince Rupert, BC, Canada	JF903378	JF903480	N.D.	N.D.
GWS005025	Ridley Island, Prince Rupert, BC, Canada	JF903369	JF903475	N.D.	N.D.
GWS005026	Ridley Island, Prince Rupert, BC, Canada	JF903362	N.D.	N.D.	N.D.
GWS006579	Island #37, Tahsis, BC, Canada	HM916793	N.D.	N.D.	N.D.
GWS006583	Island #37, Tahsis, BC, Canada	HM916794	N.D.	N.D.	N.D.



Table 1. Continued

Species and voucher	Collection site	GenBank accession <sup>1,2</sup>			
		COI-5P	UPA	ITS	LSU
GWS006627	Island #40, Tahsis, BC, Canada	JF903368	N.D.	N.D.	N.D.
GWS006628	Island #40, Tahsis, BC, Canada	JF903366	JF903474	N.D.	N.D.
GWS006749	Friendly Cove, Tahsis, BC, Canada	JF903367	N.D.	N.D.	N.D.
GWS008181	Scotts Bay, Bamfield, BC, Canada	HM915785	N.D.	N.D.	N.D.
GWS008503	Backeddy Resort, Egmont, BC, Canada	JF903389	JF903483	N.D.	N.D.
GWS008504	Backeddy Resort, Egmont, BC, Canada	JF903386	N.D.	N.D.	N.D.
GWS009992	Island #40, Tahsis, BC, Canada	JF903372	N.D.	N.D.	N.D.
GWS009994	Island #40, Tahsis, BC, Canada	JF903371	N.D.	N.D.	N.D.
GWS010144	Flower Islet, Tahsis, BC, Canada	HM917195	N.D.	N.D.	N.D.
GWS010152	Flower Islet, Tahsis, BC, Canada	HM917200	N.D.	N.D.	N.D.
GWS010787	Seppings Is., Bamfield, BC, Canada	HM917330	N.D.	N.D.	N.D.
GWS010807	Seppings Is., Bamfield, BC, Canada	HM917335	N.D.	N.D.	N.D.
GWS012982	Scudder Pt., Gwaii Haanas, Haida Gwaii, BC, Canada	HM915409	N.D.	N.D.	N.D.
GWS013010	Scudder Pt., Gwaii Haanas, Haida Gwaii, BC, Canada	HM915524	JF903472	N.D.	N.D.
GWS013335	Murchison Island Lagoon, Gwaii Haanas, Haida Gwaii, BC, Canada	HM915670	N.D.	N.D.	N.D.
GWS013336	Murchison Island Lagoon, Gwaii Haanas, Haida Gwaii, BC, Canada	HM915607	N.D.	N.D.	N.D.
GWS013400	Burnaby Narrows, Gwaii Haanas, Haida Gwaii, BC, Canada	HM915657	N.D.	N.D.	N.D.
GWS013406	Burnaby Narrows, Gwaii Haanas, Haida Gwaii, BC, Canada	HM915638	N.D.	N.D.	N.D.
GWS013407	Burnaby Narrows, Gwaii Haanas, Haida Gwaii, BC, Canada	HM915647	N.D.	N.D.	N.D.
GWS013408	Burnaby Narrows, Gwaii Haanas, Haida Gwaii, BC, Canada	HM915658	N.D.	N.D.	N.D.
GWS013409	Burnaby Narrows, Gwaii Haanas, Haida Gwaii, BC, Canada	HM915666	N.D.	N.D.	N.D.
GWS013410	Burnaby Narrows, Gwaii Haanas, Haida Gwaii, BC, Canada	HM915673	N.D.	JF903435	N.D.
GWS013576	Newberry Cove, Gwaii Haanas, Haida Gwaii, BC, Canada	HM916282	N.D.	N.D.	N.D.
GWS013577	Newberry Cove, Gwaii Haanas, Haida Gwaii, BC, Canada	HM916292	N.D.	N.D.	N.D.
GWS013578	Newberry Cove, Gwaii Haanas, Haida Gwaii, BC, Canada	HM916302	N.D.	N.D.	N.D.
GWS013580	Newberry Cove, Gwaii Haanas, Haida Gwaii, BC, Canada	HM916245	N.D.	N.D.	N.D.
GWS013591	Newberry Cove, Gwaii Haanas, Haida Gwaii, BC, Canada	HM916246	N.D.	N.D.	N.D.
GWS019399	Execution Rock, Bamfield, BC, Canada	HQ544373	N.D.	N.D.	N.D.
GWS019783	Alder Island, Haida Gwaii, BC, Canada	HQ544617	N.D.	N.D.	N.D.
GWS019821	Alder Island, Haida Gwaii, BC, Canada	HQ544637	N.D.	N.D.	N.D.
GWS019828	Alder Island, Haida Gwaii, BC, Canada	HQ544643	N.D.	N.D.	N.D.
GWS019849	Alder Island, Haida Gwaii, BC, Canada	JF903348	N.D.	N.D.	N.D.
GWS020029	Saw Reef, Haida Gwaii, BC, Canada	HQ544719	N.D.	N.D.	N.D.
GWS020076	Saw Reef, Haida Gwaii, BC, Canada	HQ544746	N.D.	N.D.	N.D.
GWS020110	Saw Reef, Haida Gwaii, BC, Canada	JF903347	N.D.	N.D.	N.D.
GWS020204	East Copper Island, Haida Gwaii, BC, Canada	HQ544793	N.D.	N.D.	N.D.
GWS020258	East Copper Island, Haida Gwaii, BC, Canada	HQ544830	N.D.	N.D.	N.D.
GWS020261	East Copper Island, Haida Gwaii, BC, Canada	HQ544832	N.D.	N.D.	N.D.
GWS020329	East Copper Island, Haida Gwaii, BC, Canada	HQ544866	N.D.	N.D.	N.D.
GWS020484	Hot Spring Island, Haida Gwaii, BC, Canada	HQ544938	N.D.	N.D.	N.D.
GWS020485	Hot Spring Island, Haida Gwaii, BC, Canada	JF903346	N.D.	N.D.	N.D.
GWS020486	Hot Spring Island, Haida Gwaii, BC, Canada	HQ544939	N.D.	N.D.	N.D.
GWS020488	Hot Spring Island, Haida Gwaii, BC, Canada	HQ544940	N.D.	N.D.	N.D.
GWS020533	Kunga Island, Haida Gwaii, BC, Canada	HQ544962	N.D.	N.D.	N.D.
GWS020534	Kunga Island, Haida Gwaii, BC, Canada	HQ544963	N.D.	N.D.	N.D.
GWS020575	Tanuu Island, Haida Gwaii, BC, Canada	HQ544989	N.D.	N.D.	N.D.
GWS021338	Pigeon Point Lighthouse, Pescadero, California, USA	JF903353	N.D.	N.D.	N.D.
GWS021340	Pigeon Point Lighthouse, Pescadero, California, USA	JF903352	N.D.	N.D.	N.D.
GWS021339	Pigeon Point Lighthouse, Pescadero, California, USA	HQ544051	N.D.	N.D.	N.D.
GWS022123	Pigeon Point Lighthouse, Pescadero, California, USA	JF903351	N.D.	N.D.	N.D.
GWS022124	Pigeon Point Lighthouse, Pescadero, California, USA	JF903350	N.D.	N.D.	N.D.
GWS022282	Stillwater Cove, Pebble Beach, California, USA	JF903349	N.D.	N.D.	N.D.
GWS022291	Stillwater Cove, Pebble Beach, California, USA	HQ544228	N.D.	N.D.	N.D.
<i>Salishia sanguinea</i> (Montagne) Clarkston & G.W. Saunders					
GWS001160	Seapool Rock, Bamfield, BC, Canada	JF903417	N.D.	N.D.	N.D.
GWS001364	Satellite Passage Reef, Bamfield, BC, Canada	JF903415	N.D.	N.D.	N.D.
GWS003051	Seapool Rock, Bamfield, BC, Canada	JF903411	N.D.	N.D.	N.D.
GWS003053	Seapool Rock, Bamfield, BC, Canada	JF903410	N.D.	N.D.	N.D.
GWS003082	Satellite Passage Reef, Bamfield, BC, Canada	JF903406	JF903487	JF903440	JF833328
GWS003142	Seapool Rock, Bamfield, BC, Canada	JF903404	N.D.	N.D.	N.D.
GWS004152	Otter Point, BC, Canada	JF903420	JF903492	JF903443	N.D.
GWS004248	Saxe Pt., Victoria, BC, Canada	JF903414	N.D.	N.D.	N.D.
GWS004251	Saxe Pt., Victoria, BC, Canada	JF903418	N.D.	N.D.	N.D.
GWS004479	Seapool Rock, Bamfield, BC, Canada	JF903394	JF903484	N.D.	N.D.
GWS004497	Seapool Rock, Bamfield, BC, Canada	JF903416	JF903490	N.D.	N.D.



Table 1. Continued

Species and voucher	Collection site	GenBank accession <sup>1,2</sup>			
		COI-5P	UPA	ITS	LSU
GWS004500	Seapool Rock, Bamfield, BC, Canada	JF903408	N.D.	N.D.	N.D.
GWS004625	Vivian Is., Comox, BC, Canada	JF903407	N.D.	JF903441	N.D.
GWS004626	Vivian Is., Comox, BC, Canada	JF903398	N.D.	N.D.	N.D.
GWS004628	Vivian Is., Comox, BC, Canada	JF903397	N.D.	N.D.	N.D.
GWS004629	Vivian Is., Comox, BC, Canada	JF903396	N.D.	N.D.	N.D.
GWS004631	Vivian Is., Comox, BC, Canada	JF903419	JF903491	N.D.	N.D.
GWS004634	Vivian Is., Comox, BC, Canada	HM918676	N.D.	N.D.	N.D.
GWS004750	Browning Wall, Port Hardy, BC, Canada	HM918693	N.D.	N.D.	N.D.
GWS004759	Browning Wall, Port Hardy, BC, Canada	JF903395	JF903485	N.D.	N.D.
GWS004766	Browning Wall, Port Hardy, BC, Canada	HM918694	N.D.	N.D.	N.D.
GWS004778	Browning Wall, Port Hardy, BC, Canada	JF903422	N.D.	N.D.	N.D.
GWS004850	Tree Knob Islands Prince Rupert, BC, Canada	JF903393	N.D.	N.D.	N.D.
GWS004868	Tree Knob Islands Prince Rupert, BC, Canada	JF903403	N.D.	N.D.	N.D.
GWS004872	Tree Knob Islands Prince Rupert, BC, Canada	JF903392	N.D.	N.D.	N.D.
GWS004908	Stenhouse Reef, Prince Rupert, BC, Canada	JF903402	JF903486	JF903439	N.D.
GWS004914	Stenhouse Reef, Prince Rupert, BC, Canada	HM918719	N.D.	N.D.	N.D.
GWS004915	Stenhouse Reef, Prince Rupert, BC, Canada	JF903401	N.D.	JF903438	N.D.
GWS004916	Stenhouse Reef, Prince Rupert, BC, Canada	JF903400	N.D.	N.D.	N.D.
GWS004917	Stenhouse Reef, Prince Rupert, BC, Canada	JF903421	N.D.	N.D.	N.D.
GWS004919	Stenhouse Reef, Prince Rupert, BC, Canada	HM918720	N.D.	N.D.	N.D.
GWS004920	Stenhouse Reef, Prince Rupert, BC, Canada	HM918721	N.D.	N.D.	N.D.
GWS004921	Stenhouse Reef, Prince Rupert, BC, Canada	JF903413	N.D.	N.D.	N.D.
GWS004923	Stenhouse Reef, Prince Rupert, BC, Canada	HM918722	N.D.	N.D.	N.D.
GWS004928	Stenhouse Reef, Prince Rupert, BC, Canada	JF903412	JF903489	JF903442	N.D.
GWS004930	Stenhouse Reef, Prince Rupert, BC, Canada	JF903399	N.D.	N.D.	N.D.
GWS008686	Seapool Rock, Bamfield, BC, Canada	JF903423	N.D.	N.D.	N.D.
GWS008920	Seapool Rock, Bamfield, BC, Canada	JF903409	JF903488	N.D.	N.D.
GWS008922	Seapool Rock, Bamfield, BC, Canada	HM918845	N.D.	N.D.	N.D.
GWS008923	Seapool Rock, Bamfield, BC, Canada	HM918846	N.D.	N.D.	N.D.
GWS008924	Seapool Rock, Bamfield, BC, Canada	JF903405	N.D.	N.D.	N.D.
GWS008926	Seapool Rock, Bamfield, BC, Canada	JF903390	N.D.	N.D.	N.D.
GWS010142	Flower Islet, Tahsis, BC, Canada	HM917193	N.D.	N.D.	N.D.
GWS010145	Flower Islet, Tahsis, BC, Canada	HM917196	N.D.	N.D.	N.D.
GWS010150	Flower Islet, Tahsis, BC, Canada	HM917199	N.D.	N.D.	N.D.
GWS010162	Flower Islet, Tahsis, BC, Canada	HM917207	N.D.	N.D.	N.D.
GWS010163	Flower Islet, Tahsis, BC, Canada	HM917208	N.D.	N.D.	N.D.
GWS010164	Flower Islet, Tahsis, BC, Canada	HM917209	N.D.	N.D.	N.D.
GWS010372	Palliser Rock, Comox, BC, Canada	HM917256	N.D.	N.D.	N.D.
GWS012551	Tcenakun Point, Chaatl Island, Haida Gwaii, BC, Canada	HM915290	N.D.	N.D.	N.D.
GWS012553	Tcenakun Point, Chaatl Island, Haida Gwaii, BC, Canada	JF903391	N.D.	N.D.	N.D.
Family Dumontiaceae					
<i>Constantinea simplex</i> Setchell					
GWS002896	Bradys Beach, Bamfield, BC, Canada	JF903295	N.D.	N.D.	N.D.
<i>Dilsea carnos</i> a (Schmidel) Kuntze					
GWS000746	Europe	N.D.	N.D.	N.D.	<b>EF033609</b>
<i>Dilsea integra</i> (Kjellman) Rosenvinge					
GWS002334	Cape Breton, Canada	<b>AY970634</b>	N.D.	N.D.	N.D.
<i>Dudresnaya verticillata</i> (Withering) Le Jolis					
GWS001090	Co. Galway, Ireland	N.D.	N.D.	N.D.	<b>GU176301</b>
<i>Dumontia alaskana</i> Tai, Lindstrom & G.W. Saunders					
G0221	Shaman Is., Alaska, USA	N.D.	N.D.	N.D.	<b>AF419122</b>
<i>Dumontia contorta</i> (S.G. Gmelin) Ruprecht					
GWS001815	Mullaghmore Head, Ireland	<b>AY970583</b>	N.D.	N.D.	N.D.
<i>Farlowia mollis</i> (Harvey & Bailey) Farlow & Setchell					
GWS000845	Seppings Is., Bamfield, BC, Canada	JF903296	N.D.	N.D.	<b>GU176299</b>
<i>Gibsmithia dotyi</i> Kraft & R.W. Ricker					
GWS002048	Islands off Balls Pyramid, Lord Howe Island, Australia	N.D.	N.D.	N.D.	<b>GU176298</b>
<i>Gibsmithia hawaiiensis</i> Doty					
GWS001343	Maunaloa Bay, Oahu, Hawaii, USA	N.D.	N.D.	N.D.	<b>GU176297</b>
<i>Neodilsea borealis</i> (I.A. Abbott) Lindstrom					
GWS001681	Bear Cove Park, Port Hardy, BC, Canada	<b>AY970614</b>	N.D.	N.D.	N.D.
<i>Neodilsea natashae</i> Lindstrom					
G0224	Shaman Is., Alaska, USA	<b>AY970624</b>	N.D.	N.D.	JF928825



Table 1. Continued

Species and voucher	Collection site	GenBank accession <sup>1,2</sup>			
		COI-5P	UPA	ITS	LSU
<i>Kraftia dichotoma</i> Shepley & Womersley GWS000924	Queenscliff Jetty, Port Phillip Heads, Victoria, Australia	N.D.	N.D.	N.D.	<b>GU176296</b>
<i>Weeksia reticulata</i> Setchell GWS001705	Gospel Reef, Haida Gwaii Islands, BC, Canada	<b>EU189325</b>	N.D.	N.D.	JF928824
Family Rhizophyllidaceae					
<i>Portieria hornemannii</i> (Lyngbye) P.C. Silva G0232	Culture isolate	N.D.	N.D.	N.D.	<b>FJ848973</b>

<sup>1</sup> N.D. = not determined.

<sup>2</sup> Accession numbers in bold type indicate sequences were acquired from GenBank.

was used per sample; ABI, Foster City, CA, USA). Forward and reverse sequence reads (excluding the PCR primer regions) were edited using Sequencher<sup>TM</sup> 4.8 (Gene Codes Corporation, Ann Arbor, MI, USA).

### Sequencing alignments

COI-5P data were generated in this study for the *Beringia*, *Callophyllis*, *Pugetia* and *Salishia* species; two *Kallymeniopsis oblongifruca* specimens; and the dumontiacean out-group species *Constantinea simplex* and *Farlowia mollis* (Table 1). COI-5P data for *Erythrophyllum delesserioides* and the remaining *K. oblongifruca* were from a previously published study (see Clarkston & Saunders 2010), while the remaining dumontiacean sequences were downloaded from GenBank (Table 1). UPA data for all species, except *E. delesserioides* and *K. oblongifruca* (see Clarkston & Saunders 2010), were generated in this study. All the data for the ITS alignment were generated in this study (see Table 1). COI-5P, UPA and ITS data were uploaded to BOLD (Ratnasingham & Hebert 2007). The LSU alignment contained newly determined sequences for *Salishia firma* (GWS003082), *Salishia sanguinea* (GWS004154), *Pugetia fragilissima* (GWS004335), *Pugetia cryptica* (GWS004125), *Beringia wynnei* (GWS004863) and *Callophyllis laciniata* (Hudson) Kützing (GWS001795) combined with previously published sequences from members of the Kallymeniaceae downloaded from GenBank (Table 1; see Harper & Saunders 2002; Clarkston & Saunders 2010). LSU sequences from 10 species of the closely related families Dumontiaceae and Rhizophyllidaceae (Tai *et al.* 2001) were downloaded from GenBank and used to root the tree (see Table 1). The COI-5P, UPA, ITS and LSU sequences were aligned manually with the assistance of MacClade version 4.08 (Maddison & Maddison 2003).

### Molecular analyses

For each of the COI-5P, UPA and ITS datasets, genetic species groups were determined by distance analyses using the neighbor-joining algorithm in PAUP\* 4.0b10 (Swofford 2002). The intra- and interspecific sequence divergence values for each species group were determined in BOLD using the Kimura 2-parameter distance model.

Phylogenetic analyses were performed on the LSU data set (20 Kallymeniaceae + 10 Dumontiaceae/Rhizophyllidaceae)

and a data set combining the LSU and COI-5P sequences (14 Kallymeniaceae + 7 Dumontiaceae) to assess interspecific relationships among the Kallymeniaceae. For both the LSU only and LSU + COI-5P data sets, a maximum likelihood analysis was performed using PhyML 3.0 (Guindon & Gascuel 2003) with a general time-reversible substitution model (selected using jModeltest version 0.1.1; Posada 2008) and a PhyML-estimated proportion of invariable sites and gamma shape parameters. The starting tree was determined using BIONJ, nearest neighbor interchanges branch swapping was in effect and tree topology and branch lengths were optimized. Branch support was estimated using both nonparametric bootstrap resampling (1000 replicates) and the Shimodaira–Hasegawa-like approximate likelihood ratio test (aLRT). The unrooted tree was imported into Archaeopteryx (version 0.955 beta) and rooted with reference to the out-group Dumontiaceae and Rhizophyllidaceae. MrBayes 3.1 (Huelsenbeck 2001) was used to complete Bayesian analyses under a general time-reversible model on both the LSU data set and the combined LSU and COI-5P data set. For the combined LSU + COI-5P analysis, the data were partitioned by gene as well as by codon for the COI-5P data. For both the LSU only and the LSU + COI-5P analyses, sampling was performed every 1000 generations, and the run was replicated twice. Each analysis was run for three million generations, and an appropriate burn-in was estimated by plotting the overall likelihood against generations prior to estimating the posterior probability distribution. The final tree topology and posterior probability values for each analysis were based on the combined results from the stationary phase of the two independent runs.

### Morphological and anatomical analyses

Tissue for anatomical work was excised from herbarium specimens and either rehydrated in a 4% formaldehyde, 1% Tween<sup>®</sup> 20 detergent solution for 30 minutes and sectioned using a freezing microtome (CM 1850; Leica, Heidelberg, Germany) or peeled using a technique similar to Hansen & Lindstrom (1984). For peeling, pieces *c.* 2 cm in diameter were cut from the apical region of a blade and soaked for 1 hour each in 5% 1 N KOH and then water. The cortices were then teased apart using a razor and soft-touch forceps. A 1% analine blue in 6% 5 N hydrochloric acid stain was used for highlighting vegetative features and structures of the female reproductive system in all sections and peels; however,



sections were assessed for pigmentation prior to staining. Male specimens were not stained because the colourless spermatangia were not as easily detected once stained. Samples were permanently mounted in 50% corn syrup (with 4% formaldehyde to prevent microbial growth). Photomicrographs were recorded on a Leica DFC480 digital camera mounted on a Leica DM5000B light microscope. All images were imported into Adobe® Photoshop® CS (Adobe Systems Inc., San Jose, CA, USA) for plate assembly.

## RESULTS AND DISCUSSION

Based on our molecular and morphological results (discussed below) the following taxonomic changes are proposed: *Salishia* Clarkston & G.W. Saunders gen. nov., *Salishia firma* (Kylin) Clarkston & G.W. Saunders comb. nov., *Salishia sanguinea* (Montagne) Clarkston & G.W. Saunders comb. nov., *Salishia chilensis* (J. Agardh) Clarkston & G.W. Saunders comb. nov., *Pugetia cryptica* Clarkston & G.W. Saunders sp. nov. and *Beringia wynnei* Clarkston & G.W. Saunders sp. nov.

### Molecular results and discussion

Specimens from British Columbia that were field identified as *P. firma* resolved as three species groups using the COI-5P marker (Fig. 1). A specimen of *Pugetia chilensis* (here referred to as *Salishia chilensis*) from Chile was also included in our analysis and resolved as a distinct genetic group associated with one of the '*P. firma*' groups (here rendered the type of *Salishia* and referred to as *Salishia firma*). Both *S. firma* and *S. chilensis* grouped with another '*P. firma*' group (here referred to as *Salishia sanguinea*), while the third '*P. firma*' group (here referred to as *Beringia wynnei*) was highly divergent (Fig. 1). Specimens identified as *P. fragilissima* resolved as two separate but closely related groups (here referred to as *P. fragilissima* and *P. cryptica*; Fig. 1). In addition, *Callophyllis laciniata* from Northern Ireland resolved as a distinct species group and was included because of its affinity to *Pugetia* species rather than *Callophyllis* (discussed below), while *Kallymeniopsis oblongifruca* and *Erythrophyllum delesserioides* were included because of their close affinities with *Beringia wynnei*.

The UPA and ITS markers each resolved the same species groups as COI-5P (not shown). The level of intra- and interspecific sequence divergence varied for each marker with ITS being the most variable marker overall (except for between *K. oblongifruca*, *E. delesserioides* and *B. wynnei*, where COI-5P was most variable) and UPA the most conserved (Fig. 2). The level of intraspecific variation in this study is consistent with previously published data for red algae (Robba *et al.* 2006; Saunders 2008; Le Gall & Saunders 2010); for all species groups and all markers, the highest intraspecific variation was lower than the lowest interspecific variation. Also, in every case, the mean interspecific variation was at least 10× greater than the mean intraspecific variation.

To resolve the interspecific relationships of the species groups determined using COI-5P, we conducted phylogenetic analyses on two data sets: an LSU-only and a

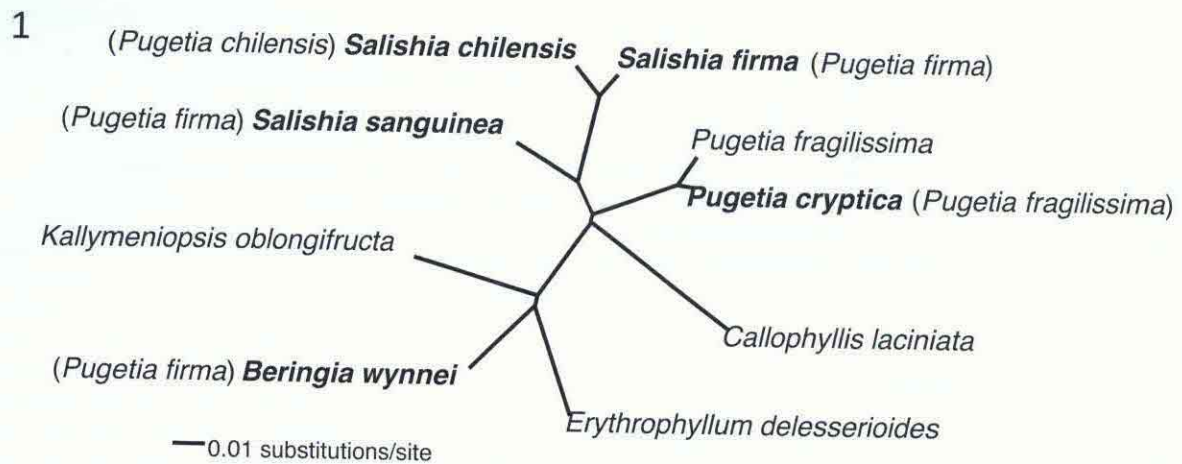
combined LSU and COI-5P data set. Phylograms inferred from Bayesian analyses are presented for the LSU ( $-\text{LnL} = 12,093.63$ ) and LSU+COI-5P data sets ( $-\text{LnL} = 13,402.84$ ) with posterior probabilities as well as bootstrap and aLRT support results for maximum likelihood analyses appended (Figs 3, 4). In all analyses, there was a fully supported alliance between *Pugetia fragilissima* and *P. cryptica* as well as a fully supported alliance between *Salishia firma*, *S. chilensis* and *S. sanguinea*. The divergence between the *Pugetia fragilissima/P. cryptica* cluster and the *S. firma/S. sanguinea/S. chilensis* cluster was greater than typically seen between genera of the Kallymeniaceae (Figs 3, 4), and the two clusters were separated by *Callophyllis laciniata* with weak to strong support, depending on the analysis (Figs 3, 4). All molecular evidence is consistent with generic division for *Pugetia* spp., which was also evident in our morphological and anatomical investigations (see *Taxonomic Results*). We consider *C. laciniata* as incertae sedis until further sampling (only one sample available here) and analyses can be conducted to determine its taxonomic affinities.

*Beringia wynnei* resolved in a strongly supported lineage with the species *Erythrophyllum delesserioides* and *Kallymeniopsis oblongifruca* in all our phylogenetic analyses (Figs 3, 4). The close allegiance of these taxa is surprising given that each is assigned to a separate genus. The traditional placement of *E. delesserioides* and *K. oblongifruca* in different genera is based on the high level of morphological divergence between them – *Erythrophyllum delesserioides* has a lanceolate blade with a midrib, filamentous medulla, and specialized papillae on the blade where reproductive structures develop (Kylin 1956), while *K. oblongifruca* has a foliose blade, filamentous medulla that also contains stellate-shaped, light-refracting cells and lacks specialized papillae (Hansen 1997). There are, however, reproductive characters that suggest a closer relationship – both are monocarpogonial and nonprocarpic, and after fertilization the fusion cell is formed from only the supporting cell and subsidiary cells (Norris 1957; Perestenko 1975). The morphology of *B. wynnei* is different from both *E. delesserioides* and *K. oblongifruca* because it has a foliose blade and compact medulla of round clear cells and small-celled filaments. Unfortunately, all of our specimens are vegetative. However, our phylogenetic analyses indicate that these are closely related species with divergent morphologies that will require taxonomic reassessment following a comprehensive sampling of the remaining species within these genera as well as other kallymeniacean genera that may fall into this cluster.

### Taxonomic results and discussion

The advent of molecular assisted alpha taxonomy and large-scale survey studies (e.g. Saunders 2008; Clarkston & Saunders 2010; Le Gall *et al.* 2010; Saunders & McDonald 2010) is quickly leading to a large number of genetically resolved species that require comparison to existing species concepts. Currently, the most reliable method for comparing type specimens to genetic species is via morphological and anatomical examinations. These examinations can be time consuming, require taxonomic expertise, and may not even be possible in some cases (e.g. specimen is degraded).





2

Species	Marker	Intraspecific divergence (%)		Interspecific divergence to nearest neighbour (%)	
		Highest	Mean	Lowest	Mean
<b><i>Salishia chilensis</i></b> ( <i>Pugetia chilensis</i> )	COI-5P (n=1)	-	-	2.47 (to <i>S. firma</i> )	2.52
	ITS	-	-	-	-
	UPA	-	-	-	-
<b><i>Salishia firma</i></b> ( <i>Pugetia firma</i> )	COI-5P (n=86)	0.31	0.02	6.75 (to <i>S. sanguinea</i> )	7.15
	ITS (n=3)	0.15	0.10	19.6	19.9
	UPA (n=12)	0	0	1.09	1.09
<b><i>Salishia sanguinea</i></b> ( <i>Pugetia firma</i> )	COI-5P (n=51)	0.31	0.03	6.75	7.15
	ITS (n=6)	0.14	0.08	19.6	19.9
	UPA (n=9)	0	0	1.09	1.09
<b><i>Pugetia fragilissima</i></b> ( <i>P. fragilissima</i> )	COI-5P (n=83)	0.33	0.09	1.39	1.58
	ITS (n=4)	0.45	0.20	1.83	2.01
	UPA (n=11)	0	0	0.54	0.54
<b><i>Pugetia cryptica</i></b> ( <i>Pugetia fragilissima</i> )	COI-5P (n=14)	0.33	0.07	1.39	1.58
	ITS (n=4)	0.36	0.18	1.83	2.01
	UPA (n=7)	0	0	0.54	0.54
<b><i>Beringia wynnei</i></b> ( <i>Pugetia firma</i> )	COI-5P (n=17)	0.62	0.15	7.70	7.94
	ITS (n=2)	0.15	-	4.60	4.68
	UPA (n=9)	0	0	1.08	1.08
<b><i>Erythrophyllum delesserioides</i></b>	COI-5P (n=15)	0.62	0.16	7.70	7.94
	ITS (n=2)	0.31	0.31	3.06	3.26
	UPA (n=12)	0.27	0.14	1.89	2.00
<b><i>Kallymeniopsis oblongifruca</i></b>	COI-5P (n=22)	0.77	0.24	8.06	8.52
	ITS (n=2)	0	-	3.06	3.26
	UPA (n=10)	0	-	1.08	1.08
<b><i>Callophyllis laciniata</i></b>	COI-5P (n=1)	-	-	10.85	10.92
	ITS (n=1)	-	-	22.87	23.25
	UPA	-	-	-	-

**Figs 1, 2.** Cryptic and overlooked diversity within the Canadian Kallymeniaceae revealed using the DNA barcode (COI-5P).

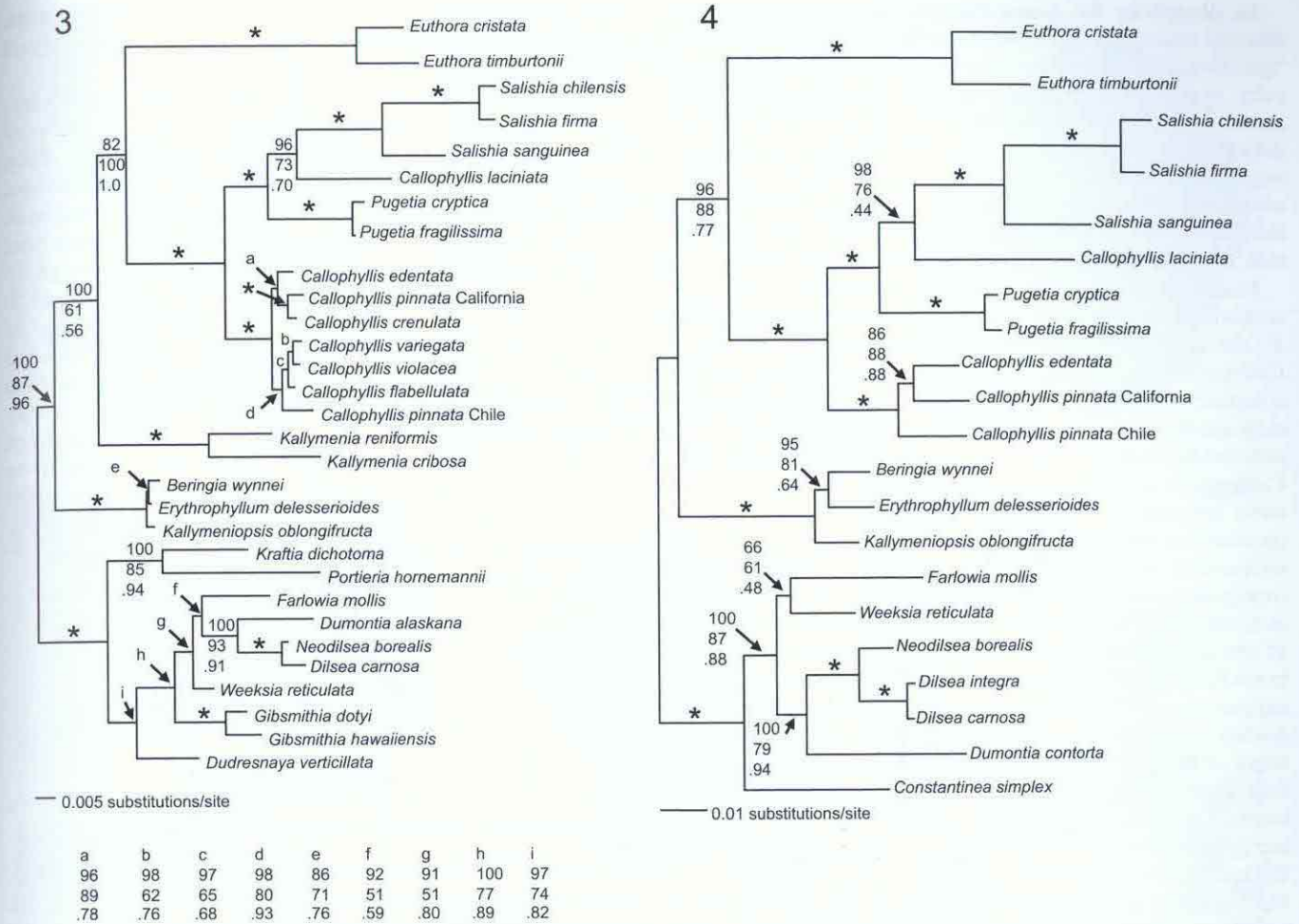
**Fig. 1.** Genetic species groups displayed as an unrooted phylogenetic tree inferred from the DNA barcode sequences.

**Fig. 2.** Intra- and interspecific divergence values (%) for each species group and molecular marker used in this study [COI-5P, the internal transcribed spacer of the ribosomal cistron (ITS), and the universal plastid amplicon (UPA)]. Where taxonomic changes are proposed, names in parentheses indicate the name assigned to each species group in the field using a taxonomic key, and names in bold indicate the taxonomic revisions made during this study. *Salishia firma* is compared to *S. chilensis*, its closest neighbour for COI-5P, and, because of missing data, to *S. sanguinea* for all three of the markers tested.

Genetic comparisons between type material and contemporary collections would be ideal, and the acquisition of viable DNA sequences from older herbarium specimens has been reported (Hughey *et al.* 2001; Gabrielson 2008).

However, a recent study concluded that the likelihood of successful amplification of (the correct) DNA from archival collections decreases as the age of the collection increases and that cross-contamination is a constant and





Figs 3, 4. Inclusion of the newly-determined species groups in phylogenetic analyses of representative Kallymeniaceae.

Fig. 3. Phylogram inferred by Bayesian analyses of LSU data (letters denote support values listed below phylogram).

Fig. 4. Phylogram inferred from a combined data set including LSU and COI-5P. Support values are listed for both phylogenies as Bayesian posterior probabilities and bootstrap and aLRT values for maximum likelihood analyses, respectively. Asterisks denote nodes that are strongly supported (posterior probability = 100%, bootstrap  $\geq$  95% and aLRT values  $\geq$  0.95) in all analyses.

serious concern (Saunders & McDevit, in press). We believe that for situations in which a species concept (type) cannot be applied unequivocally to one species among a cryptic complex, the best solution for incorporating historical information from type collections into modern species concepts is to designate an epitype from contemporary collections for which genetic data are available. In light of our molecular results, we combine detailed anatomical observations, information related to biogeography and ecology and the judicious use of epitypes to provide our best appraisal of species diversity and distribution for the genus *Pugetia* emphasizing the northeastern Pacific. This is acknowledged as only an interim step forward; the taxonomic status of many of the species will remain in question until new collections are made from the type localities that can be genetically compared to other species.

#### *Pugetia* Kylin 1925, p. 31, fig. 14

REVISED DESCRIPTION: Thallus flat, thin, delicate, roughly orbicular in shape or slightly wider than tall, typically

with ruffled or sinuate margins, sometimes with holes (ontogenetic?). Blades erupt abruptly from a small (< 1 mm) discoid holdfast or a short stipe. Medulla of relatively large round to oval, unpigmented cells interspersed with filaments of small, round to elongate, pigmented cells. Cortex two to three layers. Monocarpogonial and nonprocarpic. Carpogonial branch cells circular to slightly lobed.

Tetrasporophytes isomorphic relative to the gametophytes, with cruciately divided tetrasporangia scattered throughout the cortex.

TYPE SPECIES: *Pugetia fragilissima* Kylin.

COMMENTS: Morphological examination of the *Pugetia* and *Salishia* species revealed distinct differences between the two groups. *Pugetia fragilissima* and *P. cryptica* had thin, delicate thalli; were nonprocarpic, with round, oval or slightly lobed carpogonial branch cells; and produced small cystocarps, while *S. firma*, *S. sanguinea* and *S. chilensis* had thicker, more robust thalli (but see below regarding *S. chilensis*); were procarpic, with carpogonial branches with highly lobed supporting, subsidiary and first branch cells; and produced large cystocarps.



In describing the genus *Pugetia*, Kylin (1925) did not discuss postfertilization development except to note that 'the development [of the procarp and gonimoblast] comes very near that one in *Callophyllis*' (Kylin 1925). Kylin (1941) subsequently described *S. firma* (as *P. firma*), delimiting it from his earlier-described *P. fragilissima* using vegetative features – *S. firma* having smaller blades, more cortical cells layers, and an overall thicker and more robust thallus – with no discussion of differences in postfertilization development between the two species.

Norris (1957) emphasized female reproductive characters to distinguish *Pugetia* and *Callophyllis*, and he believed that *S. firma* (as *P. firma*) was more similar to species of *Callophyllis* than to *P. fragilissima* with regards to the structure of the female reproductive system (i.e. the carpogonial branch and auxiliary cell systems) and in the postfertilization development of the cystocarp. In both *Callophyllis* and *S. firma*, the auxiliary cell is part of the same carpogonial branch as the fertilized carpogonium (procarpic), while in *P. fragilissima* the auxiliary cell is the supporting cell of a separate carpogonial branch system (nonprocarpic) (Norris 1957). Immediate postfertilization in *Callophyllis* and *S. firma* reportedly involves the transfer of the diploid nucleus to the first cell of the carpogonial branch, and a fusion cell forms from the first branch cell, supporting cell, and any subsidiary cells (Norris 1957). The gonimoblasts develop directly from the fusion cell. The lobes of the enlarged fusion cell cut off cells at the tips (the first gonimoblast cells) that divide or greatly enlarge and eventually produce carposporangial mother cells. Immediate postfertilization in *P. fragilissima* is presumed to involve the transfer of the diploid nucleus from the carpogonium to the supporting cell, which enlarges and produces lobed protuberances that give rise to long, nonseptate connecting filaments (Norris 1957). The connecting filaments grow through the medulla and reportedly connect with auxiliary cells, which then enlarge and produce lobes from which septate gonimoblast filaments are produced. The gonimoblast filaments produce many short branches whose terminal cells repeatedly divide to produce clusters of cells that either develop directly into carposporangia or further divide and ultimately develop carposporangia. Based on his observations of female reproductive characters and postfertilization development, Norris concluded that *S. firma* was more appropriately placed in the genus *Callophyllis* than *Pugetia*, and he subsequently made the combination *Callophyllis firma* (Kylin) R.E. Norris (= *S. firma*).

We observed structures in *P. fragilissima* (presented below) consistent with what Norris (1957, p. 270, fig. 4C) diagrammed as the fusion cell producing connecting filaments and the auxiliary cell system producing septate gonimoblasts (Norris 1957, p. 271, fig. 5C), which supports Norris's interpretation that *P. fragilissima* is nonprocarpic. We observed many postfertilization structures in *S. firma* and *S. sanguinea* but saw no evidence that they are nonprocarpic, consistent with Norris's interpretation that *S. firma* is procarpic. In emphasizing vegetative attributes, Kylin (1941) was correct in associating *S. firma* with *Pugetia* rather than *Callophyllis*, while Norris (1957), in emphasizing reproductive attributes, clearly established

that an alliance with *Pugetia* was inappropriate. Here we combine the previous incongruency with molecular analyses to propose a third taxonomic hypothesis.

Harper & Saunders (2002) included the first molecular data for *S. firma* (as *C. firma*) in a phylogeny of representative Kallymeniaceae using LSU sequences. They found that *S. firma* grouped with *P. fragilissima* and not with *Callophyllis* and accordingly transferred *S. firma* back to *Pugetia*. However, they also noted that *S. firma* was quite divergent from *P. fragilissima*, perhaps enough to justify a new genus to accommodate it, but they retained *S. firma* within *Pugetia* pending a larger molecular study of the genus and anatomical assessment of the included species. Here we provide such an assessment and conclude that strong molecular and morphological differences justify the removal of *S. firma*, *S. sanguinea* and *S. chilensis* from *Pugetia* and which also argue against their transfer to *Callophyllis*. As such, the new genus *Salishia* is proposed below, leaving only *P. fragilissima*, *P. cryptica*, *P. japonica*, *P. kylinii*, *P. delicatissima*, *P. latiloba*, *P. porphyroidea*, *P. harveyana* and *P. mexicana* in the genus *Pugetia*.

#### *Pugetia fragilissima* Kylin 1925, p. 31, fig. 14

Figs 5–10

HOLOTYPE: Kylin, 1924 (LD 090095; cystocarpic; Figs 5, 6).

TYPE LOCALITY: Peavine Pass, Canoe Is., Friday Harbor, Washington State, USA, dredged (30–60 ft).

EPITYPE: B. Clarkston, G.W. Saunders & D. McDevit, June 27, 2006 (UNB GWS004559; cystocarpic) (Figs 9, 10), Satellite Passage Reef (lat. 48°51'43.2", long. 125°10'37.2"), Bamfield, British Columbia, Canada, subtidal (40 ft), on hard animals and corallines. A morphological examination of the *P. fragilissima* holotype (LD 090095; Figs 5, 6) revealed it to be nearly indistinguishable morphologically from either of our species groups; therefore, we assign the holotype to the one which is the best match in blade thickness, medullary cell size and habitat and which is also the most commonly collected of the two groups.

CONFIRMED DISTRIBUTION: Thus far known with certainty to be widely distributed only throughout British Columbia at the Haida Gwaii Islands, Prince Rupert and Vancouver Island (see Table 1), and, if our taxonomic conclusions are correct, the type locality at nearby San Juan Islands, Washington State, USA.

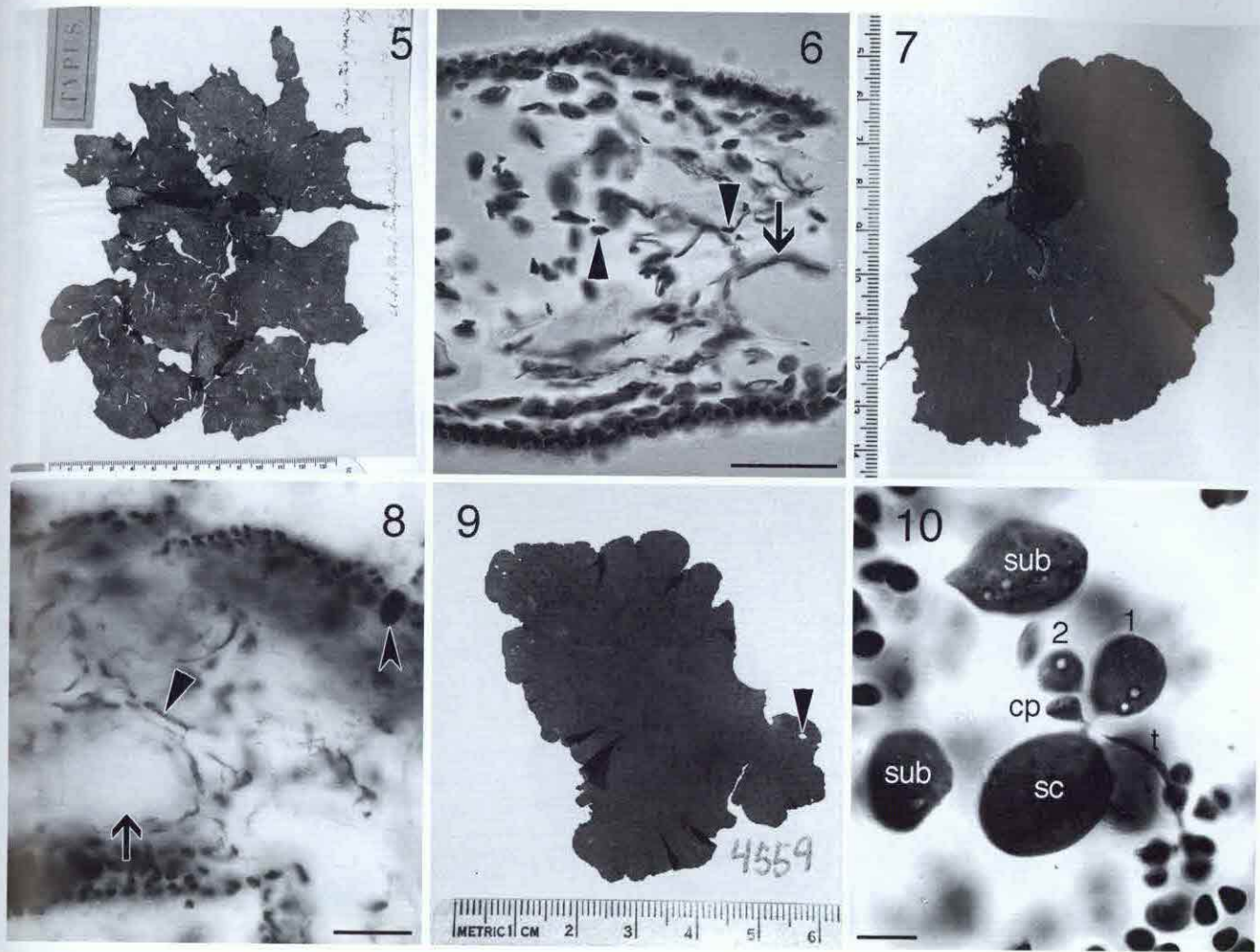
EPITYPE DNA BARCODE: JF903314.

REPRESENTATIVE DNA BARCODES: See Table 1.

REFERENCES: Kylin (1925), Doty (1947), Norris (1957), Hollenberg & Abbott (1966), Lindstrom (1977), Scagel *et al.* (1989), Hansen (1997), and Harper & Saunders (2002). It is important to acknowledge that these references (other than the type description) could apply to *P. fragilissima* and/or *P. cryptica* and must be interpreted cautiously.

HABIT AND VEGETATIVE ANATOMY: All 83 *P. fragilissima* specimens collected during this study were found in the





Figs 5–10. Morphology and anatomy of *Pugetia fragilissima* Kylin.

Fig. 5. Pressed voucher for LD 090095 (holotype; cystocarpic). Scale bar = centimeter ruler.

Fig. 6. Vegetative cross section showing large, unpigmented cells (arrow) interspersed with filaments of small, pigmented cells (arrowheads) (LD 090095). Scale bar = 50  $\mu$ m.

Fig. 7. Pressed voucher for GWS003267 (tetrasporophyte). Scale bar = centimeter ruler.

Fig. 8. Vegetative cross section showing large, unpigmented cells (arrow) interspersed with filaments of small, pigmented cells (arrowhead) and tetrasporangia scattered in the cortex (concave arrowhead (GWS003267)). Scale bar = 50  $\mu$ m.

Fig. 9. Pressed voucher for GWS004559 (epitype; cystocarpic) with possible ontogenetic holes (arrowhead). Scale bar = centimeter ruler.

Fig. 10. Close-up of single, three-celled carpogonial branch attached to a supporting cell with two detached subsidiary cells (t, trichogyne; cp, carpogonium; 1, 2 indicate branch cells progressively distal to the supporting cell; sc, supporting cell; sub, subsidiary cell) (GWS004559). Scale bar = 10  $\mu$ m.

subtidal (15–50-ft depth) growing on either rock or invertebrates such as polychaete worm tubes or barnacles.

Mature plants were up to 9 (14) cm in height and 9 cm in width (Figs 5, 7, 9). Some blades were entire, others lacinate (Fig. 5), the lacinae formed from tears in the blade, some blades had lobes and/or circular holes.

Blades were 130–250  $\mu$ m thick near the apex. The large medullary cells were 28–150  $\mu$ m wide  $\times$  32–100  $\mu$ m high, (Fig. 6). The intercalating filaments were abundant in older regions and less common in younger regions to the point of being difficult to find (Fig. 8). The cortex contained an inner layer of periclinal cells and one, rarely two, outer layers of round, pigmented cells (4–9  $\mu$ m wide  $\times$  4–6  $\mu$ m high; Figs 6, 8).

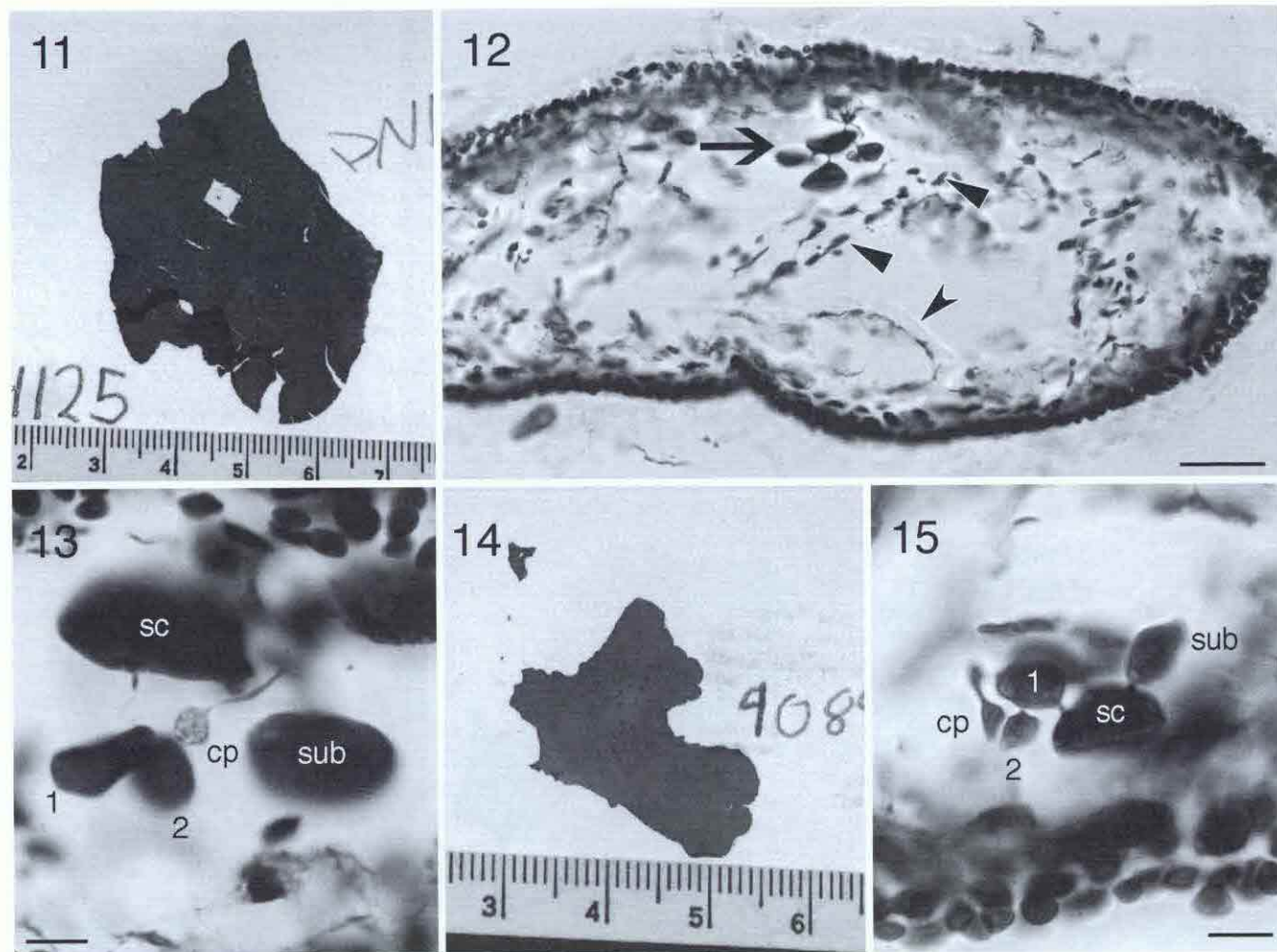
**REPRODUCTIVE ANATOMY:** Carpogonial branches were typical for the genus (Fig. 10). Mature cystocarps varied in size [190–490 (1040)  $\mu$ m, mean = 320  $\mu$ m] and had a

single ostiole. Tetrasporangia were 10–17.5  $\mu$ m wide  $\times$  12.5–30  $\mu$ m high (Fig. 8). Male gametophytes unreported in this species.

**COMMENTS:** The two closely related '*fragilissima*' species groups were easily delimited in all of our genetic analyses (Figs 1–4). However, the two species could not be differentiated based on morphology alone except in blade thickness and medullary cell size, but these differences are not absolute, as only a few blades of either species could be fully rehydrated and sectioned successfully.

Norris (1957) examined an isotype specimen of *P. fragilissima* (UC 279582), as well as several collections from the type locality and from British Columbia to California, and reported that carpogonial branch cells of *P. fragilissima*, except the carpogonium, were multinucleate and that two-celled subsidiary cell branches and two carpogonial





Figs 11–15. Morphology and anatomy of *Pugetia cryptica* Clarkston & G.W. Saunders sp. nov.

Fig. 11. Pressed voucher for GWS004125 (holotype; cystocarpic). Scale bar = centimeter ruler.

Fig. 12. Cross section showing large, unpigmented cells (concave arrowhead) interspersed with filaments of small, pigmented cells (arrowheads) and location of carpogonial branch just below cortex and extending into the medulla (arrow) (GWS004125). Scale bar = 50  $\mu$ m.

Fig. 13. Squash mount showing a single, three-celled carpogonial branch attached to a supporting cell with a detached subsidiary cell (cp, carpogonium; 1, 2 indicate branch cells progressively distal to the supporting cell; sc, supporting cell; sub, subsidiary cell) (GWS004125). Scale bar = 10  $\mu$ m.

Fig. 14. Pressed voucher for GWS009089 (cystocarpic). Scale bar = centimeter ruler.

Fig. 15. Cross section showing a single, three-celled carpogonial branch attached to a supporting cell with one subsidiary cell (abbreviations as for Fig. 13) (GWS009089). Scale bar = 10  $\mu$ m.

branches per supporting cell occurred occasionally in this species. However, these reports should be followed with caution in light of the cryptic diversity revealed in this study.

*Pugetia cryptica* Clarkston & G.W. Saunders sp. nov.

Figs 11–15

*Plantae sub recessu aestuum vel interdum modice super recessum aestuum crescentes. Thalli plani, plusminusve orbiculares, delicati, marginibus integris vel laciniatis, undulatis vel laevibus. Medulla constans e cellulis comparate magnis (25–68  $\mu$ m latis  $\times$  23–48  $\mu$ m altis), rotundis vel ovalibus, pellucidis et filamentis cellularum parvarum rotundarum vel elongatarum pigmentiferarum. Cortex e 2–3 stratis cellularum parvarum pigmentiferarum constans. Monocarpogoniales. Cellulae ramorum carpogonialium rotundae. A *P. fragilissima* sequentiis nucleotidorum COI-5P, ITS, UPA, et LSU distinguendae.*

Plants subtidal, occasionally lowest intertidal. Thalli flat, roughly orbicular, and delicate, margins entire to lacinate, ruffled or smooth. Medulla of relatively large (25–68  $\mu$ m wide  $\times$  23–48  $\mu$ m high), round to oval, clear cells with filaments of small, round to elongate, pigmented cells. Cortex two to three layers of small, pigmented cells. Monocarpogonial. Carpogonial branch cells rounded. Distinguished from *P. fragilissima* by COI-5P, ITS, UPA and LSU sequence data.

HOLOTYPE: B. Clarkston, G.W. Saunders, and D. McDevit, 18 June 2006 (UNB GWS004125; cystocarpic; Figs 11–13).

TYPE LOCALITY: Scotts Bay (lat. 48°50'6", long. –125°8'45.6"), Bamfield, British Columbia, Canada, subtidal (30 ft), on rock.

DISTRIBUTION: Throughout the Haida Gwaii Islands, Vancouver Island and the Sunshine Coast in British Columbia, Canada (see Table 1).



ETYMOLOGY: Named for its cryptic habit relative to *P. fragilissima*.

HOLOTYPE DNA BARCODE: JF903308.

REPRESENTATIVE DNA BARCODES: See Table 1.

HABIT AND VEGETATIVE ANATOMY: The majority of *P. cryptica* specimens (13) were collected in the subtidal (20–65-ft depth), and two were collected intertidally at a site that was unusual in that there were a number of typically subtidal species present in the low intertidal. Most specimens were growing on rock, a few were on polychaete worm tubes.

Most mature plants were up to 5 cm in height and 5.5 cm in width (Figs 11, 14), with one outlier that was 13 cm in height and 19 cm in width (GWS004239; Table 1). Some blades were entire, others were lacinate, the lacinae formed from tears in the blade, and others had lobes.

Blades were 98–120  $\mu\text{m}$  thick near the apex. The large medullary cells were 25–68  $\mu\text{m}$  wide  $\times$  23–48  $\mu\text{m}$  high (Fig. 12). The intercalating filaments were abundant in older regions and less common in younger regions to the point of being difficult to find. The cortex contained an inner layer of periclinal cells and one, rarely two, outer layers of round, pigmented, smaller cells (3–9  $\mu\text{m}$  long  $\times$  3–6  $\mu\text{m}$  wide; Fig. 12).

REPRODUCTIVE ANATOMY: Carpogonial branches were typical for the genus (Figs 12, 13, 15). Mature cystocarps varied in size (170–690  $\mu\text{m}$ , mean = 290  $\mu\text{m}$ ) and had a single ostiole. No tetrasporophytes or male gametophytes were observed in this study.

COMMENTS: This species was far less common in British Columbia than *P. fragilissima*, at least from the locations and seasons that sampling was conducted for this study (see Table 1 for location and <http://www.barcodinglife.org> for collection date), which could indicate a phenological difference. Two of the *P. cryptica* specimens were found in the lowest intertidal; whereas, all the *P. fragilissima* specimens were collected in the subtidal below 15 ft, suggesting these two species may have different habitat ranges.

*Pugetia latiloba* (W.R. Taylor) R.E. Norris 1957, p. 216, fig. 6A, pl 32

Fig. 16

BASIONYM: *Kallymenia latiloba* W.R. Taylor 1945, p. 216, fig. 1, pl 71.

HOLOTYPE: W.R. Taylor, 31 January 1934 (UC 1884384; cystocarpic). Taylor labeled at least three presses with the collection number 34-417, one of which was sent to AHFH and the others to MICH (Taylor 1945). There has been some confusion regarding which press should be considered the holotype for this species because there are multiple collections, and Taylor included a photograph of an isotype (MICH 1306499) in his manuscript (Taylor 1945). However, Taylor (1945, p. vi) specifically designated the collections sent to AHFH (AHFH108) as the 'technical types'

(= holotypes) for his newly described species, and there is only one press of *P. latiloba* housed at AHFH (now at UC); therefore, it is the holotype.

TYPE LOCALITY: Gardner Bay, I. Espanola, (Hood Island), Galapagos Is., Ecuador, dredged (37–55 m).

DISTRIBUTION: as *Kallymenia latiloba* W.R. Taylor: Galapagos Islands (Taylor, 1945).

REFERENCES: Taylor (1945) (as *K. latiloba*) and Norris (1957) (as *P. latiloba*).

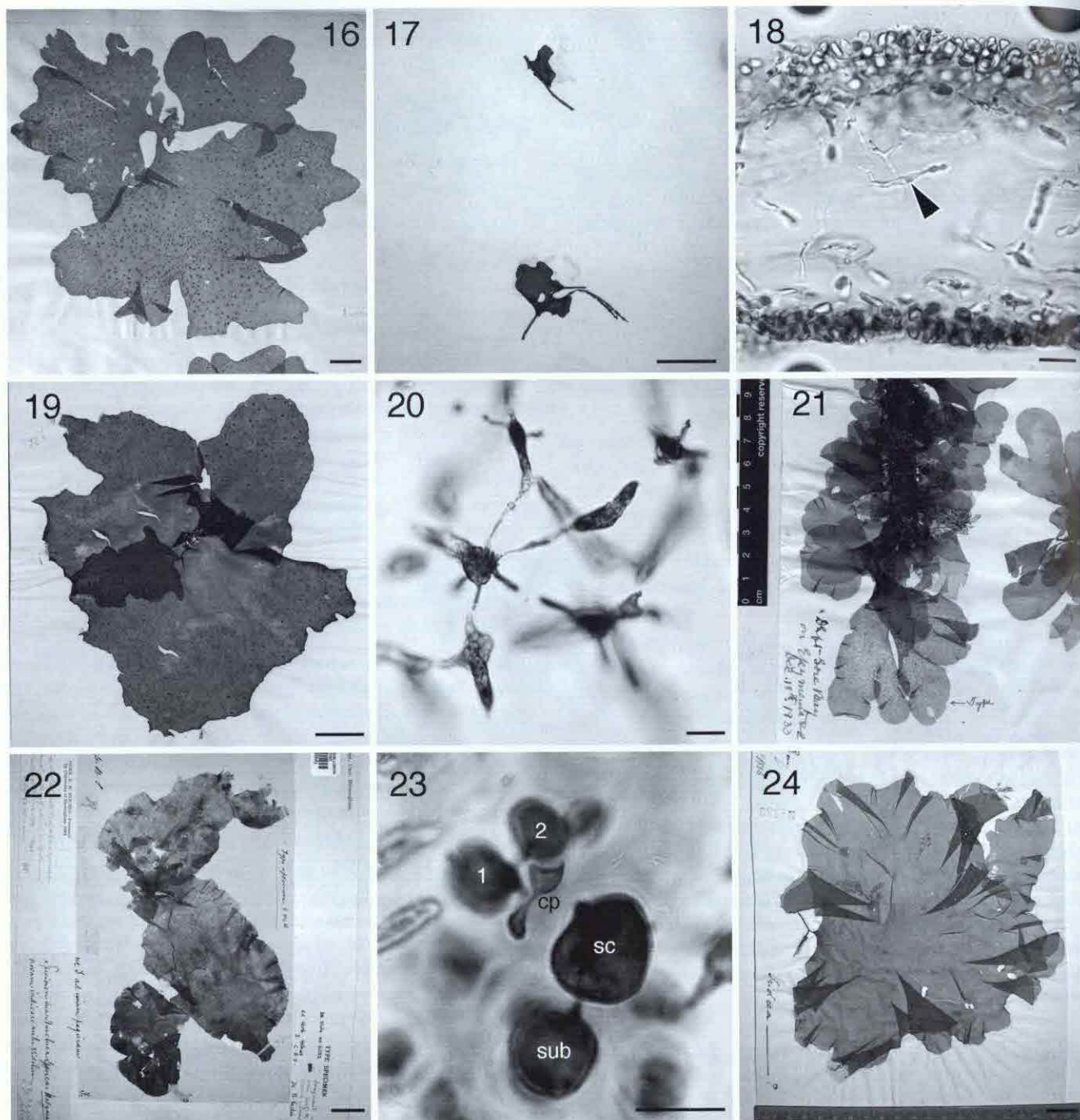
HABIT AND VEGETATIVE ANATOMY: We examined and sectioned one isotype (MICH 1309830; cystocarpic; Fig. 16) and also superficially examined another (MICH 1306499; cystocarpic). The thalli were flat and divided into two to three cuneate main segments that were bluntly lobed at the margins and substantially narrowed (to 5–8 mm wide) near the small holdfast. The isotypes examined were 10–12 cm in height, and Taylor (1945) reported mature plants up to 15 cm high and 25 cm wide. The blades were reported to be 200–300  $\mu\text{m}$  thick (Taylor 1945). We observed a sparsely filled medulla of elongate, irregularly shaped cells; however, this was likely an artifact of poor rehydration, which made intact sections difficult to obtain. Taylor (1945) reported a distinctly thick cuticle for this species, which we did not observe.

REPRODUCTIVE ANATOMY: We did not observe any intact female reproductive structures. Norris (1957) described this species as polycarpogonial, with three to six carpogonial branches produced per supporting cell, and nonprocarpic in postfertilization development. Mature cystocarps were large (1–2 mm) with large ostioles (Taylor 1945). Taylor (1945) reported tetrasporangia as 21–25  $\mu\text{m}$  wide  $\times$  28–32  $\mu\text{m}$  high. Male gametophytes unreported in this species.

COMMENTS: Taylor (1945) described *Kallymenia latiloba* from subtidal (18–55 m) collections made from several sites on the Galapagos Islands. He reported the medulla as 'a very sparse, loose arachnoid tissue ... with delicate, somewhat refractive fibers' and accordingly placed the species in *Kallymenia*. Norris (1957) examined an isotype (UC 694841) and reported an internal anatomy more typical of *Pugetia*. He subsequently made the new combination *Pugetia latiloba* (Taylor) R.E. Norris. Based on our limited morphological examination of the type material, we concluded that none of our species groups were a match to *P. latiloba*.

In an effort to determine whether *P. latiloba* should be assigned to *Pugetia* or *Salishia*, we noted that *P. latiloba* is nonprocarpic, like *Pugetia*. The auxiliary system cells, which Norris (1957, p. 274, fig. 6A) reported as identical in form to the carpogonial branch cells, are rounded to slightly oval in shape, similar to *Pugetia*. However, the blades we examined were bluntly lobed at the margins and not ruffled, which is more consistent with *Salishia*. Additionally, the report by Norris (1957) that *P. latiloba* is a polycarpogonial species is unusual for both *Pugetia* and *Salishia*. Until fresh collections of this species are examined morphologically and included in molecular analyses, we tentatively retain this species in *Pugetia*.





**Figs 16–24.** Morphology and anatomy of additional *Pugetia* species examined in this study.

**Fig. 16.** Isotype of *Pugetia latiloba* (W.R. Taylor) R.E. Norris (MICH 1309830; cystocarpic). Scale bar = 2 cm.

**Fig. 17.** Isotype of *Pugetia kylinii* Baardseth (BM 000530325; vegetative). Scale bar = 2 cm.

**Fig. 18.** Vegetative cross section of *P. kylinii* showing sparse medulla containing filaments of distinctly elongate cells (arrowhead) (BM 000530325). Scale bar = 50  $\mu$ m.

**Fig. 19.** Holotype of *Pugetia japonica* Kylin (LD 090348; cystocarpic). Scale bar = 2 cm.

**Fig. 20.** Close-up of distinctive, stellate appearance of smaller, highly pit-connected medullary cells in *P. japonica* (LD 090348). Scale bar = 10  $\mu$ m.

**Fig. 21.** Type specimen of *Pugetia delicatissima* R.E. Norris (UC 513609; cystocarpic). Scale bar = centimeter ruler.

**Fig. 22.** Type specimen of *Pugetia porphyroidea* (F. Schmitz ex Holmes) R.E. Norris (BM 000530348; cystocarpic). Scale bar = 2 cm.

**Fig. 23.** Squash mount of *P. porphyroidea* showing a three-celled carposporangial branch detached from a round supporting cell and subsidiary cell (cp, carposporangium; 1, 2 indicate branch cells progressively distal to the supporting cell; sc, supporting cell; sub, subsidiary cell) (BM 000530348). Scale bar = 10  $\mu$ m.

**Fig. 24.** Lectotype of *Pugetia harveyana* (J. Agardh) R.E. Norris (LD 090097; tetrasporophyte). Scale bar = 2 cm.



***Pugetia kylinii* Baardseth 1941, p. 63, figs. 29C, 31**

Figs 17, 18

SYNTYPE LOCALITIES: Sandy Point (st. 40), Tristan da Cunha Island; Landing (st. 117), Nightingale Island; South Coast (st. 86), Middle Island; Blenden Hall (st. 140, 142, 150), South Point (st. 156), Inaccessible Island, Tristan da Cunha.

DISTRIBUTION: Tristan da Cunha (Baardseth, 1941).

REFERENCES: Baardseth (1941) and Norris (1957).

HABIT AND VEGETATIVE ANATOMY: Baardseth (1941) described *P. kylinii* from several collections made around the Atlantic islands of Tristan da Cunha, at depths of 9–24 ft. We examined an isotype (BM 000530325; vegetative; Figs 17, 18) from Inaccessible Island, which included three plants, each epiphytic on terete algae and from a depth of 15–24 ft. The blades were small (0.5–1.5 cm; Fig. 17), roughly orbicular in shape and expanded immediately from a small discoid holdfast. The blades had previously been stained with safranin and could not be restained with aniline blue, and they rehydrated poorly, which made intact sections difficult to obtain. Baardseth (1941) reported the blades were 150 µm thick. We observed a medulla with a loose arrangement of elongate filaments (Fig. 18) and very few large round cells. Though Baardseth did not state it, his diagrams (1941, p. 64, fig. 31A, C) show a medulla less densely filled with cells compared to other *Pugetia* species, which we also observed.

REPRODUCTIVE ANATOMY: Carpogonial branches were reported to be typically three celled, but on occasion can be four or five celled, with branch cells rounded, as in *Pugetia* (Baardseth 1941). The tetrasporangia and spermatangia were both reported to be scattered throughout the cortex (Baardseth 1941).

COMMENTS: This species has not been reported since it was described, and the characters used to distinguish it are questionable. Baardseth (1941) stated that *P. kylinii* differed from *P. fragilissima* and *S. chilensis* (as *P. chilensis*) in the irregular arrangement of the large, unpigmented medullary cells; whereas, the latter two species had 'more or less regularly arranged series of these cells'. We observed the layers of large medullary cells in both *P. fragilissima* and *S. chilensis* to be irregularly arranged, with an occasional few cells from different layers appearing, by chance, to be in a regular series, and we posit that this is not a useful taxonomic character. In addition, the report of four- and five-celled carpogonial branches is atypical for the family Kallymeniaceae. This species did not match any of the species groups determined in this study and clearly requires further investigation based on fresh collections to clarify its taxonomic status.

***Pugetia japonica* Kylin 1941, p. 16**

Figs 19, 20

SYNONYM: *Callophyllis okamurae* P.C. Silva 1987, p. 32.

HOLOTYPE: K. Okamura (LD 090348; cystocarpic; Figs 19, 20).

TYPE LOCALITY: Chiba Prefecture, Japan.

DISTRIBUTION: As *Callophyllis okamurae* P.C. Silva: Japan (Yoshida *et al.* 1990; Yoshida 1998); Philippines (Silva *et al.* 1987; Yoshida *et al.* 1990; Yoshida 1998).

REFERENCES: Okamura (1899), Okamura (1900) [as *Callophyllis (Microcoelia) chilensis*], Howe (1914) (as *Callophyllis chilensis*), Kylin (1941), Norris (1957) (as *P. japonica*), Silva *et al.* (1987), Yoshida *et al.* (1990), and Yoshida (1998) (as *Callophyllis okamurae*).

HABIT AND VEGETATIVE ANATOMY: We examined the holotype (LD 090348; Figs 19, 20), which was a flat, roughly orbicular blade, divided into three main lobes that overlapped, with several tears in the blade (ontogenetic?) and smooth to slightly irregular margins (Fig. 19). There was a small holdfast and no discernable stipe. The largest lobe measured 7.5 cm in height from the holdfast. The medulla was atypical for *Pugetia*, with relatively few large (70–420 µm wide × 90–320 µm high), round, unpigmented cells, abundant filaments of long, narrow cells, and an outer medulla of stellate shaped, highly pit-connected cells that were also occasionally observed in the inner medulla. The stellate appearance of the outer medullary cells was distinctive (Fig. 20), and we did not observe anything similar in other *Pugetia* species. The cortex was composed of one to two inner layers of round cells and two to three outer layers of pigmented smaller cells (2–4 µm wide × 3–5 µm high).

REPRODUCTIVE ANATOMY: We did not observe carpogonial branches. Mature cystocarps were up to 1 mm in diameter and protruded only slightly from the blade surface. Tetrasporophytes and male gametophytes have not been reported for this species.

COMMENTS: Howe (1914) first suggested that collection No. 12 [*Callophyllis (Microcoelia) chilensis*] from Okamura's Algae Japonicae Exsiccatae (1899) might be a new species, but he did not officially designate one. Kylin (1941) examined Okamura's No. 12 in the Agardh herbarium (now LD 24893) and concluded it was a distinct species related to *S. chilensis* (as *P. chilensis*) and designated a new species, *Pugetia japonica* Kylin. Norris (1957) examined a different collection of Okamura's No. 12 (UC 688721) and reported it was a young female gametophyte with a reproductive system exactly like *S. firma* (as *C. firma*), though he did not officially transfer *P. japonica* to *Callophyllis* along with *S. firma*. Silva *et al.* (1987) later transferred *P. japonica* to *Callophyllis* as *C. okamurae* P.C. Silva because the epithet *japonica* was already in use (*C. japonica* Okamura).

We think it likely that the specimens examined by Kylin and Norris belonged to two different species that had both been identified by Okamura as *Callophyllis (Microcoelia) chilensis*. Norris (1957) remarked that the specimen he examined had similar reproductive organs and thallus construction as *S. firma*, and he even speculated that the specimen belonged in *S. firma*. The specimen examined by Kylin from the Agardh Herbarium (the specimen he used in describing *P. japonica*), which is also the specimen we examined, had stellate-shaped cells in the outer medulla and filaments of elongate, narrow cells in the inner medulla,



which is highly distinctive from *S. firma*. We believe Norris would have noted these features had they been present in the specimen he examined. From our examination of the true holotype, we concluded that *P. japonica* is a distinct species of the Kallymeniaceae that did not match any of the species groups resolved in our study. Without intact carpogonial branches, we could not determine whether *P. japonica* is better placed within *Pugetia* or *Salishia*, and so retain it within the former until new collections become available.

***Pugetia delicatissima* R.E. Norris 1957, p. 273, fig. 6 B–J, pl 31**

Fig. 21

TYPE: R.M. Laing, December 1933 (UC 513609; cystocarpic; Fig. 21).

TYPE LOCALITY: Gore Bay, Canterbury, New Zealand, drift.

DISTRIBUTION: Australia and New Zealand (Chapman & Parkinson 1974, Adams 1994); Antarctic and the sub-Antarctic islands (Ricker 1987).

REFERENCES: Norris (1957), Chapman & Parkinson (1974) and Ricker (1987).

HABIT AND VEGETATIVE ANATOMY: Norris (1957) described *P. delicatissima* from drift collections made by R.M. Laing from Gore Bay, New Zealand. We examined the type specimen (UC 513609; Fig. 21), which was a single blade on a press that contained dozens of blades, all epiphytic on other algae or seagrass (Fig. 21). The blade was flat, cuneate to obcordate with a short (1 mm) narrow stipe. Mature plants were to 12 cm in height and 7 cm in width (Norris 1957). Blades were extremely thin and delicate and reported to be 95–150  $\mu\text{m}$  thick (Norris 1957). Intact sections were difficult to obtain, as the blade virtually disintegrated upon rehydration. Norris (1957) reported the medulla as composed of very large cells sparsely interspersed with anastomosing filaments of cells. The cortex was composed of one to two layers of small, pigmented cells.

REPRODUCTIVE ANATOMY: Norris (1957) reported that this species is monocarpogonial and nonprocarpic. The auxiliary cell is the supporting cell of a separate branch system that looks similar to a carpogonial branch system but has only the supporting cell and subsidiary cells. Norris considered that the lack of complete carpogonial branches in the auxiliary cell system of *P. delicatissima* was different from *P. fragilissima*, where the auxiliary cell systems are identical in form to the carpogonial branches. We observed several potential auxiliary cell systems that lacked carpogonia and closely resembled what Norris diagramed (1957, p. 274, fig. 6F). We also observed fusion cells that closely resembled the fusion cell Norris diagramed for this species (1957, p. 274, fig. 6C). Tetrasporophytes and male gametophytes have not been reported for this species.

COMMENTS: The collections of *P. delicatissima* made by Laing were originally identified by Setchell as *Kallymenia berggrenii* J. Agardh (Norris 1957). Norris (1957) later designated Laing's collections as a new species, *P.*

*delicatissima* R.E. Norris, which he placed in *Pugetia* based on the presence of large clear cells in the medulla and a single carpogonial branch per supporting cell.

From our morphological observations alone, we were unable to determine if either of our '*P. fragilissima*' species groups matched *P. delicatissima*. However, unpublished molecular data indicate that *P. delicatissima sensu* Norris is in fact three species, none of which groups with *P. fragilissima* (R. D'Archino, personal communication). This species complex is thus not related to our *Pugetia* spp. discussed herein and is in need of taxonomic revision.

***Pugetia porphyroidea* (F. Schmitz ex Holmes) R.E. Norris 1964, pp. 113, 115–119, figs 37–45, pls 7, 8**

Figs 22, 23

BASIONYM: *Glaphyrymenia porphyroidea* Schmitz ex Holmes 1894, p. 338.

HOLOTYPE: H. Becker (BM 000530348; cystocarpic; Figs 22, 23).

TYPE LOCALITY: Cape of Good Hope, South Africa.

DISTRIBUTION: South Africa (Silva *et al.* 1996).

REFERENCES: Holmes (1894), Delf (1921), Stephenson (1947) (as *G. porphyroidea*), Norris (1964), Seagrief (1988) and Silva *et al.* (1996) (as *P. porphyroidea*).

HABIT AND VEGETATIVE ANATOMY: We examined the holotype (BM 000530348; Figs 22, 23), which consisted of two blades from a single holdfast, both roughly oval, with entire to ruffled margins (Fig. 22). Norris (1964) reported a short stipe (< 4 mm) and small, membranous holdfast. Mature specimens are reportedly up to 25 cm long and 21 cm wide and usually epiphytic on other algae (Norris 1964). We were unable to obtain intact sections of the holotype due to degradation of the specimen. Norris (1964) reported a medulla typical of *Pugetia*, with the large cells 165  $\mu\text{m}$  wide  $\times$  30  $\mu\text{m}$  high and a cortex of one to two layers of pigmented cells, the outer cells smaller.

REPRODUCTIVE ANATOMY: Carpogonial branches were similar to *P. fragilissima* (Fig. 23). Mature cystocarps were reportedly variable in size, the largest up to 1 mm in diameter (Norris 1964). The tetrasporangia were reported as relatively small (12  $\mu\text{m}$  wide  $\times$  19  $\mu\text{m}$  high) for the genus, with the tetrasporangial mother cells often produced by medullary cells instead of the inner cortical cells as is typical for other kallymeniacean species (Norris 1964). Male gametophytes have not been reported for this species.

COMMENTS: Holmes (1894) described *Glaphyrymenia porphyroidea* F. Schmitz ex Holmes from specimens collected from the Cape of Good Hope by H. Becker. Norris (1964) examined photographs and a fragment of the holotype and concluded the type matched fresh collections made by Papenfuss and Pocock from Woodstock Beach, near the type locality. He further reported the thallus structure and development of *G. porphyroidea* differed from *Glaphyrymenia* and more closely matched *Pugetia*, and, accordingly, he transferred the species (Norris 1964). From our examination, we concluded that *P. porphyroidea* did not match any



of our species groups. The location of tetrasporangial formation is atypical for the family; however, the round cells of the carpogonial branch are similar to *P. fragilissima*. Until molecular data are available for *P. porphyroidea* to resolve its taxonomic status, we retain its position within the genus *Pugetia*.

***Pugetia harveyana* (J. Agardh) R.E. Norris 1964, p. 119, figs 46–50, pl 9**

Fig. 24

BASIONYM: *Kallymenia harveyana* J. Agardh 1844, p. 40.

SYNONYM: *Euhymenia harveyana* (J. Agardh) Kützing 1849, p. 743.

LECTOTYPE: Harvey (LD 090097; tetrasporophyte; Fig. 24).

TYPE LOCALITY: Cape of Good Hope, South Africa.

DISTRIBUTION: As *Kallymenia harveyana* J. Agardh: Korea (Lee 2008); as *Pugetia harveyana* (J. Agardh) R.E. Norris: Namibia (Rull Lluich 2002; John *et al.* 2004); South Africa (Stegenga *et al.* 1997).

REFERENCES: Agardh (1844) (as *K. harveyana*), De Toni (1897), Kützing (1849, 1866) (as *E. harveyana*), Norris (1964) (as *P. harveyana*), Stegenga *et al.* (1997), Rull Lluich (2002) and John *et al.* (2004) (as *K. harveyana*).

HABIT AND VEGETATIVE ANATOMY: We examined the lectotype (LD 090097; Fig. 24).

The blade was large (15 cm high and 17.5 cm wide), epiphytic on another red alga, and resembled *P. fragilissima* in morphology (Fig. 24). The specimen did not rehydrate well, making intact sections difficult to obtain. Norris (1964) reported that the intercalating filaments of the medulla were so dense they sometimes obscured the large clear cells. The cortex was typical for the genus.

REPRODUCTIVE ANATOMY: Norris (1964) reported that this species was monocarpogonial, and the supporting cell, subsidiary cells, and first cell of the carpogonial branch were elongate and lobed as in *S. firma*. He also reported nonprocarpy for this species, with the auxiliary cell system similar in form to the carpogonial branch system, and mature cystocarps were 0.5–2 mm (Norris 1964). Tetrasporophytes had a thicker outer cortex of two to five cell layers, compared to two or three layers in female and male plants, with tetrasporangia 14–19  $\mu\text{m}$  wide  $\times$  25–30  $\mu\text{m}$  high (Norris 1964). Male gametophytes were reported as half the size of female gametophytes and tetrasporophytes, and spermatangia developed from the outer cortex in irregular sori that appeared as lighter patches on the blade (Norris 1964).

COMMENTS: J. Agardh (1844) described *Kallymenia harveyana* J. Agardh based on collections made by Harvey from the Cape of Good Hope, South Africa. Kützing (1849) transferred *K. harveyana* to *Euhymenia* as *E. harveyana* (J. Agardh) Kützing. Papenfuss later compared the lectotype of *K. harveyana* to fresh collections from the Cape Province region and concluded they were the same species (Norris 1964). Norris himself examined the Cape Province

collections and, finding them similar to *Pugetia*, transferred *Kallymenia harveyana* to *Pugetia harveyana* (J. Agardh) R.E. Norris (he made no mention of *Euhymenia harveyana*). The name *Euhymenia* is now considered to be a synonym of *Kallymenia* (Schneider & Wynne 2007).

Based on the limited information we could obtain from the tetrasporic lectotype of *P. harveyana*, we concluded that this species was not a match to any of our species groups. Additionally, we were unable to determine if *P. harveyana* is more appropriately placed in *Pugetia* or *Salishia* or if it requires transfer to a separate genus. The lobed cells of the carpogonial branch system are similar to species of *Salishia*; however, the nonprocarpic postfertilization development is similar to species of *Pugetia*. Until molecular data become available for this species, we conservatively retain it within *Pugetia*.

#### ***Salishia* Clarkston & G.W. Saunders, gen. nov.**

*Thallus comparate crassus, integer vel valde lobatus, interdum perforatus (per ontogeniam?). Laminae haptero parvo (~ 1 mm) eccentrico et interdum stipite brevi. Medulla constans e 1–4 stratis irregularibus cellularum magnarum pellucidarum et filamentarum interspersarum e cellulis parvis rotundis pigmentiferis constantium. Cortex e 2–4 (5) stratis cellularum constans, cellulis interioribus rotundis vel periclinalibus, cellulis exterioribus minoribus pigmentiferis, quo magis anticlinalibus eo propioribus strato extimo. Monocarpogoniales procarpicae. Rami carpogoniales proxime sub cortice locati, in medullam extendentes, per laminam dispersi. Cellula sustinens, cellulae subsidiariae, et prima cellula ramorum carpogonialium elongata valde lobata. Cystocarpia matura per thallum dispersa, quaeque ostiolo singulo. Tetrasporangia cruciatim divisa, per corticem dispersa.*

Thallus relatively thick, entire to highly lobed, sometimes with holes (ontogenetic?). Blades with a small (~ 1 mm) eccentric holdfast and occasionally a short stipe. Medulla of one to four irregular layers of large clear cells interspersed with filaments of small, round, pigmented cells. Cortex two to four (five) cell layers, with the inner cells round to periclinal and the outer cells smaller, pigmented and progressively more anticlinal toward the outermost layer. Monocarpogonial and procarpic. Carpogonial branches situated just below the cortex and extend into the medulla and scattered throughout the blade. Supporting cell, subsidiary cells, and first cell of carpogonial branch elongate and highly lobed. Mature cystocarps scattered throughout the thallus and with a single ostiole each. Tetrasporangia cruciately divided and scattered throughout the cortex.

TYPE SPECIES: *Salishia firma* (Kyllin) Clarkston & G.W. Saunders comb. nov.

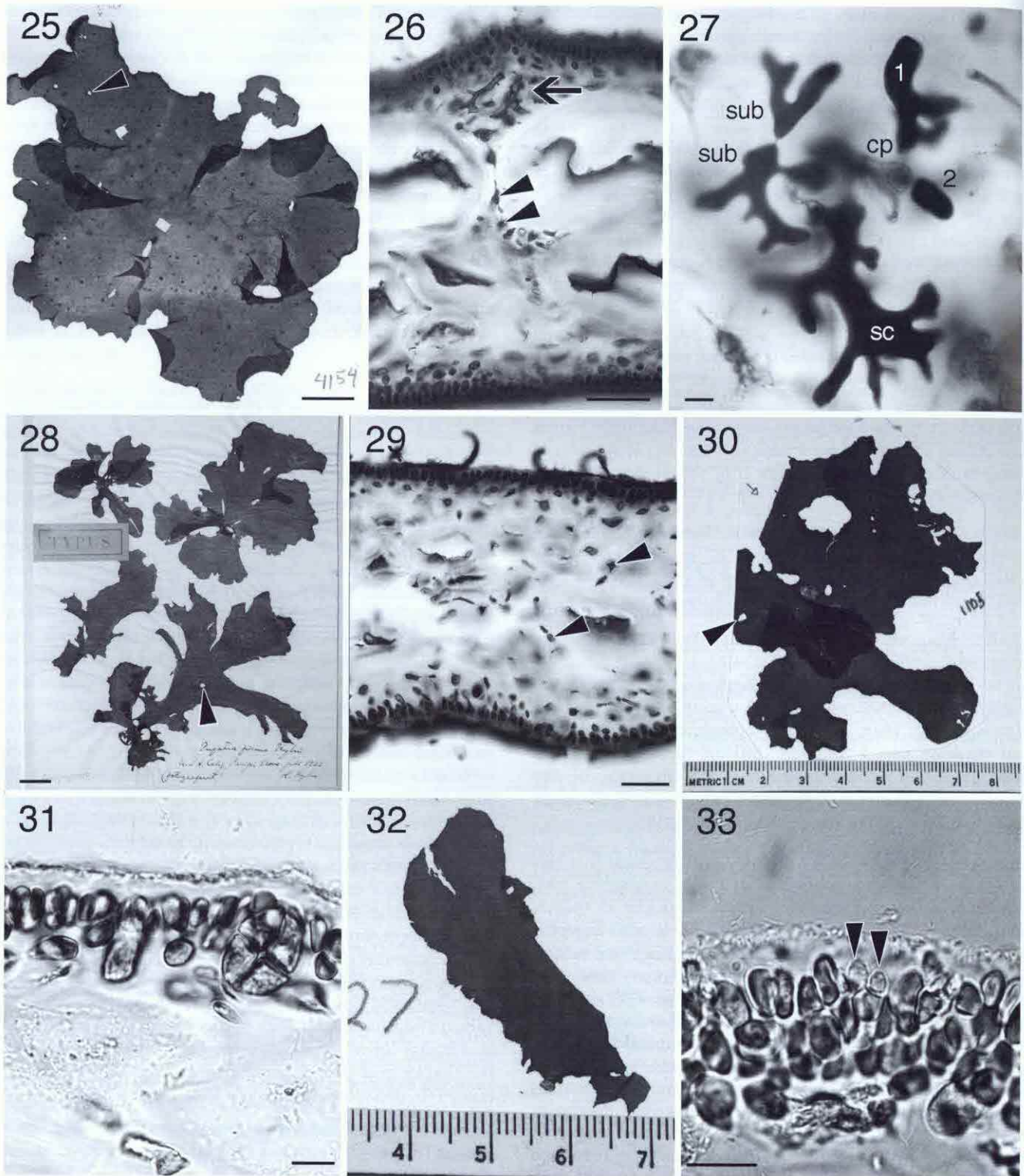
ETYMOLOGY: Named for the Salish Sea in the eastern Pacific, where members of this genus have been frequently collected.

#### ***Salishia firma* (Kyllin) Clarkston & G.W. Saunders comb. nov.**

Figs 25–33

BASIONYM: *Pugetia firma* Kyllin 1941 (Californische Rhodophyceen). Acta Universitatis Lundensis 37(2): 15, pl 4, fig. 12.





**Figs 25–33.** Morphology and anatomy of *Salishia firma* (Kylin) Clarkston & G.W. Saunders gen. & comb. nov.  
**Fig. 25.** Pressed voucher for GWS004154 (cystocarpic) with possible ontogenetic holes (arrowhead). Scale bar = 2 cm.  
**Fig. 26.** Cross section showing large, unpigmented cells interspersed with filaments of small, pigmented cells (arrowheads), and a carpogonial branch at the medulla to cortex boundary (arrow) (GWS004154). Scale bar = 50  $\mu$ m.  
**Fig. 27.** Peeled section showing a three-celled carpogonial branch attached to a lobed supporting cell, which also bore a two-celled subsidiary cell branch (cp, carpogonium; 1, indicates probable first cell of the carpogonial branch (branch cells numbered progressively distal to the supporting cell); 2, indicates second cell of the carpogonial branch; sc, supporting cell; sub, subsidiary cell) (GWS004154). Scale bar = 10  $\mu$ m.  
**Fig. 28.** Pressed voucher for LD 090094 (holotype; tetrasporic and cystocarpic) with possible ontogenetic holes (arrowhead). Scale bar = 2 cm.



SYNONYM: *Callophyllis firma* (Kylin) Norris 1957, p. 287.

HOLOTYPE: Kylin, July, 1922 (LD 090094; tetrasporic and cystocarpic; Figs 28, 29).

TYPE LOCALITY: Pacific Grove, California, USA, from the uppermost sublittoral.

REPRESENTATIVE SPECIMEN: GWS004154 (see Table 1 for collection site).

REPRESENTATIVE DNA BARCODE: JF903377.

CONFIRMED DISTRIBUTION: Thus far known with certainty to be widely distributed only throughout the Haida Gwaii Islands, Prince Rupert, Vancouver Island, and the Sunshine Coast in British Columbia, Canada, and Pigeon Point, California (near the type locality; see Table 1), suggesting a wide distribution along the Pacific coast of North America.

REFERENCES: Kylin (1941), Norris (1957) (as *Callophyllis firma*), Abbott & Hollenberg (1976), Scagel *et al.* (1989), Stewart (1991), Lee & Kang (2001) and Harper & Saunders (2002) (as *P. firma*). It is important to acknowledge that these references (other than the type description) may not apply to *Salishia firma sensu stricto* because of the cryptic diversity uncovered here.

HABIT AND VEGETATIVE ANATOMY: Of the 87 *S. firma* specimens collected during this study, the majority (74) were found in the low intertidal, with the rest (13) collected subtidally (8–32 ft) at one site in northern British Columbia (Stenhouse Reef) and five sites on Vancouver Island [Otter Point, Whiffen Spit, Dixon Is. and Tahsis (Island #37 and Flower Islet)]. Specimens were found growing on rock or occasionally on invertebrates.

The thalli were flat, robust, more or less circular in shape, and irregularly lobed with the lobes often overtopping one another and were usually procumbent (Figs 25, 30, 32). Mature plants were up to 18 cm in height.

Blades were 105–460  $\mu\text{m}$  thick near the apex. The large medullary cells were 75–300  $\mu\text{m}$  wide  $\times$  65–250  $\mu\text{m}$  high (Figs 26, 29). The intercalating filaments were common throughout the thalli, but were most abundant in older regions. The cortex was composed of one inner layer and one to three (three) outer layers of smaller cells (2–7  $\mu\text{m}$  wide  $\times$  3–8  $\mu\text{m}$  high; Figs 26, 29, 31).

REPRODUCTIVE ANATOMY: Carpogonial branches were typical for the genus (Figs 26, 27). Mature cystocarps were 540–1680 (2200)  $\mu\text{m}$ , mean = 900  $\mu\text{m}$ . The tetrasporophytes were essentially isomorphic relative to the gametophytes (Figs 28, 30), with the tetrasporangia (10–17.5  $\mu\text{m}$  wide  $\times$  22.5–27.5  $\mu\text{m}$  high) slightly smaller than in *S. sanguinea* (Fig. 31). Male gametophytes (Fig. 32) produced small,

colourless spermatangia ( $\sim$  2  $\mu\text{m}$ ) from spermatangial mother cells in the outer cortex throughout younger regions of the blade (Fig. 33).

COMMENTS: *Salishia firma* and *S. sanguinea* were easy to distinguish at the genetic level (Figs 1–4); however, they overlapped considerably in morphological characters. The only observed morphological difference was in the thickness of the outer cortex near the growing margin of the blade. All *S. firma* specimens, including the holotype (LD 090094; Figs 28, 29), had mostly two, occasionally three, outer cortical layers but never four, while some *S. sanguinea* specimens had an outer cortex with mostly three and up to four layers. This difference was not absolute, however, as other *S. sanguinea* specimens (younger?) were observed with outer cortices of mostly two and occasionally three layers. The two species also differed somewhat in habitat – *S. firma* was most often found in the low intertidal zone and rarely in the subtidal (to 32 ft deep), while *S. sanguinea* was found only in the subtidal zone below 20 ft. The holotype was collected by Kylin (1941) in July from the ‘most upper part’ of the sublittoral, which we interpreted to mean Kylin collected the holotype from at most a few feet below the low-tide mark. We collected two specimens from the low intertidal near the type locality in California (see Table 1) that had the same COI-5P sequence as our predominantly intertidal ‘*firma*’ species groups, and we assigned that species group to *S. firma* because it matched the holotype in cortical thickness, habitat and geography.

***Salishia sanguinea* (Montagne) Clarkston & G.W. Saunders  
comb. nov.**

Figs 34–41

BASIONYM: *Kallymenia sanguinea* Montagne 1852–1854 [Flora Chiliana. Plantas celulares In C. Gay, Historia física y política de Chile. Vol. 8. Paris & Santiago, pp. 1–256 (1852), 257–448 (1854)].

SYNONYMS: *Callophyllis sanguinea* (Montagne) M.A. Howe 1914, p. 118.

*Pugetia sanguinea* (Montagne) Kylin 1941: 15.

TYPE: M.Cl. Gay (PC 0097273; tetrasporic; Figs 37, 38).

TYPE LOCALITY: Southern coast of Chile.

REPRESENTATIVE SPECIMEN: GWS004152 (see Table 1 for collection site).

REPRESENTATIVE DNA BARCODE: JF903420.

CONFIRMED DISTRIBUTION: Throughout the Haida Gwaii Islands, Prince Rupert, and Vancouver Island in British

Fig. 29. Vegetative cross section showing large, unpigmented cells interspersed with filaments of small, pigmented cells (arrowheads) (LD 090094). Scale bar = 50  $\mu\text{m}$ .

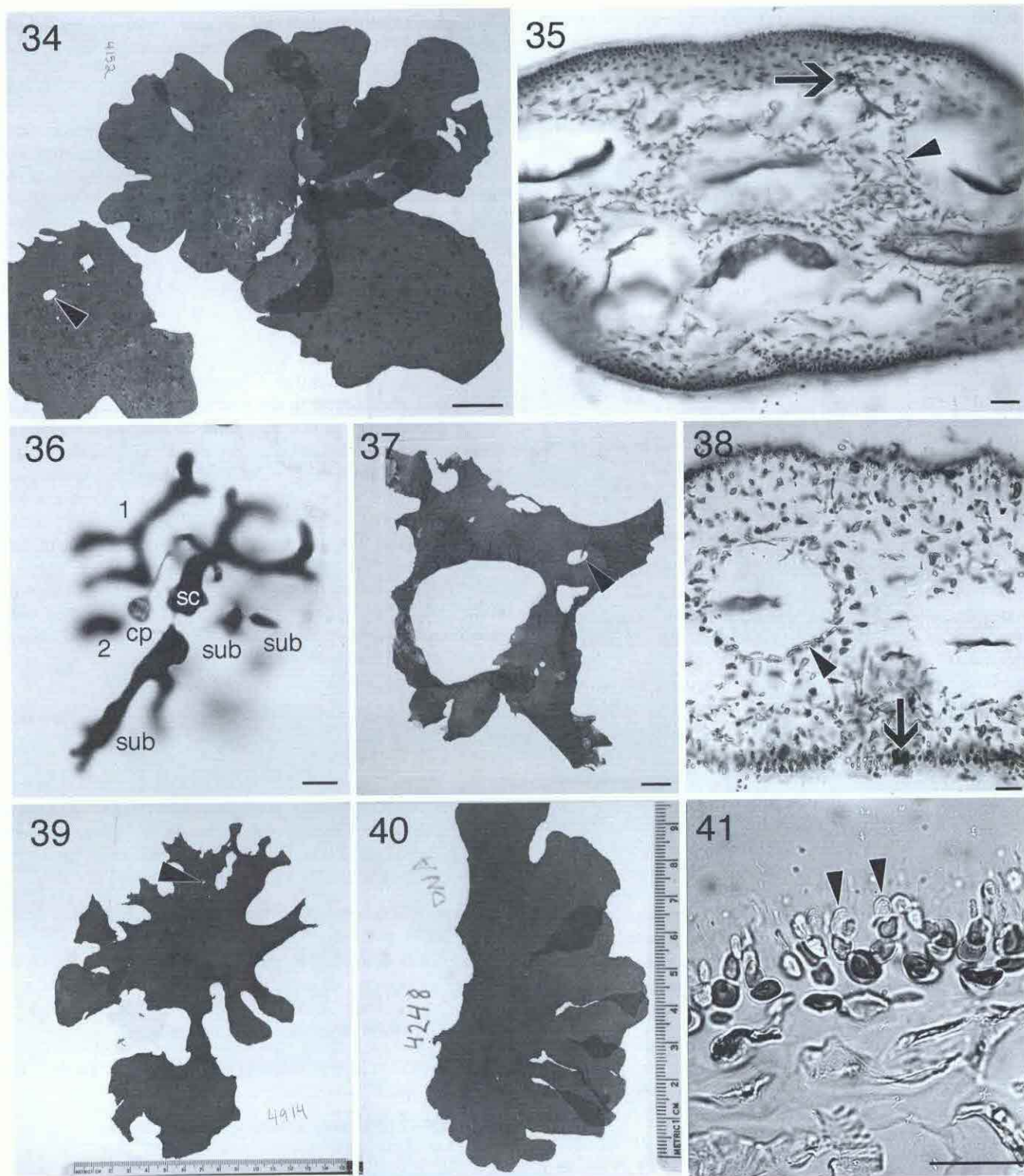
Fig. 30. Pressed voucher for GWS001105 (tetrasporic) with possible ontogenetic holes (arrowhead). Scale bar = centimeter ruler.

Fig. 31. Cross section showing cruciately divided tetrasporangium in the cortex (GWS001105). Scale bar = 10  $\mu\text{m}$ .

Fig. 32. Pressed voucher for GWS006627 (male gametophyte). Scale bar = centimeter ruler.

Fig. 33. Outer cortical cell bearing spermatangia (arrowheads) (GWS006627). Scale bar = 5  $\mu\text{m}$ .





**Figs 34–41.** Morphology and anatomy of *Salishia sanguinea* Clarkston & G.W. Saunders comb. nov.

**Fig. 34.** Pressed voucher of GWS004152 (cystocarpic) with possible ontogenetic holes (arrowhead). Scale bar = 2 cm.

**Fig. 35.** Cross section showing large, unpigmented cells interspersed with filaments of small, pigmented cells (arrowhead) and location of carpopogonial branch at the medulla to cortex boundary (arrow) (GWS004152). Scale bar = 50  $\mu$ m.

**Fig. 36.** Peeled section showing a three-celled carpopogonial branch attached to a lobed supporting cell, which also bore a one-celled subsidiary cell branch and a two-celled subsidiary cell branch (out of focus) (cp, carpopogonium; 1, indicates probable first cell of the carpopogonial branch (branch cells numbered progressively distal to the supporting cell); 2, indicates second cell of the carpopogonial branch; sc, supporting cell; sub, subsidiary cell) (GWS004152). Scale bar = 10  $\mu$ m.

**Fig. 37.** Pressed voucher of PC 0097273 (type specimen; tetrasporic) with possible ontogenetic holes (arrowhead). Scale bar = 2 cm.

**Fig. 38.** Vegetative cross section showing large, unpigmented cells interspersed with numerous filaments of small, pigmented cells (arrowhead) and tetrasporangia embedded in the cortex (arrow) (PC 0097273). Scale bar = 50  $\mu$ m.



Columbia, Canada (See Table 1). If our taxonomic conclusions are correct, there is a disjunct distribution with this species occurring along the temperate Pacific coasts of both North and South America.

REFERENCES: Montagne (1852–1854), De Toni (1897) (as *K. sanguinea*), Howe (1914) (as *Callophyllis sanguinea*), Kylin (1941) (as *P. sanguinea*), Norris (1957) (as *C. sanguinea*) and Ramírez & Santelices (1991) (as *P. sanguinea*). It is important to acknowledge that these references (other than the type description) may not apply to true *Salishia sanguinea sensu stricto* in light of the cryptic diversity uncovered here.

HABIT AND VEGETATIVE ANATOMY: The 51 specimens of *S. sanguinea* collected during this study were all subtidal (20–50 ft), growing on rock or occasionally invertebrates. We also examined two isotype specimens (PC 0097273; tetrasporic and 0097274; cystocarpic).

The thalli were flat, robust, more or less circular in shape, often highly lobed with the lobes often overtopping one another, typically procumbent, and occasionally blades had holes that appeared to be ontogenetic in origin (Figs 34, 37, 39, 40). Mature plants were up to 16 cm in height.

Blades were 150–620 µm thick near the apex. The large medullary cells were 50–145 µm wide × 55–135 µm high (Fig. 35). The intercalating filaments were common throughout thalli but were most abundant in older regions. The cortex was composed of one inner layer and one to four outer layers of smaller cells (2–7 µm wide × 4–10 µm high; Fig. 35).

REPRODUCTIVE ANATOMY: Carpogonial branches were typical for the genus (Figs 35, 36). We did not observe any carpogonial branches on the *S. sanguinea* isotype; however, Norris (1957) examined a Montagne isotype specimen (University of California Herbarium, no number given) and reported the carpogonial branch cells were lobed, similar to *S. firma*. Mature cystocarps were 435–2020 (2160) µm, mean = 1220 µm. Tetrasporophytes were isomorphic relative to the gametophytes (Fig. 37), with the tetrasporangia 14–20 µm wide × 21–32.5 µm high (Fig. 38). Male gametophytes (Fig. 39) produced small, colourless spermatangia (~ 2 µm) from spermatangial mother cells in the outer cortex throughout younger regions of the blade (Fig. 41).

COMMENTS: Howe (1914) examined two isotype specimens of *Callymenia sanguinea* (= *Salishia sanguinea*) and subsequently made the combination *Callophyllis sanguinea* (Montagne) M.A. Howe because he believed the species fell under the 'Schmitzian conception of the limits of the genus (*Callophyllis*)'. Howe also speculated whether *Microcoelia chilensis* (= *Salishia chilensis*) should be considered a synonym of *S. sanguinea* because of similar-

ities in their internal vegetative anatomy. He concluded that the cartilaginous thallus and thick, obvious walls of the large medullary cells in *S. sanguinea* were sufficiently different from *S. chilensis* to retain them as separate species. When Kylin (1941) transferred *S. chilensis* (as *M. chilensis*) to *Pugetia*, he briefly stated that *S. sanguinea* was closely related to *S. chilensis* (as *P. chilensis*) and accordingly transferred it also as *Pugetia sanguinea* (Montagne) Kylin (= *S. sanguinea*). Norris (1957) subsequently concluded that characters of the female reproductive system were more similar to *Callophyllis* than *Pugetia* and so transferred *S. sanguinea* (as *P. sanguinea*) back to *Callophyllis*.

We conclude that the isotypes of *Callymenia sanguinea* (= *S. sanguinea*) are morphologically and anatomically similar to our second 'firma' species group, in particular with respect to the thicker outer cortex, and we provisionally consider them the same species. Genetic analysis of additional collections, especially from the type locality, are necessary to test this association. In addition to assigning our species group to this taxon, we transfer the species to *Salishia* based on the robust thallus, lobed carpogonial branch cells, procarpy (as reported by Norris 1957) and the association with *S. firma* in our molecular analyses.

***Salishia chilensis* (J. Agardh) Clarkston & G.W. Saunders  
comb. nov.**

Figs 42–45

BASIONYM: *Microcoelia chilensis* J. Agardh 1876 (*species genera et ordines algarum, seu descriptiones succinctae specierum, generum et ordinum*. C.W.K. Gleerup, Leipzig, p. 227).

SYNONYMS: *Callophyllis chilensis* (J. Agardh) Okamura 1942, p. 101.

*Pugetia chilensis* (J. Agardh) Kylin 1941, p. 15.

HOLOTYPE: Harvey (LD 090096; cystocarpic; Figs 42, 43).

TYPE LOCALITY: Bay of Concepción, Chile.

REPRESENTATIVE SPECIMEN: GWS000501 (see Table 1 for collection site).

REPRESENTATIVE DNA BARCODE: JF903345.

DISTRIBUTION: As *Pugetia chilensis* (J. Agardh) Kylin: Chile (see Table 1; Ramírez & Santelices 1991); Peru (Acleto 1973; Ramírez & Santelices, 1991).

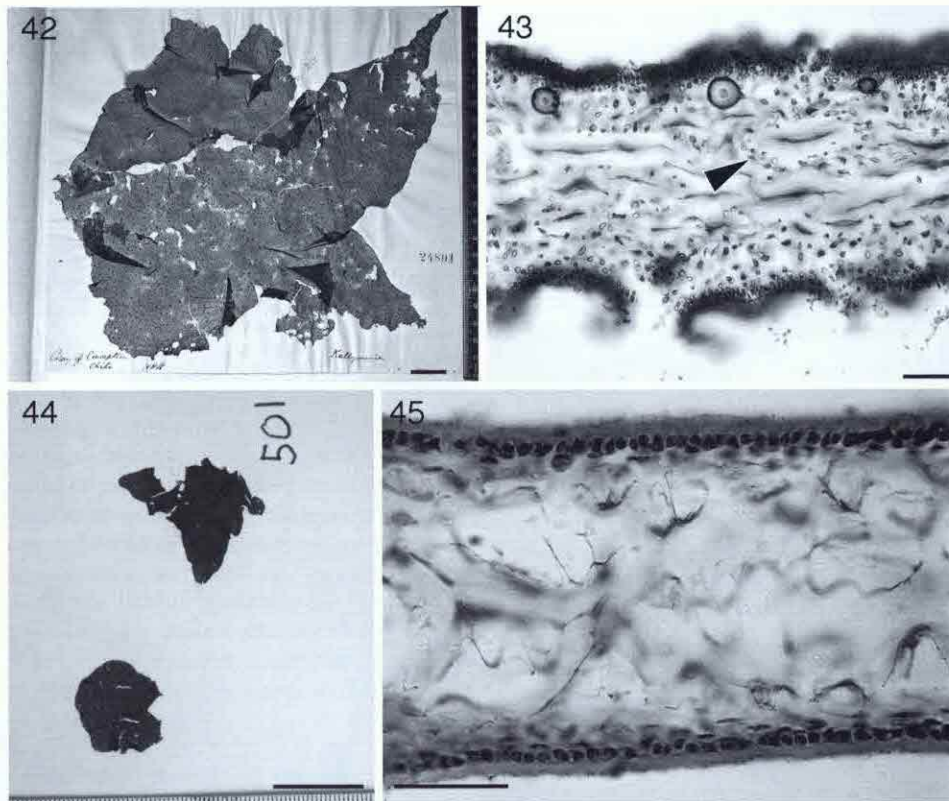
REFERENCES: Agardh (1876) (as *M. chilensis*), Schmitz & Hauptfleisch (1896) (as *Callophyllis chilensis*), De Toni (1897) (as *M. chilensis*), Okamura (1899) [as *Callophyllis (Microcoelia) chilensis*], Howe (1914) (as *C. chilensis*), Kylin (1941) (as *P. chilensis*), Norris (1957) (as *C. chilensis*),

Fig. 39. Pressed voucher of GWS004914 showing similar gross morphology to the holotype and possible ontogenetic holes (arrowhead). Scale bar = centimeter ruler.

Fig. 40. Pressed voucher for GWS004248 (male gametophyte). Scale bar = centimeter ruler.

Fig. 41. Outer cortical cells bearing spermatangia (arrowheads) (GWS004248). Scale bar = 10 µm.





Figs 42–45. Morphology and anatomy of *Salishia chilensis* (J. Agardh) Clarkston & G.W. Saunders comb. nov.

Fig. 42. Pressed voucher of *Salishia chilensis* (holotype; LD 090096; cystocarpic). Scale bar = 2 cm.

Fig. 43. Vegetative cross section (poorly rehydrated) showing large, unpigmented cells interspersed with filaments of small, pigmented cells (arrowhead) (LD 090096). Scale bar = 50  $\mu$ m.

Fig. 44. Pressed voucher for putative immature collection of *Salishia chilensis* (GWS000501; vegetative). Scale bar = 2 cm.

Fig. 45. Vegetative cross section showing single outer cortical layer and lack of obvious intercalating filaments (GWS000501). Scale bar = 50  $\mu$ m.

Acleto (1973), Ramírez & Santelices (1991) and Harper & Saunders (2002) (as *P. chilensis*).

**HABIT AND VEGETATIVE ANATOMY:** This species was described by J. Agardh (1876) from Chilean collections sent to him by Harvey. We examined the holotype (LD 090096; Figs 42, 43), which was large (~ 20 cm in diameter), lacinate and with no obvious holdfast (Fig. 42), as well as a recent collection, field identified as *P. chilensis* (GWS000501; see Table 1; Figs 44, 45).

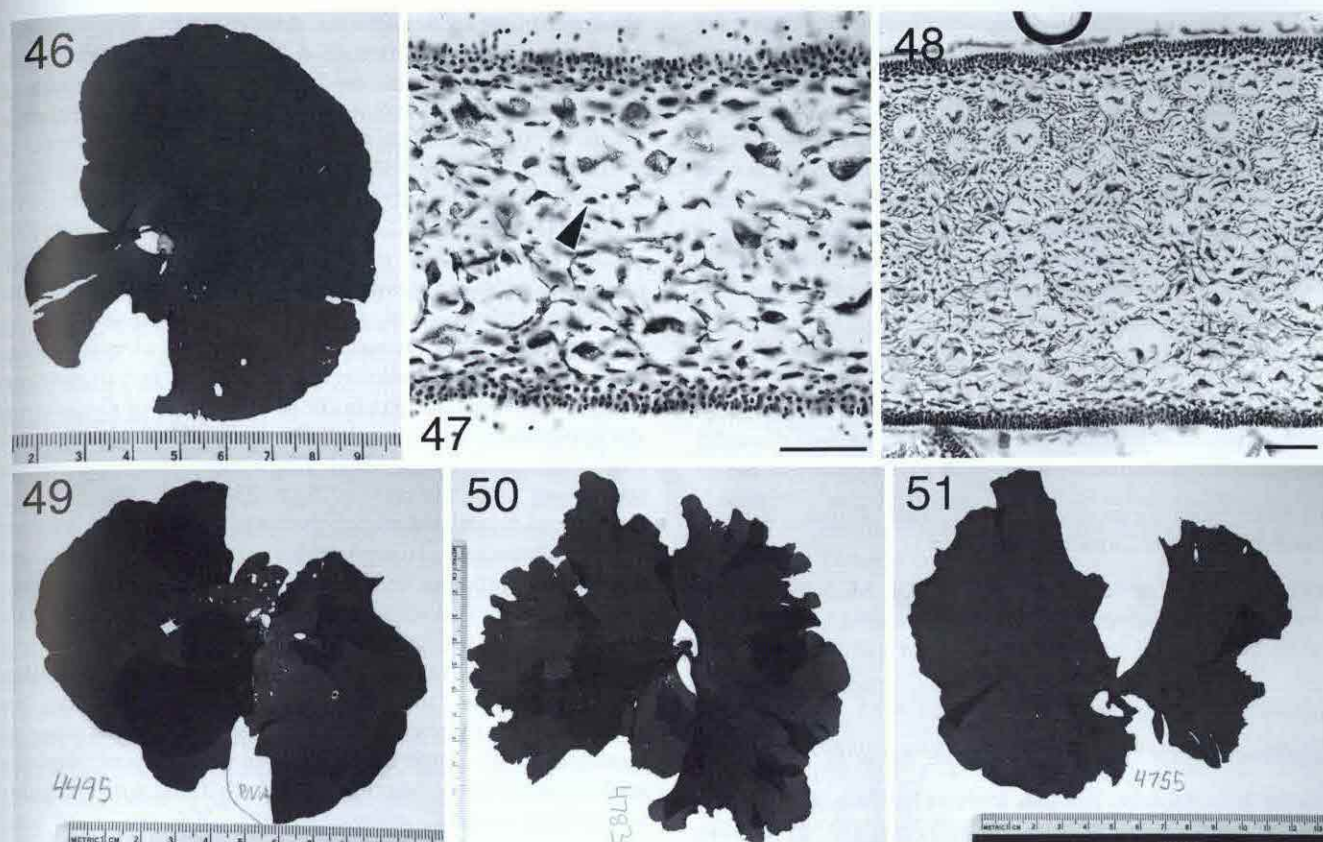
In the holotype, the medulla was composed of three to four irregular layers of round and unpigmented cells, with abundant filaments of small, round to elongate, pigmented cells interspersed among the larger medullary cells (Fig. 43). In contrast, our recent collection had two to three irregular layers of round and unpigmented cells and no obvious intercalating filaments (Fig. 45). The cortex of the holotype was composed of two to four layers – an inner layer of round cells and one to three outer layers of smaller, somewhat anticlinal, pigmented cells (2–4  $\mu$ m wide  $\times$  5–7  $\mu$ m long) (Fig. 43), while our recent collection had an inner layer of periclinal cells and a single outer layer of round, pigmented cells. The holotype blade rehydrated poorly; however, it was thicker (255  $\mu$ m) in cross section than our fully rehydrated recent collection (153  $\mu$ m; Fig. 45). The recent collection contained three vegetative

blades that were small (2–4 cm) compared to the holotype (~ 20 cm) and possibly represent immature plants of this species.

**REPRODUCTIVE ANATOMY:** Carpogonial branches were consistent with other *Salishia* species. Norris (1957) reported this species as procarpic. Tetrasporophytes and male gametophytes have not been reported for this species and were not observed in this study.

**COMMENTS:** J. Agardh (1876) described *Microcoelia chilensis* (= *S. chilensis*) as similar in general appearance to *Kallymenia* but with an internal structure more like that of *Callophyllis*. Schmitz & Hauptfleisch (1896) reduced *Microcoelia* to synonymy under *Callophyllis*, arguing that the vegetative characters used to separate the genera were insufficiently distinctive (Norris, 1957). Okamura (1899) identified (incorrectly; Kylin 1941) several Japanese collections as *M. chilensis*, but in following Schmitz and Hauptfleisch's synonymy, he labeled the collections as *Callophyllis (Microcoelia) chilensis* (J. Agardh) Okamura. Howe (1914) agreed with Schmitz and Hauptfleisch in recognizing only *Callophyllis* (though he examined only a photograph of J. Agardh's holotype) and accordingly assigned his Peruvian collections to *Callophyllis chilensis* (J. Agardh) Okamura. As well, Howe pointed out that the





Figs 46–51. Morphology and anatomy of *Beringia wynnei* Clarkston & G.W. Saunders sp. nov.

Fig. 46. Holotype (GWS004493; vegetative). Scale bar = centimeter ruler.

Fig. 47. Vegetative cross section at apex of plant showing medulla with many filaments of small, pigmented cells (arrowhead), among relatively small isodiametric to elliptical unpigmented cells (GWS004493). Scale bar = 100  $\mu\text{m}$ .

Fig. 48. Vegetative cross section at base of plant showing thick medulla that accounted for c. 90% of the blade thickness (GWS004493). Scale bar = 100  $\mu\text{m}$ .

Figs 49–51. Pressed vouchers for GWS004495, GWS004783 and GWS004755, respectively, showing variation in blade morphology. Scale bar = centimeter rulers.

name *Microcoelia* J. Agardh was a later homonym already in use for an orchid genus and therefore illegitimate.

Kylin (1941) subsequently considered *C. chilensis* as best assigned to his genus *Pugetia* and effected the transfer as *P. chilensis* (J. Agardh) Kylin. In doing this, he explicitly disagreed with Howe's opinion that *Microcoelia* and *Callophyllis* were too similar to maintain as separate genera and reaffirmed the features that distinguished *Pugetia* from *Callophyllis*, namely, an unbranched thallus and abundant intercalary filaments in the medulla.

Norris (1957) examined an isotype of *S. chilensis* (as *P. chilensis*; University of California Herbarium, no number given) and, in emphasizing reproductive attributes (i.e. procarpy and lobed carpogonial branch cells) rather than Kylin's vegetative features, transferred *S. chilensis* back to *Callophyllis*.

Our collection of *S. chilensis* (GWS000501; from Ancud Bay, Chile; Figs 44, 45), used by Harper & Saunders (2002) and in our molecular analyses (Figs 3, 4), grouped closely with *S. firma*, a relationship predicted by Kylin (1941). However, in comparison to the holotype of *Microcoelia chilensis* (= *S. chilensis*), our collection, containing three small plants (Fig. 44), is anatomically different (see above). It is possible that our plants were immature when

collected and would have resembled the holotype if they were mature (cortical and medullary cell layers, as well as the degree of intercalating filaments present, all increasing as thalli mature). Alternatively, this collection could represent a new and previously unknown species attributable to *Salishia*. Without molecular data for the *M. chilensis* holotype, we cannot conclusively link our collection to it, nor can we positively conclude the two are different species. We tentatively retain our collection as *S. chilensis* until such time that specimens from the type locality are included in molecular analyses. Regardless of the resolution of the species identity of our collection, we transfer the species to *Salishia* based on morphological and anatomical attributes of the holotype.

*Beringia wynnei* Clarkston & G.W. Saunders sp. nov.

Figs 46–51

*Plantae sub recessu aestuum crescentes. Thallus 5–10 cm planus orbicularis, integer vel profunde fissus in 2–3 segmenta primaria interdum imbricata, margine integro vel irregulariter lobato. Hapteron eccentricum crustosum; stipes nullus. Thallus prope apicem 280–310  $\mu\text{m}$ , prope basin 680–770  $\mu\text{m}$  crassus. Medulla constans e cellulis rotundis non pigmentiferis et filamentis circumcingentibus cellularum minorum rotun-*



*darum vel elongatarum pallide pigmentiferarum. Cortex e 3–5 stratis cellularum parvarum pigmentiferarum constans.*

Plants subtidal. Thallus 5–10 cm, flat, orbicular, entire or deeply cleft to form two to three main segments, which can overlap, margin smooth or irregularly lobed. Eccentric crustose holdfast, no stipe. Thallus 280–310  $\mu\text{m}$  thick near apex, 680–770  $\mu\text{m}$  near base. Medulla of round, unpigmented cells surrounded by filaments of smaller, round to elongate, weakly pigmented cells. Cortex consisting of three to five layers of small, pigmented cells.

HOLOTYPE: B. Clarkston, G.W. Saunders, and D. McDevitt, June 27, 2006 (UNB GWS004493; vegetative; Figs 46–48).

TYPE LOCALITY: Seapool Rock (lat. 48°49'8.4", long. –125°12'28.7994"), Bamfield, British Columbia, Canada, subtidal (35 ft), on rock.

DISTRIBUTION: Vancouver Island and Prince Rupert, British Columbia, Canada (see Table 1).

ETYMOLOGY: Named in honour of Dr Michael Wynne (University of Michigan, Ann Arbor), whose studies have greatly contributed to our knowledge of red algae.

HOLOTYPE DNA BARCODE: JF903287.

REPRESENTATIVE DNA BARCODES: See Table 1.

HABIT AND VEGETATIVE ANATOMY: The 17 collections of this new species were all collected subtidally (30–50 ft) from sites that experienced either high current and/or high wave exposure. Blades were thick, flat and orbicular, typically procumbent and 5–10 cm in height from holdfast to apex (Figs 46, 49–51). The medulla accounted for c. 90% of the blade thickness and was composed of many layers [four to six or more in younger regions (Fig. 47), nine or more in older regions (Fig. 48)] of round cells (42–198  $\mu\text{m}$  wide  $\times$  38–108  $\mu\text{m}$  high) interspersed with branched filaments of smaller cells (Figs 47, 48). The cortex was contained an inner layer of round to periclinal, pigmented cells, and two to four layers of round to weakly anticlinal, pigmented smaller cells (3–4  $\mu\text{m}$  wide  $\times$  5–6.5  $\mu\text{m}$  high; Fig. 47).

REPRODUCTIVE ANATOMY: None of the specimens were reproductive.

COMMENTS: In attempting to assign our divergent '*firma*' species group to a member of the Kallymeniaceae, we came across the little-known monospecific genus *Beringia* Perestenko. Perestenko (1975) described the type species *Beringia castanea* Perestenko as 'brownish-red ... ring-shaped, split into lobes ... with a medulla of ring-shaped, isodiametric, star-shaped, and large oval cells; the secondary filaments formed of long narrow cells.' Our specimens matched well with *B. castanea* in all aspects except for lacking 'star-shaped' cells in the medulla. We were unsuccessful in borrowing the holotype of *B. castanea* so instead examined a representative specimen collected subtidally (17 m) from the North Kurile Islands, Russia, in 1989, identified by O.N. Selivanova and borrowed from the University of Michigan Herbarium.

The blade was round, brown-red in colour and in overall gross morphology was similar to both our collections and

the description of *B. castanea*. Anatomically, the plant was relatively thin (125  $\mu\text{m}$ ) with a medulla loosely filled with refractive cells, irregular to distinctly stellate in shape and no large oval cells. The cortex was composed of one to two layers of small, round, pigmented cells. The presence of stellate cells in the medulla of this plant matched, in part, with Perestenko's description of *B. castanea* (Perestenko 1986). However, her description and her drawings (1986, p. 280, pl X, figs 2–4) depict a medulla densely filled with large oval cells and small-celled filaments in addition to the refractive stellate cells, which were not present in the specimen of *B. castanea* we examined. Upon closer inspection of Perestenko's drawings, we noted that no stellate cells were present in the cross section of *B. castanea* she presented, and the medulla was composed of only large oval cells and small-celled filaments. The only figure containing a stellate cell (1975, p. 280, pl X, fig. 5) was a drawing of an individual cell that was not placed in context within the medulla. It is possible that Perestenko examined two different species with similar gross morphologies when she described *B. castanea* – one with a loose medulla containing refractive stellate cells and one with densely filled medulla of larger, oval cells and filaments of smaller, irregularly round to elongate cells. If the specimen we examined is a representative of 'true' *Beringia castanea* as identified by an expert in the Russian marine flora, then the medulla is properly described as loosely filled with irregular to stellate shaped, refractive cells. We designate our specimens as a new species within *Beringia*; however, this taxonomic position is tenuous pending molecular analysis of the type species.

#### Taxa of uncertain status and not assessed in this study

##### *Hommersandia palmatifolia* (Tokida) Perestenko ex O.N. Selivanova & G.G. Zhigadlova 1997, p. 17

BASIONYM: *Pugetia palmatifolia* Tokida 1948, p. 37, figs 7–9.

TYPE LOCALITY: Higashisoia, Southern Saghalien, Japan (now Russia).

HABIT AND VEGETATIVE ANATOMY: Blades to 14 cm in height, with proliferous bladelets at the margins (Tokida 1948). The outer medulla is composed of round, unpigmented cells, with an inner medulla of filaments of thick, vertically oriented cells (Tokida 1948).

COMMENTS: The current name for this species is *Hommersandia palmatifolia* (Tokida) Perestenko ex O.N. Selivanova & G.G. Zhigadlova (Perestenko 1986; Selivanova & Zhigadlova 1997). However, the taxonomic position of *H. palmatifolia* remains uncertain, as Gabrielson *et al.* (2006) have remarked that a critical examination of the holotype is necessary to confirm its conspecificity with the generitype, *H. maximacarpa*.

##### *Pugetia mexicana* E.Y. Dawson 1966, p. 62, pl 6, figs G, H

HOLOTYPE: E.Y. Dawson, 22 November 1964 (D. 26168).

TYPE LOCALITY: Isla San Lorenzo del Sur, Mexico, dredged (19–30 m).



**HABIT AND VEGETATIVE ANATOMY:** Blades irregularly shaped and deeply lobed, with mature specimens 4–6 cm in height and a medulla of large vacuolate cells interspersed with filaments of small cells (3 µm diameter; Dawson 1966).

**COMMENTS:** *Pugetia mexicana* is known virtually only from the type specimen; more collections and a thorough examination using molecular data are necessary to determine its taxonomic placement.

**GENERAL DISCUSSION:** The advent of molecular assisted alpha taxonomy and large-scale survey studies (e.g. Saunders 2008; Le Gall *et al.* 2010; Clarkston & Saunders 2010; Saunders & McDonald 2010) is quickly leading to a large number of genetically resolved species that require comparison to existing species concepts. Currently, the most reliable method for comparing type specimens to genetic species is via morphological and anatomical examinations. These examinations can be time consuming, require taxonomic expertise and may not even be possible in some cases (e.g. specimen is degraded). Genetic comparisons between type material and contemporary collections would be ideal, and the acquisition of viable DNA sequences from older herbarium specimens has been reported (Hughey *et al.* 2001; Gabrielson 2008). However, a recent study concluded that the likelihood of successful amplification of (the correct) DNA from archival collections decreases as the age of the collection increases and that cross-contamination is a constant and serious concern (Saunders & McDevit, in press). We believe that for situations in which a species concept (type) cannot be applied unequivocally to one species among a cryptic complex, the best solution for incorporating historical information from type collections into modern species concepts is to designate an epitype from contemporary collections for which genetic data are available. For the genus *Pugetia*, the taxonomic status of many of the species will remain in question until new collections are made from the type localities that can be genetically compared to other species.

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