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RESEARCH ARTICLE

The genus *Callophyllis* (Kallymeniaceae, Rhodophyta) from the central-south Chilean coast (33° to 41° S), with the description of two new species

El género *Callophyllis* (Kallymeniaceae, Rhodophyta) de la costa central y sur de Chile (33° a 41° S), con la descripción de dos nuevas especies

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ABSTRACT

The taxonomic status of the species in the genus *Callophyllis* Kützing (Kallymeniaceae, Rhodophyta) from central-south Chile (33° to 41° S) is examined on the basis of morphological and molecular evidence. Of the four species originally cited for central Chile, *C. variegata*, *C. pinnata*, *C. atrosanguinea* and *C. laciniata*, only the presence of *C. variegata* has been confirmed in this study. *C. pinnata* reported from Chile is found to be different from *C. pinnata* described from California, and it is here newly described as *C. concepcionensis* sp. nov. *C. atrosanguinea* from southern Chile (including *C. linguata* from the Antarctic Peninsula) is distinct from the species called *C. atrosanguinea* from central-south Chile, the latter which is here described as *C. macrostiolata* sp. nov. *C. variegata*, *C. concepcionensis* and *C. macrostiolata* are distinguished from one another by their external habit, the nature and distribution of their cystocarps, and the female reproductive morphology. Comparative rbcL sequence analysis corroborates the distinction of these taxa from central-south Chile and their relationships to other species worldwide.

Key words: *Callophyllis*, Chile, Kallymeniaceae, rbcL, Rhodophyta, systematics.

RESUMEN

Se examina el estatus taxonómico de las especies del género *Callophyllis* Kützing (Kallymeniaceae, Rhodophyta) de la costa centro-sur de Chile (33° a 41° S) en base a caracteres morfológicos y moleculares. De las cuatro especies citadas para Chile central, *C. variegata*, *C. pinnata*, *C. atrosanguinea* y *C. laciniata*, solo la presencia de *C. variegata* ha sido confirmada en este estudio. *C. pinnata* de Chile muestra diferencias con *C. pinnata* descrita para California y con el resto de las especies de *Callophyllis* hasta ahora conocidas, constituyendo así una nueva especie, *C. concepcionensis* sp. nov. *C. atrosanguinea* del sur de Chile (incluyendo *C. linguata* de la Península Antártica) muestra diferencias con la especie llamada *C. atrosanguinea* de la costa centro-sur de Chile, esta última especie es descrita como *C. macrostiolata* sp. nov. *C. variegata*, *C. concepcionensis* y *C. macrostiolata* se distinguen una de otra por la morfología externa del talo, la distribución y morfología de los cistocarpos, incluyendo la morfología reproductiva femenina. El análisis comparativo de las secuencias de la subunidad mayor del gen Rubisco (rbcL), corrobora la distinción de los taxa procedentes de la zona central y sur de Chile y su relación con otras especies del género provenientes de otras partes del mundo.

Palabras clave: *Callophyllis*, Chile, Kallymeniaceae, rbcL, Rhodophyta, sistemática.

INTRODUCTION

The genus *Callophyllis* Kützing (1843) contains the largest number of species in the family Kallymeniaceae, with more than 50 species (Guiry & Guiry 2011) distributed preferentially in cold and temperate waters of both hemispheres. The distinction among *Callophyllis* species is based on comparative vegetative features such as color, size, and branching pattern, as well as reproductive characters such as number of carpogonial branches per supporting cell, position and size of cystocarps and number of ostioles, and whether or not tetrasporangia are organized in sori (Setchell 1923, Dawson 1954, Norris 1957, Abbott & Norris 1965). The morphological variability found in the genus has led many

authors to overestimate the number of species, as in the Pacific Coast of North America where most of the species are concentrated (Abbott & Norris 1965).

In the southern hemisphere, nine species of *Callophyllis* have been reported from the temperate Pacific Coast of South America, namely the generitype *C. variegata* (Bory) Kützing, *C. atrosanguinea* (J.D. Hooker & Harvey) Hariot, *C. fastigiata* (J. Agardh) J. Agardh, *C. laciniata* (Hudson) Kützing, *C. multifida* (Reinsch) Kylin, *C. pinnata* Setchell & Swezy, *C. tenera* J. Agardh, *C. violacea* J. Agardh, and *Callophyllis* sp. (Ramírez & Santelices 1991). Most of these species are reportedly distributed in the southern part of the continent (Hooker & Harvey 1847, Hariot 1889, Skottsberg 1923, Pujals 1963, Lee 1964, Ramírez 1982, Wiencke & Clayton 2002). For the central-south Chilean coast only four species have been reported: *C. variegata*, *C. pinnata*, *C. laciniata* and *C. atrosanguinea* (Ramírez & Santelices 1991). The distribution of *C. variegata* also includes the Falkland Islands, Fuegia, Iles Crozet, Iles Kerguelen, Macquarie Islands, New Zealand, South Africa, South Georgia, South Orkney Islands, and Tristan da Cunha Islands (Ricker 1987). According to Ricker (1987), large numbers of often poorly defined species of *Callophyllis* occur in New Zealand and subantarctic regions. Morphological studies on *C. variegata* by Montagne (1852) and Etcheverry (1986) emphasized a polymorphic thallus and the position of the cystocarps on the blade margins as remarkable features for this species. Hooker & Harvey (1847) described six varieties of *C. variegata* for the subantarctic region of South America, with one of them (var. *atrosanguinea*) later elevated to species level by Hariot (1889).

C. atrosanguinea is reported from both south and central Chile (Ramírez & Santelices 1991). This species shares with *C. variegata* a circumpolar distribution (Adams 1994), being cited for the Falkland Islands, Kerguelen Island, and New Zealand. According to Levring (1960) and Etcheverry (1986), *C. atrosanguinea* and *C. variegata* are conspecific based on thallus shape; Adams (1994), however, suggested that the two taxa are distinct species that could be distinguished in New Zealand by the texture and color of the thallus while downgrading the importance of whether cystocarps are scattered over the blade, as in *C. atrosanguinea*, or restricted to the margins, as in *C. variegata*.

C. pinnata was described by Setchell & Swezy in Setchell (1923) from Duxbury reef, Marin County, California, for a North Pacific species occurring from Whidbey Island, Washington, to Baja California (Abbott & Norris 1965); the species was later reported for the central-south coast of Chile (Ramírez & Rojas 1988). The description by Ramírez & Rojas (1988) of the morphology, anatomy and some reproductive attributes of *C. pinnata* from Chile agrees with the one given by Abbott & Norris (1965) and Abbott & Hollenberg (1976) for *C. pinnata* from the Pacific coast of North America. However, a molecular analysis conducted by Harper & Saunders (2002) noted that two isolated specimens identified as "*C. pinnata*" from Chile and California do not group together in a phylogenetic tree on the basis of LSU rDNA sequence analysis, suggesting that perhaps the South American taxon could represent either a previously unrecognized species or perhaps a robust form of *C. violacea* J. Agardh.

C. laciniata from the central coast of Chile (Valparaíso) and Peru (Callao) has not been reported since Montagne (1852). Ramírez & Santelices (1991) point out that Howe (1914) had already expressed doubts about the Peruvian record. The geographical distribution of *C. laciniata* is reported to be restricted to Western Europe (Bert 1967) and the Islas Orcadas del Sur (Orkney Islands, in the southern Atlantic Ocean) (Pujals 1963).

An important goal of this study is to describe and illustrate the reproductive system of *Callophyllis* species from central-south Chile as these diagnostic characters are missing in reports of the taxa from this region. Since the *Callophyllis* species from Chile have not been the subject of an up-to-date systematic revision, the present study is a critical examination of the species reported from the central-south coast of Chile (33° to 41° S), *C. variegata*, *C. pinnata*, *C. laciniata* and *C. atrosanguinea*, on the basis of a comparative study of vegetative and reproductive structures, and of rbcL sequence data.

It is especially important that the species of *Callophyllis* are well characterized as in Chile,

one to several species of *Callophyllis* identified as *C. variegata* or *Callophyllis* spp. and known collectively as "carola", are being exported as raw material for direct consumption.

METHODS

Morphological analyses

The material examined belongs to the Herbarium of the Museo Nacional de Historia Natural, Santiago, Chile (SGO), Herbarium of the Marine Science Department at University of Valparaiso, Chile (UV), and Herbarium of the University of Louisiana at Lafayette (LAF). Reference material of *C. atrosanguinea* was obtained from the Muséum National d'Histoire Naturelle in Paris, France (PC). Algal samples were collected in the Concepción-Arauco area, in Cocholgüe, Coronel and Lota (36º35' S, 72º57' W) and Isla Santa María (36º59' S, 73º32' W). Voucher specimens are also deposited in the algal collection of the Department of Oceanography at the University of Concepcion, Chile (CON) and in the LAF Herbarium. Specimens were pressed as herbarium sheets, and duplicates were wet-preserved in 10 % formalin/seawater and subsequently transferred to 5 % formalin/seawater for long-term storage. Longitudinal and cross sections were done by hand using a razor blade. Staining was performed using the modified Wittmann's aceto-iron-haematoxylin chloral hydrate (Wittmann 1965) method of Hommersand et al. (1992), with samples mounted in 50 % Hoyer's medium (Stevens 1981). Sections were also stained with 1 % aniline blue, acidified with 1 % HCl and mounted in 50 % Karo™ Syrup. Images of habit were taken using a Cannon New F-1 camera. Observations were conducted using an Olympus CHT microscope, and photographs were taken with a Nikon Coolpix 4500 and Polaroid Ie digital microscope camera, respectively.

Molecular analyses

Total DNA was extracted from silica gel-dried specimen parts of the same samples that were formalin-fixed. Voucher specimens are deposited at the University of Louisiana at Lafayette (LAF). DNA extraction was performed with a DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). Specific gene regions were amplified by PCR and prepared for sequencing following the protocols described in Gavio & Fredericq (2002). The rbcL primers used for amplification and sequencing reactions were as follows: FrbcLstart, F57, R753, R1150, RrbcSstart (Freshwater & Rueness 1994); F577, F753, F993 (Hommersand et al. 1994) and F64 (Lin et al. 2001). Sequencing reactions were set up using the Big Dye kit and protocol (Applied Biosystems, Foster City, CA, USA), and run on ABI 3100 Genetic Analyzers (DNA Analysis Core Facility, CMS; Biology Deparment, UL Lafayette). Sequence contigs were assembled using Sequencher (Gene Codes Corp., Ann Arbor, MI, USA) and sequence data sets were compiled and aligned using MacClade (v.4, Maddison & Maddison 2000). Phylogenetic analyses were conducted with the Maximum Parsimony (MP) algorithm as implemented in PAUP 4.0b 10 (Swofford 2002), the Maximum Likelihood (ML) algorithm using RAxML (Stamatakis et al. 2008), and the Bayesian (MB) inference as implemented in MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). Maximum likelihood tree was done under the General Time Reversible (GTR) + GAMMA evolutionary model of nucleotide substitution. A parametric bootstrap test was applied to the ML analysis using 100 boostrap replicates. For Bayesian trees four Metropolis-coupled Markov chain Monte Carlo (MCMC) chains (one cold and three incrementally heated) were run using the GTR + I + Γ model of sequence evolution for 5000000 generations, trees were sampled every 1000 generations, with a burn-in value of 20000 generations. Maximum parsimony trees were inferred from a heuristic search, excluding uninformative characters consisting of 1000 random sequence additions holding 10 trees at each step. MULPARS and tree-bisection-reconnection (TBR) algorithms with the MULTREES (saving multiple trees) and STEEPEST DESCENT option. Support for nodes of the MP analysis groups was determined by calculating bootstrap proportion based on 1000 replicates. Species examined, collection localities, and GenBank accession numbers for generated sequences are listed in Table 1.

RESULTS

Observations

Callophyllis specimens were collected in central-south Chile (see Appendix) and examined for their habit shape, and for their vegetative and reproductive morphology. Three distinct entities were recognizable as follows:

Species 1

Habit and vegetative morphology: Pink to intense red in color, with a membranous consistency. The holdfast is small and extends in a short stipe (Figs. 1A-1C) bearing fronds up to 30 cm long. The thallus is fan-shaped, consisting of flat, dichotomous to subdichotomous branches (6-20 or more) in a same plane; additional blades are irregularly divided and are numerous near the basal region of the thallus (Figs. 1A-1C). Cystocarps are located on the upper margins of the fronds, forming rows in some cases (Fig. 1H). Prominent cystocarps bear one (Fig. 1G) to three (Fig. 1I) ostioles. The tetrasporophytic thalli are superficially similar to the gametophytes, with reproductive structures irregularly distributed throughout the fronds (Fig. 1B). Male specimens are typically thinner than the female gametophytes (Fig. 1C).

Fronds are 50-140 µm thick, with a cortex of three layers of unordered cortical cells (Fig. 1E), the outer cells isodiametric (approximately

5 µm in diameter) to rectangular (7 µm long x 5 µm wide). The subcortex is comprised of isodiametric cells, 7 µm long x 7-15 µm wide. The medulla consists of three to four layers of ovoid cells (Fig. 1G), 65-90 µm x 35-50 µm, surrounded by rhizoidal cells (Fig. 1G, arrow), 17-25 µm long x 5-15 µm wide, that are more abundant in mature parts of the thallus.

TABLE 1

Callophyllis species and associated taxa included in the molecular analyses.

Especies de *Callophyllis* y otros taxa incluidos en los análisis moleculares.

Species	Locality and collector	Date	Collector notes	Nº Genbank
Callophyllis variegata (Bory) Kützing	Isla Santa María, Concepción, Chile, coll. N. Arakaki	28 Jul 2000	LAF-7-28-00-1-1 (N-2)	HQ910494
Callophyllis variegata (Bory) Kützing	Playa Mendieta, Ica, Perú, coll. N. Arakaki	6 Jan 2006	LAF-1-6-06-1-1 (N-46)	HQ910495
Callophyllis concepcionensis sp. nov.	Puerto Montt, Chile, Angelmo food market, coll. S. Fredericq & M.E. Ramírez	24 Feb 1994	24.ii. LAF-2-24-94-1-1	AY294397
Callophyllis concepcionensis sp. nov.	Isla Santa María, Concepción, Chile, coll. N. Arakaki	28 Jul 2000	LAF-7-28-00-1-2 (N-5)	HQ910496
Callophyllis concepcionensis sp. nov.	Isla Santa María, Concepción, Chile, coll. N. Arakaki	28 Jul 2000	LAF-7-28-00-1-3 (N-6)	HQ910497
Callophyllis pinnata Setchell & Swezy	Central Beach of Moss Beach, California, USA, coll. S. Fredericq	17 Jul 1996	LAF-7-17-96-1-1 (N-174)	HQ910498
Callophyllis edentata Kylin	Cast Ashore, Hopkins Marine Station, Pacific Grove, California, USA, coll. P. Martone	3 Oct 2005	LAF-10-3-05-1-1 (N-43)	HQ910499
Callophyllis edentata Kylin	Cast Ashore, Hopkins Marine Station, Pacific Grove, California, USA, coll. P. Martone	3 Oct 2005	LAF-10-3-05-1-2 (N-44)	HQ910500
Callophyllis macrostiolata sp. nov.	Isla Santa María, Concepción, Chile, coll. N. Arakaki	18 Mar 2001	LAF-3-18-01-1-1 (N-15)	HQ910501
Callophyllis macrostiolata sp. nov.	Isla Santa María, Concepción, Chile, coll. N. Arakaki	18 Mar 2001	LAF-3-18-01-1-2 (N-16)	HQ910502
Callophyllis sp.	Punta Santa Ana, Estrecho de Magallanes, Chile, coll. M. Núñez	15 Apr 2000	LAF-4-15-00-1-1 (N-1)	HQ910503
Callophyllis atrosanguinea (J.D. Hooker & Harvey) Hariot	Punta Dungeness, Chile, coll. M.R. Ramírez.	11 Dec 2001	LAF-12-11-01-1-1 (N-170)	HQ910504
Callophyllis crispata Okamura	Tokawa, Choshi, Chiba Prefecture, Japan, coll. M. Yoshizaki	22 May 1993	LAF-5-22-93-1-1	UO4190
Callophyllis edentata Kylin	Indian I. Jetty, WA, USA, coll. M.H. Hommersand	10 Jun 1994	LAF-6-10-94-1-1	HQ910505
Callophyllis firma (Kylin) R. Norris	Mar Vista Resort, W. San Juan I., WA, USA, coll. M. Wynne.	26 Jul 1995	LAF-7-26-95-1 (Wynne-10363)	HQ910506
Callophyllis hombroniana (Montagne) Kützing	Ringaringa, New Zealand, drift, coll. W. Nelson	6 Oct 1994	LAF-10-6-94-1-1	U21804

TABLE 1. Continuación

Species	Locality and collector	Date	Collector notes	Nº Genbank
Callophyllis japonica Okamura	Nemoto, Awa Co., Chiba Pref., Japan, coll. S. Fredericq & M. Yoshizaki	3 Sep 1993	LAF-9-3-93-2-2	HQ910507
Callophyllis laciniata Kützing	Penmarch, Brittany, France, coll. M.H. Hommersand	20 Jun 1993	LAF-6-20-93-1-1	HQ910508
Callophyllis lambertii (Turner) J. Ag.	Port MacDonnell, Australia, coll. M.H. Hommersand	29 Aug 1995	LAF-8-29-95-1-1	HQ910509
Callophyllis linguata Kylin	Pta. Adley, Bahía Fildes, King George I., Antarctic Península, coll. S. Fredericq & J. Rodríguez	8 Feb 1994	LAF-2-8-94-1-12	HQ910510
Callophyllis obtusifolia J. Agardh	Baja California, México, coll. L. & R. Aguilar	29 Sep 1995	LAF-9-29-95-1-1	HQ910511
Callophyllis rangiferinus (Turner) Womersley	Port MacDonnell, S. Australia, coll. M.H. Hommersand & G.T. Kraft	15 Jul 1995	LAF-7-15-95-1-1	HQ910512
Callophyllis violacea (J. Agardh) Kylin	Pigeon Point, San Mateo Co., CA, USA, coll. M.H. Hommersand	21 Dic 1992	LAF-12-21-92-1-1	UO4191
Callophyllis sp.	Bahía Collins, King George I., Antarctic Peninsula, coll. S. Fredericq & J. Rodríguez	10 Feb 1994	LAF-2-10-94-1-2	U21802
Callophyllis depressa (J. Agardh) Schmitz ex Laing	Chatham Island, New Zealand, coll. R. D'Archino, W. Nelson & G.C. Zuccarello			GQ376535
Callophyllis decumbens J. Agardh	Northland, Doubtless Bay, Cable Bay, New Zealand, coll. R. D'Archino, W. Nelson & G.C. Zuccarello			GQ376536
Callophyllis sp.	Chatham Island, New Zealand, coll. R. D'Archino, W. Nelson & G.C. Zuccarello		GGCZ-2009b	GQ376537
Pugetia delicatissima R. Norris	New Zealand, coll. W. Nelson	6 Apr 1996	LAF-4-6-96-1-1	HQ910513
Kallymenia reniformis (Turner) J. Agardh	Piguet, Brittany, France, coll. J. Cabioch	22 Jun 1993	LAF-6-22-93-1-1	AY294377

Reproductive morphology: Female gametophytes bear procarps on both sides of the thallus, near the frond margin (Fig. 1D). A supporting cell, originated from a subcortical cell, forms two to four filaments comprising one to three two-celled unfertile subsidiary cells (Fig. 1E). The other filament corresponds to a three-celled carpogonial branch with a carpogonium bearing a trichogyne directed towards the surface. A single carpogonial branch per supporting cell is produced (Fig. 1F) per procarp. Mature procarps measure aproximately 87 µm in diameter. The supporting cell, the subsidiary cells and the basal cell of the carpogonial branch become lobulated, the hypogynous cell remains isodiametric and smaller than the rest of the procarp cells, and a triangular-shaped carpogonium contains a distinct nucleus (Fig. 1F). A fusion cell forms after presumed fertilization, established by the fusion of the subsidiary cells, the basal cell of the carpogonial branch and the supporting cell. The fusion cell cuts off protrusions towards the medulla that continue to grow into gonimoblast filaments of uninucleate cells, 5-12 µm long x 3-5 µm wide. Gonimoblast filaments develop between medullary cell spaces.

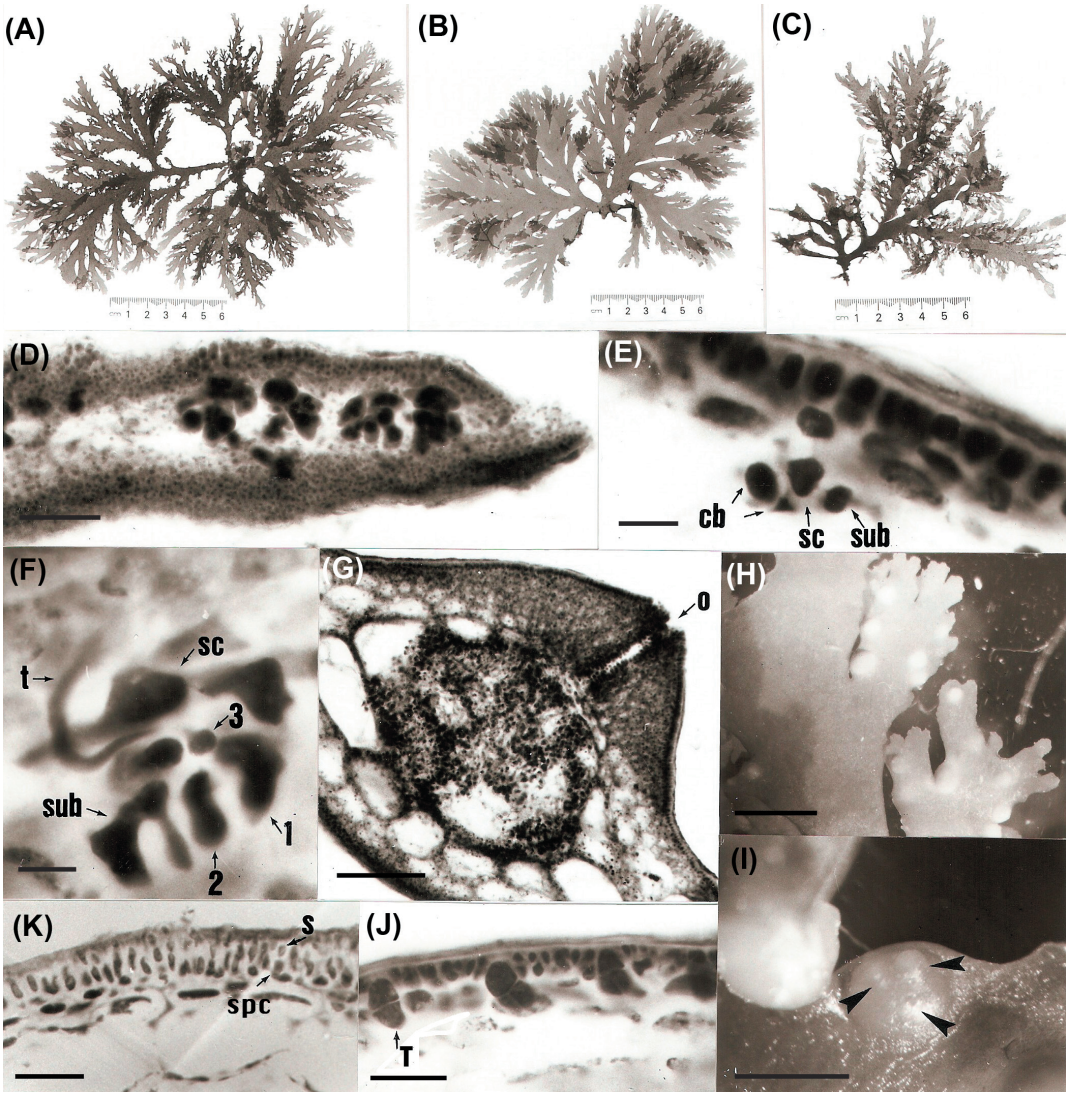

Fig. 1: Habit and reproductive structures of *C. variegata*. (A)**:** Female gametophyte, Concepción, Caleta Cocholgüe, K. Alveal, 19.i.1990 (CON 1155). (B): Tetrasporophyte, Concepción, Caleta Cocholgüe, N. Arakaki, 11.xi.2000 (CON). (C): Male gametophyte, Bahía Coliumo, Los Morros, H. Romo, 3.iv.1976 (CON 1105). (D): Longitudinal section through branch showing procarps. Scale = 50 µm. (E): Immature procarp showing supporting cell (sc) with carpogonial branch (cb) and subsidiary cell (sub). Scale = 10 µm. (F): Mature procarp (sc, supporting cell; t, trichogyne; first (1), second (2) and third (3) cells of carpogonial branch; sub, subsidiary filament). Scale = 10 µm. (G): Mature cystocarp lacking pericarp and with ostiole projecting toward outside (o). Scale = 100 µm. (H): Cystocarps distributed on upper edges of frond. Scale = 3 mm. (I): Cystocarp with three ostioles (arrowhead). Scale = 1 mm. (J): Longitudinal section showing tetrasporangia (T) located in cortex. Scale = 50 µm. (K): Longitudinal section showing spermatangia (spc, spermatangial parent cell; s, spermatium). Scale = 25 µm.

Hábito y estructuras reproductivas de *C. variegata*. (A): Talo gametofítico femenino, Concepción, Caleta Cocholgüe, K. Alveal, 19.i.1990 (CON 1155). (B): Talo tetrasporofítico, Concepción, Caleta Cocholgüe, N. Arakaki, 11.xi.2000 (CON). (C): Talo gametofítico masculino, Bahía Coliumo, Los Morros, H. Romo, 3.iv.1976 (CON 1105). (D): Corte longitudinal mostrando procarpos. Barra = 50 µm. (E): Procarpo inmaduro mostrando una célula de soporte (sc) con una rama carpogonial (cb) y una célula subsidiaria (sub). Barra = 10 µm. (F): Procarpo maduro (sc, célula de soporte; t, tricogino; 1, primera 2, segunda y 3, tercera célula de la rama carpogonial; sub, filamento subsidiario). Barra = 10 µm. (G): Cistocarpo maduro sin pericarpo y con un ostiolo proyectado en punta (o). Barra = 100 µm. (H): Cistocarpos localizados en los márgenes superiores de la fronda. Barra = 3 mm. (I): Cistocarpo con tres ostiolos (cabeza de flecha). Barra = 1 mm. (J): Corte longitudinal mostrando tetrasporangios (T) localizados en la corteza. Barra = 50 µm. (K): Corte longitudinal mostrando espermatangios (spc, célula parental espermatangial; s, espermacio). Barra = 25 µm.

The gonimoblasts cut off chains of three to four carposporangia filling part of the medulla when mature and they are distributed in groups separated by abundant vegetative filaments (Fig. 1G). The cystocarps are spherical, reaching 1 mm in diameter (Figs. 1H, 1I). Mature carpospores, about 15 µm in diameter, are surrounded by medullary filaments, about 100 µm thick, that do not contribute to a pericarp. Cortical cell layers increase in number and project towards the outside forming exit pores (Fig. 1G). Cystocarps are located near the margins of the thalli, especially in young parts (Fig. 1H). Each cystocarp is topped by one to three ostioles (Fig. 1I) located on one or both sides of the fronds, with each ostiole, up to 250 µm tall, extending inwardly in a narrow channel.

Cruciately divided tetrasporangia, measuring approximately 27 µm long x 17 µm width, are located on both sides of the tetrasporophyte fronds; they are irregularly grouped, with most concentrated near the apical region. Tetrasporangial parent cells originate from inner cortical cells. Cortical cells surrounding the tetrasporangia do not become modified and do not increase the number of cell layers (Fig. 1J). Male gametophytes cut off spermatangial parent cells (Fig. 1K), 7 µm long x 5 µm wide, that are surface cells and produce spermatangia, approximately 4 µm in diameter. The spermatia are released through a disintegrating zone at the thallus surface (Fig. 1K).

Species 2

Habit and vegetative morphology: Membranous to cartilaginous consistency and pink to dark purple in color. The stipe is generally thin and short, although in some specimens the first division is 2 or 3 cm removed from the holdfast, extending in a palmate or flabellate upright frond that reaches a height of up to 35 cm, with wide main segments usually not exceeding 3 cm (Figs. 2A-2C). Thalli are four to eight times dichotomously branched in a regular pattern. Although most specimens have narrow branch angles at the branching points and smooth margins, some have laciniate branches that only appear on the upper part of the frond. The apices are generally rounded. Fronds sometimes bear few to many pinnate proliferations 1 to 4 cm long x 1 cm wide (Fig. 2A). Female gametophytes bear barely projecting cystocarps scattered over the frond. Male gametophytes are similar to female gametophytes and tetrasporophytes (Fig. 2B) in size and shape, but are thinner and sometimes more branched (Fig. 2C). Thalli are approximately 150-500 µm thick. Longitudinal sections through the frond at mid level reveal a two-layered cortex consisting of regular, elongate surface cells 7 µm long x 3-5 µm wide. The subcortical cells are 5-10 µm long x 5-17 µm wide. The medulla consists of four layers of cells 60-80 µm long and 100-300 µm wide, surrounded by rizoidals cells, 3-15 µm wide x 12-25 µm long (Fig. 2H).

Reproductive morphology: The female gametophyte has procarps located on both surfaces of the frond, isolated from other procarps (Fig. 2D). A supporting cell originates from a subcortical cell, 7-10 µm in diameter, and cuts off three filaments, two of them comprising two-celled subsidiary cells (Fig. 2E). The other filament is a carpogonial branch of three cells with a carpogonium bearing a curved trichogyne. The procarp consists of a single carpogonial branch per supporting cell. Subsequently, cells of the procarp increase in size, most of them become lobulate with the exception of the hypogynous cell and the carpogonium. Mature procarps measure about 87 µm (Fig. 2F) in diameter. After presumed fertilization, a fusion cell, formed from the fusion of the procarp cells, cuts off gonimoblast initials towards the medulla (Fig. 2G), that continue cutting of chains of uninucleate gonimoblast cells, each 7-17 µm long x 4-5 µm wide.

Small rhizoidal cells in the medulla tend to be globose when close to gonimoblast filaments. Short three-celled carposporangial chains within cystocarps distributed over the frond surface measure 1 to 2 mm in diameter and completely fill the medulla with carpospores 12 to 17 µm in diameter. The cystocarps are surrounded by vegetative filaments, 65-125 µm thick, whose cells becoming differentiated from the medullary cells (Fig. 2H). Each cystocarp is topped by one to three ostioles (Fig. 2I), whose exit channels are surrounded by a few layers of cells resembling a simple breakage in the cortex. These channels extend to a depth of 125-250 µm

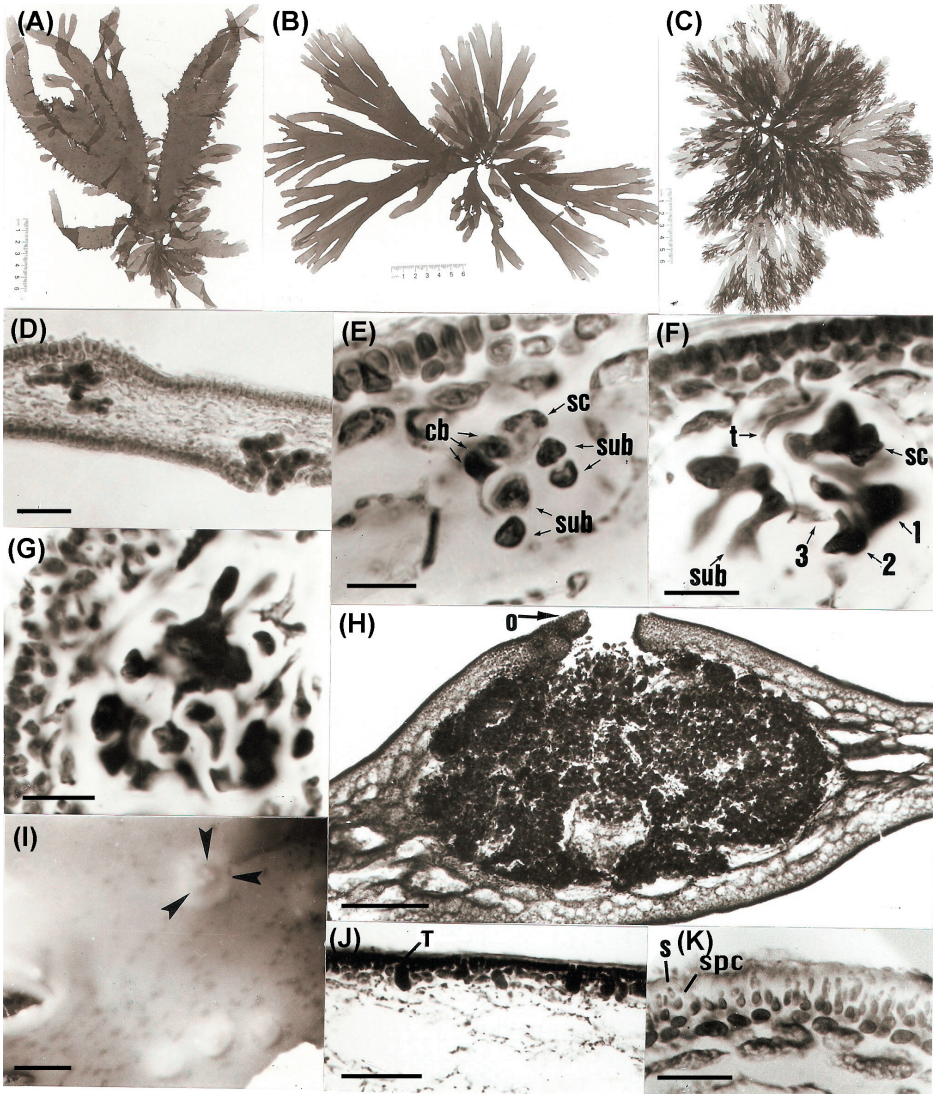

Fig. 2: Habit and reproductive structures of *C. concepcionensis*. (A): Female gametophytic thallus with proliferations, Concepción, Golfo de Arauco, J. Vásquez, i.1981 (SGO 100665, Type). (B): Tetrasporophytic thallus, Concepción, Caleta Cocholgüe, N. Arakaki, 18.xi.2000 (CON). (C): Male gametophytic thallus, Concepción, Caleta Cocholgüe, N. Arakaki, 18.xi.2000 (CON). (D): Longitudinal section showing procarps. Scale = 50 µm. (E): Inmature procarp showing supporting cell (sc) with carpogonial branch (cb) and two subsidiary filaments (sub). Scale = 10 µm. (F): Mature procarp (sc, supporting cell; t, trichogyne; 1, first 2, second and 3, third cells of carpogonial branch; sub, subsidiary filament). Scale = 20 µm. (G): Fusion cell with gonimoblast filaments. Scale = 20 µm. (H): Mature cystocarp with a simple ostiole (o). Scale = 200 µm. (I): Cystocarps distributed over entire frond. Detail of a cystocarp with three ostioles (arrowhead). Scale = 1 mm. (J): Longitudinal section of tetrasporangia (T) located in cortex. Scale = 100 µm. (K): Longitudinal section of spermatangia (spc, spermatangial parent cell; s, spermatium). Scale = 25 µm.

Hábito y estructuras reproductivas de *C. concepcionensis*. (A): Talo gametofítico femenino con proliferaciones, Concepción, Golfo de Arauco, J. Vásquez, i.1981 (SGO 100665, Tipo). (B): Talo tetrasporofítico, Concepción, Caleta Cocholgüe, N. Arakaki, 18.xi.2000 (CON). (C): Talo gametofítico masculino, Concepción, Caleta Cocholgüe, N. Arakaki, 18.xi.2000 (CON). (D): Corte longitudinal mostrando procarpos. Barra = 50 µm. (E): Procarpo inmaduro mostrando una célula de soporte (sc) con una rama carpogonial (cb) y dos filamentos subsidiarios (sub). Barra = 10 µm. (F): Procarpo maduro (sc, célula de soporte; t, tricogino; 1, primera 2, segunda y 3, tercera célula de la rama carpogonial; sub, filamento subsidiario). Barra = 20 µm. (G): Célula de fusión emitiendo filamentos gonimoblásticos. Barra = 20 µm. (H): Cistocarpo maduro con un ostiolo simple (o). Barra = 200 µm. (I): Cistocarpos distribuidos sobre la fronda. Detalle de un cistocarpo con tres ostiolos (cabeza de flecha). Barra = 1 mm. (J): Corte longitudinal mostrando tetrasporangios (T) localizados en la corteza. Barra = 100 µm. (K): Corte longitudinal mostrando espermatangios (spc, celula parental espermatangial; s, espermacio). Barra = 25 µm.

and open to a single side of the frond. In female specimens, extensive zones bearing young or immature cystocarps are color-less. Most cystocarps are located next to the margins, but in some specimens, are scattered over the entire thallus.

Tetrasporophyte specimens bear tetrasporangia that are distributed irregularly throughout the thallus, abundantly at mid-level. Cruciately divided tetrasporangia, 27-37 µm long x 15-20 µm wide, originate from tetrasporangial initials in the inner cortex on both sides of the thallus (Fig. 2J). Male gametophytes bear spermatangia, about 4 µm in diameter, cut off from isodiametric spermatangial parent cells, 5 µm in diameter, in the outer cortex on both sides of the thallus. Colorless spermatia are released at the thallus surface, surrounded by a gelatinized cuticle (Fig. 2K).

Species 3

Habit and vegetative morphology: Cartilagenous thallus, red to brown in color, subdichotomous branching. Stipe 1.5 cm long, widening gradually to 5-7 cm until the first branching point, thallus reaching 12 to 35 cm in length, branching points rounded, branch apices lightly rounded and weakly dissected (Figs. 3A-3C). Female gametophytes with abundant distal segments, narrow at the base, dividing four to 13 times, some with lateral proliferations (Fig. 3A). Cystocarps prominent over the entire frond, each with a large, centrally invaginated ostiole (Fig. 3H). Tetrasporophyte thalli (Fig. 3B) and male gametophytes (Fig. 3C) less ramified than the female gametophytes. Thalli 50-150 µm in thickness, with a cortex of four to five cell layers, surface cells isodiametric to rectangular, 5-7 µm long x 4-5 µm wide, inner cells isodiametric, 7-15 µm in diameter. Cells gradually increasing in size towards the medulla. Medulla of five to eight layers of ovoidal cells, 65-140 µm long x 30-70 µm wide. Abundant medullary filaments of small rhizoidal cells present, 7-20 µm long x 4-12 µm wide.

Reproductive morphology: Procarps originate on both sides of the thallus over the entire surface in groups and can be seen in different stages of development (Fig. 3D). Supporting cells, formed from an internal cortical cell 5-7 µm in diameter, each cut off a single three-celled carpogonial branch (Fig. 3E) and sometimes a one- or two-celled subsidiary filament. The carpogonium emits a thin trichogyne towards the surface. When the procarp matures, the supporting cell and cells of the subsidiary filament increase in size, become rounded, bulbose, elongated and oriented toward the interior of the medulla. Mature procarps measure 75 µm x 37 µm (Fig. 3F). Following presumed fertilization, the supporting cell fuses with cells of carpogonial branch forming a fusion cell that emits extensions from which initial gonimoblasts are generated. Cells of the gonimoblast filaments measure 7-12 µm in length x 2-5 µm in width and form chains of carposporangia in groups of three, carpospores measure 10 to 17 µm in diameter. Cystocarps 2 mm in diameter (Fig. 3H), with a large ostiole on only one side of the thallus to the thallus (Fig. 3G), central ostiole channel 140-400 µm long. Vegetative medullary filaments surround compact cystocarps 150-350 µm in thickness, and consisting of isodiametric cells 10-40 µm in diameter.

Tetrasporangia originate in the subcortex, and are distributed over the entire surface of the frond, on both sides of the thallus. Tetrasporangial initials developing in cruciate tetrasporangia 25-27 µm x 15-17 µm, and remaining embedded among cortical cells, surrounded by several cells, less numerous towards the apical portions of the frond (Fig. 3I). Spermatangia occur on both sides of the frond. The spermatangial parent cells, 4 µm x 5 µm, are formed in the outer cortex, and produce spermatangia 4 µm in diameter. The liberation of the spermatia occurs together with the gelatinization of the thallus' surface (Fig. 3J).

Molecular analyses

The rbcL dataset included in the analyses consists of 1419 characters for 29 taxa; *Kallymenia reniformis*, the type of the family, was selected as the outgroup. The Maximum Likelihood (ML) analysis resulted in a tree indicating that most species relationships are strongly supported (Fig. 4). The Bayesian analysis produced a majority rule consensus tree that corroborates the overall tree topology

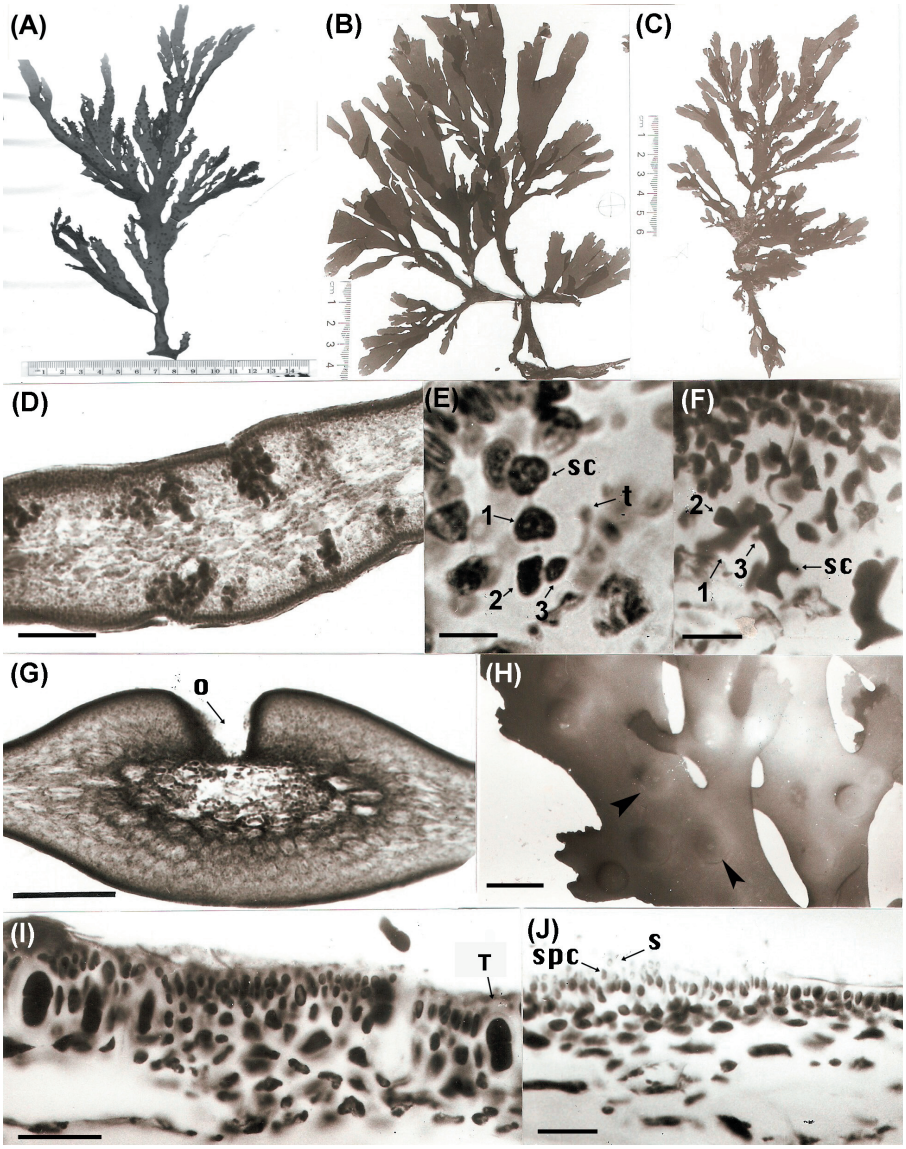

Fig. 3: Habit and reproductive structures of *C. macrostiolata*. (A): Female gametophytic thallus, Concepción, Isla Santa María, N. Arakaki, 17.iii.2001 (SGO 159068, Type). (B): Tetrasporophytic thallus, Concepción, Isla Santa María, N. Arakaki, 16.xi.2000 (CON). (C): Male gametophytic thallus, Concepción, Isla Santa María, N. Arakaki, 16.xi.2000 (CON). (D): Longitudinal section showing procarps. Scale = 100 µm. (E): Inmature procarp (sc, supporting cell; 1, first 2, second and 3, third cells of carpogonial branch; t, trichogyne). Scale = 10 µm. (F): Mature procarp (same abbreviations of Fig. 27). Scale = 25 µm. (G): Mature cystocarp with an invaginated ostiole (o). Scale = 200 µm. (H): Ostiolate cystocarps (arrowhead) distributed over entire frond. Scale = 2 mm. (I): Longitudinal section showing tetrasporangial formation, tetrasporangia (T) located in cortex. Scale = 50 µm. (J): Longitudinal section showing spermatangial organization (spc, spermatangial parent cell; s, spermatium). Scale = 25 µm.

Hábito y estructuras reproductivas de *C. macrostiolata*. (A): Talo gametofítico femenino, Concepción, Isla Santa María, N. Arakaki, 17.iii.2001 (SGO 159068, Tipo). (B): Talo tetrasporofítico, Concepción, Isla Santa María, N. Arakaki, 16.xi.2000 (CON). (C): Talo gametofítico masculino, Concepción, Isla Santa María, N. Arakaki, 16.xi.2000 (CON). (D): Corte longitudinal mostrando procarpos. Barra = 100 µm. (E): Procarpo inmaduro (sc, célula de soporte; 1, primera 2, segunda y 3, tercera célula de la rama carpogonial; t, tricogino). Barra = 10 µm. (F): Procarpo maduro procarp (abreviaturas de Fig. 27). Barra = 25 µm. (G): Cistocarpo maduro con un ostiolo invaginado (o). Barra = 200 µm. (H): Cistocarpos distribuidos sobre la fronda. Cada cistocarpo con un ostiolo (cabeza de flecha). Barra = 2 mm. (I): Corte longitudinal mostrando tetrasporangios (T) localizados en la corteza. Barra = 50 µm. (J): Corte longitudinal mostrando espermatangios (spc, célula parental espermatangial; s, espermacio). Barra = 25 µm.

resulting from the ML analysis. The Maximum Parsimony (MP) analysis also recovered twelve most parsimonious trees with similar topologies. In the dataset, 257 characters (18 % of the total) were parsimony-informative, 148 characters were parsimony-uninformative, and 1014 characters were constant. The support values for the MP, ML and Bayesian analyses (PP) are shown in Fig. 4. *C. concepcionensis* sp. nov. (a taxon previously known as *C. pinnata*) from Chile and *C. pinnata* from California are resolved into two separate clades. *C. concepcionensis* sp. nov. from Chile is sister to *C. obtusifolia* J. Agardh from Baja California (MP = 94, ML = 96, PP = 100), and *C. pinnata* from California does not group with any of the other species from California and Washington. The % rbcL sequence divergence between *C. pinnata* from California and *C. concepcionensis* from Chile is 4.8-5.

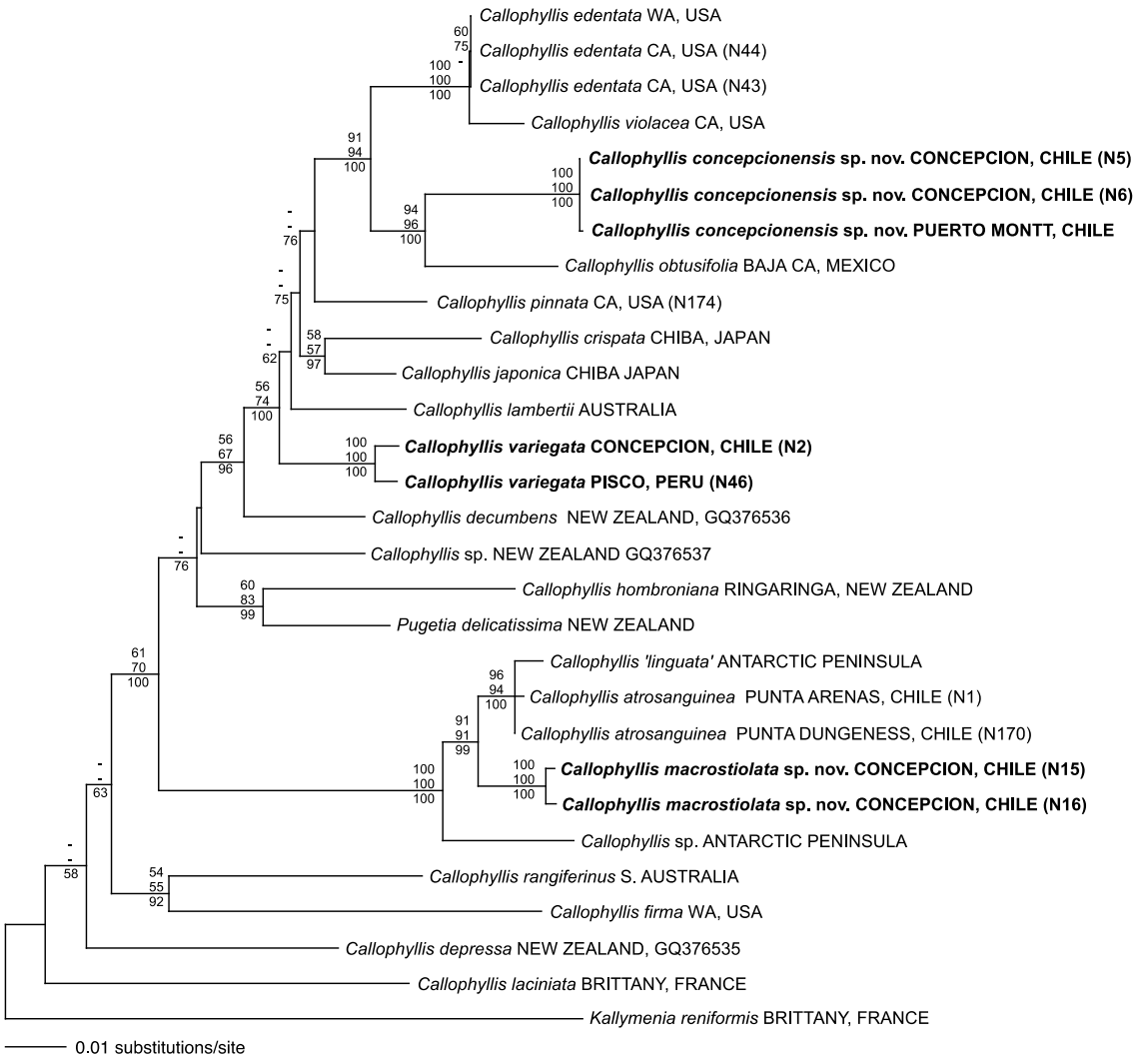

Fig. 4: Maximum-likelihood (ML) tree from the analysis of rbcL sequence data under the (GTR) + GAMMA model, for 28 taxa including *Callophyllis* from central-south Chile (33º to 44º S) (in bold letters) and *Kallymenia reniformis* as the outgroup. Bootstrap proportion values are indicated for MP (1000 replicates) and ML (100 replicates) (above branches), and Bayesian (below branches) methods when > 50.

Arbol filogenético inferido de las secuencias rbcL con un análisis de Maximum-likelihood (ML) usando el modelo (GTR) + GAMMA, para 28 taxa incluyendo *Callophyllis* de la costa centro-sur de Chile (33º to 44º S) (en negritas), y *Kallymenia reniformis* como outgroup. Valores de bootstraps están indicados para los métodos de MP (1000 repeticiones) y ML (100 repeticiones) (sobre las ramas), y Bayesian (debajo de la ramas) para valores > 50.

C. edentata Kylin from California and *C. edentata* from Washington form a clade with *C. violacea* (J. Agardh) Kylin from California (MP = 100, ML = 100, PP = 100). *C. variegata*, the type species of the genus, from Chile and Peru is resolved as a fully supported monophyletic group (MP = 100, ML = 100, PP = 100). The Japanese species *C. crispata* Okamura and *C. japonica* Okamura group together (MP = 58, ML = 57, PP = 97). A Bayesian phylogenetic reconstruction indicates that the *Callophyllis* species from Japan, USA, and Chile form a well-supported clade (PP = 75). This clade, in the Bayesian-generated tree, is weakly resolved as a sister taxon to the Australian species *C. lambertii* (Turner) J. Agardh (PP = 62), but variously supported as a sister group to *C. variegata* from Peru and Chile (ML = 56, MP = 74, PP = 100).

Taxa from New Zealand are spread across four different but closely related clades: *C. decumbens* J. Agardh (GQ376536) clusters with taxa from Japan, USA, Chile, Peru and Australia (MP = 56, ML = 67, PP = 96). The position of *Callophyllis* sp. from New Zealand (GQ376537) lacks support as a sister taxon in any phylogenetic analysis. The clade of the New Zealand taxa *C. hombroniana* (Montagne) Kützing and *Pugetia delicatissima* R. Norris (MP = 60, ML = 83, PP = 99) is sister to the previously mentioned group only in the Bayesian phylogenetic reconstruction (PP = 76). Another New Zealand species, *C. depressa* (J. Agardh) Schmitz ex Laing (GQ376535), occupies a basal position and is poorly resolved (PP = 58).

C. atrosanguinea from Punta Dungeness and Punta Arenas, southern Chile, is conspecific with *C. linguata* Kylin from the Antarctic Peninsula and forms a strongly-supported monophyletic clade (MP = 91, ML = 91, PP = 99) with *C. macrostiolata* sp. nov. from Concepción, Chile. The latter is currently reported as *C. atrosanguinea* but the rbcL sequence divergence between vouchers from central and southern Chile ranges from 2-2.5 %. This new species is clearly resolved as a sister taxon to the Antarctic and Chilean southernmost species of *Callophyllis*. This Antarctic Peninsula and Chilean clade is resolved with variously support as sister to the remainder of the *Callophyllis* taxa (MP = 61, ML = 70, PP = 100).

DISCUSSION

This study recognizes three distinct species of *Callophyllis* from central-south Chile (33º to 41º S) on the basis of comparative morphology and anatomy and sequence analysis of chloroplast-encoded rbcL: Species 1 is here confirmed as representing *C. variegata* (see Appendix), but there is incongruence with the current taxonomy (Abbott & Hollenberg 1976, Etcheverry 1986) of two additional species growing in central Chile. The two species that do not fit the concept of the type of *C. pinnata* Setchell & Swezy and of the type of *C. atrosanguinea* (J.D. Hooker & Harvey) Hariot are here assigned new species rank. Species 2 that goes under the name *C. pinnata* in central Chile is here newly described as *C. concepcionensis* sp. nov. (see Appendix). Species 3 that goes under the name *C. atrosanguinea* in central Chile is here newly described as *C. macrostiolata* sp. nov. (see Appendix).

A nuclear marker, rDNA LSU, explored by Harper & Saunders (2002) provided less resolution within species of *Callophyllis* than obtained with rbcL sequence analysis. In red algae, a rbcL sequence divergence between taxa of ≥ 2 % is the standard to recognize a separate species (see for example Gurgel & Fredericq 2004). The sequence divergence between *C. concepcionensis* and its closest sister taxon, *C. obtusifolia* is 4 %, and between *C. concepcionensis* and *C. pinnata* from type locality in California is 4.8-5 %. Hence, it is clear that *C. concepcionensis*, currently reported as *C. pinnata* in Chile, is a distinct species from true *C. pinnata*.

With a % rbcL sequence divergence of 2-2.5, *C. atrosanguinea* from Punta Dungeness in southern Chile (including *C. linguata* from the Antarctic Peninsula), is distinct from the taxon going under this name from Concepción in central Chile. We have provided a new name for the central Chilean coast, *C. macrostiolata* sp. nov.

In a taxonomic study of *Callophyllis*, *Euthora* and *Pugetia* (Kallymeniaceae) on the basis of rDNA LSU DNA sequences, Harper & Saunders (2002) also found that *C. pinnata* from Isla Chiloé (Chile) and *C. pinnata* from Piedras Blancas, California (USA) comprise different taxa. They did not determine the

identity of the Chilean taxon although based on previous morphological studies they suggested a similarity with *C. violacea* from Peru or *C. obtusifolia* from North America. Our phylogenetic analysis includes *C. obtusifolia* from Baja California and *C. violacea* from California and supports the distinction of the Chilean "*C. pinnata*" as *C. concepcionensis* sp. nov. The habit of *C. obtusifolia* has been well characterized as comprising long, linear blades that are rarely palmately divided with terminal dichotomies longer than penultimate dichotomies (Dawson 1954, Pl. 35 and Pl. 36; Abbott & Hollenberg 1976, Fig. 410); in contrast, *C. concepcionensis* sp. nov. from Concepción, Chile, has straight palmate fronds with narrow branch angles or with laciniate branches that only appear at the upper part of the dissected frond. These characters do not fit the habit pattern described for *C. pinnata* from Moss Beach, California, near to the type locality (Duxbury Reef, Marin Co.), as this species is characterized by being loosely and irregularly branched with long branches 2-3 cm wide that terminate in straight to somewhat pointed apices (Abbott & Hollenberg 1976). Since *C. concepcionensis* sp. nov. from Chile occupies a position in the rbcL tree removed from *C. pinnata* from California, the recognition of two species is fully supported.

A close relationship between *C. macrostiolata* sp. nov., *C. atrosanguinea* (including *C. linguata*) from southern Chile and the Antarctic region, and an undescribed species from the Antarctic Peninsula is found in all the phylogenetic analyses conducted. *C. macrostiolata* sp. nov. was previously identified from central Chile as *C. atrosanguinea* by Etcheverry (1986), a species reported from New Zealand (Antipodes Islands, Auckland Islands, Campbell Island), the Falkland Islands (type locality), Kerguelen Islands, and Tierra del Fuego (Hariot 1889, Levring 1960). The rhizoidal and basal cells in *C. atrosanguinea* from Punta Dungeness, Chile, are of the same size and agree with Hariot's (1889) description, a characteristic not observed in material from central Chile, which suggests that this species does not occur in the region. It is not possible to determine with the data at hand if *C. atrosanguinea* is present in other parts of the Chilean coast, but in all likelihood this species is restricted to southern Chile. Further phylogenetic and biogeographic studies are needed on the Antarctic species of *Callophyllis* that are also reported for the southern Chilean coast, such as *C. multifida*, *C. fastigiata*, and *C. tenera*. The habits of these three species are well characterized (Kützing 1867, Pl. 90; Reinsch 1888, Taf II Figs. 1-5; Levring 1960, Fig. 7), and do not correspond to any of the features characterizing *C. macrostiolata* sp. nov., in which the frond consists of numerous thin, distal bladelets with truncated, non-laciniate apices, and in which the first branching point is located a distance away from the holdfast. The cystocarpic specimens of *C. macrostiolata* sp. nov. from Isla Santa María, Concepción (36° S) consist of larger fronds and are more branched than the male gametophytes and tetrasporophytes. These differences between individuals were not noticeable in specimens from the coast near Osorno, Valdivia, or Chiloé (40° to 41° S).

The three taxa from central-south Chile, *C. variegata*, *C. concepcionensis* sp. nov., and *C. macrostiolata* sp. nov., differ in their comparative vegetative and reproductive structures (Table 2). *C. macrostiolata* sp. nov. is characterized by surface and internal cortical cells that are regularly isodiametric and more numerous than those found in *C. variegata* and *C. concepcionensis* sp. nov. which contain fewer cell layers, and in which the subcortical cells are periclinally wider than high. In agreement with Etcheverry (1986), Ricker (1987), and Ramírez & Rojas (1988), cell arrangement may be a useful characteristic in the delimitation of the species.

The number of carpogonial branches per supporting cell is an important taxonomic character to separate *Callophyllis* species; however, it is not applicable to differentiate the species from central south Chile since they are all monocarpogonial. Although Harper & Saunders (2002) suggested a close relationship between the polycarpogonial species, the rbcL-based analyses indicate that monocarpogonial and polycarpogonial species can group together, such as *C. edentata* (polycarpogonial species) from California with *C. violacea* (monocarpogonial) from California. Norris (1957) considered *C. variegata*, the type species, as monocarpogonial although in one instance he observed a procarp that had

two complete carpogonial branches attached to one supporting cell; this prompted him to view the presence of only one carpogonial branch per supporting cell as not being a reliable diagnostic character for *Callophyllis*. Later, he reconsidered his earlier opinion because Abbott & Norris (1965) reported the existence of both mono- and polycarpogonial species. The current study shows the presence of only monocarpogonial species occurring in central-south Chile (*C. macrostiolata* sp. nov., *C. variegata*, *C. concepcionensis* sp. nov.), even though Ramírez & Rojas (1988) had suggested that *C.* '*pinnata*' (= *C. concepcionensis* sp. nov.) from Chile was polycarpogonial. *C. pinnata* from Pacific North America is polycarpogonial (Abbott & Norris 1965) and occupies a different position from *C. concepcionensis* sp. nov. in the rbcL tree.

Womersley and Norris (1971) proposed that the procarps are relatively uniform in *Callophyllis* species. As shown in this study, *C. variegata* and *C. concepcionensis* sp. nov. from central-south Chile have procarps with lobulate cells and one to three subsidiary filaments, in contrast to the procarp of *C. macrostiolata* sp. nov. that is comprised of rounded, bulbose cells that are non-lobulate and have almost not subsidiary filaments. This attribute was previously used by Millar (1993) for differentiating species of *Callophyllis* and by Ganesan (1976) for differentiating species of

TABLE 2

Comparison of relevant morphological and anatomical characters of *Callophyllis* from central-south Chile (33° to 41° S).

Comparación de caracteres morfológicos y anatómicos relevantes de *Callophyllis* de centro-sur de Chile (33° a 41° S).

	C. variegata	*C. concepcionensis*	*C. macrostiolata*
Habit shape	flabellate	Palmate-flabellate	flabellate
Branching	subdichotomous	dichotomous	subdichotomous
Dichotomies (N°)	repeatedly over 6-20 segments	4-8	4-13
First dichotomy	close to holdfast	close to holdfast or a few cm above	3-7 cm from holdfast
Branching points	rounded with branch angles ample	rounded with branch angles narrow	rounded with branch angles ample
Apex	dissected	rounded and smooth, weakly dissected	truncated, rounded, weakly dentate
Cortex	three-layered unordered surface cells, subcortical cells wide	two-layered ordered surface cells, subcortical cells wide	four-five-layered compact, ordered surface cells, isodiametric subcortical cells
Medulla	three-four cell layers	four cell layers	five-eight cell layers
Procarp	solitary 87 µm x 87 µm	solitary 87 µm x 87 µm	grouped 75 µm x 37 µm
Carpogonial branch (N°) and procarp shape	monocarpogonial with one-three two-celled lobulate subsidiary filaments	monocarpogonial with two-three two-celled lobulate subsidiary filaments	monocarpogonial with zero-one two-celled non-lobulate subsidiary filaments
Cystocarp position	on thallus edges	scattered over entire frond	distributed over entire frond
Ostioles (N°)	one-three	one-three	one
Ostiole channel	projecting towards the outside (acuminate)	simple exit pore	invaginate, strongly curved margins

Kallymenia.

Whereas the distribution of cystocarps on the fronds of *Callophyllis* species was an important characteristic for Setchell (1923) and Millar (1993) to delineate species, Dawson (1954) emphasized the size and degree of cystocarp protrusion and the number of ostioles. Besides these characteristics, the present study adds information on ostiole shape and projection, and size of the ostiole channel. Thus, the cystocarps of *C. macrostiolata* sp. nov. and *C. variegata* are protuberant, while those of *C. concepcionensis* sp. nov. from Chile are extended and less protuberant. Although Millar (1993) suggested that this character depends on the position of the cystocarp on the thallus and the stage of maturity, it is here confirmed that ostiole features show up early and are maintained throughout the development of the cystocarp.

The distribution of protuberant cystocarps over the entire frond, as in *C. macrostiolata* sp. nov., has also been observed in specimens of *C. megalocarpa* Setchell & Sweezy from Mexico (Dawson 1954). However, this Mexican species bears cystocarps with one to many rostrate ostioles, a different situation from that in *C. macrostiolata* sp. nov., a species characterized by a single, notably invaginated ostiole.

The male reproductive structures of *C. macrostiolata* sp. nov. and *C. concepcionensis* sp. nov. from central-south Chile generally fit the general pattern described for the genus (Dawson 1954, Norris 1957, Womersley & Norris 1971). The disposition of the tetrasporangia in the cortex of *C. macrostiolata* sp. nov. from central Chile does not show remarkable differences to that in other species cited from this area (Etcheverry 1986, Ramírez & Rojas 1988). In *C. macrostiolata* sp. nov. the tetrasporangia are immersed in the cortex whereas in *C. variegata* and *C. concepcionensis* sp. nov. the cortical cells do not completely cover the tetrasporangia due to the smaller number of cortical cell layers. Using the presence or absence of tetrasporangial sori advocated by Dawson (1954) to characterize species of *Callophyllis* was not useful in this study, as all specimens analyzed had sori lacking a characteristic pattern.

Two tetrasporic specimens and a female individual from a Typological Series of *C. atrosanguinea* collected by J. D. Hooker in the Falkland Islands (Cape Pembroke) and Tierra del Fuego (Rade de Goreé, Point Guanaco) housed in PC, France, were examined in this study. The material analyzed by Etcheverry (1986) from Isla Santa María, Chile, and identified as *C. atrosanguinea*, does not fit the characteristics of *C. atrosanguinea* and instead corresponds to *C. macrostiolata* sp. nov. Although *C. macrostiolata* sp. nov. resembles *C. atrosanguinea* in that both are dark red, subdichotomously branched, with rounded apical regions that are poorly laciniate, and with cystocarps distributed over the entire frond, the frond of *C. macrostiolata* sp. nov. bears protuberant cystocarps, each with a well-defined and invaginated ostiole, and markedly different from *C. atrosanguinea*, a species with extended cystocarps and diffuse ostioles. A taxon identified as *C. atrosanguinea* from Punta Dungeness, Chile, is included in this study; as this material was not fertile, cystocarp comparison was not possible. In conclusion, *C. macrostiolata* is morphologically, reproductively and phylogeneticaly distinct from the other species of *Callophyllis* present in central Chile, including *C. variegata* and *C. concepcionensis*, another new species.

The current geographical distribution of *C. macrostiolata* sp. nov. and *C. concepcionensis* sp. nov. is restricted to central-south Chile, with as northern limit the Concepción region (36° S). The range for *C. variegata* is confirmed for Peru (14° S) to Magallanes, Punta Arenas (52° S). The Identification key to the species of *Callophyllis* of the central-south (33° to 41° S) coast of Chile is presented in Table 3.

Even though *Callophyllis* has been the subject of many studies, its extreme morphological variability (especially branching pattern) and the suggested inclusion of species with undivided blades (*C. firma*) makes it difficult to clarify the generic status at this point, and further phylogenetic studies are needed in this group. The intergeneric relationships among *Callophyllis* and other genera of Kallymeniaceae is beyond the scope of this study. Only after we have gained a better understanding of the genera and species relationships within the Kallymeniaceae, will the attempted reconstruction of the biogeographic history of the family be meaningful.

ACKNOWLEDGEMENTS: We thank Gloria Collantes

TABLE 3

Identification key to the species of *Callophyllis* from central-south Chile (33º to 41º S).

Clave de identificación para las especies de *Callophyllis* de centro-sur de Chile (33º a 41º S).

1a. Thalli regularly dichotomous, palmately divided up to 35 cm in height, four-eight orders of branching; branch angles narrow; ultimate branchlets rounded; with straight and smooth margins, weakly dissected; cortex of two layers of regular surface cells; cystocarps distributed over the frond, each with one to three simple ostioles..... *Callophyllis concepcionensis*

1b. Thalli subdichotomous, repeatedly branched, up to 13-20 orders of branching, branch angles wide.....2

2a. Thallus surface soft; stipe short; up to 30 cm in length, ultimate branchlets with dissected margins; cortex of three layers of unordered surface cells; cystocarps distributed on the edges of the frond, one to three acuminate ostioles per cystocarp ..... *Callophyllis variegata*

2b. Thallus surface firm and cartilaginous; short stipe lacking; 12-35 cm in length; ultimate branchlets with blunt margins; cortex of four-five layers of compact, ordered surface cells; cystocarps distributed over the entire frond, a single invaginated ostiole per cystocarp . *Callophyllis macrostiolata*

(University of Valparaiso, Chile), Bruno de Reviers (National Museum of Natural History, Paris), Patrick Martone (Stanford University), and all the collectors listed in Table I for providing specimens used in this study. This research was funded by a University of Concepcion grant (PI 96-112-036-1.0), and NSF DEB-0743024, DEB-0919508 and DEB-0936855 to SF.

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APPENDIX

Description, typification and list of representative specimens and species of *Callophyllis* examined from the central-south Chilean coast (33º to 41º S).

Species 1 = *Callophyllis variegata* (Bory) Kützing.

Basionym: *Halymenia variegata* Bory, 1826-1828: 179.

Type: Herbarium Montagne, PC. Isotype (UC 435656, ♀) collected by Binder.

Type locality: Valparaíso, Chile.

Representative specimens examined: MAGALLANES, PUNTA ARENAS: M. Codoceo, 9.ii.1954 (SGO 095804, ♀); Seno Otway, Punta Prat, U.M.A.G., 20.xi.1985 (SGO 105382); Punta Dungeness, D. Díaz, 24.iv.1986 (SGO 105352, ⊕). CHILOÉ: Abtao, I. Meza, ii.1984 (SGO 105133); Ancud, Mar Brava, M.E. Ramírez & G. Rojas, 4.xii.1990 (SGO 122249). VALDIVIA: Playa Blanca, D. Contreras, 4.i.1979 (SGO 096039, ⊕); Los Molinos, B. Parra, 20.ii.1985 (SGO 103978, ♀); Punta Misión, G. Rojas, 30.i.1987 (SGO 106151), M.E. Ramírez, 22.x.1988 (SGO 109824). CONCEPCIÓN: Bahía Coliumo, D.H. Kim, 14.v.1969 (SGO 088128, ♀); Los Morros, H. Romo, 3.iv.1976 (CON 1105, ♀), J. Cid, 3.iv.1976 (CON 1107, 1110, ♀); Bahía San Vicente, zona sur, H. Etcheverry, ix.1959 (UV, ⊕); Las Escaleras, D.H. Kim, 2.vi.1976 (CON 1767, ♀; 1109, 1115, ⊕); Golfo de Arauco, J. Vásquez, i.1981 (SGO 095732, ⊕);

Punta Lavapie, K. Alveal, 30.i.1991 (CON, ⊕); Raqui, W. Wilkomirsky, iii.1974 (CON 194, ⊕), Llico, Isla Verde, K. Alveal, 30.i.1991 (CON, ♀); Yana, K. Alveal, iv.1978 (CON 557, ⊕); Trauco, J. Cid, xi.1975 (CON 551, 559, ⊕); Lebu, A. Alveal & K. Alveal, ii.1985 (CON 526, ♀); Bahía Concepción, Tumbes, K. Alveal, xi.1972 (CON 548, 593, 1101, ⊕); Cocholgüe, K. Alveal, v.77 (CON 545, 547, 591, ⊕), L. Chuecas, 22.vi.1982 (SGO, ⊕), C. Werlinger, iv.1987 (CON 603, ♀), J. Valenzuela & M. Figueroa, 20.x.1989 (CON 1157, 1342, 2448, ⊕), K. Alveal, 19.i.1990 (CON 1102, 1155, ♀); Caleta Leandro, J. Cid, 9.vi.1976 (CON 1113, ⊕), H. Romo, 9.vi.1976 (CON 1114, ⊕); Isla Santa María, W. Wilkomirsky, iii.1974 (CON 549, ⊕); Cocholgüe, Puertos de Coronel and Lota e Isla Santa María, N. Arakaki, iii.1998, 9.x.1998, 3.xi.1998, 7.iv.2000, 25.vii.2000, 28.vii.2000, 4.x.2000, 11.xi.2000, 16.xi.2000 (CON, ♀, ⊕, 22 specimens). VALPARAÍSO: Navidad, La Boca, Punta Perros, M.E. Ramírez, 24.x.1980 (SGO 095640); Cartagena, M. Espinoza, i.1917 (SGO 081074, ⊕), Ventana, M. Figueroa, H. Romo & K. Alveal, iii.1971 (CON 534, ⊕) Maitencillo, H. Etcheverry, ii.1954 (UV, ♀). Los Vilos, Bahía Conchalí, A. Llaña, v.1945 (UV 1247, ⊕). COQUIMBO: Bahía Tongoy, H. Etcheverry, ii.1954 (UV, ♀); Puerto Aldea, J. Vásquez & P. Ojeda, 22.i.1981 (SGO 081153, ⊕); Bahía Herradura, E. Fonck, 23.ix.1976 (SGO 097104, ♀), H. Black & M.E. Ramírez, 13.xii.1982 (SGO 102514); Bahía Herradura, A. Llaña, 20.vi.1945 (UV 1208, ⊕). LA SERENA: Peñuelas, H. Etcheverry & K. Alveal, x.1959 (UV, ♀). ANTOFAGASTA: Bahía Mejillones del Sur, M.E. Ramírez, 13.i.1987 (SGO 106175). IQUIQUE: Cavancha, R. Pinto, xii.1987 (SGO 107308, ♀). Additional material: 108 specimens from different localities of central-south Chile (SGO and CON, ♀, ⊕).

Species 2 = *Callophyllis concepcionensis* Arakaki, Alveal et Ramírez sp. nov.

Thallus membranaceus usque ad cartilagineus, 4-8 dichotome ramosus ordinate ad 35 cm alt. Stipes brevis extensus in thallum palmatum aut flabellatum usque ad 35 cm longum, segmentis principalibus largis plerumque non plus quam 3 cm., angusti ramorum anguli, margines laeves, aut rami laciniati solum primi visi supra thalli, apices rotundati interdum prolificationibus pinnatis paucibus vel multibus. Cortex 2-5 stratus, cellulis isodiametricus. Cellula sustinens monocarpogonialis, procarpium 2 ramulis subsidiariis bicellularibus 3-cellulari ramo carpogoniali, cellulae procarpii lobatae. Cystocarpia plana protuberantia, 1-2 mm diam., super lamina dispersa, cum 1-3 simplicibus ostiolis. Thalli feminei zonati cystocarpiis immaturis albidis. Tetrasporangia cruciata, 27-37 µm x 15-20 µm, orienda in subcortice. Spermatangia, 4 µm, irregulariter dispersa in cortice externo.

Membranous to cartilaginous thallus, four to eight times dichotomously branched in a regular pattern. Short stipe,extending in an upright palmate or flabellate frond up to 35 cm in height, with wide main segments usually not more than 3 cm. Branch angles narrow with smooth margins, branch apices rounded, or branches laciniate at the upper part of the frond, sometimes with few to many pinnate proliferations. Cortex of two-five layers of isodiametric cells. Procarp monocarpogonial, consisting of three-celled carpogonial branch and two two-celled subsidiary filaments, procarp cells lobulated. Cystocarp flat, protuberant, 1-2 mm in diam., scattered over the frond, each topped by one to three simple exit channel ostioles. Female specimens with extensive zones bearing young or immature off-white cystocarps. Tetrasporangia cruciately divided, 27-37 µm x 15-20 µm, originating in the subcortex. Spermatangia originating in the outer cortex, irregularly distributed, 4 µm diam.

Etymology: *C. concepcionensis* is named for Concepción, Central Chile, an area in which the new species is common.

Holotype: Herbarium of Museo de Historia Natural, Santiago (SGO 100665), ♀, Concepción, Golfo de Arauco, J. Vásquez, i.1981 (Fig. 2A).

Type locality: Concepción, Chile (36º59' S; 73º32' W).

Paratypes: CHILOÉ: Abtao, I. Meza, 7.ii.1984 (SGO 105299, ♀); Ancud, Mar Brava M.E. Ramírez & G. Rojas, 4.xii.1990 (SGO 136438, ♀); Estuario Pudeto, A. Llaña, xii.1943 (UV 631, 687, ⊕). VALDIVIA: Los Molinos, B. Parra, 1.xi.1984 (SGO 103999, ⊕), 20.i.1985 (SGO 104010, ♀), B. Parra, i.1985 (CON 1773, ♀); San Carlos, Corral, B. Parra, 7.v.1985 (SGO 104008, 104009, ♀), B. Parra, iv.1983 (CON 2676, ♀). OSORNO: Playa Pucatrihue, G. Arriagada, 22.i.1981 (SGO 100661, ♀). CONCEPCIÓN: Bahía Coliumo, Los Morros,

D.H. Kim, 30.iv.1976 (CON, ♀); Dichato, K. Alveal, iii.1986 (CON 589, ⊕); Golfo de Arauco, J. Vásquez, i.1981 (SGO 100665, 100666, 100349, ♀); Trauco, K. Alveal, 30.i.1991 (CON, ♀); Punta Lavapié, K. Alveal, 30.i.1991 (CON, ♀); Lebu, A. Alveal, ii.1985 (CON 594, 1700, ⊕); Las Escaleras, D.H. Kim, 2.vi.1976 (CON 1765, ♀); Pichiraqui, W. Wilkomirsky, 5.v.1974 (CON 553, ⊕; CON, ♀); Bahía Concepción, Punta Parra, E. Rebolledo, v.1986 (SGO, ♀); Cocholgüe, K. Alveal, iii.1977 (CON 192, ⊕), H. Romo & K. Alveal, iii.1977 (CON 552, 555, ⊕), X. Silva, 14.xi.1981 (SGO 105310, ⊕), L. Ortiz, 22.vi.1982 (SGO 103986, ♀), J. Vargas, 23.vi.1982 (SGO, ♀), O. Mendoza, 2.vii.1982 (SGO 103989, ♀), K. Alveal, xii.1991 (CON 2494, ⊕), K. Alveal, 1.iii.2000 (CON, ♀); Cocholgüe, Puertos de Coronel y Lota e Isla Santa María, N. Arakaki, 7.iv.2000, 31.v.2000, 28.vii.2000, 11.xi.2000, 16.xi.2000, 18.xi.2000, 24.ii.2001, 5.iii.2001, 24.iii.2001 (CON, ♀, ⊕, 154 specimens). Additional material: 77 specimens from different localities of central-south Chile (SGO and CON, ♀, ⊕).

Species 3 = *Callophyllis macrostiolata* Arakaki, Alveal et Ramírez sp. nov.

Thallus cartilagineus, subdichotome ramosus, ad 35 cm alt. Stipites elongati ad 7 cm longi, segmentis ramulosis attenuate base, axillae rotundatae, apices rotundati vel truncati, raro dentati. Cortex 4-5 stratus, cellulis isodiametricus. Cellula sustinens monocarpogonialis, procarpium 0 vel 1 ramulis subsidiariis bicellularibus 3-cellulari ramo carpogoniali, cellulae procarpii rotundae, bulbosae non lobatae. Cystocarpia magna (ad 2 mm lat.) protuberantia, super lamina dispersa, cum ostiolo singulari profundo cum margine valde inflexo. Area circa cystocarpium 150-350 µm crassum, compactum, cellulis ovoideis. Tetrasporangia cruciata, 25-27 x 15-17 µm, in cellulis peripheriicis inclusa et ad cellulas corticales subsuperficias affixa. Spermatangia, 4 µm, in pagina thalli irregulariter dispersa efferentia.

Cartilaginous thallus weakly dentate, with subdichotomous branching, up to 35 cm in height. Stipes elongated up to 7 cm in length, distally branched segments narrow at the base, with rounded branching points and apices. Cortex of four-five layers of isodiametric cells. Procarp monocarpogonial, supporting cell bearing a three-celled carpogonial branch and zero-one two-celled subsidiary filaments; procarp cells rounded, bulbose, non-lobulate. Cystocarps large, protuberant, up to 2 mm diam., distributed over the entire frond, each bearing a single, deep ostiole with strongly curved margins. Area surrounding cystocarp 150-350 µm thick, compact, of ovoid cells. Tetrasporangia cruciately divided, 25-27 µm x 15-17 µm, originating in the subcortex. Spermatangia originating in the outer cortex, irregularly distributed, 4 µm diam.

Etymology: *C. macrostiolata* is named for its large, invaginated cystocarp ostiole.

Holotype: Herbarium of Museo de Historia Natural, Santiago (SGO 159068), ♀, Isla Santa María, Concepción, Chile, coll. N. Arakaki, 17.iii.2001 (Fig. 3A).

Type locality: Isla Santa María, Concepción, Chile (36°59' S; 73°32' W).

Paratypes: CHILOÉ: Ancud, M. Robles, xi.1997 (CON, ♀); Maullín, M. Robles, xi.1997 (CON, ♀), ix.1997 (CON, ⊕). OSORNO: Playa Pucatrihue, G. Arriagada, 22.i.1981 (SGO 105135, 105137, ♀; 105136, ⊕). VALDIVIA: San Carlos, Corral, C. Moreno, 8.iii.1980 (SGO, ♀), 10.iv.1980 (SGO, ♀). CONCEPCIÓN: Isla Santa María, N. Arakaki, 7.iv.2000, 16.xi.2000, 5.iii.2001 and 17.iii.2001 (CON, ♀, ⊕, 26 specimens).