MORPHOLOGICAL VARIATIONS OF *GRACILARIA CHILENSIS* BIRD,
MCLACHLAN ET OLIVEIRA, 1986 (RHODOPHYTA, GRACILARIALES) IN
THE SOUTHEAST PACIFIC

VARIACIONES MORFOLOGICAS DE *GRACILARIA CHILENSIS* BIRD,
MCLACHLAN ET OLIVEIRA, 1986 (RHODOPHYTA, GRACILARIALES) EN EL
PACIFICO SURESTE

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ABSTRACT

The present work is a population morphometric study of *Gracilaria* from different environments along a latitudinal gradient of the Chilean coast that cover 1,500 km. *Gracilaria* species have been extensively exploited with an average extraction of 75,000 t per year. In Chile this seaweed is usually found in sandy bottom bays between Antofagasta (23°29'S - 70°25'W) and Chiloé (42°03'0"S - 73°11'0"W). Observations were made in 51 plants. Macroscopic observations were done in fresh plants, microscopic characters were determined in transversal sections with 20% Karo syrup and stained with aniline blue. Numerical analyses were performed by one-way ANOVA and Principal Component Analysis. The latter analysis was performed for those characters with significant differences among the localities: basal constriction of branches, branch frequency, cortex cell diameter, subcortex cell diameter, thickness of the cortex and medullar cell diameter. The results of the Principal Component Analysis showed that 61% of the variance corresponded to the two first components. There were not observed groups clearly defined. The data suggest that along the coast of Chile there is only one morphotype that corresponds to the description of *Gracilaria chilensis* Bird, McLachlan et Oliveira, 1986 and it fits to the bulk of the material utilized in commercial production.

KEYWORDS: *Gracilaria chilensis*, seaweed, morphology, Chile.

RESUMEN

Este trabajo es un estudio morfológico de poblaciones de *Gracilaria* que habitan diferentes ambientes a lo largo de una gradiente latitudinal de la costa de Chile que cubre 1.500 Km. La especie de *Gracilaria* han sido extensivamente explotadas, con promedios de extracción de 75.000 toneladas anuales. El alga se encuentra en bahías de fondos arenosos, entre Antofagasta (23°29'S - 70°25'W) y Chiloé (42°03'0"S - 73°11'0"W). Fueron analizadas 51 plantas. Se realizaron observaciones macroscópicas en plantas frescas. Los caracteres microscópicos se determinaron en secciones teñidas con azul de anilina y montadas en una solución de miel Karo al 20%. Los análisis numéricos de los datos se realizaron mediante un ANOVA de una vía y un Análisis de Componentes Principales. Este último análisis se realizó para aquellos caracteres con diferencias significativas entre localidades: constricción basal de las ramificaciones, frecuencia de la ramificación, diámetro de las células de la corteza, diámetro de las células subcorticales, grosor de la corteza y diámetro de las células de la médula. Los resultados del Análisis de Componentes Principales mostraron que el 61% de la varianza correspondió a los 2 primeros componentes. No se observaron grupos claramente definidos. Los datos encontrados sugieren que a lo largo de la costa estudiada existe un solo morfotipo que corresponde a la descripción de *Gracilaria chilensis* Bird, McLachlan et Oliveira, 1986 y esta descripción concuerda con la mayoría del material que se emplea para un uso comercial.

PALABRAS CLAVES: *Gracilaria chilensis*, algas marinas, morfología, Chile.

INTRODUCTION

The genus *Gracilaria* Greville 1830 is distributed in areas with temperate and tropical waters and it is possible to find intertidal and subtidal populations (Bird *et al.* 1977). The species of this genus are highly polymorphics with variable sizes and ramifications. Therefore, several specific names have been misplaced or certain specific characters were poorly defined (Abbott 1983). *Gracilaria* has been extensively exploited along the Chilean coast with an average extraction of 75,000 t per year (Anuario Estadístico de Pesca, 2001). The development of *Gracilaria* management strategies and establishment of over 500 farming centers sustain the high production of this seaweed with important revenues for Chile (Buschmann *et al.* 2001).

Along the Chilean coast, *Gracilaria* was represented by several species. Etcheverry (1958) reported *Gracilaria lemaneiformis* (Bory) Weber von Bosse [now *Gracilariopsis lemaneiformis* (Weber von Bosse) Dawson, Acleto *et* Foldvik] in Chiloé. Levring (1960) informed the presence of *Gracilaria confervoides* (Linnaeus) Greville [now *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine *et* Farnham], *Gracilaria peruana* Piccone *et* Grunow, and *Gracilaria pulvinata* Skottsberg. Later, Kim (1970) communicated the name of five species of *Gracilaria*: *Gracilaria verrucosa* (Hudson) Papenfuss [now *G. gracilis*]; *G. lemaneiformis*, *Gracilaria edulis* (Gmelin) Silva [now *Hydropuntia edulis* (S.G. Gmelin) Gurgel & Fredericq, 2004], *G. peruana*, and *G. pulvinata*. Bird *et al.* (1986) described *Gracilaria chilensis* Bird, McLachlan *et* Oliveira as the result of carpospores cultured from plants of La Herradura Bay, Coquimbo (29°53'S - 71°18'W) and Maullín, Puerto Montt (41°30'S - 73°50'W). From these records it is important to mention that *G. peruana* has been not reported again as components of the Chilean flora. In northern Chile there is *G. chilensis* and in the southern Chilean coast *G. verrucosa*, although several studies (Bird *et al.* 1986, Candia, 1988, Möller & Westermeier 1988, Santelices & Ugarte 1990) presume the existence of *G. chilensis* also in southern Chile. Nevertheless, molecular analysis of nuclear and plastidial DNA verified that the different morphotypes found for *Gracilaria* in Chilean coast corresponded to *G. chilensis* (González *et al.* 1996, Meneses 1996, Candia *et al.* 1999).

The present work is a population morphometric study of *Gracilaria* species along the Chilean latitudinal gradient which analyzes plants from different environments where anatomical and morphological variations may exist. The morphometric analysis is a good complement of the molecular one (Muller *et al.* 1998) as it was demonstrated in the work with *Gracilaria* by Liao and Homersand (2003). This study was made between 1985 and 1991 before the global knowledge and availability of molecular techniques to investigate the algal systematic. Therefore the hypothesis was that the only species utilized in aquaculture along the coast of Chile is *G. chilensis*. The goals of this study were to evaluate the morphometric variations of some taxonomical characters in natural population of *Gracilaria*; to discriminate probable existence of morphological groups; and to determine the relative taxonomical weight of each character.

MATERIAL AND METHODS

1. STUDY AREA

The biological material was collected from 4 zones along the Chilean coast: Coquimbo (29°58'55"S - 71°22'20"W), Concepción (36°45'50"S - 73°10'18"W), Puerto Montt (41°28'54"S - 72°57'25"W) and Chiloé (41°51'S - 73°47'W) between 1985 and 1991 (Fig. 1). In each zone the seaweeds were collected according to Table I. 51 plants were selected for the study.

2. CHARACTER MEASUREMENTS

The measured characters (Table II) were done following Renfrew (1983). Each character was measured 10 times in the same plant except for characters 3 and 4 that correspond to the degree of ramification. Plants were stored in the herbarium of the Department of Marine Biology, Universidad Católica del Norte in Coquimbo, Chile (UCN 00093-00180). Observations and measurements of macroscopic characters were done in fresh plants. Microscopic characters of vegetative and reproductive structures were determined in transversal sections stained with aniline blue and mounted in 20 % Karo syrup (Best Foods C.P.C. International Inc.)

3. NUMERICAL ANALYSIS.

Each morphological character (Table II) was measured ten times from the same individual plant. The data were analyzed by a one-way ANOVA (95% of confidence limit) after the check of variance

homogeneity by Bartlett test (Sokal & Rohlf, 1981). Once the hypothesis was rejected the data were transformed according to $(X + 0.5)^{1/2}$. Characters that separated the major groups were entered to a Principal Component Analysis. The software utilized for the analysis was Statgraphics (STSC, Inc. 1991).

TABLE I. *Gracilaria* specimens, information on localities, collecting period, number of plants, and plant reproductive state.

TABLA I. *Gracilaria*, información de localidades, períodos de colecta, número de plantas y fenología.

ZONE/ LOCALITY	DATE	Nº PLANTS (*)
COQUIMBO		
Herradura Bay (29°58'55"S - 71°22'20"W)	Mar, Jun and Sep, 1986	3 (1)
	Jan and Mar, 1988	16 (2)
CONCEPCIÓN		
Lenga (36°45'50"S - 73°10'18"W)	Oct, 1985	7 (1)
Tubul, Gulf of Arauco (37°14'S)	Mar and Jul, 1991	2 (2)
PUERTO MONTT		
Tenglo Island (41°28'54"S - 72°57'25"W)	Apr, 1988	8 (2)
CHILOÉ		
Pudeto River (41°51'S - 73°47'W)	Aug and Oct, 1987	2 (2)
Quemillén (41°52'S - 73°46'W)	Aug and Oct, 1987	1 (1)
Putemún (42°29'22"S - 73°45'27"W)	Aug and Oct, 1987	9 (2)
	Apr, 1991	3 (2)

* (1) Cistocarpic; (2) Tetrasporic

RESULTS

1. Morphometric analysis in a latitudinal gradient. In *Gracilaria* plants used for the study, 11 were cystocarpic and 40 tetrasporic. Male plants were observed once in the Río Tubul estuary, Gulf of Arauco.

From 21 measured characters, only six of them presented significant differences among the localities. They were: (# 1) the basal constriction of branches; (# 3) branch frequency; (# 9) cortex cell diameter; (# 10) subcortex cell diameter; (# 11) thickness of the cortex and (# 12) medullar cell diameter (See Table III).

Basal constriction of branches (# 1) in plants from Concepción was significantly ($p < 0.05$) greater

than the other localities (Table III). Branch frequency (# 3) showed significant variation ($p < 0.05$) between the plants from Puerto Montt and Chiloé, when compared to those from Coquimbo and Concepción (average values < 1) (Table III). Cortex cell diameter (# 9) in plants from Concepción and Puerto Montt showed smaller values ($p < 0.05$) than Coquimbo and Chiloé, and subcortex cell diameter (# 10) from Concepción was lower than other localities (Table III). The thickness of the cortex (# 11) evidenced a latitudinal variation with a gradual increase towards the south (Table III). The observed medullar cell diameter (# 12) showed that southern localities (Puerto Montt and Chiloé) have lower values than Concepción and Coquimbo (Table III).

TABLE II. Description of microscopic and macroscopic characters utilized for the morphological evaluation of *Gracilaria* specimens. The number of each character represents the authentication code.

TABLA II. Descripción de caracteres macroscópicos y microscópicos empleados para la evaluación morfológica de las plantas de *Gracilaria*. El número de cada carácter representa el código de verificación.

MACROSCOPIC CHARACTERS	DESCRIPTION
1. Basal constriction of branches (mm)	Ratio of the branch width at its base to the width half way to the next fork was determined.
2. Branch apex width (mm)	Width of a branch taken 5 mm from the apex.
3. Branch frequency (n°)	Most branched apex was determined. For this axis a ratio between the number of branches and the length from the base of the plant to the tip of the axis was calculated.
4. Degree of branching (n°)	Number of times the best-developed axis of the plant branched.
5. Outer branch angles (°)	Ratio of the two abaxial angles at branch point was determined.
6. Inner branch angle (°)	Angle between the branch and main axis.
7. Flattening at the forks (mm)	Ratio of the width of the thallus fork to its thickness was measured.
8. Flattening at mid branch (mm)	Same as above was measured half a way between two forks.
9. Cortical cell diameter (µm)	Diameter of the outermost cortical cell was taken perpendicular to the thallus surface.
10. Second cortical layer cell diameter (µm)	Diameter of the second cortical layer.
11. Thickness of cortex (n°)	Number of cortex cell layers.
12. Medullar cell diameter (µm)	Diameter along the longest axis of the cell.
13. Tetrasporangium length (µm)	Length of intact tetrads was taken along their longest axis.
14. Tetrasporangium width (µm)	Width perpendicular to the length.
15. Cystocarp width (µm)	Measured at the widest point from median sections.
16. Cystocarp height (µm)	The height was determined from the base of the gonimoblast to the tip of the ostiole.
17. Basal constriction of cystocarp (µm)	Width of the cystocarp at its base.
18. Thickness of pericarp (n°)	Number of cell layers taken from a median section of a mature cystocarp at the widest point.

Continuation Table II.

MACROSCOPIC CHARACTERS	DESCRIPTION
19. Diameter of pericarp cells (im)	Diameter of pericarp cells was measured from the outermost, through to the innermost layers.
20. Width of gonimoblast cells (im)	Width of the widest point and perpendicular to the length was found.
21. Length of gonimoblast cells (im)	Length was taken along the longest axis of gonimoblast cells.

TABLE III. Morphometric summary and ANOVA (* $p < 0.05$; ** $p < 0.01$) of the analyzed characters of *Gracilaria* specimens collected in Coquimbo, Concepción, Puerto Montt and Chiloé. Values without standard deviation correspond to 1 sample.TABLA III. Resumen de la morfometría y ANDEVA (* $p < 0.05$; ** $p < 0.01$) de los caracteres analizados en la plantas de *Gracilaria* recolectadas en Coquimbo, Concepción, Puerto Montt y Chiloé. Los valores sin desviación estándar corresponden a 1 muestra.

Code N°	Coquimbo	Concepción	Puerto Montt	Chiloé	ANOVA	
					F _(d.f.)	p
1 (mm)	1.354 ± 0.163	1.558 ± 0.248	1.414 ± 0.140	1.277 ± 0.203	3.79 _(3,45)	0.0164*
2 (mm)	0.233 ± 0.076	0.181 ± 0.056	0.170 ± 0.046	0.222 ± 0.045	1.88 _(3,45)	0.1452
3 (N°/cm)	0.905 ± 0.373	0.680 ± 0.390	3.263 ± 0.458	1.912 ± 0.810	40.09 _(3,44)	<0.01**
4 (N°)	3.526 ± 0.512	3.142 ± 0.377	3.625 ± 0.517	3.500 ± 0.500	1.38 _(3,45)	0.2605
5 (°)	0.857 ± 0.075	0.816 ± 0.082	0.815 ± 0.053	0.881 ± 0.061	2.29 _(3,45)	0.0902
6 (°)	54.32 ± 8.56	58.00 ± 10.44	54.09 ± 6.37	51.06 ± 5.33	1.39 _(3,45)	0.2649
7 (mm)	1.195 ± 0.118	1.214 ± 0.114	1.276 ± 0.120	1.288 ± 0.193	1.36 _(3,45)	0.2660
8 (mm)	0.926 ± 0.306	0.998 ± 0.009	1.000 ± 0.001	1.277 ± 0.203	0.57 _(3,45)	0.6362
9 (μm)	13.04 ± 0.05	10.81 ± 0.06	10.69 ± 0.06	12.10 ± 0.08	5.47 _(3,45)	0.0027**
10 (μm)	18.90 ± 0.25	13.16 ± 0.18	23.12 ± 0.16	20.31 ± 0.19	4.35 _(3,45)	0.0089**
11 (N°)	1.18 ± 0.29	1.43 ± 0.53	1.88 ± 0.58	1.80 ± 0.82	4.35 _(3,45)	0.0089**
12 (μm)	259.1 ± 1.51	318.9 ± 1.15	117.9 ± 0.36	170.7 ± 0.35	6.49 _(3,43)	0.0010**
13 (μm)	41.36 ± 0.23	—	37.71 ± 0.26	35.05 ± 0.30	3.11 _(2,35)	0.0569
14 (μm)	32.05 ± 0.31	—	29.47 ± 0.12	29.76 ± 0.41	0.36(2,35)	0.0699
15 (μm)	1,940 ± 2.639	1,560 ± 3.681	—	1,168	0.05(2,8)	0.9502
16 (μm)	824.6 ± 1.837	891.4 ± 2.730	—	1,220	0.23(2,7)	0.7944
17 (μm)	751.3 ± 1.344	683.0 ± 0.846	—	497.0	2.45(2,8)	0.1473
18 (N°)	8.833 ± 0.763	7.833 ± 1.602	—	8.5	0.52(2,7)	0.6131
19 (μm)	13.13 ± 0.14	13.68 ± 0.24	—	13.84	0.01(2,7)	0.9890
20 (μm)	45.13 ± 0.47	27.50 ± 0.41	—	44.36	2.89(2,7)	0.1213
21 (μm)	23.84 ± 0.45	18.28 ± 0.37	—	29.97	0.68(2,7)	0.5328

2. Relative taxonomical weight of the characters. The result of the Principal Component Analysis (Table IV) showed that 61% of the variance is within the two first components, basal constriction of

branches and cortex cell diameter. When these components were graphically represented no groups were defined related to the latitudinal gradient.

TABLE IV. Principal Component Analysis for 5 characters analyzed in *Gracilaria*: (1) basal constriction of branches, (2) cortex cell diameter, (3) subcortex cell diameter, (4) cortex thickness, and (5) medullar cell diameter.

TABLA IV. Análisis de Componentes Principales para 5 caracteres analizados en *Gracilaria*: (1) contricción basal de la ramificación, (2) diámetro de las células corticales, (3) diámetro de las células subcorticales, (4) grosor de la corteza, y (5) diámetro de las células medulares.

N° COMPONENT	% VARIANCE	% CUMULATIVE
1	31.69	31.69
2	29.20	60.89
3	18.49	79.38
4	12.35	91.74
5	8.25	100.00

DISCUSSION

The morphology of *Gracilaria* populations along the Pacific coast of Chile has been scarcely documented. The presence of few specimens bearing reproductive structures (Kim 1970, Black & Fonck 1981, Fonck 1986, Pizarro 1986, Edding *et al.* 1987), high degree of phenotypic plasticity within the genus (Bird *et al.* 1977, Bird & McLachlan 1982, Abbott 1983, Bird & McLachlan 1984), and massive transplants with commercial purposes have generated confusion (Kim 1970, Bird *et al.* 1986) and made difficult a precise description of the species.

The existence of *Gracilaria verrucosa* (now *G. gracilis*) in the Pacific coast of Chile (Etcheverry 1958, Kim 1970, Kim & Candia 1977, Romo *et al.* 1979, Bird *et al.* 1986) is doubtful. Kim (1970) and Kim & Candia (1977) erroneously denominated *G. verrucosa* to plants with textorii type of spermatangia giving more importance to external vegetative characters. Between *G. chilensis* and *G. verrucosa* there are main differences that allowed Bird *et al.* (1986) to state that *G. verrucosa* is not part of the Chilean marine flora. Bird & McLachlan (1982) suggested that *G. verrucosa* from Chile correspond to plants that were incorrectly named. In this study, plants bearing verrucosa type of spermatangia and nutritive filaments, and connecting pericarp and gonimoblast were not observed, confirming the observed by Bird & McLachlan (1982).

The presence of *Gracilaria lemaneiformis* along the Chilean coast has been recorded by Etcheverry (1958), Kim (1970), Kim & Candia (1977), Romo *et al.* (1979), Bird *et al.* (1986) and Santelices (1989). Nevertheless, any of these authors described the cystocarp or spermatangia. Fredericq & Hommersand (1989) transferred this species to the genus *Gracilariopsis*. Two characters are the key to its determination: absence of nutritive filaments in the cystocarp; and chorda type of superficial spermatangia. Kim (1970) reported plants with chorda type of spermatangia from Río Tubul to indicating the presence of male plants with textorii type of spermatangia. *Gracilariopsis lemaneiformis* for Chile was latter registered by Ramírez & Tapia (1991) for Antofagasta (24° 22' S – 70° 32' W) giving a geographical range from Paita, Perú to Antofagasta. Candia (1988) and Möller & Westermeier (1988) reported *Gracilaria chilensis* in southern Chile without mentioning the presence of nutritive filaments, as is observed in the present work. This may be related to the variability of the character (Papenfuss 1967, Yamamoto 1978, Bird & McLachlan 1984, Oliveira 1984, Bird & Rice 1990). Searles *et al.* (1974) collected seaweeds by scuba dive during two consecutive years (Hero cruises 72 and 73) from Puerto Montt to Statten Island and they did not find *Gracilaria* specimens (R. Searles, pers. com.).

The phylogeny and systematic analyses of Family

Gracilariaceae from Atlantic and Pacific coast based on small subunit rDNA and its sequences (Bellorin *et al.* 2002), suggest that the main divergences in *Gracilaria* are related more to geographic isolation than to broad morphological differences. The Pacific species includes *G. chilensis* and *G. tenuistipitata*, which appear to be closely related entities, both slender and terete with textorii type spermatangial configuration despite their different respective habitats of cold and warm water (Bellorin *et al.* 2002).

The existence of few plants with reproductive structures, mainly male plants; the high degree of phenotypic plasticity in the genus and the massive transplant of *Gracilaria* that occurred in Chile as a consequence of the commercial cultivation, have generated a degree of confusion in the taxonomy of this genus. The results of this study do not establish a morphological variation that permits determining morphological groups. The variations observed in the characters: branch basal constriction, branch frequency, cortex cell diameter, subcortex cell diameter and thickness of the cortex may correspond to local phenotypical variations due to environmental or biological forces or according to Chapman *et al.* (1977), whom observed similar characters, to seasonal changes. The Principal Component Analysis confirms that the observed morphological differences are not consistent and they do not have taxonomical value. The morphological homogeneity of the examined characters defined the presence of only one morphotype that conforms with the description of *Gracilaria chilensis*. In conclusion, utilizing traditional morphometric techniques we conclude that *G. chilensis* is the main species of *Gracilaria* present along the Chilean coast. Similar results have been reported after this study by using molecular techniques (González *et al.* 1996, Meneses 1996, and Candia *et al.* 1999). The results confirm the distribution of *G. chilensis* extending its southern range down to Chiloé. Hoffmann & Santelices (1997) found *G. chilensis* in the coast of Coihaique (45°S) mainly due to an introduction for aquaculture.

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