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Bioprospecting, a tool to conserve Chilean bryophytes

Bioprospección, una herramienta para la conservación de briófitas chilenas

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ABSTRACT

Here, we present the current state of knowledge on the diversity of Chilean bryophytes (mosses, liverworts, and hornworts), the secondary metabolites present in these plants, and the biological activity of extracts from populations of Chilean species. Our goal is to establish the potential of these plants as a source of useful molecules for pharmaceutical and agricultural industries, thus promoting their conservation. Only 3,7% of Chilean bryophytes (55 spp.) have been analyzed using chemical characterization of their extracts (methanol, ether, ethanol, dichloromethane, acetone and hexane). Only four species from Chilean and Argentinian populations (*Porella chilensis* (Lehm. & Lindenb.) Trevi., *Riccardia polyclada* (Mitt. ex Thurn) Hässel, *Balantiopsis cancellata* (Nees) Stephani, and *Sphagnum magellanicum* Brid.) have been evaluated for biological activity. The majority of these studies have concentrated on liverworts widely distributed in the Southern Hemisphere and in the temperate forests of the southern half of Chile and adjacent Argentina. We briefly discuss aspects related to the use of bioprospecting as a conservation tool as well as the maintenance of *in vitro* bryophyte cultures with the goal of conservation and production.

Keywords: Mosses, liverworts, hornworts, uses, secondary metabolites, biological activity, *in vitro* cultivation.

RESUMEN

Esta revisión presenta el estado actual del conocimiento de la diversidad de las briófitas chilenas (musgos, hepáticas y antocerotas), de los metabolitos secundarios de estas plantas y de la actividad biológica de extractos obtenidos a partir de especies de poblaciones chilenas. Nuestro objetivo es determinar el potencial de estas plantas como fuente de moléculas útiles para las industrias farmacéutica y agroalimentaria, y promover su conservación. Sólo de un 3,7% de las briófitas chilenas (55 spp.) han sido analizados químicamente sus extractos (metanol, éter, etanol, diclorometano, acetona y hexano). Se ha evaluado la actividad biológica sólo de cuatro especies de poblaciones chileno-argentinas (*Porella chilensis* (Lehm. & Lindenb.) Trevi., *Riccardia polyclada* (Mitt. ex Thurn) Hässel, *Balantiopsis cancellata* (Nees) Stephani, y *Sphagnum magellanicum* Brid.). La mayor parte de los estudios se ha concentrado en hepáticas de amplia distribución en el Hemisferio Austral y en los bosques templados de Chile y Argentina. Se discuten brevemente aspectos relacionados a la bioprospección como herramienta para la conservación, y al cultivo in vitro de briófitas con fines productivos y de conservación.

Palabras clave: Musgos, hepáticas, antocerotas, usos, metabolitos secundarios, actividad biológica, cultivo *in vitro*.

INTRODUCTION

In general terms, bryophytes (mosses, liverworts, and hornworts) are flowerless photoautotrophs that exist predominantly in the gametophyte (haploid) stage. In the majority of cases, the sporophyte, produced by the fertilization of an egg cell by antherozoids, is formed by a foot, a seta, and a capsule, in which haploid spores are produced. The spores are dispersed into the environment by wind, water, or animals, after which they germinate and give rise to new haploid plants. They also spread vegetatively from buds, fragments, and rhizoids. These plants do not have roots, and as such, their nutrition is strongly influenced by weather and atmospheric deposition (Ardiles *et al*. 2009). Bryophytes can be differentiated from other plants by certain characteristics that make them excellent environmental indicators: they live in a wide range of ecosystems, habitats, and specific microhabitats, including substrates on which vascular plants could not survive. In addition, many species are able to grow in poor nutritive conditions and are adapted to rapidly initiate photosynthesis in response to intermittent periods of moisture (Slack 2011).

Approximately 20,000 species of bryophytes exist in the world (Asakawa 2001), including 14,000 species of mosses (Bryophyta), 6,000 species of liverwort (Marchantiophyta), and 300 hornworts (Anthocerotophyta). These species represent approximately 8% of the known diversity within the plant kingdom. In Chile, the bryophytes consist of approximately 1,500 species (890 mosses, 553 liverworts, and 14 hornworts) (Hässel de Menéndez & Rubies 2009, Müller 2009), with rates of endemism at a regional level that may exceed 50% (Villagrán *et al*. 2003, Bell & Cuvertino 2003). High rates of endemism occur primarily in the southern regions, which are the richest for this type of flora (Rozzi *et al*. 2008). Among the richest genera of Chilean mosses and liverworts are *Chiloscyphus* (Lophocoleaceae, Marchantiophyta), *Plagiochila* (Plagiochilaceae, Marchantiophyta), *Riccardia* (Aneuraceae, Marchantiophyta), *Bryum* (Bryaceae, Bryophyta), *Andreaea* (Andreaeaceae, Bryophyta), and *Syntrichia* (Pottiaceae, Bryophyta) (Hässel de Menéndez & Rubies 2009, Müller 2009).

Uses of bryophytes

In terms of the utilization of these plants, many researchers have highlighted traditional and commercial uses in diverse areas of the world (Saxena & Harinder 2004, Frahm 2004, Glime 2007). Frahm (2004) points out that the economic uses for bryophytes include horticultural peat (*Sphagnum* moss) for cleaning oil spills, or for flower pots, among other uses. The same author mentions the commercial development of products based on bryophyte extracts for fighting foot odor and as an antifungal cream for horses, which has been patented. Sphagnol, a product of peat distillation, is recognized for its utility in the treatment of eczema, psoriasis, itching, scabies, acne, and other skin diseases. Sphagnol is also effective at reducing the irritation produced by insect stings and bites (Grieve 1931).

Wilhelm de Mosbach (1992), in his work *Botánica Indígena* (indigenous botany), mentions some Chilean bryophyte species by their name in the Mapundungun language. Among them, he reports the use of *Funaria hygrometrica* Hedw. (hueñoquintúe) and *Marchantia polymorpha* L. (paillahue) in the preparation of love potions. There is no written evidence of medical usage in Chile. Nevertheless, in 1982, Herman Núñez (National Museum of Natural History, July 2011, personal communication) found that compresses of dry mosses were used by the inhabitants of Piruquina, near Castro (Region X), when their wounds began to show signs of infection.

Regarding the traditional uses, Glime (2007) mentions that medicinal properties have been attributed to these plants in China, India and by Native Americans. These uses include the treatment of liver diseases, ringworm, inflammation, fever, digestive and urinary problems, and skin infections, among others. Glime (2007) also mentions that the moss *Rhodobryum giganteum* (Schwägr.) Paris is frequently used in China to treat cardiovascular problems. Recently, Cai *et al*. (2011) demonstrated that p-hydroxycinnamic acid and 7,8-dihydroxycoumarin, isolated from *Rh*. *giganteum*, show protective effects against myocardial hypoxia/reoxygenation in mice.

In the biotechnology field, bryotechnologies - BryoSpeed™ and BryoMaster™, has been developed by Greenovation for the production of human therapeutic proteins through genetic manipulation of the moss *Physcomitrella patens* (Hedw.) Bruch & Schimp. and its cultivation in bioreactors (Greenovation 2011).

Biological activity of bryophytes

Secondary metabolites can be classified on the basis of the properties described in Table I. In this regard, Sabovljevic *et al*. (2009) consider bryophytes as a notable reserve of natural products and novel secondary compounds. The authors also mention that many of these plants have shown interesting biological activity. Along the same lines, Zhu *et al*. (2006) suggest that bryophytes constitute one of the most significant and promising sources of antibiotics and active biological compounds. Among the recognized biological activities in these plants are insect-repelling, insecticidal, cytotoxic, phytotoxic, allergenic (dermatitis), neurotrophic, anti-obesity, muscle-relaxing, antibacterial, antifungal, antitumor, and anti-HIV properties (Saxena & Harinder 2004, Asakawa *et al*. 2009).

With regard to insect-repelling properties, Schuster (1966) mentions that, as a general rule, liverworts are free from insect attacks. This result may be related to the presence of oilbodies, which are membrane-bound cellular organelles unique from liverworts. Recently, Asakawa (2008) mentioned that, despite the weak mechanical protection possessed by bryophytes, they are generally not attacked by microorganisms, insects and other invertebrates, or small mammals. The oilbodies contain terpenoids suspended in a matrix rich in carbohydrate and/or protein-rich matrix, and 90% of liverworts develop them (Vanderpoorten & Goffinet 2009). Furthermore, their morphology and chemical composition offer relevant taxonomic information (Vanderpoorten & Goffinet 2009).

Agricultural potential

In Peru, Bolivia, and Germany, some greenhouse and field studies have demonstrated the effectiveness of bryophyte extracts in the control of vegetable crop pathogens (Frahm 2004). *In vivo* experiments have proven that bryophyte extracts added to lettuce at different concentrations deter snails and slugs. In addition, the application of liverwort extracts to tomato, pepper, and wheat plants before and after inoculation with the fungi *Phytophthora infestans* (Mont.) de Bary, *Botrytis cinerea* Pers., and *Erysiphe graminis* DC. lead to variable positive effects depending on the species and concentration of extract (Frahm 2004).

Bioprospecting among populations of Chilean species

In Chile, studies of bioprospecting and the biological activity of bryophytes have been relatively successful. *Riccardia polyclada* (Mitt. ex Thurn) Hässel (Marchantiophyta) has shown moderate deterrent activity against *Spodoptera littoralis* (Boisduval 1833) (Lepidoptera) and also inhibits the growth of the fungus *Cladosporium herbarum* (Pers.) Link (Fungi). However, *R. polyclada* has also been shown to be lethal against *Artemia salina* (Linnaeus 1758) (Crustaceae) at lower doses than the commercial acaricide Asuntol (Labbé *et al*. 2007).

Dichloromethane extract of *Balantiopsis cancellata* (Nees) Stephani (Marchantiophyta) has shown a strong inhibition of growth of *C. herbarum* at lower concentrations than commercial fungicides. In addition, the extract has shown to be lethal against *A. salina* at similar doses than the commercial acaricide Asuntol (Labbé *et al*. 2005).

The antibacterial properties of *Sphagnum magellanicum* Brid. (Sphagnopsida) have been tested by Montenegro *et al*. (2009) using ethanol extracts of dehydrated plants collected in the province of Llanquihue (Region X). The results of their study show an inhibitory effect on the growth of certain bacterial strains, although high concentrations of extract were used. Similarly, Wallach *et al*. (2010) obtained positive results against Gram (+), Gram (–) bacteria and fungi, using fresh *S. magellanicum* plants, extracted with a mixture of acetone and hexane. Interestingly, while the extract prepared with fresh material inhibited *Staphylococcus aureus* Rosenbach, the extract from dried plants did not.

Extracts of *Porella chilensis* (Lehm. & Lindenb.) Trevis., a common species of liverwort in the temperate forests of Chile and Argentina, have shown equal or lower rates of inhibition of the cellular proliferation and formation of biofilms by *Pseudomonas aeruginosa* (Schroeter) Migula as compared with the antibiotics ciprofloxacine and azithromycin (Gilabert *et al*. 2011). Recently, Corzo *et al*. (2012) have demonstrated lethal and sublethal effects on larvae of *Spodoptera frugiperda* (J.E. Smith 1797) (Lepidoptera) fed with a diet incorporating 8 isolated and purified terpenoids from *P. chilensis* extract. The most active compounds were the sesquiterpenoids norpinguisone, norpinguisone methyl ester, norpinguisone acetate and pinguisenol. Table II summarizes these findings.

Table I. The major classes of bryophyte secondary metabolites and their respective biological activities (adapted from Chun-Feng & Hong-Xiang 2009).

Tabla I. Principales clases de metabolitos secundarios en Briófitas y actividades biológicas respectivas (adaptado de Chun-Feng & Hong-Xiang 2009).

Classes	Biological activity						
	aprox. number of molecules described	Phytotoxic	Antimicrobial	Insect antifeedant, molluscicide	UV-absorbing	Drought tolerance	Freeze tolerance
Benzenoids	150		x	x	x		
Bibenzyles y Bis-bibenzyles	270	x	x	x		x	
Fatty acid derivates	25		x				x
Flavonoids	360	x	x		x		
Phenylpropanoids	65			x	x		x
S- and N- containing compounds	20			x			
Terpenoids	1400	x	x	x			
Total number	2290						

In general, liverworts have shown greater antibiotic activity than mosses and hornwort species (Russell 2010). Among the genera of liverworts present in Chile (in addition to diverse species distributed throughout the globe) that have been tested for biological activity are the following, which are listed alongside their biological properties:

- *Frullania*: allergenic, cytotoxic, antimicrobial, antifungal
- *Porella*: antimicrobial, bitterness
- *Plagiochila*: cytotoxic, deterrent, nematicidal
- *Radula*: antifungal
- *Marchantia*: antimicrobial
- *Trichocolea*: antimicrobial

Chemicals compounds from Chilean species

Regarding Chilean bryophytes, Asakawa & Inoue (1984a, 1984b) have elucidated the chemical compositions of crude extracts of 17 species of liverwort (Table III) and 13 species of the genus *Plagiochila*. The latter contained sesquiterpenes to a large extent, whereas the levels of mono- and diterpenes were considerably reduced (Asakawa & Inoue 1984b).

Plagiochila lechleri Gottsche, *P. dura* De Not., endemic to Patagonia, and *P. gayana* Gottsche (or *P. heterodonta* (Hook.f. & Taylor) Gottsche), also present in the Antarctic islands, have diverse types of plagiochilides. One plagiochilide, isolated from *Plagiochila* species, causes the death of *Nilaparvata lugens* (Stål 1854) (Hemiptera) at a concentration of 100 $\mu g \cdot ml^{-1}$ (Asakawa *et al*. 2009). Asakawa *et al*. (2003) analyzed 25 taxa of Frullaniaceae and characterized volatile compounds from three Patagonian species of the genus *Frullania*: *F. magellanica* F. Weber & Nees, *F. lobulata* (Hook.) Dumort., and *F. microcaulis* Gola. Moreover, several unidentified sesquiterpene lactones, sesquiterpenoids and aromatic compounds have been found in theses plants. In Fueguine and Argentinian samples of *Tylimanthus renifolius* Hässel de Menéndez & Solari (endemic to the Magallanes province and Tierra del Fuego), researchers have found sesquiterpenes, diterpenes, and flavone-type flavonoids and flavanones (Feld *et al*. 2003). In *Gackstroemia decipiens* Hässel, endemic to Patagonia, Geis & Becker (2000) have identified sesqui- and diterpenes. Hertewich *et al.* (2003) isolated and characterized six new labdane type diterpenoids, two new jamesoniellides, K and L, a new chlorinated bisbibenzyl, and the previously known jamesoniellide I and the sesquiterpene waitziacuminone, from Jamesoniella colorata (Lehm.) Stephani collected near Puerto Cisnes (Region XI).

Table II. Bryophytes present in Chile (Chilean and Argentinean populations) and organisms that have been tested for biological activity. D+: positive antifeedant activity, A+: positive antimicrobial activity, A-: negative antimicrobial activity, white spaces: unreported or undetermined (source: authors).

Tabla II. Briófitas presentes en Chile (poblaciones chilenas y argentinas) y organismos sobre los cuales se ha probado la actividad biológica de sus extractos. D+: actividad antialimentaria positiva, A+: actividad antimicrobiana positiva, A-: actividad antimicrobiana nula, espacios en blanco: no reportada o no determinada (fuente: autores).

Taxon	*Spodoptera frugiperda*	*Spodoptera littoralis*	*Artemia salina*	*Cladosporium herbarum*	*Malassezia pachydermatis*	*Candida albicans*	*Erwinia carotorova*	*Vibrio cholerae*	*Salmonella typhi*	*Salmonella enteritidis*	*Escherichia coli*	*Streptococcus tipo B*	*Azotobacter vinelandii*	*Enterobacter aerogenes*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus cereus*	Ref.
Porella chilensis	D+															A+		Gilabert *et al*. 2011; Corzo *et al.* 2012
Riccardia polyclada		D+	D+	A+														Labbé *et al*. 2007
Balantiopsis cancellata		D+	D+	A+														Labbé *et al*. 2005
Sphagnum magellanicum							A+	A+	A+		A+	A+	A-	A-	A-	A-		Montenegro *et al*. 2009
Sphagnum magellanicum					A+	A+				A+	A+	A+			A+	A-	A+	Wallach *et al*. 2010

Table III. The major compounds found in Chilean liverworts (adapted from Asakawa & Inoue 1984a).

Tabla III. Principales compuestos presentes en hepáticas de Chile (adaptado de Asakawa & Inoue 1984a).

Taxon	Sesquiterpenes	Diterpenes	Monoterpenes	Benzyl benzoates	Benzoates (other)	Germacrene D	Phenyl-cinnamate	Oxygenated compounds	Bicyclogermacrene	Bibenzyles	Tylimanthins
Adelanthus bisetulus	x										
Adelanthus lindenbergianus	x	x									
Balantiopsis cancellata	x			x	x						
Balantiopsis erinacea				x	x						
Chiloscyphus hookeri	x										
Chiloscyphus pallido-virens	x										
Clasmatocolea humilis	x										
Gackstroemia magellanica	x	x				x					
Isotachis humectata	x	x	x				x				
Jamesoniella colorata	x										
Noteroclada confluens	x							x			
Riccardia prehensilis	x										
Roivainenia jacquinotii	x							x			
Schistochila laminigera	x								x		
Schistochila reflexa	x							x			
Triandrophyllum subtrifidum	x										
Tylimanthus urvilleanus										x	x

Several clerodane type diterpenoids, sesquiterpenoids, triterpenoids along with other compounds known from liverworts, flowering plants and a sea pen (Cnidaria) have been isolated from a fueguine population of Adelanthus lindenbergianus (Lehm.) Mitt. by Bläs et al. (2004).

Of the widely distributed liverworts, also present in Chile, several species have been characterized, tested for biological activity, and/or used to isolate secondary metabolites, including the following: *Lepicolea ochroleuca* (L. f. ex Spreng.) Spruce, *Dumortiera hirsuta* (Sw.) Nees, *Lunularia cruciata* (L.) Dumort. ex Lindb., *Reboulia hemisphaerica* (L.) Raddi, *Noteroclada confluens* Taylor ex Hook. f. & Wilson, *Targionia hypophylla* L., *Marchantia polymorpha*, *M. chenopoda* L., *M. foliacea* Mitt., Tylimanthus tenellus (Taylor ex. Lehm.) Mitt. *Fossombronia pusilla* (L.) Dumort., and *Ricciocarpos natans* (L.) Corda (Liu *et al*. 2000, Asakawa 2001, Asakawa *et al*. 2009, Chung-Feng & Hong-Xiang 2009 Tori *et al.* 1994, Toyota *et al.* 2004, Ludwiczuk & Asakawa 2008). Sesquiterpenoids, ledol, and fusicoccanoids have been isolated from *L. ochroleuca* (Liu *et al*. 2000), which grows frequently and abundantly on *Pilgerodendron uviferum* (D. Don) Florin and *Nothofagus* spp. in the fjords of region de Aysén. The presence of polyphenol lignans has also been reported by Cullman & Becker (1999). New compounds such as dumortenol, sesquiterpenoids, and dumortane and nordumortane derivatives have been isolated from Argentine and Japanese specimens of *D. hirsuta* (Asakawa 2001), in addition to acetogenins isolated from other specimens (Asakawa *et al.* 2009). Four components of terpenoids with activity against the bacterium *Bacillus subtilis* (Ehrenberg) Cohn have been biosynthesized from *in vitro* cultures of *F. pusilla*, in addition to two sesquiterpenoids and three diterpenoids that show activity against the fungi *Botrytis cinerea*, *Rhizoctonia solani* J.G. Kühn, and *Pythium debaryanum* R. Hesse (Chung-Feng & Hong-Xiang 2009). In addition, Grammes *et al*. (1994) identified new terpenoids in gametophytes of

F. pusilla grown *in vitro*. Furthermore, *R. natans* produces ricciocarpin A, ricciocarpin B, cuprenolide, cuprenolidol, ricciofuranol, and sesquiterpenoids, which act as deterrents against *Biomphalaria glabrata* (Say 1818) (Gastropoda) (Chung-Feng & Hong-Xiang 2009). Kunz & Becker (1992) have reported the production of bibenzyl from *in vitro* cultured gametophytes of *R. natans*.

Lunularic acid has been isolated and synthesized from *L. cruciata*. In addition to having antifungal activity, lunularic acid also shows antiallergenic activity greater than that of Tranilast, an orally administered anti-asthma drug developed in Japan (Asakawa *et al*. 2009). Riccardins C and F have been isolated from *R. hemisphaerica*, cis- and trans-pinocarveol acetates have been isolated from *T. hypophylla*, and bis-bibenzyls and marchantin A have been isolated from *M. polymorpha* and *M. chenopoda* (Asakawa *et al*. 2009). *M. polymorpha* causes dermatitis and shows diuretic activity and inhibitory activity against gram-negative bacteria. The isolated compound marchantin A has cytotoxic activity against the P388 leukemia cell line. In addition, marchantin A has antibacterial activity against *Acinetobacter calcoaceticus* (Beijerinck) Baumann *et al.*, *Alcaligenes faecalis* Castellani & Chalmers, *Bacillus cereus* Frankland & Frankland, *B. megaterium* de Bary, *B. subtilis*, *Enterobacter cloacae* (Jordan) Hormaeche & Edwards, *Escherichia coli* (Migula) Castellani & Chalmers, *Proteus mirabilis* Hauser, *Pseudomonas aeruginosa*, *Salmonella typhimurium* (Loeffler) Castellani & Chalmers, and *Staphylococcus aureus* as well as antifungal activity against *Alternaria kikuchiana* S. Tanaka, *Aspergillus fumigatus* Fresen., *A. niger* Tiegh, *Candida albicans* (C.P. Robin) Berkhout, *Cryptococcus neoformans* (San Felice) Vuill., *Microsporum gypseum* (E. Bodin) Guiart & Grigoraki, *Penicillium chrysogenum* Thom, *Pyricularia oryzae* Cavara, *Rhizoctonia solani*, *Saccharomyces cerevisiae* Meyen, *Sporothrix schenckii* Hektoen & C.F. Perkins, *Trichophyton mentagrophytes* (C.P. Robin) Sabour., and *T. rubrum* (Castell.) Sabour. (Asakawa *et al*. 2009). Marchantin P and Riccardin G have been isolated from *M. chenopoda* collected in Venezuela along with the new sesquiterpene chenopodene (Tori *et al.* 1994) *Marchantia berteroana* Lehm. & Lindenb. and *M. foliacea* Mitt. contain flavonoids, much like *Corsinia coriandrina* (Spreng.) Lindb., *Riccia crystallina* L., *Apometzgeria pubescens* (Schrank) Kuwah., and *Haplomitrium gibbsiae* (Stephani) R.M. Schust. (Chopra & Kumra 2005) Marchantin C has been detected as a major compound in *M. foliacea* and sesquiterpenes have been isolated from Tylimanthus tenellus, both from New Zealand populations (Toyota *et al.* 2004, (Ludwiczuk & Asakawa 2008). The Ecuadorian plants of *N. confluens* contain diterpenoids, sesquiterpenoids, and acetogenins (Asakawa *et al*. 2009). Sesquiterpenoids and riccardiphenols A and B have been isolated from *Riccardia crassa* (Schwägr.) Carrington & Pearson (Toyota & Asakawa 1992). *Plagiochasma rupestris* (Forst.) Steph. has been demonstrated to produce sesquiterpenoids and bis-benzyls (Bardón *et al*. 1999). Pinguisone and sesquiterpenes have been isolated from cultures of *Aneura pinguis* (L.) Dumort. (Tazaki *et al*., 1996). Terpenoids have been found in the *Barbilophozia hatcheri* (A. Evans) Loeske plants (Tori *et al*. 1993), a species frequently found on the base of *Nothofagus pumilio* (Poepp. & Endl.) Krasser in the forests of Patagonia. Analysis of the ether extract of *C. coriandrina* has shown the presence of benzofurans (corsifurans) and bis-benzyls (Von Reuss & König 2004).

Finally, Xiaowei (2007) mentions other bryophyte species also present in Chile (none of which are endemic) that have been analyzed chemically in crude extract form. These species include the following: *Campylopus clavatus* (R. Br.) Wilson (Bryophyta), *C. introflexus* (Hedw.) Brid. (Bryophyta), *Dicranoloma robustum* (Hook. f. & Wilson) Paris (Bryophyta), *Racomitrium lanuginosum* (Hedw.) Brid. (Bryophyta), *Bryum capillare* Hedw. (Bryophyta), *Hypnum cupressiforme* Hedw. (Bryophyta), *Riccardia crassa* (Marchantiophyta), *Lepidozia chordulifera* Taylor (Marchantiophyta), *Balantiopsis erinacea* (Hook. f. & Taylor) Mitt. (Marchantiophyta), and *Cryptochila grandiflora* (Lindenb. & Gottsche) Grolle (Marchantiophyta).

DISCUSSION

Most studies on bioprospecting and biological activity have been performed using species that display a wide range of distribution at a regional level (over the Southern Cone of the Americas) or in the Southern Hemisphere. Most of these studies have used dehydrated material for the extraction of secondary metabolites and diverse solvents. Additionally, these studies have primarily concentrated on liverworts, which appear to have considerable potential due to their oilbodies. The process of drying may lead to the loss of a variety of compounds, particularly in these organelles from fresh liverwort plants. Notably, dehydrated plants do not exhibit such organelles once they are rehydrated.

According to Schuster (1966) the role of oilbodies in liverworts is unclear and their presence in most of them indicates that they are an ancient cellular component. This author also suggests that the more specialized the taxonomic group (e.g., Marchantiales, complex thalloid liverworts) the more frequently these structures are not found. On the contrary, molecular methods has been useful to unraveling the liverwort phylogeny, hence Jungermanniales (leafy liverworts) are nowadays considered a more derived group (Crandall-Stottler *et al.* 2009), in which oilbodies are commonly found. Otherwise, considering that the levels of UV radiation in past geological eras was higher than present levels, it is possible that these structures play a protective role against this type of radiation. Indeed, an increase has been observed in the accumulation of resins (terpenes) in the spermatophyte *Grindelia chiloensis* (Cornel.) Cabrera (Asteraceae) under UV radiation, suggesting that these compounds play a protective role against UV (Zavala & Ravetta 2002). Although, Schuster (1966) suggested that oilbodies in perennial liverworts may play a role in overwinter.

The Chilean bryoflora, particularly that of the southern tip, has valuable characteristics. Among these benefits are the presence of ancient taxa, a strong floristic similarity to Gondwanic territories, and the remarkable degree of local endemism (Villagrán *et al*. 2003, Bell & Cuvertino 2003), even at the level of monotypic genera (*Vetaforma* - Marchantiophyta - and *Ombronesus* - Bryophyta -, among others). For these reasons, Chilean bryophytes are an important resource that must be conserved, protected, and investigated for potential applications through bioprospecting. This strategy has been recognized by the Millennium Ecosystem Assessment (2005), particularly in developing countries. An example of conservation by this tool is the "International Cooperative Biodiversity Groups" program, which is a project conducted cooperatively by diverse institutions from the United States and Panama. This project was financed from 1995 to 2008 by the National Institutes of Health (US), the National Science Foundation (US), the US Department of Agriculture, the Huntsman Cancer Institute of the University of Utah, and the Panamanian Fundación NATURA. This program conducts most of the studies in Panamanian laboratories, thus providing jobs and boosting the local research skills of students by offering training and practical research experience. This initiative has been constructive in the fields of conservation and sustainable development (Kursar *et al*. 2007).

As noted above, Patagonia is a region that exhibits high rates of endemism and richness of mosses and liverworts. In fact, Asakawa *et al*. (2009) mention the existence of 25 endemic genera of bryophytes in the temperate forests of Chile and Argentina, and Matteri (2000) mentions the "Fuegian domain" as one of the important regions for the conservation of bryophytes in southern South America. In addition, Patagonia and the Tierra del Fuego archipelago are regions with severe weather that are affected by the depletion of the ozone layer, and thus, experience abrupt increases in UVB radiation (Casiccia *et al*. 2008). At a global level, the populations of some species of bryophytes have diminished in recent times, creating concern regarding land use practices and climate change (Slack 2011). In Chile, there are no published data in this area; however, there are diverse endemic species in the mediterranean climate and temperate ecosystems, described 100 or 150 years ago, that have been probably adversely affected by decreased population size and genetic diversity.

Among the global threats facing bryophytes, Hallingbäck & Hodgetts (2000) recognize the following: the loss and degradation of habitats, intensive agriculture, sulfur anhydride contamination, waste chemicals, metals such as Hg, Pb, Cu, and Cd, invasive and introduced species, the harvesting of bryophytes for scientific and commercial purposes, a lack of inclination to conservation, and, finally, the gaps in basic knowledge about these plants. In our country, we can add the impacts of forestry, silvicultural management, and the exploitation of peatlands in regions X, XI and XII (Regions of Los Lagos, Aisén and Magallanes respectively). The latter territory is occupied by 20% wetlands (Arroyo *et al*. 2005), where bryophytes are an important component of the vegetation. Exploitation is considered to be deregulated in the peatlands of our nation, which are important carbon sinks characterized by their high productivity, their ability to purify and regulate the flow of waters, and by the specific flora, and fauna associated with these environments (Promis 2010, Ibarra *et al*. 2010). However, while bryophytes may be an essential element of these wetlands, we lack a thorough knowledge of the richness of bryophyte communities. Thus, Arroyo *et al*. (2005) highlight the need to increase our knowledge of these plants.

One of the strategies to reverse the loss of species is *ex Situ* conservation, particularly *in vitro*. There are numerous conservation initiatives focusing on bryophytes, mainly in Europe, led by Kew Gardens (England), National Botanic Gardens (Ireland), the Museum of Natural History and Archaeology (Trondheim, Norway), and the University of Belgrade (Serbia). These institutions make up the European Bryophyte *Ex situ* Conservation Network (EBESCONet 2011); the objective of this network is to develop techniques, distribute information, establish training needs, and promote collaborations (EBESCONet 2011).

The *in vitro* cultivation and propagation of bryophytes has a nearly 100-year-old history, and the cultivation of *Marchantia polymorpha* (Marchantiophyta) was a routine practice during the first quarter of the 20th century (Hohe & Reski 2005).

Considering the small size of most bryophytes and their growth in mixed populations with other organisms, cultivation is necessary to develop sufficiently pure biomass to be used in biological and pharmacological studies, as has been suggested by Sabovljevic *et al*. (2009). An increase in the yields of *in vitro* bryophyte cultures in bioreactors will be necessary for the large-scale production of valuable compounds (Hohe & Reski 2005). There is evidence that, in general, crude extracts prepared from *in vitro* bryophyte cultures show higher antifungal activity than extracts prepared from natural populations (Sabovljevic *et al*. 2011). In contrast, the antibacterial activity is higher in extracts from natural populations as compared to *in vitro* cultures (Sabovljevic *et al*. 2010). Based on the evidence presented, the production of secondary metabolites in bioreactors from plants, propagules, or spores may be possible through the establishment of culture of protoplasts or protonemata, similar to what has been done with *Anthoceros punctatus* L. (Ono *et al*. 1992) or *Physcomitrella patens*. Specifically, aquatic liverworts may be the most suitable group for *in vitro* culture, as they are free of endophytes and endosymbionts (Duckett *et al*. 2004). A further application of micropropagation of bryophytes may include restoration ecology projects.

CONCLUSIONS

Because the research in bioprospecting and related activities and benefits depend on maintaining an intact ecosystem, this activity is recognized as a ecosystem service (Kursar *et al*. 2007). Bioprospecting improves human wellbeing and highlights the value of biodiversity, as emphasized by the Millennium Ecosystem Assessment (2005). There is evidence that bryophytes can be used in agriculture and pharmaceutics by exploiting their anti-pest, anti-cancer and antimicrobial properties. However, due to their small size and restricted abundance in certain ecosystems and habitats, it will be necessary to develop production and conservation strategies compatible with their commercial and sustainable use to achieve the goals of the national agricultural innovation strategy for the production of medicinal and aromatic plants (FIA 2001). Thus, it is necessary to develop lines of work that elucidate the potential of various species (native and endemic) and promote the appreciation and stewardship of the genetic heritage that they represent.

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