

Lichen diversity associated with native forest of the Achibueno river ravine, Maule region, Chile

Diversidad líquénica asociada al bosque nativo del Cajón del río Achibueno, región del Maule, Chile

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RESUMEN

Estudiamos la diversidad de líquenes del bosque nativo del cajón del río Achibueno. Fueron identificadas 30 especies de 14 familias y 26 géneros. Un 77% de la diversidad líquénica corresponde a Clorolíquenes y un 23% a Cianolíquenes, lo que demuestra la condición prístina del lugar. Adicionalmente, *Hypogymnia fragillima* es registrada por primera vez para Chile y se extiende la distribución de *Crocodia guilleminii*.

INTRODUCTION

In Chile great efforts have been made in order to know and expand the knowledge about the distribution of lichens in the different ecoregions of the country. Some have done it through direct collections in the field (Villagra *et al.* 2020) and others based on the study of databases of different herbaria and field collections, being able to project, through models, the lichen distribution in areas without records (Vargas & Sandoval 2019). Regarding the diversity of lichens in the Maule region, it has been intensely studied, especially that occurring in national reserves and other localities within the region (Pereira *et al.* 2012; Wang *et al.* 2013; Pereira *et al.* 2014). However, the lichens of the Nature Sanctuary of the Achibueno River Ravine have not been studied to date. The sanctuary is located in the mountain zone of the Linares

province, Maule region, which has a pristine and highly complex ecosystem. The owners of the place have decided to protect this area, with the purpose of promoting knowledge of its vegetation and fauna, which include several species of vascular plants with conservation problems. The aim of this study was identify the lichen diversity associated the native forest and understory of Achibueno River Ravine that grows in diverse substrates such as; bark trunk and branches of deciduous and evergreen trees that occur within the forest, wood and rocky substrates, and in and between mosses and in the soil. The pH conditions of the diverse substrates available in the area and the foliar condition of trees, favor the presence of greater lichen diversity. Besides, we also aim to know if there are any threatened lichen species in this area, and to extend the knowledge of the distribution and ecology of the lichen species found in study area.

MATERIALS AND METHODS

STUDY AREA

The study area is located in the Linares province, Maule region, Chile (Supplementary Figure 1). The Achibueno River Ravine corresponds to a valley surrounded by mountains whereby the Achibueno River runs in the middle. It is an ecosystem composed by "Maulino forest", characterized by the presence of associations of deciduous species belonging to the genus *Nothofagus* (Southern beeches) with predominant species such as *Nothofagus obliqua* (Roble pellín) and *Nothofagus glauca* (Hualo). This forest is distinguished worldwide for its great uniqueness and biological value because it is located in a transition region between the biomes of the Valdivian Temperate Rainforest and the Chilean Sclerophyllous scrubland and forest. It is considered as number two of the two hundred most important ecoregions for conservation globally (WWF 1997). This area is also located within the 34 biodiversity "hotspots" recognized worldwide as being of global importance for the conservation of biodiversity (Myers *et al.* 2000; Arroyo *et al.* 2006). This place is dominated by Angiosperm trees species such as *Nothofagus glauca* (Phil.) Krasser, *N. obliqua* (Mirb.) Oerst. and *N. dombeyi* (Mirb.) Oerst. In this ecosystem, *Persea lingue* (Miers ex Bertero) Nees, *Lomatia ferruginea* (Cav.) R. Br., *L. dentata* (Ruiz & Pav.) R.Br., *Schinus molle* L. and Gymnosperms like *Austrocedrus chilensis* (D. Don) Pic-Serm. & Bizzarri, *Prumnopitys andina* (Poepp. ex Endl.) de Laub. and *Podocarpus saligna* D. Don can be found as accompanying species to the *Nothofagus* trees. In this zone, the presence of pristine primary forest, of high complexity and presence of endangered woody species could be of interest in finding a possibly high associated lichen diversity.

SAMPLING

Sampling was made at random considering the great diversity of available substrates: soil, rocks, among mosses, trunks and branches bark of deciduous and evergreen trees and shrubs. The collection of lichens in the arboreal substrates was carried out from the base of the trees to breast height both in the main trunk or secondary logs and in the branches; these lichens were obtained manually or by means of a spatula. To obtain lichens from rocks and soil, a chisel and hammer were used. The samples were transported in paper envelopes, duly labeled with data of the locality, geographical coordinates, altitude, substrate type, name of the phorophyte in the case of epiphytes species or other substrates. Each site was georeferenced with a GPS Model: eTrex Vista® HCx company: Garmin. Samples were collected in four different sites of the Nature Sanctuary of the Achibueno

River Ravine: (1) Punta Tricahue (740 m a.s.l., and 36°08' S; 71°14' W), (2) Las Mulas (790 m a.s.l., and 36°06' S; 71°13' W), (3) Las Animas (810 m a.s.l., and 36°05' S; 71°12' W) and (4) Altos Las Mulas (840 m a.s.l., and 36°06' S; 71°12' W) (Supplementary Figure 1). In the laboratory, the samples were dried at room temperature and finally they were herborized and incorporated into the UTAL herbarium database and collection.

TAXONOMIC IDENTIFICATION

For the taxonomic identification of the lichen species, we considered morphological, anatomical and reproductive characters and spot tests. For the observation of morphological and anatomical characters, a stereoscopic magnifying glass was used (Clauzade & Roux 1985). For the observation of reproductive characters such as apothecia, spores, etc., freehand cuts were made using a razor blade, which were put in glass slides and observed by means an optical microscope (Nikon Ophthophot) equipped with a graduated ocular. The spot tests, were applied in thallus cuts, independently, using the following reagents: K (saturated solution of KOH), C (aqueous solution of sodium hypochlorite at 50 %), KC (application of potassium hydroxide (KOH)) and then aqueous solution of sodium hypochlorite (NaOCl) and finally P (ethanolic solution of paraphenyldiamine) with the purpose of determining the presence or absence of certain substances in different structures of the lichen thallus (Orange *et al.* 2001). The reaction N (aqueous solution of concentrated nitric acid, in a proportion of 1:3 acid/water) was applied in the ephythecium of some apothecia). We also identified the photobiont of all samples to generic level. For that, it was necessary make sections or macerates of the lichen thallus to analyse the following morphological traits: colour, form and cell size of algae in the lichen thallus. Then, we followed published keys and descriptions for algae or cyanobacteria using the following literature: Bourrelly (1966, 1970); Letrouit-Galinou (1968). The major part of the taxonomic identifications of lichens was conducted mainly with the help of available specialized bibliography (Clauzade & Roux 1985; Galloway 1985, 1992b, 1995; Galloway & Jorgensen 1995; Galloway *et al.* 2006; Jorgensen & James 1990; Kashwadani 1990; Krog 1976; Messuti 2005; Stenross 1995; Nash & Elix 2002a, 2002b, 2002c; Nash *et al.* 1995, 2002; etc.). Lastly, taxonomic classification of lichens was adjusted to follow Index Fungorum. Vouchers of specimens studied were deposited in UTAL Herbarium (herbarium of Talca University, Talca, Chile), whose records range from N° 591 to 608 and from 977 to 995.

RESULTS AND DISCUSSION

A total of 30 lichen taxa were found in the study area, 29 of which were identified at the species level, and only one as *cf.* Of the total taxa found, 23 corresponded to chlorolichens (77%) and 7 to cyanolichens (23%) (Table 1, Supplementary Figure 2). Out of the 30 taxa, 22 were corticolous, representing 73 %, 7 saxicolous (23 %) and 1 terricolous (4 %) (Table 1, Supplementary Figure 2). Regarding the growth forms, 47% of lichen taxa were foliose, 33% crustose, 10% fruticose, 7% dimorphic, and 3% squamulose (Supplementary Figure 2).

Among the cyanolichens species, 6 corresponded to the Lecanoromycetes class including the families Collemataceae (2 species), Lobariaceae (3 species) and Stereocaulaceae (1 species), and 1 to the Basidiomycetes class, in the Hygrophoraceae family (Table 2).

The best-represented family in the area was Parmeliaceae with 9 species, followed by Lobariaceae, Physciaceae and Stereocaulaceae with 3 species, Collemataceae and Lecanoraceae with 2 species and Candelariaceae, Chrysothricaceae, Cladoniaceae, Haematommaceae, Hydrophoraceae, Ochrolechiaceae, Ramalinaceae and Rhizocarpaceae with 1 species each (Table 2, Supplementary Figure 2).

Platismatia glauca grew only on *Austrocedrus chilensis* bark and *Protousnea poeppigii* also on bark of the same tree species and also on bark of *Lomatia dentata*. On *Nothofagus obliqua* forests occurred a greater number of species, among them *Candelariella reflexa*, *Hypogymnia fragillima*,

Hypotrachyna laevigata, *Lecanora albella*, *Lepraria incana*, *Parmotrema perlatum*, *Ramalina chilensis*, *Rinodina anomala* and *R. sophodes*, all of them chlorolichens and corticolous, which could be related to the foliar condition (deciduous) and the pH of the barks of this tree species. On the other hand, *Cladonia chlorophaea* was found growing on the base of the trees, where large amounts of nutrient and moisture accumulate. On *N. dombeyi* bark, appeared *Platismatia glauca*, chlorolichen and *Leptogium cochleatum* and *Pseudocyphellaria nudata* cyanolichens, between 740-810 m a.s.l., in the soil between mosses.

On metamorphic rocky substrates near the edge of “wells” grew species like: *Haematomma fenizianum*, *Rhizocarpon geographicum* and *Stereocaulon alpinum*, 790 m above sea level, acid substrates with sufficient light and as epiphyte appeared *Crocodia guilleminii* (Supplementary Figure 3) growing in *N. dombeyi* bark.

Among corticolous species, there were some species that grows on branches of Gymnosperms phorophytes like *Podocarpus saligna* or *Austrocedrus chilensis* as is the case of *Hypogymnia fragillima* (Supplementary Figure 3). This is the first record of the species for Chile, at an altitude of 740-810 m above sea level.

Among mosses and rocks with some of soil and decomposition of organic matter appeared cyanolichens species such as *Dictyonema glabratum*, *Leptogium cochleatum* and *L. brebissoni* and occasionally *Parmotrema perlatum*, chloroliquen species that occurs commonly as epyphyte in trees species of native forests at 810 m above sea level.

Table 1. List of lichen species found in forest and understory of Achibueno River Ravine, Maule region, Chile. / Listado de especies líquénicas encontradas en bosque y sotobosque del Cajón Achibueno, región del Maule, Chile.

Lichen species	Substrates			Locality	Photobiont	Growth form	Distribution in Chile
	Cort	Sax	Terr				
1.- <i>Candelariella vitellina</i> (Hoffm.) Müll. Arg.	+			2	Chloroli.	Crustose	Tarapacá and Maule regions (Pereira 2019).
2.- <i>Chrysothrix granulosa</i> G. Thor	+			3	Chloroli.	Crustose	Cuesta Buenos Aires, 29°35' S, 71°14' W, 257 m a.s.l., on branch, November 13 2013, S.-O. Oh & J. S. Hur CL130420; Caleta Tototal Baja, 28°17' S, 71°10' W, 203 m a.s.l., on branch, November 14 2013, S.-O. Oh & J. S. Hur CL130527; Fray Jorge National Park, La Serena.
3.- <i>Cladonia chlorophaea</i> (Flörke ex Sommerf.) Sprengel		+	+	3	Chloroli.	Dimorphic	Coquimbo, Valparaíso, Biobío, La Araucanía, Los Lagos, Aysén and Magallanes regions (Stenross, 1995).

Lichen species	Substrates			Locality	Photobiont	Growth form	Distribution in Chile
	Cort	Sax	Terr				
*4.- <i>Crocodia guilleminii</i> (Mont.) Nyl	+			1	Cyanoli.	Foliose	Juan Fernández Archipelago, and from La Araucanía to Magallanes regions (Galloway 1992b).
5.- <i>Dictyonema glabratum</i> (Spreng.) D. Hawksw.		+		1	Cyanoli.	Foliose	Los Lagos region, Parque Katalapi, (Pereira 2007) Lago Todos Los Santos (Redón 1972a, Redón & Quilhot 1977) Maule region, Ramadillas de Tregualemu (Pereira 2002) and Los Ruiles Reserve (Pereira et al. 2015).
6.- <i>Flavoparmelia caperata</i> (L.) Hale	+			3	Chloroli.	Foliose	Tarapacá, Maule and Biobío regions (Quilhot et al. 2012).
7.- <i>Haematomma fenizianum</i> A. Massal.		+		2	Chloroli.	Crustose	Valparaíso, RM, Maule and Biobío regions (Quilhot et al. 1998).
*8.- <i>Hypogymnia fragillima</i> (Hillmann) Rass.	+			1,3	Chloroli.	Foliose	Not cited for Chile.
9.- <i>Hypotrachyna laevigata</i> (Sm.) Hale	+			3	Chloroli.	Foliose	Talca to Magallanes (Quilhot et al. 2012).
10.- <i>Lecanora albella</i> (Pers.) Ach.	+			3	Chloroli.	Crustose	Maule region: Cerro El Roble de Libún (Zalbruckner 1933); Los Ruiles reserve (Pereira & San Martín 1998; Pereira et al. 1998); Biobío region, El Carmín y Ramadillas de Tregualemu (Pereira et al. 2002); Chillán (Pereira et al. 2016).
11.- <i>Lecanora chlorotera</i> Nyl.	+			3	Chloroli.	Crustose	Maule and Biobío regions (Quilhot et al. 1998); Maule region: Ramadillas de Tregualemu (Pereira et al. 2002).
12.- <i>Lepraria incana</i> (L.) Ach.	+			1	Chloroli.	Crustose	Maule region (Herbarium University of Talca).
13.- <i>Leptogium brebissonii</i> Mont	+			1	Cyanoli.	Foliose	Aysén region, Laguna San Rafael National Park (Galloway 1995).
14.- <i>Leptogium cochleatum</i> (Dicks.) P.M. Jørg. & P. James	+			3	Cyanoli.	Foliose	Maule region: El Carmín (Pereira et al. 2002), and Los Lagos and Aysén regions (Galloway & Jørgensen 1995).
15.- <i>Parmotrema perlatum</i> (Hudson) M. Choisy	+			1	Chloroli.	Foliose	Chillán to Magallanes (Nash III & Elix 2002b; Villagra et al. 2009).
16.- <i>Physconia muscigena</i> (Ach.) Poelt	+			3	Chloroli.	Foliose	Putre, Portillo, and from Llanquihue to Isla Navarino and Antarctic Territory (Elvebakk & Moberg 2002; Galloway 1998; Øvstedal & Lewis Smith 2001).
17.- <i>Platismatia glauca</i> (L.) Culb. & C. Culb.	+			1,3	Chloroli.	Foliose	La Campana National Park, and from Talca to Isla Navarino and Antarctic Territory (Culberson & Culberson 1968; Øvstedal & Smith 2001; Redón & Quilhot 1977).
18.- <i>Protousnea poeppigii</i> (Nees & Flot.) Krog.	+			3,4	Chloroli.	Fruticose	In Chile: Valdivia; Ñuble region, Recinto Las Trancas, 1200-1300 m a.s.l. (Krog 1976).

Lichen species	Substrates			Locality	Photobiont	Growth form	Distribution in Chile
	Cort	Sax	Terr				
19.- <i>Pseudocyphellaria coriifolia</i> (Müll. Arg.)	+			3	Cyanoli.	Foliose	Juan Fernández Archipelago, and from Chillán to Isla Navarino. (Galloway 1992a, 1992b; Redón & Quilhot 1977). Biobío region: Quebrada Honda (Pereira <i>et al.</i> 2002) Maule region: Ramadillas de Tregualemu (Pereira <i>et al.</i> 2002).
20.- <i>Pseudocyphellaria nudata</i> (Zahlbr.) D.J. Galloway	+			3	Cyanoli.	Foliose	Chillán to Aysén (Galloway 1992b); Maule region: Ramadillas de Tregualemu, (Pereira <i>et al.</i> 2002).
21.- <i>Ramalina chilensis</i> Bertero ex Nyl.	+			3	Chloroli.	Fruticose	In coastal areas of middle Chile, such as Provinces Aconcagua, Coquimbo and Valparaíso (Kashiwadani 1990).
22.- <i>Rhizocarpon geographicum</i> (L.) DC.		+		2	Chloroli.	Crustose	Biobío region (Pereira <i>et al.</i> 2016).
23.- <i>Rimelia reticulata</i> (Taylor) Hale & A. Fletcher	+			3	Chloroli.	Foliose	Talca to Aysén (Louwhoff & Elix 2002; Nash III & Elix 2002c), Maule (Pereira <i>et al.</i> 1998) and Aysén regions (Quilhot <i>et al.</i> 1998).
24.- <i>Rinodina anomala</i> (Zahlbr.) H. Mayrhofer & Giralte	+			3	Chloroli.	Crustose	Maule region, Altos de Lircay National Park and Los Ruiles reserve (Pereira <i>et al.</i> 2015).
25.- <i>Rinodina sophodes</i> (Ach.) A. Massal.	+			3	Chloroli.	Crustose	Maule region, Altos de Lircay National Park and Los Ruiles reserve (Pereira <i>et al.</i> 2015).
26.- <i>Squamarina squamulosa</i> (Nyl.) Follmann		+		2	Chloroli.	Squamulose	Biobío region (Pereira <i>et al.</i> 2016).
** 27.- <i>Stereocaulon alpinum</i> Laurer		+		2	Cyanoli.	Dimorphic	From Cautín Province to Isla Navarino and Antarctic Territory (Lamb 1977; Redón & Quilhot 1977; Øvstedal & Lewis-Smith 2001). Not cited for Maule region.
28.- <i>Usnea aff. igniaria</i> Motyka	+			3	Chloroli.	Fruticose	Metropolitan region, Santiago, (Mahu 1989).
29.- <i>Varicellaria velata</i> (Turner) Schmitt & Lumbsch	+			3	Chloroli.	Crustose	Maule region (Pereira & San Martín 1998; Pereira <i>et al.</i> 2002).
30.- <i>Xanthoparmelia submougeotii</i> Hale		+		3	Chloroli.	Foliose	In Chile, the type locality is placed on the Juan Fernández Island (Nash <i>et al.</i> 1995; Calvelo & Adler 2001).

*New record for Chile / Nuevo registro para Chile.

**New record for Maule region / Nueva cita para la región del Maule.

Abreviations: Cort: Corticolous, Sax: Saxicolous; Terr: Terricolous, Chloroli: Chlorolichen, Cyanoli: Cyanolichen.

Localities: (1) Punta Tricahue, 740 m a.s.l., (2) Las Mulas, 790 m a.s.l., (3) Las Animas, 810 m a.s.l., and (4) Altos Las Mulas, 840 m a.s.l.

Table 2. Family and distribution of found lichen species. / Familia y distribución de las especies de líquenes encontradas.

Family	Lichen name	Worldwide Distribution
Candelariaceae	<i>Candelariella vitellina</i>	Cosmopolitan (Kirk <i>et al.</i> 2001).
Chrysothricaceae	<i>Chrysothrix granulosa</i>	Bipolar. This species is known previously from South (Australia and New Zealand) and North America (Thor 1988; Tønsberg 2004).
Cladoniaceae	<i>Cladonia chlorophaea</i>	All continents, mainly temperate to boreal.
Collemaaceae	<i>Leptogium brebissonii</i>	Bipolar. North and South America (Galloway & Jørgensen 1995; Quilhot <i>et al.</i> 2010).
	<i>Leptogium cochleatum</i>	Widespread in the tropics and warm temperate regions (Galloway & Jørgensen 1995; Quilhot <i>et al.</i> 2010). Cosmopolitan.
Haematommaceae	<i>Haematomma fenizianum</i>	Pantropical to subtropical or Mediterranean in southwestern North America, South America, Europe, South Africa, and Australasia.
Hydrophoraceae	<i>Dictyonema glabratum</i>	Central and South America and the Caribbea and south Africa.
Lecanoraceae	<i>Lecanora albella</i>	Widespread in temperate to boreal regions of the Holarctic, including Africa, Asia, Europe, North America and South America.
	<i>Lecanora chlarotera</i>	A subcosmopolitan species occurring in Africa, Asia, Europe, and North and South America.
Lobariaceae	<i>Crocodia guilleminii</i>	Endemic to southern South America (Galloway 1992b).
	<i>Pseudocyphellaria coriifolia</i>	Endemic to southern South America (Galloway 1992a, 1992b; Redón & Quilhot 1977).
	<i>Pseudocyphellaria nudata</i>	Endemic to southern South America (Galloway 1992b).
Ochrolechiaceae	<i>Varicellaria velata</i>	Cosmopolitan.
Parmeliaceae	<i>Flavoparmelia caperata</i>	North and South America, Europe, Asia, Africa.
	<i>Hypogymnia fragillima</i>	NE Asia; so far unknown from SW China and Russia.
	<i>Hypotrachyna laevigata</i>	Cosmopolitan (Hale 1974; Nash III <i>et al.</i> 2002). Tropics and extending into some temperate regions in Europe, North and South America and Australasia.
	<i>Parmotrema perlatum</i>	Widespread in temperate region of the Northern and Southern Hemispheres.
	<i>Platismatia glauca</i>	Circumpolar and circumboreal in the Northern Hemisphere, Macaronesia; southern South America and East Africa.
	<i>Protousnea poeppigii</i>	Endemic to southern South America (Krog 1976; Calvelo <i>et al.</i> 2005).
	<i>Rimelia reticulata</i>	Neotropics from Mexico to northern Argentina and southern Africa.
	<i>Usnea cf. igniaria</i>	In Chile, Prov. de Santiago, 8 Km to west of Tilttil, in shrub chaparral, on east slope of Cuesta la Dormida, 1000-1100 m a.s.l.
Physciaceae	<i>Xanthoparmelia submougeotii</i>	Tasmania, south-eastern Australia, New Zealand, South America (in Chile and Argentine) (Kantvilas <i>et al.</i> 1996).
	<i>Physconia muscigena</i>	North America, Europe, Africa, Asia, South America.
	<i>Rinodina anomala</i>	North and South Hemispheres.
Ramalinaceae	<i>Rinodina sophodes</i>	Bipolar. North and South Hemispheres.
Ramalinaceae	<i>Ramalina chilensis</i>	South America, Argentine and Chile.
Rhizocarpaceae	<i>Rhizocarpon geographicum</i>	Cosmopolitan.
Stereocaulaceae	<i>Lepraria incana</i>	Cosmopolitan, but most reports are from the Northern Hemisphere and the species is rarely reported from the tropics (Flakus & Kukwa 2007).
	<i>Squamarina squamulosa</i>	Biobío region (Pereira <i>et al.</i> 2016).
	<i>Stereocaulon alpinum</i>	Bipolar (Lamb 1977; Øvstedal & Lewis Smith 2001; Redón & Quilhot 1977).

CONCLUSIONS

- Of a total of 30 species found in the Achibueno River Ravine, a 23% corresponded to Cyanolichens (7) which allowing to conclude that this area still holds a pristine, old forests.
- Lichen diversity in the Achibueno river ravine is mostly corticolous.
- Most species found have a cosmopolitan distribution, followed by endemic, bipolar, tropical and subtropical distributions.
- The meridional distribution of *Crocodia guilleminii* is extended and *Hypogymnia fragillima* is recorded for the first time in Chile.
- We suggest that there should be new sampling of lichens in this area, with the purpose to increase the knowledge about lichen diversity, their ecology, especially considering that possible new records could be found.

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