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Vertical Diversity of Beetles (Insecta: Coleoptera) Associated with *Lithraea caustica* (Anacardiaceae) in Patches of Sclerophyllous Forest in Central Chile

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ABSTRACT

Forest stratum are important ecotones representing transition zones between open, sunlit, and drier habitats rich in herbaceous plants and shady, cooler and more humid forests with woody plants and possibly rich in dead wood. *Lithraea caustica* (Mol.) an endemic tree of Chile, is the least disturbed taxon in these fragments, probably due to the presence of litrol, a highly irritant oleoresin, due to which its wood has not been used for fuel. This study evaluated the species richness, abundance and composition of the beetle assemblages in different strata (foliage, epigeum and hypogeum) at intervals of fifteen and thirty days, in a sclerophyllous plant formation in the Escuadrón farm, Coronel, Chile. Our results show that in the studied sector, 27 families, 57 genera and 69 species are associated with the soil under *L. caustica*, which represents 31.3%, 4.2% and 1.7%, respectively, of the total diversity of coleopterans known for Chile .

Key words: Epigeum coleoptera, Hypogeum coleoptera, Foliage, Mediterranean region.

INTRODUCTION

The sclerophyllous forests of central Chile, notable for their biological singularity, currently are a biodiversity reserve because their biota is rich in endemic species and genera. The native forest has been reduced to small, dispersed fragments in a matrix of forest plantations (Arroyo, 1999; Myers *et al*., 2000). Forest stratum are important ecotones representing transition zones between open, sunlit, and drier habitats rich in herbaceous plants and shady, cooler and more humid forests with woody plants and possibly rich in dead wood (Wermelinger *et al.,* 2007). In addition, the forest mantle represents some kind of vertical canopy, i.e. a vegetation stratum with leaves, twigs and thin branches, which are all food sources for beetles specially saproxylics. For these reasons, forest edges have been recognized in biological conservation as an important habitat (Temple and Flaspohler, 1998; Wermelinger *et al.,* 2007). Studies of the floristic composition of these forests have found a tendency to a few dominant species and a large number of species with low values of relative cover (Gajardo, 1994, Luebert and Pliscoff, 2005). The same situation has been found for the ensembles of epigeum insects associated with these formations, that is, few abundant species and numerous scarce and rare species (Solervicens and Estrada, 2002). Thus there appears to be a consistency between the vegetation structure and the corresponding structure of epigeum insects in the Mediterranean eco-region of Chile.

Lithraea caustica (Mol.) Hook. et Arn., an endemic tree to Chile, is the least disturbed taxon in these fragments, probably due to the presence of litrol, a highly irritant oleoresin, due to which its wood has not been used for fuel (Kalergis *et al*., 1997). It is also highly allelopathic; it has a characteristic substratum in its perimeter. For these reasons it is considered to be a good substrate to study the composition and community structure of its associated coleoptera (Briones and Jerez, 2004).

Hutcheson (1990) indicated that terrestrial insect communities integrate in the environment in which they develop and respond rapidly to environmental changes. The coleoptera of the foliage have a relation with the phenology of the *Lithraea*. Under this premise we hypothesized that in fragments in which *L. caustica* is the dominant species, the community structure of the foliage, epigeum and hypogeum (under the canopy) beetles will have high dominance and a correlation between the diversity and temperature increment (Solervicens and Estrada, 2002). The purpose of this study is to investigate the species richness, abundance and composition of the beetle assemblages in different stratum of *L. caustica* describing (i) the vertical structure of the coleoptera associated with *L. caustica*, and (ii) determine if there is a relation between species richness and abundance in the ensemble of coleoptera with environments variables.

MATERIALS AND METHODS

Study site

The study was performed from August to December, 2003, in a sclerophyllous plant formation in the Escuadrón farm, Coronel commune in the province of Concepción, Chile (36°56’21’’S; 73°08’93’’W). Escuadrón forms part of the northern chain of the Cordillera de Nahuelbuta (Coast Range) and is somewhat greater than 2100 hectares (Briones and Jerez, 2004); the majority of the area is occupied by forest plantations, especially *Pinus radiata* D. Don.

The dominant trees in the native forest fragment are *L. caustica*, *Peumus boldus* Mol., *Cryptocarya alba* (Mol.) Looser and, to a lesser degree, *Schinus polygamus* (Cav.) Cabr. The most important shrub is *Puya chilensis* Mol.. The herbaceous stratum includes *Lapageria rosea* Ruiz and Pavon, *Alstroemeria hookeri* Schultes and *Loasa tricolor* Ker-Gawl, among others. A notable characteristic of this forest fragment is the abundance of epiphytes and vines, including ferns and climbers such as *Proustia pyrifolia* D.C.*, Luzuriaga radicans* Ruiz and Pavon. The substrate has few rocks, is well drained and has mulch coverage greater than 40%. The origin of the soil includes andesitic, basaltic and granitic rocks (Zevallos and Matthei, 1992; Zevallos and Matthei, 1994; Briones and Jerez, 2004).

Sampling procedure

The study was performed in all strata (e.g., foliage, epigeum and hypogeum) at intervals of fifteen and thirty days, from August to December, 2003. At the beginning of the study we selected 30 individuals of *L. caustica* with a shrubby form, with similar volume and size. Both the canopy and the soil surface around each individual were considered in four quadrants (diameter is 5m).

Coleoptera were collected from the foliage by shaking the branches, being careful to use the same sampling effort for each tree, using the methodology applied by Vergara and Jerez (2010) . For the first sampling we chose 15 of the trees; beginning with the second sample we made sure the branches shaken were different from those of the previous sample.

Epigean coleopteran were collected using pitfall traps, one next to the trunk and the other about 1.50 m away from the trunk; these traps were left *in situ* for 15 days and in the following samplings they were changed every 15 days to the remaining quadrants, using the methodology of De los Santos *et al*. (2002a; 2002b). We used a mixture of water and formalin as preserving liquid.

From the soil around the other 15 *L. caustica*, every 30 days we removed 200 cc of mulch (Howard 1975). The mulch was stored in plastic bags; in the laboratory the hypogeal fauna was separated using *Berlesse-Tullgren* funnels under a stereoscopic microscope. The coleoptera collected were stored in ethanol, identified with locality, capture date and collector. Part of the collected material was deposited in the Museum of Zoology, University of Concepción (MZUC).

Identification and Composition

Coleoptera were identified to genus or species based on comparisons with specimens of the MZUC, the National Museum of Natural History (MNHN-CL) and Institute of Entomology at the Metropolitan University of Educational Sciences (IEUMCE). The information on taxonomic composition and abundance was compiled for each stratum, calculating mean abundance of each species per sample and per stratum. Since these are abundance estimations expressed as numbers of individuals, all means were increased by one in order not to have numerical values less than one, and then rounded off to the nearest integer value.

The assemblages were described hierarchically from family to genus and species. We evaluated the completeness of species sampling as the percentage of observed species with respect to the number of species predicted by an abundance-based estimator (Chao 1) with the EstimateS 8.20 program (Colwell, 2006). Spatial variation of beetle diversity was measured as the difference in species composition of beetle assemblages between types of strata by calculating the Bray-Curtis index of Analyses of similarity (ANOSIM).Pseudoreplication was evaluated through the Mantel test (Pardo and Ruiz, 2002). First used ANOVA and then, when significant results is used multiple comparison. The LSD test differences were analyzed using LSD and multivariate regression was used to learn more about relationships between variables. (i.e. fragment size, tree mean age, tree maximum age, dead wood, vegetation cover) in PAST 1.68 (Hammer *et al*., 2001).

Diversity of beetles

The diversity of beetles was calculated by the following descriptors of diversity: (1) richness or number of species S; (2) dominance *D* (Magurran, 1988) and (3) diversity using the Shannon-Weaver *H'* (Shannon and Weaver, 1949). The 95% of confidence was estimated for each descriptor using bootstrapping (Manly, 1997) with 1000 iterations, using the software PAST 1.68 (Hammer *et al.,* 2001). The relation between number of species and abundance of soil coleoptera associated with *L. caustica*, was graphed and adjusted a tendency curve to estimate the distribution of these parameters. A linear regression was used to examine the relations between specific richness, abundance and the environmental variables (Statsoft, 1998).

The degree of structuring by season and by sampling site (replica) was determined with non-metric multidimensional scaling (nMDS) (Field *et al.,* 1982; Clarke, 1993), using the similarity matrix of Bray-Curtis (Bray and Curtis, 1957) and the similarity matrix of Jaccard (1901), based on species presence-absence. The Bray-Curtis index was used to measure the differences produced by the abundance and presence of the species, and Jaccard's index as a measure of the differences due only to the presence of species independent of their abundance, which allows an estimation of the faunistic exclusivity of the seasons of the year and the sampling sites. In this analysis we determined the groupings by adjusting graphs of control polygons or convex hulls and calculating the stress parameter of Kruskal (1964); when its value is less than 0.01 it is considered adequate to define groupings (Johnson and Wichern, 1992).

To evaluate the statistical significance of the differences between seasons and between strata, one-way similarity analysis ANOSIM based on the distance measures of the Bray-Curtis and Jaccard indexes was used (Clarke, 1993). In ANOSIM, analogously to analysis of variance, distances among groups are compared with the distances within groups, using the statistics rb or mean ranking of all distances among groups and rw or mean ranking of all the within-group distances, calculating $R = (rb-rw)/(N(N-1)/4$, which evaluates the dissimilarity among groups. Positive values of R ($0 < R \leq 1$) indicate differences among groups, whose significance we evaluated using 50 000 permutations of the observed values. When significant differences were observed, we used paired ANOSIMS among seasons and stratum as an *a posteriori* test. However, considering that the data of the paired analyses are not independent, we used Bonferroni corrected probability values (p) in order not to overestimate the significance of the differences, to be able to corroborate the existence of structuring of seasons and stratum.

Additionally, to characterize the assemblage of saproxylic beetles based on species abundance and stratum we used canonical correspondence analyses (CCA) performed with the Canoco 4.5 software (Ter Braak and Smilauer, 1998). Data were standardized by exploratory statistical analyses in order to avoid large values improperly influencing the sorting and to consider zero values. The CCA allowed us to analyze the influence of the ecological variables considered on the structure of the saproxylic beetle assemblage. The significance of each factor in the CCA was obtained by a Monte Carlo test. To compare the ecological similarity among the different stratum and plant species, we calculated the Bray-Curtis index with the program CANOCO version 4.0 (Ter Braak and Smilauer, 1998). A value of 50% was considered as an arbitrary limit to unify the different conglomerates, a value which is fairly conservative (Jaksic and Medel, 1990). This index is relatively unaffected by sample size, although it is not statistically as robust as others.

RESULTS

Taxonomic composition and abundance

In total 838 specimens were collected belonging to 29 families, 57 genera and 69 species of coleoptera associated L. caustica. 55% of the species belong to four families (Staphylinidae, Curculionidae, Chrysomelidae and Carabidae) and 85% of the abundance was due to 7 species (Table 1).

Foliage coleoptera

A total of 323 individuals belonging to 13 families, 40 genera and 21 species were captured. The most dominant species was *Cyphon sp.*, which had 35% of the total relative abundance of this ensemble. This species together with *Adalia deficiens* had the greatest constancy, being present in 80% of the samples. *Cyphon* sp. was the dominant species in both winter and spring. The estimators of diversity indicated that the pattern of species richness for foliage Coleoptera were those expected by chance, both in winter and spring.

Epigeum coleoptera

A total of 2 343 individuals belonging to 26 families, 55 genera and 65 species were captured (Table1 2). Four families (Staphylinidae, Carabidae, Curculionidae and Chrysomelidae) had 60% of the species found in this ensemble. In relation to dominance, five species (*Eupelates transversestrigosus* (Fairmaire and Germain), *Martinezostes asper* (Philippi), *Bolitobius seriaticollis* (Coiffait and Saiz), *Baeocera nonguensis* Löbl and *Mylassa crassicollis* Blanchard (larval state)) together had 70% of the total relative abundance of this ensemble. With respect to constancy, about 25% of the species of this ensemble (*E. transversestrigosus, Eunemades chilensis* Portevin, *B. seriaticollis*, *Golasina* sp, *M. asper*, *B. nonguensis*, Scirtidae sp. 1, *Acalles* sp. *Eucalles* sp., *Listroderes* sp, *M. crassicollis*, *Longitarsus* sp., *Pseudocnides monoleus* (Putzeys) and *Trirammatus aereus* Dejean) were recorded in 100% of the samples.

The dominant species in winter was *E. transversestrigosus* (45.25%, n = 700), followed by *M. asper* with 13.05%. The rest of the species had percentages ≤ 4%. In spring, 11.5% of the individuals were *Cryptophagidae* sp. 1, followed by *M. crassicollis* (larvae) with 11.4% and *E. chilensis* with 9.3%. The rest of the species had less than 4%. Thus a few species were predominant in this ensemble, both in relative abundance and in constancy. We found some species with low relative abundance but with high constancy, that is, species which maintained a small but stable population during the sampled months.

Table 1. Families, species richness and abundance of beetles collected in vertical stratum of *Lithraea caustica.*

Family	Foliage	Epigeous	Hipogeous	Total
Anobiidae	0	1	0	1
Anthribidae	1	0	1	1
Bostrichidae	1	0	0	1
Bruchidae	1	1	0	1
Carabidae	0	7	0	7
Cerambycidae	2	2	0	2
Ceratocanthidae	0	1	1	1
Cleridae	1	0	0	1
Chrysomelidae	4	7	2	7
Coccinellidae	4	3	0	4
Cryptophagidae	2	1	0	2
Curculionidae	5	7	4	8
Elateridae	3	2	1	3
Lampiridae	2	2	0	2
Languriidae	1	1	0	1
Lathridiidae	3	4	2	4
Leiodidae	1	2	1	2
Melyridae	0	1	0	1
Mordellidae	0	1	0	1
Mycetophagidae	0	1	0	1
Nitidulidae	0	1	0	1
Protocucujidae	0	1	0	1
Ptiliidae	1	1	1	1
Ptinidae	6	0	0	1
Scarabeidae	0	1	1	2
Scirtidae	2	2	1	2
Staphylinidae	1	10	2	10
Tenebrionidae	0	3	1	3
Trogidae	0	1	1	1
Zopheridae	0	1	0	1

Hypogeum coleoptera

A total of 172 individuals belonging to 12 families, 17 genera and 19 species were captured (Table 1). Two families (Lathridiidae and Chrysomelidae) had 25 of the species found in this ensemble. In terms of dominance, three species (*M. crassicollis* (larvae), *Acalles* sp. and *Listroderes* sp.) together had 60% of the total relative abundance of this ensemble. Only two species had total constancy; (*B. nonguensis* and *B. signaticollis*) were present in 100% of the samples, while *Listroderes* sp. and *E. chilensis* were present in 80%.

The dominant species in both winter and spring was *M. crassicollis* (larvae, 45% relative abundance and 100% constancy. The rest of the species had percentages ≤ 4% (Table 1). This reflects the association between *M. crassicollis* and *L. caustica*, since this Chrysomelid completes its entire life cycle connected to the three stratum of *Lithraea* (hypogeum, epigeum and foliage (Jerez and Briones, 2010).

Community structure

The estimators of diversity indicate that during winter and spring, the pattern of specific richness for the hypogeum coleoptera were those expected by chance.

However, in winter there was greater specific richness (S) and diversity along with less dominance (abundances more nearly equal) (Fig. 1 and 2).

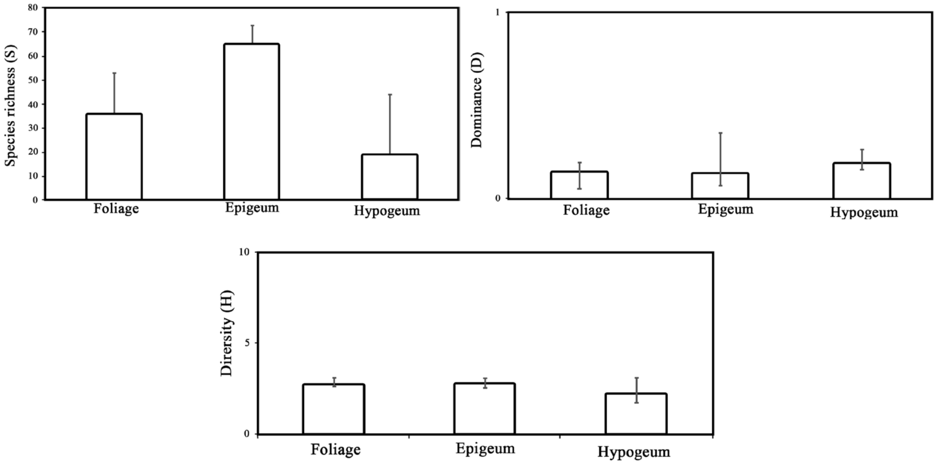

Fig. 1. The bars indicate the number of species in stratum: A) species richness (S); B) Dominance (D) and C) Shannon-Weaver diversity (H ‘). The gray lines represent the 95% confidence interval of diversity values.

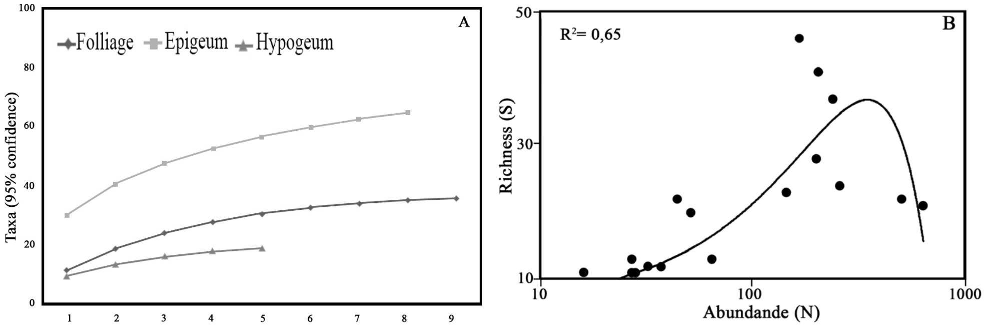

Fig. 2. A) Rarefaction curves of beetles species richness (plus one standard deviation) for the vertical stratum. The solid curves to refer to the value of the Chao 1 richness estimator. B) Analysis of species richness (S) *versus* abundance (N) of epigeous, hipogeous and foliage species adjusted to a curve of distribution of abundances.

The comparison of specific richness with abundance showed the existence of a large number of species with low abundance values and a small number of species in high abundance; this dispersion of the data was adjusted to a lognormal distribution curve ($R^2 = 0.67$, $p < 0.05$), which indicates that this ensemble is structured based on various axes of the niche (Fig. 2A). That is, there are few competitive interactions and the partition of the niche is random, in which some species occupy greater portions of the lognormal distribution (Preston, 1962) (Fig. 2B). The regression analysis of the community parameters specific richness as a function of the temperature, for each ensemble, showed a significant relation between specific richness and temperature in both ensembles ($R^2 = 0.87$; $P < 0.001$) (Fig. 3). The nMDS showed structure in all three strata with both the Jaccard and Bray-Curtis indexes. The principal determinants of the community were temperature (increment) and stratum (arboreal and terrestrial (Fig. 4A-B). The temperature was positively correlated with species richness in the three strata ($R^2 = 0.6$; $P < 0.001$). This variable along with the zero horizon and fermentation layer of the soil explained 80% of the richness according to the canonical correspondence analysis (Table 2).

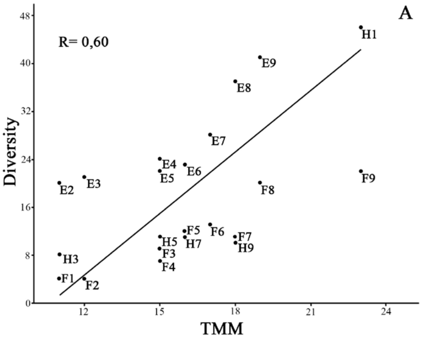

Fig. 3. Multivariate regression of community parameters (Diversity and TMM) ($R^2 = 0.6$; $P < 0.001$).

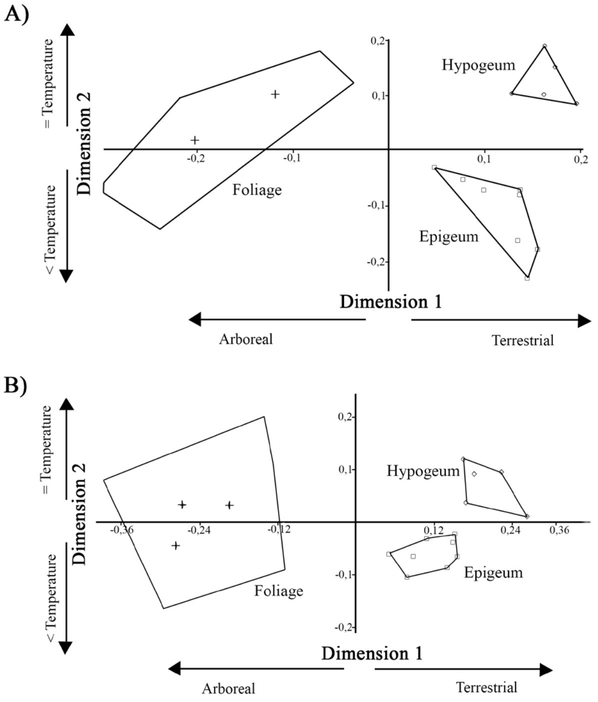

Fig. 4. A) Ordination analysis (nMDS) and value of Kruskal's Stress parameter based on the similarity matrix of A) Jaccard and B) Bray-Curtis for the vertical stratum. In the graph, the lines that enclose each group are the curves of convex hull.

Table 2. Characterization of ecological variables of each vertical stratum. (F: foliage, E: epigeum, H: hypogeum)

Ecological variables	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**E2**	**E3**	**E4**	**E5**	**E6**	**E7**	**E8**	**E9**	**H1**	**H3**	**H5**	**H7**	**H9**
TMM (°C)	16	17	16	18	19	20	21	22	20	13	12	11	14	13	13	13	13	10	9	10	11	11
PP (cc)	16.2	60.4	236	106	60.6	96.6	84.3	99.8	18	60	236	106	61	97	84	100	18	16	236	61	84	18
Ages trees mean	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60
Dead wood floor (m3/ha)	0.90	0.77	0.70	0.63	0.60	0.60	0.53	0.50	0.50	0.77	0.70	0.63	0.60	0.60	0.53	0.50	0.50	0.90	0.70	0.60	0.53	0.50
Tree cover (%)	70	70	70	75	75	80	80	85	85	70	70	75	75	80	80	85	85	70	70	75	80	85
Herbaceous cover (%)	10	15	30	40	60	70	75	80	80	15	30	40	60	70	75	80	80	10	30	60	75	80
Canopy cover (%)	15	15	15	20	30	35	45	50	60	15	15	20	30	35	45	50	60	15	15	30	45	60
Zero horison (m3/ha)	0.7	0.7	0.78	0.85	0.95	1.2	1.5	2	2.5	0.7	0.8	0.9	1	1.2	1.5	2	2.5	0.7	0.8	1	1.5	2.5
Litter laver (m3/ha)	0.4	0.4	0.42	0.46	0.5	0.6	0.8	1.1	1.6	0.4	0.4	0.5	0.5	0.6	0.8	1.1	1.6	0.4	0.4	0.5	0.8	1.6
Fermentation layer (m3/ha)	0.2	0.2	0.26	0.27	0.3	0.35	0.5	0.6	0.6	0.2	0.3	0.3	0.3	0.4	0.5	0.6	0.6	0.2	0.3	0.3	0.5	0.6
Humus layer (m3/ha)	0.1	0.1	0.1	0.12	0.15	0.2	0.2	0.3	0.3	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.1	0.1	0.2	0.2	0.3

DISCUSSION

Our results show that in the studied sector, 27 families, 57 genera and 69 species are associated with the soil under *L. caustica*, which represents 31.3%, 4.2% and 1.7%, respectively, of the total diversity of coleopterans known for Chile (Elgueta, 2000). This result is relevant due to the high diversity of families of coleoptera found only in this not previously investigated forest fragment. Although the percentages for genera and species are low compared to the country total, these species represent 55.5%, 36% and 28% of the coleoptera described for the Región del Biobio (Vergara *et al*., 2006). There were few dominant species in the stratum, a large percentage of scarce species (dominance < 10%) and rare species (dominance < 4%, constancy < 20%); this was corroborated by the dominance values. The graph of species richness *vs*. abundance approximated a lognormal distribution, which indicates that the community niche is partitioned sequentially and randomly among the species. The ensemble appears to be structured on various axes of the niche, thus there would be no competition for resources or niche space. This is supported by the diversity values (specific richness and the Shannon-Weaver index), which, although they had low values, are within the range expected by chance (Preston, 1962). Solervicens and Estrada (2002) indicated that the presence of scarce and rare species in sclerophyllous forests is a characteristic and constant pattern of the insect communities of central Chile, due to the constancy of the Mediterranean climate (Di Castri and Hayek, 1976). Montenegro *et al.,* (1980) suggested that constant environments or predictable conditions favor the maintenance of population dynamics over time. According to this, scarce and rare species should have biological strategies with low reproductive rates, low immigration and/or other attributes which maintain small local populations (Solervicens and Estrada, 2002; Saiz *et al.,* 2000; Schowalter, 2012; Joern and Laws 2013; Barton *et al*. 2013; Saether *et al.,* 2013). The Crysomelids *Jansonius aeneus* and *Jolivetia obscura* were rare species for the epigeum ensemble and *Lathridius minutus* (Latridiidae) for the hypogeum ensemble. *Jansonius aeneus* and *J. obscura* are endemic to Chile and apterous, favoring a restricted geographic distribution (Jerez and Briones, 2010); however, there are no biological data about these species. In the hypogeum ensamble, the several larval stages of *M. crassicollis* accounted for 40% of the total abundance, while as adults they are associated with the foliage (Jerez and Briones, 2010). Soil coleoptera require a large quantity of proteins and amino acids to develop; these have both animal and vegetal origin (Saiz *et al*., 2000). The native forest of *L. caustica* and *Peumus boldus* in the study area produces a layer of leaf litter which maintains humidity, provides shade and organic material (Muñoz-Schick *et al.,* 2000).

We found that the epigeum ensemble had greater abundance and constant values of specific richness in winter. The increase in richness and abundance in spring was due mainly to the presence of carabids (predators) and tenebrionids (detritus feeders). Covarrubias (1991) suggested that there are vertical seasonal migrations of fauna from the soil to the leaf litter as a result of the increase in temperature. This may explain the relation found between environmental temperature and specific richness.

This pattern of structure and composition of insect communities appears to be characteristic of central Chile, in which the vegetation shows the same community organization (e.g. Solervicens and Estrada, 2002; Saiz *et al.,* 2000). Both plants and insects are thus responding to climatic, relief and other phenomena in a very extensive time scale, and slowly producing the biodiversity of this Mediterranean region of central Chile.

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