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FACULTAD DE CIENCIAS BIOLÓGICAS
PROGRAMA DE DOCTORADO EN CIENCIAS BIOLÓGICAS
MENCIÓN ECOLOGÍA

TESIS DOCTORAL

*GRADO DE ESPECIALIZACIÓN ECOLÓGICA Y FILOGEOGRAFÍA
COMPARADA ENTRE EL HERBÍVORO ESPECIALISTA SCURRIA
SCURRA Y SUS DOS ALGAS HOSPEDEROS EN LA COSTA
CHILENA*

POR

ANDRÉS MEYNARD

Junio 2014



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POR

ANDRÉS MEYNARD

Directores de Tesis: Sylvain Faugeron y Juan Correa M.

Comisión de Tesis: Claudio Latorre

David Véliz

Evie Wieters

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HOSPEDEROS EN LA COSTA CHILENA”

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Mención Ecología

SR. ANDRÉS PHILLIPE MEYNARD SALINAS

Ha sido aprobada por el Tribunal Examinador, constituido por los profesores abajo firmantes, calificándose el trabajo realizado, el manuscrito sometido y la defensa oral con nota 6,0 (seis).

Dr. Claudio Latorre H.
Jefe Mención Ecología
Facultad de Ciencias Biológicas-PUC

Dr. Juan A. Correa M.
Decano
Co-Director de Tesis
Facultad de Ciencias Biológicas-PUC

Dra. Evie Wieters B.
Miembro Comité de Tesis
Facultad de Ciencias Biológicas-PUC

Dr. Sylvain Faugeron W.
Director de Tesis
Facultad de Ciencias Biológicas-PUC

Dr. David Véliz B.
Miembro Externo Comité de Tesis
Universidad de Chile

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TABLE OF CONTENTS

Agradecimientos.....	iii
Table of contents.....	iv
Index of figures.....	vii
Index of tables.....	x
Resumen.....	xii
Abstract.....	xiv
CHAPTER I: GENERAL INTRODUCTION	1
1 Theoretical background on ecological specialization: Why are there so few specialist herbivores in marine environments?	2
1.1 The specialist-generalist continuum in marine environments:	2
1.2 Section I. Key concepts to define degrees of ecological specialization:	4
1.3 Section II. Main hypotheses on the origin of herbivore specialization in marine communities:	18
1.4 Biological model and main hypotheses of this thesis:	24
2 Referencias:.....	29
CHAPTER II: ABUNDANCE AND DEGREE OF SELECTIVITY OF A SPECIALIST MARINE HERBIVORE CORRELATED TO AVAILABILITY OF MICROHABITATS OF SPECIFIC SHAPE AND INCREASING SIZE WITHIN ITS TWO HOST KELPS	38
1 Introduction:	39
2 Material and Methods:	44
2.1 Study sites and data collection	44
2.1.1 Host selectivity: standardized degree of utilization according to host availability and structural complexity (Component 2 of ecological versatility)	45
2.1.2 Relative performance in different hosts (Component 3 of ecological versatility)	46

3	Results:	47
3.1	Regional picture of component 2	47
3.1.1	Component 2: standardized degree of utilization according to host availability	49
3.1.2	Component 2: standardized degree of utilization according to host structural complexity 51	
3.1.3	Component 3: Relative performance in different hosts.....	57
4	Discussion	59
5	References:	63
CHAPTER III: COMPARATIVE PHYLOGEOGRAPHY OF A SPECIFIC BUT RECENT ASSOCIATION BETWEEN A SPECIALIST MARINE HERBIVORE AND ITS HOST KELP IN THE SOUTHEAST PACIFIC COAST: CONCORDANT SPATIAL GENETIC STRUCTURES AND SIMILAR DEMOGRAPHIC TRAJECTORIES SINCE THE LAST GLACIAL MAXIMUM.		
		69
1	Introduction:	70
2	MATERIAL AND METHODS	77
2.1	Sampling for COI and AFLP genotyping.....	77
2.2	DNA extraction and COI amplification.....	78
2.3	AFLP genotyping	78
2.4	Population genetic analyses:	80
2.5	Phylogeographic inferences:	82
2.6	Correlation of pairwise population genetic distances between <i>S. scurra</i> and <i>L. spicata</i> : 83	
2.7	Searching for loci under selection in <i>S. scurra</i> :	83
3	RESULTS	84
3.1	Identification of the true number of <i>S. scurra</i> populations and degree of similarity with <i>Lessonia</i> phylogeographic structure:	84
3.1.1	Genetic diversity of <i>S. scurra</i> revealed by the COI and AFLP's:.....	84
3.1.2	Population genetic structure of <i>S. scurra</i> and its host <i>L. spicata</i> :	87

3.2	Evaluation of contrasting demographic scenarios since the LGM in <i>S. scurra</i> populations	93
3.2.1	Reconstruction of the effective population sizes of <i>S. scurra</i> clusters using the COI:	93
3.2.2	Simulations of Biogeographic scenarios for <i>S. scurra</i> and <i>L. spicata</i> populations since the LGM:	95
3.3	Correlation of pairwise population genetic distances between <i>S. scurra</i> and <i>L. spicata</i>	101
3.4	Searching for loci under selection in <i>S. scurra</i> inhabiting <i>L. spicata</i> or <i>D. antarctica</i>:	103
4	DISCUSSION AND PERSPECTIVES:	104
4.1	Common phylogeographic structure in <i>S. scurra</i> and its host kelp	104
4.2	Shared demographic history between <i>S. scurra</i> and its host kelp.....	108
4.3	Role of selection in the genetic structure of <i>S. scurra</i>	110
4.4	Comparative phylogeography of <i>S. scurra</i> and <i>L. spicata</i>	111
5	References:	114
	CHAPTER IV: FINAL DISCUSSION	122
1	Some logistic issues and perspectives in the study of ubiquity and ecological versatility	123
1.1	Concepts previously developed for terrestrial systems as criteria to evaluate the degree of specialization in the marine environment:	123
1.2	Concluding remarks about degree of specialization of <i>S. scurra</i> to <i>Lessonia</i> :	125
1.3	Origin of specialization in <i>S. scurra</i>	125
1.4	Some Likely mechanisms of marine herbivores to circumvent environmental stochasticity and evolve specialization to host algae	128
2	Some preliminary surveys about mobility and specific behavior of <i>S. scurra</i>:	131
3	References:	133

INDEX OF FIGURES

CHAPTER I.

- Figure 1. Niche delineation through intersecting the specific ranges and frequency of utilization of two resources (R1 and R2) by an avian herbivore, which determine a variable fitness surface (W) and an optimum range of resource categories utilized..... 6
- Figure 2. (a) Fitness earnings of a specialist (S) and generalist (G) organisms along a resource axe having from “easy” (-) to “difficult” (+) categories a resource (e.g. food, nesting sites)... 8
- Figure 3. Different combinations between cosmopolitanism (the extension of geographic range) and ubiquity (range or number of habitats occupied).....11
- Figure 5. Low ecological versatility of a specialist herbivore related to an uneven (skewed) performance or fitness when consuming the resources harbored in different host-plants, with a high performance exclusively when exploiting resources available in its preferred host-plant.13
- Figure 4. Restricted (specialized) use of one or a few host-plants or habitats, or ubiquitous (generalized) use of several host-plants or habitats types.12
- Figure 6. High ecological versatility of a generalist herbivore related to an even and positive (mean) fitness when consuming a relatively wide spectrum of resources harbored within different host plants.13
- Figure 7. Steps in measuring Ubiquity and Ecological versatility of an herbivore..16
- Figure 8. Functional dependence of versatility on the level of population saturation of an environment, where saturation is gauged by the ratio of current population density to nominal carrying capacity. (a) three strategies: an obligatory specialist, a generalist and a facultative exploiter. (b) three levels of evenness of efficiency of resource use in the facultative strategy, extremely even, intermediate and uneven efficiencies (from MacNally 2009).18

CHAPTER II.

- Figure 1. Prevalence of *S. scurra* on *L. spicata* and *D. antartica*, relative to density of ratio of *D. antartica* to *L. spicata* numbers, in Central Chile.51
- Figure 2. Number of *S. scurra* per disc (A and C) and probability of *S. scurra* presence in discs of *L. spicata* and of *D. antartica* (B and D) of different sizes, according to odds ratio of logistic regressions.55
- Figure 3. Predictive relationship between the size of *L. spicata* structural traits and the number of *S. scurra* per plant (left) and the probability of *S. scurra* presence (right), respectively (in discs and stipes, or exclusively in stipes).56
- Figure 4. Density of *S. scurra* as a fraction of disc volume...57
- Figure 5. Relationship between shell size of *S. scurra* and the size of structural traits of its hosts. Figures 5 A and 5 B show log-transformed data ($\text{Log}_e(x+1)$) of the *S. scurra* shell diameter and its better correlated structural trait in *L. spicata* (the stipe diameter, in B) and in *D. antartica* (the disc height, in D) to which Pearson correlations were fitted. C show the same data than in A but averaging the *S. scurra* shell size per plant (rather than per stipe)58

CHAPTER III.

- Figure 1. COI haplotype frequencies in *S. scurra* populations of Chile..86
- Figure 2. Graphical illustration of some likely number of *S. scurra* clusters inferred in Structure from AFLP data (N=319 individuals)..89
- Figure 3. Plots of (a) $L(K)$ as the first order rate of change of the likelihood for two successive K 's and (b) Standardized second order rate of change of the likelihood respect to K , in order to determine the true number of clusters of homogeneous populations in *S. scurra*.....90
- Figure 4. Comparative maps showing the numbers of clusters found with Geneland software in Chile for: (a) *S. scurra* (using AFLP markers) (b) *L. spicata*, (using the atp8S marker), (c) *L. berteriana* (using the atp8S marker), and (d) *D. antartica*, (using the COI marker)..92

Figure 5. (a) Map of <i>S. scurra</i> clusters with Geneland from COI data and (b) effective population size (N_e) reconstructions of each of these four clusters (indicated by arrows) using the Beast software program..	94
Figure 6. Phylogeographic scenarios for <i>S. scurra</i> clusters simulated in DYABC.	97
Figure 7. Posterior probabilities for estimation of the best fit scenario of historical demography in <i>S. scurra</i> . (a) Direct estimate and (b) Logistic regression.	98
Figure 8. Phylogeographic scenarios for <i>L. spicata</i> populations simulated in DYABC.	99
Figure 9. Posterior probabilities for estimation of the best fit scenario of historical demography in <i>L. spicata</i> (first run of analyses) (a) Direct estimate and (b) Logistic regression.	100
Figure 10. Posterior probabilities for estimation of the best fit scenario of historical demography in <i>L. spicata</i> (second run of analyses) (a) Direct estimate and (b) Logistic regression.	100
Figure 11. (a) Mantel test between Nei pairwise distances in <i>L. spicata</i> (atp8S) and Nei pairwise distances in <i>S. scurra</i> (AFLP), in six sites of the IA, and (b) Mantel test between genetic distances in <i>S. scurra</i> ($F_{ST}/1-F_{ST}$) and geographic distances, in eight populations of the IA.	102

INDEX OF TABLES

CHAPTER II

Table 1. Scaling of the mean <i>S. scurra</i> abundance in relation to structural traits in <i>L. spicata</i> and <i>D. antartica</i>	50
Table 2. Coefficients and Wald tests for logistic regression on the <i>S. scurra</i> presence and size of structural traits.....	54

CHAPTER III

Table 1. Geographical locations of the sampled sites in <i>S. scurra</i> for the COI and AFLP markers. N, number of samples per site and marker sequenced or genotyped. In parentheses are indicated the number of <i>S. scurra</i> found in a kelp different than <i>L. spicata</i> , i.e. <i>D. a</i> or <i>D. antartica</i>	80
Table 2 a. Sample size (N) and standard genetic diversity indexes of <i>S. scurra</i> from Chile using the COI marker: H (number of haplotypes), Hd (haplotype diversity), π (nucleotide diversity), Π (mean number of pairwise differences.	85
Table 2 b. Within-population genetic diversity indices of <i>S. scurra</i> in Chile using AFLP markers.....	85
Table 3 a. Pairwise FSTs between <i>S. scurra</i> populations of the PP and IA, using the mitochondrial COI marker.	87
Table 3 b. Pairwise FSTs between <i>S. scurra</i> populations of the PP and IA, using AFLP markers.	87
Table 4. Analysis of molecular variance (AMOVA) for 12 populations of <i>Scurria scurra</i> . For the nested analyses, populations were divided into two (PP vs IA) or into seven groups based on clusters inferred using Geneland and Structure softwares.	91
Table 5. Analyses of molecular variance (AMOVA) for populations of <i>S. scurra</i> inhabiting <i>L. spicata</i> or <i>D. antartica</i> kelps. For the nested analyses, <i>S. scurra</i> of two	

neighbor localities were nested within the two host kelp groups (*S. scurra* from *L. spicata* or *D. antarctica*); or alternatively, *S. scurra* inhabiting the two host kelps were nested within each of the two localities.. 104

FINAL DISCUSSION:

Table 1. Gain and losses after 11 days in the *S. scurra* abundance in three groups of *L. spicata* algae subjected to an exclusion treatment (*S. scurra* removed), overload treatment (overloading with *S. scurra* from the exclusion treatment), and an untreated group of algae (only *S. scurra* marked and presence recorded) 133

RESUMEN

Esta tesis tuvo como primer objetivo evaluar el nivel de especialización de un herbívoro marino, la patela *Scurria scurra*, en sus dos algas hospederos *Lessonia spicata* y *Durvillea antarctica*. A partir de una revisión de conceptos y criterios, se evidenció (en el Capítulo Introductorio) que un herbívoro especialista es adecuadamente caracterizado basándose en el grado de ubicuidad y versatilidad ecológica en su uso de recursos. El término versatilidad ecológica se refiere al rango de recursos utilizados y desempeño particular mostrado por una especie cuando explota estos recursos, en comparación a otras especies. El término ubicuidad se refiere al rango de hábitats o de plantas utilizadas en el caso de un herbívoro especialista, y las densidades relativas del herbívoro en cada una de estas plantas o hábitats.

En el segundo capítulo de esta tesis, se realizó una estimación del grado de ubicuidad y versatilidad ecológica de *S. scurra* respecto de sus dos algas hospederos *L. spicata* y *D. antarctica* en Chile Central. Nuestra premisa inicial fue que debido a que un alga hospedero suele ser alimento y refugio para pequeños herbívoros, el mayor tamaño y complejidad estructural de *L. spicata* ofrecería más espacio para habitar y albergaría mayores densidades de *S. scurra* y de mayores tamaños que *D. antarctica*. Se evaluó si la prevalencia de *S. scurra* en *L. spicata* y *D. antarctica* se correlaciona con la disponibilidad relativa de estos hospederos en ocho sitios. En segundo lugar, se evaluó si la probabilidad de presencia y tamaños de *S. scurra* se correlacionan con el tamaño y forma de distintas estructuras (principalmente discos y estipes) de *L. spicata* y *D. antarctica*. Nuestros datos sugieren que la ocurrencia de *S. scurra* es verdaderamente selectiva respecto de su huésped principal *L. spicata*, mientras que tiene un uso oportunista de *D. antarctica*. Concordantemente, la prevalencia de *S. scurra* en *L. spicata* osciló entre 0.7 y 1.0, mientras que en *D. antarctica*, la prevalencia de *S. scurra* aumentó con el aumento de las densidades de *D. antarctica* hacia el Sur. Regresiones logísticas sobre la presencia y el tamaño de *S. scurra* de acuerdo con el tamaño de diferentes estructuras de sus algas hospederos mostraron que la presencia de *S. scurra* está más correlacionada con el diámetro de estipes en *L. spicata*, y con la altura del disco en *D. antarctica*. *S. scurra* alcanzó una mayor abundancia por alga y mayores tamaños en *L. spicata* en comparación con *D. antarctica*. Los resultados anteriores sugieren que los rasgos morfológicos y disponibilidad de micro-hábitat en macroalgas posibilitarían alcanzar mayores abundancias en herbívoros especialistas al conferir mayor espacio para refugiarse contra enemigos o condiciones ambientales adversas.

Esta tesis tuvo como segundo objetivo general evaluar el grado de concordancia de las estructuras genéticas espaciales y las señales genéticas de cambios demográficos pasados entre *S. scurra* y el complejo *L. nigrescens* (*Lessonia berteroa* y *Lessonia spicata*) en la costa Chilena (26°S to 42°S). Un primer objetivo consistió en evaluar si la diversidad genética en *S. scurra* muestra los mismos quiebres filogeográficos mayores verificados previamente en *Lessonia* para la costa de Chile: específicamente, las discontinuidades genéticas a los 30°S y a los 27°S. En segundo lugar, se investigaron los posibles cambios demográficos experimentados por ambas especies (principalmente *S. scurra* y *L. spicata*) debido a la transición Glacial-Interglacial. De hecho, un desplazamiento hacia el Norte de los Vientos de Deriva del Oeste parece haber modificado la intensidad de la surgencia al Sur de 30°S, lo que

redujo la productividad primaria durante el Último Máximo Glacial (UMG). Nuestros análisis empleando marcadores AFLP confirmaron la ocurrencia de una discontinuidad genética mayor en la estructuración de las poblaciones de *S. scurra* consistente con la ruptura biogeográfica propuesta a los 30°S. Esta ruptura también fue demostrada anteriormente en otros vertebrados e invertebrados a lo largo del intermareal de Chile y tiene probablemente un origen extrínseco o ambiental (diferentes condiciones oceanográficas a ambos lados de los 30°S). Además, la sub-estructuración de las poblaciones de *S. scurra* ubicadas al norte de 30°S coincidió con el segundo quiebre genético observado en el complejo *L. nigrescens* con marcadores de ADN mitocondrial a los 27°S. Esta ruptura genética a 27°S es única de *L. berteroana* y *S. scurra*. En segundo lugar, de acuerdo a nuestros análisis con el marcador COI (reconstrucción de tamaños efectivos poblacionales en el software Beast, y simulaciones de escenarios en DYABC), el escenario demográfico más probable para *S. scurra* es la extinción de sus poblaciones al Sur de los 34°S durante el UMG, seguida de una recolonización postglacial de esta zona desde refugios ubicados más al norte. Para *L. spicata*, el escenario más probable en virtud de simulaciones en DYABC indica que las poblaciones al Sur de los 34°S también se vieron afectados por algún cambio ambiental mayor durante el UMG y experimentaron una recuperación demográfica post-Glacial desde entonces. Este patrón, no reportado aún para especies que no fueron afectadas por la capa de hielo en América del Sur, es probablemente el resultado de un desplazamiento post-Glacial progresivo hacia el sur de los Vientos de Deriva del Oeste y el restablecimiento asociado del sistema de surgencia costera. Todo lo anterior pone de manifiesto la estrecha interacción con *L. spicata* y *L. berteroana* y la alta especialización de *S. scurra*, la cual no fue capaz de persistir en otros sustratos o algas durante el UMG en el sur de Chile Central. Lo anterior también es coherente con estudios recientes que apoyan la idea que la concordancia en los patrones filogeográficos y demográficos es más probable entre organismos que mantienen interacciones ecológicas fuertes y específicas.

ABSTRACT

This thesis aimed to assess the level of specialization of a marine herbivore, the patella *Scurria scurra*, mainly on its two host kelps *Lessonia spicata* and *Durvillea antarctica*. From a revision of concepts and criteria, it appeared (in the Introductory chapter) that a specialist herbivore is properly characterized based on the degree of ubiquity and ecological versatility in its use of resources. The term ecological versatility refers to the range of resources used and particular performances experienced by a species when exploiting these resources, in comparison to other species. The term ubiquity refers to the range of habitats, or host plants used for a specialist herbivore, and relative densities of the herbivore in each of these plants or habitats. In the second chapter of this thesis, we performed an estimation of the degree of ubiquity and ecological versatility of *S. scurra* to its two host kelps *Lessonia spicata* and *Durvillea antarctica* in Central Chile. Our assumption was that because host kelps are the habitat and food for small herbivores, the bigger and structurally more complex *L. spicata* would offer more space to inhabit and would harbor superior densities of *S. scurra* and of bigger sizes than the smaller *D. antarctica*. We evaluated if the prevalence of *S. scurra* in *L. spicata* and *D. antarctica* is correlated to the relative availability of these two hosts in eight sites. In the second place, we assessed if the *S. scurra* presence and sizes were correlated to the size and shape of different structures (mainly disc and stipes) of *L. spicata* and *D. antarctica*. Our data suggest that the occurrence of *S. scurra* is truly selective of its main host *L. spicata* whereas it has an opportunistic use of *D. antarctica*. Accordingly, the prevalence of *S. scurra* in *L. spicata* ranged between 0.7 and 1.0, while in *D. antarctica*, the prevalence of *S. scurra* increased with increasing densities of *D. antarctica* southward. Logistic regressions on the presence and size of *S. scurra* according to the size of different kelp structures showed that *S. scurra* occurrence was more correlated to the diameter of stipes in *L. spicata*, and to the disc height in *D. antarctica*. *S. scurra* reached a higher abundance per alga and bigger size in *L. spicata* in comparison to *D. antarctica*. The above results suggest a likely important role of morphological features and availability of microhabitat in kelps allowing specialist herbivores to reach superior abundances and having more space for sheltering against enemies or harsh environmental conditions.

This thesis had as a second general objective to evaluate the degree of concordance of spatial genetic structures and signals of past demographic changes between *S. scurra* and the *L. nigrescens* complex (*Lessonia berteriana* and *Lessonia spicata*) in the Chilean coast (26°S to 42°S). A first objective consisted in evaluating if *S. scurra* genetic diversity shows the same major phylogeographic breaks verified previously in *Lessonia* for the coast of Chile: specifically, the 30°S or the 27°S genetic discontinuities. In the second place, we investigated the potential demographic changes experienced by both species (mainly *S. scurra* and *L. spicata*) due to the Glacial-Interglacial transition. Indeed, a northward shift of the West Wind Drift apparently modified the intensity of upwelling south of 30°S, reducing primary productivity during the Last Glacial Maximum (LGM). Our analyses employing AFLP markers confirmed the occurrence of a major genetic discontinuity in the structuring of *S. scurra* populations consistent with the proposed biogeographic break at 30°S in the Chilean coast. This break was also verified previously in other vertebrate and invertebrate taxa along the Chilean intertidal and has probably an extrinsic, environmental origin (different

oceanographic conditions at both sides of 30°S). In addition, the sub-structuring of *S. scurra* populations located north of 30°S matched the second genetic discontinuity observed in *L. nigrescens* complex with mtDNA markers at 27°S. This genetic break at 27°S is unique to *L. berteroana* and *S. scurra*. In the second place, according to our analyses with the COI marker (reconstruction of effective population sizes in Beast software, and scenario simulations in DYABC), the most likely demographic scenario for *S. scurra* is the extinction of its populations south to 34°S during the LGM, followed by a post-glacial recolonization of this area from refugia located further north. For *L. spicata*, the most likely scenario under DYABC simulations indicates that more southern populations were also affected by some major environmental change during the LGM and experienced a post-glacial demographic recovery since then. This pattern, yet unreported for species non-affected by ice cover in South America, is likely the result of a progressive southward shift of the West Wind Drift and the associated reestablishment of the coastal upwelling system. All the above reveals the close interaction with *L. spicata* and *L. berteroana* and high specialization of *S. scurra*, which was unable to persist in other substrata or alga during the LGM in the southernmost IA. The above is also coherent with some recent surveys supporting the view that a concordant phylogeographic and demographic patterns is more likely between organisms having very strong and specific ecological interactions.

CHAPTER I: GENERAL INTRODUCTION

1 THEORETICAL BACKGROUND ON ECOLOGICAL SPECIALIZATION: WHY ARE THERE SO FEW SPECIALIST HERBIVORES IN MARINE ENVIRONMENTS?

1.1 THE SPECIALIST-GENERALIST CONTINUUM IN MARINE ENVIRONMENTS:

In terrestrial communities, there is an abundant and highly diversified array of herbivore insects specialized to angiosperm plants (Root, 1973; Bernays, 1989). Insects use particular taxa of angiosperms both as habitat, food, as a mating, egg laying or nesting site. On the contrary, marine herbivores are generally thought as being mainly generalists, a small percentage being considered as dietary specialists (e.g. Lubchenco & Gaines, 1981; Hay et al. 1988, 1989, 1990a,b, Stachowicz & Hay 2000, Long et al. 2007). Most specialist marine herbivores correspond to Opisthobranch gastropods in the order Ascoglossa (mainly some sea slugs and sea hares) which maintain highly specialized associations with their algal host (Carefoot 1987, Pennings 1990b, Trowbridge 1994, 2004). During the past twenty years, few additional examples of herbivore specialized to algae have been reported in the seas, most being small herbivores (mesoherbivores) like small crabs, amphipods, and small gastropods (Hay et al. 1988, Paul & Alstyne 1988; Pavia & Toth 2000).

One basic question in ecology is answering why there exists a vast spectrum or continuum of different degrees of ecological specialization on earth, from very specialized and obligatory species to very generalist organisms, while the spectrum seems skewed towards generalists in the sea. There is a need to appropriately use available conceptual tools to place particular herbivore species along this specialist-generalist continuum existing in nature. Even in terrestrial communities, in comparison to insect herbivores, mammalian herbivores eat a rather wide spectrum of different host plant types or taxa (Shipley 2009). Nonetheless, among mammalian herbivores, there are herbivore species which use a relatively reduced set of host

plants and could be classified as showing intermediate level of specialization. This kind of terminological ambiguities arise from the lack of consideration of the basic levels necessary to characterize the degree of specialization of organisms, already available in the scientific literature (i.e. the rigorous review of MacNally 2009 about ecological specialization). We argue that researchers of marine biological sciences misleadingly characterize some herbivores species as being generalists because they usually compare them to (terrestrial) insect herbivore specialists.

Common ideas state that the generalist would be in advantage in a heterogeneous environment because it would benefit from being able to adapt to a wide spectrum of biotic and abiotic conditions and ensure a broad distribution range. Another common idea is that marine herbivores commonly have long distance propagules, which would preclude local adaptation and specialization to host algae in such heterogeneous environments. We show here that such common ideas do not take into account at least: a) a proper characterization of all potential axes of specialization (resources and habitat types) of candidate specialist marine herbivores based on tools already available in the literature, and b) that in spite of environmental heterogeneity, there are likely mechanisms by which specialist herbivore candidates could cope with environmental and community heterogeneity of marine rocky coastal landscapes. The biological model employed in this thesis was the patellogastropod *Scurria scurra*, a specialist herbivore which inhabits a restricted range of host kelps, the brown algae *Lessonia* (*L. spicata* and *L. berteroana*, in northern and Central Chile, respectively) and *Durvillea antarctica* in the Chilean coast. *S. scurra* has an indirect life cycle (lecitotrophic larvae) which according to the classical paradigm would preclude specialization to host algae. Nevertheless, a number of experimental studies suggest that larvae of diverse marine

gastropods show a very specific, preferential settlement onto their final habitat or host, or a gregarious settlement among conspecific adults (Proctor, 1968; Breen, 1972; Scheibling et al., 1990; Corpuz, 1981; Dixon, 1981; McGrath, 1992; Delaney et al. 1998; Graham & Fretter, 1947; Kay, 2002; Krug & Manzi 1999; Krug & Zimmer 2000). For instance, there are examples of specialist marine herbivores (mainly opisthobranch sea slugs) having direct and indirect developmental mode, which selectively recruit onto their specific host-alga (Krug & Manzi 1999; Krug & Zimmer 2000).

The initial focus of this chapter was to summarize key concepts in the study of ecological specialization (Section I) and then review the most robust hypotheses advanced in order to understand what factors more likely promote the existence of specialist herbivores in terrestrial, and especially, in marine environments (Section II). Finally, we present theoretical models by which marine herbivores having lecithotrophic larvae, through the evolution of host preference, could circumvent environmental heterogeneity of marine environments, and specialize to a relatively narrow range of host algae. Then, we present our specialist marine herbivore model, *S.scurra*, its host kelps, and the main hypotheses of this thesis.

1.2 SECTION I. KEY CONCEPTS TO DEFINE DEGREES OF ECOLOGICAL SPECIALIZATION:

Ecological specialization is usually equated to a restricted niche breadth, or the variety of resources and habitats used by a given species; conversely, generalist species have wide niche breadths, being able to proliferate in a wide range of environmental conditions, and utilize a vast number of resource types. The ecological niche concept is then fundamental when evaluating ecological specialization. The niche, after Hutchinson (1957), would have to

be, ideally, characterized by the hypervolume or aggregation of niche axes describing: (i) the range of tolerances to environmental variables within which the existence of a given species is possible; as well as by (ii) the range of resources allowing this existence. Nevertheless, the niche of a species is generally represented by the range and frequency of utilization (and indirect measure of fitness) of only a few niche axes; particularly, those niche axes or resources (R) which are important for the fitness (W) of individuals or populations of a species (Fig. 1).

These definitions of ecological specialization rest on the ecological niche concept as originally defined by Grinnell (1917). Recent propositions have highlighted the need to define ecological specialization both in terms of Grinnell (1917) and Elton (1927) ecological niche concepts (Chase and Leibold 2003, Devictor 2010). Grinnell concept focuses on the particular requirements (or habitats) needed by a focal species to exist in a given environment. On the other hand, for Elton (1927) the niche of a species corresponds to its role in the trophic web, or more specifically, to the impacts that it has on other species with which it interacts, or to the impacts it exerts on the environmental conditions in which it lives. Biological interactions of different trophic levels (e.g. predators and preys, or competitor interactions) and different signs (+, -, 0), or even indirect interactions (e.g. facilitation) should be included among the effects that a species has on, or suffer from interacting taxa. We agree that ecological specialization should ideally be characterized by both the breath of resources or requirements and the breath of impacts of a focal species (Devictor et al. 2010). Nevertheless, we consider herethat the selective forces and stochastic factors shaping the genetic background of

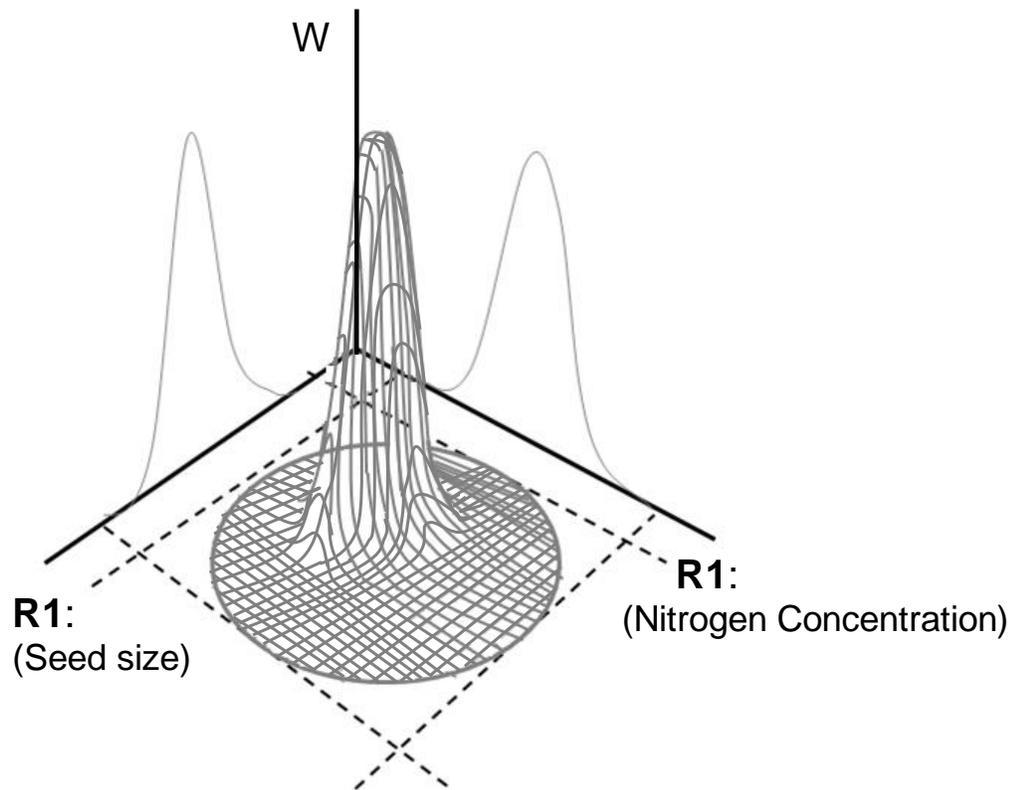


Figure 1. Niche delineation through intersecting the specific ranges and frequency of utilization of two resources (R1 and R2) by an avian herbivore, which determine a variable fitness surface (W) and an optimum range of resource categories utilized (the optimum optimum being defined by the intersection of resources categories allowing to attain a maximum fitness). The herbivore, in this case, is able to consume seeds of specific sizes (e.g. intrinsic constraints due to size and morphology of its masticatory organs) and requires specific amounts of nitrogen content in the plants consumed to reach fitness gains (adapted from Jaksic and Marone 2006).

adaptation depends more directly on the response of the focal species to constraints exerted from other species or the environment, rather than those exerted by the species on other species. Furthermore, the quantification of the impacts of a species is mostly an emergent property of communities (rather than an inherent property of a single organism) resulting from direct and indirect interactions between several species. It incorporates properly interaction dynamics which demands other types of methodological and conceptual tools than those required at the level of the organism and population level of a species. Following Berryman

(1993) we think there is always one (or at most two) dominant limiting factor(s) (i.e. true resources, or environmental constraints) that control the maximum intrinsic growth rate of a population (R_m), or the maximum fitness of an individual organism (W_m). We propose here that the specialist frequently corresponds to an organism able to reach fitness earnings from using a relatively narrow range of extreme categories along a resource or abiotic axis, and which are “difficult” to most other species: for example, habitats with extreme temperatures (e.g. extremophile bacteria) or plants with high concentrations of a secondary compound (e.g. specialist herbivores) (Fig. 2a). By evolving specialization to these extreme categories along one or more resource axes, a specialist organism would avoid environmental constraints most likely present in other environments (e.g. predators, competitors or abiotic stresses); we postulate that these constraints would be usually higher for small, slower and neurologically simpler organisms than for bigger, more mobile and neurologically more complex generalist organisms (Fig. 2b). The generalist would reach a positive fitness using categories of a resource which would not allow the specialist even to survive (high fitness losses in Fig. 2b). On the contrary, the specialist would reach a higher fitness than the generalist in extreme categories of a resource axis (Fig. 2a). Trade-offs in the performance of the specialist in our proposition would not result necessarily from fitness losses when exploiting categories along the same resource axis: for example, using a plant with low toxicity for a specialist herbivore. It would frequently ensue due to higher impacts or constraints resulting from

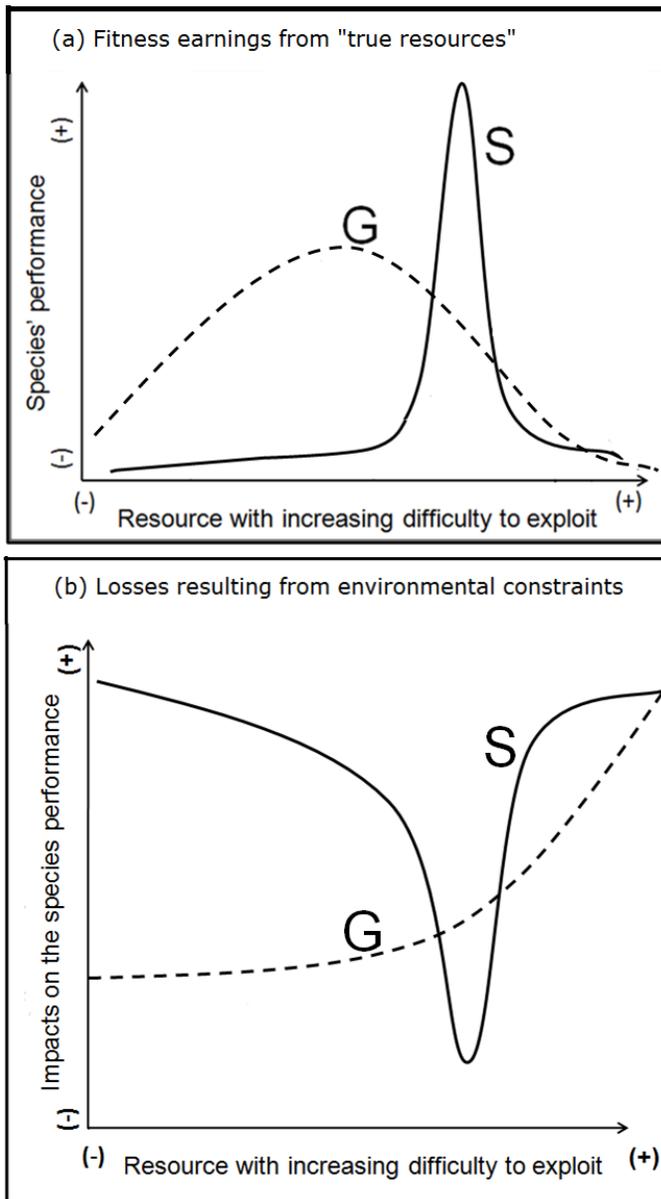


Figure 2.(a) Fitness earnings of a specialist (S) and generalist (G) organisms along a resource axis having from “easy” (-) to “difficult” (+) categories of a resource (e.g. food, nesting sites). (b) Fitness losses of a specialist and generalist organisms resulting from impacts or constraints along the same or a different niche axis (e.g. predation, competition, abiotic stressors).

the exploitation along a different niche axis, for example, a habitat or a resource that implies more competition, predation or physical stresses.

In the literature, there is some confusion among qualitatively different levels needed to determine the degree of ecological specialization of organisms, which lead to some methodological difficulties when measuring it. Some authors consider (as we do) that the Grinnellian niche breadth is a too general concept, and they propose to methodologically separate the diversity of resources used by a species from the diversity of habitats under which a species is able to exist (Petraitis 1979, Morrow 1981, Mac Nally 2009). *Ecological versatility* is the term proposed by MacNally (2009) to refer particularly to the range of resources used by a species; while the term *ubiquity* refers particularly to the range of habitats used by a particular species. A species showing a high ecological versatility is then a species exploiting a wide range of resource types, whereas a non-versatile organism is able to exploit a relatively narrow range of different categories of resources and can be considered as a specialist. On the other hand, an organism having the skills to occupy a vast array of different habitats is considered as being *ubiquitous*, while the contrary is the *restricted* organism, able to exist in one or a few habitat types. An alternative way authors distinguish ubiquitous from restricted species is by saying that: a ubiquitous species is evenly spread, in terms of densities, among the different habitats it occupies; whereas a restricted species shows a biased distribution, being more abundant in one or a few kinds of habitats. A second confusion in the literature when describing the degree of ecological specialization arises from the lack of clear distinction between the ubiquity (in particular, range of habitats used) of a species and the geographic range of the species distribution (Hesse et al. 1951). Here the appropriate terms are *cosmopolitan* for geographically widespread species, versus *localized* species. MacNally (2009) contends that suitable habitats for a particular species could span a small area, or at the contrary correspond to one or several patches spanning vast geographical areas, depending on

the vagility, scale of perception and versatility of the organism. Then we could find different combinations of cosmopolitan and localized species, showing variable degrees of ubiquities in habitat use (Fig.3). In particular, a specialist herbivore whose specific algal host occupies patches of intertidal rocky coast spread over large geographical extensions would, by this logic, be characterized as a cosmopolitan organism restricted to a single (narrow) habitat.

Over-abundant resources in the environment, often called *superabundant resources* (Real 1975), do not cause species competition and therefore do not generate resources optimization trade-offs. Only those environmental components which are relatively scarce and to which the species can gain exclusive access for some period of time (termed *true resources* by McNally 2009) are crucial components for the relative fitness of individuals. Therefore, specialization is not expected to evolve along a superabundant resource axis.

Then, a prerequisite for characterizing ecological versatility, and therefore the level of ecological specialization, is to distinguish between a *true resource* and *superabundant resources*. More formally, a true resource is any environmental component (e.g. food, refuges, nesting sites, environmental gradients or patches of light, space and time, etc.) to which an organism can gain exclusive access for some periods of time, and by which it enhances its fitness (Mac Nally 2009). Environmental components that reduce the fitness of an organism are termed constraints (i.e. biotic and abiotic impacts suffered by individuals of the focal species, like predators or stressful physical conditions). Finally, a habitat is a collection of true resources and constraints that determinethe performance of organisms and populations. A microhabitat refers to that particular habitat occupied and exploited by individual organisms in discrete time periods (like a particular host-plant and its immediate surrounding conditions). In

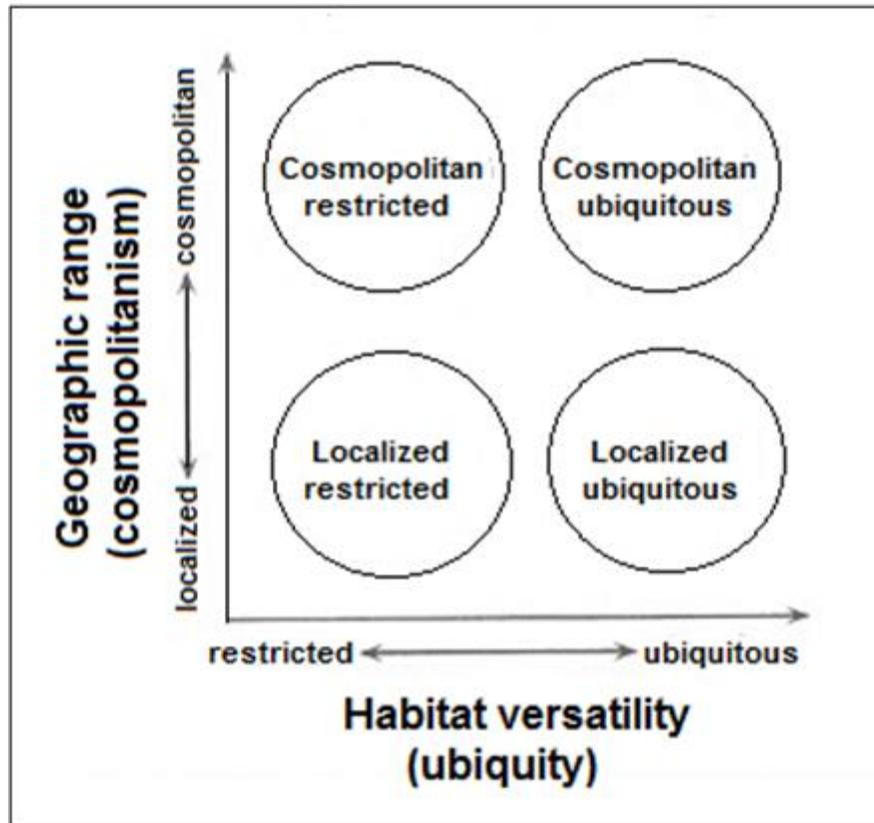


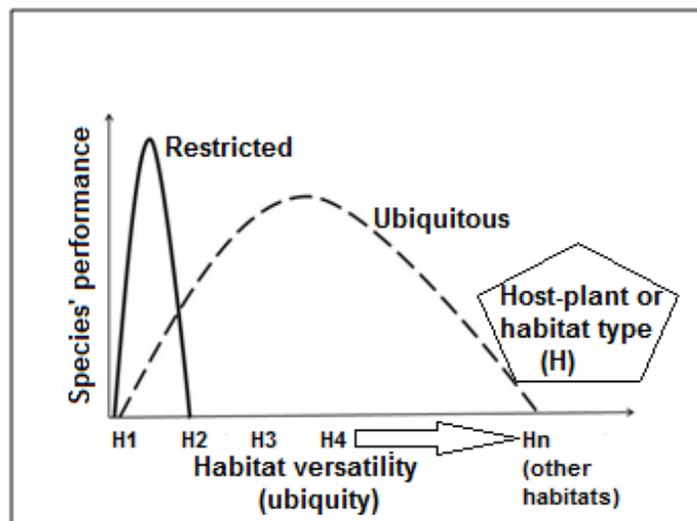
Figure 3. Different combinations between cosmopolitanism (the extension of geographic range) and ubiquity (range or number of habitats occupied). Four extreme conditions are shown (from Mac Nally 2009).

this sense, host plants used as main habitat or microhabitat by specialized herbivores species are collections of resources. It is worth mentioning that the term habitat refers to that part of the physical and ecological environment where the individuals of a species live; thus, it encompasses only a fraction of all possible environmental variables considered in biogeography or macroecology.

In summary, herbivores could show, in the first place, variable levels of ubiquity (or specialization to habitat) depending on the range of host plants used as habitat(s) or microhabitat(s) (Fig.4). In the second place, being host plants potential habitat(s) or

collections of true resources, herbivores could also manifest variable levels of ecological versatility for particular true resources offered by plants, such as food, protection against enemies or physical stresses, or mating and nesting sites. For example, whereas a first herbivore could be extremely specialized to several resources supplied by a single (or a few) host-plant (Fig.5); a second herbivore could be specialized to use as shelter a particular host-plant, and be generalist for other resources offered by other hosts or habitats (Fig.6).

Figure 4. Restricted (specialized) use of one or a few host-plants or habitats, or ubiquitous (generalized) use of several host-plants or habitats types.



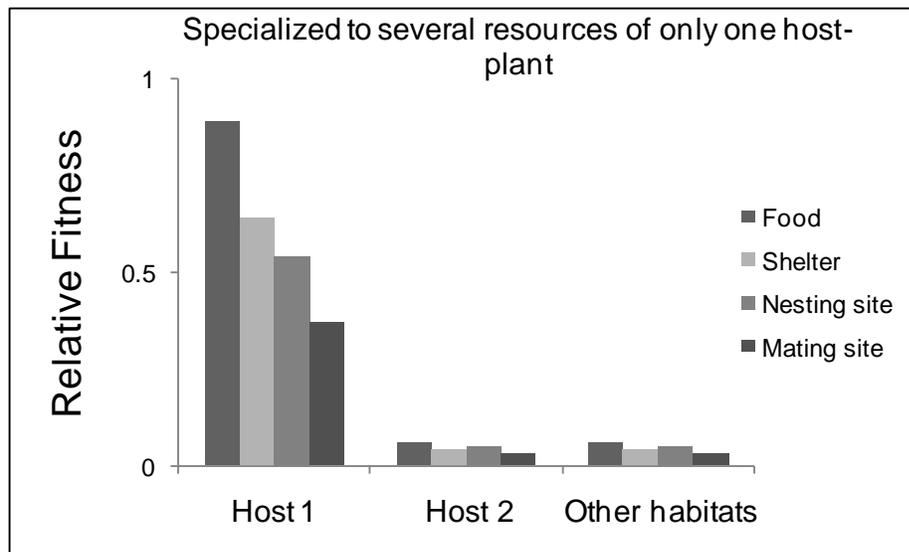


Figure 5. Low ecological versatility of a specialist herbivore related to an uneven (skewed) performance or fitness when consuming the resources harbored in different host-plants, with a high performance exclusively when exploiting resources available in its preferred host-plant (Host 1).

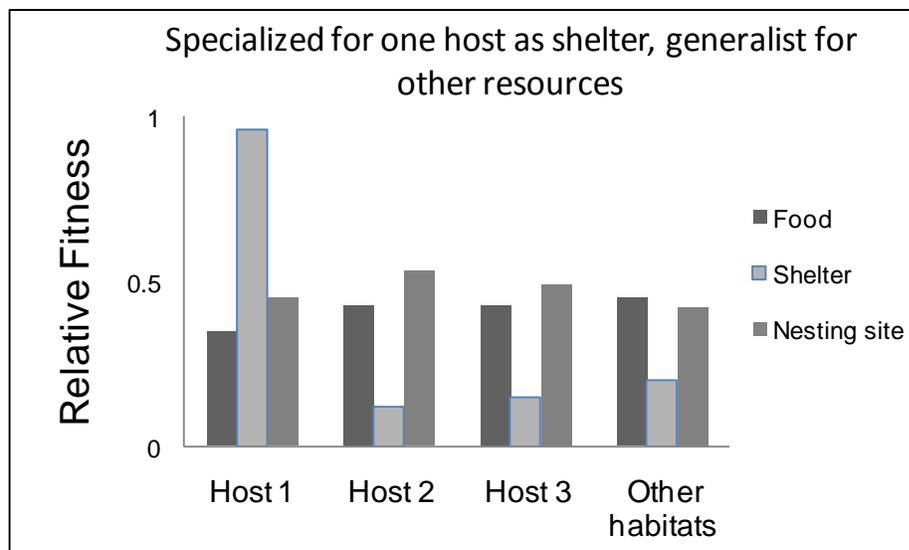


Figure 6. High ecological versatility of a generalist herbivore related to an even and positive (mean) fitness when consuming a relatively wide spectrum of resources harbored within different host plants.

Once we have preliminarily described which are the most important true resources and habitat types for a focal species, the next step would be to quantify which is its degree of

specialization (ecological versatility and ubiquity) in terms of its performance in a particular habitat or true resource. Indeed, Mac Nally (2009) asserts that the degree of ecological versatility has to be determined by matching the fitnesses experienced by individuals of a species when exploiting alternative resources (e.g. alternative food items) to the relative availabilities of these alternative resources in the environment. Accordingly, a species is increasingly versatile as long as its performance matches availabilities of resources in local environments; and it has low ecological versatility when it is intrinsically able to exploit only a subsample of the (local) available resources. On the other hand, as previously mentioned, host-plants are collections of true resources. Then, the evaluation of the ubiquity (through measuring the evenness of densities of the focal herbivore in different host-plant types) is a global characterization of its fitness at the population level. The characterization of the degree of specialization requires nonetheless standardizing the density of the herbivore on each host-plant by the relative availability of the host (i.e. standardizing by the proportional density of each host plant type, in relation to the total density of acceptable host-plants, in local environments) or standardizing by the type and quantity of a true resource available in each host-type (for example, different secondary compounds, nutrients, or microhabitats with a particular architecture used as shelter).

In order to establish the degree of specialization of a specialist marine herbivore, we propose to follow the three following methodological steps: the first two for determining its ubiquity, and the third one to determine its ecological versatility when using a true resource offered by its host-plants (see Fig.7, in the next page). These three steps consist in determining: (i) the range of all potential categories of (acceptable) host-plants that the focal species is intrinsically able to exploit, or at least, with which the species is in natural contact

(ii) the rough pattern of utilization or prevalence of the herbivore (selective or opportunist) in each host-plant, in relation to the relative availabilities of the hosts, in spatially or temporally different environments, and (iii) the even or uneven performance (for example, in terms of organism growth, population growth, reproductive success or survival) of the focal herbivore species, when exploiting different categories and quantities of a true resource offered by particular host-plants. Points (ii) and (iii) are tightly connected, because a host-plant is frequently a microhabitat that potentially harbors one or several true resource(s) for a specialized herbivore. Then, evaluating the selectivity of an herbivore for each host-plant (point ii) for example in terms of its local prevalence in each host-plant type or taxa, is a rough but efficient way to quantify the global preference of the herbivore for the ensemble of true resources offered by particular host-plants. The utility of such a methodology would be that one would expect: (a) that the relative performance of the focal herbivore on different host-plants (measured in point iii) would depend on the differential supply of the true resource(s) in each host-plant type, and (b) that the relative performance (e.g. degree of evenness of population growths) of the focal herbivore when using the true resource on different host-plant types, would have to conform to the pattern of selectivity previously verified in point ii.

Finally, a major issue when studying ecological specialization is how to place particular herbivore species along the generalist-specialist continuum existing in nature. Authors tend to misleadingly classify an herbivore as a generalist as soon as it uses more than two plant species belonging to different families (using terrestrial insect obligatory herbivore specialists as absolute standards). A general way to describe the continuum in the degree of

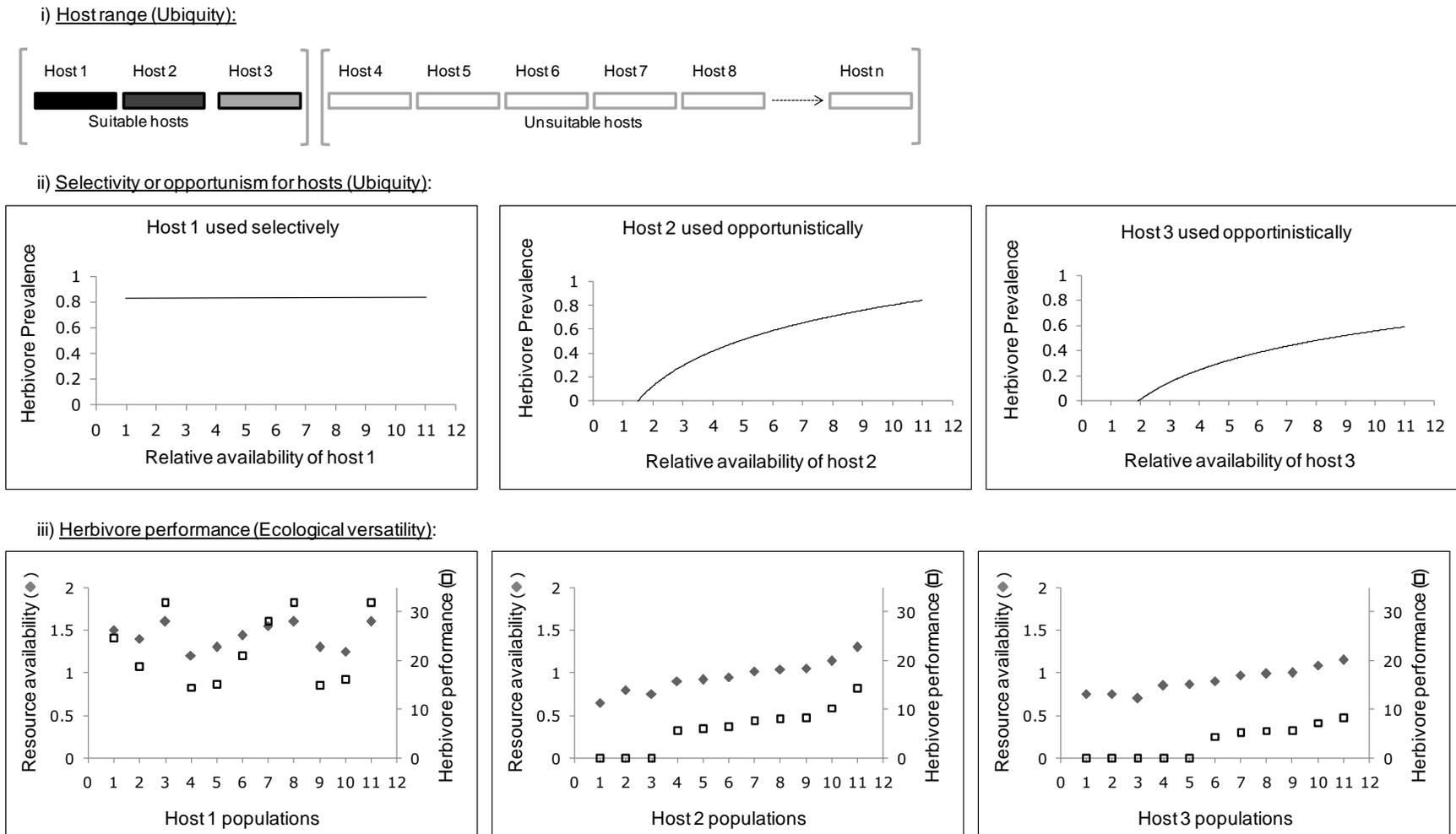


Figure 7. Steps in measuring Ubiquity and Ecological versatility of an herbivore: (i) Determining the range of suitable host plants for the focal herbivore species. (ii) Measuring the prevalence of the herbivore in plants of each suitable host according to relative availability of the host (in relation to total availability of suitable hosts) in different environments or sites. (iii) Measuring the performance of the herbivore when confronted to different availabilities of a “true resource” in each suitable host in different environments.

versatility existing in nature is to distinguish organisms by the evenness by which they use resources and on the level of population saturation of the environment. A graphical representation of the latter is figure 8 (of Mac Nally 2009, see next page), where an obligatory specialist shows an uneven use of resources, the obligatory generalist performs well and uses evenly a wide spectrum of resources. At an intermediate position is the facultative user, with a moderately uneven use of resources. This case would be classified as a facultative specialist or a facultative generalist according to their relative range of resource use and according to some general rules, such as the classificatory scheme proposed by Shipley (2009) for mammal herbivores. Shipley (2009) argues that the adequacy of plants for herbivore consumption would be globally described according to two main plant characteristics: first, by being “difficult” or not in terms of their mechanical and chemical difficulty of being ingested and/or digested; and, secondly, by their nutrient quality. Accordingly, Shipley proposes a classificatory scheme producing four general categories of herbivores, where the terms *specialist* or *generalist* are complemented with the terms *obligatory* or *facultative*. Indeed, whereas an *obligatory specialist* herbivore is able to use only a very particular plant type, chemically *difficult* and generally *nutrient-poor*, the *facultative specialist* herbivore prefers to use a particular type of *difficult plant* but is also able to exploit two or a relatively bounded additional number of difficult plant types (Shipley, 2009). On the other hand, in comparison to the obligatory generalist herbivore, which is able to exploit a wide range of plant taxa but only of chemically easy and nutrient rich plants; the facultative generalist use efficiently the last category of plants but performs well also when exploiting difficult and nutrient poor plants (Shipley, 2009). We consider the previous classificatory scheme of Mac Nally 2009 and that proposed by Shipley (2009) for mammal herbivores to be an appropriate way of solving the

issue of placing a particular herbivore species along the generalist-specialist continuum existing in nature.

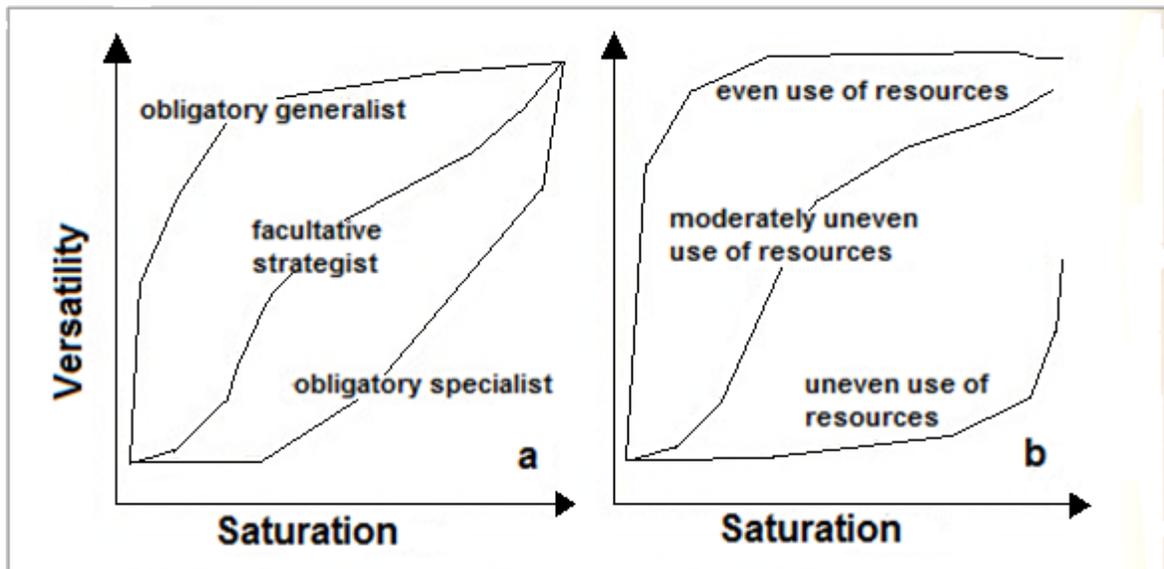


Figure 8. Functional dependence of versatility on the level of population saturation of an environment, where saturation is gauged by the ratio of current population density to nominal carrying capacity. (a) three strategies: an obligatory specialist, a generalist and a facultative exploiter. (b) three levels of evenness of efficiency of resource use in the facultative strategy, extremely even, intermediate and uneven efficiencies (from MacNally 2009).

1.3 SECTION II. MAIN HYPOTHESES ON THE ORIGIN OF HERBIVORE SPECIALIZATION IN MARINE COMMUNITIES:

In this section, we summarize the main hypotheses proposed to explain the origin of ecological specialization of insect herbivores in terrestrial environments, and we will discuss how they can be applied to understand specialization of marine herbivores. By presenting different examples of marine herbivores, we show how studies usually fail to properly distinguish ecological versatility, ubiquity, and geographic range, all needed to characterize the degree of ecological specialization.

In terrestrial environments, four general hypotheses have been advanced to explain the predominance of a restricted host range and specialization of insect herbivores to specific angiosperm plants: (1) the physiological-efficiency hypothesis, (2) the enemy-free space hypothesis, (3) the optimal-foraging hypothesis, and (4) the neural-constraints hypothesis.

(1) Two important assumptions of the physiological-efficiency hypothesis (Dethier 1954) are that: host-plant specificity of insect herbivores arises from their adaptation to the specific nutritional quality and secondary chemistry of their host plants as food; and that specialists perform better than generalists consuming their specific host plants.

(2) According to the enemy-free space hypothesis (Bernays & Graham 1988), host plant specificity of insect herbivores has its main origin in the evolution of a specific use of host plants, generally rich in secondary compounds, as refuge (among “toxic” plant structures) or defense against generalist predators (by sequestering plant secondary compounds).

(3) The optimal-foraging hypothesis posits that host plant specificity stems from herbivores maximizing different adult fitness components (e.g. mating success, oviposition preference and suitability of host for juvenile development) through using as resources the same high quality plants (eaten by and) ensuring a high adult performance (Scheirs & De Bruyn 2002, after Stephens & Krebs 1986 for host-parasitoid interactions).

(4) The “neural-constraints” or “information-processing” hypothesis (Bernays & Weislo 1994; Bernays 2001) affirms that simpler neural systems, like those of insect herbivores, are restricted by design to perform more complex behaviors. This would not allow them recognizing and efficiently exploiting a vast number of different host plant taxa. In this case, the only evolutionary stable strategy would be to efficiently perceive and exploit a single or a

few specific host plants. A specialist would do more accurate and more rapid decisions in selecting a host, because it focuses its attention on a restricted set of informative stimuli than a generalist (which has to distinguish among a broader array of acceptable hosts and stimuli).

Whereas the first three hypotheses focus more on fitness and the particular selective pressures shaping a specialized behavior, morphology or physiological tolerance to secondary plant chemistry, the neural-constraints hypothesis is more like a design restriction, or phylogenetic constraint, determining the biological framework on which selective pressures operate. On the other hand, contrary to hypothesis 2 and 4, hypotheses 1 and 3 assume that there is a trade-off in fitness currency, between exploiting efficiently a restricted set of resources (specialists) versus exploiting less efficiently a broader array of resources (generalists).

In the marine realm, design restrictions have also been advanced to explain the more likely occurrence of herbivore specialization in certain kinds of marine taxa. For Steneck (1982), there is a greater chance of finding specialists among small marine herbivores, with low vagility, and inhabiting large and long lived algal hosts. At the scale of perception of small herbivores, to move from plant to plant may be costly and risky, so they would spend most of their life in one or a few algal hosts (Steneck, 1982). Hay (1988) also proposed to focus our attention on slow motion, small marine herbivores termed “mesoherbivores” or “insect like” (like amphipods, isopods, polychaetes, small crabs and gastropods) which he opposes to large highly mobile herbivores termed “macroherbivores” (like fishes and large urchins).

More importantly, a series of studies have demonstrated that some sedentary, small marine herbivores would be attracted to and tolerate seaweeds that are unpalatable to their predators, a strategy oriented to gain “enemy free space” (Price et al. 1980; Vermeij, 1983; Vermeij et al., 1987; Hay et al., 1987, 1988, 1989, 1990a, 1990b, 1992, 2009). In most cases, specialist herbivores have resistance to chemical host defenses, but the type of resource of the host alga exploited to deter or avoid predators varies for particular specialists. For example, while the sea slugs *Elysia halimeda* and *Costasiella ocellifera* (Opisthobranchia) deter predators through sequestering secondary compounds of their preferred host algal type (*Halimeda* species, and *Avrainvillea longicaulus*, respectively); the crab *Thersandrus compressus* and the amphipod *Pseudoamphitoides incurvaria* mostly avoid predation building a protective home with the tissues of their host-algae (*Avrainvillea longicaulus* and *Dyctiota* spp., respectively) that deter predators (Hay et al. 1990b).

We would like to emphasize that studies aiming at characterizing ecological specialization of herbivores in the sea usually do not distinguish among the main axes needed to define specialization (ecological versatility, ubiquity, and geographic extension) or resources types harbored by host. There are facultative herbivore specialists having specificity for a specific algal host (which they use to avoid or deter predators) but which frequently use also other algal hosts for activities other than escape from enemies. For example, the polychaete *Platynereis dumerilii* and the amphipod *Amphitoe tea* are very selective when building a refuge in specific algal host types (*Dictyota* mostly, and *Pelvetia*, respectively) to protect themselves from predators but are opportunist exploiting other algae species as food. Like obligatory specialists, these latter facultative specialists also use some specific algae to avoid predation that are probably usually apparent and sometimes even cosmopolitan.

Exceptionally in the case of particular sea slugs, nonetheless, their preferred host alga is frequently highly variable both spatially and temporally. These groups of sea slugs has probably phylogenetically determined skills like: 1) sequestering the chloroplasts of their specific host-alga into their tissues, and being then able to lead an independent life from their host-alga for some periods; or 2) produce a combination of lecitotrophic and planktotrophic larvae (as in *Alderia modesta*), providing variable dispersing capacities of larvae.

The most supported and proximate cause explaining the occurrence of herbivore specialists in the sea is the enemy-free space hypothesis. Nevertheless, we state that the physiological-efficiency and the neural-constraints hypotheses are also likely factors explaining herbivore specialization in the sea, and are in fact complementary with the latter hypothesis. The “neural constraints” hypothesis has received some support in terrestrial systems (Janz & Nylin 1997, Bernays 1999, Bernays & Funk 1999, Nylin et al. 2000, Egan & Funk 2006; in Tosh et al. 2009); moreover, neural network simulations showed that this hypothesis is theoretically concordant with the “enemy-free space” hypothesis in predicting specialization whenever an increased cost of searching an acceptable host is associated to an increased risk of predation (Tosh et al. 2009). More importantly, both hypotheses are coherent with the fact that many specialists can survive well exploiting in the laboratory resources they usually don't exploit in nature (potential versus realized niche concepts of Hutchinson, 1959).

There is also growing evidence that the physiological-efficiency hypothesis and the enemy-free space hypothesis are complementary. While most marine seaweeds show innate (constitutive) chemical responses to macroherbivore damage, only grazing by small herbivores has been shown to induce chemical responses by algae (Paul & Fenical 1987, Van Alstyne

1988, Cronin & Hay 1996, Pavia & Toth 2000). Only small sedentary herbivores would stay enough time in particular plants and consume them slowly enough to produce inducible chemical responses. On the contrary, to respond to the large bites and superior vagility of macroherbivores, only innate chemical responses, structural complexity and tissue toughness or calcification would be effective (Hay 1996, Duffy & Hay 2001).

Finally, we emphasize that there is an excessive focus of marine studies in trophic resources, and an almost absence of research concerning the behavioral, morphological or physiological adaptations of herbivores to stay and exploit the particular “habitat architectures” of algal hosts. Indeed, structurally complex algae such as kelps harbor faunal organisms which distribute themselves according to their differential selectivity for different vertical positions along the plants, which offer particular structures or microhabitats (Christie et al. 2009). More structurally complex habitats would allow the partitioning of resources and shelter (among potentially competing species) through the use of separate microhabitats (Beukers & Jones 1997). Then, it is likely that macroalgae evolving highly effective secondary compounds or physical barriers against most consumers could constitute an empty niche for herbivores which could specialize to unexploited macroalgal microhabitats. This is particularly true for small, specialized herbivores able to tolerate specific macroalgal chemicals and/or behaviorally adapted to stay and exploit the enemy-free and stress-free spaces of the microhabitat (following Bernays and Graham 1988 for terrestrial insects, and Hay 1987, 1988 for small specialist marine herbivores).

1.4 BIOLOGICAL MODEL AND MAIN HYPOTHESES OF THIS THESIS:

Several theoretical models have shown the antagonistic roles of gene flow and the strength of selection in the adoption of specialist (adaptation to local habitat) or generalist strategies in biological organisms. While low gene flow and strong selective regimes would favor adaptation to local habitat; high migration rates benefit the genotype having the highest mean phenotype in most habitat(s), i.e. the generalist (e.g. Lenormand 2002). An example of organisms having extremely low dispersive capacities and manifesting both high ecological specialization and co-speciation with their hosts are parasitic lice and their vertebrate hosts (with vertical transmission among generations of the host). When there is migration, there are still simple classical models (Levene 1953, Dempster 1955) for the evolution of specialization on two alternative habitats. Ravné et al. (2003, 2009) have shown that when organisms evolve habitat choice, the evolution of one-habitat specialist or two-habitat specialist are both possible and more likely than the evolution of a generalist. For instance, there are examples of specialist marine herbivores (mainly sea slugs) having direct and indirect developmental mode, and which selectively recruit into their specific host-alga, as previously said.

There are potentially specialized herbivores with intermediate dispersal potential whose specific habitats are kelps having a distribution across a large geographic extension. Kelps afford them a relatively stable and predictable habitat at the regional level compensating for great environmental heterogeneity in the sea. Such cosmopolitan herbivores would manifest both a restricted ubiquity and/or narrow ecological versatility. Among this category of likely specialist marine herbivores, there are patellogastropods with distinctive shell morphology which form close associations with kelps or seagrasses (Estes and Steinberg 1988).

Particularly, there are six limpet species with saddle-shaped shells, known to form close associations with cosmopolitan laminarian or furoid kelps (e.g. Branch 1975, Black 1976, Choat & Black 1979, Estes & Steinberg 1988, Muñoz & Santelices 1989): two from the North Pacific (*Lottia instabilis*, *Lottia incessa*, *Rhodapatela rosea*), one from the North Atlantic (*Patella pellucida*), one from South Africa (*Patella compressa*), and one from South America (*Scurria scurra*), our marine specialist model used here. Kelps could provide to limpets both chemically defended food resources deterring generalist predators and a particular habitat architecture (defined by the number, size, shape and arrangement of habitable spaces and structures for a given organism, after Hacker & Steneck 1990) to avoid enemies or attenuate physical stresses. Some difficult kelp microhabitats (like fronds or stipes) would harbor both underexploited food items (few trophic competitors for the herbivore specialist) and constitute “enemy-free space”, or “stress-free space” in highly physically perturbed marine coastal environments for limpets with saddle-shaped shells. Christie et al. (2007) demonstrated that kelp fauna of different kinds selectively distribute in different vertical positions on kelp, which have different habitat architectures. Nonetheless, the importance of kelp microhabitats and food resources has not been evaluated rigorously for specialist marine herbivores. In the case of specialized herbivore limpets, they would have had to evolve both: (1) a specialized behavior and morphology (saddle-shaped shell) enabling them to stay in underexploited kelp structures, usually stipes or fronds, and (2) detoxifying mechanisms to process and consume the chemically defended tissues of its kelp-host (s).

In the next two chapters of this thesis, my main objective consisted in defining some important dimensions of the degree of ecological specialization and spatial genetic structure of the candidate specialist patella *Scurria scurra* in the Chilean coast. *S. scurra* is a

patellogastropod herbivore having saddle-shaped shell, and inhabiting preferentially the stipes and discs of its specific host the giant kelp *Lessonia* (*Lessonia berteroana* and *L. spicata*) along the temperate east Pacific coast in South America. It is the only species in its genus having evolved specialization to kelps, the remaining species living on rocks of the intertidal zone. The origin of *S. scurra* has been calculated to be 2 MYA by Espoz (2002), relatively contemporaneous to the arrival of kelps (Laminariales) to the Southeast Pacific coast (Estes and Steinberg 1988) but posterior to the divergence calculated for *Lessonia* in two species, *L. berteroana* and *L. spicata* (Martin & Zuccarello 2012). Moreover, Espoz (2002) has shown that the more derived species in the phylogeny of the genus *Scurria* (like *S. scurra*) lack of an active antipredator response to starfishes. *S. scurra* individuals have been shown to stay in scars or “homes” that they make in the proximal parts of *L. spicata* stipes (Muñoz & Santelices 1989). In contrast, most other herbivores of the rocky environment are removed from the reach of the stipes and fronds of *L. spicata*, as a consequence of the whiplash effect, a strong movement of fronds and stipes agitated by waves that hits continuously the undercover (Ojeda and Santelices 1984). Then *S. scurra* is able to occupy a difficult microhabitat in *L. spicata* where it can have exclusive access to food resources and avoid enemies.

In the intertidal marine environment, the ubiquity and ecological versatility of specialist herbivores has not been evaluated taking into account the relative availabilities of their algal hosts and the resources that they harbor in different environments. In chapter II, I will then characterize globally the degree of ubiquity and ecological versatility of this patella for its host *Lessonia spicata*, along the biogeographic area called Intermediate Area (Camus 2001), spanning from 30° to 42°S. The main focus in chapter II will be to execute a

correlational study to test the hypotheses that: (1) *Scurria scurra* is a facultative herbivore specialist using selectively its preferred host *Lessonia spicata*, and using opportunistically its alternative host the fucoid kelp *Durvillaea antarctica*; and (2) one of the main true resources for *S. scurra* is the structural complexity or shape and size of specific microhabitats of its host-kelps, used as an enemy-free space, or as a “stress-free space”.

As mentioned previously, there is new evidence of preferential settlement of larvae in their algal host in the marine environment. Some theoretical models show that under some circumstances and two habitats the evolution of one specialist is more likely (one habitat) than the evolution of a generalist (two habitats), especially when it is coupled with habitat choice (Ravigné 2009). For instance, it has been shown that, in the sea, local adaptation is not more common in direct developers than in planktonic dispersers (Sotka 2012). Limpet species with saddle-shaped shells are then good models of planktonic dispersers (with intermediate dispersal potential) that likely have evolved specialization to their host algae.

Our initial expectation, consistent with some recent surveys, was that a concordant phylogeographic and demographic patterns are more likely between organisms having very strong and specific ecological interactions (e.g. Smith et al. 2011). Thereafter, in chapter III, my main aim was to determine if there is concordant demographic history and population genetic structure between *S. scurra* and *Lessonia* spp., along most of the Peruvian Province and the Intermediate Area in Chile (26° to 41°S). More specifically, we hypothesized that, because *S. scurra* shared similar environments with its host *Lessonia* during most of the Pleistocene, *S. scurra* genetic diversity shows the same phylogeographic breaks verified previously in *Lessonia* for the coast of Chile: specifically, the 30°S or the 27°S genetic

discontinuities. At a more restricted geographical scale, my main expectation was that the likely evolution of host preference for *Lessonia* spp. and low gene flow in *S. scurra* would translate into a concordant pattern of spatial genetic structures between *S. scurra* and *L. spicata*. My main objective then consisted in establishing the degree of similarity concerning the number and boundaries of spatial genetic clusters existing in *S. scurra* and *L. spicata*. In the second place, we investigated the potential demographic changes experienced by both species due to the Glacial-Interglacial transition. Indeed, a northward shift of the West Wind Drift apparently modified the intensity of upwelling south of 30°S, reducing primary productivity during the Last Glacial Maximum (Klump 1999, Williams & Bryan 2006, Toggweiler et al. 2006). Therefore, coastal populations likely experienced demographic changes associated with the reduction of primary productivity during the glacial period, and a population growth after the reestablishment of upwelling. Here, we hypothesized that both *S. scurra* and *L. spicata* experienced similar and contemporaneous demographic changes during the Late Pleistocene. It is worth mentioning that phylogeographic studies deal with genealogical relationships across landscapes of individuals within and between populations that relate to more contemporaneous events than those implied in historical biogeography and co-phylogenetic inference. At the macroevolutionary scale, genetic concordance between tightly associated species, especially plant-herbivore associations, is more infrequent because of the occurrence of numerous past demographic changes and vicariant events having taken place. Moreover, co-phylogenetic concordance is very infrequent between plants and their specialist herbivores, because it requires that specialized organism fulfill a series of conditions that specialist herbivores generally don't meet completely (eg. vertical transmission among generations of the host, and having only a unique and obligatory host).

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**CHAPTER II: ABUNDANCE AND DEGREE OF
SELECTIVITY OF A SPECIALIST MARINE
HERBIVORE CORRELATED TO
AVAILABILITY OF MICROHABITATS OF
SPECIFIC SHAPE AND INCREASING SIZE
WITHIN ITS TWO HOST KELPS**

1 INTRODUCTION:

Generalist and specialist species are usually defined in relation to the breadth of resource types they utilize or by the range of environmental conditions under which they can thrive (Richmond et al. 2005). A generalist species is able to proliferate in a wide range of environmental conditions, and utilize a vast number of resource types, the opposite being stated for a specialist. Specialist and generalist are extreme cases in a continuum of patterns of resource and habitat use, corresponding then to conceptual tools to compare and classify the diversity of nature (Fox & Morrow 1981, cited by Mac Nally 2009). To refer to this specialized-generalized continuum, we adopt here the term ecological versatility, which corresponds to the range of resources used and particular performances experienced by a species when exploiting these resources, in comparison to other species (MacNally 2009). A related but hierarchically different concept is that of ubiquity, which refers to the range of habitats occupied by a species and its performances when using these different habitats (MacNally 2009). Species are said to be highly ubiquitous when there is evenness of densities of its populations among the different habitat they use. On the contrary, they are said to be restricted (or less ubiquitous) when showing an uneven pattern of densities of its populations among the different habitat they exploit. Ecological versatility is logically nested within ubiquity, because habitats are collections of resources. It is important to highlight that populations of a species are more or less versatile depending on particular biotic or abiotic variables determining a high fitness, not necessarily to all resource axes (Futuyma 1988). In measuring ecological versatility, one needs then to distinguish between true resources, which are main environmental components determining the fitness of a particular organism (e.g. food, refuges, nesting sites, inorganic nutrients, shelter from biotic and abiotic threats, etc.),

from superabundant resources that are likely extensively occupied simply as a density dependent process (after Real 1975, in McNally 2009).

Host plants are probably the main selective environment, or habitat, for specialist grazers of small size (Poor & Steinberg 2001). We suggest then that a major issue when characterizing the ubiquity and ecological versatility of herbivores inhabiting or consuming large host algae is the confusion among three of its basic components. These three components consist in: (1) characterizing the range of hosts (or true resources) exploited by a species; (2) standardizing the degree of utilization of each host (or true resources) by the availabilities of the different suitable hosts (or true resource types) present in local environments, and (3) evaluating varying performances, in terms of the differential fitness reached by individuals of a species when using different hosts (or true resources). In characterizing component 2, we suggest to expand the application of the related terms opportunism and selectivity. Opportunist consumers are said to vary their consumption of different trophic items and use those resources (host plants, in our case) that are most abundant in local environments. On the contrary, a very selective herbivore will consume preferentially one type of trophic item, even if the local relative abundance of this item (the host plant) is low in comparison to other trophic items. There are no studies evaluating rigorously if marine herbivores occupy their host plants according to the relative availabilities of its host kelps or the resources that they harbor; say if herbivores settle (the larvae) or forage (the adults) following a selective or opportunist pattern of use of their host plants.

In terrestrial communities, there is an abundant and highly diversified array of insect herbivores specialized to angiosperm plants (Root 1973, Bernays 1989). Insects use particular taxa of angiosperm plants both as habitat, as food, as mating, and as egg laying or nesting

sites. On the contrary, marine herbivores are generally thought as being mainly generalists, a small percentage being considered as dietary specialists (Lubchenco & Gaines 1981; Hay et al. 1988, 1989, 1990a,b). Most specialist marine herbivores correspond to Opisthobranchs gastropods in the order Ascoglossa (mainly some sea slugs and juvenile sea hares) which maintain highly specialized algal host associations (Carefoot 1987, Pennings 1990, Trowbridge 1994, 2004). In fact, the last twenty years few additional examples of herbivores specialized to algae have been reported in the seas, most being mesoherbivores, like small crabs, amphipods, and small gastropods. Moreover, marine studies dealing with ecological specialists have focused on trophic or chemical adaptations of mesoherbivores to algae (Paul & Alstyne 1988; Pavia & Toth 2000). These studies have demonstrated that specialists correspond mostly to small herbivores inhabiting and consuming chemically-rich algae frequently avoided by their predators, which then function as an “enemy-free space”; they showed also that some small herbivores sequester secondary compounds from their host algae which deter predators from consuming them (Hay et al. 1989, 1990a, 1990b, 1996, 2009, Stachowicz & Hay 2000).

Structural complexity of an algal host potentially constitutes a basic environmental component to which small herbivores can be specialized and occupy with exclusivity in time and space (not available to its enemies). Such a permanent “true resource” can allow reaching a high performance. This is indeed very important, because the scale of spatial variability for a small sized specialized herbivore with low mobility is coarse-grained, i.e. it perceives and experiences its host plant as its almost unique habitat (Levins 1962). In terrestrial systems, for example, the specialist mutualist bug *Pameridea roridulae* has been shown to circumvent glandular trichomes of its sticky hairy host plant *Roridula gorgonias* (which serve as

mechanical defenses against most other insect herbivores) by having evolved long legs, among other specialized anatomical features, which allow them to walk and eat freely among these “death traps” (Voigt & Gorb 2008, but see also Gross & Price 1988, for another obligate specialist). Only for opportunistic fauna living on kelps, several studies have shown that the big size and a greater heterogeneity of the structure of kelps would explain their great diversity and abundance (Chemello & Milazzo 2002, Hauser et al. 2006, Christie et al. 2003, 2007, Norderhaug & Christie, 2007, 2011). Nevertheless, these same studies suggest that some species of the mobile fauna are highly selective and associated exclusively to kelp microhabitats like the holdfast or the stipes of *L. hyperborea*. There is no such study, except Hacker & Steneck (1990), for the dependence of habitat architecture and body size. Then, our main suggestion here is that the size and shape of specific kelp microhabitats or structures (disc, stipes, or fronds) are likely unexplored axes of specialization for marine herbivores of small to intermediate size, which probably act as an “enemy-free space” or “stress-free space” to avoid predators, competitors or physical stresses of the coastal environment. More generally, we suggest here that an herbivore specialized to stay in a specific microhabitat within a kelp could have exclusive access to the trophic resources harbored in this kelp microhabitat achieving a greater survival, growth and reproduction.

Host versatility was investigated here in the very specialized patellogastropod *Scurria scurra* (Lesson 1830), reported to live almost exclusively on stipes and/or the holdfast of the two most important intertidal canopy forming seaweeds in the rocky shores of Chile, formerly known as *Lessonia nigrescens* Bory (renamed by Gonzales et al. 2012 as *Lessonia berteriana* and *Lessonia spicata*) and *Durvillaea antarctica* (Chamisso) Hariot. Very rarely, this patella is observed living on stipes of a third kelp species, the subtidal *Lessonia trabeculata*, or on rocky

substrate (Espoz 2002). As shown by Espoz (2002), *S. scurra* has a latitudinal range that span between 24°S and 42°S along the lineal coast of Chile where the intertidal *Lessonia nigrescens* complex is also present (*L. berteriana* and *L. spicata* species, distributed in the coast north and south of 30°S, respectively), but where *D. antarctica* only co-occur between 32°S and 42°S. In the more structurally complex *L. spicata*, *S. scurra* individuals have been shown to stay in scars or “homes” that they make in the proximal parts of stipes (Muñoz & Santelices 1989). In contrast, most other herbivores remain out of the reach of the stipes and fronds of this kelp, as a consequence of the whiplash effect of fronds and stipes agitated by waves (Ojeda & Santelices 1984) also reported for other large kelps of wave exposed coasts (Connell 1975, Dayton 1975a). On the other hand, analyzing stomach content, Camus et al. (2008) found that *S. scurra* ingests no more than seven dietary items, a narrow variety in comparison to most other marine herbivores analyzed (83% having between 25 to 99 food items, and 17%, between 7 to 21 items). Therefore, *S. scurra* seems to be specialized to both the habitat type and the dietary resources.

The range of hosts used by *S. scurra* (component 1 of ubiquity) has been previously established by Espoz (2002). Then we globally characterized host versatility of *S. scurra* in terms of component 2 (level of opportunism or selectivity) and a rough description of component 3 (matching of *S. scurra* size categories as an evaluation of fitness, and availability of microhabitat structures). Component 2 of ubiquity was assimilated here to the degree of selectivity versus opportunism of *S. scurra* for *L. spicata* and *D. antarctica* hosts, as well as for the specific microhabitats that these hosts harbor. In a first approach, this has been done through characterizing the *S. scurra* prevalence in *D. antarctica* and *L. spicata*, in relation to varying relative densities of these hosts in different sites. In a second approach, we evaluated

if component 2 of *S. scurra* is a function of the sizes and specific structural complexity offered by its two host kelps. In accordance with the above, component 2 was quantified here in terms of the probability of the *S. scurra* presence when occupying *D. antarctica* or *L. spicata* structures of different sizes using logistic regressions: we intended to assess how the degree of evenness of densities changes with sizes and shape of structures. Our main assumption was that larger and more structurally complex structures of *L. spicata* and *D. antarctica* (mainly discs and stipes) are true resources conferring space for refuge or protection. This available space would probably allow *S. scurra* to reach a greater survival and higher success in reaching sexual maturity. Our related predictions were that we would find (1) a greater abundance and sizes of *S. scurra* in specific and bigger algal structures of both *L. spicata* and *D. antarctica* (2) but a higher *S. scurra* load (number of individuals per algal host) and/or larger sizes of *S. scurra* in the larger and more structurally complex *L. spicata* than in the smaller and simpler *D. antarctica*. Finally, we performed a rough description of component 3, through correlating the sizes reached by *S. scurra* and the sizes of specific structures available in *D. antarctica* or *L. spicata*.

2 MATERIAL AND METHODS:

2.1 STUDY SITES AND DATA COLLECTION

Eight sites along the Chilean coast situated between 33°S and 40°S, and where both *L. spicata* and *D. antarctica* co-occurred abundantly, were sampled from June to September 2010

(Table 1). In each site, we counted the number of *D. antartica* and *L. spicata* individuals within fourteen quadrants of 1m². Quadrants were placed approximately every 1 m, along a linear 40 m transect in the low intertidal level, parallel to the coastal border. Eleven to fourteen adult individuals of *L. spicata* and *D. antartica* were removed in each site (except in an additional site, Infiernillo, where *D. antartica* was too scarce to fulfill this sampling scheme, see Table 1). Only one individual was sampled in each quadrant for an appropriate representation of spatial heterogeneity within sites and to avoid creating clearings in the kelp populations. Adult individuals were selected as representative of the mean adult size within a quadrant, with the aim to be representative of the mean size of algal hosts of the site.

For the average 14 *L. spicata* and 14 *D. antartica* sampled in each site, we measured or counted the following structural characters: maximum diameter of the holdfast, number of stipes (branches), maximum diameter of the stipes, the distance between the holdfast and the first bifurcation of stipes (not measured in *D. antartica* because its stipes don't bifurcate). Then, we cut each holdfast into five to eight transverse segments and counted the number of *S. scurra* individuals present both at the level of the holdfast and the stipes of each alga. Moreover, we measured the shell diameter of all *S. scurra* shells found on each alga, as proxy of fitness.

2.1.1 *Host selectivity: standardized degree of utilization according to host availability and structural complexity (Component 2 of ecological versatility)*

We tested the degree of host selectivity of *S. scurra* for its hosts, in the different localities sampled through verifying the steepness and significance of a simple regression model between the prevalence of *S. scurra* per site in *L. spicata* or *D. antartica* as the

dependent variable, and the relative densities of *L. spicata* and *D. antarctica* as the independent variable. By relative densities of *D. antarctica* host per locality, for example, we mean the ratio between the mean number of *D. antarctica* divided by the addition of the mean number of both *D. antarctica* and *L. spicata* .

Therefore, we evaluated if the degree of host-selectivity of *S. scurra* would also depend on the availability of structural complexity in its two host-kelps. For this purpose, we first performed partial correlations searching for the size of the structural characters (in 113 *L. spicata* and 105 *D. antarctica* individuals) showing the highest positive correlation with the number of found *S. scurra*. Then, logistic binary regressions were performed between the size of the measured kelp structural character(s) and the presence of *S. scurra* in discs, stipes, or both.

In five of the eight localities, we further determined the holdfast volume of 70-77 individuals for each algal host, by measuring the volume of water displaced by each disc when it was introduced into a 3,000 cm³ tail. Our intention was to evaluate if the size and shape of the holdfast were a better predictor of the *S. scurra* load than its volume, considered here to be a proxy of the quantity of tissue available for food. Then, additionally to the previous regressions, we performed simple regressions between: the *S. scurra* load and the disc volume, in the 70 *D. antarctica* and 77 *L. spicata* algae, respectively.

2.1.2 *Relative performance in different hosts (Component 3 of ecological versatility)*

Globally, we measured the *S. scurra* shell diameter as a proxy of relative fitness in *L. spicata* and *D. antarctica*. The minimum size of a fertile female *S. scurra* has been reported to

be 10 mm (Cancino & Santelices, 1984); thus, we assumed adults to have a shell diameter equal or superior to this value. In the 8-9 sites sampled, we calculated and compared the mean proportion of adult *S. scurra* inhabiting discs, stipes or both of *D. antartica* and *L. spicata* having different disc diameters. More specifically, the kelp structural characters better correlated to the adult *S. scurra* size in *L. spicata* and *D. antartica* were the stipe diameter and the disc height, respectively. Then, simple regressions between stipe diameter or disc height (and other kelp traits, in *L. spicata* and *D. antartica*) and *S. scurra* shell size were fitted. All this was done in order to confirm that the rate of increase of *S. scurra* size depends on the size of kelp structures. All statistical analyzes were performed in Statistica 7.0 (Statsoft. Inc.) and Minitab V13 (Minitab Inc., USA).

3 RESULTS:

3.1 REGIONAL PICTURE OF COMPONENT 2

Over eight sites sampled, *L. spicata* presented lower mean densities but higher disc sizes than *D. antartica* (see Table1). In both *L. spicata* and *D. antartica*, the mean density per site showed a clear negative correlation with the mean disc diameter: in *L. spicata*, the (Pearson) correlation was high and marginally significant ($r=-0.63$, $p=0.069$) and in *D. antartica* it was even higher and significant ($r=-0.81$, $p=0.016$). In *L. spicata*, the mean *S. scurra* load displayed a high positive correlation ($r=0.93$, $p<0.001$) with the mean disc diameter, and a negative relationship with the mean density of algae per site ($r=-0.65$, $p=0.056$). Then, in *L. spicata*, there was a trend over the sites having the largest disc diameters to have the highest

S. scurra densities. For example, Las Docas, El Canelo and Infiernillo which had the largest disc diameters (20 to 26 cm) showed also the highest mean of *S. scurra* loads (8.3 to 9.6 *S.scurra*/alga); whereas the opposite was verified with Tirua and Pelancura (1.6 and 3.9 *S.scurra*/alga), exhibiting the smaller mean disc diameter (14.1 and 11.8 cm). In *D. antartica*, on the contrary, *S. scurra* load was not clearly related to either the mean disc diameter or the mean density of plants per site. On the other hand, the prevalence of *S. scurra* in *L. spicata* (always high) did not vary with *L. spicata* densities. On the contrary, in *D. antartica*, the southern sites displaying the highest algal densities (Caleta Piure, Tirua and Calfuco) were those with the highest prevalences (Table 1 and Fig. 1). Neither the prevalence nor the *S. scurra* load showed a clear increase with a variation in *D. antartica* mean disc diameter.

Regionally, the mean *S. scurra* load was nearly three times larger in *L. spicata* than in *D. antartica*: 5.8 (± 7.0) patella versus 2.3 (± 3.2) per alga, respectively. Very infrequently a *S. scurra* individual was found in stipes of *D. antartica* (only two *D. antartica* out of 112 plants surveyed). The mean *S. scurra* load in stipes of *L. spicata* (4.1 ± 5.8 *S.scurra*/alga) was at least two times larger to both: a) the mean *S. scurra* load (1.8 ± 3.0) observed in the discs of these same *L. spicata* (One-Way Anova, $F_{1, 194}=11.62$, $p=0.001$), and b) to the *S. scurra* load quantified in the discs of *D. antartica* (2.3 ± 3.2 ; (One-Way Anova, $F_{1, 194}=7.72$, $p=0.006$). When focusing on individual plants, the maximum observed values were 15 and 41 *S. scurra* per plant in *D. antartica* and *L. spicata*, respectively (see Fig. 2c and 3a).

3.1.1 Component 2: standardized degree of utilization according to host availability

The proportion of *L. spicata* having *S. scurra* ranged between 0.7 and 1.0 (Fig.1 and Table 1). There was no correlation between the prevalence of *S. scurra* and the relative density of *L. spicata* ($R^2=0.001$, $p= 0.79$). In *D. antartica*, there was a significant regression between the prevalence of *S. scurra* and the relative density of *D. antartica* ($R^2= 0.83$, $p=0.003$), with a relatively steep and positive slope ($b=0.67$). The steepness of this regression slope is intermediate between the behavior of a selective and an ideal “fully opportunist” herbivore (see Fig. 1) but is closer to the opportunist. For *D. antartica*, we only considered for this regression seven localities from the eight sampled. These eight data produced a non significant ($p= 0.16$) flat slope regression. This was because, the *D. antartica* holdfasts of the eighth site (Las Salinas; empty rhombus in Fig. 1) were practically all colonized by different species of crabs and barnacles (93% and 100% of discs inspected, respectively) and most lacked *S. scurra* (71%).

Table 1. Scaling of the mean *S. scurra* abundance in relation to structural traits in *L. spicata* and *D. antarctica*.

Site	Latitude	Density of algae		Disc Diameter of algae		Prevalence <i>S.s</i>		<i>S.s</i> /alga		Proportion of adult <i>S.s</i>		
		<i>D.a</i>	<i>L.s</i>	<i>D.a</i>	<i>L.s</i>	<i>D.a</i>	<i>L.s</i>	<i>D.a</i>	<i>L.s</i>	<i>D.a</i> (discs)	<i>L.s</i> (discs)	<i>L.s</i> (stipes)
Las Salinas	32° 59' S	6.8 (± 4.8)	4.0 (± 2.8)	15.6 (± 5.0)	15.4 (± 5.7)	0.29	0.85	0.57 (± 1.1)	4.4 (± 3.5)	0.75	0.00	0.83
Las Docas	33° 08' S	1.5 (± 1.6)	4.0 (± 3.5)	16.7 (± 4.4)	22.1 (± 7.0)	0.44	0.82	1.9 (± 3.0)	10.6 (± 10.5)	0.41	0.14	0.88
El Canelo	33° 21' S	2.4 (± 1.9)	3.9 (± 2.3)	16.7 (± 7.3)	20.0 (± 9.4)	0.57	0.85	1.9 (± 2.3)	8.3 (± 10.7)	0.80	0.15	0.80
Isla Negra	33° 26' S	2.2 (± 1.6)	6.6 (± 1.4)	19.4 (± 8.7)	16.6 (± 4.9)	0.50	0.75	2.2 (± 3.8)	2.4 (± 2.0)	0.69	0.08	0.50
Pelancura	33° 34' S	5.1 (± 2.3)	9.5 (± 2.5)	12.4 (± 4.4)	14.1 (± 2.5)	0.47	1.00	1.5 (± 2.3)	3.9 (± 2.9)	0.86	0.74	1.00
Infiernillo	34° 23' S	n.a	4.3 (± 2.2)	n.a	25.9 (± 5.0)	n.a	1.00	n.a	10.9 (± 7.2)	n.a	1.0	0.66
Caleta Piure	37° 14' S	13.6 (± 5.7)	3.4 (± 1.3)	14.0 (± 4.6)	18.5 (± 6.5)	0.80	0.93	1.9 (± 2.1)	5.2 (± 3.2)	0.85	0.47	0.76
Tirua	38° 20' S	15.8 (± 9.9)	7.2 (± 3.2)	6.6 (± 1.5)	11.8 (± 4.9)	0.73	0.71	1.5 (± 1.4)	1.6 (± 1.8)	0.17	0.14	0.25
Calfuco	39° 46' S	7.4 (± 4.1)	5.6 (± 3.5)	13.8 (± 5.2)	17.5 (± 5.5)	0.79	0.93	5.1 (± 5.1)	4.8 (± 4.2)	0.64	0.35	0.88
Mean (Total)		7.5 (± 4.1)	5.4 (± 2.5)	14.4 (± 3.8)	18.0 (± 5.7)	0.57	0.87	2.0 (± 3.2)	5.8 (± 7.0)	0.65	0.31	0.77

Da : *D. antarctica*; *L.s*: *L. spicata*; *S.s*: *S. scurra*; *S.s*/alga: number of *S. scurra* per alga.

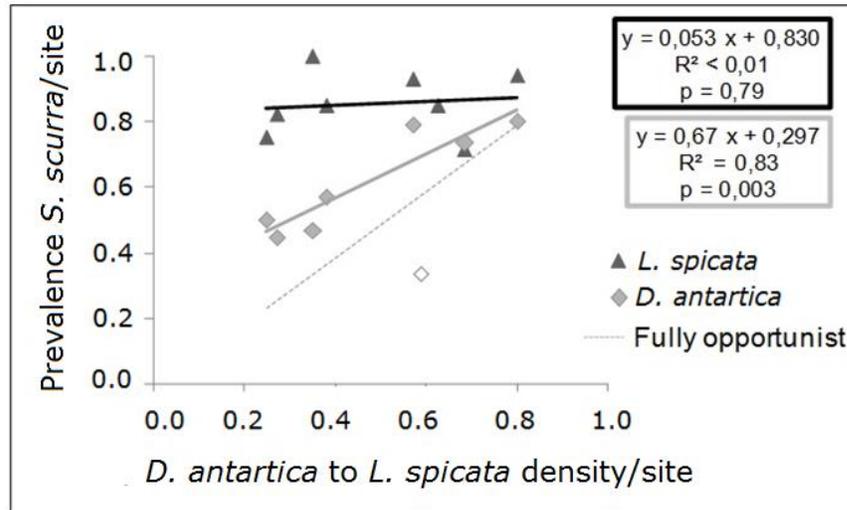


Figure 1. Prevalence of *S. scurra* on *L. spicata* and *D. antarctica*, relative to density of ratio of *D. antarctica* to *L. spicata* numbers, in Central Chile. Empty rombus indicates the *S. scurra* prevalence in Las Salinas, which was not considered in correlation because *D. antarctica* discs were unusually dominated by different species of crabs. A fully opportunist herbivore uses the different resources exactly in proportion to their relative availabilities in the environment (in this case, the relative availabilities of *L. spicata* and *D. antarctica*).

3.1.2 Component 2: standardized degree of utilization according to host structural complexity

The disc height or the disc diameter were the most correlated disc traits (Pearson partial correlations) associated to: 1) the number of *S. scurra* per disc (mostly in *D. antarctica*; $r = 0.61$, $p < 0.001$); and 2) the number of *S. scurra* per disc and stipes in *L. spicata* ($r = 0.63$, $p < 0.001$); and 3) the size of other structural traits measured in both algal species (not shown). Then, the disc diameter or the disc height (in *L. spicata* and *D. antarctica*, respectively) were used as general predictors of the *S. scurra* load in both hosts.

A structure that is comparable in *L. spicata* and *D. antarctica* in terms of space available to inhabit for *S. scurra* is the disc. The increase of the most important disc trait (the

disc height in *D. antarctica*, and the disc diameter in *L. spicata*) was a well predictor of the probability of *S. scurra* presence in discs of each species (predicted values of logistic binary regressions seen in Fig. 2B and D and estimated coefficients in Table 2, plus rough data of the *S. scurra* load and kelp disc sizes in Fig. 2 A and C). Every one centimeter increase of the disc height of *D. antarctica* implied a 2.05-fold (IC95% = 1.50-2.78) increase of the odds ratio of *S. scurra* being present in those discs. *D. antarctica* disc heights of 3.4 cm or higher translated into a probability of 50% or higher of observing *S. scurra* in the disc (Fig. 2D). In *L. spicata* , the odds ratio of observing an increase of the *S. scurra* presence in the disc with every 1 cm increase of the disc diameter was only of 1.09 (IC95%:1.01-1.19). Most *S. scurra* in *L. spicata* were juveniles. Nonetheless, the probability of *S. scurra* being present in the discs of *L. spicata* (Fig. 2B) was always higher than 0.4 (and higher than 0.5 for discs larger than 7.4 cm). This trend arose probably because most *L. spicata* reach bigger sizes than *D. antarctica* and most sampled sites, except one, showed mean disc diameters larger than 12 cm (Table 1).

In *L. spicata*, the *S. scurra* load was higher in stipes than in the disc. It was more correlated to the mean stipe diameter than to the disc diameter or to the distance of the first bifurcation in stipes, and was not correlated to the number of stipes (logistic regressions in Table 2, and plots of the predicted probabilities of *S. scurra* presence in Fig. 3A to 3J). Every cm increase of the maximum disc diameter exhibited a significant and constant increase of the probability of observing *S. scurra* both in stipes and the disc (IC95%:1.12-1.65, Fig. 3B) or only in stipes (IC95%:1.43-1.69, Fig.3D). *L. spicata* discs with a diameter equal or larger than 5.3 cm exhibited a probability of 50% or higher of *S. scurra* being present. When evaluating the effect of an increase of every 10 cm of disc diameter, the odds ratio of the *S. scurra* presence both in discs and stipes or only in stipes was even more evident (IC95%:3.12-

148.53, and IC95%:6.70-192.14, respectively). Every cm increase of the mean stipe diameter was also a good predictor of the probability of the *S. scurra* presence in *L. spicata* (IC95%:1.93-11.84, Fig. 3F). The mean distance to the first bifurcation of stipes had also a significant predictive power of the *S. scurra* presence probability but considerably lower than the other *L. spicata* traits (IC95%:1.12-1.24, Fig. 3H). On the contrary, an increase of the number of stipes didn't translate into a rise of the probability of the *S. scurra* presence. Indeed, there was a high probability of *S. scurra* being present both in plants with few or high numbers of stipes (Fig. 3J). As revealed by the odds ratio of the previous logistic regressions, the number of *S. scurra* per plant (disc and stipes) seemed to steadily increase more in *L. spicata* than in *D. antartica* (only discs) along with the rise of the kelp size (comparing the maximum numbers of *S. scurra* per alga, of figures 2C and 3A). This steady increase is most probably explained by the larger sizes (Table 1) and structural complexity of *L. spicata* compared to *D. antartica*.

When focusing exclusively at the level of the kelp disc, the *S. scurra* loads (Fig. 2A and C) and the probabilities of *S. scurra* presence (Fig. 2B and D) were better predicted in *D. antartica* than in *L. spicata*. The net density of *S. scurra* per unit of disc volume (number of *S. scurra* per cm³) was considerably greater in *D. antartica* than in *L. spicata* discs, specifically for *D. antartica* discs smaller than 500 ml. Nevertheless, both in *D. antartica* and *L. spicata*, the net density of *S. scurra* decreased with increasing disc volume (Fig. 4A and 4B). On the contrary, *S. scurra* loads (number of *S. scurra* per disc) were positively correlated to the disc volume, in both *L. spicata* and *D. antartica*.

Table 2. Coefficients and Wald tests for logistic regression on the *S. scurra* presence and size of structural traits of host-kelps.

Kelp species	Algal trait and constant	Coefficient	SE	Wald	df	p	OR	95% CI for OR	
								Lower	Upper
<i>D. antarctica</i> (<i>S.s</i> in discs)	Disc height	0.716	0.157	20.851	1	0.000	2.05	1.50	2.78
	Constant	-2.450	0.625	15.369	1	0.000			
<i>L. spicata</i> (<i>S.s</i> in discs)	Disc diameter	0.090	0.042	4.653	1	0.031	1.09	1.01	1.19
	Constant	-0.665	0.590	1.271	1	0.259			
<i>L. spicata</i> (<i>S.s</i> in discs and stipes)	Disc diameter	0.306	0.098	9.706	1	0.001	1.36	1.12	1.65
	Constant	-1.620	1.013	2.568	1	0.109			
<i>L. spicata</i> (<i>S.s</i> only in stipes)	Disc diameter	0.357	0.085	17.494	1	0.000	1.43	1.21	1.69
	Constant	-3.182	0.953	11.191	1	0.000			
<i>L. spicata</i> (<i>S.s</i> only in stipes)	Stipe diameter	1.565	0.462	11.452	1	0.001	4.78	1.93	11.84
	Constant	-1.132	0.633	3.203	1	0.073			
<i>L. spicata</i> (<i>S.s</i> only in stipes)	Distance 1st bifurcation	0.113	0.053	4.467	1	0.034	1.12	1.01	1.24
	Constant	0.059	0.516	0.014	1	0.909			
<i>L. spicata</i> (<i>S.s</i> only in stipes)	Number of stipes	0.016	0.016	0.000	1	0.994	1.02	0.98	1.05
	Constant	0.776	0.381	0.000	1	0.994			

CI, confidence interval; df, degrees of freedom; OR, odds ratio; SE, standard error; *S.s*, *S. scurra* presence; 1st, first.

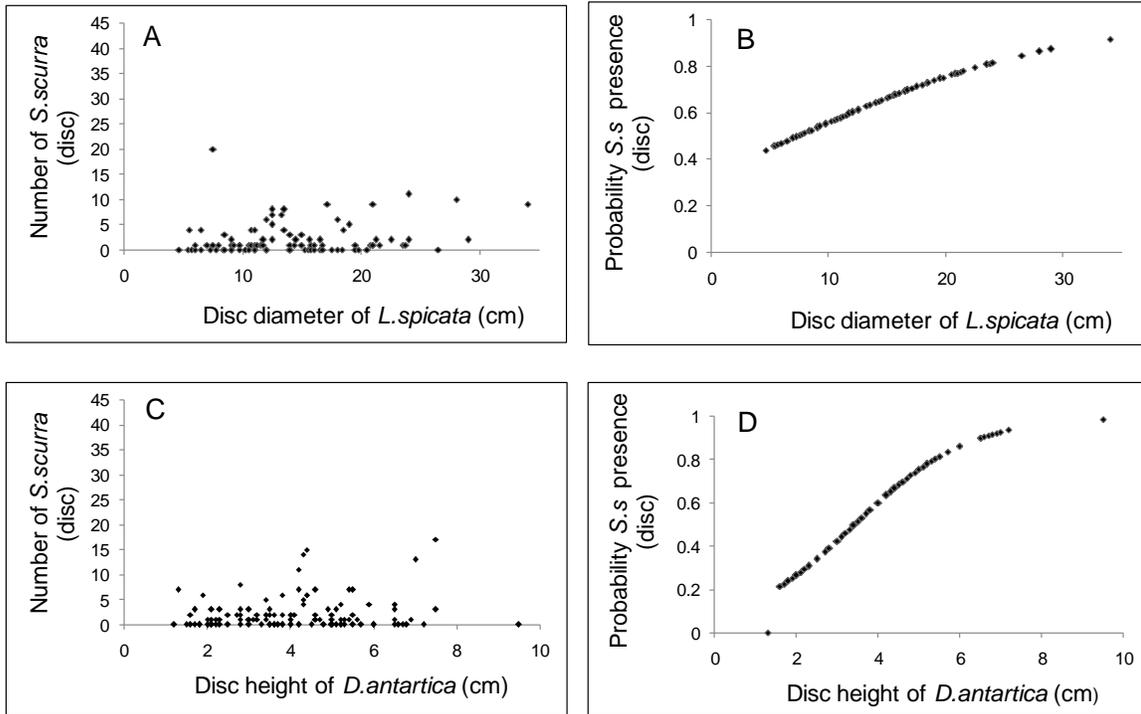


Figure 2. Number of *S. scurra* per disc (A and C) and probability of *S. scurra* presence in discs of *L. spicata* and of *D. antarctica* (B and D) of different sizes, according to odds ratio of logistic regressions. S.s: *S. scurra*.

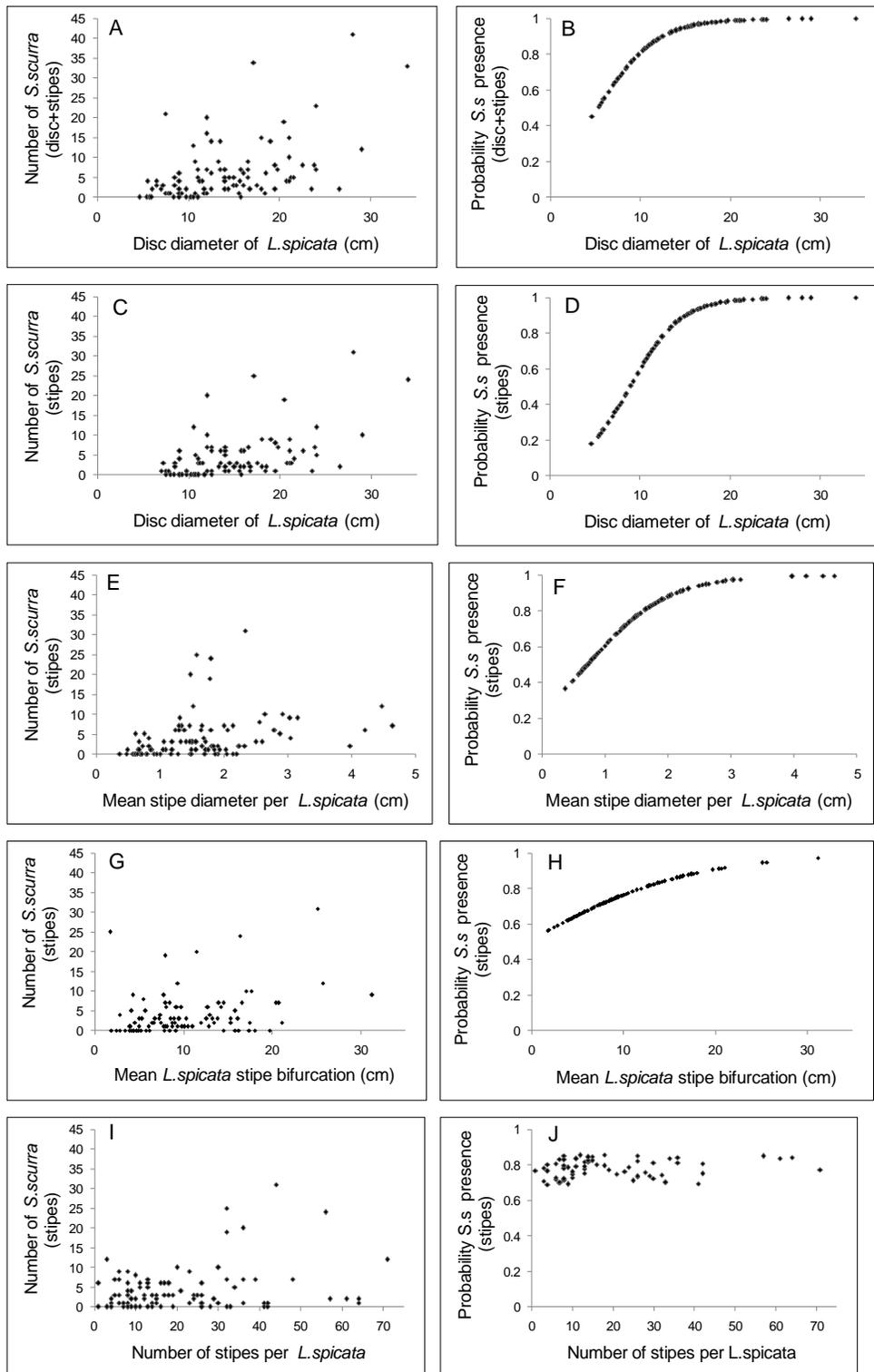


Figure 3. Predictive relationship between the size of *L. spicata* structural traits and the number of *S. scurra* per plant (left) and the probability of *S. scurra* presence (right), respectively (in discs and stipes, or exclusively in stipes). *S.s*: *S. scurra*; stipe bifurcation: distance from the base to the first bifurcation in a stipe.

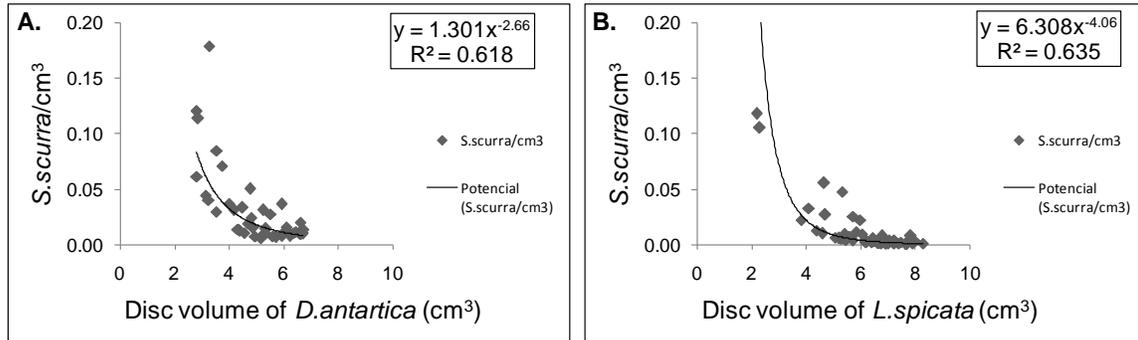


Figure 4. Density of *S. scurra* as a fraction of disc volume. Points correspond to the density of *S. scurra* for discs of different volumes. A potential fitted curve is shown for *D. antartica* and *L. spicata*.

3.1.3 Component 3: Relative performance in different hosts

While regionally the prevalence of *S. scurra* was clearly lower in *D. antartica* than in *L. spicata*, the mean shell diameter of *S. scurra* in stipes and discs of *L. spicata* ($1.21 \text{ cm} \pm 0.63$) was not statistically different from the one observed in *D. antartica* discs (1.25 ± 0.69) (GLM test, $F_{1, 537} = 0.47$, $p = 0.49$). On the contrary, the shell diameter in *L. spicata* discs ($0.87 \pm 0.48 \text{ cm}$) was significantly smaller from that in *D. antartica* discs (GLM test, $F_{1, 332} = 29.62$, $p < 0.001$). In *L. spicata*, juveniles (shell diameter $< 10 \text{ mm}$) seem to inhabit mostly the disc (mean proportion of 0.69 juveniles), whereas adults occur mostly in stipes (mean proportion of 0.77 adults). Accordingly, the mean shell diameter of *S. scurra* found in the stipes of *L. spicata* ($1.42 \text{ cm} \pm 0.60$) was almost two times higher than that at the disc of the same species ($0.87 \pm 0.48 \text{ cm}$) (GLM test, $F_{1, 327} = 72.13$, $p < 0.001$). The mean shell diameter of *S. scurra* in stipes of *L. spicata* was also superior and statistically different from the one observed in *D. antartica* discs (GLM test, $F_{1, 413} = 6.77$, $p = 0.01$). On the other hand, when focusing on individual algae, *S. scurra* shell diameter reached a potentially greater maximum

size in *L. spicata* stipes (up to 4 cm, Fig. 5A) than in *D. antarctica* discs (2.8 cm). Nonetheless, in *L. spicata* two sites exhibited relatively high proportions of *S. scurra* adults inhabiting the discs (Pelancura and Caleta Piure, Table 1). In *D. antarctica*, most discs harbored a high proportion (0.65) of adult *S. scurra* (Table 1).

A significant correlation between the shell size of *S. scurra*, and the size of morphological traits in *L. spicata* and in *D. antarctica* (the stipe diameter, or the disc height, respectively) seems to exist (Fig 5A and B). In *L. spicata*, the correlation is still significant when averaging the *S. scurra* size per plant (Fig 5C) rather than by stipe. On the other hand, in *L. spicata*, when performing a stepwise regression adding as predictive variables other morphological traits additionally to the stipe diameter, the R^2 value didn't vary considerably.

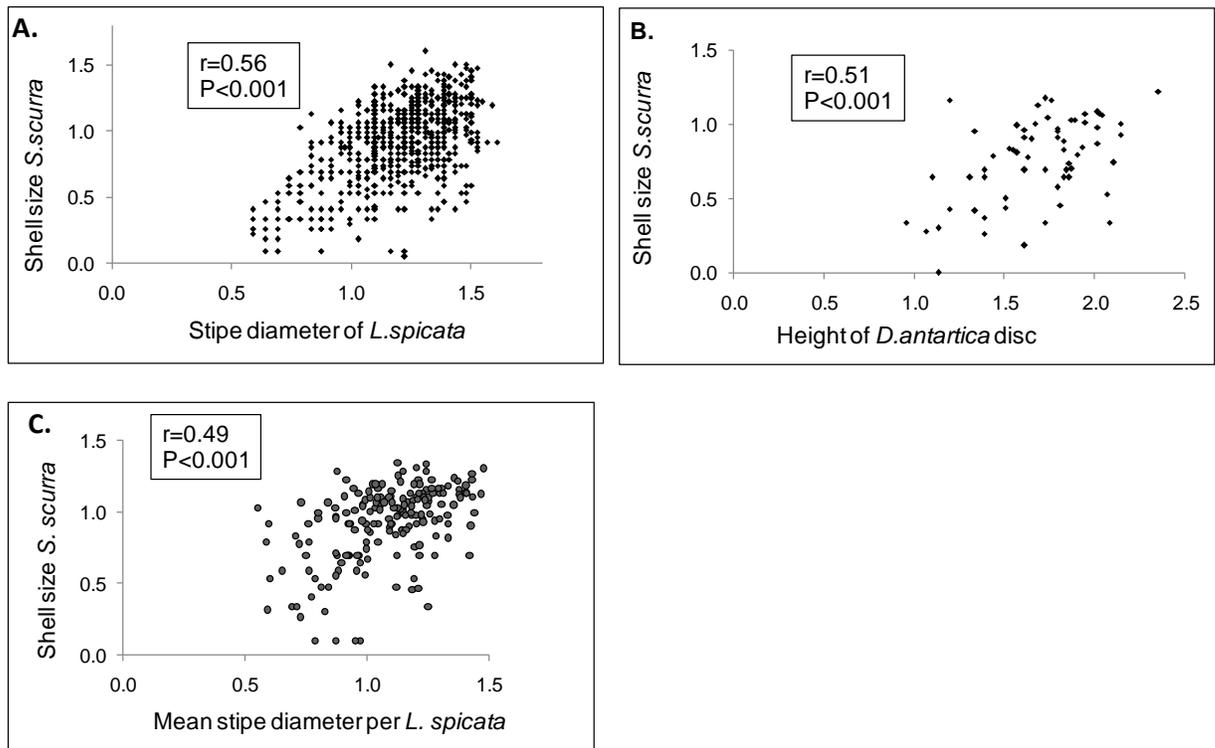


Figure 5. Relationship between shell size of *S. scurra* and the size of structural traits of its hosts. Figures 5 A and 5 B show log-transformed data ($\text{Log}_e(x+1)$) of the *S. scurra* shell diameter and its better correlated structural trait in *L. spicata* (the stipe diameter, in B) and in *D. antarctica* (the disc height, in D) to which Pearson correlation were fitted. C show the same data than in A but averaging the *S. scurra* shell size per plant (rather than per stipe).

4 DISCUSSION

The previous study of Espoz (2009) demonstrated that *S. scurra* has a very narrow range of algae used as hosts (e.g. only the *L. nigrescens* complex and *D. antartica*), corresponding to component 1 of ubiquity. Our rough characterization of component 2 and 3 in *S. scurra* allowed us to go a step further in the determination of the level of ecological specialization of this patella. We demonstrated that, in terms of prevalence in its two host-kelps (component 2, degree of ubiquity) (i) *S. scurra* has a very selective use of *L. spicata* as host whereas it has an opportunistic use of *D. antartica* as its second most preferred host; (ii) *S. scurra* exhibits an uneven density in its two host kelps, reaching higher loads in *L. spicata* than in *D. antartica*, which means it is selective, or restricted, in terms of its degree of ubiquity. Going a step further, our characterization of component 2 concerning microhabitat or structure complexity showed that: (iii) the odds ratio of the *S. scurra* presence increases significantly with increasing size of specific structural traits related to kelp shape in *L. spicata* (mainly the mean stipe diameter) and in *D. antartica* (the disc height). This last trend is apparently in contradiction with prevalence data, in the sense that *S. scurra* occupies opportunistically structures of both *L. spicata* and *D. antartica* according to their sizes and shape. Nevertheless, *S. scurra* presence (and thus prevalence) is more likely in *L. spicata* mainly because of its higher structural complexity, larger sizes, and longer lifespan than *D. antartica* (with 2-3 times higher maximum *S. scurra* loads per plant in *L. spicata* than in *D. antartica*). Finally the survey of *S. scurra* shell sizes (our proxy of performance or component 3) suggests that larger *S. scurra* sizes are reached in *L. spicata* than in *D. antartica*. On the other hand, size categories distribution indicates that *S. scurra* is specialized to occupy

different structures in its two hosts: whereas both adult and juvenile *S. scurra* individuals occur in the disc of either *L. spicata* or *D. antarctica*, only adults were observed in the stipes of *L. spicata*. Thus, this survey highlights the leading role played by particular structures in kelps as a resource determining a high fitness for specialized herbivores. This is complementary and concordant to previous studies demonstrating the important role played by the size of kelp structures in the distribution pattern and higher abundance of the opportunistic fauna living associated to them (Christie et al. 2003, 2007; Norderhaug & Christie, 2007, 2011).

Hacker and Steneck (1990) demonstrated that the distribution and abundance of an algal amphipod depends on the scaling between its body size and sizes and habitat architecture of its host algae. Our study also indicates that there is a scaling between the body size of *S. scurra*, and the size and shape of specific structures that it occupies in *L. spicata* and *D. antarctica* (stipes and disc, respectively, in Figures 5A and B). This scaling in body-size between the patella and size and shape of kelp structures would also explain its presence, abundances and distribution in specific structures of *L. spicata* and *D. antarctica* (Figures 3A-J). We would add that *S. scurra* is able to occupy with exclusivity and select as habitat a stressful structure such as the stipes of *L. spicata*. As previously explained, most competitors or predators, like other patellae and crabs, remain out of the reach of the stipes and fronds of this kelp as a consequence of the whiplash effect of fronds and stipes agitated by waves (Ojeda and Santelices 1984). This selectivity and exclusivity in habitat use defines precisely the specialization of *S. scurra* to *L. spicata*. It enables *S. scurra* exploiting resources within this microhabitat without the constraints imposed by competition and predation. Along with the

larger sizes and longevity reached by *L. spicata* in comparison to *D. antarctica* algae, it would also explain why prevalences and abundances are larger in *L. spicata* than in *D. antarctica*.

The above results should be nonetheless interpreted with caution, because they were inferred based on indirect estimations of the *S. scurra* behaviour and the shape of kelp structures. Microhabitat specialization in *S. scurra* would require directly testing of its specialized behaviour that determines host/habitat preference. For instance, the potential occurrence of “kairomones” (i.e.chemical host-recognition cues governing preference) could allow the patella to recruit or move to *L. spicata* or *D. antarctica*. It is yet unclear whether juveniles recruit on the disc and larger and mature *S. scurra* migrate onto the stipes of *L. spicata* (as suggested by Santelices 1980), or they recruit evenly in larger *L. spicata* but survive and grow better on stipes. A survey of recruitment and growth of different *S. scurra* would help discriminate between these two options.

Our study is in agreement with previous experiments performed by Muñoz and Santelices who showed that *S. scurra*: (i) stay mainly in the proximal parts of *L. spicata* stipes (before the first bifurcation, close to the holdfast). Moreover, these authors demonstrated that (ii) the main factor governing this distribution is the friction generated by waves between stipes of *L. spicata*, which is stronger above the first stipe bifurcation, and that (iii) *S. scurra* only moves from its home-cavity during high tide, and stay in the cavity during low tide. Then *S. scurra* has probably a tuned behaviour. Indeed, we performed two preliminary experiments inquiring about mobility of *S. scurra* in *L. spicata*. In the first experiment we removed all *S. scurra* individuals from a first group of *L. spicata*, and overloaded an adjacent group of *L. spicata*, employing a third untouched group of *L. spicata* algae as control. After 11 days, three out of five plants of the removal treatment and two of five plants of the control group acquired

new *S. scurra* individuals not present at the beginning of the experiment. In the second experiment, we only marked and counted all *S. scurra* found on stipes of 15 adult *L. spicata* without removing any individual. After 11 days, about 94% *S. scurra* individuals of this almost unaltered system had remained in the same home-cavity. These results suggest that *S. scurra* can be highly mobile to allow colonization of empty stipe cavities, but they display a high fidelity to their cavity when undisturbed. On the other hand, as previously mentioned Camus et al. (2008) showed that *S. scurra* living in *L. berteroana*, the sister species of *L. spicata*, have a very narrow diet. Preliminary observations under microscope made by us of the content of the digestive tract of *S. scurra* in Central Chile indicated that most tissues in its diet belong to the host alga from where the animals were collected (*L. spicata* or *D. antartica*). Therefore, the movements of *S. scurra* are not leading to new habitat where alternative resources can be consumed, but rather to explore, within the same host, alternative stipes and cavities where they can eat and escape from predators and competitors at the same time.

The different distributions of adults and juveniles of *S. scurra* quantified in the disc and stipes of these two kelps might suggest that *S. scurra* has a different degree of dietary specialization for the food offered by *L. spicata* in comparison to *D. antartica*. However, the availability of microhabitat for *S. scurra*, which is higher in *L. spicata* than in *D. antartica*, seems a better explanatory variable for the presence of the herbivore. Even though trophic resources provided by *D. antartica* discs should allow reaching larger sizes, *S. scurra* is apparently limited in growth because of the high risk of dying together with its host *D. antartica* whose lifespan is shorter and probability of detachment is higher than *L. spicata* (Santelices et al. 1980). Indeed, most *L. spicata* live more than 20 months whereas a maximum of 6 months is reported for *D. antartica* (Santelices et al. 1980). Then, *S.*

scurra inhabiting and eating in *L. spicata* could likely grow, survive and reproduce more than in *D. antarctica* simply as a matter of the longer lifespan of the host.

Accordingly, the higher densities of *S. scurra* quantified here in *L. spicata* over *D. antarctica* would have probably translated into higher selective pressures and better adaptation to *L. spicata* microhabitats and its resources than to *D. antarctica*. The related expectation is then that demographic fluctuations of *S. scurra* over the long term scale would follow *L. spicata* population fluctuations rather than those of *D. antarctica*. A second likely expectation is that local adaptation of *S. scurra* to *L. spicata* is probably operating; thus, one would envision a high concordance of the spatial genetic structures at the geographical scale (or phylogeographic clustering) between this two interacting organisms.

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**CHAPTER III: COMPARATIVE
PHYLOGEOGRAPHY OF A SPECIFIC BUT
RECENT ASSOCIATION BETWEEN A
SPECIALIST MARINE HERBIVORE AND ITS
HOST KELP IN THE SOUTHEAST PACIFIC
COAST: CONCORDANT SPATIAL GENETIC
STRUCTURES AND SIMILAR DEMOGRAPHIC
TRAJECTORIES SINCE THE LAST GLACIAL
MAXIMUM.**

1 INTRODUCTION:

The main goal of phylogeography is to describe the distribution of genealogical lineages across geographical landscapes (Avice et al. 1987). The joint survey of the phylogeography of co-distributed species, or comparative phylogeography, involves establishing to what degree common historical events similarly shaped the genetic structure in populations of these co-distributed species (Arbogast & Kenagy 2001). Co-distributed species may just share the geographic range, and therefore be under the influence of the same macro-climatic events. But they can also be more tightly interacting species, such as mutualists, a parasite and its main host, a specialist herbivore and its main plant resource. In this case, there may be in addition a demographic interaction that can further lead to congruent phylogeographic patterns. The degree of phylogeographic congruence, and indirectly common demographic patterns between interacting species is an issue that fall into what are currently the major issues of comparative phylogeography (e.g. Zink 1986, Avice 2000). The inclusion of demographic patterns is justified because, as noted by Zink (2002), comparative phylogeography is not only concerned with inferring isolating barriers of environmental or ecological origin, but also with recent population contractions or expansions.

Partially concordant phylogeographic patterns have been observed in terrestrial systems for some plant-insect associations having experienced the great variability of quaternary climatic cycles, and forced to modify their distribution range during the successive warm and glacial cycles (herbivores tracking their plants; e.g. Downie et al. 2001, Ahern et al. 2009, DeChaine and Martin 2006, or Aoki et al. 2011). Most of these studies have found concordance between phylogeographic structures at the geographical scale but not at the local

scale. Such is the case, for example, in the survey of DeChaine and Martin (2006) who found concordant phylogeographic clusters among an alpine specialist herbivore (a butterfly) and its plant. Most of these surveys comparing the phylogeography of specialist insect herbivores or the insect and the plant have employed mainly mitochondrial or chloroplastic markers, which have a small effective population size (N_e) and relatively slow mutation rates. More generally, the disparate levels of phylogeographic and demographic concordance found to date among closely associated and co-distributed taxa have been interpreted as resulting from at least three factors (according to Smith et al. 2011): 1) the interacting species have different dispersal potentials or niche requirements (e.g. Carsten & Richards 2007); 2) numerous refugia and corridors for dispersal existed in the past, depending on the specific local and geographical landscapes, which precluded them having common genetic structures shaped exclusively by isolating barriers; or 3) the degree of phylogeographic concordance depends on the strength and specificity of the ecological interactions (e.g. Jackson & Overpeck 2000, Whiteman et al. 2007).

A recent survey of the comparative phylogeography among four obligate specialist yucca moths and their Joshua trees revealed that they experienced concerted population expansions during the Pleistocene (Smith et al. 2011). The comparative phylogeography of four other specialized yucca moth species suggested that phylogeographic histories are influenced by both the strength of interactions (e.g. mutualistic or commensalistic) and specifically where do insects feed on their host plants (Althoff et al. 2007). Then, major environmental changes affecting populations of a host plant could have had similar demographic consequences on their specialist herbivores, depending on the strength and specificity of their ecological interactions, and on the relative strength of intrinsic and extrinsic

factors. At a microevolutionary scale, a high ecological dependence of a specialist herbivore on its host plant(s) could likely lead to an important degree of dependence between its effective population size and the demographic dynamics of its host plant populations. Indeed, several ecological surveys have found that the density and connectivity among herbivore populations (above all specialist herbivores) depends on the size and stability of the host plant patches, on distances between these patches, and on patch quality (Nieminen 1996, Debinski & Holt 2000, Chapman et al. 2007, Ekroos et al. 2010). Moreover, being largely accepted that population dynamics of herbivores are density dependent; in specialist herbivores, recent studies support the fact that the shape of this density dependence is controlled by important plant quality traits (i.e. reproductive effort, or concentration of some secondary compounds) that are correlated with herbivore performance (e.g. Larsson et al. 2000, Rotem & Agrawal 2003, Miller 2007). Therefore, major climatic or oceanographic events that affected primary productivity could also have affected patch quality and population dynamics of both the plant and its specialist herbivore(s).

We used as biological model the patellogastropod *Scurria scurra* specialized preferentially to the intertidal kelp species complex *Lessonia nigrescens* Bory, recently recognized as two parapatric species *Lessonia berteroana* Montagne and *Lessonia spicata* (Suhr) Santelices (Tellier et al. 2009; González & Reviers 2012). *Lessonia berteroana* is distributed in the lineal south-eastern Pacific coast from 12°S to 30°S, while *Lessonia spicata* is mainly distributed from 30°S to at least 42°S. A few isolated *L. spicata* populations are scattered between 29°S and 30°S, in the middle of an area dominated by *L. berteroana*, with no evidence however of gene flow and hybridization between the two species (Tellier et al. 2011). Between 32°S and 42°S, *S. scurra* also inhabits the kelp *Durvillea antarctica*, which

occupies opportunistically and gradually toward southern areas, as stands of *D. antarctica* increase and *L. spicata* lower their relative densities (Espoz 2002). Nevertheless, the *L. nigrescens* complex constitutes the main habitat for *S. scurra*. It has been proposed that this preference is due to the structure of the kelp where *S. scurra* finds a habitat complexity able to accommodate its highest prevalence and abundance per alga (Muñoz & Santelices 1989, Espoz 2002, Meynard et al. unpublished). A specialization to *Lessonia* as the main trophic resources has also been proposed (Muñoz & Santelices 1989, Camus 2008). On the other hand, the interaction between *S. scurra* and *Lessonia* is relatively recent (2 MYA, according to Espoz 2002) and its evolution seems posterior to the diversification of the 2 *Lessonia* species (estimated at 2-4.5 MYA; Martin & Zuccarello 2012). Due to its specialized use of the intertidal kelp (see Chapter 2), and exclusive interaction north of 32°S, *S. scurra* is then good model to search for recent and concordant phylogeographic and demographic patterns with *Lessonia*.

Phylogeographic analysis of Tellier et al.(2009) showed the presence of a major genetic break within *Lessonia* at 30°S (separating *L. berteriana* from *L. spicata*) and splitting the coast on two biogeographic areas, the Peruvian Province or PP (12°S-30°S) and the Intermediate Area or IA (30°S-42°S) (sensu Camus 2001). This genetic discontinuity was observed in the same region for a number of invertebrate species (*Crepidula dilatata*, Brante et al. 2012; *Excrolana braziliensis*, Varela & Hayes 2012; *Tegula atra*, Hayes & Faugeron, pers. com.). The 30-33°S zone corresponds to a biogeographic transition along the Chilean coast where the range limits of a large number of species have been reported to occur (Santelices 1980, Camus 2001). There is uncertainty about the factors (historical events or current oceanography) that originated this discontinuity in community composition. The most

likely factor is an oceanographic discontinuity in eddy kinetic activity and degree of variability in equatorward wind stress affecting the intensity and variability of upwelling forcing, reported at approximately 30°S by Hormazabal et al. (2004, but see Villagran 1995 for the advance of glaciers at 30°S as an historical factor). This biogeographic and genetic discontinuity could also have its origin on a displacement of the West Wind Drift north of its current position during the LGM. The displacement of this major driver of ocean circulation would have induced a northward shift of the Humboldt current system and its accompanying upwelling regimes, with a likely location at 30°S during the LGM (Klump 1999, Williams & Bryan 2006, Toggweiler et al. 2006). A number of species do not show any evidence of genetic discontinuity in this region, suggesting that the phylogeographic break could depend on life-history characteristics related to development type and dispersal potential. Indeed, while both a direct developer (*C. dilatata*, Brante et al. 2012) and a low dispersive invertebrate having lecithotrophic larvae (*Notochamalus scabrosus*, Zakas et al. 2009) showed a genetic break at 30°S as did *L. nigrescens*, the genetic structure of other invertebrates (*Concholepas concholepas*, Cardenas et al. 2009, and *Jehlius cirratus*, Zakas et al. 2009), a vertebrate (*Lontra felina*, Vianna et al. 2010) and the kelp *Macrocystis pyrifera* (Macaya and Zuccarrello 2010), with higher dispersal potentials, do not show any evidence of a genetic break.

Based on the previous knowledge, we hypothesize that, because *S. scurra* probably shared similar environments with its host *Lessonia* during most of the Pleistocene (Espoz 2002, Macaya and Zuccarrello 2010), *S. scurra* genetic diversity shows the same phylogeographic breaks verified previously in *Lessonia*, at the 30°S or the 27°S. To answer the latter hypothesis, we genetically characterized individuals of *S. scurra* along the Chilean coast from 26°S to 42°S with the mitochondrial COI marker and the highly variable dominant

markers AFLP. Our first objective entailed establishing the number of genetic (COI and AFLP) clusters or demes in *S. scurra* along the Chilean coast and verifying if *S. scurra* genetic structure reproduces one of the former genetic breaks of biogeographic origin (30°S) or unique to its host kelp phylogeography (27°S).

In the second place, we investigated the potential demographic changes experienced by both species due to the Glacial-Interglacial transition. Indeed, the northward shift of the West Wind Drift apparently modified the intensity of upwelling south of 30°S, reducing primary productivity (Klump 1999, Williams and Bryan 2006, Toggweiler et al. 2006). Therefore, coastal populations likely experienced demographic changes associated with the reduction of primary productivity during the glacial period, followed by a population growth after the reestablishment of the upwelling. Such a signature of recent demographic growth was detected for *L. spicata* (“southern clade” in Tellier et al. 2009), although the precise date of the event could not be inferred. Unfortunately, in-depth inferences of demographic changes was not performed in any of the published phylogeographic studies of the Chilean coast, and the question about the role of post-glacial demographic dynamics remains unanswered. Here, we hypothesize that both *S. scurra* and *L. spicata* experienced similar and contemporaneous demographic changes during the Late Pleistocene. Our second objective then involved reconstructing the changes in the effective sizes of each of the geographical clusters or populations of *S. scurra* previously defined in objective one. A second related section of this objective involved simulating different biogeographic scenarios and assessing basically if: i) if *S. scurra* and *L. spicata* followed a classic scenario of extinction of its southernmost populations during the LGM, and post-glacial recolonization from northern refugees or if ii) *S.*

scurra and *L. spicata* geographic clusters of the southernmost populations have contracted during the LGM, and independently recovered post-glacially after the LGM.

In the third place, as previously explained, *S.scurra* displays a high degree of specialization and habitat specificity to the intertidal *Lessonia*. Moreover, demographic changes and probably at some levels phylogeographic structuring of *S. scurra* have reproduced those of its host *L. spicata* . Dispersal potential is higher in *S. scurra* (pelagic larvae in the Lottiidae spend from three days to two weeks in the water column, Kolbin and Kulikova 2011, Gilman 2006) than in *L. spicata* (spores last no more than a day in the water column according Martinez and Santelices 1998, although occasional rafting has been observed when individuals are entangled with floating kelps such as *Macrocystis pyrifera* or *Durvillaea antarctica*, Martin Thiel pers. com.), and therefore genetic differentiation between populations could be less intense in the former species. However, because the specialization of *S. scurra* to its host implies some role of selective evolutionary process, genetic divergence in the herbivore could be expected to match the divergence of its host if, for example, speciation between *L. spicata* and *L. berteroana* involved some evolution in its response to herbivory. Indeed, because both species are located on both sides of the 30°S biogeographic transition, the invertebrate community interacting with each kelp species is different and could have played a role during the parapatric speciation. Accordingly, we hypothesize that the spatial genetic structure in *S. scurra* would be highly correlated to that of *L. spicata* . Our third objective then consisted in establishing the degree of similarity between geographical clusters in *S. scurra* and *L. spicata* (established using the COI or AFLP's, and the *atp8S*, respectively). We further assessed the degree of correlation of genetic distances between pairs of populations of the herbivore and its kelp (correlating pairwise distances in *S. scurra* to *L.*

spicata pairwise distances). Similar approaches have been undertaken previously in order to assess the interconnection between host plants and their specialized insect herbivores and evaluate their potential for co-differentiation (e.g. Michalakis et al. 1993, Anderson et al. 2004, Ahern et al. 2009, or Nieberding et al. 2004 for a nematode parasite and its mouse host). Finally, as a fourth objective, we explored the possibility of *S. scurra* populations being genetically differentiated between its two main habitats, *L. spicata* and *D. antarctica*. Under strong selection between habitat, some genomic regions can be under positive selection and show a higher than expected genetic differentiation (Beaumont and Balding 2004). Therefore, the objective was to look for candidate loci likely under selection and compare the purely spatial genetic structure with the genetic differentiation between host species.

2 MATERIAL AND METHODS

2.1 SAMPLING FOR COI AND AFLP GENOTYPING

Tissue samples of 377 patellae, to be sequenced with the mitochondrial COI marker, were collected from twelve locations along the lineal Chilean coast (26°S to 42°S) during 2009 and 2012. We used some of the latter and new samples collected from different localities during 2009, to perform a population genetic study employing AFLP's markers, with tissue of 321

patellae belonging to twelve sites in Chile (Table 1). Tissue was taken from the locomotive foot and placed in 95% ethanol.

2.2 DNA EXTRACTION AND COI AMPLIFICATION

Total DNA of *S. scurra* was extracted using the saline extraction protocol (Aljanabi & Martinez, 1997). A partial sequence of the mitochondrial Citochrome Oxydase I gene (COI) was amplified using the universal primers LCO1490 and HCO2198 of Folmer et al. (1994). Polymerase chain reaction (PCR) were carried out in a 25 μ l reaction volume consisting of 2.5 μ l 10X buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.0), 1.0 μ l of 50 mM of MgCl₂, 2 μ l dNTPs (10 μ M), 0.5 μ l of each primer (10 pg/ μ l), 1 U Taq (Invitrogen), 17.5 μ l of ultrapure water plus 100 ng of DNA. Cycle parameters consisted of an initial denaturation step at 95°C for 3 min, followed by 35 cycles at 94°C for 40 s, 48°C for 40 s, and 72°C for 90 s, and performing a final extension at 72°C for 7 min. PCR products were purified and automatically sequenced (Macrogen Inc, Korea). Sequences were 586-bp to 672-bp long and were edited and aligned by eye in BioEdit version 7.1.3 (Hall, 1999).

2.3 AFLP GENOTYPING

Genomic DNA was extracted as previously mentioned for the COI. Restriction and ligation were performed in a single step, using 100 ng of DNA according to Vos et al. (1995). Pre-amplification of fragments was done using the primer combination EcoRI+A/MseI+C. For selective amplification four fluorescently labeled MseI primer sets were used: EcoRI+CTA/MseI+ACC, EcoRI+CTC/MseI+ACT, EcoRI+CTT/MseI+AGC and

EcoRI+CAC/MseI+AGG. To check for reproducibility of fragment detection, DNA extraction and the previous reaction steps were replicated for 5% of individuals. Peak Scanner software v1.0 (Applied Biosystems, Foster City, California, USA) was used to obtain the size, height and area under the curve (in fluorescence units) for each peak of each individual. Selection of candidate loci was done through developing a macro in Excell (Microsoft, Washington, USA, 2003) modified following Rees (2004) which calculated the total area of peaks for each individual, and pre-selected those peaks having an area superior to 0.5 % of the total area for each individual. The total area of the remaining peaks was recalculated and, through a rounding function, the peak sizes were converted to the nearest integer value (e.g. 105.5 base pair rounded to 106 bp). Loci which were either present or absent in less than 5% of the samples were discarded from subsequent analyses (following Mock et al. 2007). The final step consisted of corroborating by visual inspection in Peak Scanner the fragments selected in the matrix previously generated. Only clear, intense polymorphic peaks between 75bp and 500bp and belonging to unambiguous size categories among all sampled individuals were considered. The presence or absence of bands in each individual was scored as 1 and 0, to generate the binary data matrix used for posterior analyses.

Table 1. Geographical locations of the sampled sites in *S. scurra* for the COI and AFLP markers. N, number of samples per site and marker sequenced or genotyped. In parentheses are indicated the number of *S. scurra* found in a kelp different than *L. spicata*, i.e. *D.a* or *D.antartica*

Sampled sites	Abbreviation	Coordinates (latitude, longitude)	NCOI	NAFLP's
Pan de Azucar	Paz	26°08'S/70°39'W	50	27
Carrizal Bajo	CB	28°04'S/71°08'W	15	25
Isla Damas	ID	29°13'S/71°31'W	6	33
Punta Choros	PCH	29°14'S/71°27'W	32	36
Rio Limari	RL	30°44'S/71°42'W	38	24
Los Vilos	LV	31°54'S/71°30'W	0	20
Las Docas	LD	33°08'S/71°42'W	0	30 (12 <i>D.a</i>)
Quintay	Qui	33°10'S/71°42'W	0	47 (24 <i>D.a</i>)
Canelillo	CA	33°21'S/71°41'W	70	0
Pelancura	PEL	33°34'S/71°37'W	0	19
La Boca	LBo	33°37'S/71°52'W	2	0
Talcahuano	TAL	36°59'S/73°11'W	33	0
Lebu	LB	37°08'S/73°35'W	35	0
Caleta Piure	CP	37°14'S/73°39'W	23	21
Valdivia	VA	39°46'S/73°23'W	40	19
Ancud	AN	41°48'S/74°01'W	33	20
<i>N</i> Total			377	321

2.4 POPULATION GENETIC ANALYSES:

Nei unbiased genetic distances between sampled populations (of the COI and AFLP markers) were generated in GenAlex 6.5 b3 (Peakall & Smouse 2006, 2012). We used Arlequin (Excoffier et al., 2005) to calculate pairwise F_{ST} values of AFLP markers between sampled populations, and to test for their significance values following the non-parametric permutation method of Excoffier et al. (1992).

For objectives one and three, data of COI and AFLP's were searched for the actual number of population clusters in *S. scurra* (across geographical space) using mainly Geneland software (or Structure, only applied for AFLPs). Results from the latter analyses were compared to the same analysis made on mitochondrial atp8S sequences of *L. spicata* (and *L. berteroana*, or on COI sequences in *D. antarctica*). Geneland not only allows clustering of sampled populations into a certain number of groups according to their homogeneity; it also allows incorporating the coordinates of samples using a spatial model. The statistical reliability of the clusters inferred previously were further tested through analyses of molecular variance (AMOVA's) in Arlequin (Excoffier et al. 2005) assuming different partitioning of such geographical clusters. In particular, the total number of clusters inferred using Geneland, or alternatively, populations placed to the north and south of the 30°S biogeographical break, were compared searching for (statistically significant) hierarchical differences of their molecular variances (among clusters, among populations within clusters, within populations). All analyses in Geneland and Structure for both *S. scurra* and *L. spicata* and the different markers assumed the Admixture and Correlated frequencies options. Runs of 480,000 simulations (atp8S, *L. spicata* and *L. berteroana*), of 530,000 simulations (AFLP's, *S. scurra*) and of 25,000,000 simulations (COI, *S. scurra*) were done in Geneland. For the analyses under Structure, we applied Evanno's et al. (2005) graphical method (shown in Figure 4a and 4b) in order to determine the uppermost level of genetic structure (Evanno's method employ results from Structure analyses). Specifically, the plot of the second order Evanno's statistic respect to K (in Figure 4b) make prominent the break in slope of the distribution of L(K) at the true K.

2.5 PHYLOGEOGRAPHIC INFERENCES:

To evaluate the second hypothesis, we were only able to infer the demographic changes occurred in *S. scurra* populations revealed by the mitochondrial COI marker. This was done through reconstructing, in Beast software, the changes of the (COI) effective sizes of the different geographical clusters (previously inferred with Geneland).

Simulations of alternative biogeographic scenarios for the origin of the *S. scurra* (COI geographic) and *L. spicata* (atp8S) clusters were performed, and determination of the most likely among them was done through Approximate Bayesian methods, both options available in the DYABC software. The scenarios evaluated basically whether there has been demographic stability, contraction, or extinction of the southernmost populations of *S. scurra* and *L. spicata*, during the LGM, on the one hand; and if there has been an *in-situ* post-glacial expansion, or alternatively, a post-glacial recolonization of the southern areas of the IA from northern populations or from the PP, on the other hand. A graphical explanation of these alternative scenarios and population genetic parameters is shown in Figure 6 and 8, for *S. scurra* and *L. spicata*, respectively. In the case of *L. spicata*, two runs of analyses were performed. In a first run, scenarios 1, 2 and 3 were tested. The best fit scenario was scenario 1 (see Results). In a second run of analyses, scenario 1 was contrasted against scenario 2', which presumes a demographic stability for the northernmost clusters and a demographic expansion for the four southernmost clusters of the IA (following results observed for *S. scurra*). DYABC assess the likelihood of the different scenarios using two methods. The "direct estimate" (following Miller et al. 2005 and Pascual et al. 2007) calculates which scenario has the highest posterior probability when using the closest simulated data sets compared to the observed data

sets. And the “logistic regression” method (second criterion, following Fagundes et al. 2007 & Beaumont 2008) consists in calculating logistic regression of each scenario’s probability on the deviations between simulated and observed summary statistics.

2.6 CORRELATION OF PAIRWISE POPULATION GENETIC DISTANCES BETWEEN *S. SCURRA* AND *L. SPICATA* :

Nei genetic distances between pairs of populations in *S. scurra* (calculated in GenAlex6.5 b3) were contrasted to Nei’s genetic distances between the same pairs of populations of *Lessonia* using a paired Mantel test in GenAlex 6.5 b3. Nei’s genetic distance matrix was calculated using separately the COI and AFLP markers in *S. scurra*, and the mitochondrial atp8S marker in *Lessonia*. We also tested for isolation by distance (IBD) comparing matrices of Euclidian geographic distances and genetic distances ($F_{ST}/1-F_{ST}$) performing Mantel tests, within *S. scurra*.

2.7 SEARCHING FOR LOCI UNDER SELECTION IN *S. SCURRA*:

We conducted an outlier detection of AFLP loci potentially under selection using the program DFIST available in the selection detection workbench for dominant markers MCHEZA (Antao & Beaumont 2011), with a level of detection of 0.99. This was replicated in two neighbor localities. To assess the significance of the AFLP differentiation of *S. scurra* inhabiting *L. spicata* or *D. antartica*, populations were partitioned adopting two nesting strategies: *S. scurra* present in *L. spicata* or *D. antartica* hosts, nested within locality; and *S. scurra* individuals present per locality, nested within *L. spicata* or *D. antartica* hosts. Moreover, population

comparisons were made between the four groups sampled (two groups per locality, inhabiting *L. spicata* or *D.antartica*) through calculating F_{ST} indexes among them in Arlequin.

3 RESULTS

3.1 IDENTIFICATION OF THE TRUE NUMBER OF *S. SCURRA* POPULATIONS AND DEGREE OF SIMILARITY WITH *LESSONIA* PHYLOGEOGRAPHIC STRUCTURE:

3.1.1 Genetic diversity of *S. scurra* revealed by the COI and AFLP's:

Standard genetic diversity indices calculated from the 587 nucleotides sites recovered from the COI (Table 2a) indicated that northern populations of *S. scurra* were generally more diverse than the southernmost populations: North of 33°S, the number of polymorphic sites varied between six and seven, while in the South variation ranged from one to two. The southernmost population (Ancud or AN in Fig.1) was clearly the least diverse (e.g. mean number of pairwise differences of 0.06, ten times lower than all other sites). This was also corroborated by distribution of COI haplotype frequencies (Fig.1), with the northern populations displaying the highest number of haplotypes per site sampled (three to eight). Nonetheless, in this Fig. 1, the northernmost site, Paz, (north of 27°S) showed the highest number of COI unique haplotypes (five out of seven haplotypes), with only two of its haplotypes common and shared with the rest of the region (north of 30°S), one of which was present in most of the studied range.

Standard diversity indices calculated from the 88 polymorphic loci recovered from AFLP profiles (Table 2b) did not display such marked differences in the within population diversity as the COI.

Table 2 a. Sample size (N) and standard genetic diversity indexes of *S. scurra* from Chile using the COI marker: H (number of haplotypes), Hd (haplotype diversity), π (nucleotide diversity), Π (mean number of pairwise differences).

Location	Coordinates	N	H	S	Hd	π	Π
Pan de azucar	26°08 S/70°39'W	50	7	7	0.2620	0.000542	0.3184
Carrizal Bajo	28°04'S/71°08'W	15	3	2	0.2571	0.000649	0.3810
Isla Damas	29°13'S/71°31'W	6	4	3	0.8000	0.002044	1.2000
Punta Choros	29°14'S/71°27'W	32	8	7	0.4839	0.001103	0.6472
Rio Limari	30°44'S/71°42'W	38	7	6	0.2945	0.000538	0.3158
Canelillo	33°21'S/71°41'W	70	7	6	0.3089	0.000602	0.3532
Talcahuano	36°59'S/73°11'W	33	3	2	0.4773	0.000884	0.5189
Lebu	37°08'S/73°35'W	35	2	1	0.5109	0.000870	0.5109
Caleta Piure	37°14'S/73°39'W	23	2	1	0.4743	0.000808	0.4743
Valdivia	39°46'S/73°23'W	40	3	2	0.4449	0.000819	0.4808
Ancud	41°48'S/74°01'W	33	2	1	0.0606	0.000103	0.0606

Table 2b. Within-population genetic diversity indices of *S. scurra* in Chile using AFLP markers. Standard deviations in parentheses.

Location	Coordinates	N	H	% Polimorphic sites	Nei's diversity index (H_E)	Theta S
Pan de azucar	26°08 S/70°39'W	24	88	75.00	0.21 (0.17)	17.67 (6.04)
Carrizal Bajo	28°04'S/71°08'W	24	88	70.45	0.21 (0.20)	16.60 (5.69)
Isla Damas	29°13'S/71°31'W	30	88	82.95	0.24 (0.18)	18.43 (5.99)
Punta Choros	29°14'S/71°27'W	36	88	82.95	0.25 (0.18)	17.60 (5.52)
Rio Limari	30°44'S/71°42'W	23	88	81.82	0.25 (0.18)	19.51 (6.69)
Los Vilos	31°54'S/71°30'W	21	88	72.73	0.24 (0.19)	17.79 (6.26)
Las Docas	33°08'S/71°42'W	30	88	69.32	0.21 (0.19)	15.40 (5.06)
Quintay	33°10'S/71°42'W	47	88	88.64	0.22 (0.18)	17.66 (5.26)
Pelancura	33°34'S/71°37'W	24	88	78.41	0.24 (0.18)	18.48 (6.30)
Caleta Piure	37°14'S/73°39'W	21	88	70.45	0.23 (0.20)	17.23 (6.07)
Valdivia	39°46'S/73°23'W	19	88	75.00	0.24 (0.19)	18.88 (6.77)
Ancud	41°48'S/74°01'W	20	88	76.14	0.24 (0.19)	18.89 (6.69)

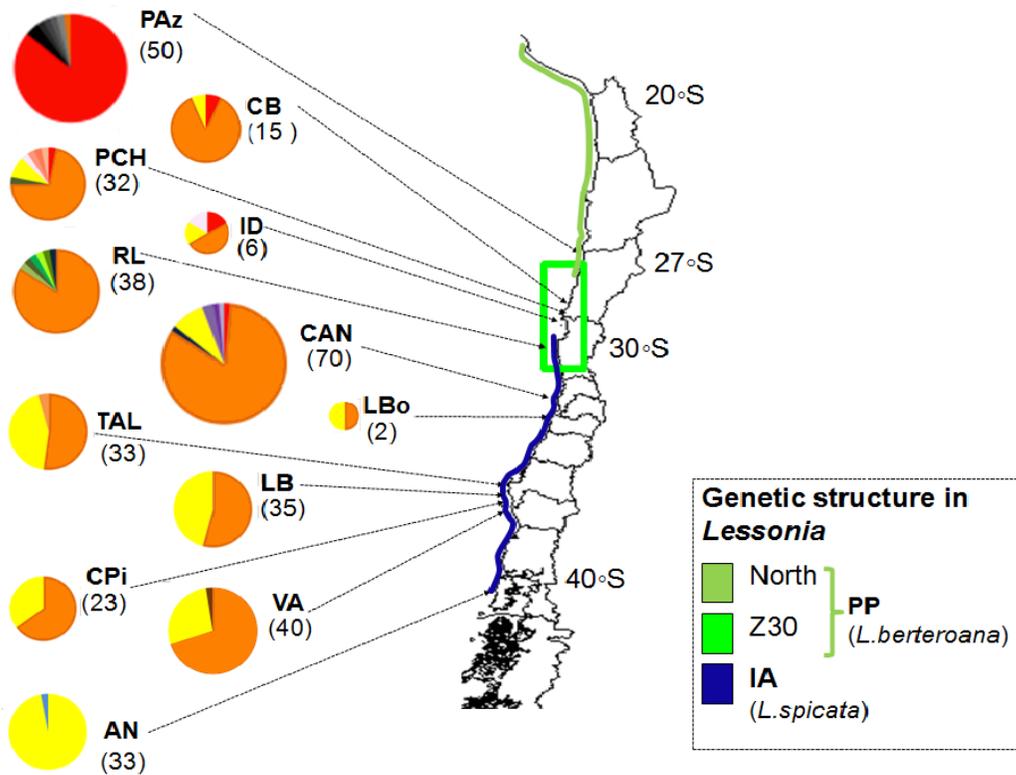


Figure 1. COI haplotype frequencies in *S. scurra* populations of Chile. Each color in each circle indicates the relative frequency of a specific COI haplotype in a specific site (number of samples per site are in parentheses). Paz: Pan de Azúcar; CB: Carrizal Bajo; PCH: Punta Choros; ID: Isla Damas; RL: Río Limarí; CAN: Canelillo; LBo: La Boca; TAL: Talcahuano; LB: Lebu; CPi: Caleta Piure; VA: Valdivia; AN: Ancud; PP: Peruvian Province; IA: Intermediate Area.

Table 3a. Pairwise F_{ST} between *S. scurra* populations of the PP and IA, using the mitochondrial COI marker.

Locality	PP				IA							
	P.Azucar	C.Bajo	I.Damas	P.Choro	R.Limari	L.Vilos	Canelo	Talcahuano	Lebu	C.Piure	Valdivia	Ancud
P.Azucar	0.00000											
C.Bajo	0.82251*	0.00000										
I.Damas	0.72851*	0.01354	0.00000									
P.Choro	0.77944*	-0.03859	0.00041	0.00000								
R.Limari	0.81591*	0.01928	0.07361	0.02922	0.00000							
L.Vilos	0.84368*	-0.08986	0.00734	-0.07091	-0.02588	0.00000						
Canelo	0.83432*	-0.02639	0.14524*	-0.00080	0.05400*	-0.06732	0.00000					
Talcahuano	0.74679*	0.15672*	0.00414	0.12218*	0.23641*	0.13674	0.24494*	0.00000				
Lebu	0.75548*	0.15501*	0.02025	0.12086*	0.25516*	0.13775	0.22919*	-0.03281	0.00000			
C.Piure	0.78660*	0.05670	-0.01364	0.03531	0.15763*	0.03282	0.11969*	-0.00864	-0.01210	0.00000		
Valdivia	0.78413*	0.02577	0.00598	0.02041	0.14141*	-0.00490	0.07784*	0.02992	0.02380	-0.02897	0.00000	
Ancud	0.81587*	0.82567*	0.69097*	0.66965*	0.77527*	0.88920*	0.75591*	0.48235*	0.49518*	0.64580*	0.62394*	0.00000

Table 3b. Pairwise F_{ST} s between *S. scurra* populations of the PP and IA, using AFLP markers.

Locality	PP				IA							
	P.Azucar	C.Bajo	I.Damas	P.Choro	R.Limari	L.Vilos	Quintay	L.Docas	Pelancura	C.Piure	Valdivia	Ancud
P.Azucar	0.00000											
C.Bajo	0.08403*	0.00000										
I.Damas	0.10665*	0.03886*	0.00000									
P.Choro	0.05596*	0.02410*	0.02476*	0.00000								
R.Limari	0.16323*	0.16307*	0.12438*	0.14371*	0.00000							
L.Vilos	0.18340*	0.16217*	0.12438*	0.12991*	0.06849*	0.00000						
Quintay	0.19536*	0.17504*	0.15209*	0.16247*	0.10590*	0.06196*	0.00000					
L.Docas	0.19777*	0.18187*	0.14793*	0.16775*	0.10541*	0.09926*	0.03667*	0.00000				
Pelancura	0.14696*	0.13492*	0.11629*	0.11401*	0.08678*	0.06129*	0.11157*	0.14003*	0.00000			
C.Piure	0.21008*	0.18030*	0.15035*	0.16829*	0.08696*	0.12731*	0.12376*	0.15742*	0.06664*	0.00000		
Valdivia	0.18805*	0.16497*	0.13954*	0.14249*	0.06692*	0.11112*	0.10218*	0.12801*	0.05357*	-0.00473	0.00000	
Ancud	0.17717*	0.16654*	0.14225*	0.14184*	0.07687*	0.11843*	0.10242*	0.12042*	0.06242*	-0.00811	0.01365	0.00000

Asterisks (*) indicate significantly differentiated population pairs at $p < 0.001$. PP: Peruvian Province; IA: Intermediate Area.

3.1.2 Population genetic structure of *S. scurra* and its host *L. spicata* :

When focusing on the pairwise F_{ST} matrix between *S. scurra* populations, AFLP markers exhibited a better resolution than the COI of the phylogeographic structure of *S. scurra* populations around the 30°S biogeographic break previously confirmed in *Lessonia* (Table 3a and 3b, for the COI and AFLP's, respectively). Indeed, (AFLP's) pairwise distances between pairs of PP populations were always smaller than any PP-IA population pair. As with the COI (and within the PP), the site north of 27°S was the most differentiated from the rest of PP populations (at least twice). Moreover, AFLP markers allowed distinguishing, better than

the COI, that the three *S. scurra* populations from the southern tip of the IA were the only Chilean populations not being significantly differentiated.

In order to determine the true number of clusters of *S. scurra* populations in Chile, we focused on the analyses performed in Structure and Geneland only using AFLP's. This is because AFLP loci in *S. scurra* were highly polymorphic and resolved the genetic population differentiation better than the COI, as indicated above.

The number of clusters as revealed in Structure with the greatest likelihood ($L P(D)$, or $L(K)$) was $K=8$ (Fig. 2, 3a). Nonetheless, as it is evident from the top plots of Fig.2, the uppermost hierarchical level of structure in *S. scurra* was two or three, following Evanno's method (Fig.3b). With $K=2$, the two clusters corresponded to the North (in green in Fig.2) and the South (in red) of the 30°S. With $K=3$, the spatial structure grouped individuals North of 30°S in one cluster, those from 30°S to 37°S in a second cluster, and those from 37°S to 42°S in a third cluster (Fig. 2).

Geneland analysis of AFLP markers allowed clustering the twelve *S. scurra* populations not only according to their homogeneity; it also allowed incorporating the geographic coordinates of the 319 *S. scurra* samples using a spatial model. The "true" number of clusters obtained with Geneland was seven ($K=7$) instead of the eight found with Structure. Geneland inferred a probable separation between populations from 30-27°S and the northernmost PAZ(Fig.4). Nested AMOVA's assuming two or seven groups were highly significant ($p<0.001$) at all hierarchical levels (Table 4). The percentage of the total variance contributed by the within populations level was the highest (83.91% to 86.71%), followed by the among groups level in

the second place (9.04% to 11.29%) and the among populations level with the lowest contribution (2.00% to 7.05 %).

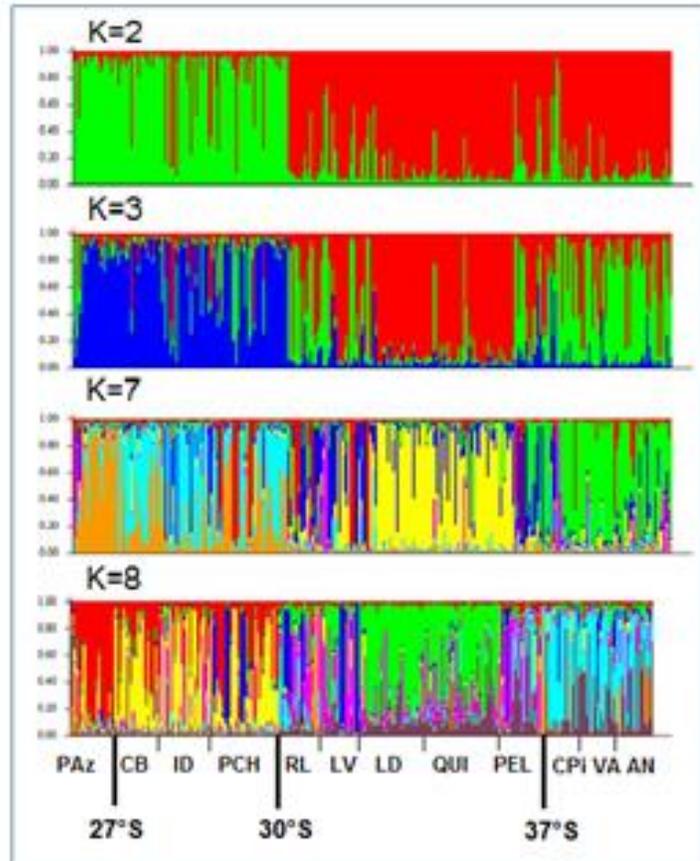


Figure 2. Graphical illustration of some likely number of *S. scurra* clusters inferred in Structure from AFLP data (N=319 individuals). Each color indicates a different cluster, and each individual (vertical lines) is assigned a given probability of belonging to one or more clusters. The true number of clusters and displaying the greatest posterior probability of the data ($\ln P(D)$) or $L(K)$ in Structure was with $K=8$. Nevertheless, the separation in two clusters ($K=2$, and separating the PP, in green, from the IA populations, in red, in the superior plot) or three clusters corresponded to the uppermost levels of population structure. Paz: Pan Azúcar; CB: Carrizal Bajo; ID: Isla Damas; PCH: Punta Choros; RL: Río Limarí; LV: Los Vilos; LD: Las Docas; QUI: Quintay; PEL: Pelancura; Cpi: Caleta Piure; VA: Valdivia; AN: Ancud.

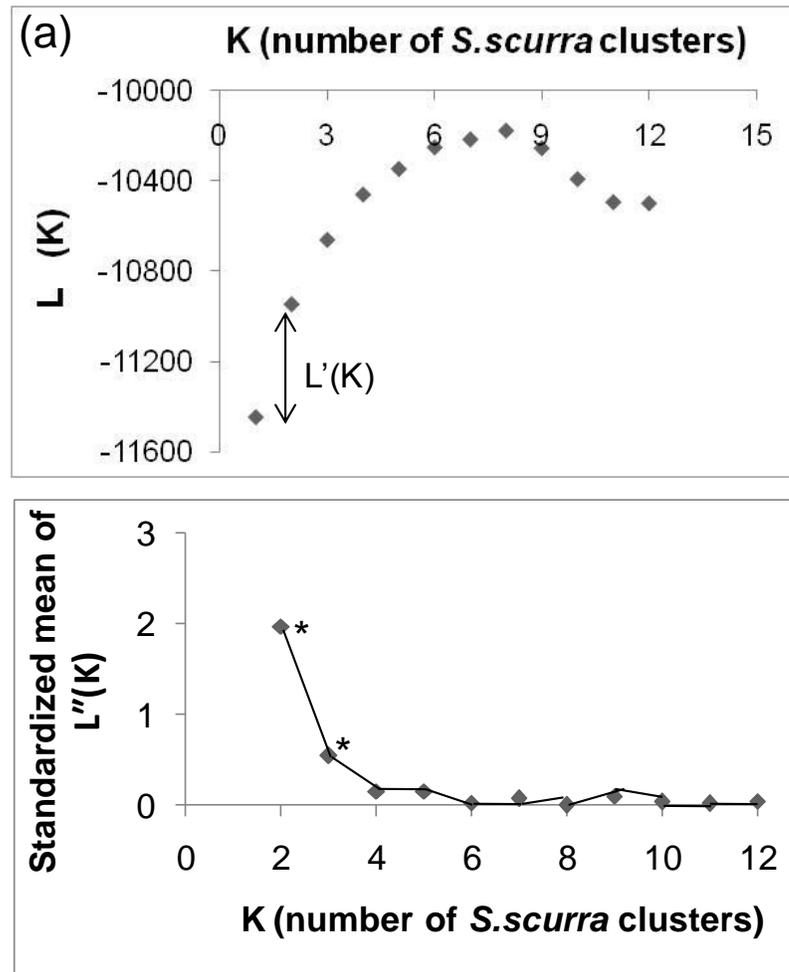


Figure 3. Plots of (a) $L(K)$ as the first order rate of change of the likelihood for two successive K 's and (b) Standardized second order rate of change of the likelihood respect to K , in order to determine the true number of clusters of homogeneous populations in *S.scurra*. $K=2$ (PP versus IA) or $K=3$, according to Evanno's et al. (2005) method, as indicated with the symbol * in Fig. 4b.

Table 4. Analysis of molecular variance (AMOVA) for 12 populations of *Scurria scurra*. For the nested analyses, populations were divided into two (PP vs IA) or into seven groups based on clusters inferred using Geneland and Structure softwares. Degrees of freedom (d.f), sums of square deviations (SSD), variance component estimates, the percentages of the total variance (% Total) contributed by each component, and the probability (P-value)

	d.f.	SSD	Variance Components	% Total	P-value
Population					
Among populations	11	518.574	1.40296	12.16	p<0.001
Within populations	307	3112.260	10.13765	87.84	p<0.001
Total	318	3630.834	11.54061		p<0.001
Nested level (Two groups)					
Among groups	1	194.972	1.09253	9.04	p<0.001
Among populations	10	323.602	0.85124	7.05	p<0.001
Within populations	307	3112.260	10.13765	83.91	p<0.001
Total	318	3630.834	12.08142		
Nested level (Seven groups)					
Among groups	6	436.140	1.31952	11.29	p<0.001
Among populations	5	82.434	0.23379	2.00	p<0.001
Within populations	307	3112.260	10.13765	86.71	p<0.001
Total	318	3630.834	11.69096		

The comparison of the number of clusters inferred in Geneland between *S. scurra* (using AFLP's) and its main hosts *L. spicata* and *L. berteroana* (using the atp8S), suggests that they follow a similar genetic structuring along the IA and the PP (Figures 4a, 4b, and 4c respectively). Indeed, both *S. scurra* and *L. spicata* displayed a similar clustering of their populations, with more clusters found from 30°S to 36°S (four and five clusters, respectively) than from 36°S to 42°S (one and two clusters, respectively). Then, both in *S. scurra* and *L. spicata*, the northern IA was more heterogeneous than the southern half of the IA, where a widely distributed cluster was present. A similar pattern of contrasting latitudinal structure was also verified in *D. antarctica*, but with a latitudinal shift southward (Fig. 4d).

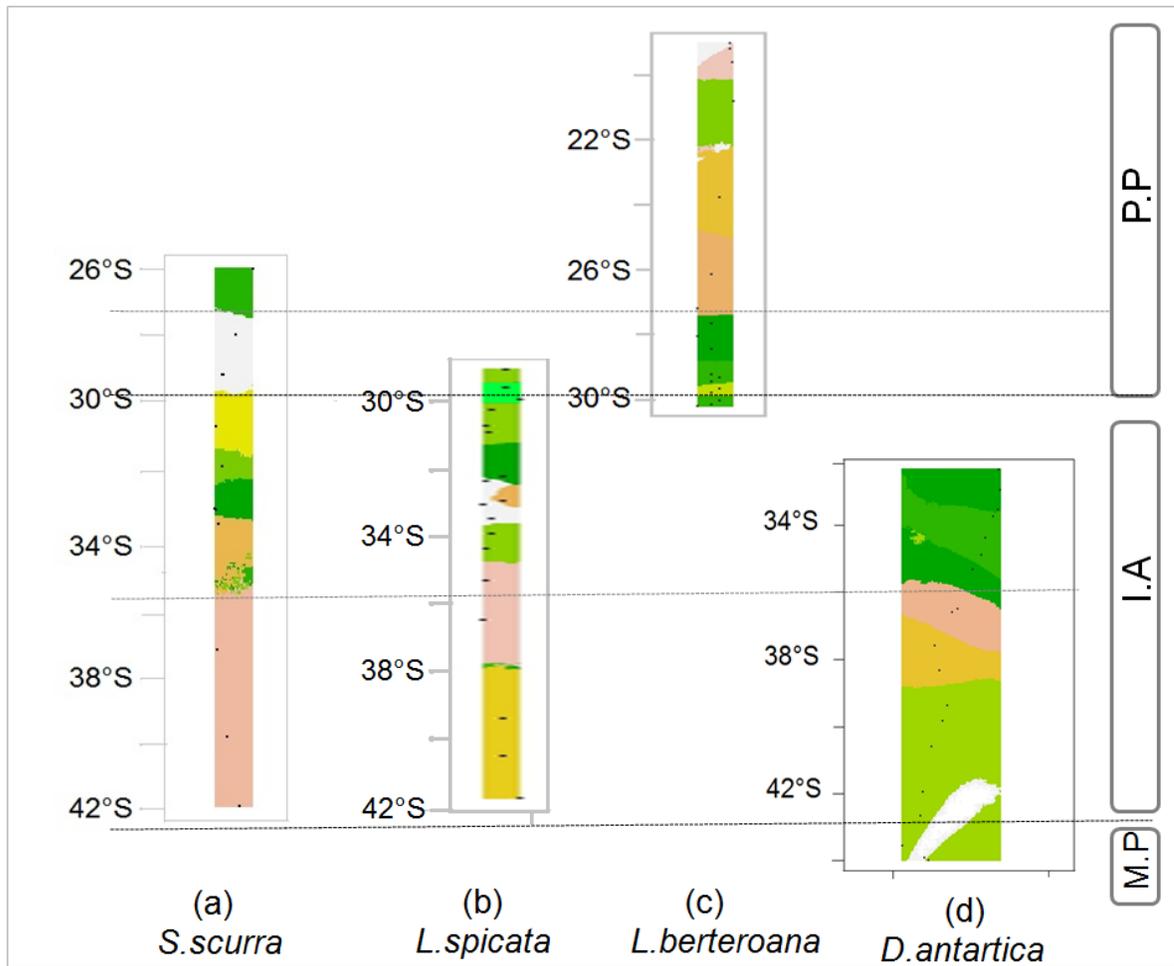


Figure 4. Comparative maps showing the numbers of clusters found with Geneland software in Chile for: (a) *S. scurra* (using AFLP markers) (b) *L. spicata*, (using the atp8S marker), (c) *L. berteroana* (using the atp8S marker), and (d) *D. antarctica*, (using the COI marker). Each color represents a different cluster. P.P: Peruvian Province, I.A: Intermediate Area, M.P: Magellan Province, according to Camus (2001).

3.2 EVALUATION OF CONTRASTING DEMOGRAPHIC SCENARIOS SINCE THE LGM IN *S. SCURRA* POPULATIONS

3.2.1 Reconstruction of the effective population sizes of *S. scurra* clusters using the COI:

Bayesian Skyline analyses for the reconstruction of the effective population sizes (N_e) are not possible under Beast software program using dominant markers (AFLP's). Then, we used only COI sequences of *S. scurra* to perform Bayesian Skyline analyses and generated Skyline Plots of each of the population clusters previously inferred in Geneland (also from COI data).

Both *S. scurra* clusters 1 and 2 (Fig.5a) experienced similar growths of their effective population sizes from about 20,000-50,000 to approximately 700,000 in both cases (Skyline Plots of Figure 5b). Nevertheless, while population growth in cluster 1 initiated closer to the end of the LGM (e.g. 20,000 years before present), cluster 2 population growth started later, around 10,000 years before present. Effective population sizes began increasing very recently in cluster 3 (approximately 3-4,000 years ago), whereas no clear signal of population expansion was detectable in cluster 4, which displayed a small N_e (approximately 12,000). More specifically, effective population size's medians and their HPD 95% (lower and upper) were as follows: for cluster 1, the median N_e was 719,140 (13,508-1,943,200); for cluster 2, the median N_e was 748,360 (29,843-2,307,000); for cluster 3, the median N_e was 181,660 (377-1,376,000); and for cluster 4, the median N_e was 11,966 (9-37,922). Both Fu's F_s and

Tajima's D indices (not shown) were negative and significant for clusters 1 and 2, and negative but not significant for clusters 3 and 4.

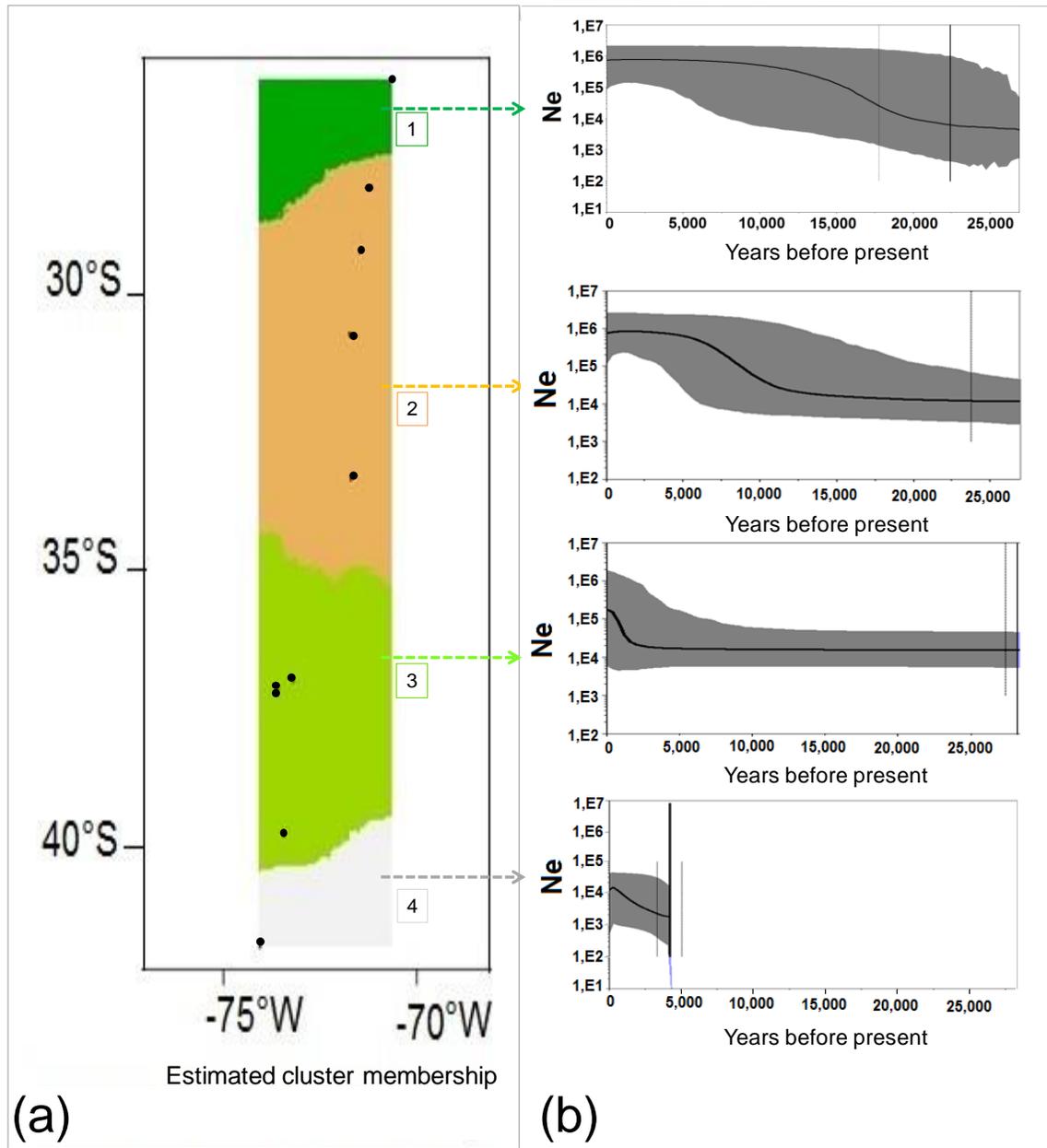


Figure 5.(a) Map of *S. scurra* clusters with Geneland from COI data and (b) effective population size (N_e) reconstructions of each of these four clusters (indicated by arrows) using the Beast software program. Numbers below arrows identify clusters.

3.2.2 *Simulations of Biogeographic scenarios for S. scurra and L. spicata populations since the LGM:*

3.2.2.1 **Simulations of Biogeographic scenarios for *S.scurra*:**

Both criteria incorporated in DYABC software to establish the most likely phylogeographic scenario supported scenario 3 (Fig.7) as having the highest posterior probabilities and explaining the origin of *S. scurra* populations. The “direct estimate” of the posterior probabilities of scenarios (shown in Fig.7a, first criterion, following Miller et al. 2005 & Pascual et al. 2007) demonstrated that scenario 3 had the highest posterior probability when using the 500 simulated data sets closest to the observed data. The logistic regressions (Fig.7b) also supported scenario 3, which is then the most likely phylogeographic scenario containing demographic steps that led to the genetic diversity and spatial structure of *S. scurra* populations.

3.2.2.2 **Simulations of Biogeographic scenarios for *L. spicata* :**

In a first run in DYABC, scenarios 1, 2 and 3 were compared (Figure 8). The best fit scenario was scenario 1 according to both the “direct estimate” (Figure 9a) and the “logistic regressions” criteria (Fig.9b). In a second run, scenario 1 was contrasted against scenario 2’ (Fig. 8). Both criteria incorporated in DYABC software to establish the most likely phylogeographic scenario supported the scenario 2’ as having the highest posterior probability

and explaining the demography of *L. spicata* populations. The “direct estimate” of the posterior probabilities of scenarios (Fig. 10a) demonstrated that scenario 2’ exhibited the highest posterior probability based on the 500 simulated data sets closest to the observed data. Similarly, the logistic regressions (Fig. 10b) supported scenario 2’ as the most likely phylogeographic scenario that led to the spatial structure of the genetic diversity of *L. spicata* populations.

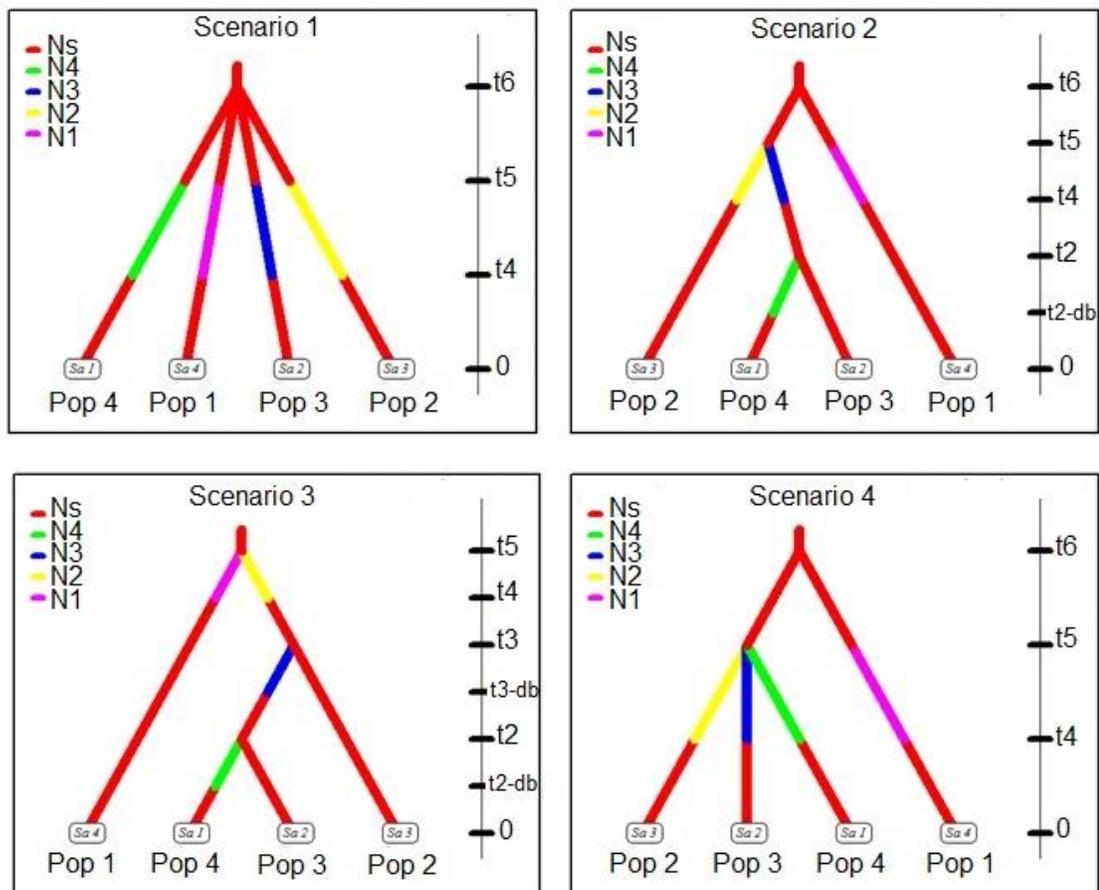


Figure 6. Phylogeographic scenarios for *S. scurra* clusters simulated in DYABC. Scenarios 1 and 4 assume no population extinction of southern populations. Scenarios 3 and 4 during the LGM, and a population size change since the LGM for all populations; the only difference in Scenario 4 is that the divergence of Pop 1 is more ancient in comparison to Scenario 1 which states a synchronous divergence for all populations. Scenarios 2 and 3, presume that there has been an extinction of the southernmost IA clusters (4 and/or 3) during the LGM, and a post-glacial recolonization from northernmost populations. Effective population sizes were set: for N_s (100,000 to 1,000,000 individuals), and for N_4 , N_3 , N_2 and N_1 (10 to 10,000 individuals). Timing of events was as follows: t_6 (60,000 to 80,000 years ago), t_5 (20,000 to 26,500 years ago), t_4 (18,000 to 20,000 years ago), t_3 (10,000 to 16,000 years ago) and t_2 (5,000 to 11,000). The duration of bottleneck (db) was set from 1 to 50 years, and a prior condition in the order of occurrence of events was set with $t_3 > t_2$.

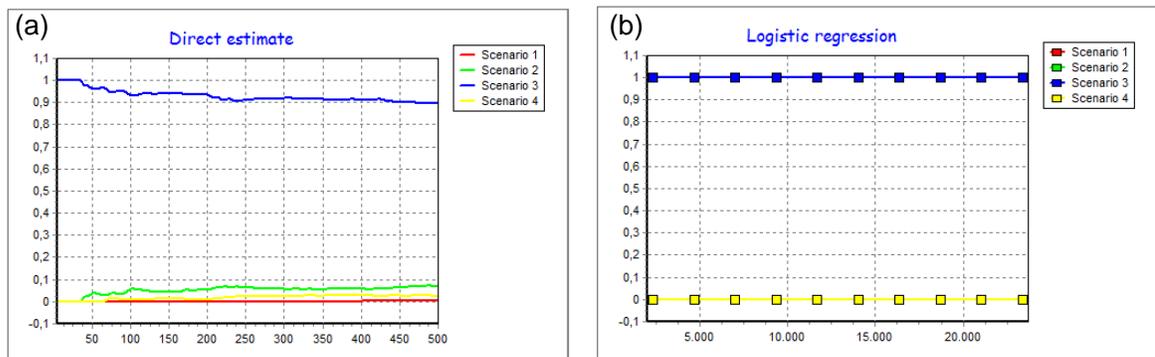


Figure 7. Posterior probabilities for estimation of the best fit scenario of historical demography in *S. scurra*. (a) Direct estimate and (b) Logistic Regression. Both the direct estimate and the logistic regression methods supported scenario 3 as having the highest probability and generating the genetic structure and diversity observed in *S. scurra* sampled populations. Scenario 3 assumes that there was an extinction of the southernmost populations of the IA during the LGM, and a postglacial recolonization or expansion from northern populations of *S. scurra*.

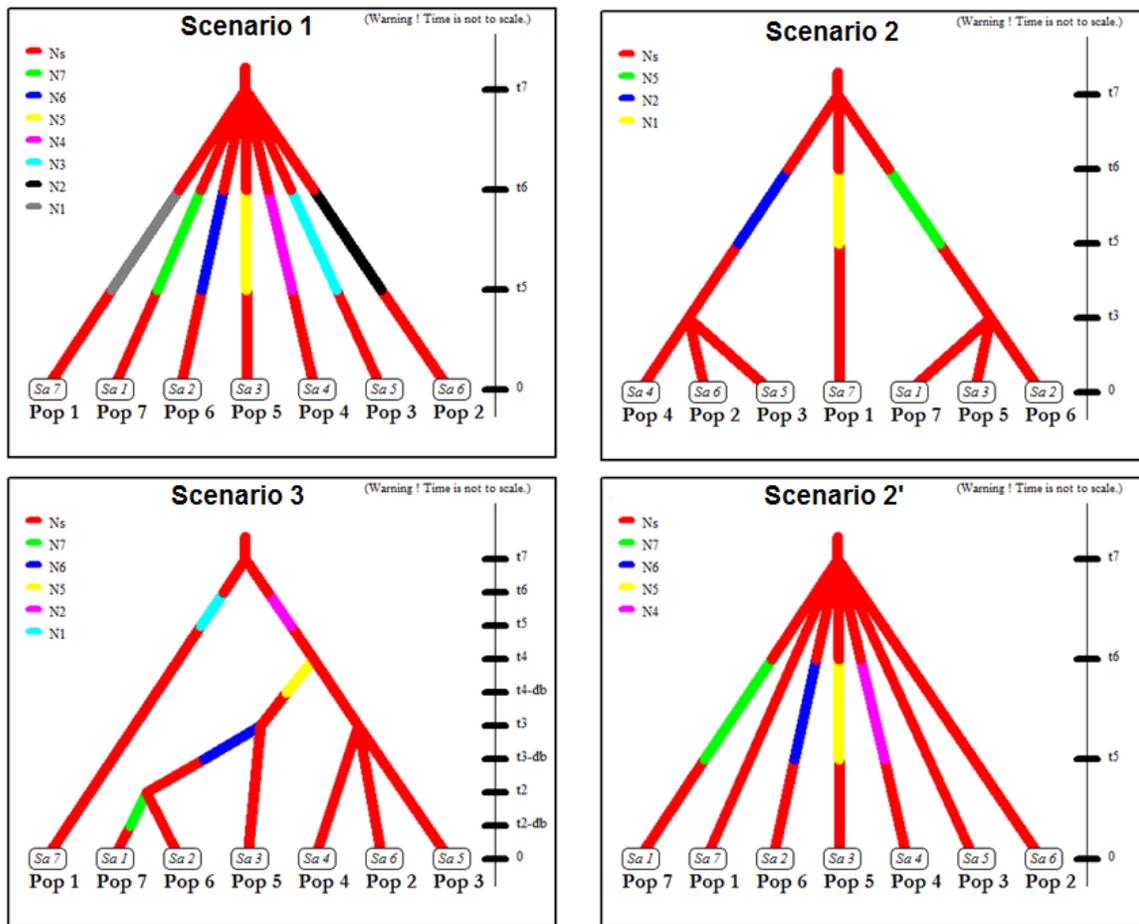


Figure 8. Phylogeographic scenarios for *L. spicata* populations simulated in DYABC. Pop 1 to Pop 7 correspond to clusters 1 to 7 shown in figure 5b (Pop 1-3 span 30°-33°S, and Pop 4-7 span 33°-42°S). Scenarios 1 assume a demographic contraction of all IA populations during the LGM, and a population size change or recovery since the LGM for all populations. Scenario 2 presume that there has been an extinction of the southern tip populations of the IA and a postglacial range expansion from northern populations from the PP; and scenario 3 assume a contraction during the LGM and a demographic recovery since the LGM for three ancestral populations, which diverged more recently originating the seven populations sampled. In a first run, scenarios 1, 2 and 3 were tested. The best fit scenario was scenario 1 (see Results). In a second run of analyses, scenario 1 was contrasted against scenario 2', which presume a demographic stability for the northernmost clusters and a demographic expansion for the four southernmost clusters of (following results observed for *S. scurra*). Effective population sizes were set: for Ns (1 to 10,000 individuals), and for N1 to N7 (1 to 1,000 individuals). Timing of events were as follows: t7 (80,000 to 1,200,000 years ago), t6 (21,000 to 25,000 years ago), t5 (17,000 to 21,000 years ago), t4 (6,000 to 17,000 years ago), t3 (6,000 to 17,000 years ago) and t2 (6,000 to 17,000). Duration of bottleneck (db) was set from 1 to 50 years, and a prior condition in the order of occurrence of events was set with $t_2 < t_3$, $t_4 > t_3$ and $t_4 < t_5$.

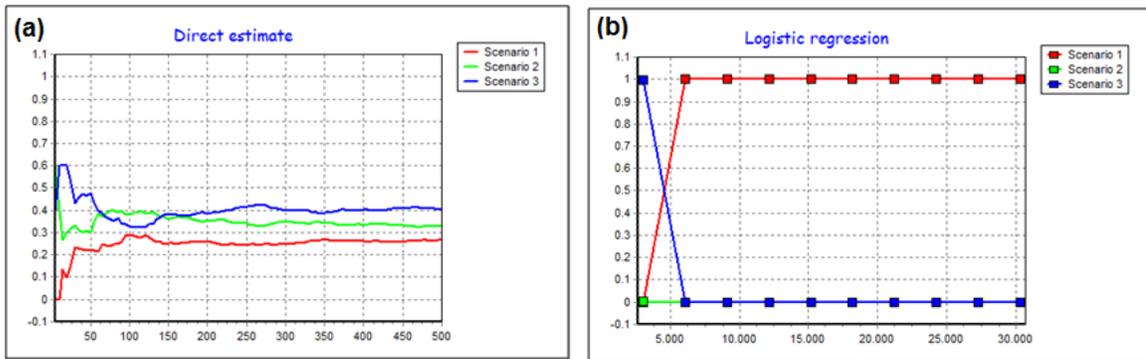


Figure 9. Posterior probabilities for estimation of the best fit scenario of historical demography in *L. spicata* (first run of analyses) (a) Direct estimate and (b) Logistic regression. Both the direct estimate and the logistic regression methods supported scenario 1 as having the highest probability and allowing generating the genetic structure and diversity observed in *L. spicata* sampled populations. Scenario 1 assumes that there has been demographic change over all northern and southern populations of the IA during and since the LGM; it assumes that there has been probably a contraction of populations of the IA during the LGM, with a postglacial demographic recovery since the LGM.

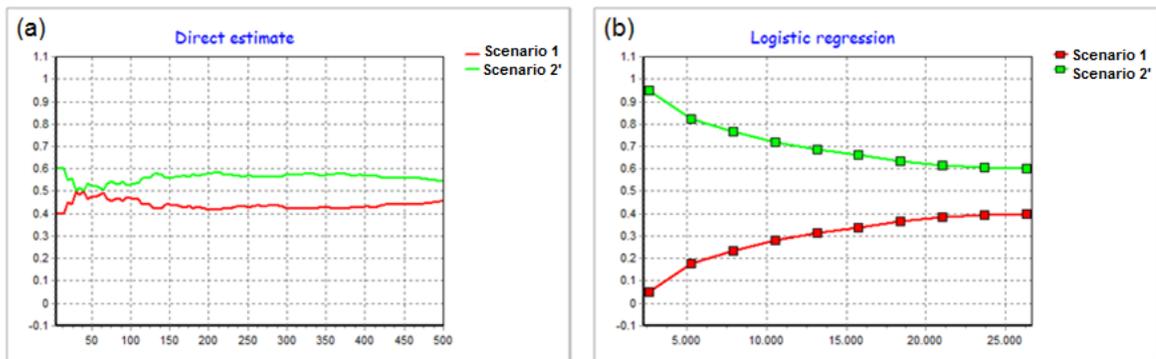


Figure 10. Posterior probabilities for estimation of the best fit scenario of historical demography in *L. spicata* (second run of analyses) (a) Direct estimate and (b) Logistic regression. Both the direct estimate and the logistic regression methods supported scenario 2' as having the highest probability and allowing generating the genetic structure and diversity observed in *L. spicata* sampled populations. Scenario 2' assumes that there has been demographic stability of the northernmost populations of the IA during and since the LGM; on the contrary, it assumes that there has been probably a contraction of the southernmost populations of the IA during the LGM, with a postglacial demographic recovery since the LGM.

3.3 CORRELATION OF PAIRWISE POPULATION GENETIC DISTANCES BETWEEN *S. SCURRA* AND *L. SPICATA*

As shown in Figure 11 a, the Mantel test on Nei distance matrix in *L. spicata* and in *S. scurra* (atp8S, and AFLP's, respectively) displayed a significant and high Mantel value of 0.637 ($p=0.004$) (six populations of the IA contrasted between *S. scurra* and *L. spicata*). The Mantel test searching for isolation by distance (IBD) in *S. scurra* (Fig. 11b) displayed an almost flat non significant relationship between pairwise differentiation indexes ($F_{ST}/1-F_{ST}$) and geographic distances (Mantel value of 0.257, $p=0.125$, for eight populations). On the contrary, a significant and important IBD was demonstrated in *L. spicata* populations, as reported previously by Tellier et al. (2009). In *S. scurra*, the pairwise genetic distances using the COI were not correlated neither with genetic distances in *L. spicata* nor with geographic distances between sites.

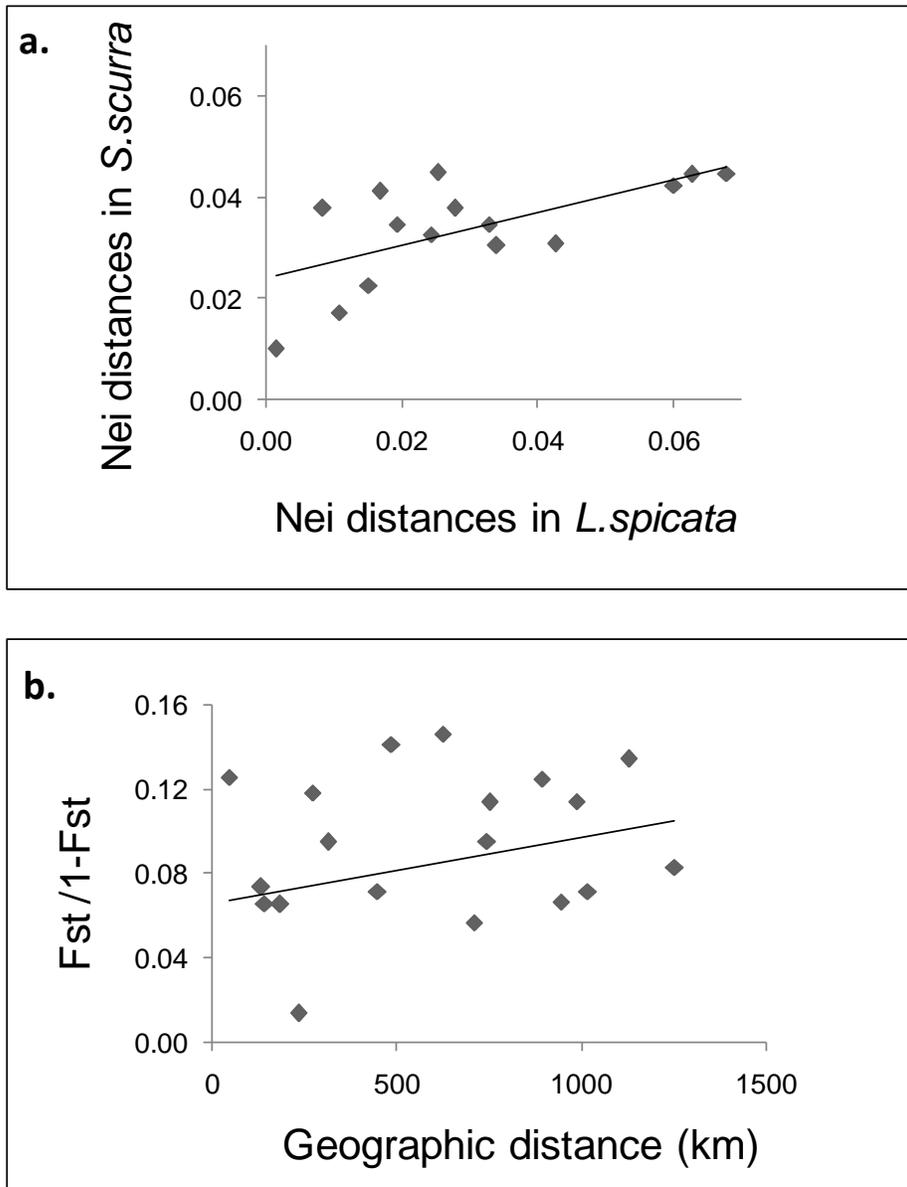


Figure 11.(a) Mantel test between Nei pairwise distances in *L. spicata* (atp8S) and Nei pairwise distances in *S. scurra* (AFLP), in six sites of the IA, and (b) Mantel test between genetic distances in *S. scurra* (FST/1-FST) and geographic distances, in eight populations of the IA. Grey dots represent pairwise comparisons; grey line is the best fit regression line. Mantel values were 0.637 ($p=0.004$) in (a), and 0.257 ($p=0.125$) in (b). Mantel tests were computed using 9999 permutations with GenAlex V6.5.

3.4 SEARCHING FOR LOCI UNDER SELECTION IN *S. SCURRA* INHABITING *L. SPICATA* OR *D.*

ANTARTICA:

The analysis performed in MCHEZA for the ensemble of *S. scurra* inhabiting either *L. spicata* or *D. antarctica* (two localities grouped), detected 3 AFLP candidate loci for positive selection out of 88 loci, and 6 candidate loci for balancing selection. Genetic differentiation between *L. spicata* and *D. antarctica* varied from $F_{ST} = 0.02$ ($p < 0.001$) when all loci were included, to $F_{ST} = 0.002$ ($p = 0.31$) when the 3 selected loci were excluded. When the same analyses were performed on only one locality (Quintay, with the largest number of samples per host) 3 AFLP candidate loci for positive selection were also detected but they were different from those found when the full set of data (two sites) was included. In this case again, F_{ST} values decreased from 0.028 ($p = 0.001$) to 0.018 ($p = 0.027$). When genetic differentiation was evaluated comparing the four *S. scurra* populations inhabiting one or the other host kelp (separated localities), all pairwise F_{ST} were significant except with *S. scurra* inhabiting *D. antarctica* in Las Docas (where only twelve *S. scurra* were sampled). This small *D. antarctica* group of *S. scurra* was not statistically different from any of the three other sampled populations. Nested AMOVA (Table 5) showed significant p values of differentiation for all hierarchical levels when host kelps were nested within localities; on the contrary, when localities were nested within host kelps, the superior level (between hosts) displayed a non significant p value.

Table 5. Analyses of molecular variance (AMOVA) for populations of *S. scurra* inhabiting *L. spicata* or *D. antarctica* kelps. For the nested analyses, *S. scurra* of two neighbor localities were nested within the two host kelp groups (*S. scurra* from *L. spicata* or *D. antarctica*); or alternatively, *S. scurra* inhabiting the two host kelps were nested within each of the two localities. Degrees of freedom (d.f), sums of square deviations (SSD), variance component estimates, the percentages of the total variance (% Total) contributed by each component, and the probability (p-value).

	d.f.	SSD	Variance Components	% Total	P-value
Population (<i>L. spicata</i> versus <i>D. antarctica</i>)					
Among populations	1	17.244	0.19984	2.04	p<0.001
Within populations	75	718.704	9.58272	97.96	
Total	76	735.948	9.78256		
Nested level (localities within host kelps)					
Among groups	1	17.244	-0.00273	-0.03	p=0.66
Among populations	2	32.918	0.39037	3.99	p<0.001
Within populations	73	685.786	9.39432	96.04	p<0.001
Total	76	735.948	9.78197		
Nested level (host kelps within localities)					
Among groups	1	22.767	0.24927	2.53	p=0.33
Among populations	2	27.395	0.22715	2.30	p<0.001
Within populations	73	685.786	9.39432	95.17	p<0.001
Total	76	735.948	9.87075		

4 DISCUSSION AND PERSPECTIVES:

4.1 COMMON PHYLOGEOGRAPHIC STRUCTURE IN *S. SCURRA* AND ITS HOST KELP

In line with Smith et al. (2011) and other surveys demonstrating a concordant spatial genetic structure and demography among plants and their specialized herbivores, this study supports the hypothesis that phylogeographic concordance is more likely when the strength and specificity of ecological interactions are higher. Nevertheless, divergence between both sides of the 30°S and the 27°S phylogeographic breaks observed in the host kelp and the

specialist herbivore have probably been shaped by the action of extrinsic factors (common environment) or by the strength of the ecological interaction, respectively. Our analyses employing AFLP markers confirmed the occurrence of a major genetic discontinuity in the structure of *S. scurra* populations, consistent with the proposed biogeographic break at 30°S in the Chilean coast. Both the plotting of results from Structure assuming two clusters (K=2 based on Evanno's method or three clusters, based on the standardized second order rate of likelihood change) showed a clear separation at 30°S, matching the genetic discontinuity observed in the *L.nigrescens* species complex (Tellier et al. 2009). As previously mentioned, other invertebrate and vertebrate organisms also display a major genetic discontinuity between both sides of the 30°S biogeographic break, and probably have been under the influence of the same macro-climatic events. In this case, the most likely factor is an oceanographic discontinuity in eddy kinetic activity and degree of variability in equatorward wind stress affecting the intensity and variability of upwelling forcing, reported at approximately 30°S by Hormazabal et al. (2004). On the other hand, the sub-structuring of populations located North of 30°S seems to match the second genetic discontinuity observed in the *L.nigrescens* complex with mtDNA markers at 27°S (Tellier et al. 2009). Indeed, with both COI and AFLP markers, PAzucar (north of 27°S) displayed an always significant genetic differentiation with both the 27-30°S cluster and the South 30°S cluster. Both AMOVA and GENELAND analyses further support this sub-structuring of the northern populations of *S.scurra*, confirming a tight co-divergence between the specialist herbivore and its main host. This second phylogeographic break at 27°S is unique to *S. scurra* and *L. berteriana*. It is then more parsimoniously explained by the strength and specificity of the ecological interaction between the host kelp and the specialist herbivore. On the other hand, AFLP, as fast evolving markers, may have

accumulated strong genetic divergence between populations and regions, as the result of both historical and contemporary differentiating processes. Whereas COI, as a slow evolving marker, display incomplete lineage sorting which mainly reflects historical processes. Therefore, it seems that genetic divergence between both sides of the 30°S and the 27°S phylogeographic breaks observed in the host kelp and the specialist herbivore may be of recent origin.

Despite dispersal potential of *S. scurra* is higher than that of *L. spicata* , AMOVA and GENELAND analyses indicate that *S. scurra* is highly structured, showing a number of clusters similar to its host *L. spicata* . Accordingly, while *S. scurra* did not show a pattern of IBD using AFLP's, genetic distances between *S. scurra* and *L. spicata* population pairs were significantly correlated (Fig. 11). Moreover, evaluation of genetic differentiation through the F_{ST} index was significant even for nearby localities using AFLP's (Table 3b). Nonetheless, differences arise probably because of the different dispersal capacities of the patella and its host kelp. In sites that are geographically close, pairs of populations of *S. scurra* are more genetically differentiated than equivalent *L. spicata* population pairs (points to the left in Figure 11 a); with increasing geographical distance, pairwise Nei distances between pairs of populations in *L. spicata* become proportionally larger than in *S. scurra* (right points in Fig. 11a). The above confirms the existence of a concordant pattern of spatial genetic structure between the specialist herbivore and its main algal host. The high genetic structure in neighbour localities revealed by the F_{ST} index also supports the role of selective evolutionary forces in shaping this spatial co-divergence, as will be discussed latter.

Even though *S. scurra* and *L. spicata* genetic structures were similarly shaped at the landscape and geographical scale, *S. scurra* did not diverge in two species as did *Lessonia* on both sides of the 30°S biogeographic break. Co-speciation, defined as co-phylogenetic divergence, is not however a frequent evolutionary outcome of the interaction between specialist herbivores and their host plants. According to Price et al. (2011), phytophagous specialist herbivores or the rare known marine specialist herbivores do not always meet two necessary conditions for cospeciation. The main unfulfilled condition is the vertical transmission across generations of a single host species. Indeed, most of these species have dispersive phases, such as larvae with high dispersal potential, highly mobile adults, or eventually transmission vectors, which exclude vertical transmission. The existence of dispersive phases such as larvae in most marine herbivores implies that the organism is exposed during its life cycle to a set of diverse environmental conditions and then to selective pressures different to those found on its host plant. The second commonly unfulfilled condition is that specialist parasites or herbivores that co-speciate are generally obligatory specialists i.e. organisms having a unique host like some parasitic lice and their vertebrate hosts. Accumulated knowledge supports the view that common chemicals mediate host plant selection and utilization by some phytophagous insects, which would also favor host specialization and speciation (Menken and Roessingh 1998; Linn et al. 2003). Nonetheless, these host plant recognition systems have been shown to be plastic and probably promote host switching (e.g. Roessingh et al. 2007). The above would explain why specialist herbivores frequently manifest both: i) a horizontal transmission through more than one generation or population of its host plant at the ecological level and, ii) a frequent switching to different host-plant taxa, which prevents phylogenetic co-divergence (Tilmon 2008, Winkler and Mitter

2008, Medel et al. 2009). Moreover, gene trees of the herbivore and its host plant may differ because their loci have experienced different mutation rates, migration patterns and selective regimes (Nieberding and Olivieri, 2007).

4.2 SHARED DEMOGRAPHIC HISTORY BETWEEN *S. SCURRA* AND ITS HOST KELP

Globally, demographic inferences employing genetic data suggest that *S. scurra* and its host kelp *L. spicata* have been under the influence of the same contrasting environmental conditions during the late Pleistocene, north and south of approximately 34-36 °S. According to our analyses with the COI marker, the most likely biogeographic and demographic scenario for *S. scurra* is the extinction of its southernmost populations (south of 34°S) during the LGM, followed by a post-glacial recolonization of this area from refugia located further north. Both the reconstruction of effective population sizes of *S. scurra* clusters using Beast and the estimated posterior probabilities of alternative scenarios using DYABC support this contention. Interestingly, it seems that the demographic changes have been progressive and spread over several thousand years. As shown in Fig.5b, the signature of population growth started early after the LGM in the northernmost part of the studied range. Whereas it was estimated at around 3-4,000 years ago in the cluster 3, the southernmost populations seem to have started the population expansion only very recently, with a modest increase in effective population size compared to other populations. This pattern, yet unreported for species not affected by ice cover in South America, is likely the result of a progressive southward shift of the West Wind Drift and the associated reestablishment of the coastal upwelling system.

Simulations of demographic scenarios in DYABC were only partially concordant in *S. scurra* and *L. spicata*, but advocates for a major influence on effective sizes of both organisms of this unreported northward and posterior southward displacement of the coastal upwelling system during and since the LGM, respectively. The most likely scenario according to DYABC analyses was scenario 3 in which *S. scurra* expanded its distribution southward after LGM. This scenario is consistent with clusters 1 and 2 being the oldest, and having probably diverged from an ancestral population after a demographic change took place in both of them during, or since the end of the LGM. Unlike the other scenarios, scenario 3 supports the hypothesis of a post-glacial origin of cluster 3 from cluster 2, and the subsequent southward range expansion and divergence of cluster 3 giving rise to cluster 4. Both the smaller and more recent recovery of the effective population sizes of cluster 3 and 4 would indicate a major shift of ecological or environmental conditions affecting *S. scurra* during the LGM. For *L. spicata*, the most likely was scenario 2', which indicates that southernmost populations were also affected by some major environmental change during the LGM and experienced a post-glacial demographic recovery since then. Nonetheless, our data indicate that *L. spicata* populations from 30° to 33°S have been demographically stable during and since the LGM. This would indicate that *L. spicata* populations in the southern IA were affected, but were more resilient than *S. scurra* populations and were not extinct. In this case, the likely displacement of the West Wind Drift to the north during LGM probably reduced the availability of resources for *L. spicata* in the southern tip of the IA diminishing its population sizes but not to the extinction. For instance, prolonged development of kelp microscopic stages (particularly gametophytes, or young sporophytes) is critical to their population persistence (e.g. McConnico and Foster 2005). Moreover, long distance dispersal by rafting

adults has been shown in kelps (e.g. Smith 2002). Adhesion discs of *L. spicata* frequently fuse to discs of other algae such as *Durvillea sp* which have an internal honeycombing of their fronds responsible for their buoyancy. Notwithstanding, a higher demographic instability, smaller sizes of *L. spicata* populations along with a reduction in patch quality are to be expected if upwelling centers disappeared from the southern tip of the IA during the LGM. Such a fragmentation and lower availability of its host probably affected mainly the dispersive phase of *S. scurra*. In fact, recruitment and habitat limitation have been shown to trigger a decreasing distribution range for another member of the Lottiidae in the northern hemisphere in recent times (Fenberg & Rivadeneira 2011). This idea is not necessarily in contradiction with our previous hypothesis about a higher likelihood of concordance of spatial genetic structures when the strength and specificity are higher between associated organisms. This only suggests that probably there were thresholds in the size of (or distances among) populations of *L. spicata* below (or beyond) which *S. scurra* southernmost populations in the IA were unable to locally recruit and where extrinsic or vicariant forces started to determine demographic trajectories and shape genetic structures.

4.3 ROLE OF SELECTION IN THE GENETIC STRUCTURE OF *S. SCURRA*

Finally, we explored the possibility of genetic structure in *S. scurra* at small spatial scales using AFLP data, coupling it to the host occurrence. Our survey was suggestive but not conclusive. F_{ST} index was significant between *S. scurra* inhabiting *L. spicata* or *D. antarctica* when all data from two neighbour sites were pooled. Moreover, this differentiation vanished when deleting the candidate loci for positive selection from the data set. Nonetheless, when the two *L. spicata* and two *D. antarctica* groups were analyzed taking into account their site of

origin (Quintay or Las Docas), the *D. antartica* group belonging to Las Docas (with only twelve *S. scurra* sampled) was not significantly different from the other three groups. Unravelling this interesting and promising avenue of research will require replicating this analysis in several sites along the Chilean coast to sort loci that are under selection by the host kelp environment, and those outlier loci that were shaped by other demographic and genetic forces (Bonin et al. 2006). Moreover, as pointed by Bonin et al. (2007), dominant biallelic markers like AFLP's require an additional sampling effort and more loci to attain the same level of accuracy in estimates of genetic structure, and especially in the detection of outlier loci under selection. On the other hand, in highly heterogeneous environments, as the intertidal, a migration load is expected due to arrival of unfavorable mutations from other localities and may cause mal-adaptation to the local environment. Theoretical predictions state that in this case, selection should shape traits that reduce gene flow, including the evolution of habitat preference (Lenormand 2002). In fact, the high genetic structure observed with AFLP's in *S. scurra* in geographically close sites suggests that this patella has probably evolved some mechanisms to reduce gene flow, and especially to track and locally adapt to its host *L. spicata*.

4.4 COMPARATIVE PHYLOGEOGRAPHY OF *S. SCURRA* AND *L. SPICATA*

Contrary to studies of historical biogeography and co-speciation inference, comparative phylogeography deals with more contemporaneous events of genetic structuring that take place at the level of individuals within and between populations of co-distributed species (Zink 2002). Increasingly more sophisticated coalescent and computation methods allow uncovering recent structuring and demographic events at the intraspecific level, that are

more easily erased from the genome of organisms when searching for concordance at higher taxonomic level and macroevolutionary time. Following Zink (2002), our general aim was assessing whether the demographic changes that took place since the LGM in populations of a specialist herbivore could complement the genetic patterns observed at the level of its phylogeographic structuring. More indirectly, we intended to show how the comparative phylogeography of a specialist herbivore and its alga could reveal past demographic changes in the host alga.

The integration of both the genetic structure and genetic signs of demographic changes inferred in *S. scurra* and *L. spicata* populations allowed a better delineation of their recent and common biogeographical history. In both organisms, a higher genetic structure was verified in northern populations (north of 34°S) than in the more homogeneous southern populations of the IA. This is in agreement with Paleocceanographic models and Paleoproductivity data suggesting an equatorward shift of the WWD during the LGM and a weakening of upwelling south of the 30°S (Williams and Bryan 2006, Toggweiler et al. 2006, Toggweiler & Russell 2008), and more generally with a decreased productivity in the southeastern Pacific Ocean during this period (Klump 1999). Moreover, the similar spatial genetic structure of *D. antarctica* with both *L. spicata* and *S. scurra* (Fig.4), with more genetic homogeneity and a predominant cluster in the southern tip of the IA, argue for a major environmental change having affected the demography of several co-distributed organism in this area of the Southeast Pacific coast. Effective population size changes were also consistent between *L. spicata* and *S. scurra* south to 34°S, during and since the LGM. Our data support the view that *S. scurra* populations became extinct during the LGM along with a demographic contraction of its host kelp *L. spicata* in the southern IA. Moreover, our demographic inferences suggest

that *S. scurra* populations in the southern IA started recovering only after *L. spicata* populations expanded post-glacially. *S. scurra* was then unable to persist by colonizing a new host or habitat during the LGM; except perhaps *D. antarctica*, but this host kelp also experienced a demographic contraction. In addition, it seems that *S. scurra* populations recover very slowly after the fragmentation and reduction of *L. spicata* populations, as evidenced by the recent and small effective sizes of its two southernmost populations in the IA (Fig. 5B). This high dependence of *S. scurra* for its intertidal *Lessonia* sp. hosts is also supported by the almost absence of *S. scurra* (personal observations) in the northernmost PP populations of *L. berteroana*, which first disappeared from and then spatially re-colonized the north since the strong El Niño Event of 1982-83 (Martinez and Cardenas 2003). Then, all the above support the view that *S. scurra* is highly ecologically specialized to *L. spicata*.

Finally, the significant correlation of genetic distances between *S. scurra* and its host *L. spicata*, along with the significant differentiation revealed by F_{ST} indexes even between neighbour *S. scurra* populations, also support the thesis about a higher probability of spatial genetic concordance when ecological interactions are strong and specific (specialists herbivores) rather than weak and opportunist (generalists). Nevertheless, a deeper understanding of the phenotypic traits and fitness consequences implied in local adaptation and their potential role in determining barriers to dispersal (versus extrinsically originated barriers) is required. Unlike generalist and plastic strategies, specialization requires local adaptation which, contrary to the most current ideas, is not restricted to direct developers but is almost as frequent in those having planktonic larvae (Sotka 2012). Moreover, as pointed by Lenormand (2002), excessive levels of gene flow would imply a migration load from the arrival of deleterious mutations causing maladaptation to the local habitat and favoring the

evolution of low gene flow. The evolution of low gene flow is more probable when it is coupled to habitat preference by which the locally adapted organism (especially planktonic dispersers) can track and recruit on its specific habitat (Lenormand 2002). For instance, as pointed by Sotka (2012) differences in locally adapted phenotypes across the landscape could in fact explain some frequently assumed extrinsic barriers to dispersal. In this context, extrinsic factors such as climate change during glacial ages could have affected sequentially and similarly both the plant and its specialized herbivore triggering a similar “local scale” of adaptation. In the case of *S. scurra*, the phylogeographic break at 27°S dividing *L. berteriana* in two different clades could have caused first maladaptation to local hosts, but then favoured the evolution of low gene flow and local adaptation for hosts located north and south of 27°S.

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CHAPTER IV: FINAL DISCUSSION

1 SOME LOGISTIC ISSUES AND PERSPECTIVES IN THE STUDY OF UBIQUITY AND ECOLOGICAL VERSATILITY

1.1 CONCEPTS PREVIOUSLY DEVELOPED FOR TERRESTRIAL SYSTEMS AS CRITERIA TO EVALUATE THE DEGREE OF SPECIALIZATION IN THE MARINE ENVIRONMENT:

This thesis aimed at assessing the level of specialization of a marine herbivore, the patella *S. scurra*, on its two host kelps *L. spicata* and *D. antartica*. From a revision of concepts and criteria, it appeared (in the Introductory chapter) that a specialist is properly characterized based on the degree of ubiquity and ecological versatility in its use of resources. In the second chapter of this thesis, we performed an estimation of the degree of ubiquity and ecological versatility of *S. scurra* to its two host kelps. Our data suggest that the occurrence of *S. scurra* is truly selective of its main host *L. spicata* whereas it has an opportunistic use of *D. antartica*. Then we described only one potential true resource able to regulate fitness gains in *S. scurra* when exploiting these algae: the shape and size of the kelp structures (stipes and discs) which would constrain the occurrence, densities and size of *S. scurra* found in different groups of algae. Our general description through logistic regressions on the presence/absence of *S. scurra* occurring in structures of different size and shape of *L. spicata* and *D. antartica* hide the influence of other confounding factors likely to promote a higher density and occurrence in larger algae such as: (i) the availability of food resources, (ii) the intrinsic behavioral capacity and chemical sensory organs allowing *S. scurra* to stay in proximal parts of stipes, as well as (iii) thresholds in size, quality or distances among the patches constituted by *L. spicata* and *D. antartica* algae and potentially determining immigration and emigration rates between them

(as shown for terrestrial specialist herbivores; e.g. Ekroos et al. 2010, Miller 2007, Chapman & Dytham 2007).

A selective organism would show an uneven performance when exploiting different food resources, showing high or positive fitness for a single or a few dietary items, while having zero benefits or costs when eating non-suitable food. On the contrary, a dietary generalist would show an opportunistic pattern of exploitation of dietary items according to their availabilities in the environment. Theoretically then, the opportunist is able to obtain similar earnings from the different items available in the environment, which would translate into similar fitness benefits. Nonetheless, these two criteria (selectivity and opportunism) are generally employed in a correlational way, as we did in chapter II. These criteria are then usually applied as an indirect way to describe positive relationships between fitness benefits and biotic or abiotic variables able to favor these improvements in performance. On the other hand, when such an approach is combined with experiments in the field and in the laboratory contrasting proximal factors explaining these improvements in fitness, a better understanding can be reached. In our case, the higher occurrence of *S. scurra* and their larger sizes when living in the larger structures of *L. spicata* and *D. antartica*, suggest that the availability of space, in terms of refuge against enemies or physical constraints, is more important than the availability of food (an over-abundant resource, taking into account the small size of the herbivore in comparison to its host kelps).

Nevertheless, from the application of some simple concepts already available in the terrestrial literature, I could verify if some particular resource axes (in this case, the structural complexity of host algae) constitute a main axis of specialization determining a high fitness for

a marine herbivore. More generally, it can be concluded about the applicability of concepts already developed for terrestrial systems in order to characterize more formally the degree of specialization of marine organisms. Moreover, it can be concluded about the need to define the degree of specialization in relation to resource axes other than food. This could in fact broaden the actual number of herbivore specialists known from the sea.

1.2 CONCLUDING REMARKS ABOUT DEGREE OF SPECIALIZATION OF *S. SCURRA* TO *LESSONIA*:

Both the ecological (as shown previously) and the genetic studies of this thesis support the hypothesis that *S. scurra* maintains a strong and specific ecological interaction with the two intertidal *Lessonia* species found along the Chilean coast. In fact, *S. scurra* populations located south of 34°S in the Chilean coast were not able to switch to another host alga or habitat during the LGM and extinguished locally. Moreover, the recovery of these southern populations of *S. scurra* is relatively recent and of small magnitude, concordant with a post-glacial demographic recovery of its host *L. spicata*, as previously shown with DYABC analyses in Chapter III. On the other hand, in Northern Chile, *S. scurra* shows a concordant genetic discontinuity at 27°S with its host *L. berteriana*, not verified in other organisms. This suggests that *S. scurra* followed the footsteps in the genetic structuring of its host *L. berteriana*, which probably resulted from the adaptation to different selective regimes in either sides of 27°S of latitude.

1.3 ORIGIN OF SPECIALIZATION IN *S. SCURRA*

Some hypotheses can be advanced in relation to the factors that could have facilitated the ecological specialization of *S. scurra* to *Lessonia*. The interaction between *S. scurra* and kelps

is relatively recent in geological time, approximately 2 mya (Espoz 2002), and is relatively coincident with the likely arrival of large Laminarian kelps in the Southeast Pacific coast, between 1,367- 4,340 mya (Martin & Zuccarello 2012). Among the eight species in the *Scurria* genus, *S. scurra* is the only one to have evolved specialization to an alga (Espoz 2009). The locomotive feet of patellae imply a high fitness cost and allow the organism to move slowly and relatively short distances. Both intertidal marine fishes and coastal bird predators have been reported to predate on *S. scurra* and other patellae in the coast of Central Chile (e.g. Bahamondes & Castilla 1986). Moreover, derived species in the genus *Scurria* like *S. scurra* have been shown to lack antipredator responses (Espoz 2002). Thus, the arrival to the Southeast Pacific coast of kelps like *Lessonia* could have afforded to the ancestor of *S. scurra* to adapt to this novel refuge against predators and to have access to stable food resources. Therefore, in the case of *S. scurra*, the hypothesis of enemy free space is the most likely.

All the accumulated knowledge about the use of microhabitat within *L. spicata* structures by *S. scurra*, -with the need to avoid being dislodged by the friction between stipes- and the preliminary surveys about its tuned behavior in *L. spicata*, would indicate the evolution of specialized traits allowing this herbivore to exploit an almost unoccupied niche. The skill for using a difficult microhabitat and give *S. scurra* exclusive access to true resources i.e. resources not available to its enemies and conferring important advantages in using an exclusive space for extensive periods of time, as defined previously in the introductory chapter. In relation to the versatility of habitats used or *ubiquity*, specialist organisms exploit a very narrow range of habitats (with skewed positive densities within one or a few habitats) and generally have an extreme *position* along environmental gradients (Mac Nally 2009). For example, extremophile bacteria live in a very restricted range of habitats like hydrothermal

vents but are adapted to survive and reproduce under physically or biochemically extreme conditions, not suited to most other organisms. Such is the case of *S. scurra* because of the reason previously mentioned.

The frequently observed trade-offs in herbivores between food acquisition and predation risk (e.g. Lima & Dill, 1990; Byrom et.al 2000 for terrestrial systems, or Hamilton & Heithaus 2001, in a marine environment), or between other important activities (eg. mating or breeding strategies) and predation risk (Hamel & Côté 2007 ; Cooper et al. 2009) constitute a promising and scarcely explored area of research in the marine environment (except Utne etal. 1993, 2001), particularly for small specialized herbivores. In fact, depending on factors such as size, mobility and chemical weaponry against enemies, organisms would be more or less constrained to exploit a narrow range of habitats and resources. Among opisthobranchia gastropods, the highly specialized small marine sea slugs are generally restricted to a single or a few different algae exploited as habitat and food. On the contrary, the larger opisthobranchia gastropods, or sea hares, exploit a wider range of algal species as food but some of them, mainly juveniles, specialize in specific algal species as refuge. A deeper understanding of the more specific circumstances under which local adaptation and specialization of marine herbivores to host algae could proceed are detailed in the next section.

1.4 SOME LIKELY MECHANISMS OF MARINE HERBIVORES TO CIRCUMVENT ENVIRONMENTAL STOCHASTICITY AND EVOLVE SPECIALIZATION TO HOST ALGAE

Recent studies among marine organisms show that dispersal distances (direct, genetic and invasion estimates) do not correlate linearly with pelagic propagule duration. This is due probably because of the difficulty of effectively measuring propagule duration in the sea, and because dispersal distance, when inferred from population genetic data, is highly dependent on a series of factors independently the duration of the pelagic phase (Weersing and Toonen 2009; Riginos et al. 2011). Still, there is a widespread agreement in that particular life history features (e.g. development mode, demersal or pelagic eggs, and particular dispersal behaviors) are correlated to the deterministic components, explaining the discrete categories of propagule dispersal distances estimated. There is also consensus about the major influence that oceanographic processes have in determining dynamics of populations (Jackson and Strathmann 1981, Rougharden et al. 1988, Gaylord and Gaines 2000). Indeed, recent studies (e.g. Riginos et al. 2011, and Weersing and Toonen 2009) demonstrate that genetic structure does not always relate linearly with geographic distance along the coast. These authors highlighted that population structure can be highly unpredictable, due to unaccounted physical deterministic parameters like currents or coastal topography. Moreover, bottom-up processes act at large spatial scales such that a great environmental heterogeneity and variability in population and community structures are to be expected.

Despite the few specialist herbivores reported to date, we suggest that other lines of evidence support the idea that there are probably more specialist herbivores in coastal benthic marine areas. In the first place, literature from the sixties and more recent evidence demonstrate the

existence, for some marine invertebrates, of a preferential settlement of larvae and development of recruits into specific host-algae (Proctor, 1968; Krug & Manzi 1999; Krug & Zimmer 2000). In the second place, contrary to the classical paradigm of almost exclusively long dispersal distances in the sea; recent knowledge shows that many marine organisms belonging to different taxonomic groups would effectively disperse at short distances: i.e. marine algae, some invertebrates and some reef fishes (e.g. Kinlan et al. 2003). This would foster the local adaptation to specific host algae. Herbivores with relatively intermediate dispersal distance would have to settle preferentially into their specific cosmopolitan host alga, and be highly specialized to exploit its host as habitat or as an aggregate of true resources (e.g. food, brooding site for females, or shelter against physical stress or enemies). In the third place, it has been shown that local adaptation is as common in direct developers as in planktonic dispersers (Sotka 2012). The theory of environmental “grain” predicts that the evolutionary responses of a species (e.g. local adaptation, phenotypic plasticity, generalism, and balanced polymorphism) depends on the scale of environmental variations relative to the scale that the organism is able to disperse (Sotka 2012).

Several theoretical models have shown the antagonistic roles of gene flow and the strength of selection in the adoption of specialist or generalist strategies in biological organisms. While low gene flow and strong selective regimes would favor adaptation to local habitat; high migration rates benefit the genotype having the highest mean phenotype in most habitat(s), i.e. the generalist (e.g. Lenormand 2002). An example of organisms having extremely low dispersive capacities and high specialization to their hosts are parasitic lice and their vertebrate hosts (with vertical transmission among generations of the host). In this case, the low migration rate and strong selective regimes within the host ensures not only local adaptation

(obligatory specialization) in ecological time, it also usually enables the coevolution or even cospeciation (co-phylogenetic divergence) with its host.

Nevertheless, even when gene flow is not low, there are still simple classical models (Levene 1953, Dempster 1955) for the evolution of specialization on two alternative habitats. Indeed, these theoretical models show that under some circumstances and two habitats the evolution of one specialist is more likely than the evolution of a generalist (two habitats), especially when it is coupled with habitat choice (Ravigné 2009). These models are based on varying combinations of density regulation (local or global) and relative contribution of each habitat to the next generation (constant or variable output). Modelisations of Ravigné et al. (2003, 2009) have shown that the evolution of specialization is more likely when there is strong selection and adaptation to a specific habitat (i.e. “hard selection”, currently associated to Dempster’s model). More importantly, Ravigné et al. (2003, 2009) showed that when organisms evolve habitat choice, the evolution of one specialist is more likely than the evolution of a generalist. Indeed, many experimental studies suggest that larvae of many marine gastropods show a preferential, specific settlement, into their final habitat or host, or a gregarious settlement among conspecific adults (Delaney et al. 1998; Kay, 2002; Krug & Manzi 1999; Krug & Zimmer 2000). For instance, there are examples of specialist marine herbivores (mainly opisthobranch sea slugs) having direct and indirect developmental mode, and which selectively recruit into their specific host-alga. For example, both the sea slugs *Elysia halimeda* and *Costasiella ocellifera* and the amphipod *Pseudoamphitoides incurvaria*, have direct development and adult females lay directly their eggs and/or brood them into their obligatory host alga (Hay & Fenical 1988). More importantly, in the case of the marine obligatory specialist *Alderia modesta* whose host-alga is *Vaucheria longicaulis*, both its lecithotrophic and

planktotrophic larvae have been demonstrated to exclusively metamorphose in response to carbohydrate cues released from its host alga. These same studies demonstrated that *A. modesta* larvae change its swimming behavior in accordance with a preferential settlement into this specific alga (Krug & Manzi 1999; Krug & Zimmer 2000).

The previous theoretical models and recent discoveries on host specific recruitment of larvae in marine organisms highlight that local adaptation to host is a likely evolutionary outcome in marine organisms with planktonic dispersive phases. Moreover, as is likely the case with *S. scurra*, such adaptation would result from the need to evolve low gene flow and avoiding migration load. This has interesting consequences on the inference of phylogeographic patterns and the need to unravel if some barriers to dispersal are explained by locally adapted phenotypes unable to cross to foreign environments or, on the contrary, have been shaped by extrinsic climatic or physical factors.

2 SOME PRELIMINARY SURVEYS ABOUT MOBILITY AND SPECIFIC BEHAVIOR OF *S. SCURRA*:

We performed exploratory surveys about the behavior and diet versatility of *S. scurra*, not detailed previously. Microscopic inspection of the content of the digestive tract in *S. scurra* individuals (six and five, in *L. spicata* and *D. antarctica*, respectively) revealed that almost all food material corresponded to tissues of the host kelp species from which *S. scurra* were collected. On the other hand, we performed two experiments in the field which support a high

specificity in behavior and mobility of *S. scurra* within patches of its host *L. spicata*. In a first experiment, we excluded *S. scurra* individuals from stipes of a group of five big adult *L. spicata* (exclusion treatment), and transferred most of them to stipes of an adjacent group of five *L. spicata* (overload treatment). A third group of unaltered five adjacent *L. spicata* were used as control. Measures of the maximum diameter kelp sizes and number of *S. scurra* within each alga were recorded. As shown in Table 1, several *S. scurra* individuals were lost from the overloaded group after 11 days. On the contrary, three out of five algae of the exclusion treatment and two out of five of the control group acquired new *S. scurra* individuals not present on their stipes at the beginning of the experiment. In the exclusion group, five new juvenile individuals of *S. scurra* were found (and one in the control group). Results from this overload treatment indicates that there is a threshold in the *S. scurra* density above which the available structural space and food resources of *L. spicata* are no longer sufficient to sustain the presence of additional *S. scurra* individuals; alternatively, competition and density dependent processes start to predominate. The new juvenile *S. scurra* individuals observed after 11 days in the exclusion treatment (and control) probably came from the overloaded treatment or from unrecorded individuals from the holdfast; it would have been necessary to repeat the experiment marking *S. scurra* individuals to ascertain this hypothesis. Nevertheless, our second experiment about mobility in *S. scurra* suggests that such statement is probably correct. We marked 53 *S. scurra* belonging to 15 adult *L. spicata*, registering their position on their respective stipe during 11 days. Our intention was to evaluate if *S. scurra* migrate or stay in their same home-cavities when densities were not experimentally altered. After 11 days, 94% of *S. scurra* individuals remained in their same cavity-home (Table 1), supporting the hypothesis of a high behavioral specificity of *S. scurra* to *L. spicata*.

Table 1. Gain and losses after 11 days in the *S. scurra* abundance in three groups of *L. spicata* algae subjected to an exclusion treatment (*S. scurra* removed), overload treatment (overloading with *S. scurra* from the exclusion treatment), and an untreated group of algae (only *S. scurra* marked and presence recorded)

Treatment	Disc diameter of kelp	<i>N Sscurra</i> before treatment	<i>N Sscurra</i> beginning treatment	<i>N Sscurra</i> after 11 days	Gain or Loss in <i>N Ss</i>	Average earnings per treatment
Overload	34	4	14	7	-7	
Overload	32	9	11	10	-1	
Overload	34	3	7	5	-2	
Overload	45	16	22	22	0	
Overload	27	8	15	6	-11	-4,2
Exclusion	27	5	0	0	0	
Exclusion	35	12	0	2 (+3 juveniles)	2	
Exclusion	20	2	0	1	1	
Exclusion	29	13	0	5	5	
Exclusion	50	10	0	0 (+2 juveniles)	0	1,6
Control	19	1	1	6 (+1 juvenile)	5	
Control	20	5	5	7	2	
Control	23	8	8	8	0	
Control	27	3	3	3	0	
Control	27	3	3	4	0	1,4
Average	29,93	6,8	5,93	5,73	-0,4	

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