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# Convergent evolution in the colour polymorphism of *Selkirkiella* spiders (Theridiidae) from the South American temperate rainforest

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Convergent evolution tends to lead to similar phenotypic responses to the same selective pressures. However, when the phenotypic response is polymorphic, it is less clear how evolutionary convergence can lead to parallel diversity across species and populations. The present study focuses on South American spiders in the genus *Selkirkiella* (Theridiidae), which are shown to be polymorphic for colour. We (1) examine the number of morphs and their correspondence across taxa and (2) place the phenomenon in a phylogenetic context to determine whether the colour polymorphism in *Selkirkiella albogutatta* from the Juan Fernández archipelago represents a case of independent evolution and convergence, or whether there is common ancestry with other colour-polymorphic theridiids. Regarding the latter question, we also examine colour morphs in a related species (*Selkirkiella luisi*) from continental areas. We show that *S. alboguttata* and *S. luisi* have six and two morphs, respectively. The rank-order of morph frequencies in both species is approximately similar to that reported in other polymorphic theridiids. A molecular phylogeny supports previous work and shows that the colour polymorphism in *Selkirkiella* species appears to be a case of convergent evolution of a diverse colour polymorphism at the family level. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2017, **120**, 649–663.

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## INTRODUCTION

Convergent evolution occurs at different levels of biological complexity, from molecules to behaviour, as well as across scales, from species, to genera and families (Wake, Wake & Specht, 2011). Some of the best examples of convergence are found in archipelago settings, where similar sets of ecological forms

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have evolved mostly independently on the constituent islands. This is well-illustrated by cichlid fish in Nicaraguan (Muschick *et al.*, 2011) and African (Muschick, Indermaur & Salzburger, 2012) lakes, *Anolis* lizards in the Caribbean (Losos, 2009), sticklebacks in post-glacial lakes (Schluter & Nagel, 1995), and Hawaiian spiders (Gillespie, 2004). However, convergence does not need to be associated with diversification. In situations where single species are characterized by diverse phenotypes (polymorphism) with their frequencies maintained by balancing selection, convergence can occur within species, as well as between species and genera, as illustrated in butterfly mimicry complexes (Nijhout, 2003; Kapan *et al.*, 2006).

Polymorphism is generally defined as discrete variation that is determined genetically at a small number of major loci (Ford, 1940). Because they are visible, colour polymorphisms are perhaps the most thoroughly characterized. Some of the best known examples of balanced colour polymorphisms are found in butterflies in the genera *Papilio* (Nijhout, 2003) and *Heliconius* (Kapan *et al.*, 2006), as well as in snails of the genus *Cepaea* (Surmacki, Ożarowska-Nowicka & Rosin, 2013), in which morph frequencies can remain constant over long periods of time.

There are several ways in which natural selection can operate to maintain colour variation within populations, including heterozygote advantage (Kalmus, 1945; Battaglia, 1958), disruptive selection between populations with migration between them (Spieth, 1979), and various forms of negative frequency-dependent selection (Takahashi *et al.*, 2010). Heterozygote advantage has been demonstrated in very few cases (Kalmus, 1945; Battaglia, 1958). Disruptive selection in different environments with gene flow between them is a probable explanation for the distribution of pale and melanic forms of the peppered moth *Biston betularia* in rural and industrial areas of Britain (Majerus, 1998). Some form of negative frequency-dependent selection is likely to be responsible for the maintenance of the majority of conspicuous polymorphisms in the wild (Gigord, Macnair & Smithson, 2001; Takahashi *et al.*, 2010; Ajuria Ibarra & Reader, 2013; Iserbyt *et al.*, 2013). However, using stochastic multilocus simulations, genetic polymorphism on resistance for plant–pathogen interactions was shown to be maintained by genetic drift, without invoking selection (Salathé, Scherer & Bonhoeffer, 2005).

In spiders, colour polymorphisms have now been characterized in crab spiders (*Synaema globosum*; Ajuria Ibarra & Reader, 2014) and orb weaving species (*Agalenatea redii*: Geay *et al.*, 2012; *Gasteracantha minax*: Waldock, 1991) amongst others (Oxford & Gillespie, 1998). One of the most spectacular examples is provided by the endemic Hawaiian happy-face spider *Theridion grallator* (Theridiidae) across the islands of O'ahu, Moloka'i, Maui, and Hawai'i. In any one population, this small spider occurs in approximately eight distinct colour morphs (Gillespie & Tabashnik, 1989; Gillespie & Oxford, 1998). Laboratory rearing experiments have shown that this polymorphism is inherited in a Mendelian fashion, with the phenotypes exhibiting a dominance hierarchy that reflects the extent of expressed pigmentation (Oxford & Gillespie, 1996a, b, c). In almost all populations, the polymorphism comprises a common, cryptic, plain yellow morph and numerous rarer *patterned* morphs, and appears to be maintained by balancing selection (Gillespie & Oxford, 1998). Remarkably, a fundamental change appears to have occurred in the mechanism of inheritance of the colour polymorphism on the island of Hawai'i (compared to Maui), with the most common morphs being sex limited (unlike Maui) (Oxford & Gillespie, 1996a, c). This suggests that the colour polymorphism, or at least many of the rarer *patterned* morphs, may have been 'reinvented' in different island populations (Croucher *et al.*, 2012).

Similar exuberant colour polymorphisms have been documented in other members of the Theridiidae, most recently in *Theridion californicum*, a North American west-coast species that extends from southern California to British Columbia (Oxford, 2009). Less variable but equally well-studied is the polymorphism in *Enoplognatha ovata* (Oxford, 1983, 1985a, b; Wise & Reillo, 1985; Reillo & Wise, 1988a, b). This comprises three morphs: *lineata* (all yellow), *redimita* (yellow with two dorsolateral carmine stripes), and *ovata* (yellow with a solid shield of carmine on the dorsal surface) (Oxford, 1983). Breeding experiments have shown that the colour polymorphisms in *T. californicum* (Oxford, 2009) and *E. ovata* (Oxford, 1983), like to that of *T. grallator* (Gillespie & Tabashnik, 1989; Oxford & Gillespie, 1996a, b), are all genetically determined. Intriguingly, not only have there been independent origins of the polymorphisms in the three species, but also, in some cases, the evolution of almost identical colour and pattern morphs (Oxford, 2009).

Ecologically, the theridiid spiders that display colour polymorphism tend to be found exposed under leaves and display black or red patterns on top of a background colour, which is often translucent, green or yellow (Oxford & Gillespie, 1998; Oxford, 2009). In *T. grallator*, the frequency of the bottom recessive phenotype (plain Yellow) is remarkably constant among populations both within and between islands (Gillespie & Tabashnik, 1990), possibly as a result of selection by gleaning birds (Gillespie & Oxford, 1998). Many bird species have been shown to be

capable of developing search images depending on the frequency and/or abundance of prey in a given area (Bond, 2007). However, on their own, variation in search rates and apostatic selection do not appear to be able to generate highly diverse colour polymorphisms; dietary wariness (the tendency to avoid novel food items) is likely to play a key role (Franks & Oxford, 2009). Carduelinae birds (Fringillidae) coexist with *T. grallator* in Hawai'i and have long been recognized as important predators of spiders (Perkins, 1913).

The present study builds on an observation in 2011 of colour polymorphism in *Selkirkiella alboguttata*, a representative of the family Theridiidae that is endemic to the islands of the Juan Fernández archipelago, 680 km off the Chilean coast (Berland, 1924) (Fig. 1). The goal was to quantify similarities in the colour polymorphisms between *S. alboguttata* and other colour-polymorphic theridiids and to map the phenomenon within a phylogenetic context, aiming to determine the nature of convergence, as well as the potential roles of selection and constraint leading to its repeated demonstration across the family. We can thus determine whether the colour polymorphism in *S. alboguttata* represents another case of independent evolution and convergence. We note that there are two levels of convergence. One is at the population level, where convergence in similar habitats leads to similar population characteristics; in the case of the theridiids, this would be the demonstration of colour polymorphism, with similar overall frequencies. A second level of convergence is at the level of the individual morphs, where specific morphs within the colour polymorphism are convergent. In the present study, we address both levels for the *Selkirkiella* species in the context of the family Theridiidae.

We addressed three specific questions: (1) is the colour polymorphism in *S. alboguttata* convergent to those of *E. ovata*, *T. grallator*, and *T. californicum* in terms of the number of morphs and their colours/patterns; (2) are the frequencies of colour morphs in *S. alboguttata* convergent to those in the other polymorphic species; and (3) do all polymorphic species form a monophyletic clade on a molecular phylogeny?

## MATERIAL AND METHODS

### The genus *Selkirkiella*

The genus *Selkirkiella* was originally proposed on the basis of a specimen of *S. alboguttata* from the Juan Fernández archipelago, Chile (Berland, 1924). All other species currently in this genus are confined to the Falkland Islands and the temperate rainforests of southern Chile and Argentina (Agnarsson, 2004; Platnick, 2014). Variability in abdominal colour and pattern was noted in the original description (Berland, 1924) and subsequent examination of related specimens in the genus (Levi, 1963). However, colour tends to be leached when specimens are stored in ethanol, making identification of discrete morphs difficult in long-preserved material. The best-documented colour polymorphism in *Selkirkiella* is that of *Selkirkiella purpurea* in which the original description explicitly states that there are five variants: (1) Red abdomen with black spots and black on the lateral sides of the ventral area, (2) Dark on the dorsal area of the abdomen with small red lateral markings and two white spots on the base of the markings, (3) Red abdomen with white spots and lateral markings almost absent, (4) Dark abdomen with lateral markings that are white in the anterior area and red in the posterior, and (5) Smaller in size, dark brown with white and red spots in the dorsal area (Nicolet, 1849). Phylogenetic analyses place the genus *Selkirkiella* as a basal lineage within the Theridiidae, close to *Pholcomma*, although morphological (Agnarsson, 2004) and molecular (Arnedo *et al.*, 2004) analyses differ in its placement relative to the genus *Enoplognatha*.

### Collecting sites and specimens

The material for the present study came from two field collections on Robinson Crusoe Island, Juan Fernández Archipelago (*S. alboguttata*), one museum collection (California Academy of Sciences) from the Valdivia area (Chile) (*S. luisi*) (Fig. 1), and a field collection from Berkeley, California (USA) (*T. californicum*). The first field collection on Robinson Crusoe Island was conducted in August 2011 (DCC, 14 individuals), whereas the second (48 individuals) was conducted in April 2012 (Gustavo Hormiga and Miquel A Arnedo). The spiders were collected during the day and at night, by hand, and with a beating sheet. The specimens collected in 2011 were photographed before preservation. All the samples were preserved in 95% ethanol at −20 °C for molecular work.

A total of eight jars of unsorted arachnid material from the California Academy of Sciences collection were examined. This material came from tree fogging in the Valdivia area of Chile (Elizabeth Arias in February 2008). From this collection, a total of 80 *Selkirkiella* specimens were identified. Lastly, specimens of *T. californicum* were collected in the Berkeley Hills, California (USA) (PJPC in 2011). These specimens were also preserved for molecular work. Collecting sites for all samples are provided in the Supporting information (Table S1).


**Figure 1.** Field sites. A, the Juan Fernández Archipelago is composed of three volcanic islands [Robinson Crusoe, Santa Clara (small), and Alejandro Selkirk] and located 680 km from the coast of central Chile. The Valdivian forest is marked in green (images and map created on www.esri.com). B, forests on Robinson Crusoe Island showing the general habitat occupied by *Selkirkiella alboguttata*. The vegetation present in the Valdivian forest is similar to that on Robinson Crusoe Island.

### Colour polymorphims

The colour morphs of *Selkirkiella* were scored using a classification from previous studies of colour polymorphisms in theridiid spiders (Reillo & Wise, 1988a; Oxford & Gillespie, 1996a, b; Oxford, 2009). Exact contingency tables were used to test for homogeneity in morph frequencies between the sexes (http://vassarstats.net).

### DNA sequences

DNA was extracted from four legs from the right side of each spider using the DNeasy kit (Qiagen). Mitochondrial [cytochrome oxidase subunit I (COI) and the large ribosomal subunit (16S)] and nuclear [two ribosomal subunits (28S and 18S) and histone 3 (H3)] genes were amplified so that these samples could be combined with previously published data sets (Arnedo *et al.*, 2004; Arnedo, Agnarsson & Gillespie, 2007) (see Supporting information, Table S2). We sequenced one individual of each of the following species: *S. alboguttata*, *S. luisi*, and *T. californicum* (see Supporting information, Table S3). The respective GenBank accession numbers are provided in the Supporting information (Table S2).

The master mix for all the polymerase chain reaction (PCR) reactions consisted of: 1 μL of each primer at 10 μM, 2 μL of AmpliTaq buffer 10×, 0.5 μL of $MgCl_2$ 25 mM, 1.6 μL of dNTP 10 mM, 11.7 μL of $H_2O$, 1 μL of bovine serum albumin 1×, 0.2 μL of DreamTaq (ThermoFisher Scientific, Waltham, MA USA), and 1 μL of DNA extraction. The amplification profile for COI (HCO : LCO; Folmer *et al.*, 1994) started with 2 min at 95 °C, followed by 35 repetitions of a cycle that started with 30 s at 95 °C, then 45 s at 42 °C, and, finally, 1 min at 72 °C; there was an extra extension step at 72 °C for 10 min. The same profile was also used for 16S, 18S, 28S, and H3, and the only elements that differed were the annealing temperatures and the number of cycles. For 16S, we used the primers LR-N-13398 and LR-J-12864 (Simon *et al.*, 1994), an annealing temperature of 47 °C, and 25 cycles. For 18S, we used the primers 18S-5F and 18S-9R (Giribet *et al.*, 1999), an annealing temperature of 59 °C, and 40 cycles. For 28S, we used the primers 28SA and 28SB (Whiting *et al.*, 1997), an annealing temperature of 48 °C, and 40 cycles. Finally, for histone 3, the primers H3F and H3R were used (Colgan *et al.*, 1998), with an annealing temperature of 48 °C and 40 cycles.

The PCR products were verified on an agarose/TBE gel. PCR products were cleaned using an AxyPrep MagPCR Clean-up kit (Axygen). DNA was sequenced directly in both directions using the cycle sequencing method with dye terminators and BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies). Sequenced products were cleaned using AxuPrep Mag DyeClean kit (Axygen) and analyzed on an 3730XL DNA Analyzer automated sequencer (ABI). The sequences were assembled into specimen- and gene-specific contigs using GENEIOUS PRO, version 5.6.7 (Biomatters) (Drummond *et al.*, 2012) and edited by hand. Multiple sequence alignments were produced using the algorithm MAFFT (Katoh *et al.*, 2002) with the default parameters.

### Phylogenetic reconstruction

Model parameters for estimating molecular evolution were determined using PARTITIONFINDER (Lanfear *et al.*, 2012). The concatenated sequence (2426 bp) had eight partitions, including codon positions. The estimated models were: H3 position 1: SYM+G; H3 position 2: GTR+G; H3 position 3 and COI position 3: JC+I+G; 16S position 1, 16S position 2, and 16S position 3: GTR+I+G; 18S position 1, 18S position 2, 18S position 3, 28S position 1, 28S position 2, and 28S position 3: SYM+I+G; COI position 1: HKY+I+G; and COI position 2: GTR+I+G. *Argiope argentata* (Araneidae) was used as an outgroup *sensu* Arnedo *et al.* (2004). In addition to the sequenced species from the present study, we used other representatives from the family (see Supporting information, Table S2). Among them is an undescribed specimen of *Selkirkiella* from Osorno in the southern Chilean region of Los Lagos.

Phylogenetic reconstruction was performed with MrBayes, version 3.2.6 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), as made available from the remote server CIPRES Science Gateway, version 3.1 (Miller, Pfeiffer & Schwartz, 2010). The reconstruction utilized two runs of four independent chains each. Each run comprised 10 000 000 generations sampling every 1000. Convergence was determined by combining both runs in TRACER, version 1.7.5. (Rambaut *et al.*, 2014). The burn-in was set at 25%. Parameter convergence was determined by checking the potential scale reduction factor (1.0), the SD of split frequencies (< 0.01), and the estimated effective sample size (> 200). A final consensus tree and parameters were determined using the 'sumt' and 'sump' command of MrBayes respectively, again discarding 25% as burn-in. The phylogenetic tree was visualized with FIGTREE, version 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree). Ancestral character state reconstruction was performed using parsimony with unordered characters in MESQUITE, version 3.1 (Maddison & Maddison, 2016).

## RESULTS

### Colour polymorphism

A total of six variants of *S. albogutatta* were identified from 63 specimens from five different localities on the island of Robinson Crusoe (Fig. 2; see also Supporting information, Table S1). A guanine layer is present beneath red and black pattern elements but not under the dorsolateral rows of black spots. There is also a black bar on the cephalothorax, which is more pronounced in some morphs than others. Colour morphs are discrete, allowing classification to follow the scheme used in other polymorphic theridiid spiders (Oxford, 1983, 2009; Gillespie & Tabashnik, 1989, 1990; Oxford & Gillespie, 1996a,b,c).

The frequencies of the different morphs are summarized in Table 1. All colour morphs were found in males, whereas two were absent from females and juveniles (Red front and Belt red front). In males, the three most common morphs were Yellow, Arch, and Two spots, each representing 28.6% of the population. The Yellow morph occurred in 50% of the females, with Belt morph being the second most abundant (25%). Although we could not distinguish the sexes in juveniles, most were of the Yellow morph (62.1%), followed by the Two spots morph (24.1%). Overall, the Yellow morph was the most common (48.4%), followed by the Two spots morph (24.2%). The sample sizes from each location and among mature males, females, and juveniles are so small that separate statistical analyses are not possible. However, if all patterned morphs are combined and compared with the number of Yellow specimens, a $2 \times 3$ exact contingency table (which allows working with small samples sizes) among males, females, and juveniles shows no significant difference ($P = 0.060$, two-tailed).

The museum specimens from the tree-fogging collections from the Valdivian rainforest (mainland Chile) were all identified as *S. luisi* based on the morphological key of Levi (1967). There are two morphs from a total of 80 specimens collected in three different locations (see Supporting information, Table S1). The variants recorded were Yellow and Red bands (for descriptions, see Fig. 3). In males, the Yellow morph represented an overwhelming majority (90.5%), whereas the Yellow morph frequency was 67.7% for females and 75% for juveniles (Table 2). As before, sample sizes are small but, if homogeneity of morph frequencies across sample sites is assumed, a $2 \times 3$ exact contingency table among males, females, and juveniles shows that there are no significant

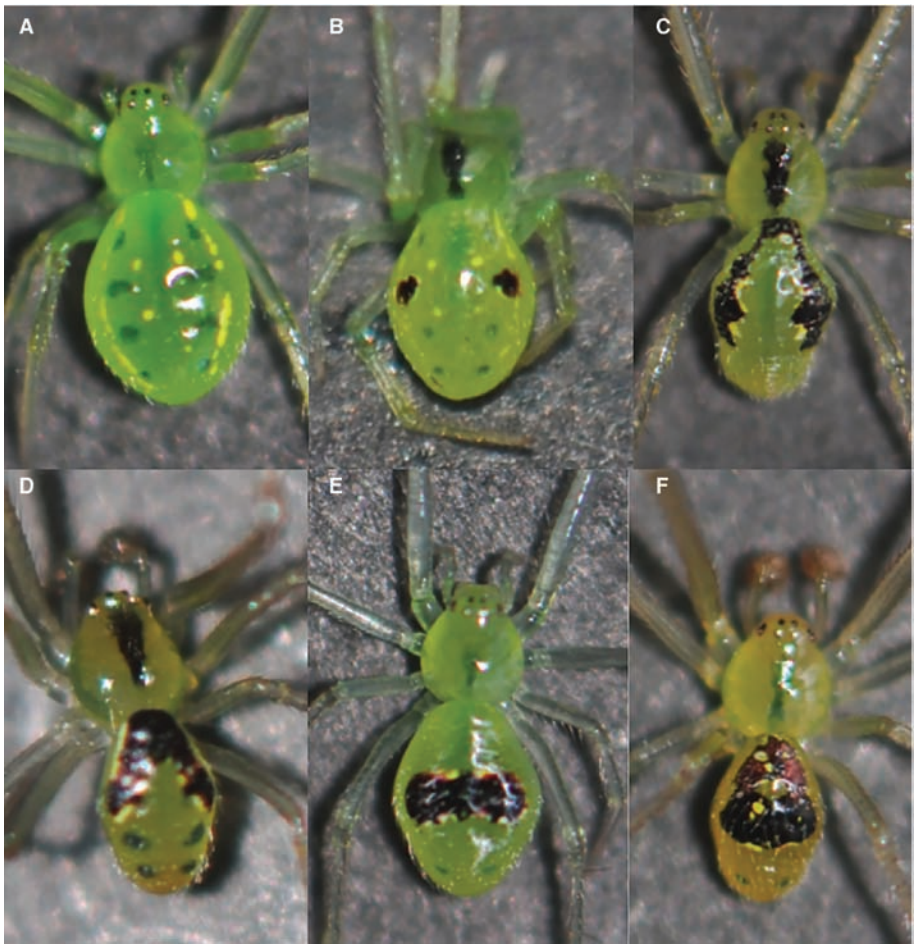


**Figure 2.** Colour polymorphism in *Selkirkiella alboguttata*. The species occurs as six different variants: Yellow morph with four pairs of dorsolateral black spots (A); Two spots morph with patches of red and black pigment in the position of the second pair of spots from the anterior end (B); Arch morph with a black U-shape mark in the anterior part of the abdomen and no separate black spots (C); Red front morph with a U-shape red/black mark in the anterior part of the abdomen and two pairs of spots in the posterior part (D); Belt morph with a horizontal dark mark in the middle of the abdomen and two very faint black spots at the posterior end (E); and Belt red front morph, which looks like the Belt morph but with a red crescent in the anterior part of the abdomen (F).

differences in the morph frequencies of these groups ($P = 0.150$, two-tailed). These samples were preserved in 95% ethanol at −20 °C, and so it is possible that some of the original pigments were degraded; however, the similarity of the percentage Yellow in *S. luisi* compared to that reported in other polymorphic theridiids is reassuring.

Finally, when comparing the percentage of Yellow morphs vs. other variants between *S. alboguttata* and *S. luisi*, a $2 \times 2$ exact contingency table shows a highly significant difference ($P = 0.00014$, two-tailed).

### Phylogenetic reconstruction

After completion and combination of both runs, all parameters had a potential scale reduction factor equal to 1.0 and the effective sample size for the combined runs was > 200. The SD of split frequencies between runs was 0.006068. A value of < 0.01 is considered to be 'very good' (Ronquist, Huelsenbeck & Teslenko, 2011). As in the original analysis that included most of these specimens (Arnedo *et al.*, 2004), the phylogeny recovered a clade that includes the *Selkirkiella* species at the base of the Theridiidae (Fig. 4). Also connecting to the polytomy at the base of the family are the Latrodectines and a large clade including several groups (Hadrotarsines, Spintharines, Lost Colular Setae clade, *Anelosimus* s.s. and Argyrodines).

The new sequences generated from the present study indicate that the genus *Selkirkiella* is monophyletic and the island species have a relatively long

**Table 1.** Frequencies of colour variants in *Selkirkiella alboguttata*

| Location | Yellow | Arch | Belt | Two spots | Red front | Belt red front |
|---|---|---|---|---|---|---|
| | Males ($N$ = 21) | | | | | |
| Plazoleta El Yunque | 3 | 5 | 1 | 5 | 1 | 0 |
| Cerro Damajuana | 0 | 1 | 0 | 1 | 0 | 0 |
| Cordón Salsipuedes | 0 | 0 | 0 | 0 | 0 | 0 |
| Salto de la Pulga | 2 | 0 | 0 | 0 | 0 | 1 |
| Valle Villagra | 1 | 0 | 0 | 0 | 0 | 0 |
| Total per category | 6 | 6 | 1 | 6 | 1 | 1 |
| Percentage per category | 28.6 | 28.6 | 4.8 | 28.6 | 4.8 | 4.8 |
| | Females ($N$ = 12) | | | | | |
| Plazoleta El Yunque | 3 | 0 | 2 | 2 | 0 | 0 |
| Cerro Damajuana | 2 | 0 | 1 | 0 | 0 | 0 |
| Cordón Salsipuedes | 1 | 0 | 0 | 0 | 0 | 0 |
| Salto de la Pulga | 0 | 1 | 0 | 0 | 0 | 0 |
| Valle Villagra | 0 | 0 | 0 | 0 | 0 | 0 |
| Total per category | 6 | 1 | 3 | 2 | 0 | 0 |
| Percentage per category | 50.0 | 8.3 | 25.0 | 16.7 | 0 | 0 |
| | Juveniles ($N$ = 29) | | | | | |
| Plazoleta El Yunque | 11 | 2 | 1 | 6 | 0 | 0 |
| Cerro Damajuana | 2 | 0 | 0 | 1 | 0 | 0 |
| Cordón Salsipuedes | 0 | 0 | 0 | 0 | 0 | 0 |
| Salto de la Pulga | 0 | 0 | 0 | 0 | 0 | 0 |
| Valle Villagra | 5 | 1 | 0 | 0 | 0 | 0 |
| Total per category | 18 | 3 | 1 | 7 | 0 | 0 |
| Percentage per category | 62.1 | 10.3 | 3.4 | 24.1 | 0 | 0 |
| Total (M+F+J) | 30 | 10 | 5 | 15 | 1 | 1 |
| Percentage (M+F+J) | 48.4 | 16.1 | 8.1 | 24.2 | 1.6 | 1.6 |

M, male; F, female; J, juvenile.

branch, perhaps as a result of their isolation. The unidentified *Selkirkiella* species [labelled as '*Anelosimus* (*Selkirkiella*) spp.'] in GenBank was not close to *S. luisi* and *S. alboguttata*, suggesting that this is a different species. This specimen was collected in Osorno (Chile). Levi (1967) did not have samples from Osorno for his revision work, and so it is difficult to suggest a species identification based solely on location.

As in all previous studies (Agnarsson, 2004; Arnedo *et al.*, 2004, 2007), the genus *Theridion* was not recovered as monophyletic. Congruent with that shown by Arnedo *et al.* (2004, 2007), in the present phylogeny it is possible to recognize three groups: (1) Hawai'i (*T. grallator*, *Theridion kauaiense* and *Theridion posticatum*); (2) New World [*Theridion* cf. *frondeum* (USA and Bahama Islands), *Theridion longipedatum* (Colombia), and *T. californicum* (California, USA)]; and (3) *Theridion varians* (Holartic) (Platnick, 2014).

The species that present colour polymorphism are marked in red on Fig. 4 (*S. alboguttata*, *S. luisi*, *T. grallator*, *T. californicum*, and *T.* cf. *frondeum*). Note that polymorphisms have been also described for the genus *Enoplognatha* (Oxford, 1983, 1985a, 1992) and *Chrysso* (R. G. Gillespie, pers. comm.). The common ancestor of the clade including *T. californicum*, *T.* cf. *frondeum*, and *T. longipedatum* presents a reconstructed state that cannot be determined with certainty because of the nonpolymorphic condition of *T. longipedatum* (see Supporting information, Fig. 1S). A similar situation occurs at the base of the genus *Selkirkiella* but, in this case, the situation of '*Anelosimus* (*Selkirkiella*) spp.' was coded as unknown (see Supporting information, Fig. 1S).

## DISCUSSION

### COLOUR MORPHS OF *SELKIRKIELLA*

*Selkirkiella alboguttata* exhibits a total of six morphs (Fig. 2), whereas *S. luisi* has two morphs: Yellow and Red bands (Fig. 3). These morphs show remarkable similarity to those already described in other

well-studied polymorphic species (Reillo & Wise, 1988a; Oxford & Gillespie, 1996a,b; Oxford, 2005, 2009). The colour morphs described in the present study for *Selkirkiella* appear to represent an evolutionary convergence with those of other species in the Theridiidae. In all these species, a Yellow morph is always present. The black spots present on the Yellow morph of *S. alboguttata* are similar to those of *T. grallator*, *E. ovata*, and *T. californicum*. The Arch and Red front morphs of *S. alboguttata* are also present in *T. grallator*. Indeed, the Red front morph gives the latter species its common name: the Hawaiian happy-face spider. The Red blob morph, found in *T. grallator*, *T. californicum*, *E. ovata*, and *E. latimana*, has not been reported for any *Selkirkiella*. However, this morph is usually rare in the well-studied polymorphic theridiids (Gillespie & Tabashnik, 1990; Oxford, 2005, 2009) and the overall sampling of *Selkirkiella* is far from complete. In *S. luisi*, the Red bands morph is similar to Red lines, described from the polymorphic *Theridion* spp., *E. ovata* and *E. latimana* (Gillespie & Tabashnik, 1989; Oxford, 2009), although not in *S. alboguttata*. In *S. luisi*, the bands/stripes are shorter than in other polymorphic species in the Theridiidae.

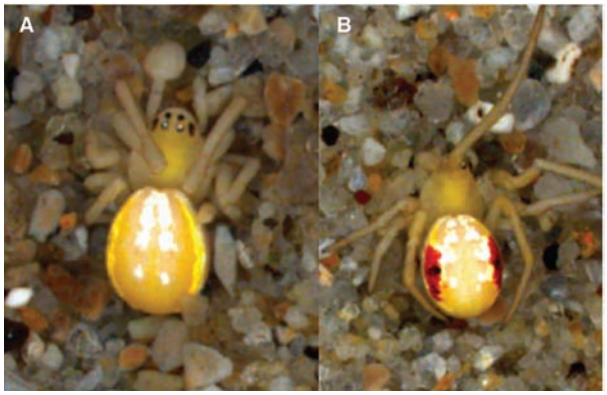


**Figure 3.** Colour polymorphism in *Selkirkiella luisi*. The species occurs as two different variants. They correspond to: Yellow morph, which is very similar to the one in *Selkirkiella alboguttata* (note there are two dark yellow lateral bands, which are where the yellow pigment is not underlain by guanine) (A) and Red bands morph, which has two red markings on the sides that do not extend for the full extension of the abdomen (B). The pictures were taken from spiders preserved in 95% ethanol in −20°C, and so the original colours of these specimens could have been altered.

The most common colour morph in *S. alboguttata* is Yellow, which also appears (albeit based on human perception) to be the most cryptic. This bias towards the most cryptic morph matches that found in the other colour-polymorphic theridiids. In *E. ovata*, morphs exhibit a highly consistent rank-order of frequencies within populations, with Yellow being the most common (Oxford, 2005). In *T. grallator*, the frequency of Yellow is remarkably constant, at approximately 70% among populations both within and between islands (Gillespie & Tabashnik, 1990). Similarly, in *T. californicum*, the Yellow morph makes up 60% or so of the population, with the remainder comprising up to 10 *patterned* morphs (Oxford, 2009). In future studies, it will be important to test the reflectance of all these species with respect to the leaves on which they live with the aim of obtaining a more precise idea about the degree of crypsis of the Yellow morph from a predator's perspective.

In *S. alboguttata*, the overall ratio of Yellow : *patterned* was lower than that documented in other taxa, with only 48% being Yellow overall. These differences in the total frequency of the Yellow morph in *S. alboguttata* with respect to the other four

**Table 2.** Frequencies of colour variants in *Selkirkiella luisi*

| | Males (21) | | Females (31) | | Juveniles (28) | |
|---|---|---|---|---|---|---|
| Location | Yellow | Red bands | Yellow | Red bands | Yellow | Red bands |
| Reserva Costera Valdiviana (*Nothofagus nitida*) | 4 | 1 | 3 | 1 | 2 | 3 |
| Fundo Paipahueño (*Aextoxicon punctatum*) | 5 | 0 | 8 | 3 | 7 | 3 |
| Fundo Paipahueño (*Podocarpus nubigenus*) | 8 | 0 | 6 | 3 | 8 | 2 |
| Fundo Paipahueño (*Myrceugenia planipes*) | 0 | 1 | 3 | 2 | 3 | 0 |
| Fundo Manchao (*Myrceugenia planipes*) | 2 | 0 | 1 | 1 | 0 | 0 |
| Total per category | 19 | 2 | 21 | 10 | 20 | 8 |
| Percentage per category | 90.5 | 9.5 | 67.7 | 32.2 | 71.4 | 28.6 |
| Total (M+F+J) | 60 | 20 | | | | |
| Percentage (M+F+J) | 75 | 25 | | | | |

The specimens were obtained by fogging from the trees species indicated in parenthesis. M, male; F, female; J, juvenile.

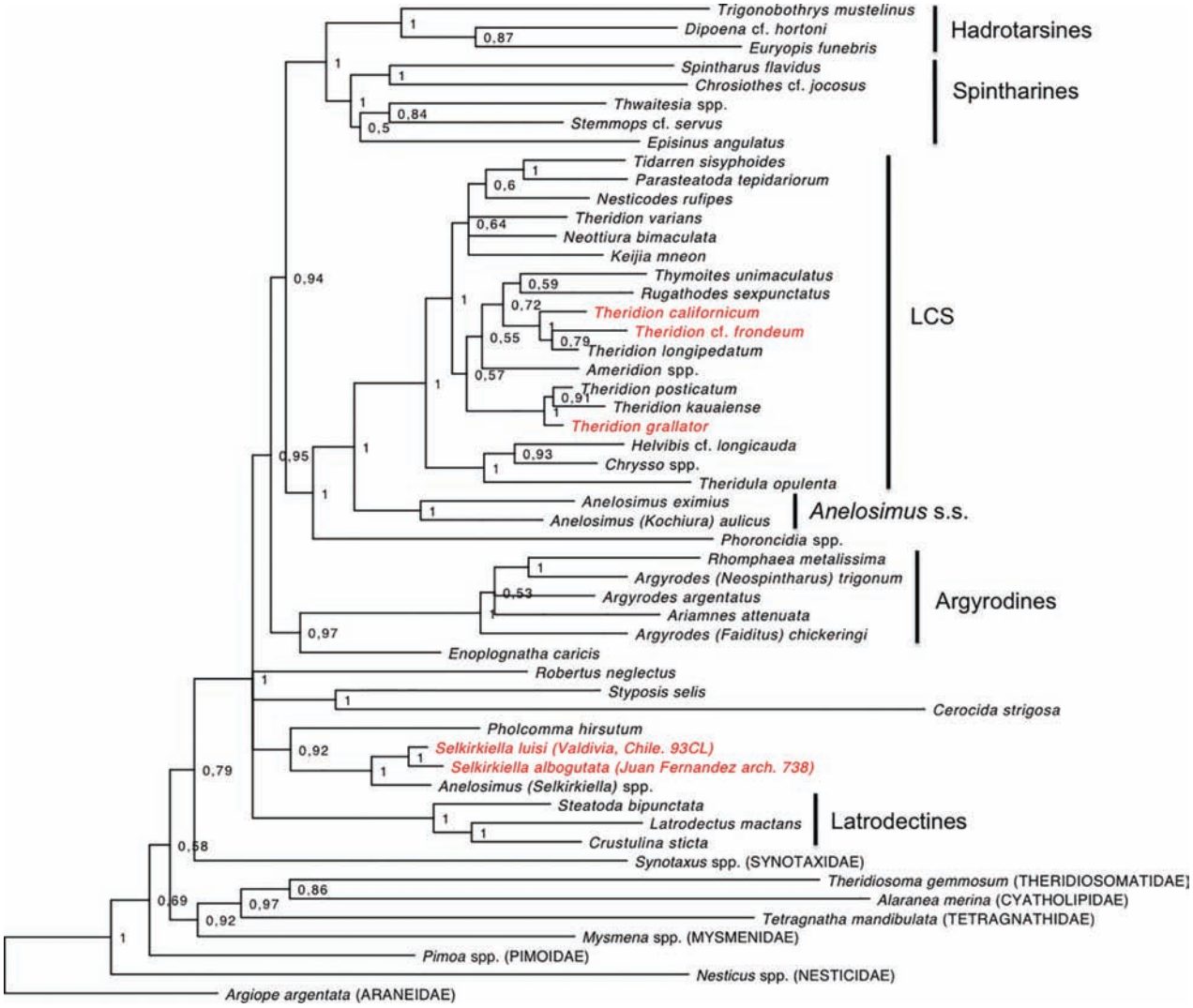


**Figure 4.** Bayesian phylogenetic reconstruction. The molecular phylogeny is based on five concatenated genes (COI, 16S, 18S, 28S, and H3). The sequenced specimens (see Supporting information, Table 2S) were included with the specimens included in Arnedo *et al.* (2007) and some from Arnedo *et al.* (2004). Nodes with posterior probabilities < 0.5 were collapsed into polytomies. All species with described colour polymorphism are shown in red.

polymorphic species might be explained by differences in the selective forces that maintain the polymorphism in each species. Given that males tend to wander actively when they are searching for mates, they may suffer higher rates of predation and so the lower percentage of Yellow individuals in males might not be surprising (Gillespie & Oxford, 1998). When excluding males from the analysis, the frequency of Yellow is 59%, which is very close to the approximately two-thirds Yellow : *patterned* in other taxa. Additionally, we cannot rule out stochastic effects on local morph frequencies (Oxford, 2005) or artefacts resulting from the under-sampling of both individuals and populations.

The two morphs found in the mainland species *S. luisi*, Yellow and Red bands (Fig. 3), are present in both males and females. In this species, the Yellow morph is found at a frequency (75%) similar to those in other colour-polymorphic theridiids. The high frequency in this species could also explain why previous descriptions make no mention of the polymorphism (Levi, 1967).

Across the genus *Selkirkiella*, there are three truly polymorphic species (*S. alboguttata*, *S. luisi*, and *S. purpurea*) and two others (*Selkirkiella magallanes* and *Selkirkiella ventrosa*) with a lesser degree of variability, which may or may not correspond to discrete polymorphic states. In particular,

it is possible that one of the variants of *S. magallanes* corresponds to the Belt morph of *S. alboguttata*. For *S. magallanes*, Levi (1967) describes the presence of a 'broad transverse band'; however, he stated that it was 'made up of white pigment spots' and further noted that the colours are altered after 1 year in alcohol. These 'white pigment spots' are likely to be guanine, which is not lost on preservation, and possibly lay under the original pigment. This phenomenon had been previously demonstrated in museum specimens of *T. californicum* (Oxford, 2009).

The other three species in the genus, *Selkirkiella carelmapuensis*, *Selkirkiella wellingtoni*, and *Selkirkiella michaelseni* have not been reported to have colour variation. The description of *S. carelmapuensis* and *S. michaelseni* appears to represent a Yellow morph and it is possible that, as in *S. luisi*, the type specimen corresponds to the most common morph. In general, to properly recognize a polymorphic species two conditions are required: (1) a robust sample size and (2) proper preservation of the pigments. These conditions may not have been met with some of the described *Selkirkiella* species.

## Genetic basis for colour polymorphism

In all theridiid species for which the genetic basis for the colour polymorphism has been documented, which includes *E. ovata* (Oxford, 1983), *Enoplognatha latimana* (Oxford, 1992), *T. grallator* (Oxford & Gillespie, 1996a,b,c), and *T. californicum* (Oxford, 2009), the Yellow morph is homozygous recessive. At present, the genetic basis for the colour polymorphism in *Selkirkiella* is unknown; rearing experiments are necessary to identify the inheritance of the different colour morphs and to estimate the number of loci that control the polymorphism.

There is no strong evidence for sex linkage or limitation in the colour polymorphism in either species. In *S. alboguttata*, even though two morphs present in males are absent from juveniles and females, these differences are not significant and most of the morphs are present in both sexes. However, in both *T. grallator* and *E. ovata*, sex-limitation of some morphs has been demonstrated: (1) the Big Island (Hawai'i) population of *T. grallator* (Oxford & Gillespie, 1996b) and (2) some populations of *E. ovata*/*E. latimana* (Oxford, 1983, 1985b, 1992). There is also variation in the timing of pigment deposition during development in *E. ovata* (Oxford, 1983). At present, we cannot eliminate the possibility of sex differences in *S. alboguttata*.

The question as to why the Yellow morph is the most common is still unresolved. However, the situation where the bottom recessive morph is more frequent than more dominant morphs has been recognized previously (Haldane, 1939). Indeed, Clarke (1964) noted that models of frequency-dependent selection also lead to the bottom recessive morph being the most frequent. These ideas still need to be tested in a comparative framework within the polymorphic species of Theridiidae spiders. An empirical comparative test would clarify, for all of the species, whether the same genetic pathways are involved in the polymorphic condition, or whether is produced by different developmental processes but triggered by similar selective forces.

## Balanced colour polymorphism

The dual role of spiders as both predators and prey means that selection can operate in different ways: whether to conceal them from predators or to make them more attractive to prey (Tso, Lin & Yang, 2004). Among theridiid spiders, the consistency of the relative proportions of the Yellow and combined *patterned* morphs between species and across isolated populations (Gillespie & Oxford, 1998; Croucher *et al.*, 2011) provides evidence for a polymorphism maintained by natural selection. For *T. grallator*, it has been hypothesized that predatory pressures (birds) are responsible for this condition (Gillespie & Oxford, 1998). Moreover, for other polymorphic species in the family (*E. ovata*: Oxford, 2005; *T. californicum*: Croucher *et al.*, 2011), there is also evidence that natural selection and not drift/migration underlies these exuberant polymorphisms.

As in other colour-polymorphic theridiids, *Selkirkiella* lives under the leaves of trees and shrubs, potentially exposing them to the activity of foliage-gleaning birds (Fig. 5). Given the similar niche, it is likely that similar selective pressures are also in operation. Predators such as birds are known to develop search images for the most common prey type in their habitat, resulting in the exposure of this form to the highest predatory pressure; in such situations, an individual that looks different from the most common form will avoid detection (Bond & Kamil, 1998). This apostatic selection and other forms of predator-mediated, negative frequency-dependent selection can maintain stable colour polymorphisms in populations (Franks & Oxford, 2009; Ajuria Ibarra & Reader, 2013). In simple simulation models, this pattern can readily be reproduced under assumptions of negative frequency-dependent selection imposed by predators, either through the development of search images (apostatic selection) or, even more effectively, by dietary wariness in naïve individuals (Franks & Oxford, 2009). In this scenario, new morphs are selected merely because they are visibly different from existing morphs. We suggest

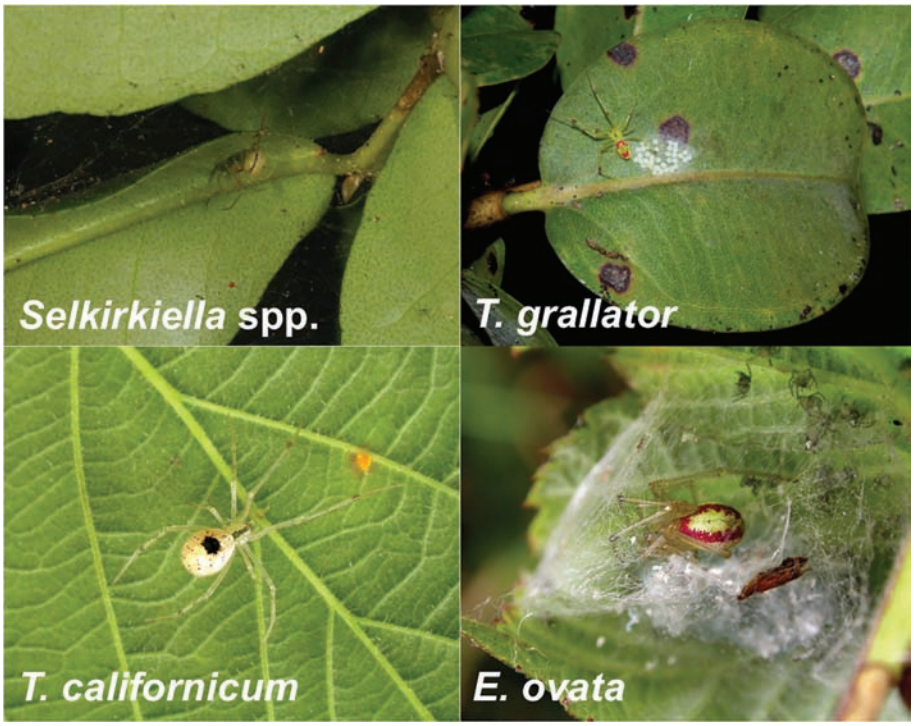


**Figure 5.** Habitat of polymorphic species. All the presented species live and spin their webs under leaves exposing themselves to predators in a very similar way. Locations: *Selkirkiella* spp., Zapallar (Chile); *Theridion grallator*, Maui (Hawai'i); *Theridion californicum*, Northern California (USA); and *Enoplognatha ovata* (UK). All the images were photographed by the authors of the present study, except by *Selkirkiella* spp. (photographed by Miquel Arnedo).

that similar processes might also operate in the case of *Selkirkiella*.

The presence of balancing selection on the colour polymorphism in *Selkirkiella* still needs to be tested. For example, it has been shown for butterflies (*Papilio*: Nijhout, 2003; *Heliconius*: Kapan *et al.*, 2006), *Cepaea* snails (Surmacki *et al.*, 2013), and the mottled rock rattlesnake, *Crotalus lepidus lepidus* (Farallo & Forstner, 2012). The occurrence of convergent colour polymorphisms in the Theridiidae family presents an ideal system with which to examine the generalities and particularities of this phenomenon.

### Phylogenetic reconstruction

Our phylogenetic analysis is consistent with previous studies showing the *Selkirkiella* species as a sister group to *Pholcomma hirsutum* (Arnedo *et al.*, 2004) and other related pholcommatines (Agnarsson, 2004) and also showing that it appears at the base of the tree of the family Theridiidae (Arnedo *et al.*, 2004). Importantly, the reconstruction suggests that, at the family level, *Selkirkiella* represents another independent origin of colour polymorphism in the Theridiidae because it appears to be phylogenetically distantly related to other well-described examples, such as *T. grallator* or *T. californicum*. However, a comprehensive phylogenetic reconstruction is needed for a more conclusive demonstration of the number of independent origins of the polymorphic condition. Three of the four reported cases of colour polymorphism in the family Theridiidae occur among species present in different genera (*Enoplognatha*, *Selkirkiella*, and *Theridion*), within each of which the colour polymorphism is an infrequent condition. Thus, the number of independent origins of colour polymorphism could well increase as more species are examined and included in the phylogeny. At the genus level, the fact that both *Selkirkiella* species are polymorphic may suggest that the condition pre-dates their separation as distinct species, as in *E. ovata* and *E. latimana* (Tan, Gillespie & Oxford, 1999). However, to test rigorously the polymorphic condition of the common ancestor of all the *Selkirkiella* it will be necessary to add the other continental species to the analysis, as well as any specimens from Santa Clara and Alejandro Selkirk islands, which are also in the Juan Fernández archipelago (Fig. 1).

The phylogenetic positions of the other polymorphic species indicate that *T. grallator* and

*T. californicum* are not sisters and actually belong to separate subclades (Fig. 4). As shown by Arnedo *et al.* (2004), the closest relative to *T. grallator* is the *T. kauaiense*/*T.posticatum* pair. *Theridion californicum* appears sister to the species pair *T.* cf. *frondeum* and *T. longipedatum*. Although there are no reports of visible colour polymorphism in *T. longipedatum*, *T. frondeum* displays a colour polymorphism similar to that documented in other theridiids (N. Breidenbaugh, pers. comm.), suggesting a potential common ancestor that could have shared the polymorphic condition. However, because of the lack of colour polymorphism in *T. longipedatum*, the character reconstruction of the ancestor of the clade presents a reconstructed state that cannot be determined with certainty (see Supporting information, Fig. 1S). If the genus *Enoplognatha* is monophyletic, then the polymorphic sister species *E. ovata* and *E. latimana* (Tan *et al.*, 1999) should cluster with *E. caricis* (Fig. 4) and represent yet another colour polymorphism convergence.

Colour polymorphisms themselves are not uncommon in spiders, being present in many other families (Araneidae, Tetragnathidae, Linyphiidae, Oxyopidae, Pisauridae, Salticidae, and Gnaphosidae) (Oxford & Gillespie, 1998). However, there is little evidence for frequency dependence in the colour morphs. Thus, within the Theridiidae, the genus *Latrodectus* shows colour polymorphism with variation so high that, initially, 22 species were described based in coloration and setae patterns; these have subsequently been synonymized into just six species using genital morphology (Levi, 1959). The situation in *Latrodectus* spiders has been hypothesized to be an example aposematic coloration (Kaston, 1970).

## CONCLUSIONS

The presence of colour polymorphism in *Selkirkiella* appears to be an example of convergent evolution at the family level. At least three of the eight described *Selkirkiella* species have distinct colour polymorphisms and two others, as far as is known, show some degree of variability.

We can conclude based on this analysis that (1) there are remarkable parallels in the colour polymorphism in spiders in the family Theridiidae, resulting in suites of matched colour morphs across different species, and (2) the taxa that display these similar colour morphs are only distantly related. The key feature uniting these species is the similarity in their niches, reinforcing the view that living under the leaves of shrubs and trees (Fig. 5) is conducive to the evolution of colour/pattern polymorphisms, with the most likely selective agent being predation by gleaning birds. Moreover, the frequency and morphology of the most cryptic morphs is remarkably similar in all situations where the phenomenon has now been reported, with sites ranging from Europe, North America, Hawai'i, and now Chile and the Juan Fernández islands. This last element is likely a result of developmental constraints within the group.

Colour polymorphisms are well known among both plants and animals, with the diversity frequently arising from sexual selection (Gray & McKinnon, 2007). Among colour polymorphisms that show convergent evolution, the selective pressure imposed by predators is most commonly invoked. However, the context differs. Thus, the mimicry rings of *Heliconius* and other butterflies lead to geographically-defined colour morphs as the product of Müllerian mimicry (Bates, 1862). Here, local predator memory and perception lead to selection for regional geographically defined differences in colour morphs (Mallet & Joron, 1999). Likewise, colour morphs of the peppered moth, *Biston betularia*, appear to be maintained by selective pressures that differ in both space and time (Majerus, 1998). Varying selective pressures over space appear also to be involved in the colour polymorphisms of spiders, most notably the apparently aposematic colouration of widow spiders (*Latrodectus* spp., Theridiidae) (Brandley, Johnson & Johnsen, 2016). In contrast, colour polymorphism in theridiid spiders that live exposed under leaves is associated with predator behaviour that is geographically homogeneous (Fig. 5). For these spiders, the main mechanisms that have been invoked to explain the maintenance of the exuberant colour polymorphism are apostatic selection (Gillespie & Oxford, 1998) and/or dietary wariness in naïve predators (Franks & Oxford, 2009). In these scenarios, selection would act on novel morphs merely because they are visibly different from existing morphs. The inference, which remains to be tested, is that avian predators are actively involved in selection for novel morphs across these polymorphic theridiids that live under leaves. Remarkably, as shown in the present study, this kind of selective pressure, apparently arising from frequency-associated changes in predator perception within a given geographical area, could be associated with the evolution of similar arrays of colour morphs across vast areas and in phylogenetically disparate taxa. If the behavioural/cognitive mechanisms operating in these situations can be validated, then it is possible that an explanation can be provided for many of the documented invertebrate visible polymorphisms.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1.** Ancestral character reconstruction of the polymorphic condition. The reconstruction method used was parsimony with a model of unordered characters. The analysis shows three independent (or four, if *Theridion californicum* and *Theridion* cf. *frondeum* are independent) origins of the condition. Note that the specimen '*Anelosimus* (*Selkirkiella*) spp' was coded as unknown.

**Table S1.** Collecting sites and samples used in the present study. All the samples from *Selkirkiella alboguttata* were collected on the Robinson Crusoe Island (Juan Fernández Archipelago, Chile). The general location for the samples of *Selkirkiella luisi* corresponds to Los Rios District, Chile.

**Table S2**. Sequences downloaded from GenBank.

**Table S3.** Sequenced specimens.