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The invasion of southern South America by imported bumblebees and associated parasites

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and associated parasites

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Summary

1. The palaearctic *Bombus ruderatus* (in 1982/83) and *B. terrestris* (1998) have both been

introduced into South America (Chile) for pollination purposes. We here report on the

results of sampling campaigns in 2004, and 2010-2012 showing that both species have

5   established and massively expanded their range.

2. *B. terrestris*, in particular, has spread by some 200 km /year and had reached the Atlantic

coast in Argentina by the end of 2011. Both species, and especially *B. terrestris*, are

infected by protozoan parasites that seem to spread along with the imported hosts and

spill over to native species.

10 3. Genetic analyses by polymorphic microsatellite loci suggest that the host population of *B.

terrestris* is genetically diverse, as expected from a large invading founder population,

and structured through isolation by distance. Genetically, the populations of the

trypanosomatid parasite, *Crithidia bombi*, sampled in 2004 are less diverse and distinct

from the ones sampled later. Current *C. bombi* populations are highly heterozygous,

15   and also structured through isolation by distance correlating with the genetic distances

of *B. terrestris*, suggesting the latter's expansion to be a main structuring factor for the

parasite.

4. Remarkably, wherever *B. terrestris* spreads, the native *B. dahlbomii* disappears although

the reasons remain unclear. Our ecological and genetic data suggest a major invasion

20   event that is currently unfolding in southern South America with disastrous

consequences for the native bumblebee species.

Introduction

Today, the invasion of species into foreign regions, that is, the spread of a species in an area

where it was previously not found, and the resulting mixing of the world's biota are on the

5   rise, being greatly facilitated by human travel and international trade (PYŠEK *et al.* 2010). In

addition, animals or plants are purposefully imported into a new area by man because of

their anticipated beneficial effects for agricultural produce or to control pests. Such

biological invasions can become detrimental when the imported species escapes control and

subsequently causes economic losses to crops or livestock (VILA *et al.* 2010) or starts

10   threatening the native fauna and flora. However, whether or not an invasion is successful is

assumed to depend on various factors, such as resource supply (PYŠEK *et al.* 2010),

introduction effort and founder diversity (LOCKWOOD, CASSEY & BLACKBURN 2005), or the

invader's affiliation with human activities (JESCHKE & STRAYER 2006). A more recent

emphasis is on the effects of parasites, e.g. when resident parasites select against invaders,

15   novel parasites select against residents, or when invaders leave behind their parasite fauna

("enemy release hypothesis") (CROWL *et al.* 2008; PRENTER *et al.* 2004).

Cases of purposeful introductions of foreign animals and plants are often well documented

and the agenda associated with the introduction is clear. Such cases can therefore elucidate

20   patterns and, sometimes, factors that mediate the invasion process. Here, we report on one

such case - the planned introduction of European bumblebees for pollination services into

southern South America. Bumblebees are important pollinators in their natural range

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(BINGHAM & ORTHNER 1998). As commercially produced pollinators they add substantially

to the economic value of agricultural products (GOULSON 2003; VELTHUIS & VAN DOORN

2006). The commercial trade is mainly in two species, *Bombus terrestris* L. in Europe and *B.*

impatiens Cresson (*B. occidentalis* Greene to a lesser extent) in North America, both of

5   which are bred in the hundreds of thousands of colonies mainly for greenhouse pollination of

valuable crops (VELTHUIS & VAN DOORN 2006). At the same time, the natural abundance

and diversity of bumblebee species is in decline in many parts of the world (BIESMEIJER *et al.*

2006; GOULSON, LYE & DARVILL 2008; WILLIAMS 1982), with parasites being identified as

drivers of these declines (CAMERON *et al.* 2011). There is therefore growing concern about

10   pathogen transport with imports and spill-over to resident pollinator communities due to the

fact that non-native, commercially raised bumblebees are being exported and have now

established in various regions of the world (MEEUS *et al.* 2011). These colonies commonly

contain parasites (MURRAY *et al.* 2013). Cases in point are the import and subsequent

naturalization of *B. terrestris* into Northern Japan (Hokkaido) in the 1990s (INOUE,

15   YOKOYAMA & WASHITANI 2008) with concerns of parasite spill-over (GOKA, OKABE &

YONEDA 2006; NIWA *et al.* 2004). Several *Bombus* species from England were introduced

and established in New Zealand and have brought parasites with them (HOPKINS 1914;

MACFARLANE & GRIFFIN 1990).

20   Here, we report on the case of two European *Bombus* having been introduced for pollination

in southern South America (Chile) in the 1980s and 1990s. We aimed at analysing the

historical spread of these species, their population genetic structure and the presence of two

common parasites, *Crithidia* (Trypanosomatidae) and *Nosema* (*Paranosema,* Microsporidia)

to scrutinize the extent of the invasion and to elucidate the possible role of parasitic

infections in this process. In fact, concerns over parasitism as a cause for the displacement of

native species have recently resurfaced (AIZEN, LOZADA & MORALES 2011). However, prior

5   to our first campaign (2004) next to nothing was known about the parasites and diseases of

Bombus in southern South America, but reports have multiplied since (ARBETMAN *et al.*

2013; MAGGI, LUCIA & ABRAHAMOVICH 2011; PLISCHUK & LANGE 2009b; PLISCHUK *et al.*

2009; PLISCHUK *et al.* 2011a). Yet, this report represents the first systematic survey to date.

10   **Introduction of *Bombus* spp. into South America**

Of the 250 species of bumblebees (*Bombus* spp.) (CAMERON, HINES & WILLIAMS 2007), a

total of 24 species have naturally reached South America (CAMERON & WILLIAMS 2003;

HINES 2008). Here, we focus on the Patagonian region (the south of Argentina and Chile)

15   that harbours five native species. We did not encounter *B*. *atratus* Franklin*, B. bellicosus*

Smith, or *B funebris* Smith in this study, either because their known distributional ranges do

not, or only marginally overlap, with our sampling locations (ABRAHAMOVICH & DÍAZ 1982;

ABRAHAMOVICH, DIAZ & LUCIA 2007; ABRAHAMOVICH, DIAZ & MORRONE 2004) but we

did find *B. dahlbomii* Guérin-Méneville and *B. opifex* Smith.

20

INTRODUCTION OF *B. RUDERATUS*

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For importation, European *B. ruderatus* Fabricius were collected in New Zealand (where it

had previously been introduced in 1882) in December 1982 (199 queens) and November

1983 (192 queens), and shipped to Chile (ARRETZ & MACFARLANE 1986). New Zealand was

chosen due to the synchronicity in the breeding cycle with South America and the assumed

5   low abundance of parasites at sampling locations. The sampling sites were believed to be

free of the nematode, *Sphaerularia bombi* Dufour 1837, and the tracheal mite, *Locustacarus*

buchneri Stammer 1951. They were also assumed to be free of the protozoan parasite

Apicystis (*Mattesia*) *bombi* Lipa & Triggiani 1996, as this gregarine is reported to be missing

in New Zeland (see MACFARLANE, LIPA & LIU 1995, for a review). Several other mite

10   species in New Zealand were also known from the native South American *B. dahlbomii*

(ARRETZ & MACFARLANE 1986) and thus not considered a problem. Queens of the first

shipment were released on 23 December 1982 east of the city of Temuco (100 queens

released near Coipue; 45 released near Cunco) in an area of red clover production. One

hundred queens of the second shipment were released on 6 December 1983 near Coipue, and

15   an additional 69 queens on 7 December 1983 near Cunco (Figure 1) (ARRETZ &

MACFARLANE 1986). Introductions were successful because workers of *B. ruderatus* were

seen near Coipue in February and March 1983 and in the following southern spring

(November/December 1983) about 7 km south of Cunco.

20   INTRODUCTION OF *B. TERRESTRIS*

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The information on the release of *B. terrestris* in Chile is much less detailed (RUZ 2002; L.

Ruz, pers. comm). One source was from Israel, likely from the Mt. Carmel region, where *B.*

terrestris became the dominant species (DAFNI & SHMIDA 1996) after having been first

recorded in the early 1930s in the North of Israel. A possible second source was from

5   Belgium but details of this shipment and its release are missing. Imports were authorized by

the Servicio Agrícola y Ganadero (SAG, Chile) in 1998 (RUZ 2002). A company (Xilema

S.A.) handled the actual introductions from Israel (see reports by the FIA, Fundación para la

Innovación Agraria; FIA-GOBIERNO DE CHILE 2000). Xilema also ran tests, on behalf of the

FIA, for pollination efficiency on tomato in the experimental station of the Pontificia

10   Universidad Católica de Valparaíso 's School of Agriculture near Quillota (*c.f.* Figure 1)

during 1997 /1998 using both closed and open glasshouses (FIA-GOBIERNO DE CHILE 2000;

project code FIA-PI-C-1997-2-A-009). According to FIA, Xilema had continued to import

bumblebees after the termination of the project (i.e. after 1998); further companies are said to

have applied to SAG for import permissions but no further information is available. The FIA

15   had furthermore run a project to breed *B. terrestris* in the years 2008-2009 (project code

08CS-1111). According to Prof. Eugenio Lopez (School of Agronomy, Valparaíso

University; contacted in early 2012; L. Ruz, pers.comm.) the first shipment from Israel in

1997 was used within the FIA-project in Quillota; the second shipment from Israel in 1998

was distributed to farmers across Chile in Arica, Copiapó, San Felipe, Quillota, Limache,

20   Santiago, and Los Angeles (MONTALVA *et al.* 2011). Given this historical information, we

tentatively set the introduction point of *B. terrestris* relevant for this study to the year 1998 in

Quillota. In addition to the two European species, the North American *B. impatiens* was also

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imported by farmers but no further details are known (RUZ 2002). No specimens of this

species were encountered during our studies; hence it probably did not establish.

5   **Material and methods**

SAMPLING

We did several explicit sampling campaigns (Figure 1). *Campaign (1)*: In January 2004, one

10   of us (RS) sampled the area around Villarrica (Chile). *Campaign (2)*: In January - February

2010, sampling was done by PSH and RSH in Chile from ca. 100 km north of Copiapó

(administrative Region III) to the island of Chiloé (Region X). *Campaign (3)*: In January

2011, PSH and RSH surveyed Patagonia (along Ruta Nacional, R.N. 40) from San Carlos de

Bariloche, Argentina, to Punta Arenas (Chile) and along the Atlantic coast (R.N. 3 to

15   Comodoro Rivadavia). *Campaign (4)*: in October - November 2011, SP checked the area

around Comodoro Rivadavia for *B. terrestris*. *Campaign (5)*: In January 2012, DG and JS

surveyed the eastern slopes of the Argentina Andes, from Bariloche north towards Mendoza.

For details see Table S1, Supporting Information. Generally, we aimed at around 50

specimens per site, but this could not always be achieved due to weather and local conditions.

20

Sampling locations were grouped into larger geographical regions: (1) "Chile Central"

(sampled in 2004: "Chile Central 2004") representing the core Chilean mainland from

Santiago south to Puerto Montt; (2) "Andes East" - the eastern slopes of the Andes, from San

Martín de los Andes (where *B. terrestris* was first reported in Argentina) south to

Gobernador Costa; (3) "Argentina Atlantic": the reach from inland, Sarmiento, to the

Atlantic coast at Comodoro Rivadavia; (4) "Patagonia West": the Patagonian region on the

5   western slope of the Andes; and (5) "Patagonia South": the southern tip of South America,

especially the area from around Puerto Natales to Punta Arenas. The sampling periods

(January-March, except campaign 4 to the Atlantic coast) coincided with the known

phenology of *Bombus* in these areas, e.g. ABRAHAMOVICH & DIAZ 2001; AIZEN 2001.

10   During the sampling campaigns, sites along the major roads with abundant flowers typically

visited by bumblebees were searched. These sites consisted of flowering patches either

directly along the road or land that could easily be reached by foot in the vicinity. The

decisions were made on the spot, such as to sample native bumblebee flowers known to be

visited by the native *Bombus* species. Examples include amancay (*Alstroemeria aurea*),

15   almost exclusively having been visited by the native *B. dahlbomii* (AIZEN 2001) especially in

forested or bushy areas, *Fuchsia* (*magellanica*), e.g. abundant in many places along Lago

General Carrera, various Fabaceae (e.g. *Adesmia*), other flowering plants (e.g. *Phacelia*,

Boringiaceae), but also more bush-like vegetation (*Luma* spp.). In addition, imported plants

such as clover (*Trifolium*) and Asteraceae (*Carduus*) were also prominent at many places.

20   Note that *B. dahlbomii* was also found in large numbers along our "road sites" in the very

same vegetation as *B. terrestris*, such that the differences reported here are not simply due to

a possible sampling bias.

Sites were separated by at least 10 - 20 km, but generally separated by larger distances

(coordinates, see Table S1, Supporting Information). Bees were collected at random by

walking slowly through a site and captured when visiting flowers, either by directly pushing

5   them into sampling vials or by netting as appropriate. The individuals were put into highly

concentrated alcohol (> 90 % ethanol) either immediately after sampling, or first individually

stored in a cooler and put into alcohol in the evenings of every sampling day. Species and

sex/caste was identified at capture but verified in the laboratory later. However, all species

sampled here (*B. terrestris*, *B. ruderatus*, *B. dahlbomii*, and *B. opifex*) are readily

10   distinguishable from one another by visual inspection alone (ABRAHAMOVICH, DIAZ &

LUCIA 2007).

DNA EXTRACTION AND HOST GENOTYPING

15

Only genetic data from *B. terrestris* females are reported here due to their abundance

(sample sizes), wide distribution, and the availability of many polymorphic microsatellite

loci. Genomic DNA (also used for parasite identification, see below) was extracted after

dissection from whole guts using the DNeasy 96 Blood & Tissue Kit [®](Qiagen GmbH,

20   Hilden, Germany) following the instructions by the manufacturer. Note that no *B. terrestris*

were present in the 2004 samples. For *B. terrestris*, we used seven microsatellites developed

by (ESTOUP *et al.* 1993) but adapted the protocol as follows. We ran two multiplex PCR

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reactions: "BB-Msat1" combined the PCR-reaction for primers B100, B118, B124, B126 and

B132 (annealing temperature = 57° C); "BB-Msat2" combined the reaction for primers B10

and B11 (annealing temperature = 52°). BB-Msat1 and BB-Msat2 were mixed before

loading the reactions onto an ABI 3730 sequencer (Applied Biosystems). Fragments were

5   further analysed with the software Peak Scanner™ (Applied Biosystems), and all results

inspected visually and checked for consistency and clarity of signal.

PARASITE IDENTIFICATION AND GENOTYPING

10

Infections by the gut parasite, *Crithidia* (Trypanosomatidae) were genotyped from all host

species. We checked for infection status (yes/no) with the Cyt b sequence (SCHMID-HEMPEL

& TOGNAZZO 2010)*,* and infection status for *Nosema bombi* Fantham & Porter 1914

(Microsporidia) with the small subunit rRNA (using the primers 18f and 1537r from BAKER

15   *et al.* 1995). For this, PCR products from all bees were visualized on PCR CheckIT® gels

(Elchrom Scientific, Baar, Switzerland) together with the necessary controls. Presence of a

band was taken as positive for infection, absence as negative. PCR-products from positive

infections were purified (ExoSAP method; HANKE & WINK 1994; WERLE *et al.* 1994), and

directly sequenced using BigDye® chemistry on a 3130/3730 ABI Sequencer (Applied

20   Biosystems). Sequences were edited and blasted for parasite identification using the software

MacVector 12.5.1 (Mac Vector Inc., Cary, NC). For *Crithidia*, all positives were of Cyt b-

type "A1", defining them as species *C. bombi* Lipa & Triggiani 1988 (SCHMID-HEMPEL &

TOGNAZZO 2010). Therefore, a subsequent PCR was performed for the five *C. bombi*

microsatellites loci Cri4G9, Cri4, Cri16, Cri2.F10, and Cri1B6 (REBER FUNK, SCHMID-

HEMPEL & SCHMID-HEMPEL 2006) to define the genotype. Two multiplex PCR (CB-Msat1

and CB-Msat2) reactions were done as described in (SCHMID-HEMPEL *et al.* 2011). The two

5   multiplex reactions were genotyped separately on an ABI 3730 (Applied Biosystems).

Fragments were analysed using the software Peak Scanner^{TM} (Applied Biosystems) and

again visually inspected. DNA samples from 2004 had been extracted during an earlier study

(SALATHÉ ZEHNDER 2007) using 10 % Chelex$^{®}$100 (BioRad), mixed with an equal volume

of Ringer solution (Merck). At the time, the samples were analysed for infection status

10   (yes/no) by one of us (RS); these infection status data were used here. For the current

combined study, these extractions were retrieved from storage but, unfortunately, could no

longer be used for a re-analysis. Therefore, we had to use a sub-sample of the infected bees

from 2004 for which the guts had been stored at -20 ˚C (hence, not all infected bees of 2004

could be fully genotyped).

15

STATISTICAL ANALYSES OF GENETIC DATA

To analyse the genetic data we used GENEPOP (RAYMOND & ROUSSET 1995), R-packages

20   pegas (PARADIS 2010), adegenet (JOMBART 2008), hierfstat (GOUDET 2005), and the program

STRUCTURE (PRITCHARD, STEPHENS & DONNELLY 2000); for geographical rendering: R

package maptool (LEWIN-KOH *et al.* 2009). For the genetic analyses of *B.terrestris*,

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populations with less than five individuals were merged with a neighbouring one whilst

respecting geographic barriers (e.g. not across the Andes; *c.f.* Table 2). This left us with a

total of 28 populations. Re-running the major analyses by simply excluding all small

populations did not change any of the conclusions. Because not all individuals were infected,

5   sample sizes for the *Crithidia* data are smaller. We again merged small populations into

neighbouring larger ones in a similar vein as above. Given the generally smaller samples, we

required at least three individuals per population for genetic analyses. (*c.f.* Table S2,

Supporting Information). In a number of cases, the genotyping of infections showed more

than two alleles at one or several loci. Since *C. bombi* is strictly diploid, this indicates a

10   multiple genotype infection. We have used the method used in (SALATHÉ & SCHMID-

HEMPEL 2011) to resolve multiple genotype infections into the single genotypes co-infecting

a host, which was possible in all cases. We have then used these resolved single genotypes

for the analyses reported here. An alternative method (Majority Rule) used the most frequent

genotypes as defined by allele frequencies in region. The corresponding details of method

15   and the results are given in the Supplementary Information for comparison. The results from

both methods were qualitatively the same.

20   **Results**

THE DISTRIBUTION AND SPREAD OF THE IMPORTED SPECIES

The original distribution of the native species, especially *B. dahlbohmii*, and prior to the

1990's is summarized in (MONTALVA *et al.* 2011) and shown in Figure 1. Furthermore, *B.*

ruderatus had crossed the Andes and was first reported from the Río Negro province,

Argentina, in 1996 (ROIG ALSINA & AIZEN 1996; cited in ABRAHAMOVICH, TELLERIA &

5   DIAZ 2001). Our first campaign of 2004 was restricted to the Chilean Lake district (region IX,

around Villarrica and Pucón); a total of 291 bees could be collected. Only two species were

found (*B. dahlbomii*, *B. ruderatus*). In the subsequent campaigns of 2010 and later, *B.*

terrestris was found over a wide area (Figure 2, Table 1). In particular, *B. dahlbomii*

common in 2004 in the Lake district had in the meantime disappeared from this area. During

10   the 2010 campaign the area from Santiago north to Copiapó was also visited (during January

2010), but no *Bombus* specimen could be found there.

In the campaign of 2011, *B. terrestris* was found on the Chilean side of the Andes as far

south as Villa O'Higgins (Aysén region; Figure 2). In January/February 2011, the invasion

15   front of *B. terrestris* was likely spreading along the river Baker and the shores of Lake

General Carrera, as suggested by the many sexuals found there (see Table S1 Supporting

Information; males in *B. terrestris*, young queens in *B. dahlbomii*). Moreover, whereas only

B. dahlbomii were found at one sampling point along Baker (site Río Baker I), only *B.*

terrestris were found 10 - 20 km further downstream (Río Baker II), thus forming a

20   relatively sharp boundary in an apparently homogeneous habitat. *B. terrestris* seemed to be

spreading eastward along the northern shore of lake General Carrera and had just reached

Los Antiguos (Argentina) at its eastern end. Along the northern lake shore only *B. terrestris*

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were found (sites Lago Gral Carrera I, II; 70 - 90 % being males); and only *B. dahlbomii*

along the southern shore (connecting to the Río Baker valley; sites Lago Gral Carrera III, IV).

Sampling in a corridor crossing the Patagonian steppe suggested that the spread of *B.*

terrestris in early 2011 had reached a point between Sarmiento and the Atlantic port of

5   Comodoro Rivadavia. No specimens of the invader were found at this time on the coast itself

despite intensive searching. Yet, sampling in October-November 2011 showed that *B.*

terrestris was now present in large numbers around Comodoro Rivadavia (Table 1, Figure 2).

In the campaign of 2012, *B. terrestris* was found on the eastern slopes of the Andes at a

considerable distance north of its first sighting near San Martín de los Andes (Neuquén

10   province) in 2006 (TORRETTA, MEDAN & ARAHAMOVICH 2006) (Table 1, Figure 2). The

native species *B. opifex* was only met around San Rafael where *B. terrestris* was not present

at that time.

Our campaigns of 2004 and 2010 confirmed the distributional status of *B. ruderatus* as

15   described by MONTALVA *et al.* 2011; specimens were collected in an area reaching from

Temuco to Puerto Montt, Chile. In 2011 we collected *B. ruderatus* in small numbers also on

the eastern slopes of the Andes, from Bariloche south to Corcovado (Table 1, Figure 2).

PARASITE INFECTIONS

20   *Crithidia bombi.* Among all species, the average *C. bombi* infection prevalence was 14.3 %,

with a peak value of 80 % for population Los Angeles, and substantial infections in Central

Chile and the region of Puerto Montt - Bariloche (Figure 3; Table S2 Supplementary

Information). A total of 183 hosts infected by *C. bombi* could be fully genotyped with

microsatellites. Of those, 41.5 % contained multiple infections; this proportion was not

different among host species (*B. dahlbomii:* 44 %, *B. ruderatus:* 36 %, *B. terrestris* 41 %;

Table S3, Supporting Information).

5

Host species differed in infection prevalence ($X^2 = 17.22$, $P < 0.001$, $df = 3$, $n = 1'620$) with

18.5 % for *B. dahlbomii* ($n = 378$), 7.7. % for *B. ruderatus* ($n = 196$), 14.4 % for *B.

terrestris* ($n = 1'018$), and none in *B. opifex* (n = 28). Thus, 70 of the total of 232 infections

(30.2%) were carried by the native species *B. dahlbomii*. Significant differences were also

10   found among populations ($X^2 = 351.04$, $df = 49$, $P < 0.0001$; $n = 50$ populations) and regions

($X^2 = 93.77$, $df = 5$, $P < 0.0001$; $n = 6$ regions; see Table S2 Supplementary Information for

additional GLM-analyses), but these effects are partly confounded with species.

Nosema bombi. Microsporidian infections were identified by rRNA sequences. The closest

15   matches were to *N. bombi* that was prevalent in the Chile samples, matches to *N. thomsoni*

and *N. portugal* were mainly found in Argentina, and a few matches to *Vairimorpha* sp. at

several locations. Overall, 2 % of the host bees carried a microsporidian infection ($n = 1,295$

bees; Table S2, Supporting Information); none was found in *B. dahlbomii* ($n = 198$) or *B.

opifex* ($n = 28$), but 6.5 % ($n = 77$) in *B. ruderatus*, and 2.0 % ($n = 992$) in *B. terrestris*

20   (among species $X^2 = 12.95$, $df = 3$, $P = 0.005$). All *Nosema* infections were thus contained

within the imported species. Although *N. bombi* was too rare for reliable analyses, nominally,

populations and regions showed significant differences (Table S2, Supporting Information).

Region "Patagonia West" (prevalence 10.9 %, $n = 128$ hosts) stood out due to high infection

levels in *B. terrestris* that were sampled along lake General Carrera. In all, prevalences of *C.*

bombi and *N. bombi* were not associated ($X^2 = 0.034$, $df = 1$, $P = 0.85$; for population means:

Spearman's r $= 0.249$, $n = 37$, $P > 0.1$).

5

GENETIC POPULATION STRUCTURE OF *B. TERRESTRIS*

A total of 818 individuals could be successfully genotyped out of 1,018 collected female *B.*

10   *terrestris*, representing 28 populations with at least 5 individuals (Table 2). Some specimen

could not be typed because of technical non-amplification (not null alleles); in some cases,

locus B118 did not amplify. All of the seven loci were polymorphic and, in any population,

had an average of around 5 alleles, with a conspicuously low value (3.2 ± 0.77 alleles) for

the Atlantic coast population of Comodoro Rivadavia. Similarly, observed heterozygosity

15   value, Hobs, and genic diversity, Hs, were relatively high (Table 2, Table S4 Supporting

Information). Almost all loci, in all populations, were in HW-equilibrium; only 11 out of the

231 tested values (4.8 %) were significantly deviating at the $P < 0.0001$ level (Bonferroni-

corrected threshold), presumably chance significances. Thus, *B. terrestris* populations in

South America are genetically variable and nominally in HW-equilibrium.

20

The populations of *B. terrestris* show some differentiation among one another (Gst $=$

$1,233.85$, $X^2 = 1,528.6$, $P < 0.001$) (GOUDET 2005; GOUDET *et al.* 1996). Analysis by the

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program STRUCTURE (PRITCHARD, STEPHENS & DONNELLY 2000) using the method of

EVANNO, REGBNAUT & GOUDET (2005) generated K = 3 sub-populations, primarily with

bees from the Atlantic coast (Comodore Rivadavia) and, to some degree, from central Chile

being separated from the remaining ones (App S1, Suppplementary Information). No

5   structuring was visible when using the ratio of pairwise Fst/(1-Fst) as suggested by

FRANCOIS *et al.* (2010) for a Principal Component Analysis of genetic distance (JOMBART,

DEVILLARD & BALLOUX 2010) (Figure S1, Supplementary Information). Similarly,

Genetical Correspondance Analysis (JOMBART, DEVILLARD & BALLOUX 2010), and the

Monmonier algorithm (R package hierfstat) also failed to identify clear boundaries between

10   populations. A nearest-neighbour joining tree suggested that the geographically far spread

populations were indeed also genetically far away (Figure S2, Supplementary Information).

Instead of separated populations, differences among the 28 populations seem to result from

an effect of isolation-by-distance (Fig. 4a). We also checked for a relationship of genetic

distance, the Fst/(1-Fst) ratio, with the physical distance from the likely source of

15   introduction (here: population Docas). But neither populations of *B. terrestris* (Spearman's *r*

= 0.04, *P* = 0.83, *n* = 25 populations) nor those of *C. bombi* (Spearman's *r* = -0.32, *P* = 0.13 *n*

= 23 populations) showed any such correlation.

GENETIC POPULATION STRUCTURE OF *C. BOMBI*

20

The population genetic structure of *C. bombi* infections collected in the 2004 campaign

showed lower genetic variation as compared to infections from the later campaigns (Table 3).

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For example, allelic richness for 2004 (average 1.95 ± 1.00 S.D. alleles, $n = 5$ populations, rarefaction minimum size) was lower than in the populations sampled later (campaigns 2010-2012: 2.63 ± 0.81 S.D., $n = 19$; Man-Whitney $U = 435$, $p = 0.003$): differences were visible even in the same location (e.g. the Pucón sample in 2004 vs. 2010, Table 3). An

5   exception was locus Cri16 that was completely monomorphic with only one allele (size 119) throughout all samples (Table S5, S6 Supplementary Information). All further genetic analyses were therefore run with locus Cri16 excluded. Apart from two out of 70 tests, all loci and populations were in HW-equilibrium (at a Bonferroni-corrected value of P < 0.0001); three significant values likely are chance positives.

10

Overall, the population of *C. bombi* in the area is structured (Gst $= 60.64$ ($X^2 = 73.1$, $P < 0.001$), and an analysis by the program STRUCTURE resulted in three populations. However, only the population of the 2004 campaign was clearly different from the remaining ones (see Appendix S1, Supplementary Information). Therefore, rather than having clearly

15   distinct sup-populations, isolation-by-distance seems the most defining feature of *C. bombi* populations; this is true for the later campaigns (see Figure 4b) but also when tested for all samples (Spearman's $r = 0.138$, $P = 0.022$, $n = 276$; $m = 24$ populations). A neighbour-joining tree suggested that the samples collected in 2004 are set apart from those collected later and that infections from the eastern slopes of the Andes were close to those from

20   southern Central Chile (Figure 5). In explicit tests, *C. bombi* infections were genetically differentiated between the 2004 campaign (all hosts, $n = 39$ infections) and later (2010-2012) campaign, but not different among host species; *B. terrestris* ($n = 134$), *B. dahlbomii* ($n = 3$),

or *B. ruderatus* ($n = 4$). A G-test also separated early and late (in GENEPOP) at every locus

and overall ($P < 0.0001$ in all cases).

Finally, we looked at the association of hosts and parasite genetic distances, which was

possible for a subset of $n = 106$ infected host individuals from 22 populations. No evidence

for any association between genotypes *per se* was found - as is to be expected for neutral

markers. However, the pairwise genetic distances of hosts or parasites from the same

populations correlated rather well (Figure S3, Supplementary Information).

Discussion

The introduction of two palaearctic bumblebee species into South America - *B. ruderatus* in

1982/83 and *B. terrestris* in 1998 - has led to a dramatic invasion process as both are now

found far from their introduction sites. *B. ruderatus* is now well established in mainland

Chile as well as on the eastern slopes of the Andes (Table 1, Figure 2). It crossed into

Argentina around the Bariloche region in 1994 (ROIG ALSINA & AIZEN 1996) and coexisted

with the native species until *B. terrestris* arrived there in 2006 (TORRETTA, MEDAN &

ARAHAMOVICH 2006). The situation for *B. terrestris* is much more dramatic. By the

beginning of 2010, it was by far the dominant species in the agricultural mainland of Chile,

from Santiago to Puerto Montt and, possibly, Chiloé Island (where we spotted a few

B.dahlbomii and *B. terrestris* in the 2010 campaign). By 2011/12, it had spread in Patagonia

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south to a point near Villa O'Higgins in Chile, and had reached the northern and eastern

shores of Lago General Carrera (named Lago Buenos Aires in Argentina). Furthermore, by

February 2011 - despite intensive monitoring - no *B. terrestris* was found along the R.N. 26

between Comodoro Rivadavia and Sarmiento in Argentina. Yet, by October 2011 it had

5   reached the Atlantic coast (Figure 2). *B. terrestris* was first seen east of the Andes in early

2006 in the region of San Martín de los Andes (TORRETTA, MEDAN & ARAHAMOVICH 2006),

presumably having crossed the mountains there as the passes to the north, reaching over

4,000 m, are presumably impossible to cross. By 2012, *B. terrestris* had spread further north

and south along the eastern slopes of the Andes (Figure 2).

10

The fate of the native *B. dahlbomii* is especially remarkable as our distributional data suggest

a rapid displacement by *B. terrestris*. A few examples illustrate this. In 2004, the native *B.*

dahlbomii and the introduced *B. ruderatus* were abundant around the Chilean Lake district of

Villarrica - Pucón (Table 1, Figure 2). But by 2010, *B. terrestris* had become the dominant

15   species whereas *B. dahlbomii* was no longer found. During the 2010/2011 campaigns we

noticed clear boundaries between the advancing *B. terrestris* and the presence of the native *B.*

dahlbomii in Southern Patagonia and around Lake General Carrera. Furthermore, *B.*

ruderatus was present around Bariloche and San Martín de los Andes, but had become much

less abundant after *B. terrestris* had arrived (TORRETTA, MEDAN & ARAHAMOVICH 2006). It

20   seems therefore that wherever *B. terrestris* appears, the native *B. dahlbomii* disappears whilst,

interestingly, *B. ruderatus* manages to persist at low abundance. Most spectacularly, the

advance of *B. terrestris* has been in the order of 200 km per year - an astonishing figure even

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given the vast spatial scale of Patagonia. Since not only sexuals but also workers are found in

all locations (Table S1, Supplementary information), viable colonies must have established

in the advancing front as workers are assumed not to venture farther than 1 - 2 km from their

nest (DARVILL, KNIGHT & GOULSON 2004; KNIGHT *et al.* 2005; OSBORNE *et al.* 1999).

5

On the western (Chilean) side of the Andes studied here, the habitats are generally temperate

in climate, covered with vegetation, forests, and agricultural lands. Hence, *B. terrestris* will

find suitable conditions there and had established in most of this area around 2008 at the

latest (Figure 2). Along the eastern slopes of the Andes, *B. terrestris* is found where similar -

10   albeit generally drier than in the West - habitat conditions prevail. By contrast, virtually all

of Patagonia between the foothills of the Andes and the Atlantic coast is dry steppe with

scarce floral resources. Given where we found the bees during the 2011 campaign, *B.*

terrestris seems to spread across the steppe along the major river systems draining towards

the Atlantic. These river systems offer moist conditions and more abundant floral resources.

15   The prevailing (and sometimes very strong) westerly winds across the Andes no doubt aid

this dispersal. Note that in its European native range, *B. terrestris* does not extend into the far

North (this is, only up to ca. 60˚ N in Scandinavia) but is widespread throughout the

Mediterranean, a region with dry summers and abundant floral resources in a moist winter.

Furthermore, our genetic analyses suggest that virtually all populations of *B. terrestris* are as

20   genetically diverse as the core populations of mainland Europe (ESTOUP *et al.* 1996;

SCHMID-HEMPEL *et al.* 2007) (Table 2). As of 2012, the overall population of *B. terrestris* is

not separated into distinct sub-populations but shows isolation by distance (Figure 4).

Based on our data, we can therefore sketch a likely expansion scenario for *B. terrestris*

(Figure 6). After an initial expansion across central Chile, which it dominated less than ten

years after its release, multiple crossings across the Andes are likely to have occurred. Likely,

5   a first crossing around 2006 in the region of San Martín de los Andes, followed by others in

the South, notably around L Gral Carrera. At the same time, *B. terrestris* spread farther south

along the western side of the Andes, and later north and south along the eastern side. Finally,

B. terrestris now rapidly expands east across the Patagonian steppe. Given the current speed

of dispersal, it will probably reach the Strait of Magellan in a few years, perhaps only slowed

10   down by the subantarctic climate of Southern Patagonia, as one might conclude by its

absence from subarctic Scandinavia.

Elucidating a possible role of parasites for the invasion process, we found that parasites,

notably *C. bombi*, is present at high prevalence. Furthermore, populations of *C. bombi*

15   infections are genetically similarly structured as its main host, *B. terrestris* and a historical

spread is suggested because infections were considerably less diverse in 2004 than later

(Table 3) and can also be separated on a phylogeographic tree (Figure 5). Also, *B. terrestris*

is known to have carried the infection in 2009 samples from around Bariloche, Argentina, at

high levels (21.6 %) (PLISCHUK & LANGE 2009a), whereas it was not present in four native

20   species collected in several parts of Argentina a few years earlier. Based on Figure 5,

perhaps, *C. bombi* infections could have been brought in first by *B. ruderatus*, and then

enriched in diversity by the later introduction of *B. terrestris*, with which it now spreads

rapidly across vast scales. Genetic distances among populations of *C. bombi* do indeed co-vary with the genetic distances of its now main host, *B. terrestris* (Figure S3 Supporting Information).

5   The apparently quick and irreversible suppression of *B. dahlbomii* by the advancing *B. terrestris* is an enigma and has been commented upon earlier by other observers (ARBETMAN *et al.* 2013; MORALES 2007). What possible processes could account for this? Ecological competition between the native species and the invaders occurs (e.g. MADJIDIAN, MORALES & SMITH 2008; MORALES & AIZEN 2006 for *B. ruderatus*; MONTALVA *et al.* 2011 for *B.*

10   *terrestris*). For example, *B. terrestris* could ecologically outcompete the native *B. dahlbomii* through its more generalized flower use (MONTALVA *et al.* 2011) or by being more adapted to fragmented habitats (AIZEN & FEINSINGER 2003). It remains unclear though whether ecological competition can account for such a rapid displacement, and, at least for *B. ruderatus*, this has been considered unlikely (AIZEN, LOZADA & MORALES 2011).

15   Nevertheless, the role of ecological effects for the displacement of *B. dahlbomii* remains to be studied.

As outlined above, a factor for displacement could be novel, introduced parasites. Is this likely in this case? Experimental evidence is lacking and will be difficult to establish for

20   such large scales. However, our data together with literature reports can sketch some possibilities. For example, we found that *N. bombi* only occurs in the introduced species. PLISCHUK *et al.* (2009) reported that *N. ceranae* Fries 1996 - an emerging parasite of

honeybees in Europe (KLEE *et al.* 2007; PAXTON *et al.* 2007) - is present in native bumblebee

species in Argentina (*B. atratus, B. morio, B. bellicosus*). So far, only the pathology of *N.*

bombi is confirmed, but the generally very low prevalence of *Nosema* in our samples (Table

S2 Supporting Information) should speak against a major role for this pathogen. It is

5   conceivable that the low prevalence of *N. bombi* is due to non-sampling of heavily affected

(and non-flying) bees. Note, however, we detected *Nosema* by molecular markers, which

identify the presence of the parasite long before any spores can be seen or any pathological

effects are noticeable. No infections by the gregarine, *Apicystis (Mattesia) bombi* (which we

have not investigated here), were found in native species in Argentina sampled between

10   2005 and 2009 outside Patagonia (*n* = 441, *B. atratus, B. morio*, *B. bellicosus* , *B. opifex*, *B.*

tucumanus; (PLISCHUK & LANGE 2009a), nor in samples of *B. dahlbomii* (*n* = 52) and *B.*

ruderatus (*n* = 30) (ARBETMAN *et al.* 2013) collected between 1994 and 2012 in the area

around Bariloche and San Martín de los Andes but before *B. terrestris* arrived. After the

arrival of *B. terrestris* (before 2009), the prevalence of *A. bombi* for *B. terrestris* in January

15   2009 was 3.6 % (*n* = 111 bees; *C. bombi*: 21.6 %) (PLISCHUK & LANGE 2009a). By 2010 in

the same area 12.1 % (*n* = 107) of *B. terrestris* were infected (PLISCHUK *et al.* 2011b). By

2012 (ARBETMAN *et al.* 2013) prevalence had increased to 47 % in *B. terrestris* (*n* = 30),

56 % in *B. ruderatus* (*n* = 9); and 11 % in *B. dahlbomii* (*n* = 9). By 2009, the parasite also

occurred in honeybees in the Bariloche region (10.1 %, *n* = 138 for 2009/10) whereas it

20   appears to be absent in the Pampas region further northeast (PLISCHUK *et al.* 2011b). The

pathology of *A. bombi* parasite is not well researched (e.g. LIU, MACFARLAND & PENGELLY

1974) but it is considered virulent (SCHMID-HEMPEL 1998).

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We now show that *C. bomb* is very widespread and abundant wherever *B. terrestris* is found (Figure 3, Table S2 Supplementary Information). Furthermore, its population genetic structure seems to match that of *B. terrestris*. According to PLISCHUK & LANGE (2009a), *C.*

5   *bombi* appears not to be present in native species outside Patagonia. The primary pathogenic effect of *C. bombi* is to sterilize founding queens (BROWN, SCHMID-HEMPEL & SCHMID-HEMPEL 2003 for *B. terrestris*), which leads to failure during colony founding and severely compromised reproductive success. This effect might be stronger in a species like *B. dahlbomii* that likely have never encountered this parasite before (OTTERSTATTER &

10 THOMSON 2008); yet, this remains to be tested. Whatever further studies will find, only a novel, rather abundant sufficiently virulent parasite should have those rapid effects that could indeed reduce the native population in a short matter of time. Both, *A. bombi* and *C. bombi* would qualify for this.

15

CONCLUSION

The introduction into South America of *B. ruderatus* and, especially of *B. terrestris,* for pollination purposes is having massive consequences. *B. terrestris* forms a healthy,

20 genetically diverse population that is currently spreading at high rates and already has settled in a vast area, along with parasites, such as *Crithidia bombi*, *Noema bombi* and *Apicystis bombi*. In its wake, native *B. dahlbomii* populations diminished dramatically. Given its

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biology, *B. terrestris* has the potential to spread further north and south along the Andes and

likely will reach Tierra del Fuego (where currently nothing is known about the native

bumblebees) in a few years. Currently, *B. terrestris* spreads eastwards in Argentina along

major river systems towards the Atlantic. *B. ruderatus* spreads much less aggressively and

5   manages to co-exist with *B. terrestris* in places. The current changes in South America

clearly are a stunning example of a rapid invasion event with potentially devastating

consequences for the only remaining native *Bombus* species in Patagonia and a lesson for

possible future introductions.

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Supporting Information

Appendix S1. Analysis of genetic data of *B. terrestris* and *C. bombi* with the program

STRUCTURE.

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Introduced bumblebees in South America   35

Figure legends

Figure 1. Original distribution of the native *B. dahlbomii* in southern South America, according to

ABRAHAMOVICH, DIAZ & LUCIA (2007) and MONTALVA *et al.* (2011); this species occurs south and

west of the dashed line except for the interior of the Patagonian steppe (are marked by the dotted line).

5   *B. dahlbomii* is typically associated with more or less forested areas. Release sites of imported

bumblebees in Chile are indicated by arrows. The current invasion by *B. terrestris* most likely started

from the research facilities at Quillota (based on RUZ 2002, MONTALVA *et al.* 2011); for *B. ruderatus*

three release sites were used - (Coipue), Malleco, and Cunco, based on ARRETZ & MACFARLANE

(1986) (arrows). No records are available for Tierra del Fuego. Sampling sites for the four

10   campaigns are indicated by their population id (see Table 1, and Table S1, Supplementary

Information).

Figure 2. Presence of the native species, *B. dahlbomii*, and the two imported, *B. terrestris* and *B.*

ruderatus in Chile. Data for *ruderatus* and *dahlbomii* are from the 2004 sampling campaign; pie size

15   proportional to sample size (see Table 1). Approximate data for the range of *terrestris* in 1998/99 are

taken from MONTALVA *et al.* (2011). Asterisks indicate release sites for the two successfully

imported species (open symbol: *B. ruderatus*; closed symbol: *B. terrestris*).

Figure 3. Prevalence of *C. bombi* infections in all hosts for the 2004 and later campaigns; pie size

20   proportional to sample size (see Table S2 Supporting Information). Infections in the 2004 campaign

(Lake district, Chile) are in *B. ruderatus* and *B. dahlbomii* (no *B. terrestris* present at the time);

infections of later campaigns in all species but mostly in *B. terrestris*.

Figure 4 Isolation by distance in specimens collected during the campaigns of 2012 - 2102 in Chile and Argentina. Geographical distance is given by angular degrees (1 degree approx. 111 km) along the shortest distance; genetic distance is given by the ratio of pairwise Fst/(1-Fst) -values for

5 polymorphic loci ($k = 7$ in *B. terrestris*, $k = 5$ *C. bombi*). **(a)** Pairwise isolation by distance in *B. terrestris* (Spearman's $r = 0.609$, $P < 0.001$, $n = 799$; $m = 28$ populations). **(b)** Pairwise isolation by distance in *C. bombi* (Spearman's $r = 0.482$, $P < 0.001$, $n = 171$; $m = 19$ populations).

Figure 5. Neighbour-joining tree for pairwise genetic distances (Fst/ (1-Fst)) of *C. bombi* infections.

10 Infections from the 2004 campaign (dashed rectangle) separate. No *B. terrestris* were sampled in this campaign. Location 'Docas' (rectangle) is close to the introduction site of *B. terrestris*. Locations on the eastern slope of the Andes (Argentina) are in italics. Location 'C Rivadavia' is on the Argentinian Atlantic coast (*c.f.* Figure 1).

15 **Figure 6.** The putative invasion of *B. terrestris* into South America. The species was imported into Chile (asterisk) in 1998/99 and escaped soon afterwards. The lines and yearly progress depict a likely scenario according to the results of the sampling campaigns reported here. Note that low passes of the Andes between Chile and Argentina can probably not be crossed north of approximately the Temuco-San Martín de los Andes line.

20

Table 1 Number of individuals sampled per host species and population.

id [1]	Population, Region	Date	*B.dahlbomii*	*B opifex*	*B ruderatus*	*B terrestris*	Sample N
	Chile Central 2004						
05	Aguas grandes	16.1.2004	85	0	38	0	123
02	Campo Anders	18.1.2004	50	0	18	0	68
06	Cunco	17.1.2004	15	0	19	0	34
07	Pucón I	10.1.2004	14	0	0	0	14
01	Valdivia	15.1.2004	13	0	39	0	52
	Chile Central						
09	Docas	7.1.2010	5	0	0	7	12
10	Farellones	17.1.2010	29	0	0	0	29
11	Los Angeles	21.1.2010	0	0	0	30	30
12	Mininco	21.1.2010	0	0	4	48	52
13	Nahal 1	23.1.2010	0	0	1	4	5
14	Los Sauces	23.1.2010	0	0	32	25	57
15	Pucón	26.1.2010	0	0	12	41	53
16	Puerto Montt	27.1.2010	1	0	0	48	49
17	Ensenada	1.2.2010	1	0	8	5	14
18	Octay	1.2.2010	0	0	6	21	27
19	Talhuaca	4.2.2010	1	0	12	44	57
20	Cobquecura	6.2.2010	4	0	0	18	22
	Andes East						
22	Bariloche	14.1.2011	0	0	1	2	3
23	El Manso	14.1.2011	0	0	1	61	62
24	El Rincón	14.1.2011	0	0	0	61	61
25	Trevelin I	16.1.2011	0	0	0	33	33
26	Trevelin II	17.1.2011	0	0	3	42	45
27	Corcovado	17.1.2011	0	0	2	62	64
43	Gobernador Costa	7.2.2011	0	0	0	27	27
44	Villavicencio	4.1.2012	0	10	0	0	10
45	Luján de Cuyo	5.1.2012	0	9	0	0	9
46	San Rafael	6.1.2012	0	9	0	0	9
47	Malargue	7.1.2012	0	0	0	7	7
48	Pehuenches	7.1.2012	0	0	0	42	42
49	Chos Malal	8.1.2012	0	0	0	25	25
50	Picunches	8.1.2012	0	0	0	1	1
51	Zapala	9.1.2012	0	0	0	29	29
52	Lacar	10.1.2012	0	0	0	68	68
53	S. Martín de los Andes	10.1.2012	0	0	0	52	52
	Argentina Atlantic						
21	Comodoro Rivadavia	4.11.2011	0	0	0	96	96
41	Sarmiento	7.2.2011	0	0	0	10	10
42	Rio Saghuer	7.2.2011	0	0	0	1	1
	Patagonia West						
28	Coyhaique	18.1.2011	0	0	0	1	1
29	LGral Carrera I	19.1.2011	9	0	0	45	54
30	LGral Carrera II	19.1.2011	0	0	0	21	21
31	Rio Baker I	19.1.2011	6	0	0	0	6
32	Rio Baker II	20.1.2011	0	0	0	27	27
33	LGral Carrera III	20.1.2011	3	0	0	0	3
34	LGral Carrera IV	20.1.2011	2	0	0	0	2
35	Los Antiguos	21.1.2011	0	0	0	14	14
	Patagonia South						
36	El Chaltén	23.1.2011	1	0	0	0	1

37	El Calafate	26.1.2011	12	0	0	0	12
38	Puerta Natales	29.1.2011	63	0	0	0	63
39	Punta Arenas I	31.1.2011	7	0	0	0	7
40	Punta Arenas II	31.1.2011	57	0	0	0	57
	Total		378	28	196	1,018	1,620

[1] Population id (running number) used in the maps (*c.f.* Figure1).

Table 2 Genetic data for *B. terrestris* females for the 2010-2012 campaign. Only populations with ≥ 5 individuals included. No *B. terrestris* were found at the sampling locations during the 2004 campaign or in region South Patagonia.

id [1]	Population, Region [1]	Sampled [2]	Typed [3]	n Allels [4] (S.D.)	Hobs [5] (S.D.)	Hs [6] (S.D.)
	Chile Central (2010)					
09	Docas	7	6	5.27 (1.21)	0.762 (0.131)	0.798 (0.088)
17	Ensenada	5	5	5.43 (1.40)	0.828 (0.214)	0.818 (0.107)
14	Los Sauces	25	20	5.13 (0.58)	0.805 (0.078)	0.803 (0.058)
12	Mininco	48	45	5.38 (0.84)	0.785 (0.083)	0.822 (0.046)
11, 13	Nahal *	7	7	5.51 (1.45)	0.762 (0.189)	0.838 (0.066)
18	Octay	21	21	5.21 (0.85)	0.801 (0.095)	0.808 (0.053)
15	Pucón	41	41	5.33 (0.58)	0.831 (0.053)	0.823 (0.036)
16	Puerto Montt	48	31	4.99 (0.81)	0.743 (0.111)	0.799 (0.059)
20	Cobquecura	18	16	4.98 (0.72)	0.830 (0.129)	0.791 (9.066)
19	Talhuaca	44	40	5.46 (0.84)	0.791 (0.101)	0.825 (0.053)
		264	232			
	Andes East					
49	Chos Malal	25	9	4.61 (0.82)	0.873 (0.149)	0.744 (0.108)
27	Corcovado	62	57	4.86 (0.89)	0.723 (0.103)	0.772 (0.093)
22, 23	El Manso *	63	54	5.47 (0.72)	0.803 (0.049)	0.833 (0.039)
24	El Rincón	61	58	5.10 (0.61)	0.836 (0.057)	0.805 (0.039)
43	Gobernador Costa	27	27	5.12 (0.87)	0.787 (0.109)	0.794 (0.086)
52	Lacar	68	33	5.78 (0.70)	0.801 (0.071)	0.853 (0.038)
47	Malargue	7	7	3.49 (0.77)	0.857 (0.184)	0.702 (0.092)
48	Pehuenches	42	41	5.23 (0.71)	0.815 (0.067)	0.817 (0.050)
53	S Martín Andes	52	47	5.34 (0.66)	0.814 (0.093)	0.829 (0.037)
25	Trevelin I	33	8	4.48 (1.06)	0.724 (0.142)	0.710 (0.145)
26	Trevelin II	42	42	4.72 (0.66)	0.735(0.123)	0.754 (0.075)
50, 51	Zapala *	30	17	5.15 (0.93)	0.781 (0.116)	0.814 (0.056)
		639	400			

1		2	3	4	5	6
	Argentina Atlantic					
21	Comodoro Rivadavia	96	96	3.20 (0.77)	0.615 (0.187)	0.595 (0.156)
41, 42	Sarmiento *	11	11	5.07 (0.97)	0.584 (0.165)	0.782 (0.096)
		107	107			
	Patagonia West					
29	LGral Carrera I	45	39	4.54 (0.52)	0.777 (0.072)	0.742 (0077)
30	LGral Carrera II	21	14	6.10 (3.10)	0.687 (0.193) [$]	0.728 (0.073) [$]
35	Los Antiguos	14	9	4.07 (0.81)	0.816 (0.115)	0.722 (0.083)
31	Rio Baker II	27	13	5.53 (3.40)	0.731 (0.187) [$]	0.786 (0.082) [$]
		107	75			
	Total	1,117	818			

1) Population id as in map (Figure 1). Note that populations with an asterisks were merged to contain ≥ 5 individuals: "El Manso" with "Bariloche" , "Sarmiento" with "Rio Saghuer" , "Zapala" with "Picunches", "Nahal" with "Los Angeles"; population "Coyhaique" removed (only 1 individual). Other populations with no *B. terrestris* not listed.

2) Number of *B. terrestris* females sampled in population.

3) Number of *B. terrestris* females genotyped for this study.

4) Average allelic richness (S.D.) over all loci; rarefaction for minimum sample sizes.

5) Hobs: fraction of heterozygotes observed over all loci, according to {Nei, 1987 #9783}.

6) Hs: Genic diversity (expected heterozygosity).

$) In these samples, Locus B118 missing (non-amplifying) in population.

Table 3 Genetic data for *C. bombi* Only populations with ≥ 3 infections included, based on multiple infections.
(for single infections, see Supplementary Material).

id [1]	Population, Region [1]	Sampled [2]	Typed [3]	Genotypes [4]	Richness [5] (S.D.)	*H*obs [6] (S.D.)	*H*s [7] (S.D.)
	Chile Central 2004						
05	Aguas grandes	15	15	22	1.86 (1.00)	0.443 (0.520)	0.319 (0.368)
02	Campo Anders	6	6	10	1.67 (0.83)	0.500 (0.577)	0.283 (0.331)
06	Cunco	4	4	5	1.94 (1.30)	0.450 (0.526)	0.319 (0.384)
07	Pucón I	6	6	9	1.67 (0.82)	0.500 (0.577)	0.281 (0.329)
01	Valdivia	9	9	12	1.82 (0.67)	0.542 (0.529)	0.328 (0.283)
	Chile Central						
09	Docas	5	5	7	2.69 (1.13)	0.750 (0.500)	0.539 (0.359)
17	Ensenada	6	6	8	2.73 (0.93)	0.750 (0.319)	0.568 (0.218)
14	Los Sauces	3	3	4	2.49 (0.77)	0.812 (0.375)	0.521 (0.200)
11	Los Angeles	24	23	40	2.50 (0.57)	0.756 (0.311)	0.551 (0.177)
12	Mininco	8	3	5	2.71 (0.75)	0.700 (0.346)	0.562 (0.164)
13	Nahal 1	4	2	3	2.25 (0.50)	0.833 (0.333)	0.500 (0.136)
18	Octay	8	7	12	2.82 (1.04)	0.625 (0.369)	0.570 (0.292)
15	Pucón	3	3	3	2.75 (1.26)	0.750 (0.500)	0.542 (0.369)
16	Puerto Montt	10	10	13	2.61 (0.68)	0.827 (0.346)	0.574 (0.208)
20	Cobquecura	9	9	13	2.61 (0.57)	0.769 (0.294)	0.580 (0.174)
19	Talhuaca	4	4	4	2.30 (0.50)	0.750 (0.353)	0.489 (0.168)
	Andes East						
27	Corcovado	4	4	4	3.00 (1.08)	0.687 (0.314)	0.635 (0.269)
23	El Manso	17	16	20	2.83 (0.91)	0.750 (0.373)	0.597 (0.272)
24	El Rincón	26	25	37	2.87 (0.85)	0.824 (0.333)	0.615 (0.231)
48, 53	S Martín Andes *)	3	3	4	3.01 (0.92)	0.875 (0.250)	0.646 (0.172)
25	Trevelin I	7	7	9	2.78 (0.66)	0.833 (0.213)	0.621 (0.144)
26	Trevelin II	3	3	4	2.91 (1.01)	0.812 (0.375)	0.594 (0.246)
	Argentina Atlantic						

21	Comodoro Rivadavia	6	5	5	1.75 (0.50)	0.750 (0.500)	0.375 (0.250)
	Patagonia West						
29, 35	LGral Carrera I*)	5	5	6	2.36 (1.17)	0.542 (0.459)	0.446 (0. 363)
	Total	192	183		2.45 (0.88)	0.701 (0.383)	0.503 (0.260)

1) Population id as in map (Figure 1). Note that populations with an asterisks were merged to contain ≥ 3infections: "Pehuenches" merged to "San Martín de los Andes", "Los Antiguos" to "LGral Carrera I".

2) Number of infected *B. terrestris* females sampled in population.

3) Number of *B. terrestris* females genotyped for this study.

4) Number of *C. bombi* genotypes analysed (with multiple infections)

5) Average allelic richness (S.D.) over all loci; rarefaction for $n = 10$ (5 diploid individuals).

6) *H*obs: fraction of heterozygotes observed over all loci, according to {Nei, 1987 #9783}.

7) *H*s: Genic diversity (expected heterozygosity).
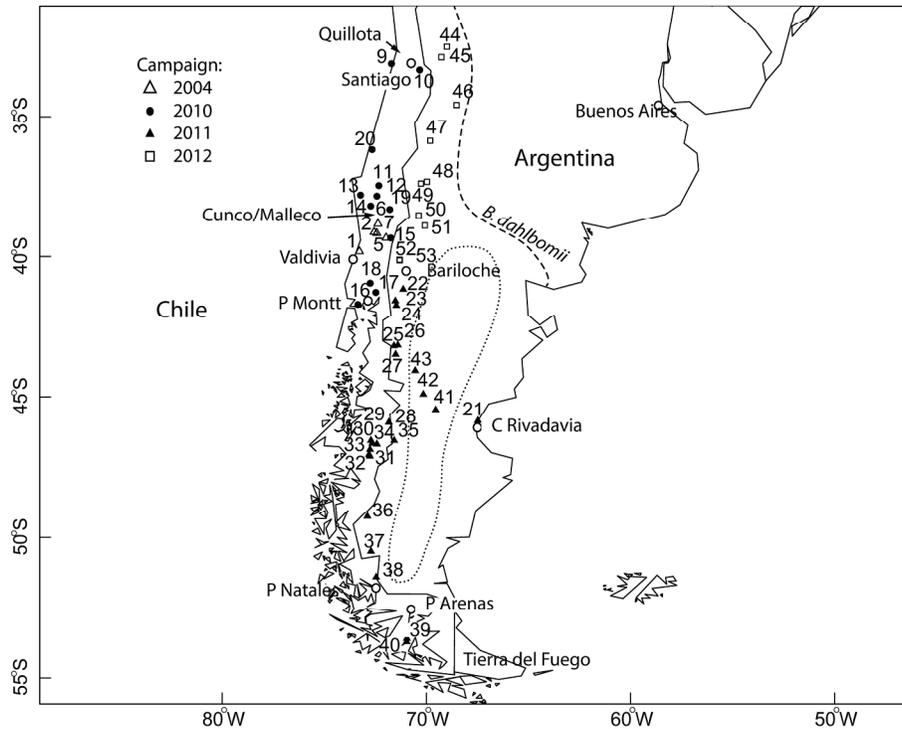
Original distribution of the native B. dahlbomii in southern South America, according to ABRAHAMOVICH, DIAZ & LUCIA (2007) and MONTALVA et al. (2011); this species occurs south and west of the dashed line except for the interior of the Patagonian steppe (are marked by the dotted line). B. dahlbomii is typically associated with more or less forested areas. Release sites of imported bumblebees in Chile are indicated by arrows. The current invasion by B. terrestris most likely started from the research facilities at Quillota (based on RUZ 2002, MONTALVA et al. 2011); for B. ruderatus three release sites were used - (Coipue), Malleco, and Cunco, based on ARRETZ & MACFARLANE (1986) (arrows). No records are available for Tierra del Fuego. Sampling sites for the four campaigns are indicated by their population id (see Table 1, and Table S1, Supplementary Information).
228x201mm (300 x 300 DPI)
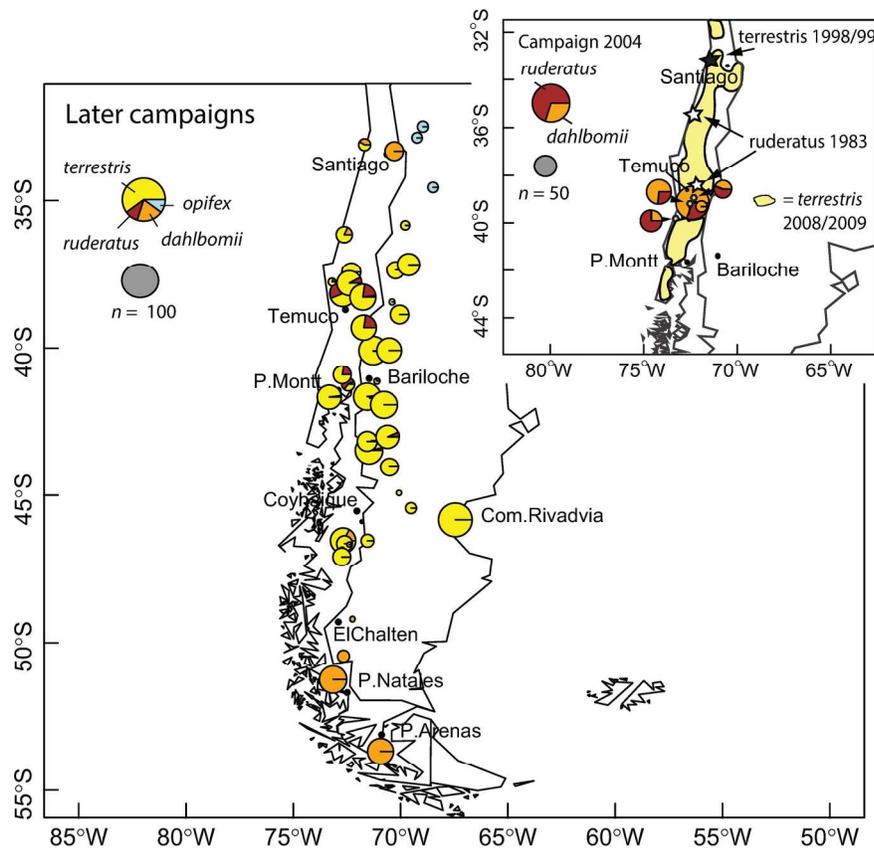
Presence of the native species, B. dahlbomii, and the two imported, B. terrestris and B. ruderatus in Chile. Data for ruderatus and dahlbomii are from the 2004 sampling campaign; pie size proportional to sample size (see Table 1). Approximate data for the range of terrestris in 1998/99 are taken from MONTALVA et al. (2011). Asterisks indicate release sites for the two successfully imported species (open symbol: B. ruderatus; closed symbol: B. terrestris).
177x177mm (300 x 300 DPI)
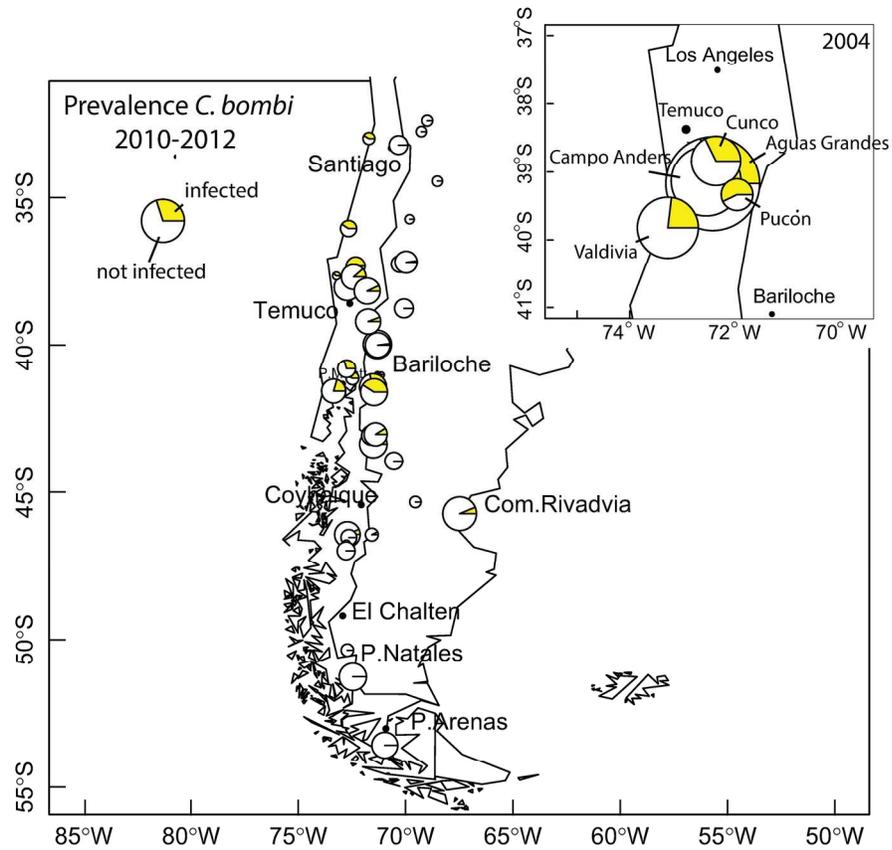
Prevalence of C. bombi infections in all hosts for the 2004 and later campaigns; pie size proportional to sample size (see Table S2 Supporting Information). Infections in the 2004 campaign (Lake district, Chile) are in B. ruderatus and B. dahlbomii (no B. terrestris present at the time); infections of later campaigns in all species but mostly in B. terrestris.

177x177mm (300 x 300 DPI)
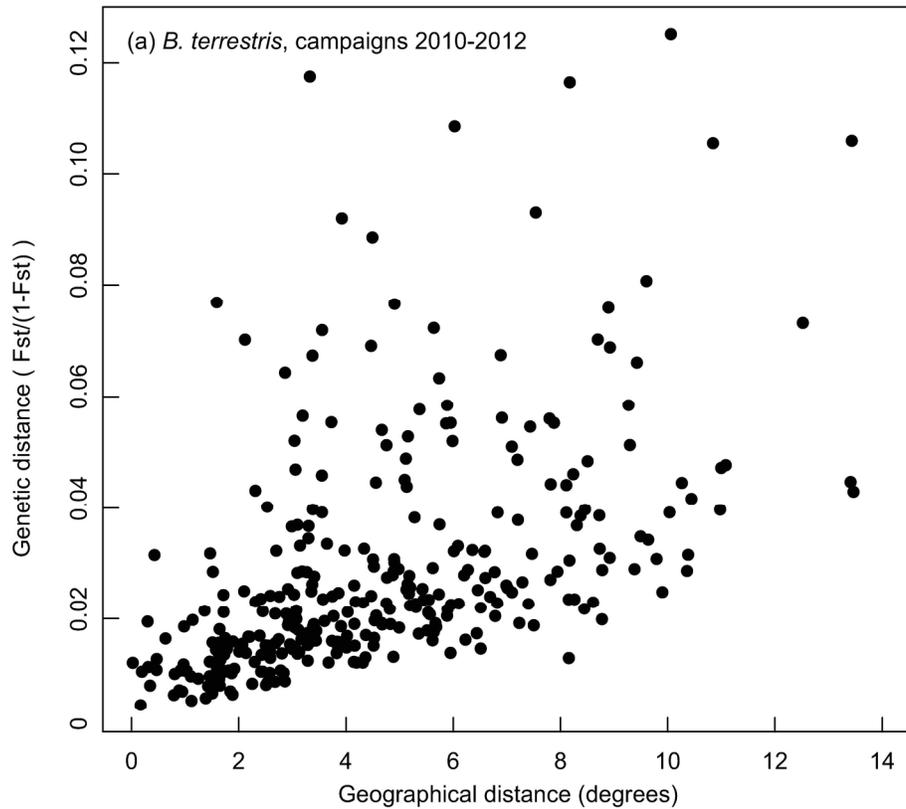
Isolation by distance in specimens collected during the campaigns of 2012 - 2102 in Chile and Argentina. Geographical distance is given by angular degrees (1 degree approx. 111 km) along the shortest distance; genetic distance is given by the ratio of pairwise Fst/(1-Fst) -values for polymorphic loci (k = 7 in B. terrestris, k = 5 C. bombi). (a) Pairwise isolation by distance in B. terrestris (Spearman's r = 0.609, P < 0.001, n = 799; m = 28 populations). (b) Pairwise isolation by distance in C. bombi (Spearman's r = 0.482, P < 0.001, n = 171; m = 19 populations).
184x188mm (300 x 300 DPI)
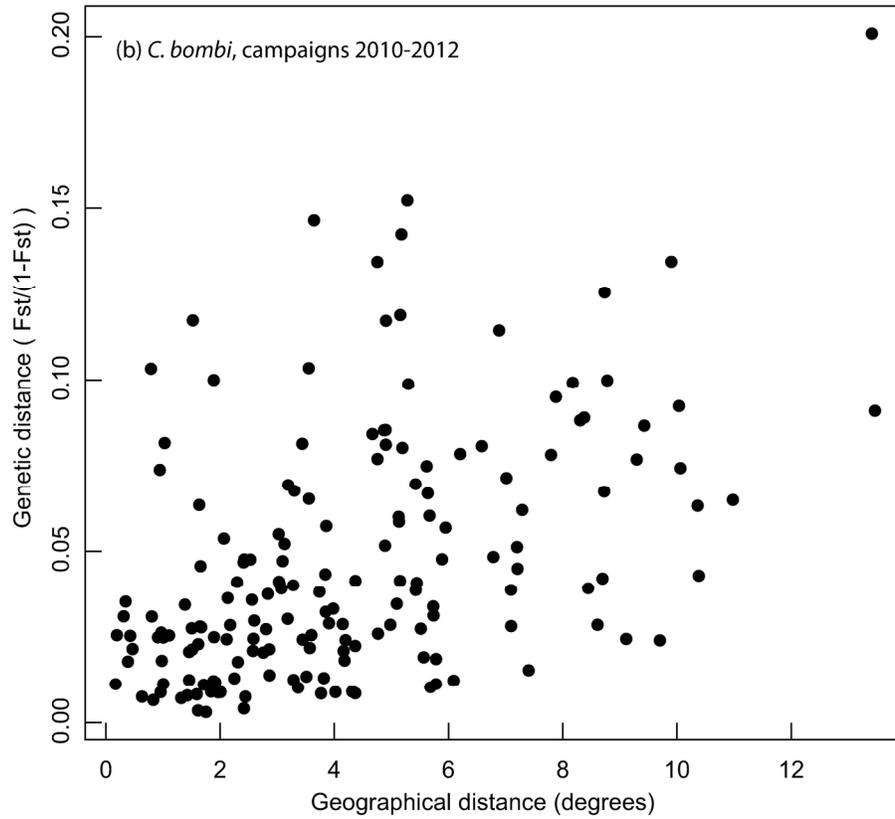
Isolation by distance in specimens collected during the campaigns of 2012 - 2102 in Chile and Argentina. Geographical distance is given by angular degrees (1 degree approx. 111 km) along the shortest distance; genetic distance is given by the ratio of pairwise Fst/(1-Fst) -values for polymorphic loci (k = 7 in B. terrestris, k = 5 C. bombi). (a) Pairwise isolation by distance in B. terrestris (Spearman's r = 0.609, P < 0.001, n = 799; m = 28 populations). (b) Pairwise isolation by distance in C. bombi (Spearman's r = 0.482, P < 0.001, n = 171; m = 19 populations).
177x177mm (300 x 300 DPI)
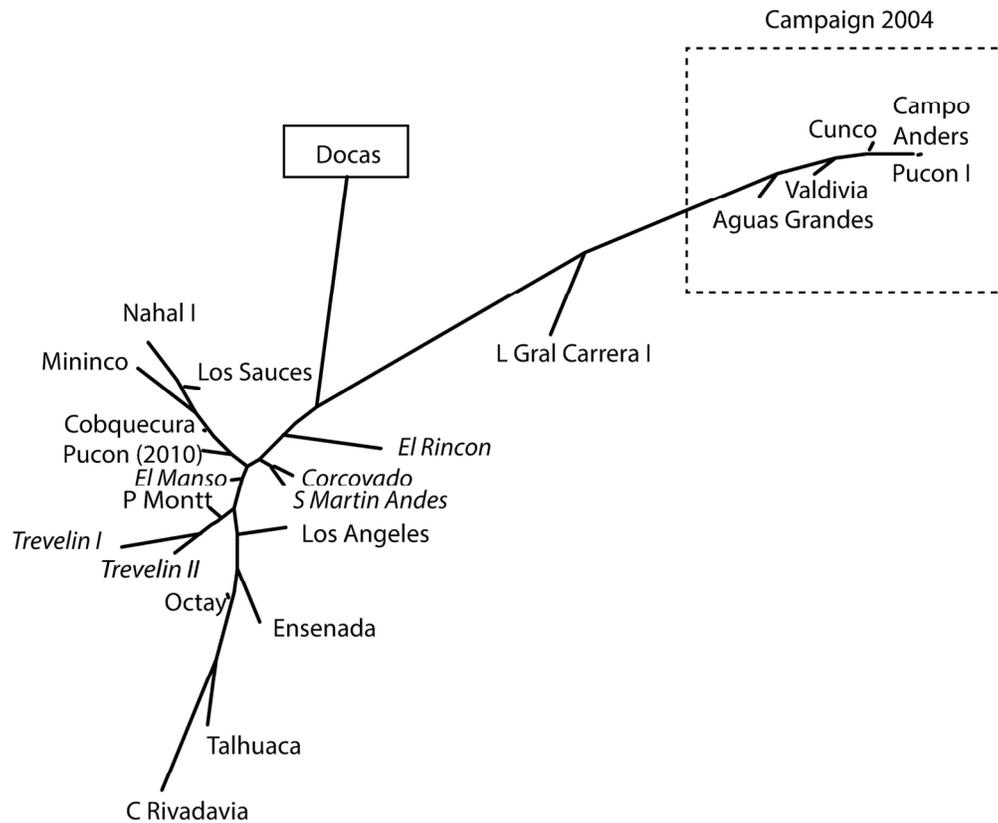
Neighbour-joining tree for pairwise genetic distances (Fst/ (1-Fst)) of C. bombi infections. Infections from the 2004 campaign (dashed rectangle) separate. No B. terrestris were sampled in this campaign. Location 'Docas' (rectangle) is close to the introduction site of B. terrestris. Locations on the eastern slope of the Andes (Argentina) are in italics. Location 'C Rivadavia' is on the Argentinian Atlantic coast (c.f. Figure 1). 141x123mm (300 x 300 DPI)
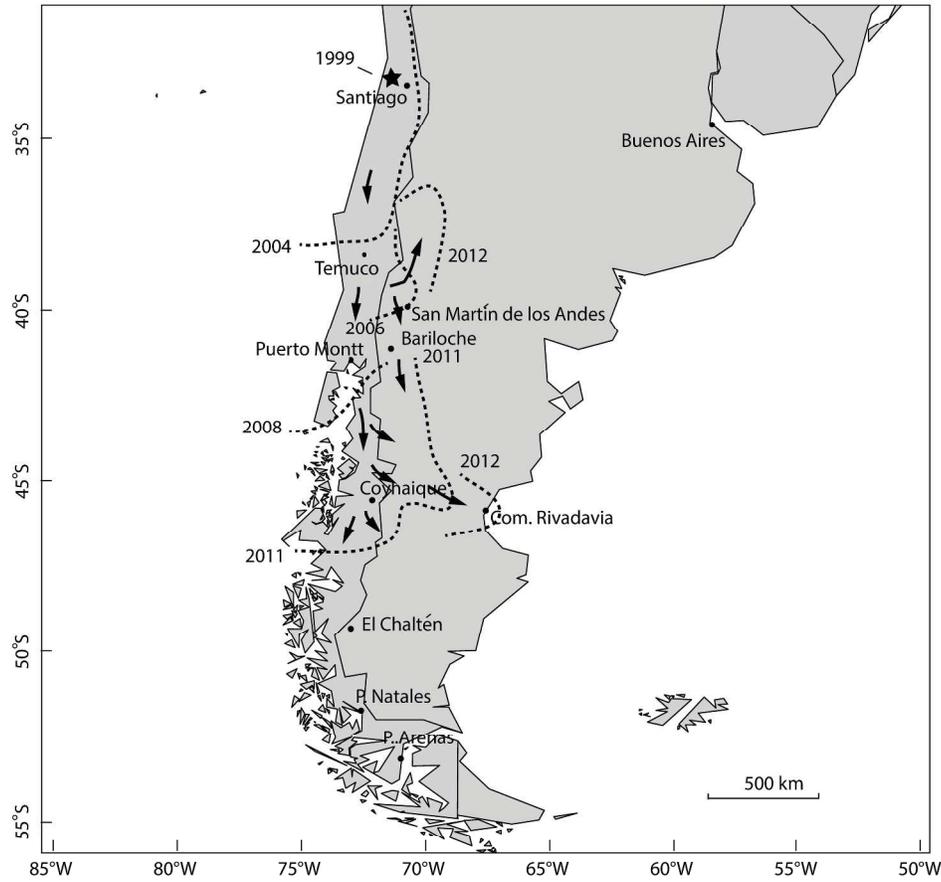
The putative invasion of B. terrestris into South America. The species was imported into Chile (asterisk) in 1998/99 and escaped soon afterwards. The lines and yearly progress depict a likely scenario according to the results of the sampling campaigns reported here. Note that low passes of the Andes between Chile and Argentina can probably not be crossed north of approximately the Temuco-San Martín de los Andes line.
258x263mm (300 x 300 DPI)

<div align="center">

(1) STRUCTURE analysis for *C. bombi*

</div>

Results for multiple genotype infection resolved (all hosts)

Data are based on resolve multiple genotype infections; see Salathé et al. (2011) for details of the method.

Parameters for the STRUCTURE analysis:
A priori: 24 populations with ≥ 3 individuals
(corresponding to the populations of the text, Table 1 main text, with populations merged as in Table 4)
n = 183 individuals, typed at 5 loci
Parameters of the simulation:

       Burn-in period: 10'000; Run period: 100'000;

       12 replicates per K (number of populations assumed)

       Settings: default
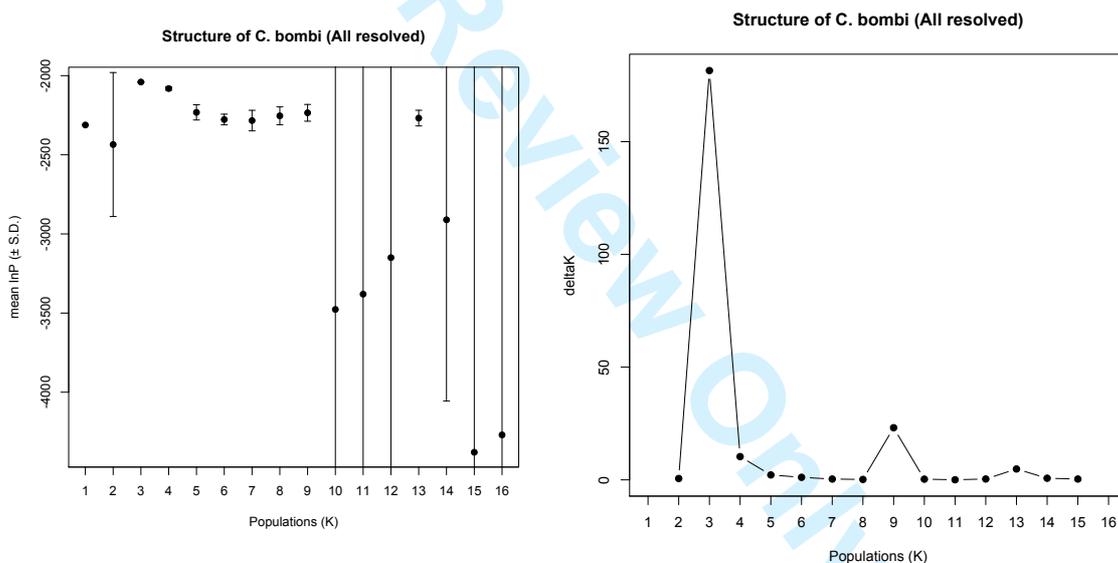
STRUCTURE Fig.1: **(a)** Likelihood-estimate, ln(P), of *C. bombi* being separated into K populations. Shown is the average (± 1 S.D.) for 12 replicate runs. **(b)** The value of ΔK according to the method of Evanno et al. (2005), K = 3 is the most likely case.

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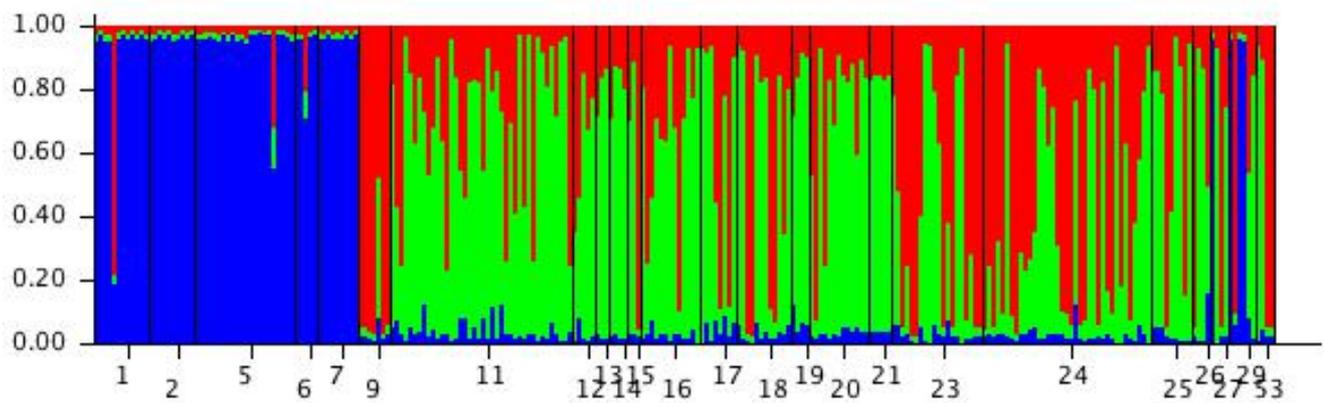
STRUCTURE Fig.2: Bar plot of the STRUCTURE analysis for K = 3 populations of *C. bombi*. Y-axis: bar length is likelihood of belonging to a given population (colour coded); X-axis: numbers refer to the prior populations. Example shown is run no. 27: *ln P(D)* = - 2042.4, α1 = 0.0437, F_{st}1 =0.0262. Structure: 'Blue': populations of Chile 2004, plus Corcovado. 'Green': primarily assigned to population nos. 11 - 21 (Central Chile, later campaigns) and no. 25 (Trevelin I). 'Red': remaining populations (*c.f.* Table 1, main text).

Results for Majority Rule (all hosts)

Data are based on Majority Rule to resolve multiple genotype infections. This simplest possible approach was done as follows: We only consider single infections, i.e. a status with maximally two alleles at any one locus. For this, multiple genotype infections were forced into plausible single ones by the Regional Majority Rule. This is, only a single infection genotype out of the many possible ones of a multiple genotype infection was retained. This selected single infection satisfied the condition that the genotype's ambiguous loci had the two alleles that were most common in the respective geographic region (ranked without the alleles of the individual in question). This procedure loses information on multiple genotype infections and is conservative but has the advantage of simplicity.

Parameters for the STRUCTURE analysis:

A priori: 26 populations with $\geq$ 3 individuals

(corresponding to the populations of the text, Table 1 main text)

n = 183 individuals, typed at 5 loci

Parameters of the simulation:

Burn-in period: 10'000; Run period: 100'000;

12 replicates per K (number of populations assumed)

Settings: default

(a) (b)
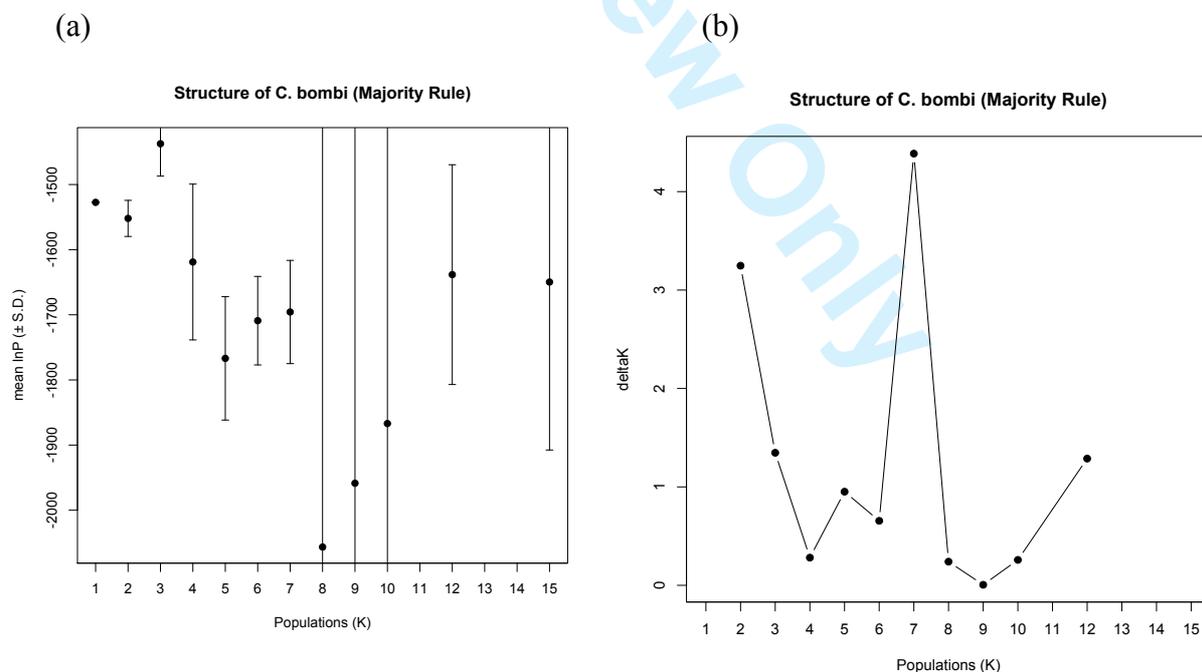
STRUCTURE Fig.3 **(a)** Likelihood-estimate, ln(P), of *C. bombi* being separated into K populations. Shown is the average (± 1 S.D.) for 12 replicate runs. **(b)** The value of ΔK according to the method of Evanno et al. (2005), K = 7 is the most likely case, but K = 2 is also plausible

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STRUCTURE bar plots for the cases of highest likelihood, K = 7, K = 2, are given in Fig.4.

(a)
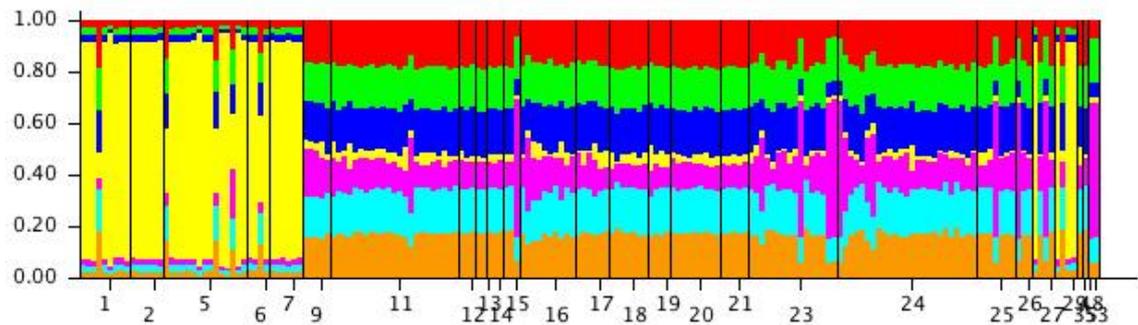
(b)
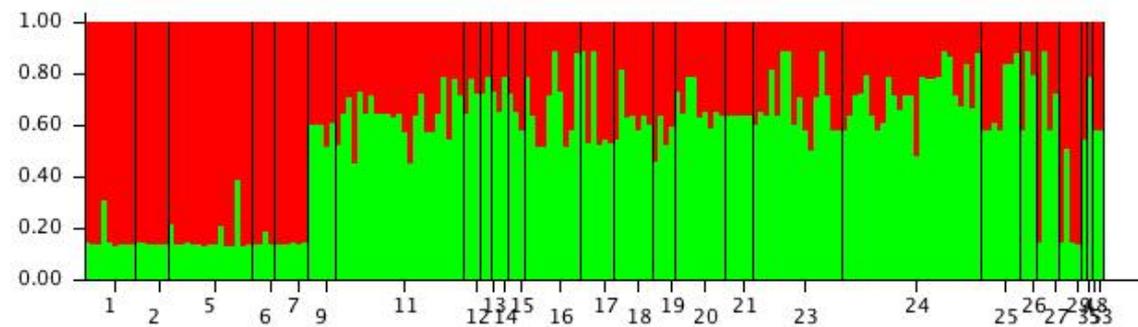
STRUCTURE Fig.4: (a) Bar plot of the STRUCTURE analysis for K = 7 populations of *C. bombi*. Y-axis: bar length is likelihood of belonging to a given population (colour coded); X-axis: numbers refer to the prior populations. Example shown is run no. 85: *ln P(D)* = - 1630.0, $\alpha 1$ = 0.0439, $F_{st}1$ =0.0103. Structure: 'Yellow' is populations nos. 1-7 from the 2004 campaign together with population no. 29 (L Gral Carrera). Although the program here identified the most likely subdivision, the remaining populations are hard to separate, and the case shown in the lower graph is perhaps more appropriate. **(b)** The second likeliest case of K =2 (see Fig.1). Example shown is run no. 27: *ln P(D)* = - 1549.6, $\alpha 1$ = 0.730, $F_{st}1$ =0.179. Structure: 'Red' is populations nos. 1-7 from the 2004 campaign, 'Green' essentially all other populations with the possible exception population no. 29 (L Gral Carrera).

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(2) STRUCTURE analysis for *B. terrestris*

Data: all available genotyped infections.

A priori: 26 populations with ≥ 5 individuals *(c.f.* Tab. 1 main text).

n = 183 individuals, typed at 5 microsatellite loci.

Parameters of the simulation:

        Burn-in period: 10'000; Run period: 100'000;

        12 replicates per K (number of populations assumed)

        Settings: default

(a)   **(b)**
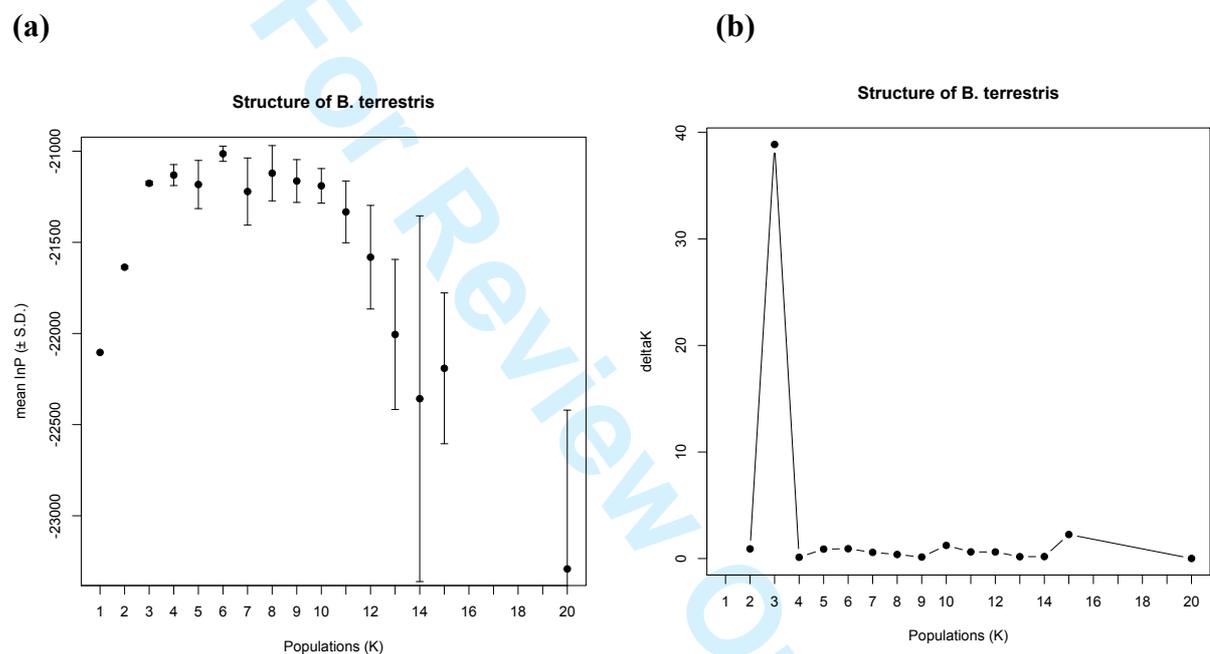
STUCTURE Fig.5: (a) Likelihood-estimate, ln(P), of *B. terrestris* being separated into K populations. Shown is the average (± 1 S.D.) for 12 replicate runs. **(b)** The value of ΔK according to the method of Evanno et al. (2005). K = 3 is the most likely case.

A STUCTURE bar plot for the grouping with the highest likelihood for more than wto subpopulations (i.e., K = 3) is given in Fig

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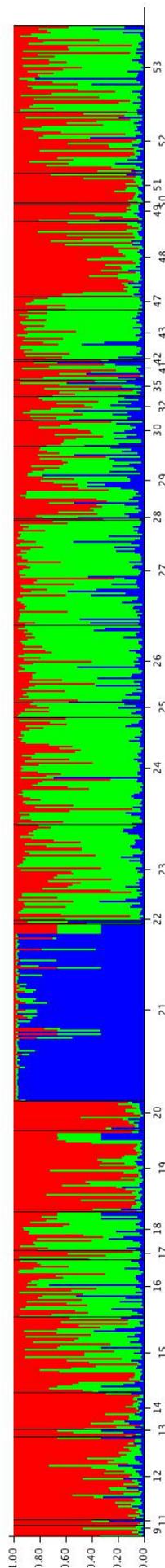
STUCTURE Fig.6: Bar plot of the STRUCTURE analysis for K = 3 populations of *B. terrestris* during th elater campaigns (no specimen found in 2004). Y-axis: bar length is likelihood of belonging to a given population (colour coded); X-axis: numbers refer to the prior populations. Example shown is run no. 47 *ln P(D)* = - 21189.8, $\alpha 1 = 0.1020$, $F_{st}1 = 0.0047$. Sub-structures: 'Blue' is Population no. 21 is Comodoro Rivadavia. 'Red' is mainly dominated by populations nos. 9-14, 19-20, which essentially represent Central Chile, and nos. 48-51, which represent the northern parts of the east slope of the Andes. 'Green' are the remaining populations. See Tab. S1, Suppl. Information for population codes.

Literature:

EVANNO, G., REGBNAUT, S. & GOUDET, J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14,** 2611-2610.

SALATHE R. & SCHMID-HEMPEL, P. (2011) Genotypic structure of a multi-host bumblebee parasite suggests a major role for ecological niche overlap. *PLoS One*, **6,** e22054.

Fig S1 Principal Component Analysis for pairwise genetic distances (ration Fst/(1-Fst)) of *B. terrestris* populations sampled in the 2010-2012 campaigns. A few populations, such as "Docas" (near the major release site of 1998), "L Gral Carrera" and "Rio Baker" (to the far south-east), and "Malargue" on the north-eastern slopes of the Andes, appear separated even if not statistically so (Discriminant Analysis yields 19 populations, with *P* = 0.78). In all, the population did not split into clearly defined sub-populations. Fraction of variation explained is 0.280 for PCA 1, and 0.208 for PCA 2.
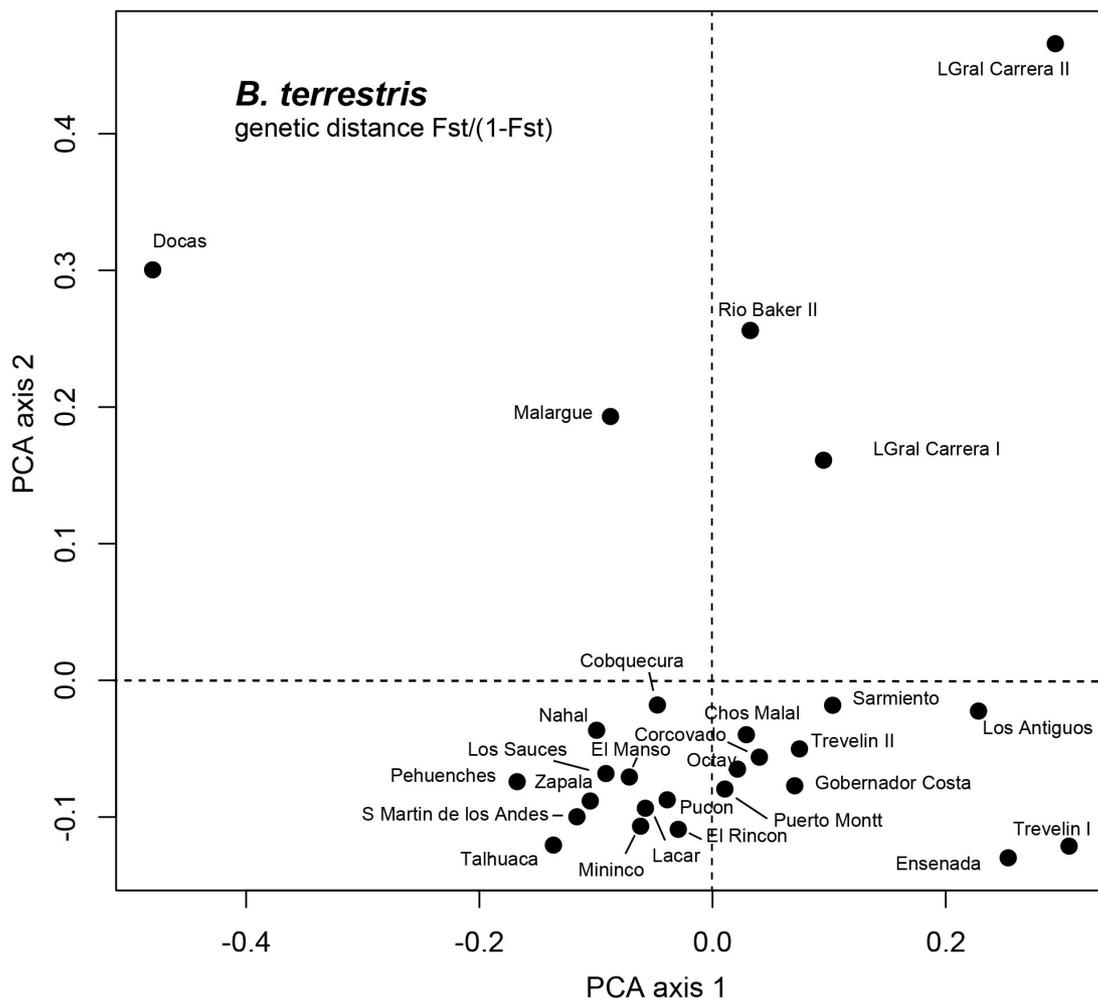
Figure S2. Unrooted neighbour-joining trees based on pairwise genetic distances among the populations of *B. terrestris*. Data are from sampling campaigns 2010-2012; population "Docas" is near the original introduction site, "Com Rivadavia" is on the Atlantic coast. **(a)** Genetic distances assessed as the ratio of pairwise Fst/(1-Fst). **(b)** Genetic distances assessed with Cavalli-Sforza distances. Calculated with R package 'ape'.

(a)

B. terrestris
NJ-tree Fst/(1-Fst)
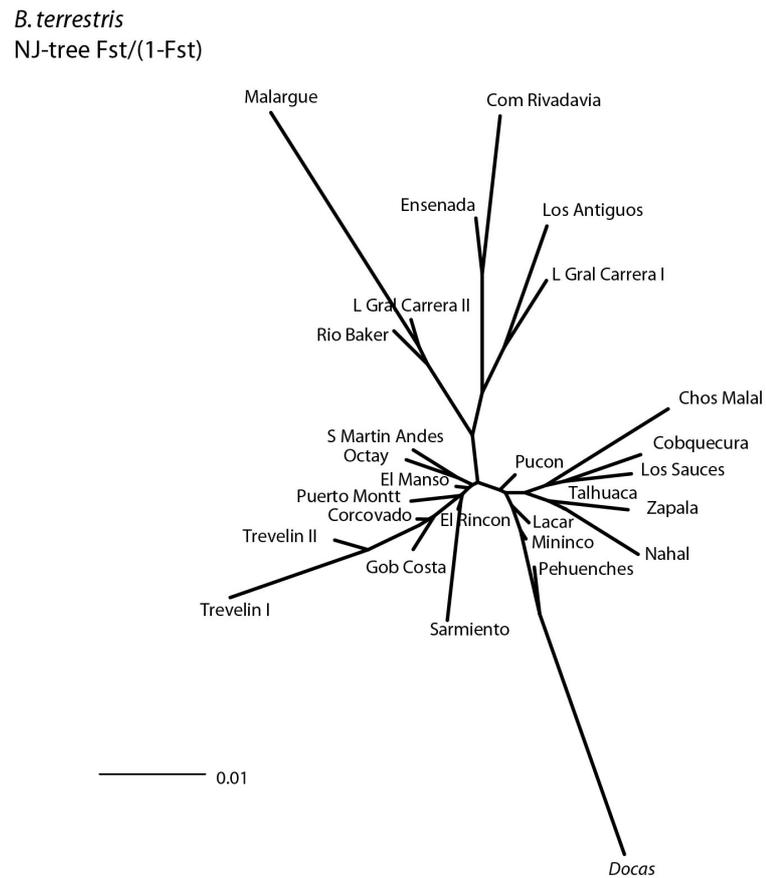
(b)

B. terrestris
NJ-tree Cavalli-Sforza
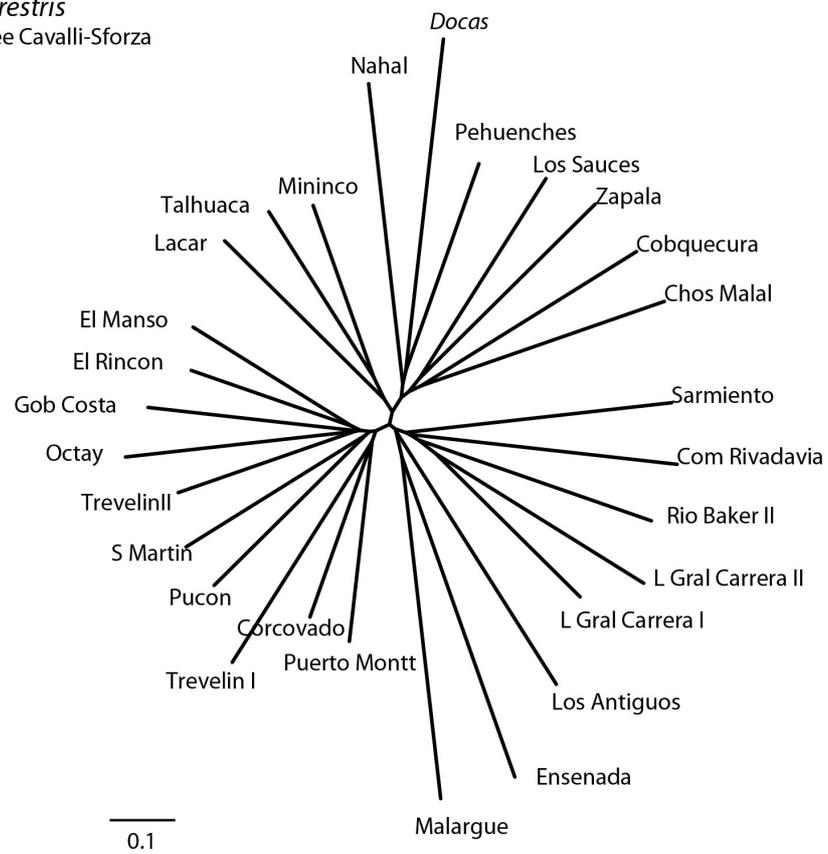
Figure S3 Pairwise genetic distances (*F*st/(1-*F*st)) for the host, *B. terrestris* and its corresponding *C. bombi* infections of the same populations. A total of *n* = 106 pairwise comparisons could be made (Spearman's *r* = 0.593, *P* < 0.001, *n* = 231; *m* = 22 populations).
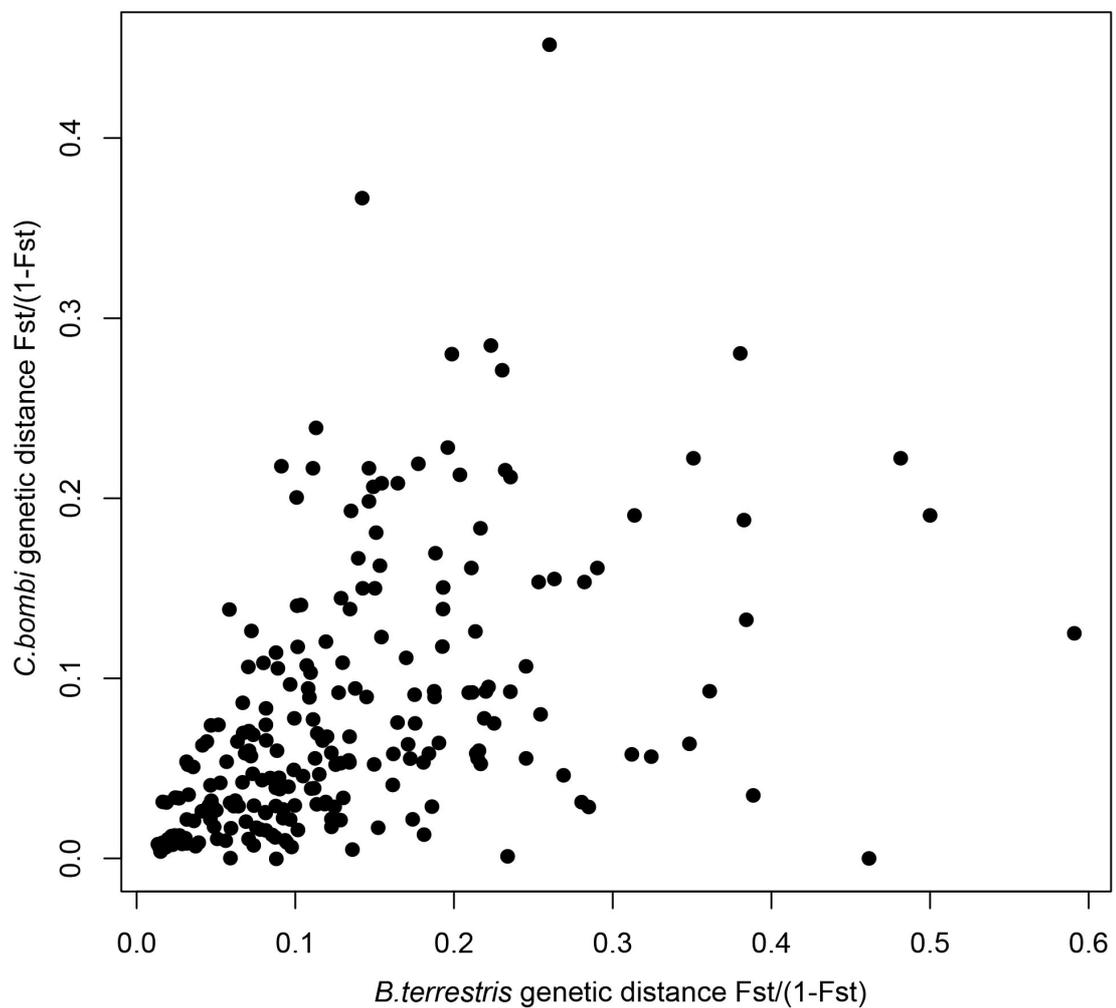
Table S1 Synopsis of collection data. For better readability dashes indicate that no specimen of given species was found in this population.

| no.[1]	Population, Region	Date	Latitude W	Longitude S	B.dahlbomii W	Q	M	n	B. opifex W	Q	M	n	B. ruderatus W	Q	M	n	B. terrestris W	Q	M
	Chile Central 2004																		
01	Valdivia	15.1.2004	39.82083	73.28083	13	0	0	13	-	-	-	-	39	0	0	39	-	-	-
02	Campo Anders	18.1.2004	39.13500	72.55800	50	0	0	50	-	-	-	-	14	4	0	18	-	-	-
05	Aguas grandes	16.1.2004	39.17200	72.43200	79	6	0	85	-	-	-	-	33	5	0	38	-	-	-
06	Cunco	17.1.2004	38.83800	72.37000	13	2	0	15	-	-	-	-	12	7	0	19	-	-	-
07	Pucón I	10.1.2004	39.33500	71.97200	8	6	0	14	-	-	-	-	-	-	-	-	-	-	-
	Total Region				163	14	0	177	-	-	-	-	98	16	0	114	-	-	-
	Chile Central																		
09	Docas	7.1.2010	33.13595	71.70783	0	5	0	5	-	-	-	-	-	-	-	-	1	5	1
10	Farellones	17.1.2010	33.35327	70.32563	28	1	0	29	-	-	-	-	-	-	-	-	-	-	-
11	Los Angeles	21.1.2010	37.46087	72.32730	-	-	-	-	-	-	-	-	-	-	-	-	3	0	27
12	Mininco	21.1.2010	37.83595	72.42110	-	-	-	-	-	-	-	-	4	0	0	4	43	2	3
13	Nahal 1	23.1.2010	37.79992	73.22475	-	-	-	-	-	-	-	-	1	0	0	1	3	1	0
14	Los Sauces	23.1.2010	38.18975	72.72617	-	-	-	-	-	-	-	-	8	0	24	32	21	0	4
15	Pucón	26.1.2010	39.33937	71.74587	-	-	-	-	-	-	-	-	11	1	0	12	40	1	0
16	Puerto Montt	27.1.2010	41.70433	73.35548	0	1	0	1	-	-	-	-	-	-	-	-	29	2	17
17	Ensenada	1.2.2010	41.27403	72.47528	0	1	0	1	-	-	-	-	8	0	0	8	5	0	0
18	Octay	1.2.2010	40.94647	72.74880	-	-	-	-	-	-	-	-	5	1	0	6	17	4	0
19	Talhuaca	4.2.2010	38.31043	71.78938	1	0	0	1	-	-	-	-	12	0	0	12	39	5	0
20	Cobquecura	6.2.2010	36.18277	72.65720	4	0	0	4	-	-	-	-	-	-	-	-	16	0	2
	Total Region				33	8	0	41	-	-	-	-	49	2	24	75	217	20	54
	Andes East																		
22	Bariloche	14.1.2011	41.16580	71.13218	-	-	-	-	-	-	-	-	1	0	0	1	0	2	0
23	El Manso	14.1.2011	41.57835	71.51672	-	-	-	-	-	-	-	-	1	0	0	1	51	1	9
24	El Rincón	14.1.2011	41.74113	71.47098	-	-	-	-	-	-	-	-	-	-	-	-	58	0	3
25	Trevelin I	16.1.2011	43.19490	71.58782	-	-	-	-	-	-	-	-	-	-	-	-	7	1	25
26	Trevelin II	17.1.2011	43.16553	71.39708	-	-	-	-	-	-	-	-	3	0	0	3	42	0	0
27	Corcovado	17.1.2011	43.49465	71.50325	-	-	-	-	-	-	-	-	1	1	0	2	54	3	5
43	Gobernador Costa	7.2.2011	44.07347	70.54620	-	-	-	-	-	-	-	-	-	-	-	-	25	2	0
44	Villavicencio	4.1.2012	32.53403	68.99202	-	-	-	-	10	0	0	10	-	-	-	-	-	-	-
45	Luján de Cuyo	5.1.2012	32.90320	69.26193	-	-	-	-	9	0	0	9	-	-	-	-	-	-	-
46	San Rafael	6.1.2012	34.58657	68.51932	-	-	-	-	9	0	0	9	-	-	-	-	-	-	-
47	Malargue	7.1.2012	35.86758	69.81120	-	-	-	-	-	-	-	-	-	-	-	-	7	0	0
48	Pehuenches	7.1.2012	37.32520	69.97270	-	-	-	-	-	-	-	-	-	-	-	-	41	0	1

1)	Location	Date	Lat	Long															
49	Chos Malal	8.1.2012	37.39122	70.26542	-	-	-	-	-	-	-	-	-	-	-	-	9	0	16
50	Picunches	8.1.2012	38.52538	70.37215	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0
51	Zapala	9.1.2012	38.89583	70.07405	-	-	-	-	-	-	-	-	-	-	-	-	16	0	13
52	Lacar	10.1.2012	40.11537	71.31240	-	-	-	-	-	-	-	-	-	-	-	-	33	0	35
53	S. Martín (Andes)	10.1.2012	40.13592	71.30175	-	-	-	-	-	-	-	-	-	-	-	-	47	0	5
	Total Region				-	-	-	-	28	0	0	28	6	1	0	7	391	9	112
	Argentina Atlantic																		
21	Comodoro Rivadavia	4.11.2011	45.86389	67.48083	-	-	-	-	-	-	-	-	-	-	-	-	95	1	0
41	Sarmiento	7.2.2011	45.46787	69.54290	-	-	-	-	-	-	-	-	-	-	-	-	0	10	0
42	Rio Saghuer	7.2.2011	44.91397	70.14578	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0
	Total Region				-	-	-	-	-	-	-	-	-	-	-	-	96	10	0
	Patagonia West																		
28	Coyhaique	18.1.2011	45.92165	71.83737	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0
29	LGral Carrera I	19.1.2011	46.56417	72.71338	9	0	0	9	-	-	-	-	-	-	-	-	39	0	6
30	LGral Carrera II	19.1.2011	46.66470	72.64425	-	-	-	-	-	-	-	-	-	-	-	-	14	0	7
31	Rio Baker I	19.1.2011	47.05705	72.80502	2	4	0	6	-	-	-	-	-	-	-	-	-	-	-
32	Rio Baker II	20.1.2011	47.11708	72.77115	-	-	-	-	-	-	-	-	-	-	-	-	11	2	14
33	LGral Carrera III	20.1.2011	46.88095	72.75928	3	0	0	3	-	-	-	-	-	-	-	-	-	-	-
34	LGral Carrera IV	20.1.2011	46.69328	72.42240	1	1	0	2	-	-	-	-	-	-	-	-	-	-	-
35	Los Antiguos	21.1.2011	46.56935	71.57338	-	-	-	-	-	-	-	-	-	-	-	-	9	0	5
	Tota Region				15	5	0	20	-	-	-	-	-	-	-	-	74	2	32
	Patagonia South																		
36	El Chaltén	23.1.2011	49.24390	72.88993	1	0	0	1	-	-	-	-	-	-	-	-	-	-	-
37	El Calafate	26.1.2011	50.49112	72.71242	12	0	0	12	-	-	-	-	-	-	-	-	-	-	-
38	Puerta Natales	29.1.2011	51.40990	72.45868	63	0	0	63	-	-	-	-	-	-	-	-	-	-	-
39	Punta Arenas I	31.1.2011	53.60250	70.95362	2	5	0	7	-	-	-	-	-	-	-	-	-	-	-
40	Punta Arenas II	31.1.2011	53.71920	70.96630	56	1	0	57	-	-	-	-	-	-	-	-	-	-	-
	Total Region				134	6	0	140	-	-	-	-	-	-	-	-	-	-	-
	Total				345	33	0	378	28	0	0	28	153	19	24	196	778	41	198

1) Running number used in the maps.

Table S2 Number of infected and non-infected individuals sampled per parasite species and host population.

Population, Region [1]	Sample N	Crithidia [4]				Nosema [5]			
		Screened	Not inf.	Infected	Prevalence	Screened	Not inf.	Infected	Prevalence
Chile Central 2004 [2]		291	212	79	0.271		-	-	-
Aguas grandes	123	123	87	36	0.293	-	-	-	-
Campo Anders	68	68	56	12	0.176	-	-	-	-
Cunco	34	34	23	11	0.324	-	-	-	-
Pucón I	14	14	6	8	0.571	-	-	-	-
Valdivia	52	52	40	12	0.231	-	-	-	-
Chile Central 2010		407	327	80	0.197		368	5	0.013
Docas	12	12	7	5	0.417	5	5	0	0.000
Ensenada	14	14	8	6	0.429	14	14	0	0.000
Farellones	29	29	29	0	0	29	29	0	0.000
Los Angeles	30	30	6	24	0.800	29	29	0	0.000
Los Sauces	57	57	54	3	0.053	49	44	5	0.102
Mininco	52	52	46	6	0.115	51	51	0	0.000
Nahal	5	5	3	2	0.400	5	5	0	0.000
Octay	27	27	19	8	0.296	26	26	0	0.037
Pucón	53	53	50	3	0.057	47	47	0	0.000
Puerto Montt	49	49	39	10	0.204	44	44	0	0.000
Cobquecura	22	22	13	9	0.409	22	22	0	0.000
Talhuaca	57	57	53	4	0.070	52	52	0	0.000
Andes East		547	485	62	0.113		541	6	0.011
Bariloche	3	3	2	1	0.333	3	3	0	0.000
Chos Malal	25	24	25	0	0.000	25	25	0	0.000
Corcovado	64	64	60	4	0.063	64	63	1	0.016
El Manso	62	62	44	18	0.290	62	60	2	0.032
El Rincón	61	61	36	25	0.410	61	59	2	0.033
Gobernador Costa	27	27	27	0	0.000	27	27	0	0.000
Lacar	68	68	67	1	0.015	68	68	0	0.000
Luján de Cuyo	9	9	9	0	0.000	9	9	0	0.000

Malargue	7	7	7	0	0.000	7	7	0	0.000
Pehuenches	42	42	41	1	0.024	42	42	0	(0.000)
Picunches [3]	1	1	1	0	(0.000)	1	1	0	0.000
San Rafael	9	9	9	0	0.000	9	9	0	0.000
S Martín Andes	52	52	51	1	0.019	52	51	1	0.019
Trevelin I	33	33	26	7	0.212	33	33	0	0.000
Trevelin II	45	45	41	4	0.089	45	45	0	0.000
Villavicencio	10	10	10	0	0.000	10	10	0	0.000
Zapala	29	29	29	0	0.000	29	29	0	0.000
Argentina Atlantic		107	101	6	0.056		107	0	0.000
Comodoro Rivadavia	96	96	90	6	0.063	96	96	0	0.000
Rio Saghuer [3]	1	1	1	0	(0.000)	1	1	0	(0.000)
Sarmiento	10	10	10	0	0.000	10	10	0	0.000
Patagonia West		128	123	5	0.039		114	14	0.109
Coyhaique [3]	1		1	0	(0.000)	1	1	0	(0.000)
LGral Carrera I	54	54	50	4	0.073	54	47	7	0.127
LGral Carrera II	21	21	21	0	0.000	21	17	4	0.190
LGral Carrera III	3	3	0	0	0.000	3	3	0	0.000
LGral Carrera IV [3]	3	2	2	0	(0.000)	2	2	0	(0.000)
Los Antiguos	14	14	13	1	0.071	14	14	0	0.000
Rio Baker I	14	6	6	0	0.000	6	6	0	0.000
Rio Baker II	27	27	27	0	0.000	27	24	3	0.111
Patagonia South		140	140	0	0.000		140	0	0.000
El Calafate	12	12	12	0	0.000	12	12	0	0.000
El Chaltén [3]	1	1	1	0	(0.000)	1	1	0	(0.000)
Puerta Natales	63	63	63	0	0.000	63	63	0	0.000
Punta Arenas I	7	7	7	0	0.000	7	7	0	0.000
Punta Arenas II	57	57	57	0	0.000	57	57	0	0.000
Total	1,620	1'620	1,388	232	0.143	1,295 [2]	1,270	25	0.019

1) GLM-models: *C. bombi* infection status /yes/no) as binomial variate.

- factors species and population, additive (deviance = 1,330.8, df = 1'619; error = 943.2, df = 1,568), significant main effects of species (*B. ruderatus* under-infected), and populations (C Rivadavia, Corcovado, Lacar, L Gral Carrera I, Los Sauces, Pehuenches, Pucon, S Martin Andes, Talhuaca, Trevelin; all under-infected).

- factors species and region, additive (deviance = 1,330.8, df = 1'619; error =1,154.8, df = 1,611):), significant main effects of species and regions (Chile over-infected, West Patagaonia under-infected).

2) Populations Chile Central 2004 (*n* = 291) without data for *Nosema*.

3) Merged for ecological analyses (to yield n ≥ 3) "Coyhaique" to "LGral Carrera I", "Rio Saghuer" to "Sarmiento", "El Chalten" to "El Calafate", "Picunches" to "Zapala", "LGral Carrera IV" to" LGral Carrera III"

4) Contingency tests for *Crithiida* infections:
- by species: $X^2 = 17.22$, $df = 3$, $P = 0.0006$.
- by population: $X^2 = 351.04$, $df = 49$, $P < 0.0001$.
- by region: $X^2 = 93.77$, $df = 5$, $P < 0.0001$.

5) Contingency tests for *Nosema* infections:
- by species: $X^2 = 12.96$, $df = 3$, $P = 0.005$.
- by population: $X^2 = 115.95$, $df = 44$, $P < 0.0001$.
- by region: $X^2 = 62.40$, $df = 4$, $P < 0.0001$.

Table S3. Single and multiple infections by *C. bombi* (all infected hosts). Multiple infections have more than two alleles for at least one locus of the infection (an infection refers to a given host individual). A total of 183 hosts could be reliable typed.

(a) Populations, merged to ≥ 3 infections per location

Location 1	multiple	single	Sum	P (multiple)
Aguas grandes	7	8	15	0.47
Campo Anders	4	2	6	0.67
Cobquecura	4	5	9	0.44
Corcovado	0	4	4	0.00
Cunco	1	3	4	0.25
Docas	2	3	5	0.40
El Manso	4	12	16	0.25
El Rincón	12	13	25	0.48
Ensenada	2	4	6	0.33
L Gral Carrera I [2]	1	4	5	0.20
Los Angeles	17	6	23	0.74
Los Sauces	1	2	3	0.33
Mininco [2]	3	2	5	0.60
Octay	5	2	7	0.71
Pucón	0	3	3	0.00
Pucón I (2004)	3	3	6	0.50
Puerto Montt	3	7	10	0.30
S Martín de los Andes [2]	1	2	3	0.33
Talhuaca	0	4	4	0.00
Trevelin I	2	5	7	0.29
Trevelin II	1	2	3	0.33
Valdivia	3	6	9	0.33
Total	76	107	183	0.41

Test for independence from population: $X^2 = 31.85$, $n = 183$, $df = 22$, $p = 0.080$

[1] for locations, see Table S1

[2] for merging of populations, see main text (Material and methods).

(b) Infections per species

Species	multiple	single	Sum	P(multiple)
B. dahlbomii	16	20	36	0.44
B. ruderatus	4	7	11	0.36
B. terrestris	56	80	136	0.41
Sum	76	107	183	0.41

Test for independence from species: $X^2 = 0.25384$, $n = 183$, df = 2, p = 0.881

Table S4. Genetic data per locus for *B. terrestris.* Only populations with ≥ 5 individuals included.[*][7]

Locus	*H*o [1] (S.D.)	*H*s [2] (S.D.)	*D*st [3]	*F*st [4]	*F*is [5]	Allelic Richness [6] (S.D.)
B10	0.852 (1.03)	0.847 (0.094)	0.048	0.054	-0.006	11.66 (5.66)
B11	0.791 (0.091)	0.784 (0.066)	0.042	0.051	-0.006	7.92 (3.06)
B100	0.670 (01.76)	0.697 (0.124)	0.032	0.044	0.039	8.27 (4.14)
B118	0.753 (0.103)	0.788 (0.057)	0.024	0.029	0.045	8.44 (3.27)
B124	0.764 (0.161)	0.773 (0.110)	0.024	0.031	0.010	9.54 (4.55)
B126	0.802 (0.117)	0.809 (0.049)	0.031	0.036	-0.009	9.28 (4.04)
B132	0.796 (0.110)	0.774 (0.066)	0.026	0.032	-0.028	7.31 (3.42)
Total	0.776 (0.048)	0.782 (0.046)	0.034	0.041	0.009	8.92 (1.43)

* The following populations with < 5 few individuals were merged: "Bariloche" merged into "El Manso", "Rio Saghuer" with "Sarmiento", "Picunches" with "Zapala", and "Los Angeles" with "Nahal_1"; population "Coyhaique" removed (only 1 individual):

1) *H*obs: observed proportion of heterozygotes
2) *H*s, *H*exp: genic diversity (expected heterozygosity)
3) Amount of gene diversity among samples.
4) *F*st = *D*st/*H*exp
5) *F*is = 1- *H*obs/*H*exp
6) Average allelic richness (S.D.) across all populations; rarefaction for *n* = 20 (10 diploid individuals).
7) Chi-square values (d.f.) for deviation from HWE; all values are P < 0.0001.

Table S5. Genetic data per locus for *C. bombi.* Only populations with ≥ 3 individuals included. Multiple genotype infections resolved as in Salathé et al. (2011).

Locus	Hobs [1] (S.D.)	Hs [2] (S.D.)	Dst [3]	Fst [4]	$F$is [5]	Allelic Richness [6] (S.D.)
Cri2F10	0.206 (0.177)	0.184 (0.150)	0.031	0.016	-0.126	1.45 (0.38)
Cri16 *)	0	0	0	-	-	1.00 (-)
Cri4	0.749 (0.410)	0.501 (0.270)	0.096	0.161	-0.497	2.39 (0.80)
Cri4G9	0.867 (0.180)	0.631 (0.840)	0.059	0.085	-0.366	2.77 (0.44)
Cri1B6	0.982 (0.060)	0.693 (0.124)	0.082	0.106	-0.419	3.18 (0.78)
Total	0.701 (0.382)	0.502 (0.259)	0.060	0.106	-0.395	2.45 (0.88)

*) Locus Cri16 is monomorphic with one allele (size 119).
1) Hobs: observed proportion of heterozygotes.
2) Hs, Hexp: genic diversity (expected heterozygosity).
3) Amount of gene diversity among samples .
4) Fst = Dst/Hexp
5) $F$is = 1- Hobs/Hexp
6) Average allelic richness (S.D.) across all populations