

# **ELECTRONIC APPENDIX**

This is the Electronic Appendix to the article

**The dynamics of genetic and morphological variation on volcanic islands**

by

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Electronic appendices are refereed with the text; however, no attempt is made to impose a uniform editorial style on the electronic appendices.

## Supporting information

### DNA extraction, amplification and sequencing

Whole genomic DNA was extracted from the *T. boettgeri* and *T. delalandii* samples using either standard protocols (1) or the Chelex method (2). A fragment of the mitochondrial *cytochrome b* gene was amplified via the polymerase chain reaction (PCR) (3) using Taq DNA polymerase (GibcoBRL) with the modified versions of primers L14841 5' CTC CCA CCC CAT CAA CAT CTC AGC ATG ATG AAA CTTC 3' (4) and MVZ16 5' AAA TAG GAA (A/G)TA TCA (T/C)TC TGG TTT 3' (5). The magnesium concentration was 3.5mM and thermo-cycling conditions were 94°C for 3 minutes, 30 cycles of 92°C for 1 minute, 48°C for 30 seconds, and 72°C for 1 minute, followed by a final step of 72°C for 3 minutes. Purified PCR products were sequenced directly with the Sequenase kit (Amersham Life Science Inc., Cleveland, OH) according to the manufacturer's protocol. Primers used for sequencing were the modified versions of L14841 and MVZ16.

Accuracy of sequence data was checked in the following ways. Sequences were aligned and checked for reading frame shifts and nonsense codons. The heavy and light strands were sequenced for a number of PCR products with substantial amount of overlap. Some individuals were sequenced several times from different PCR products, which allowed checking for PCR errors. No stop codons were detected.

### **Phylogenetic analysis**

Bayesian analysis was conducted with a chain of 1.000.000 generations and a burn-in of 30.000 generations. ML analysis was conducted with the evolutionary model and parameters selected by MODELTEST. MP analysis was conducted with the following search parameters: all characters unordered, equal weighting, the "multrees" option in effect, and TBR branch swapping. The consensus tree obtained from sampling the posterior distribution of the Bayesian analysis has a log likelihood of  $-\ln 2876.55977$  with an estimated transition/transversion ratio of 3.845118, a gamma rate correction of 0.768981 and 0.459668 invariant sites.

Maximum likelihood reconstruction with the parameters suggested by MODETEST and PAUP transition/transversion ratio of 3.6752, gamma rate correction of 0.7150 and 0.4358 invariant sites the yielded a tree with a likelihood score of  $-\ln 2865.34126$ .

There are 144 parsimonious informative characters in the data set of which 44 are variable. Heuristic search with the MP criterion found an island with 224 most parsimonious trees of a length of 410 steps.

### **Population growth simulation**

The settings for the FLUCTATE runs were 10 short chains with 20 short sample increments 2000 steps long and two long chains with 20 long sample increments 20,000 steps long.

### **Matrix correspondence test**

The hypothesis that the phylogeography of *T. boettgeri* on Gran Canaria is caused by the successive volcanic eruption of the Roque Nublo and the Llanos de la Paz formation was tested by regressing the observed (dependent) matrix (encoded as the average patristic distance of haplotypes between sampling localities) against the hypothetical (independent) matrix (expressing the locality differences between the five expected vicariance sectors as in Fig. 2 and Fig. S1A,B and Tab. S1).

### **Morphology**

Data on body dimension measurements and scalation counts was collected from 471 adult specimen from 30 localities around Gran Canaria (Fig. 2). Sampling localities were too distant for allowing (current) exchange of individuals, that is each locality provides an independent sample. Metric (body dimension) measures were taken with precision calipers to the nearest tenths of a millimeter. Meristic (scalation) characters were recorded using a dissection microscope with magnifications of 10x and 20x.

The body dimension characters recorded were (see also Fig. S2 for illustration):

- 1) Snout-vent length (**SVL**) measured from anterior tip of the snout to the anterior edge of the vent,
- 2) Mental length (**ML**)
- 3) Head length (**HL**), measured from ear to anterior tip of the snout,
- 4) Head depth (at the posterior edge of eye) (**HD**),
- 5) Head width (**HW**), measured at the widest point,
- 6) Leg length (**LL**), measured from knee to knee,
- 7) Fourth toe length of the right hind foot (**TOL**),
- 8) Trunk length (**TRL**), measured from front leg axis to hind leg axis,

- 9) Snout width (**SNW**), measured anterior to eye,
- 10) Skull width (**SKW**), measured posterior to eyes,
- 11) Mouth to ear length (**MEL**),
- 12) Mouth to snout (**MS**),
- 13) Ear length (**EL**),
- 14) Eye length (**EYL**),
- 15) Depth of snout (**SN**), measured at nostril.

The following scalation characters were recorded (see also Fig. S3):

- 16) number of transversal rows of dorsal tubercles (**DTR**),
- 17) number of large lamellae (at least two times as wide as long) on the fourth toe of the right hind foot (**LAL**),
- 18) number of small lamellae and scales on the fourth toe of the right hind foot (**SML**),
- 19) number of scales between eye and nostril (**SNEY**),
- 20) number of scales between nostrils (**SNOST**),
- 21) number of "pre-cloacal" scales (**PRECL**), counted from leg axis to cloaca
- 22) number of eye cilia scales (**EYCI**),
- 23) number of sublabial scales (**SUBLAB**),
- 24) number of tubercles on the femur of the right hind leg (**TF**),
- 25) number of upper labial scales on right and left sides (**UPLAB**),
- 26) number of lower labial scales on right and left sides counted from the middle of the eye to the mental scale (excluding the mental) (**LOLAB**).

Analysis of variance (ANOVA) for scalation characters and SVL, and analysis of covariance (ANCOVA) for body dimensions using SVL as covariate were used as heuristic tools for exploring the effects of group (locality) and sex. All ANOVA and ANCOVA tests were run on BMDP using program 2V. The results suggested significant effects of group and sex (Tab. S2A, B).

Sexual variation was dealt with by treating sexes separately. Ontogenetic effects in form of growth have been accounted for by using the gradient of pooled least-square

regression of the characters on snout vent length (SVL) after linearising the interrelationship of individual characters by log-transformation.

Variation in each character systems was summarized by canonical variate analysis. Contour plots of the first canonical variate in scalation and body dimensions are shown in Fig. S4 and Fig. S5, respectively.

Tab S1. Characterization of *Tarentola boettgeri* sampling localities on Gran Canaria giving the locality identity number, altitude (m), annual rainfall, climate type, potential natural vegetation type as hierarchical categories, Northeast –Southwest (NE-SW) habitat hypothesis (0 indicates NE, 1 indicates SW), phylogeographic clade identity as in Fig1, and hypothetical vicariance pattern as in FigS1.

Locality	Altitude	Rainfall	Climate	Vegetation	NE-SW	Clade	Vicariance pattern
2	850	2	2	2	0	A2	2
3	250	1	1	1.5	1	A3	3
5	1000	3	3	3	0	A3	2
6	140	1	1	1	1	A3	2
7	1300	3	4	3	1	A3	3
8	550	1	2	2	1	B1	1
9	220	1.5	1.5	2	0	B1	5
10	100	1	1	1	1	A1	1
11	150	1	1	2	0	B2	5
12	80	1	1	1	0	B2	5
13	220	1.5	2	2	0	B2	5
14	140	1	2	1	0	A2	2
15	80	1	2	1	0	A2	2
16	400	2.5	2	2	0	A2	2
17	160	1.5	2	1	0	A2	2
18	30	1	1	1	0	B1	4
19	75	1	1	1	1	B1	4
20	180	1	1	1	1	A1	1
21	360	1	2	2	1	B1	4
22	10	1	1	1	1	A1	1
23	880	2	3	2.5	1	B1	1
24	70	1	1	1	1	A3	3
25	680	2	2	2	1	A3	3
26	270	1	1	1	1	A3	3
27	900	2	3	3	1	A3	3
28	550	2	2.5	2	0	B1	4
29	450	1	1	1	1	A1	1
30	1200	2.5	3	3	0	B1	4
31	1780	4	4	3	0	A3	5
32	300	2	3	2	0	A2	2

Tab. 2A. Analysis of variance and covariance of body dimension characters

Variable	Locality	Sex	Locality x Sex
SVL	10.28***	44.20***	0.87 <sup>ns</sup>
ML	4.3***	7.40**	1.13 <sup>ns</sup>
HL	4.84***	39.25***	0.72 <sup>ns</sup>
HD	4.86***	105.83***	1.64*
HW	5.46***	86.42***	0.65 <sup>ns</sup>
LL	4.89***	2.49 <sup>ns</sup>	0.76 <sup>ns</sup>
TOL	2.35***	12.65***	1.02 <sup>ns</sup>
TRL	4.55***	67.27***	1.06 <sup>ns</sup>
SNW	6.03***	6.55*	0.78 <sup>ns</sup>
SKW	5.85***	6.77**	1.15 <sup>ns</sup>
MEL	4.24***	17.69***	1.22 <sup>ns</sup>
MS	4.10***	4.96*	1.25 <sup>ns</sup>
EL	4.19***	7.29**	0.80 <sup>ns</sup>
EYL	8.23***	8.32**	0.75 <sup>ns</sup>
SN	7.09***	13.48***	1.33 <sup>ns</sup>

The F-ratios are given for a two-way analysis of variance for snout-vent length (SVL) and a two-way analysis of covariance for the remaining characters (with SVL as covariate). The factors are locality (29 d.f.) and sex (1 d.f.).

ns indicates not significant, \* indicates  $p < 0.05$ , \*\* indicates  $p < 0.01$  and \*\*\* indicates  $p < 0.001$ .

Tab. S2B. Analysis of variance of scalation characters

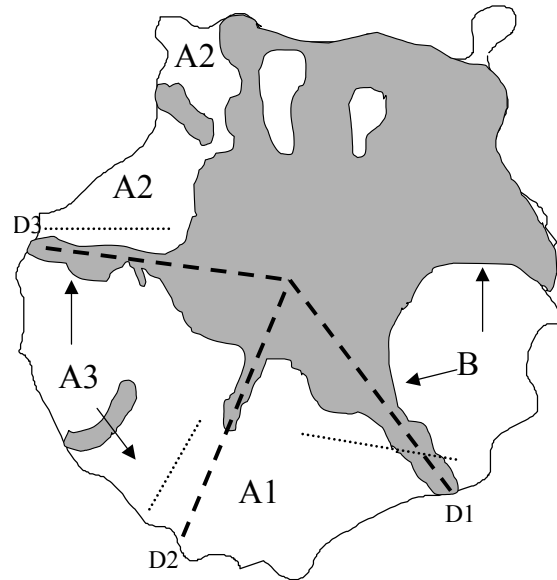
Variable	Locality	Sex	Locality X Sex
DTR	1.48 <sup>ns</sup>	1.02 <sup>ns</sup>	1.34 <sup>ns</sup>
LAL	6.94***	0.12 <sup>ns</sup>	1.44 <sup>ns</sup>
SML	1.92**	0.00 <sup>ns</sup>	0.69 <sup>ns</sup>
SNEY	2.13***	12.12***	1.05 <sup>ns</sup>
SNOST	2.63***	7.19**	1.41 <sup>ns</sup>
PRECL	3.76***	1.86 <sup>ns</sup>	0.96 <sup>ns</sup>
EYCI	2.27***	1.66 <sup>ns</sup>	1.07 <sup>ns</sup>
SUBLAB	3.15***	0.25 <sup>ns</sup>	0.96 <sup>ns</sup>
TF	3.76***	7.55**	0.51 <sup>ns</sup>
UPLAB	3.39***	0.09 <sup>ns</sup>	1.16 <sup>ns</sup>
LOLAB	6.90***	0.47 <sup>ns</sup>	0.81 <sup>ns</sup>

The F-ratios are given for a two-way analysis of variance. The factors are locality (29 d.f.) and sex (1 d.f.).

ns indicates not significant, \* indicates  $p < 0.05$ , \*\* indicates  $p < 0.01$  and \*\*\* indicates  $p < 0.001$ .



A)



B)

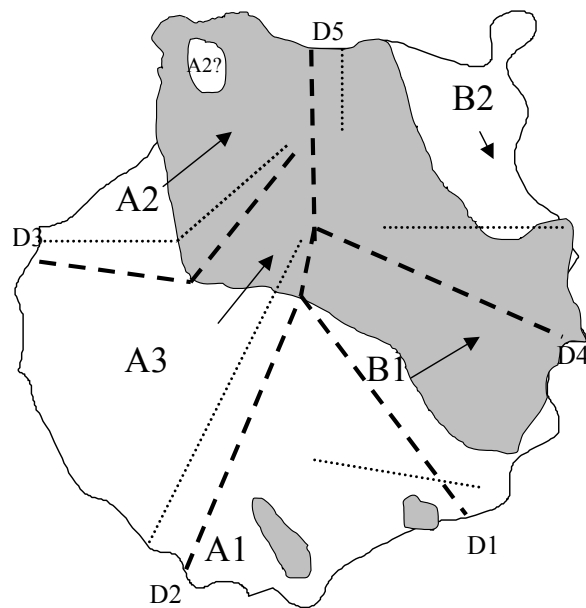


Fig S1A, B. Schematic representation of the present extension of volcanic flows (grey areas) of the Roque Nublo group (A) and the Llano de la Paz Formation (B) (2.2-2.9 Mya) on Gran Canaria (grey areas). Putative range expansions in *Tarentola boettgeri* following the end of volcanic activities are indicated by arrows and putative refugia are indicated by question marks, dashed lines and dotted lines indicate expected and actual delineation of mt haplotype distribution.

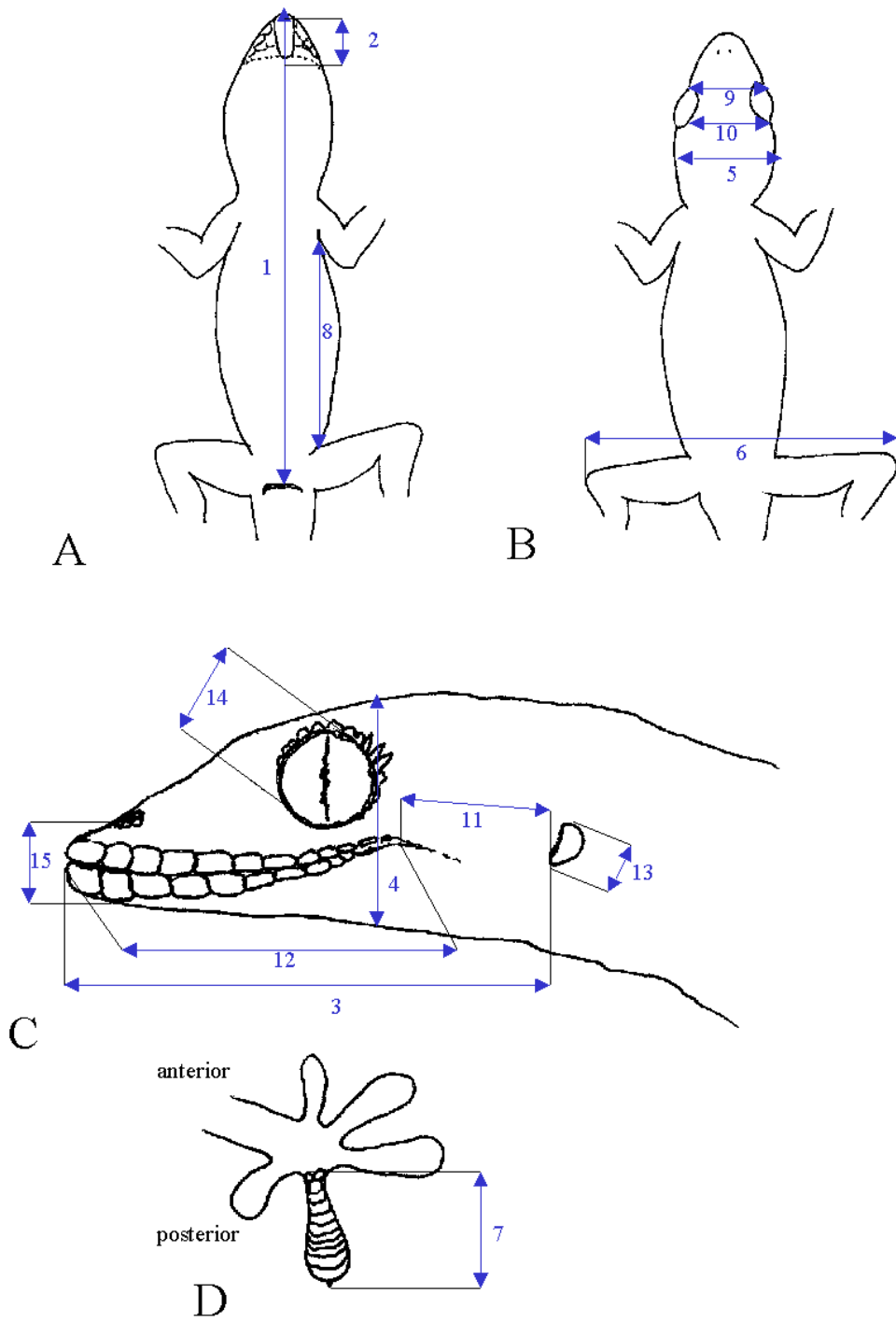


Fig. S2. Body-dimension measurements indicated by arrows. The numbers correspond to those given in the text. A) ventral view, B) dorsal view, C) lateral view of head and D) ventral view of hind foot.

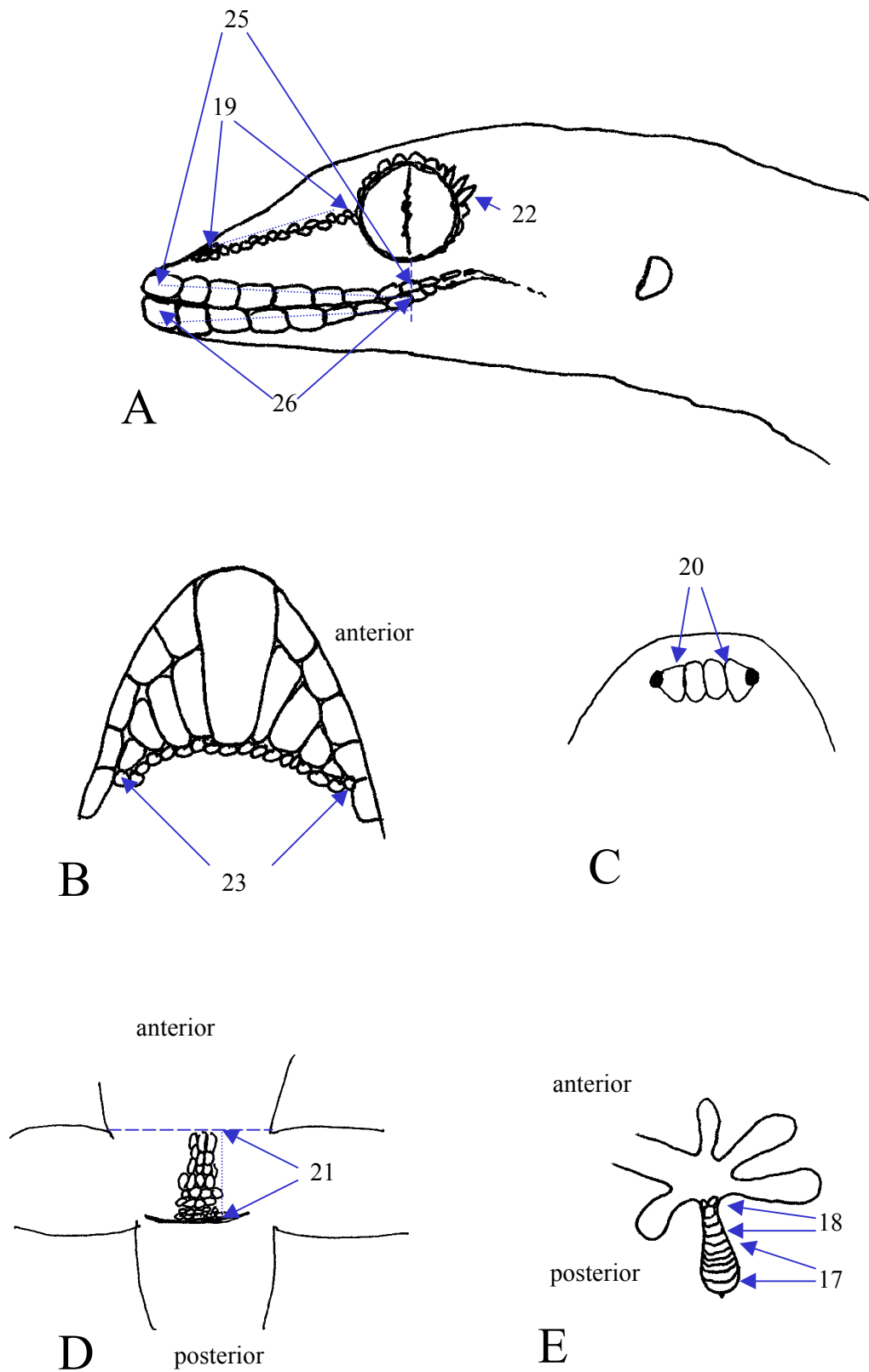


Fig. S3. Scalation characters measured. The numbers correspond to those given in the text. A) Lateral view of head, B) ventral view of snout, C) Dorsal view of snout, D) ventral view of abdomen and E) ventral view of hind foot.

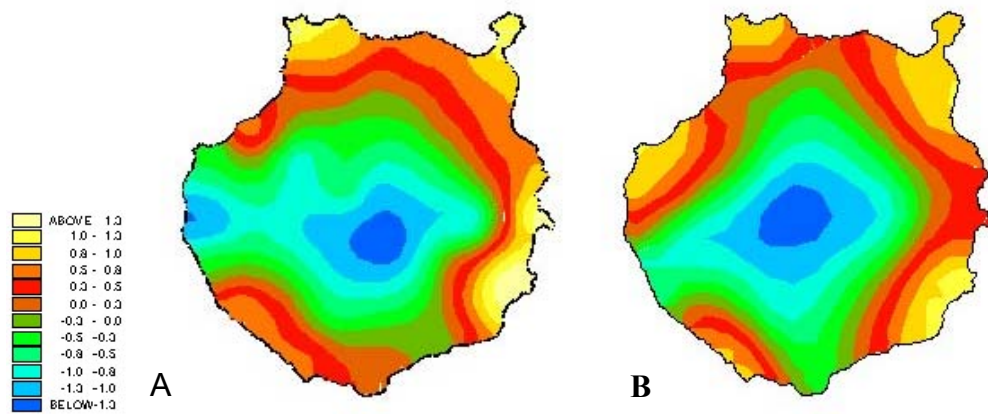


Fig. S4. Contour plots of first canonical variate in scalation in females (A) and males (B) of *T. boettgeri* on Gran Canaria

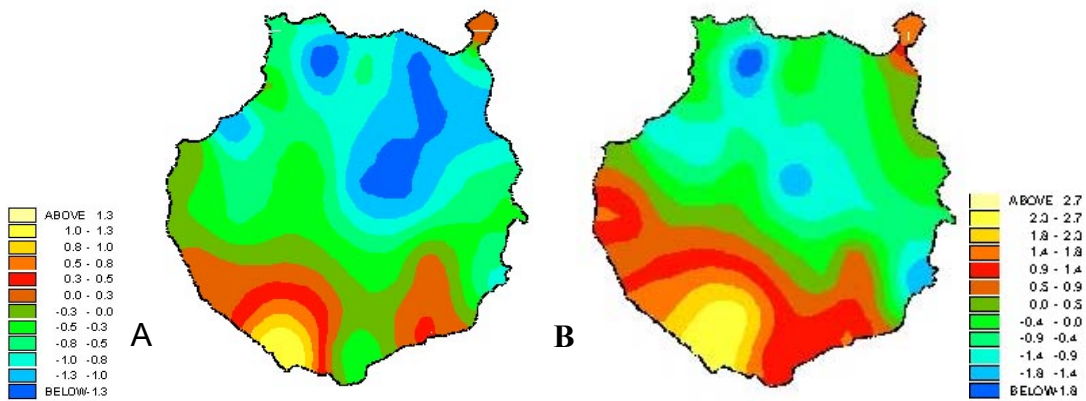


Fig. S5. Contour plots of first canonical variate in body dimensions in females (A) and males (B) of *T. boettgeri* on Gran Canaria

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