Original investigation

Cranial morphological variation of *Dromiciops gliroides* (Microbiotheria) along its geographical distribution in south-central Chile: A three-dimensional analysis

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ABSTRACT

We analyzed the variation in cranial morphology of the marsupial *Dromiciops gliroides* along its distribution in south-central Chile. We evaluated whether the cranial morphological variation is congruent with the phylogeographic structure previously observed in this species. We built three-dimensional models of 69 crania on which we digitized 30 landmarks. We used standard geometric morphometric methods to extract and analyze the shape and size components of the crania. Our data showed a subtle but consistent cranial size and shape variation along the studied distributional range, suggesting a geographic variation pattern rather than a phylogeographic structuring. Indeed, our multivariate analyses recovered a subtle morphological differentiation between island and mainland populations, contrary to what is suggested by a former phylogeographic study. We detected that either the cranial size variation, as well as the insularity and the latitude could be important factors underlying the cranial shape changes. We suggest that an interplay of historical and contemporary processes could be shaping the morphological pattern observed in this marsupial.

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Introduction

Dromiciops gliroides, "monito del monte", is a small marsupial (16–32 g, Palma and Valladares-Gómez, 2015) endemic of the temperate forests of Southern South America. This marsupial, scansorial although mostly arboreal, is a nocturnal form that inhabits the *Nothofagus-Chusquea* native forests (Hershkovitz, 1999; Mann, 1978). However, it has been observed in exotic plantations of *Eucalyptus* with dense native understory (Fontúrbel et al., 2014a; Salazar and Fontúrbel, 2016). In the native forests, *D. gliroides* displays an omnivorous diet, eating mainly insects and flesh fruits (Amico et al., 2009; Celis-Diez et al., 2012). As a consequence of this, *D.*

gliroides plays a key role as a seed disperser, helping to maintain the overall ecosystem functions (Amico and Aizen, 2000; Mora and Soto-Gamboa, 2011). However, the forests have been dramatically reduced during the last centuries due to human activities (Armesto et al., 2010). Consequently, a potential decreasing of *D. gliroides* populations could be taking place due to the susceptibility of forest fragmentation (Fontúrbel et al., 2014b; Rodríguez-Cabal et al., 2007). This is the reason why this species is currently considered by The IUCN as "Near Threatened" (Martin et al., 2015).

Dromiciops is the unique extant genus of the ancient order Microbiotheria (Reig, 1955). Molecular phylogenetic evidences have shown that the genus is more related to Australian than to American marsupial taxa (Palma and Spotorno, 1999; Spotorno et al., 1997). Traditional literature has recognized *D. gliroides* (Thomas, 1894) as the single species within the genus, although two new species have been recently proposed based on cranial morphology (D'Elía et al., 2016). Besides the latter proposition, we, hereafter, will consider *D. gliroides* as the unique living species of

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the genus. We argue about reasons and comments to D'Elía's et al. (2016) proposal in the Discussion section of this paper.

Dromiciops gliroides is distributed across the Chilean temperate forests and adjacent areas of Argentina. In Chile, its distribution extends between 35 and 43°S (Martin, 2010). In the southernmost part of its geographic range, populations are found both in the mainland and the Chiloé Island (41–43°S, 73–74°30'W). The modern distribution of *D. gliroides* has been strongly determined by the glacial cycles of the Pleistocene and the postglacial events, as occurred with great part of the Chilean south-central biota (Moreno et al., 1994; Villagran, 1991). Such historical processes have shaped the geographic genetic structure of *D. gliroides* (Himes et al., 2008), and that of other vertebrate sympatric species and plants (Formas and Brieva, 2000; Palma et al., 2012; Segovia et al., 2012). A former phylogeographic study based on mtDNA sequences detected three phylogroups along *D. gliroides* distribution, being two of those widespread and containing most of the haplotypes: a northern clade (or clade A, Himes et al., 2008) located between 36 and 39°S, and a southern clade (or clade C, Himes et al., 2008) between 40 and 43°S. Each of these clades included forms from both sides of the Andes Mountains. A third phylogroup (or clade B, Himes et al., 2008) was geographically intermediate between clades A and C showed high levels of divergence from northern and southern phylogroups (15.1% and 8.2% between A and C, respectively). Although the southern clade encompassed individuals from Chiloé Island and the mainland of Chile and Argentina, no significant genetic differences were observed between them. The latter result was associated to Pleistocene genetic connections between both areas that precluded their genetic differentiation. Nevertheless, based on external morphological characters, Osgood (1943) proposed that the Chiloé forms would constitute a different subspecies of *Dromiciops* with respect to mainland forms. However, no further studies have evaluated the latter hypothesis.

Incongruent patterns of molecular and morphological data can emerge because geographical variation of morphology could mainly respond to local adaptation processes (Maldonado et al., 2004). Thus, both genetic and morphologic analyses are critical to achieve a better understanding of the geographical differentiation of *D. gliroides* (Méndez et al., 2004). Unlike the efforts spent on genetic attributes (Himes et al., 2008), patterns of morphological variation are poorly known in *D. gliroides*. Morphological studies have been fundamental to evaluate the geographic variation in small mammals (D'Anatro and Lessa, 2006; Fonseca and Astúa, 2015). This is a major issue for understanding the evolutionary processes affecting the species variation (Gould and Johnston, 1972; Thorpe, 1987). Here, we have a valuable and stimulating opportunity to partially fill those gaps for *D. gliroides*.

As an approach focused to understand the geographical variation of *D. gliroides*, we evaluated if the cranial morphology of this marsupial shows a pattern of variation congruent with its phylogeographic structure previously observed by Himes et al. (2008). If the latter proposition is true, we expect to find: a) significant morphological differences among the specimens belonging to each phylogroup or clade as understood by Himes et al. (2008), and; b) within the southern clade, we should not observe significant differences between insular and mainland individuals, as the mtDNA data have shown. Additionally, we expect to obtain evidence about the relative importance of the shape and size components of morphological change that eventually are explaining such differences. Considering that geometric morphometric methods (GMM) represent one of the most successful tools used during the last decades to evaluate these components of morphological variation, GMM based on three-dimensional landmark coordinates were applied to fulfill the major goals of this work (Adams et al., 2013; Benítez and Püschel, 2014).

Table 1

Grouping of samples for statistical analyses, indicating correspondence with mtDNA clades after Himes et al. (2008): a) "North" (Nahuelbuta, Malleco, Cautín and Villarrica), b) "Center" (San José de la Mariquina and Valdivia), c) "South" (Puerto Octay, La Picada, Peulla and Palena) and, d) "Island" (Senda Darwin, Caulín, Llanquihue, Quilar, Ancud and Chiloé National Park).

| Geographical group	N	mtDNA clade	Clade location
North	11	Northern (A)	36–39°S
Center	8	Intermediate (B)	39–40°S
South	31	Southern (C)	40–43°S
Island	19	Southern (C)	
Total	69		

Material and methods

Samples and study site

We analyzed sixty-nine crania of adult specimens of *D. gliroides* (Astúa, 2010) from insular and mainland localities distributed along south-central Chile (Fig. 1; Appendix A, Supplementary material). Crania were deposited at the Mammal Collection of The Field Museum of Natural History (FMNH), Chicago, United States; at the Colección de Flora y Fauna "Prof. Patricio Sánchez-Reyes" (SSUC), Pontificia Universidad Católica de Chile, Santiago, Chile; and at the Colección de Mamíferos de la Universidad Austral de Chile (UACH), Valdivia, Chile. We registered information of total length (mm) for all possible samples (from skin tags). Since skull morphology of *D. gliroides* does not present sexual dimorphism (Astúa, 2010), we pooled both sexes for analyses. We grouped specimens from different localities into geographical groups according to the three major clades described for *D. gliroides* (Himes et al., 2008). Samples from the southern clade were divided in mainland and insular specimens (Table 1).

Three-dimensional geometric morphometric analyses

We analyzed crania using GMM based on three-dimensional (3D) landmark coordinates as raw data. Since these coordinates contained both "shape" and "size" information, independent analyses can be performed for both components (Zelditch et al., 2004). The 3D models of crania were obtained using a Nextengine 3D laser scanner and the software Scanstudio v. 2.0.0. Later, 30 landmarks were digitized by the same observer (AVG) on each cranium in Landmark v. 3.0 (Whiley, 2002–2005). Landmarks' location and description mainly followed Astúa (2015), Bennett and Goswami (2013) and Fonseca and Astúa (2015) (Table 2; Fig. 2). In order to quantify error in landmarks digitization ("measurement error"), the same observer digitized three times the whole sample set of crania and the resulting replicates were submitted to Procrustes Analysis of Variance (Procrustes ANOVA) according to Klingenberg et al. (2002). This procedure allowed us to estimate simultaneously the effect of "Group" (i.e., shape variation among geographical groups: North, Center, South and Island). Analyses were performed on the symmetric component of shape variation, as it was recommended by Klingenberg et al. (2002) for symmetric structures like that of the vertebrate's skulls (Savriama and Klingenberg, 2011). Changes due to scale, position and orientation of landmarkís configuration were eliminated by Procrustes fit (Zelditch et al., 2004). The resulting Procrustes coordinates matrices and their covariance matrices were analyzed following the standard multivariate methods included in the software MorphoJ v. 1.06d (Klingenberg, 2011). First, we performed a Principal Component Analysis or PCA as an exploratory evaluation of the overall shape variation among specimens. Later, to assess the shape differences between geographical groups we conducted a Canonical Variate Analysis or CVA (Fonseca and Astúa,
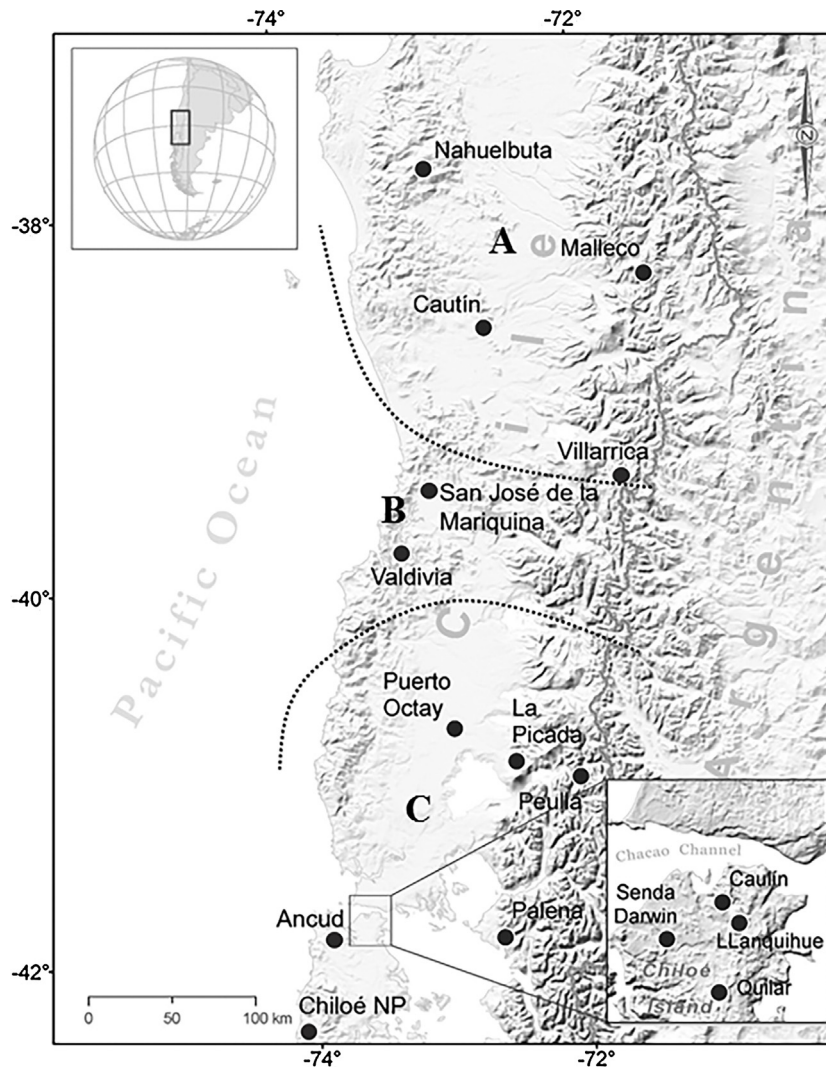
Fig. 1. Map of study sites including mainland and insular localities sampled along south-central Chile. Dotted lines indicate the approximate geographic extension of clades A, B and C after Himes et al. (2008).

Table 2

Landmarks used in this study. Identification of cranial structures of *D. gliroides* followed Hershkovitz (1999). * Bennett and Goswami (2013), ** Fonseca and Astúa (2015), *** Astúa (2015), **** this study.

Number	Landmark description	Ref.
1	Midline anterior-most point of the nasal-nasal suture	*
2	Midline posterior point of the nasal in contact with frontals	*
3	Midline posterior point of the frontals in contact with parietals	*
4	Midline posterior point of the parietals in contact with interparietal	****
5	Midpoint at posterior-most extent of vault	*
6 and 7	Anterior point of canine insertion	****
8 and 9	Anterior-most point of the orbit	***
10 and 11	Posterior end of the zygomatic arch	***
12	Midline point between the upper central incisors	*
13 and 14	Anterior lateral extent of molar row	*
15 and 16	Ventral-most point of the jugal-maxilla suture	*
17 and 18	Posterior extent of molar row	*
19 and 20	Posterolateral tip of palate	**
21	Midline posterior point of the palatine-palatine suture	*
22 and 23	Jugal-squamosal (ventral zygomatic arch)	*
24 and 25	Anterior-most central point of the tympanic process of alisphenoid	****
26 and 27	Inner point of the suture between tympanic process of alisphenoid and entotympanic	****
28	Anterior-most point of the foramem magnum, at the basioccipital	**
29 and 30	Dorsal-most occipital condyle-foramen magnum margin	*
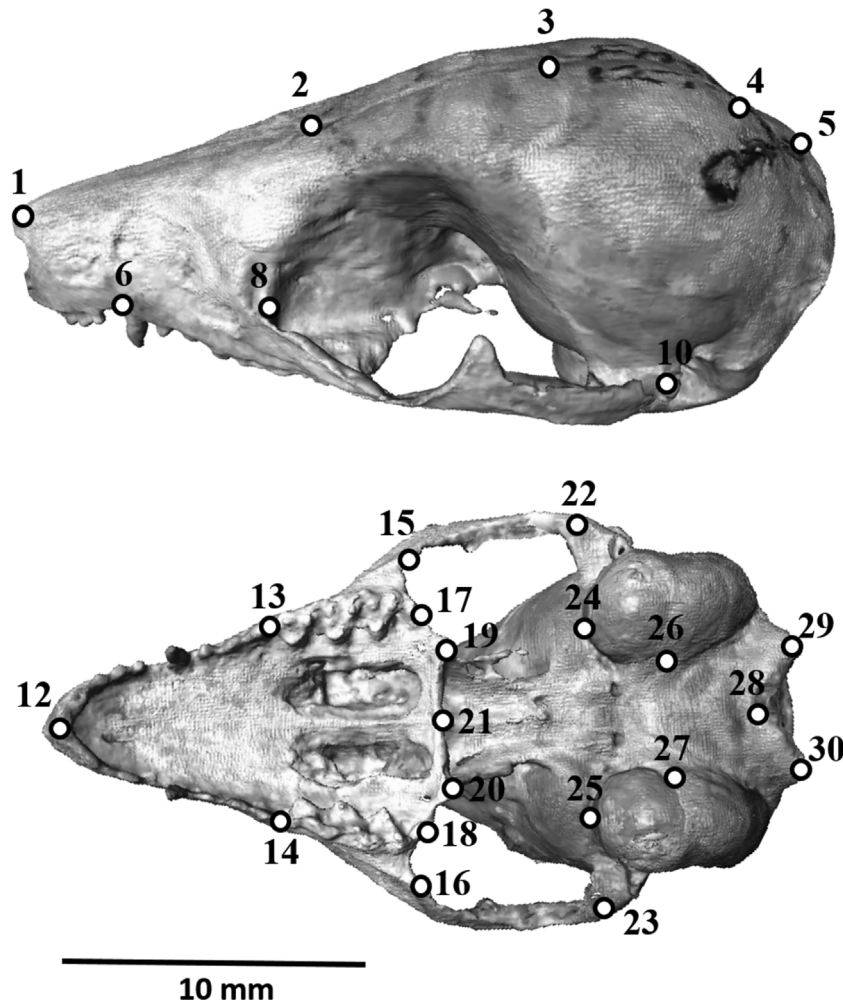
Fig. 2. Three-dimensional model of *D. gliroides* cranium indicating the 30 landmarks used in this study. Landmarks 7, 9 and 11 (not shown) are the correspondent landmarks on the right side of landmarks 6, 8 and 10, respectively.

2015). Procrustes distances among groups (North, Center, South and Island) were calculated and tested through permutation tests with 10,000 permutation rounds in MorphoJ v. 1.06d. As mentioned by Dryden and Mardia (1998) and Klingenberg and Monteiro (2005), for a small shape variation the Procrustes method produces shape coordinates with a degree of separation depending only on the distances between groups. We performed Procrustes instead of the Mahalanobis distances, because the latter measure the differences between groups in relation to the level of within group variation. Additionally, the Procrustes ANOVA model from MorphoJ v. 1.06d software works with fewer parameters than other models (like MANOVA and Mahalanobis distance approaches), requiring bigger sample sizes than the Procrustes approach (Klingenberg, 2011). An additional discriminant function analysis (DFA; Klingenberg et al., 2011) with 1,000 permutation runs was performed to test the differences between the "Island" and the "South" groups, the southern clade *sensu* Himes et al. (2008). We analyzed the size variation among groups (North, Center, South and Island) by performing a one-way ANOVA on cranial centroid size, followed by a *post-hoc* Tukey HSD test (Fonseca and Astúa, 2015). Finally, we tested the effect of size variation on shape changes with regression analyses between cranial centroid size vs. scores of PCs that explained the major variance in overall data (Astúa, 2015). The latter analyses were performed in the statistic software JMP v. 13.0.0 (SAS Institute Inc., 2016).

Results

Cranial shape variation

After performing the Procrustes and Principal Component analyses, a total number of 45 shape components were obtained, and the first 20 explained the 90.42% of overall variance (Table 3) (PC1 + PC2 = 31.09%). In general, we observed a high overlapped distribution of specimens along the first two PC axes. The phylogeographic structure revealed by the mtDNA data (Himes et al., 2008) was not recovered in our study. Moreover, contrary to what was suggested by mtDNA data, a subtle differentiation between specimens of the "southern clade" was observable along PC2. Thus, insular individuals from Himes's et al. (2008) southern clade ("Island") were mainly distributed along negative scores of PC2. Conversely, mainland individuals from southern clade ("South") were mainly distributed along positive scores of PC2 (Fig. 3). Additional discriminant function analysis confirmed differences between "Island" and "South" groups (cross-validation comparing "Island"/63.16% of true matching/vs. "South"/90.32% of true matching/; Procrustes distances *p* < 0.0001). Procrustes ANOVA indicated that the "Group" variable had a significant effect on total shape variance (Tabla 4). Additionally, measurement error explained the smaller portion on shape variation compared with all remaining factors displayed by Procrustes ANOVA. Moreover, the relative effect of "measurement error" to "Group" was only 4.8%. There-

Table 4
Procrustes ANOVA of cranial shape variation of *D. gliroides*, evaluating effects of "Group" and "Measurement error". Sums of squares (SS), mean squares (MS), degree of freedom (d.f.), F-statistic (F) and *p*-values (*p*) are showed (Reflection, Individual x Reflection and Measurement Error for testing intraobserver error).

| Effect	SS	MS	d.f.	F	*p*
Group	0.03327613	0.000246490	135	3.47	<0.0001
Individual	0.20791329	0.000071082	2925	3.48	<0.0001
Reflection	0.01400556	0.000368567	38	18.07	<0.0001
Individual × Reflection	0.05270610	0.000020397	2584	1.71	<0.0001
Measurement error	0.13638100	0.000011907	11454		

Table 5
Procrustes distances between pairwise geographical groups (bottom diagonal) and its respective *p*-values after permutation test with 10,000 permutation rounds (upper diagonal).

| Group	North	Center	South	Island
North	–	0.0223	0.0006	0.0001
Center	0.0250	–	0.0012	0.0083
South	0.0208	0.0239	–	<0.0001
Island	0.0208	0.0222	0.0208	–

Table 3
Percent of variance contributed by each shape component (PC), and cumulative variance obtained after applying Procrustes analysis to the 3D landmark coordinates data.

PCA	% Variance	Cumulative%
1	21.01	21.01
2	10.08	31.09
3	9.02	40.11
4	7.09	47.20
5	5.02	52.22
6	4.96	57.18
7	4.76	61.93
8	4.11	66.04
9	3.33	69.37
10	3.12	72.49
11	2.75	75.23
12	2.66	77.89
13	2.08	79.96
14	1.88	81.85
15	1.80	83.65
16	1.59	85.24
17	1.52	86.76
18	1.33	88.09
19	1.19	89.28
20	1.14	90.42
21	1.06	91.48
22	0.99	92.47
23	0.89	93.36
24	0.79	94.14
25	0.71	94.85
26	0.64	95.49
27	0.54	96.03
28	0.51	96.53
29	0.45	96.99
30	0.40	97.39
31	0.39	97.78
32	0.31	98.09
33	0.29	98.38
34	0.24	98.62
35	0.24	98.87
36	0.23	99.10
37	0.21	99.31
38	0.17	99.48
39	0.11	99.59
40	0.11	99.70
41	0.08	99.79
42	0.08	99.87
43	0.06	99.92
44	0.05	99.97
45	0.03	100.00
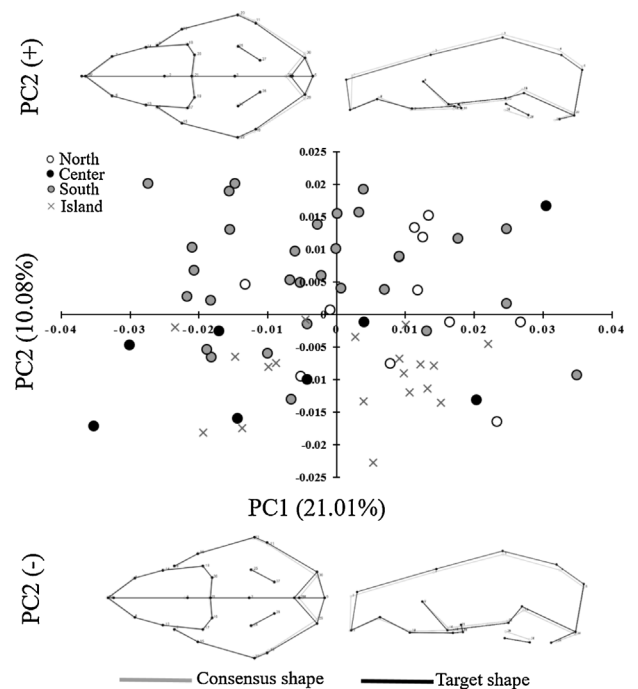
Fig. 3. Principal component analysis (PCA) of cranial shape variables. Wireframes show relative landmarks displacement along extremes PC2 scores (magnified 0.05 set scale factor).

fore, this "measurement error" was not a substantial concern in our data (Table 4). CV1 and CV2 explained 62.19% and 25.91% of the among-groups variation, respectively. When we applied this analysis assuming an *a-priori* sample assignment, geographical groups were observable on the multivariate axes (Fig. 4). Procrustes distances between all paired group comparisons after permutation test were significant (Table 5). Additionally, a multiple comparisons correction test (Bonferroni correction) was applied to the *p*-values of the results for the Procrustes distance test giving non significant scores (Appendix B, Supplementary material). Despite the latest results, the major pattern of geographical group structuration was not associated with the phylogeographic structure. For example, CV1 separated the geographical groups into two major divisions, one of these corresponding to the "South" group (negative CV1 scores) and the second pooling the remaining groups (North, Center and Island, positive CV1 scores). Additionally, CVA confirmed the separation between insular and mainland specimens
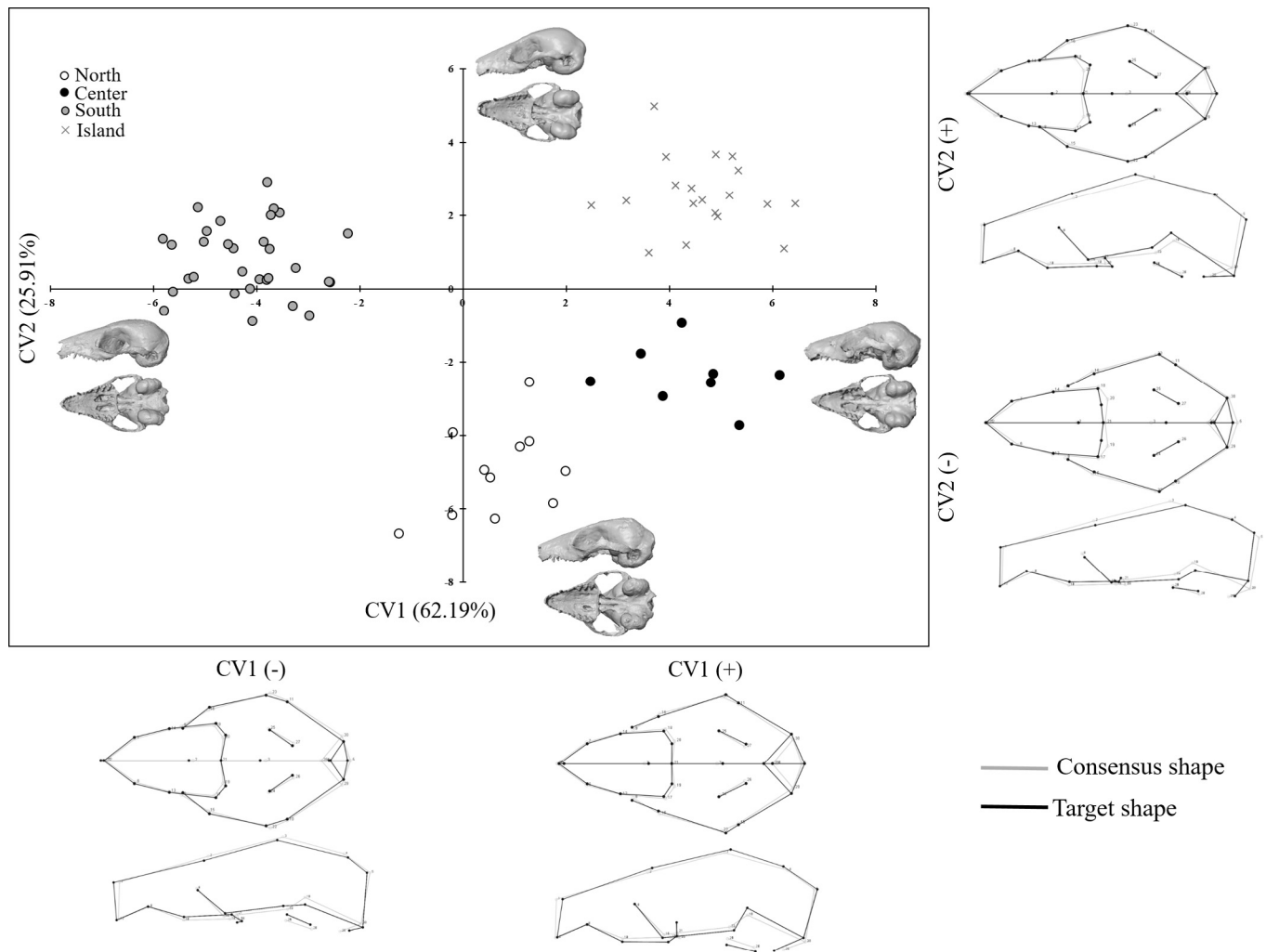
Fig. 4. Canonical variate analysis showing samples distribution on CV1 and CV2. Three-dimensional images of shape change are showed (in lateral and ventral view) to the extremes distribution of each axis (inside of box). Besides, wireframes showing the relative landmarks displacement to the extreme distribution of each axes (target shape) from the consensus shape are presented (outside and lateral to the box). Wireframe were magnified to 30 set scale factor to facilitate visualizing of shape changes.

("Southern clade"), as suggested the former PCA. Along CV2 groups scattering seem to reflect a latitudinal structure, from the northermost to the southernmost geographically distributed groups (from negative CV2 scores to positive CV2 scores, respectively). Negative CV1 scores ("South" group morphotype) were associated with both an overall elongate viscerocranium (nasal and mandibular region) and neurocranium (Fig. 4). Positive CV1 scores were associated with shorter viscerocranium and wider neurocranium (remaining group morphotype). Negative CV2 scores (a "more mainland" morphotype) showed an elongated nasal region and distal location of the end of molar row and the end of palate region, while the neurocranium showed an elongated shape. Positive CV2 scores (a "more insular" morphotype) showed a shorter nasal region and proximal location of the end of molar row and the end of palate region, while the neurocranium was wider shaped.

Cranial size variation

Cranial centroid size was normally distributed (Kolmogorov-Smirnov test $D_{69} = 0.074$, $p = 0.200$) and it was highly correlated with total length ($n = 44$, $r = 0.80$, $p < 0.0001$). Taking into account this result, we used cranial centroid size as an estimator of body size. Details of descriptive statistic of centroid size variation among groups are given in Table 6. Variation of centroid size among

geographical groups was statistically significant (one-way ANOVA $F_{(3,68)} = 6.21$, $p = 0.0009$). The *post-hoc* Tukey HSD test showed that the "Center" group, had the smaller size compared to the other groups (Fig. 5). Insular specimens resulted to be of a bigger size in average with respect to mainland individuals. The latter findings were supported by standard body measurement (total length) that we recorded from skin tags. Thus, individuals from the "Center" group showed a smaller total length compared with the other groups and insular individuals showed a bigger total length compared to overall mainland specimens (Table 6). Furthermore, cranial centroid size and total length size showed a slight increase with latitude.

Regarding allometry, we detected a strong effect of cranial centroid size on cranial shape variation (Fig. 6). PC1 (20.01% of overall shape variance) showed a strong and highly statistical significant association with cranial centroid size ($n = 69$, $r = 0.68$, $p < 0.0001$). PC2 (10.08%), and PC4 (7.09%) also showed a significant association with cranial centroid size. However, the latter correlations were considerable weak ($n = 69$, $r = 0.26$, $p = 0.0293$, and $r = -0.24$, $p = 0.0476$, respectively) (details of the allometric analysis are shown in Appendix C, Supplementary material). According to these results, we only assessed graphically the shape changes of PC1 on centroid size variation (Fig. 7). Relative landmarks displacement along PC1 related to variation in centroid size were mainly

Table 6
Size variation of *D. gliroides*. Sample size of geographical group (*n*), mean, standard deviation (SD) and 95% confidence interval (CI) are showed for cranial centroid size and total length. Tukey HSD show differences between pairs of group means. Groups not connected by the same letter are significantly different.

Size variable	Group	N	Mean	SD	95% CI		Tukey HSD	
					Lower	Upper		
Cranial centroid size	Center	8	46.67	1.23	45.64	47.70	A	
	North	11	48.62	1.16	47.84	49.39		B
	South	31	48.90	1.56	48.33	49.47		B
	Island	19	49.24	1.52	48.50	49.97		B
	Total	69						
Total length	Center	7	200.21	7.97	192.85	207.58		B
	North	7	210.43	13.71	197.75	223.11	A	B
	South	28	211.46	10.52	207.39	215.54	A	B
	Island	2	230.00	4.23	191.88	268.12	A	
	Total	44						

associated to the end of molar row and the tip of palate in rostrum (landmarks 17–18 and 19–20 respectively), and to the zygomatic arch (landmarks 10–11, 22–23), the auditory bullae (landmarks 24–25, 26–27), the vault (landmark 5) and the foramen magnum region (landmarks 28, 29, 30) in the neurocranium (Fig. 7).

Discussion

This study represents one of the first efforts devoted to quantitatively analyze the morphological variation of *D. gliroides* in a geographic context. In general, our results showed a subtle but consistent morphological variation, both in cranial shape and size traits. Furthermore, body size represents a trait that affects the cranial shape variation in this species, as it has been observed in didelphid marsupials (Astúa, 2015). Geographic variation in morphology is a common phenomenon in nature, often complex, displaying a wide range of patterns as clinal, categorical, mosaic (Thorpe, 1987), and several examples have been documented in mammals (Gallardo and Palma, 1990; Lalis et al., 2009; Motokawa et al., 2004). In particular, cranial morphology in marsupials shows similar geographic variability (Dawson and Milne, 2012; Fonseca and Astúa, 2015), as we observed in *D. gliroides*. Usually, an interplay of ecological, historical and genetic causes has been evoked to explain the geographic variation in the morphology of mam-
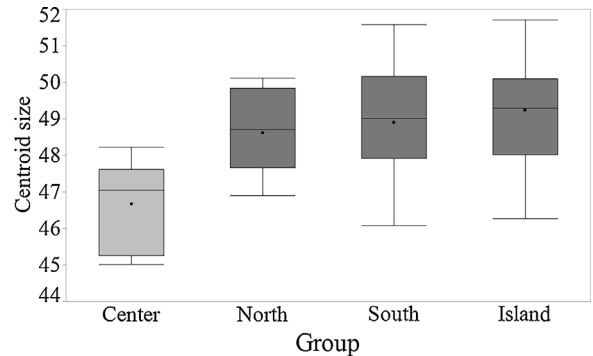

Fig. 5. Cranial centroid size variation among geographical groups. Groups not connected by the same color are significantly different (solid black correspond to group mean).

mals (Hadley et al., 2009; Maestri et al., 2016). We suspect that this variation in *D. gliroides* could require similar interpretations.

Our study suggest that the cranial shape variation observed in *D. gliroides* cannot be understood in terms of the phylogeographic structure recovered for this species based on the mtDNA results (Himes et al., 2008). Rather, our data suggest that the cranial shape variation could be determined partially by historical processes, in a pattern that not necessarily is correlated with the variation observed at the molecular level (Maldonado et al., 2004). During the
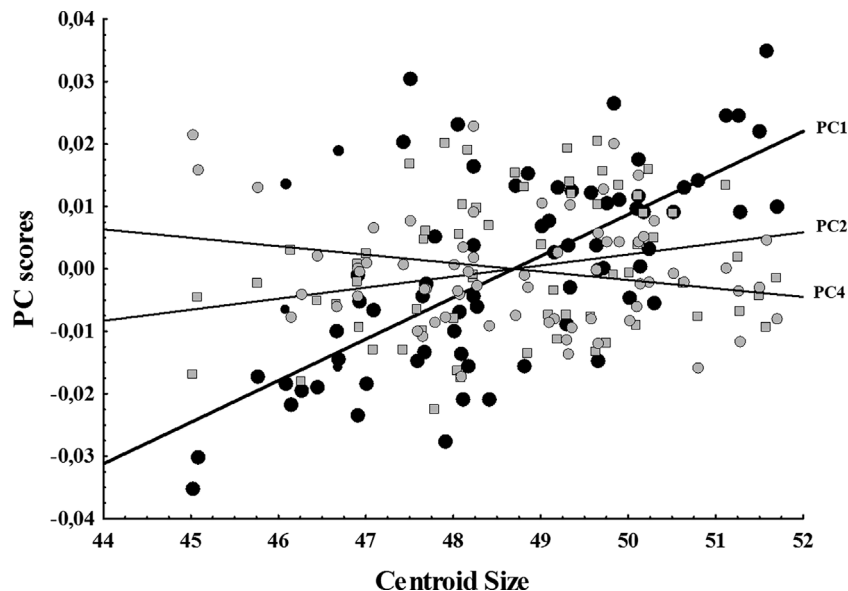
Fig. 6. Allometric analysis of the shape components showing significant *r*-Pearson values (PC1, black circles, *r* = 0.68, *p* < 0.0001; PC2, grey squares, *r* = 0.26, *p* = 0.0293; PC4, grey circles, *r* = -0.24, *p* = 0.0476).
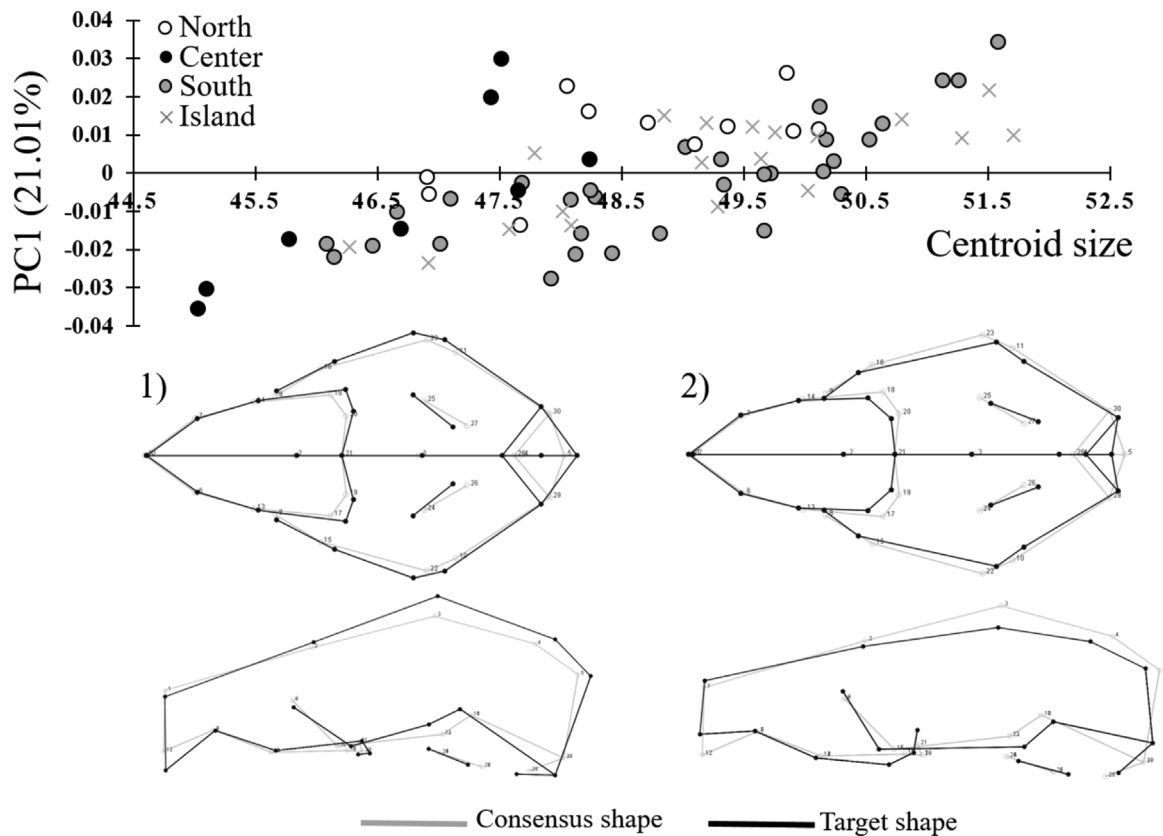
Fig. 7. Regression of PC1 scores versus cranial centroid size. Below the scatter plot, wireframes of crania are shown indicating the relative landmarks displacement of extreme PCs score along the centroid size axis. 1) Target shape of minimum and 2) Target shape of maximum PC1 score, respectively.

Last Glacial Maximum (LGM, 19,000–23,000 cal years BP, Hulton et al., 2002), the ice shield partially covered the Chilean continental territory including the Temperate Rainforests, reaching the sea level south of 43°S (Villagran, 1991). To the north, ice-free areas were located across the northwest of Chiloé Island, and in the longitudinal valley and coastal mountains in the mainland (Denton et al., 1999). We observed a significant separation between "South" and the remaining groups analyzed in our study (North, Center and Island). Such separation seems to follow the longitudinal limit of ice coverage described previously. Thus, the "South" group encompassed specimens from localities that were covered by the ice sheet during LGM, conversely to North, Center and Island localities that were free of ice. Such ice-free zones probably constituted major refuges of Temperate Rainforests (Segovia et al., 2012; Villagrán and Armesto, 2005) and associated vertebrates (Palma et al., 2012; Vera-Escalona et al., 2012). Therefore, the cranial shape differentiation of populations in the southern continental distribution of *D. gliroides* could be reflecting a morphological imprinting of the Pleistocene cycles, that deeply affected their biogeographical history and post-glacial recolonization dynamics (Himes et al., 2008).

Our results suggest that insular individuals of *D. gliroides* could represent a distinct morphological group with respect to their nearest mainland conspecifics, contrary to the pattern shown by mtDNA (southern clade *sensu* Himes et al., 2008). These findings highlight the potential effect of the postglacial insular isolation of *D. gliroides* populations, after the interruption of terrestrial connection between Chiloé Island and mainland during LGM. Molecular and morphological data support such isolation scenario in local vertebrates (Napolitano et al., 2014; Vidal et al., 2006) and we suspect that a similar process could be affecting to *D. gliroides*, which was not evidenced by mtDNA data (Himes et al., 2008), but recovered by cranial morphology. Moreover, as it has been demonstrated in

the felid *Leopardus guigna*, mtDNA may not be sensible enough to detect the modern isolation of Chiloé island populations and the use of a more variable molecular marker (e.g., microsatellites) could be more appropriate to reveal such process (Napolitano et al., 2014). Additionally, microevolutionary changes due to local adaptation, or to genetic drift and founder effect (Hadley et al., 2009; Maldonado et al., 2004), could be reinforcing the differentiation of populations of *D. gliroides* after of Pleistocene connection. For example, differences in cranial and mandibular traits have been widely associated with dietary changes (Nogueira et al., 2009; Prevosti et al., 2012). Moreover, dietary divergence can be an important factor promoting insular/mainland morphological differentiation (Clegg and Owens, 2002). Indeed, several studies have reported dietary changes between Chiloé Island and mainland populations in the Darwin's fox *Lycalopex fulvipes* (Jaksić et al., 1990; Jimenez et al., 1991; Jiménez, 2007) and the lizard *Liolaemus pictus* (Vidal et al., 2006; Willson et al., 1996). Therefore, similar ecological responses could be expected for *D. gliroides*.

In relation to cranial size changes, our study showed that individuals from the Coastal Mountains range of Valdivia were consistently smaller in size traits (cranial centroid size and total length) compared to specimens of the remaining distribution of *D. gliroides*. Besides the latter finding in the cranial size of this species, we think that these differences are not sufficient to ascribe this population to a different cluster (or clade) as proposed by Himes et al. (2008). Indeed, the latter authors did not make any major conclusions regarding clade B due to the small number of specimens available for that population. Without considering clade B, the centroid size variation and the total length slightly increased with latitude. On the other hand, insular specimens were bigger compared with the mainland individuals in both size traits (cranial centroid size and total length). The latest general tendencies

of size variation seem to fit with classical biogeographic patterns. For example, increased size of insular individual of *D. gliroides* (Chiloé Island) seems to be in agreement with predictions of the "Island rule" for small mammals (Foster, 1964; Lomolino, 1985), as well as it has been corroborated for other small marsupials (Dawson and Milne, 2012). Furthermore, latitudinal changes of body size in mammals have been widely understood evoking the Bergmannís rule, which predicts larger individuals in cooler environment than those from warmer areas (Yom-Tov and Nix, 1986). Several examples of Bergmannís rule have been documented in marsupials (Dawson and Milne, 2012; Meiri and Dayan, 2003), and a similar response has been suggested for *D. gliroides* (Patterson and Rogers, 2007). In addition to these classical explanations, geographical body size variation in mammals has been related for example, to prey size and competition (Mcnab, 1971), food availability (Yom-Tov and Geffen, 2006), phylogenetic community composition and environmental factors (Meastri et al., 2016), and forest fragmentation (Lomolino and Perault, 2007). The relative effect and interplay of such causes on size variation in *D. glirodies* make further studies necessary.

Whereas we were writing this paper, D'Elía et al. (2016) published a work in which they proposed two new species of the genus *Dromiciops*. They analyzed the variation on qualitative and quantitative cranial traits, assuming an *a priori* classification of the sp. nova specimens considering the phylogeographic structure suggested by mtDNA data (Himes et al., 2008). D'Elía et al. (2016) concluded that given the agreement found between the cranial morphology observed in their own data and the phylogeographic pattern reported by Himes et al. (2008), *Dromiciops* would be currently represented by three different species, whose distributions match the mtDNA clades: *D. bozinovici* n. sp. (Clade A, Himes et al., 2008), *D. mondaca* n. sp. (Clade B, Himes et al., 2008), and *D. gliroides* now restricted to Chiloé Island and the southern part of the continent in Chile and Argentina (Clade C, Himes et al., 2008). However, we have our reservations about D'Elía's et al. (2016) interpretation. First, we disagree as enough evidence, in describing a new species, to use a previous study that suggests a phylogeographic structuring. It has been demonstrated that such kind of structuring does not necessarily reflects the morphological variation (Maldonado et al., 2001, 2004). Secondly, cranial shape and size changes can be strongly dependent on latitudinal environmental variation (Dawson and Milne, 2012). Latitudinal environmental variation is a major feature within *D. gliroides* distribution (Di Castri and Hajek, 1976). Therefore, we could expect that its cranial morphology reflects such variation. Furthermore, D'Elía et al. (2016) claimed consistent morphological differences among groups (species *sensu* D'Elía et al. (2016)), but overall distances in cranial ventral view and a particular comparison of lateral view failed to distinguish such groups. Moreover, the discriminant analysis performed by D'Elía et al. (2016) seems to discretely separate each species, but such phenomena could be the result of methods that assume an *a priori* assignment. Indeed, supposing that the *a priori* approaches are adequate for alpha taxonomy research, from a strictly methodological point of view, discriminant function analysis is designed for testing dichotomic classifications. When there are three or more groups, as is the case in D'Elía et al. (2016) study, and in our work, the a priori multifactorial method recommended is Canonical Variate Analysis, as the approach that we used in this study. Despite this fact, to avoid the *a priori* assumptions about the similarity relationships among the groups under study, we also applied *a posteriori* and more conservative method such as Principal Component Analysis (PCA), using Procrustes coordinates as raw data. This approach is broadly used in geometric morphometrics to explore the main patterns of morphological (shape and size) variation (Adams et al., 2013). Third, the use of partial warps to assess morphological change, as did D'Elía et al. (2016), has been

thoroughly discussed and criticized, because the pattern of partial warp variation is determined by the landmark configuration *a priori* defined in the map of homologous landmarks and not by the patterns of covariation in the data (Adams and Rosenberg, 1998; Rohlf, 1998). In addition, D'Elía et al. (2016) did not provide analysis to evaluate an eventual insular differentiation of *D. gliroides* probably assuming the results of molecular data. Nevertheless, our results showed that insularity could be an important factor affecting the cranial morphology in *D. gliroides*. This finding is in agreement with earlier observations on this marsupial (Osgood, 1943), and in other sympatric vertebrates such as the cervid *Pudu puda* (Fuentes-Hurtado et al., 2011), the felid *Leopardus guigna* (Napolitano et al., 2014), and the lizard *Liolaemus pictus* (Vidal et al., 2006). Finally, D'Elía et al. (2016) followed Himes' et al. (2008) arguments who compared nucleotide distance values of partial cytochrome *b* and Control Region mitochondrial sequences between *Dromiciops* clades and recognized species of the didelphid mouse opossums of the genus *Thylamys* (Palma et al., 2002). Besides, Himes et al. (2008) did not recognize the *Dromiciops* phylogroups as different species, D'Elía et al. (2016) presented nucleotide distances among *Dromiciops* clades (citing Himes et al., 2008) as an additional antecedent for the proposed new species of "monitos". However, we consider that to contrast nucleotide distances between species that correspond to far divergent lineages is a mistake, as is the case of *Thylamys* species and the *Dromiciops* clades. *Thylamys* is part of the order Didelphimorphia included in the Ameridelphia radiation of marsupials, whereas *Dromiciops* is part of the Microbiotheria, an order included in the Australidelphia radiation. Millions of years (approximate 70 Mya) separate didelphimorphs from microbiotheres, thus nucleotide substitution rates should not be contrasted between these far divergent marsupial taxa (Springer et al., 2009).

Taking into account our findings, we consider that understanding the cranial morphology observed in *D. gliroides* in terms of the occurrence of three phylogeographic clades is not adequate. Consequently, we cannot support the recognition of two new species of *Dromiciops* as proposed by D'Elía's et al. (2016). Furthermore, the latter work reveals methodological problems and unfounded assumptions about the genetic structure and the ecological scenario where *D. gliroides* variation occurs. However, D'Elía's et al. (2016) and our study highlight that *D. gliroides* is morphologically variable along its geographic distribution in terms of cranial traits. Our data seem to reflect intraspecific geographic variation associated to the complex environment along the distribution range of *D. gliroides*, the merging of recent geographical barriers in its southernmost distribution, and the partial effect of historical processes. We encourage future studies generating new molecular and morphological data conducted to better understand the population variation in *D. gliroides*. Finally, we highlight the successful use of 3D geometric morphometric methods to elucidate the intraspecific variation in this microbiotheriid marsupial.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mambio.2017.07.003.

References

Adams, D.C., Rosenberg, M.S., 1998. Partial warps, phylogeny, and ontogeny: a comment on Fink and Zelditch (1995). Biol 47, 1–68.

Adams, D.C., Rohlf, J.F., Slice, D.E., 2013. A field comes of age: geometric morphometrics in the 21st century. Assoc. Teriologica Ital. Hystrix Ital. J. Mammal. 24, 7–14, http://dx.doi.org/10.4404/hystrix-24.1-6283.

Amico, G., Aizen, M.A., 2000. Mistletoe seed dispersal by a marsupial. Nature 408, 929–930.

Amico, G.C., Rodríguez-Cabal, M.A., Aizen, M.A., 2009. The potential key seed-dispersing role of the arboreal marsupial *Dromiciops gliroides*. Acta Oecol. 35, 8–13, http://dx.doi.org/10.1016/j.actao.2008.07.003.

Armesto, J.J., Manuschevich, D., Mora, A., Smith-Ramirez, C., Rozzi, R., Abarzúa, A.M., Marquet, P.A., 2010. From the Holocene to the Anthropocene: a historical framework for land cover change in southwestern South America in the past 15,000 years. Land Use Policy 27, 148–160, http://dx.doi.org/10.1016/j.landusepol.2009.07.006.

Astúa, D., 2010. Cranial sexual dimorphism in New World marsupials and a test of Rensch's rule in Didelphidae. J. Mamm. 91, http://dx.doi.org/10.1644/09-MAMM-A-018.1.

Astúa, D., 2015. Morphometrics of the largest new world marsupials, opossums of the genus *Didelphis* (Didelphimorphia, Didelphidae). Oecologia Aust. 19, 117–142, http://dx.doi.org/10.4257/oeco.2015.1901.08.

Benítez, H., Püschel, T., 2014. Modelando la varianza de la forma: morfometría geométrica aplicaciones en biología evolutiva. Int. J. Morphol. 32, 998–1008.

Bennett, C.V., Goswami, A., 2013. Statistical support for the hypothesis of developmental constraint in marsupial skull evolution. BMC Biol. 11, 52, http://dx.doi.org/10.1186/1741-7007-11-52.

Celis-Diez, J.L., Hetz, J., Marín-Vial, P.A., Fuster, G., Necochea, P., Vásquez, R.A., Jaksic, F.M., Armesto, J.J., 2012. Population abundance, natural history, and habitat use by the arboreal marsupial *Dromiciops gliroides* in rural Chiloé Island, Chile. J. Mamm. 93, 134–148, http://dx.doi.org/10.1644/10-mamm-a-406.1.

Clegg, S.M., Owens, P.F., 2002. The island rule in birds: medium body size and its ecological explanation. Proc. R. Soc. B Biol. Sci. 269, 1359–1365, http://dx.doi.org/10.1098/rspb.2002.2024.

D'Anatro, A., Lessa, E.P., 2006. Geometric morphometric analysis of geographic variation in the Río Negro tuco-tuco, *Ctenomys rionegrensis* (Rodentia: ctenomyidae). Mamm. Biol., 288–298.

D'Elía, G., Hurtado, N., D'Anatro, A., 2016. Alpha taxonomy of *Dromiciops* (Microbiotheriidae) with the description of 2 new species of monito del monte. J. Mamm. 97, 1136–1152, http://dx.doi.org/10.1093/jmammal/gyw068.

Dawson, R., Milne, N., 2012. Cranial size and shape variation in mainland and island populations of the quokka. J. Zool. 288, 267–274, http://dx.doi.org/10.1111/j.1469-7998.2012.00952.x.

Denton, G.H., Heusser, C.J., Lowel, T.V., Moreno, P.I., Andersen, B.G., Heusser, L.E., Schluhter, C., Marchant, D.R., 1999. Interhemispheric linkage of paleoclimate during the Last Glaciation. Geogr. Ann. Ser. A Phys. Geogr., 81A, http://dx.doi.org/10.1111/j.0435-3676.1999.00055.x.

Di Castri, F., Hajek, E.R., 1976. Bioclimatología de Chile. Vicerrectoría Académica de la Universidad Católica de Chile.

Dryden, I.L., Mardia, K.V., 1998. Statistical Analysis of Shape. Wiley, Chichester, UK.

Fonseca, R., Astúa, D., 2015. Geographic variation in caluromys derbianus and *Caluromys lanatus* (Didelphimorphia: Didelphidae). Zool 32, 109–122, http://dx.doi.org/10.1590/s1984-46702015000200002.

Fontúrbel, F.E., Candia, A.B., Botto-Mahan, C., 2014a. Nocturnal activity patterns of the monito del monte (*Dromiciops gliroides*) in native and exotic habitats. J. Mamm. 95, 1199–1206.

Fontúrbel, F.E., Candia, A.B., Salazar, D.A., Malebrán, J., González-Browne, C., Botto-Mahan, C., 2014b. How forest marsupials are affected by habitat degradation and fragmentation? A meta-analysis. Naturwissenschaften 101, 599–602, http://dx.doi.org/10.1007/s00114-014-1193-z.

Formas, J.R., Brieva, L.M., 2000. Population genetics of the Chilean frog *Batrachyla leptopus* (Leptodactylidae). Genet. Mol. Biol. 23, 43–48, http://dx.doi.org/10.1590/s1415-47572000000100007.

Foster, J.B., 1964. The evolution of mammals on islands. Nature 202, 234–235.

Fuentes-Hurtado, M., Marín, J.C., González-Acuña, D., Verdugo, C., Vidal, F., Vianna, J.A., 2011. Molecular divergence between insular and continental Pudu deer (*Pudu puda*) populations in the Chilean Patagonia. Stud. Neotrop. Fauna Environ. 46, 23–33, http://dx.doi.org/10.1080/01650521.2010.537906.

Gallardo, M.H., Palma, E., 1990. Systematics of *Oryzomys longicaudatus* (Rodentia: Muridae) in Chile. J. Mamm. 71, 333–342.

Gould, S.J., Johnston, R.F., 1972. Geographic variation. Annu. Rev. Ecol. Syst. 3, 457–498.

Hadley, C., Milne, N., Schmitt, L.H., 2009. A three-dimensional geometric morphometric analysis of variation in cranial size and shape in tammar wallaby (*Macropus eugenii*) populations. Aust. J. Zool. 57, 337–345, http://dx.doi.org/10.1071/zo08098.

Hershkovitz, P., 1999. *Dromiciops gliroides* Thomas, 1894, last of the Microbiotheria (Marsupialia), with a review of the family Microbiotheriidae. Fieldiana Zool. 93, 1–60.

Himes, C.M.T., Gallardo, M.H., Kenagy, G.J., 2008. Historical biogeography and post-glacial recolonization of South American temperate rain forest by the relictual marsupial *Dromiciops gliroides*. J. Biogeogr. 35, 1415–1424, http://dx.doi.org/10.1111/j.1365-2699.2008.01895.x.

Hulton, N.R.J., Purves, R.S., McCulloch, R.D., Sugden, D.E., Bentley, M.J., 2002. The last glacial maximum and deglaciation in southern South America. Quat. Sci. Rev. 21, 233–241, http://dx.doi.org/10.1016/s0277-3791(01)00103-2.

Jaksić, F.M., Jiménez, J.E., Mendel, R.G., Marquet, P.A., 1990. Habitat and diet of Darwinís fox (*Pseudalopex fulvipes*) on the Chilean mainland. J. Mamm. 71, 246–248.

Jiménez, J.E., 2007. Ecology of a coastal population of the critically endangered Darwin's fox (*Pseudalopex fulvipes*) on Chiloé Island, southern Chile. J. Zool., http://dx.doi.org/10.1111/j.1469-7998.2006.00218.x.

Jimenez, J.E., Marquet, P.A., Medel, R.G., Jaksić, F.M., 1991. Comparative ecology of Darwin's fox (*Pseudalopex fulvipes*) in mainland and island settings of southern Chile. Rev. Chil. Hist. Nat. 63, 177–186.

Klingenberg, C., Monteiro, L., 2005. Distances and directions in multidimensional shape spaces: implications for morphometric applications. Syst. Biol. 54, 678–688, http://dx.doi.org/10.1080/10635150590947258.

Klingenberg, C.P., Barluenga, M., Meyer, A., 2002. Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. Evolution (N.Y). 56, 1909–1920.

Klingenberg, C.P., 2011. MorphoJ: an integrated software package for geometric morphometrics. Mol. Ecol. Resour. 11, 353–357, http://dx.doi.org/10.1111/j.1755-0998.2010.02924.x.

Lalis, A., Baylec, M., Cosson, J.F., Makundi, R.H., Machang'u, R.S., Denys, C., 2009. Cranial morphometric and fine scale genetic variability of two adjacent Mastomys natalensis (Rodentia: Muridae) populations. Acta Theriol. 54, 171–181.

Lomolino, M.V., Perault, D.R., 2007. Body size variation of mammals in a fragmented, temperate rainforest. Conserv. Biol. 21, 1059–1069, http://dx.doi.org/10.1111/j.1523-1739.2007.00727.x.

Lomolino, M.V., 1985. Body size of mammals on islands: the Island Rule reexamined. Am. Nat. 125, 310–316, http://dx.doi.org/10.1086/284343.

Méndez, M.A., Soto, E.R., Correa, C., Veloso, A., Vergara, E., Sallaberry, M., Iturra, P., 2004. Morphological and genetic differentiation among Chilean populations of *Bufo spinulosus* (Anura: Bufonidae). Rev. Chil. Hist. Nat. 77, 559–567, http://dx.doi.org/10.4067/S0716-078x2004000300014.

Maestri, R., Luza, A.L., de Barros, L.D., Hartz, S.M., Ferrari, A., de Freitas, T.R.O., Duarte, L.D.S., 2016. Geographical variation of body size in sigmodontine rodents depends on both environment and phylogenetic composition of communities. J. Biogeogr. 43, 1192–1202, http://dx.doi.org/10.1111/jbi.12718.

Maldonado, J.E., Vilà, C., Wayne, R.K., 2001. Tripartite genetic subdivisions in the ornate shrew (*Sorex ornatus*). Mol. Ecol. 10, 127–147, http://dx.doi.org/10.1046/j.1365-294X.2001.01178.x.

Maldonado, J.E., Hertel, F., Vilà, C., 2004. Discordant patterns of morphological variation in genetically divergent population of ornate shrews (*Sorex ornatus*). J. Mamm. 85, 886–896.

Mann, G., 1978. Los pequeños mamíferos de Chile (marsupiales, quirópteros, edentados y roedores). Gayana Zool. 40, 1–342.

Martin, G.M., Flores, D., Teta, P., 2015. Dromiciops gliroides. The IUCN Red List of Threatened Species (e.T6834A22180239. http://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T6834A22180239.en. Downloaded on 20 May 2017).

Martin, G.M., 2010. Geographic distribution and historical occurrence of *Dromiciops gliroides* Thomas (Metatheria: Microbiotheria). 10.1644/.

Mcnab, B.K., 1971. On the Ecological Significance of Bergmann's Rule. Source Ecol. 52155117, 845–854. J. Biogeogr. 30, 331–351.

Meiri, S., Dayan, T., 2003. On the validity of Bergmann's rule.

Mora, J.P., Soto-Gamboa, M., 2011. Legítima dispersión de semillas Ugni molinae Turcz. (Myrtaceae) por monito del monte, *Dromiciops gliroides*. Gayana Bot. 68, 309–312.

Moreno, P.I., Villagrán, C., Marquet, P.A., Marshall, L.G., 1994. Quaternary paleobiogeography of northern and central Chile. Rev. Chil. Hist. Nat. 67, 487–502.

Motokawa, M., Lin, L.K., Lu, K.H., 2004. Geographic variation in cranial features of the polynesian rat *Rattus exulans* (Peale, 1848) (Mammalia: rodentia: muridae). Raffles Bull. Zool. 52, 653–663.

Napolitano, C., Johnson, W.E., Sanderson, J., O'Brien, S.J., Rus Hoelzel, A., Freer, R., Dunstone, N., Ritland, K., Ritland, C.E., Poulin, E., 2014. Phylogeography and population history of *Leopardus guigna*, the smallest American felid. Conserv. Genet. 15, 631–653, http://dx.doi.org/10.1007/s10592-014-0566-3.

Nogueira, M.R., Peracchi, A.L., Monteiro, L.R., 2009. Morphological correlates of bite force and diet in the skull and mandible of phyllostomid bats. Funct. Ecol. 23, 715–723, http://dx.doi.org/10.1111/j.1365-2435.2009.01549.x.

Osgood, W.H., 1943. The mammals of Chile. Field Mus. Nat. Hist. Zool Ser. 30, 1–268.

Palma, R.E., Spotorno, A.E., 1999. Molecular systematics of marsupials based on the rRNA 12S mitochondrial gene: the phylogeny of Didelphimorphia and of the living fossil microbiotheriid *Dromiciops gliroides* Thomas. Mol. Phylogenet. Evol. 13, 525–535.

Palma, R.E., Valladares-Gómez, A., 2015. Family microbiotheriidae. In: Wilson, D.E., Mittermeier, R.A. (Eds.), Handbook of the Mammals of the Word: Monotremes and Marsupials. Lynx Edicions, Barcelona, España, pp. 200–208.

Palma, R.E., Boric-Bargetto, D., Torres-Pérez, F., Hernández, C.E., Yates, T.L., 2012. Glaciation effects on the phylogeographic structure of *Oligoryzomys longicaudatus* (Rodentia: Sigmodontinae) in the southern Andes. PLoS One 7 (3), http://dx.doi.org/10.1371/journal.pone.0032206.

Prevosti, F.J., Turazzini, G.F., Ercoli, M.D., Hingst-Zaher, E., 2012. Mandible shape in marsupial and placental carnivorous mammals: a geometric morphometric comparative study using geometric morphometrics. Zool. J. Linn. Soc., http://dx.doi.org/10.1111/j.1096-3642.2011.00785.x.

Reig, O.A., 1955. Noticia preliminar sobre la presencia de microbiotherinos vivientes en la fauna sudamericana. Invest. Zool. Chil. 2, 121–130.

Rodríguez-Cabal, M.A., Aizen, M.A., Novaro, A.J., 2007. Habitat fragmentation disrupts a plant-disperser mutualism in the temperate forest of South America. Biol. Conserv. 139, 195–202, http://dx.doi.org/10.1016/j.biocon.2007.06.014.

Rohlf, F.J., 1998. On applications of geometric morphometrics to studies of ontogeny and phylogeny. Syst. Biol. 47, 147–158, http://dx.doi.org/10.1080/106351598261094.

JMP v. 13.0.0. Software. SAS Institute Inc., 2016. www.jmp.com.

Salazar, D.A., Fontúrbel, F.E., 2016. Beyond habitat structure: landscape heterogeneity explains the monito del monte (*Dromiciops gliroides*) occurrence and behavior at habitats dominated by exotic trees. Integr. Zool. 11, 413–421, http://dx.doi.org/10.1111/1749-4877.12198.

Savriama, Y., Klingenberg, C., 2011. Beyond bilateral symmetry: geometric morphometric methods for any type of symmetry. BMC Evol. Biol. 11, 280, http://dx.doi.org/10.1186/1471-2148-11-280.

Segovia, R.A., Pérez, M.F., Hinojosa, L.F., 2012. Genetic evidence for glacial refugia of the temperate tree *Eucryphia cordifolia* (Cunoniaceae) in southern South America. Am. J. Bot. 99, 121–129.

Spotorno, A.E., Marin, J.C., Yevenes, M., Walker, L.I., Fernandez-Donoso, R., Pincheira, J., Soledad Berrios, M., Palma, R.E., 1997. Chromosome divergences among American marsupials and the Australian affinities of the American *Dromiciops*. J. Mamm. Evol. 4.

Springer, M.S., Krajewski, C.W., Meredith, R.W., 2009. Marsupials (Metatheria). In: Hedges, S.B., Kumar, S. (Eds.), The Timetree of Life. Oxford University Press, pp. 466–470.

Thorpe, R.S., 1987. Geographic variation: a synthesis of cause, data, pattern and congruence in relation to subspecies, multivariate analysis and phylogenesis. Bolletino di Zool. 54, 3–11, http://dx.doi.org/10.1080/11250008709355549.

Vera-Escalona, I., D'Elía, G., Gouin, N., Fontanella, F.M., Muñoz-Mendoza, C., Sites, J.W., Victoriano, P.F., 2012. Lizards on ice: evidence for multiple refugia in *Liolaemus pictus* (Liolaemidae) during the Last Glacial Maximum in the Southern Andean beech forests. PLoS One 7 (11), http://dx.doi.org/10.1371/journal.pone.0048358.

Vidal, M.A., Veloso, A., Méndez, M.A., 2006. Insular morphological divergence in the lizard *Liolaemus pictus* (Liolaemidae). Amphib-Reptil 27, 103–111.

Villagrán, C., Armesto, J.J., 2005. In: Smith-Ramírez, C., Armesto, J.J., Valdovinos, C. (Eds.), Fitogeografáa histírica de la Cordillera de la Costa de Chile), Historia, biodiversidad y ecologáa de los bosques costeros de Chile. Editorial Universitaria, Santiago, Chile, pp. 99–116.

Villagran, C., 1991. Historia de los bosques templados del sur de Chile durante el Tardiglacial y Postglacial. Rev. Chil. Hist. Nat. 64, 447–460.

Whiley, D.F., 2002–2205. Software. Landmark v. 3.0.0.6. Institute for Data Analysis and Visualization IDAV, Davis, United Stated. http://www.idav.ucdavis.edu/research/EvoMorph.

Willson, M.F., Sabag, C., Figueroa, J., Armesto2, J.J., Caviedes, M., 1996. Seed dispersal by lizards in Chilean rainforest. Rev. Chil. Hist. Nat. 69, 339–342.

Yom-Tov, Y., Geffen, E., 2006. Geographic variation in body size: the effects of ambient temperature and precipitation. Oecologia 148, 213–218, http://dx.doi.org/10.1007/s00442-006-0364-9.

Yom-Tov, Y., Nix, H., 1986. Climatological correlates for body size of five species of Australian mammals. Biol. J. Linn. Soc. 29, 245–262, http://dx.doi.org/10.1111/j.1095-8312.1986.tb00278.x.

Zelditch, M.L., Swiderski, D.L., Sheets, H.D., Fink, W.L., 2004. Geometric Morphometrics for Biologist: a Primer. Elsevier Academic Press, San Diego.