Variation in vertebral number and its morphological implication in *Galaxias platei*

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Variation in the vertebral number of the puyen grande *Galaxias platei* was examined for specimens from 22 localities that span the entire distribution range of the species (from 40° to 55° S). The mean vertebral number (N_{MW}) increases towards high latitudes, *i.e.* Jordan's rule is applicable to this species. Owing to the wide geographic variation of the species, not only in latitude but also in altitude, the most explicative variable for N_{MW} was mean winter air temperature, showing negative dependence. Morphological data suggest that the increment in vertebral number lies in the pre-pelvic region of the trunk and in the caudal region, but not in the segment between pelvic-fin insertion and the origin of the anal fin. As these alterations in body shape have important consequences for hydrodynamics and swimming performance, vertebral number variation in *G. platei* also holds implications for both individual and population fitness.

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Key words: body shape; Galaxiidae; Jordan's rule; landlocked populations; vertebral number; Patagonia; South America.

INTRODUCTION

Vertebral number is a major trait of fish structure, related mainly to swimming, and therefore also to feeding and predator avoidance (Abrahams, 2006). Variation in the number of vertebrae is very common both within and among fish species (Nelson, 2006). The most frequent pattern of variation is associated with latitude, also known as Jordan's rule (McDowall, 2007). Jordan (1892) pointed out that populations at higher latitudes have a higher vertebral number than those at lower latitudes. This biogeographical phenomenon has been attributed to the effect of temperature on developmental processes, either as a phenotypic response to environment (Lindsey, 1988; Brooks & Johnston, 1994) or to the genetic control of vertebral number as a result of adaptive processes (Yamahira *et al.*, 2006; Yamahira & Nishida, 2009) or both simultaneously (McDowall, 2003*a*, *b*).

McDowall (2003*a*) carried out an exhaustive review of the causes and consequences of variation in vertebral number in fishes. In particular, McDowall (2003*b*)

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examined in detail a number of cases from the family Galaxiidae. One of the larger members of this family is the puyen grande *Galaxias platei* Steindachner 1898, a benthic freshwater fish widely distributed throughout the Andean lakes of southern Chile and Argentina (McDowall, 1971; Cussac *et al.*, 2004). McDowall (2003*b*) noted that *G. platei* has the highest within-species vertebral range (50–62) among landlocked species of the Galaxiidae. There could be several reasons for this variation, such as large body size, egg size, broad latitudinal distribution and wide thermal range (McDowall, 2003*a*). This species has witnessed the successive consequences of the Andean uplift and the Pleistocene glaciations (Ruzzante *et al.*, 2008; Zemlak *et al.*, 2008; Fraser *et al.*, 2012). The wide latitudinal range in the distribution of *G. platei* is attributed to specific characteristics such as retinal adaptation for vision in the dark, presence of a cephalic lateral line, gill protection against abrasion, strong resistance to low oxygen availability, low metabolic rate and a strong conformational ability for aerobic metabolism (Cussac *et al.*, 2004). A recent phylogenetic study by Burridge *et al.* (2012) recovered *G. platei* as a sister group of the genus *Neochanna*, shedding new light on the significance of the adaptations that allowed the species to endure glacial periods (Shuter & Post, 1990; Ruzzante *et al.*, 2008; Zemlak *et al.*, 2008).

Milano *et al.* (2002, 2006) have pointed out the strong relationship that exists between latitude and morphology within *G. platei*. This morphological variation was found in head dimensions, caudal morphology and fin length. Moreover, this finding was associated with river-basin origin, latitude, predation risk and diet (Milano *et al.*, 2006). The main goals of the present work were firstly to investigate variation in the vertebral number of *G. platei* with respect to its geographic (latitudinal and altitudinal) distribution throughout Patagonia, and secondly, as morphology is strongly related to fitness (Aguirre, 2009; Langerhans, 2009; Leinonen *et al.*, 2011), to explore possible relationships between morphological traits and vertebral variation.

MATERIALS AND METHODS

VERTEBRAL COUNTS

Vertebral counts of *G. platei* were obtained from the literature ($n = 147$; McDowall, 1971) and from 181 individuals captured in several sampling programmes carried out between 1996 and 2009 (Milano *et al.*, 2002, 2006), spanning the entire distribution range of the species (from 40° to 55° S; Fig. 1). Fish were collected from both deep and shallow Andean lakes in Patagonia (Table I). In deep lakes, sampling was performed using gillnets (15, 20, 30, 50, 60 and 70 mm bar mesh size, each 10 m long and 2 m high) placed at depths of 2, 10, 20, 30 and 50 m, resting at the lake bottom. Gillnets were set parallel to the coast and left in place overnight. In shallow lakes, fish were collected with a seine (25 m long, 1·5 m high and 5 mm mesh size) or by means of a 24 V d.c. backpack electrofishing unit (model 12-B Smith-Root, Inc.; www.smith-root.com). Individuals were killed by an overdose of benzocaine and fixed with 4% formaldehyde. In the laboratory, fish were dissected under a stereoscopic microscope (Wild M3C Leica AG Corp.; www.leica.com) and the vertebrae were counted, excluding all modified caudal centra, following the criteria of McDowall (1971). As the data taken from McDowall (1971) provided only total vertebral number, a more detailed analysis per body region could not be performed, *i.e.* caudal *v*. abdominal regions (Yamahira & Nishida, 2009).
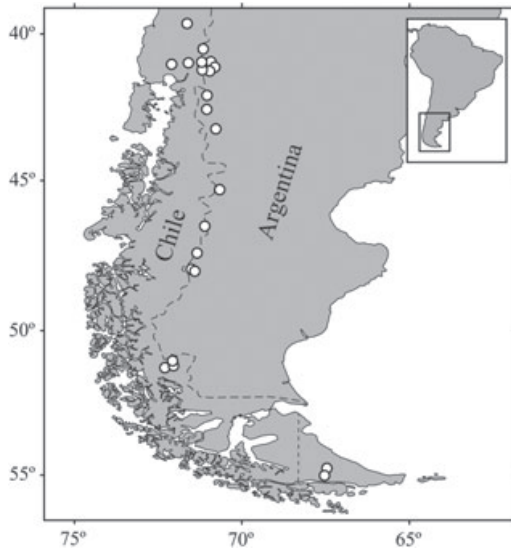
Fig. 1. Populations (○) of *Galaxias platei* in Patagonia, South America (inset box), included in the vertebral number analysis. ----, border between Chile and Argentina.

MORPHOLOGICAL DATA

To explore the relationship between vertebral number and body shape, 27 morphological characteristics were considered (Fig. 2), measured in 158 adult specimens of *G. platei*, following McDowall (1971). Measurements were taken using a digital calliper.

PHYLOGEOGRAPHIC INFORMATION

Zemlak *et al.* (2008), working with Chilean and Argentinean individuals of *G. Platei*, the latter being the same individuals as used by Milano *et al.* (2002, 2006), observed two main clades, corresponding to east and west sides of the Andes, according to the ancient (late Pleistocene-early Holocene; del Valle *et al.*, 2007) ocean drainage of each lake (Pacific and Atlantic Oceans). In order to consider phylogeographic relationships as suggested by these authors, the ancient ocean drainage of each sampling site (Table I) was included in the analyses.

STATISTICAL ANALYSIS

Firstly, the relationship of mean vertebral number (N_{MW}) with latitude, longitude and altitude was evaluated by means of a multiple linear regression to explore geographical patterns, and specifically to test whether Jordan's rule is valid for *G. platei*. Following this, simple and multiple linear regressions were performed to find the dependence of N_{MW} on mean austral annual air temperature (MAAT), mean austral summer air temperature (MSAT) and mean austral winter air temperature (MWAT). Mean air temperature data for the period 1950–2000 were extracted from the WorldClim global climate database (www.worldclim.org). The spatial resolution of these data is 30 arc s, equivalent to $1 \, km^2$ spatial resolution (Hijmans *et al.*, 2005). MAAT was considered as an estimation of the temperature of a lake's hypolimnion (Quirós & Drago, 1985; Quirós, 1991) and MSAT as an estimation of the surface water temperature during summer (Livingstone & Lotter, 1998). MWAT was considered in the analysis because winter is the most frequent spawning season for *G. platei* (Cussac *et al.*, 2004). Finally, a stepwise regression was performed to determine the most explicative variables. Variables were entered or removed from the model depending on the significance (probability) of the F value. These probabilities were 0·05 and 0·1 for entering and removing the

TABLE I. Variation in vertebral number in *Galaxias platei*. Lakes are ordered from north to south. Current and ancient drainages are also indicated as Pacific (P) or Atlantic (A)

Lake	Drainage		Lati-tude (°S)	Longi-tude (°W)	Altitude (m a.s.l.)	Number of vertebrae				
	Ancient	Current				Mean	s.d.	Mini-mum	Maxi-mum	Indi-viduals
Riñihue*	P	P	39·8369	72·3028	106	54·56	0·70	53	56	18
Espejo	A	A	40·6253	71·7558	786	58·00	1·00	57	59	3
Nahuel Huapi*	A	A	41·0633	71·7994	778	57·88	1·13	56	59	8
Moreno	A	A	41·0728	71·5058	768	56·50	1·31	54	58	12
Todos los Santos*	P	P	41·0967	72·2478	189	53·00	0·59	52	54	18
Llanquihue*	P	P	41·1333	72·8000	52	53·10	1·12	50	55	49
Gutierrez	A	A	41·2097	71·4131	807	57·50	1·00	57	59	4
Fonck	A	P	41·3125	71·7825	776	57·60	1·14	56	59	5
Mascardi	A	P	41·3500	71·5450	806	57·60	0·55	57	58	5
Puelo	A	P	42·1536	71·6389	191	54·42	1·36	51	59	26
Rivadavia	A	P	42·6092	71·6647	520	58·50	0·76	57	60	14
Rosario	A	P	43·2586	71·3464	662	57·29	0·76	56	58	7
Coyte	A	A	45·2639	71·2164	793	58·00	0·85	56	59	15
Buenos Aires	P	P	46·4505	71·7050	201	57·90	0·72	56	59	20
Pueyrredon	A	P	47·2936	71·9700	151	56·69	1·14	54	59	16
Belgrano	A	P	47·8575	72·1347	809	58·33	1·18	56	60	15
Mie	A	A	47·9053	72·0447	873	56·74	1·77	52	59	27
Azul*	P	P	50·8819	72·7744	223	57·67	0·71	57	59	9
Figueroa*	P	P	50·9753	72·7478	66	56·63	1·09	55	59	16
Pehoe*	P	P	51·0939	73·0331	33	58·56	1·01	57	60	9
Yehuin	A	P	54·4128	67·7208	42	58·00	1·07	56	60	22
Escondido	A	P	54·6431	67·7928	117	58·20	0·63	57	59	10

m a.s.l., m above sea level.

*Data from McDowall (1971).

variables (Norusis, 1986). The significance of each regression coefficient was tested using ANOVA. To test N_{MW} differences between Pacific and Atlantic populations (both ancient and current drainages), a non-parametric ANOVA (Kruskal–Wallis) was applied, as a result of the failure of the parametric assumptions (normality and homoscedasticity) of the data.

Morphological measurements were $\log_{10}$ transformed and adjusted to a common fish size by regression of the $\log_{10}$ measurement against $\log_{10} L_S$ (Milano *et al.*, 2006). Regression residuals were used in a principal component analysis (PCA) in order to obtain measurements of the total morphological variation that could be related to the vertebral number. Each principal component (PC) was then correlated with vertebral number using Spearman's coefficient ρ (Norusis, 1986) to quantify the percentage of the total morphological variation related to the vertebral number variation. Finally, the relationship between the vertebral number and each morphological characteristic (regression residual) was again evaluated applying the Spearman's ρ.

RESULTS

GEOGRAPHIC AND THERMAL PATTERNS

Using a geographical approach, the multiple linear regression indicates that the two geographical variables that explain N_{MW} in *G. platei* are latitude and altitude (ANOVA, $F_{2,19} = 13·48$, $P < 0·001$; Fig. 3 and Table II), but not longitude (ANOVA,
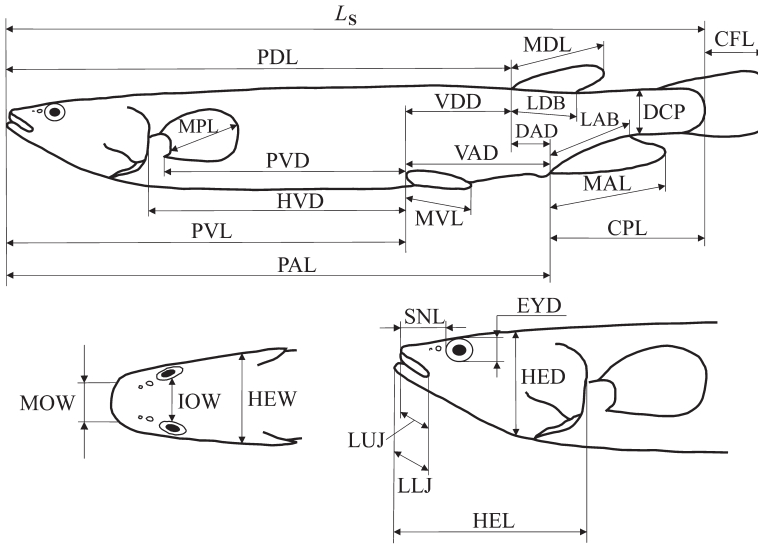
FIG. 2. Dimensions measured in *Galaxias platei* for the morphological analysis: CFL, caudal-fin length; CPL, caudal-peduncle length; DAD, dorsal-anal-fin distance; DCP, depth of the caudal peduncle; EYD, eye diameter; HED, head depth; HEL, head length; HEW, head width; HVD, head-pelvic-fin distance; IOW, interorbital width; LAB, length of the anal-fin base; LDB, length of the dorsal-fin base; LLJ, length of the lower jaw; L_S, standard length; LUJ, length of the upper jaw; MAL, maximum anal-fin length; MDL, maximum dorsal fin length; MOW, mouth width; MPL, maximum pectoral-fin length; MVL, maximum pelvic-fin length; PAL, pre-anal length; PDL, pre-dorsal length; PVD, pectoral-pelvic-fin distance; PVL, pre-pelvic length; SNL, snout length; VAD, pelvic-anal-fin distance; VDD, pelvic-dorsal-fin distance. MOW was measured ventrally as an intercommisural width.

$P > 0.05$). The close relationship between temperature data and these geographical variables must be noted (latitude and altitude are included as independent variables): MAAT (ANOVA, $F_{2,19} = 204.81$, $P < 0.001$), MWAT (ANOVA, $F_{2,19} = 172.82$, $P < 0.001$) and MSAT (ANOVA, $F_{2,19} = 115.43$, $P < 0.001$).

Simple linear regressions demonstrate significant dependence of N_{MW} with MWAT (ANOVA, $F_{1,20} = 30.91$, $P < 0.001$), MAAT (ANOVA, $F_{1,20} = 18.45$, $P < 0.001$) and MSAT (ANOVA, $F_{1,20} = 12.84$, $P < 0.05$) as independent variables (Table II). On the other hand, no differences were found between N_{MW} clustering populations in relation to either ancient drainage (Kruskal–Wallis, $n = 22$, $P > 0.05$) or present drainage (Kruskal–Wallis, $n = 22$, $P > 0.05$).

Finally, taking all variables into account together using a stepwise multiple linear regression to summarize these relationships, only one (negative) dependence was found, between N_{MW} of *G. platei* and MWAT (ANOVA, $F_{1,20} = 30.91$, $P < 0.001$; Fig. 4 and Table II).

BODY SHAPE

The PCA was obtained from 158 individuals using regression residuals of 26 morphological variables. The first six components account for 78.27% of the variation (Table III). Two of these components are significantly correlated with vertebral number: PC 4 ($\rho = -0.201$, $P < 0.05$) and PC 5 ($\rho = -0.222$, $P < 0.05$). A low
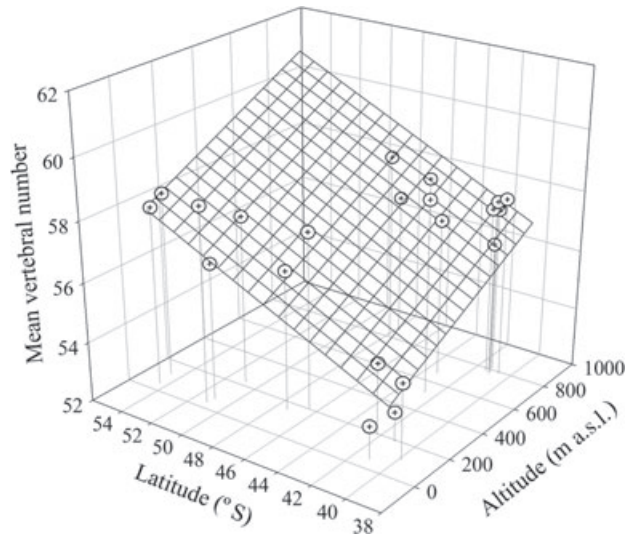
FIG. 3. Relationship between mean vertebral number (N_{MW}) of *Galaxias platei*, latitude (L) and altitude (A). Reticulated plane represents a multiple linear regression with the most explicative geographical variables, $N_{MW} = 43 \cdot 575 + 0 \cdot 261L + 0 \cdot 004A$ ($n = 22$, $r^2 = 0 \cdot 59$).

percentage of the total morphological variation is related to the vertebral number (PC 4 and PC 5 accounted for 13·32%). Seven morphological variables (regression residuals) are related to vertebral number. Pre-pelvic length (PVL) ($\rho = 0 \cdot 199$, $P < 0 \cdot 05$), caudal-fin length (CPL) ($\rho = 0 \cdot 185$, $P < 0 \cdot 05$), head-pelvic-fin distance (HVD) ($\rho = 0 \cdot 167$, $P < 0 \cdot 05$), maximum pectoral-fin length (MPL) ($\rho = 0 \cdot 159$, $P < 0 \cdot 05$) and pre-dorsal length (PDL) ($\rho = 0 \cdot 159$, $P < 0 \cdot 05$) correlate positively with vertebral number, whereas pelvic-anal-fins distance (VAD) ($\rho = -0 \cdot 159$, $P < 0 \cdot 05$) and dorsal-anal-fins distance (DAD) ($\rho = -0 \cdot 183$, $P < 0 \cdot 05$) correlate in a negative manner (Fig. 5).

DISCUSSION

The geographic analysis of vertebral number indicates that Jordan's rule is valid for *G. platei*. The N_{MW} shows a clear direct relationship with latitude, but altitude also contributes to explaining N_{MW} variation among populations (Fig. 3). This represents the first evidence of the relationship between vertebral number and altitude, as a proxy of temperature conditions, for a species of the Galaxiidae. McDowall (2003*a*) suggested this possibility but failed to find this relationship for landlocked galaxiid species from Australia or New Zealand (*e.g. Galaxias brevipinnis* Günther 1866). Both geographic variables (*i.e.* latitude and altitude) prove to be closely related to the thermal gradient. This is in agreement with Baumann *et al.* (2012), who have provided evidence supporting the hypothesis that temperature gradients are the ultimate basis for the Jordan's rule.

Regression analyses performed on N_{MW} and environmental variables show negative dependence of vertebral number on temperature in *G. platei*, in particular with

TABLE II. Relationship between mean vertebral number (N_{MW}) of *Galaxias platei*, mean austral annual air temperature (MAAT), mean austral summer air temperature (MSAT), mean austral winter air temperature (MWAT), latitude (L, ° S) and altitude (A, m a.s.l.). Dependent and independent variables, regression coefficients, d.f., F value, determination coefficient (r^2) and P value of ANOVA are indicated for each simple or multiple linear regression

Dependent variable	Regression coefficients, 95% C.I. (lower–upper) and independent variables	F	d.f.	r^2	P
N_{MW}	43·575 (37·709–49·441) 0·261 (0·140–0·382) L 0·004 (0·002–0·005) A	13·48	2; 19	0·59	<0·001
N_{MW}	58·740 (57·914–59·567) −0·616 (−0·848−−0·385) MWAT	30·91	1; 20	0·61	<0·001
N_{MW}	61·797 (59·375–64·219) −0·587 (−0·872−−0·302) MAAT	18·45	1; 20	0·48	<0·001
N_{MW}	63·067 (59·451–66·683) −0·476 (−0·754−−0·199) MSAT	12·84	1; 20	0·39	<0·05

mean winter temperature (Fig. 4). As winter is the most frequent spawning season for *G. platei* (Cussac *et al.*, 2004), the dependence of vertebral number to temperature during embryonic and larval development (McDowall, 2003*a*) is important. In this sense, Hubbs (1922) suggested that the number of vertebrae in a fish depends on the duration of the embryogenesis period, during which the vertebrae primordia are laid down. Temperature is related to both growth and development. Both processes, however, do not necessarily change at the same rate; consequently, a change in temperature may decouple growth and differentiation resulting in, for example, higher vertebral number under lower developmental temperature. This variation has been modelled by Lindsey & Arnason (1981) in their Atroposic model.

Morphological results indicate that almost all measurements related to the vertebral number are parallel to the body axis, with the exception of MPL. Individuals with higher vertebral counts have longer pre-pelvic, pre-dorsal and head-pelvic lengths, longer caudal peduncles, shorter pelvic-anal and dorsal-anal-fin distance and longer pectoral fins. This suggests that the increment in the vertebral number would be in the pre-pelvic region of the trunk and in the caudal region but not in the segment between the pelvic-fin insertion and the origin of the anal fin (Fig. 2). Although these measurements represent a low percentage of the total morphological variation (<14%), all these traits have strong implications in hydrodynamics and swimming performance (Videler, 1996). The hydrodynamic requirements for swimming performance of larvae change throughout the latitudinal gradient, due to an increase in water viscosity with low temperature (Hunt von Herbing, 2002). At high latitude,

TABLE III. Principal component analysis (PCA) based on 26 morphological variables recorded in 158 individuals of *Galaxias platei*. The highest contributions of variables for each PC are indicated in bold

| Morphological trait	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Head length (HEL)	**0·92**	0·05	0·16	0·07	0·08	−0·15
Mouth width (MOW)	**0·90**	−0·08	0·16	−0·02	0·05	−0·02
Length of the upper jaw (LUJ)	**0·88**	−0·06	0·15	0·12	0·25	−0·06
Head depth (HED)	**0·85**	−0·06	0·25	−0·01	0·04	0·02
Depth of the caudal peduncle (DCP)	**0·83**	0·06	−0·13	0·11	−0·13	0·04
Head width (HEW)	**0·82**	−0·07	0·32	−0·08	−0·06	−0·07
Snout length (SNL)	**0·81**	−0·10	0·13	−0·03	0·22	−0·13
Interorbital width (IOW)	**0·80**	−0·16	0·31	0·08	0·03	0·02
Length of the lower jaw (LLJ)	**0·80**	−0·02	0·09	0·19	0·32	0·10
Length of the anal-fin base (LAB)	**0·75**	0·02	−0·08	−0·15	−0·31	0·25
Maximum anal-fin length (MAL)	**0·63**	0·41	−0·36	0·08	−0·37	−0·04
Pre-anal length (PAL)	0·21	**0·83**	−0·01	−0·25	0·33	0·06
Pre-pelvic length (PVL)	0·08	**0·82**	0·42	−0·29	−0·03	0·05
Maximum pelvic-fin length (MVL)	0·09	**0·74**	−0·04	0·27	−0·26	−0·32
Head-pelvic-fin distance (HVD)	−0·56	**0·65**	0·24	−0·31	−0·07	0·15
Pre-dorsal length (PDL)	0·43	**0·64**	−0·25	−0·52	0·19	−0·03
Maximum pectoral-fin length (MPL)	0·38	**0·60**	−0·31	0·32	−0·26	−0·28
Pectoral-pelvic-fin distance (PVD)	−0·52	**0·55**	0·29	−0·27	0·03	0·03
Maximum dorsal-fin length (MDL)	0·45	**0·53**	−0·19	0·32	−0·11	0·15
Caudal-fin length (CPL)	−0·34	**0·43**	−0·04	0·40	0·19	0·09
Pelvic-dorsal-fin distance (VDD)	0·41	−0·11	**−0·73**	−0·30	0·24	−0·10
Eye diameter (EYD)	0·01	0·07	**0·55**	−0·12	0·18	−0·40
Caudal-peduncle length (CPL)	0·43	−0·22	−0·03	**−0·57**	−0·09	0·35
Dorsal-anal-fin distance (DAD)	−0·41	0·19	0·37	**0·52**	0·20	0·14
Pelvic-anal-fin distance (VAD)	−0·10	0·25	−0·44	0·22	**0·71**	0·15
Length of the dorsal-fin base (LDB)	0·35	0·21	0·16	0·33	−0·13	**0·60**
Eigenvalues	9·31	4·30	2·22	2·00	1·46	1·06
Variance explained (%)	35·81	16·54	8·52	7·69	5·63	4·07
Total variance explained (%)	35·81	52·36	60·88	68·57	74·2	78·27
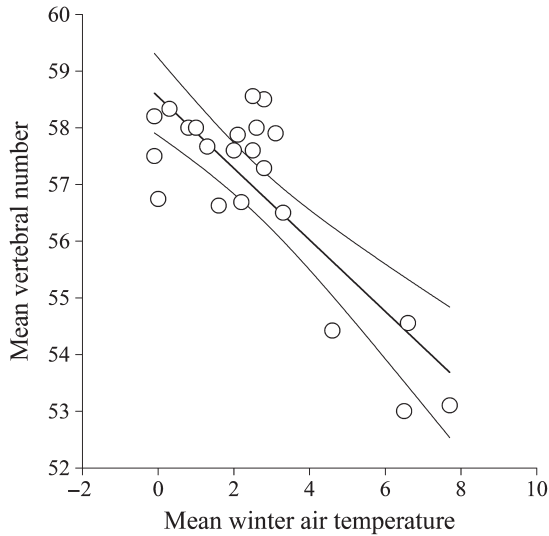
FIG. 4. Relationship between mean vertebral number of *Galaxias platei* and mean winter air temperature at each location (data from www.worldclim.org). Linear regression ($y = 58\cdot740 - 0\cdot616x$; $r^2 = 0\cdot607$) and 95% c.i. are indicated.

water viscosity is higher and, consequently, more vertebrae could confer a swimming advantage during the larval period.

The adaptive value of both vertebral number (Swain, 1992) and caudal-peduncle length (Langerhans & Makowicz, 2009) has mainly been related to skills employed in avoiding predators. A longer caudal peduncle enhances survival chances owing to the improvement in swimming performance, *e.g.* faster burst swimming (Langerhans *et al.*, 2004). In general, a better swimming performance could also enhance foraging activity. Moreover, McDowall (2003*b*) pointed out that the critical adaptive feature of vertebral number in landlocked species of the Galaxiidae is related to optimal flexibility for performing anguilliform swimming during larval life. In this context, *G. platei* is landlocked and has a lacustrine life history where the larvae spend up to 6 months in the limnetic zone of the lakes, and juveniles and adults live in benthic, usually deep, habitats (Barriga *et al.*, 2002; Cussac *et al.*, 2004). Recently, both the slenderness of the body and the length of the caudal peduncle were found to be related to food availability and predation risk in larval *Galaxias maculatus* (Jenyns 1842) (Barriga *et al.*, 2012). This among-population phenotypic variation during the limnetic period is also strongly related to swimming performance and has implications in two areas. One is the intraspecific competition for food, where those larvae with the skill for finding zooplankton patches quickly would have an advantage, thus incrementing their food intake. The second is the ability to escape from an encounter with a predator (Barriga *et al.*, 2012).

Yamahira *et al.* (2006) have pointed out another explanation for selection of vertebral number in temperate fishes. They suggest that the size-dependent overwinter mortality could be another selective agent. It is known that overwinter mortality is higher in smaller individuals (Schultz & Conover, 1997, 1999; Hurst & Conover, 2002). The main requirement is that temperate fishes must reach a minimum size
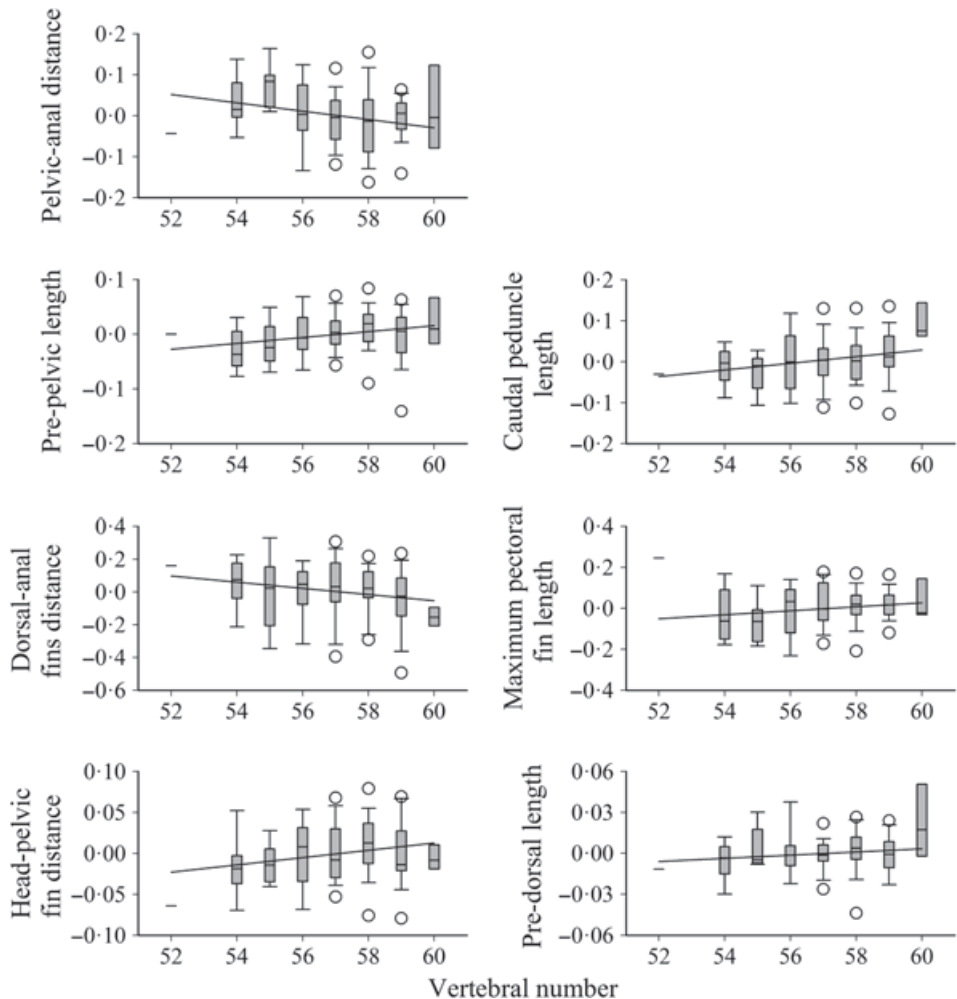
FIG. 5. Relationship between seven morphological measurements (regression residuals): (a) pelvic-anal-fin distance, (b) pre-pelvic length, (c) dorsal-anal-fin distance, (d) head-pelvic-fin distance, (e) caudal-peduncle length, (f) maximum pectoral-fin length and (g) pre-dorsal length and vertebral number of *Galaxias platei* ($n = 158$). Median, quartiles and data outside of 10th and 90th percentiles for each vertebral number are indicated. ____, correlation tendencies (Spearman, $P < 0.05$).

before their first winter in order to minimize specific metabolic rate, maximize stored energy and in consequence be able to endure periods of resource scarcity (Shuter & Post, 1990). Thus, thermal constraints towards high latitudes would promote populations with larger larvae (*i.e.* with higher vertebral number), and with better chances of surviving. Owing to the tendency for N_{MW} to be higher in populations with greater maximum body lengths (*i.e.* pleomerism; McDowall, 2003*b*), the latter will be positively selected at high latitudes.

Zemlak *et al.* (2008) have pointed out that historic drainage patterns explain geographical patterns of genetic variation better than current drainage patterns. No differences between vertebral number of Atlantic and Pacific populations were found,

whether clustered by current or ancient drainages. Hence, the number of vertebrae in *G. platei* does not seem to be related to the phylogeography of the species. In order to test both genetic and environmental effects on vertebral number, further experiments similar to those carried out with the silverside *Menidia* spp. (Billerbeck *et al.*, 1997; Yamahira *et al.*, 2006; Baumann *et al.*, 2012) will be required. At this point, however, it should be noted that performing such experiments becomes extremely difficult in *G. platei*, partly owing to the small number of individuals per lake captured for artificial spawning, but mainly because of the high mortality rate usually associated with the early ontogeny of this species (Ortubay & Wegrzyn, 1991; Barriga *et al.*, 2002).

In conclusion, Jordan's rule does apply to *G. platei*. The among-population vertebral variation in this species can be explained by the mean winter air temperature because of the wide geographic variation of the populations studied, not only regarding latitude but also altitude. The vertebral variation is also related to some morphological traits that are directly involved in hydrodynamics and swimming performance. Hence, the vertebral number variation in *G. platei* has implications in both individual and population fitness. Further studies are required to elucidate the influence of genetic and environmental factors on the vertebral variation of this species.

References

Abrahams, M. (2006). The physiology of antipredator behaviour: what you do with what you've got. In *Behaviour and Physiology of Fish* (Sloman, K. A., Balshine, S. & Wilson, R. W., eds), pp. 79–108. London: Elsevier Academic Press.

Aguirre, W. E. (2009). Microgeographical diversification of threespine stickleback: body shape-habitat correlations in a small, ecologically diverse Alaskan drainage. *Biological Journal of the Linnean Society* **98**, 139–151.

Barriga, J. P., Battini, M. A., Macchi, P., Milano, D. & Cussac, V. E. (2002). Spatial and temporal distribution of landlocked *Galaxias maculatus* and *Galaxias platei* (Pisces, Galaxiidae) in a lake in the South American Andes. *New Zealand Journal of Marine and Freshwater Research* **36**, 345–359.

Barriga, J., Battini, M., Garcia-Asorey, M., Carrea, C., Macchi, P. & Cussac, V. (2012). Intraspecific variation in diet, growth, and morphology of landlocked *Galaxias maculatus* during its larval period: the role of food availability and predation risk. *Hydrobiologia* **679**, 27–41.

Baumann, H., Rosales-Casián, J. A. & Conover, D. O. (2012). Contrasting latitudinal variations in vertebral number and sex determination in Pacific versus Atlantic silverside fishes. *Copeia* **2012**, 341–350.

Billerbeck, J. M., Ortí, G. & Conover, D. O. (1997). Latitudinal variation in vertebral number has a genetic basis in the Atlantic silverside, *Menidia menidia*. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 1796–1801.

Brooks, S. & Johnston, I. A. (1994). Temperature and somitogenesis in embryos of the plaice (*Pleuronectes platessa*). *Journal of Fish Biology* **45**, 699–702.

Burridge, C. P., McDowall, R. M., Craw, D., Wilson, M. V. H. & Waters, J. M. (2012). Marine dispersal as a pre-requisite for Gondwanan vicariance among elements of the galaxiid fish fauna. *Journal of Biogeography* **39**, 306–321.

Cussac, V., Ortubay, S., Iglesias, G., Milano, D., Lattuca, M., Barriga, J., Battini, M. & Gross, M. (2004). The distribution of South American galaxiid fishes: the role of biological traits and post glacial history. *Journal of Biogeography* **31**, 103–122.

Fraser, C. I., Nikula, R., Ruzzante, D. E. & Waters, J. M. (2012). Poleward bound: biological impacts of Southern Hemisphere glaciation. *Trends in Ecology and Evolution* **27**, 462–471. doi: 10.1016/j.tree.2012.04.011

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25**, 1965–1978.

Hubbs, C. L. (1922). Variations in the number of vertebrae and other meristic characters of fishes correlated with the temperature of water during development. *American Naturalist* **56**, 360–372.

Hunt von Herbing, I. (2002). Effects of temperature on larval fish swimming performance: the importance of physics to physiology. *Journal of Fish Biology* **61**, 865–876.

Hurst, T. P. & Conover, D. O. (2002). Interaction of temperature and salinity in determining mortality and optimal overwintering habitats of young-of-the-year Hudson River striped bass (*Morone saxatilis*). *Canadian Journal of Fisheries and Aquatic Sciences* **59**, 787–795.

Jordan, D. S. (1892). Relations of temperature to vertebrae among fishes. *Proceedings of the United States National Museum* **1891**, 107–120.

Langerhans, R. B. (2009). Morphology, performance, fitness: functional insight into a post-Pleistocene radiation of mosquitofish. *Biology Letters* **5**, 488–491. doi: 10.1098/rsbl.2009.0179

Langerhans, R. B. & Makowicz, A. M. (2009). Shared and unique features of morphological differentiation between predator regimes in *Gambusia caymanensis*. *Journal of Evolutionary Biology* **22**, 2231–2242.

Langerhans, R. B., Layman, C. A., Shokrollahi, A. M. & DeWitt, T. J. (2004). Predator-driven phenotypic diversification in *Gambusia affinis*. *Evolution* **58**, 2305–2318.

Leinonen, T., Herczeg, G., Cano, J. M. & Merilä, J. (2011). Predation-imposed selection on threespine stickleback (*Gasterosteus aculeatus*) morphology a test of the refuge use hypothesis. *Evolution* **65**, 2916–2926.

Lindsey, C. C. (1988). Factors controlling meristic variation. In *Fish Physiology*, Vol. 11 (Hoar, W. S. & Randall, D., eds), pp. 197–274. London: Academic Press.

Lindsey, C. C. & Arnason, A. N. (1981). A model for responses of vertebral numbers in fish to environmental influences during development. *Canadian Journal of Fisheries and Aquatic Sciences* **38**, 334–347.

Livingstone, D. M. & Lotter, A. F. (1998). The relationship between air and water temperatures in lakes of the Swiss Plateau a case study with palaeolimnological implications. *Journal of Paleolimnology* **19**, 181–198.

McDowall, R. M. (1971). The galaxiid fishes of South America. *Zoological Journal of the Linnean Society* **50**, 33–73.

McDowall, R. M. (2003a). Variation in vertebral number in galaxiid fishes (Teleostei: Galaxiidae) a legacy of life history, latitude and length. *Environmental Biology of Fishes* **66**, 361–381.

McDowall, R. M. (2003b). Variation in vertebral number in galaxiid fishes, how fishes swim and a possible reason for pleomerism. *Reviews in Fish Biology and Fisheries* **13**, 247–263.

McDowall, R. M. (2007). Jordan's and other ecogeographical rules, and the vertebral number in fishes. *Journal of Biogeography* **35**, 501–508.

Milano, D., Cussac, V. E., Macchi, P. J., Ruzzante, D. E., Alonso, M. F., Vigliano, P. H. & Denegri, M. A. (2002). Predator associated morphology in *Galaxias platei* in Patagonian lakes. *Journal of Fish Biology* **61**, 138–156.

Milano, D., Ruzzante, D. E., Cussac, V. E., Macchi, P. J., Ferriz, R. A., Barriga, J. P., Aigo, J. C., Lattuca, M. E. & Walde, S. J. (2006). Latitudinal and ecological correlates of morphological variation in *Galaxias platei* (Pisces, Galaxiidae) in Patagonia. *Biological Journal of the Linnean Society* **87**, 69–82.

Nelson, J. S. (2006). *Fishes of the World*, 4th edn. New York, NY: Wiley.

Norusis, M. J. (1986). *SPSS/PC+Advanced Statistics*. Chicago, IL: SPSS Inc.

Ortubay, S. & Wegrzyn, D. R. (1991). Fecundación artificial y desarrollo embrionario de *Galaxias platei* Steindachner (Salmoniformes, Galaxiidae). *Medio Ambiente* **11**, 84–89.

Quirós, R. (1991). Factores que afectan la distribución de salmónidos en Argentina. *COPESCAL, FAO Technical Document* **9**, 163–173.

Quirós, R. & Drago, E. (1985). Relaciones entre variables físicas, morfométricas y climáticas en lagos patagónicos. *Revista de la Asociación de Ciencias Naturales del Litoral* **16**, 181–199.

Ruzzante, D. E., Walde, S. J., Gosse, J. C., Cussac, V. E., Habit, E., Zemlak, T. S. & Adams, E. D. M. (2008). Climate control on ancestral population dynamics: insight from Patagonian fish phylogeography. *Molecular Ecology* **17**, 2234–2244.

Schultz, E. T. & Conover, D. O. (1997). Latitudinal differences in somatic energy storage: adaptive responses to seasonality in an estuarine fish (Atherinidae: *Menidia menidia*). *Oecologia* **109**, 516–529.

Schultz, E. T. & Conover, D. O. (1999). The allometry of energy reserve depletion: test of a mechanism for size-dependent winter mortality. *Oecologia* **119**, 474–483.

Shuter, B. J. & Post, J. R. (1990). Climate, population viability, and the zoogeography of temperate fishes. *Transactions of the American Fisheries Society* **119**, 314–336.

Swain, D. P. (1992). The functional basis of natural selection for vertebral traits of larvae in the stickleback *Gasterosteus aculeatus*. *Evolution* **46**, 987–997.

del Valle, R. A., Tatur, A. & Rinaldi, C. A. (2007). Cambios en lagos y circulación fluvial vinculados al calentamiento climático del Pleistoceno Tardío-Holoceno Temprano en Patagonia e Isla 25 de Mayo, Islas Shetland Del Sur, Antártida. *Revista de la Asociación Geológica Argentina* **62**, 618–626.

Videler, J. J. (1996). *Fish Swimming*. London: Chapman & Hall.

Yamahira, K. & Nishida, T. (2009). Latitudinal variation in axial patterning of the medaka (Actinopterygii: Adrianichthyidae): Jordan's rule is substantiated by genetic variation in abdominal vertebral number. *Biological Journal of the Linnean Society* **96**, 856–866.

Yamahira, K., Lankford, T. E. & Conover, D. O. (2006). Intra and interspecific latitudinal variation in vertebral number of *Menidia* spp. (Teleostei: Atherinopsidae). *Copeia* **2006**, 431–436.

Zemlak, T. S., Habit, E. M., Walde, S. J., Battini, M. A., Adams, E. D. M. & Ruzzante, D. E. (2008). Across the southern Andes on fin: glacial refugia, drainage reversals and a secondary contact zone revealed by the phylogeographical signal of *Galaxias platei* in Patagonia. *Molecular Ecology* **17**, 5049–5061.