Cephalic morphological variation in freshwater silversides *Odontesthes hatcheri* and *Odontesthes bonariensis* in Patagonia: introgression and ecological relationships

S. Crichigno*, C. Conte-Grand, M. Battini and V. Cussac

Instituto de Investigaciones en Biodiversidad y Medioambiente (INIBIOMA), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) - Universidad Nacional del Comahue, San Carlos de Bariloche, Río Negro 8400, Argentina

Based on geometric morphometrics and discriminant analysis, the percentage of silverside *Odontesthes hatcheri* and *Odontesthes bonariensis* individuals identified by a taxonomic key and misclassified by discriminant analysis was obtained and a negative correlation between the percentage of misclassified individuals of *O. hatcheri* and the distance to the nearest hatchery stocking silversides was found, suggesting a genetic introgression. Morphological analyses between species, between populations and within populations pointed to the same anatomical structures, suggesting a nested variation related to environmental cues such as availability of littoral shelter. The dependence between the cephalic morphology of *O. hatcheri* and body size would be in agreement with the trophic niche shifts of the species. Introgression adds a new threat to the already observed decline of populations of *O. hatcheri* and suggests that this species deserves particular consideration in terms of conservation guidelines.

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Key words: Atherinopsidae; fish stocking; geometric morphometrics; head shape; hybrids.

INTRODUCTION

The present status of freshwater silversides in Patagonia (Pascual *et al.*, 2007) involves an ensemble of geological and historical causes: the temperature decrease linked to the establishment of the Antarctic Circumpolar Current (Cione *et al.*, 2007), the consequent decline of the Neotropical fish fauna (Cione & Báez, 2007), the succession of Pleistocene glacial events leading to extirpation and re-colonization (Ruzzante *et al.*, 2008), fish species translocation, exotic fish introduction (Aigo *et al.*, 2008) and global warming (Cussac *et al.*, 2009).

It is not surprising that colonization of postglacial lakes (Ruzzante *et al.*, 2006, 2008) has led to conspicuous oropharyngeal morphological variation in other Patagonian fishes in relation to the usage of different feeding resources (Cussac *et al.*, 1998; Ruzzante *et al.*, 1998, 2003; Logan *et al.*, 2000). The silversides of the genus *Odontesthes* present in Patagonia, however, show a different situation. Both species, the

*Author to whom correspondence should be addressed. Tel.: +54 294 4423374; email: soacri@yahoo.com.ar

native Patagonian pejerrey *Odontesthes hatcheri* (Eigenmann 1909) and the trans-located Bonaerensean pejerrey *Odontesthes bonariensis* (Valenciennes 1835), do not have conspicuous morphological variations. The references to oropharyngeal vari-ation are limited to the observation of Dyer (2000*a*, 2006), regarding the slight prognathism of the lower jaw in *O. bonariensis* and upper jaw prognathism in *O. hatcheri*. Crichigno *et al.* (2012) were able to induce phenotypic plasticity in juve-niles of *O. hatcheri*, adding new morphological variation to wild juveniles by means of controlled feeding experiments.

The genus *Odontesthes* (family Atherinopsidae) can be found in southern South America and includes freshwater silversides such as *O. hatcheri* and *O. bonariensis* (Dyer, 2006), with the most ancient records on the genus reaching the early Miocene (Cione & Báez, 2007). Both species (*O. hatcheri* and *O. bonariensis*) have disjunct original distributions: *O. hatcheri* in the south-west (the Andean Cuyan and Patag-onian Provinces; Dyer, 2000*b*, 2006; Aigo *et al.*, 2008; López *et al.*, 2008) and *O. bonariensis* in the north-east (the Pampean Province; Marrero, 1950; Dyer, 2000*b*, 2006; Baigún & Ferriz, 2003).

Odontesthes hatcheri shows a strong preference for animal prey (Ferriz, 1987; Grosman & Rudzik, 1990; Bello *et al.*, 1991; Macchi *et al.*, 1999), and large-sized individuals can be piscivorous (Bello *et al.*, 1991; Macchi *et al.*, 1999). Early life-history studies identified largely zooplanktivorous larvae in the littoral zone of lakes during the summer (Ferriz, 1987; Battini *et al.*, 1995) and ontogenetic shifts in diet have been documented in relation to habitat shifts from littoral to limnetic and from limnetic to benthic zones (Cussac *et al.*, 1992; Cervellini *et al.*, 1993).

Odontesthes bonariensis is a very popular freshwater fish among commercial and sport fishermen, with a long history of domestic and international introductions, which attests to the high quality and market value of its flesh, as well as its attrac-tiveness as a game fish (Somoza *et al.*, 2008). *Odontesthes bonariensis* is a dominant species in the limnetic zone of the Pampean lagunas. It is a limnetic swimmer (Freyre & Protogino, 1993) with a high metabolic rate (Gómez & Ferriz, 2001).

The first man-made translocations of *O. bonariensis* occurred in the last century, between 1938 and 1941, from the Pampean Province to the Andean-Cuyan and Patagonian Provinces, the original distribution of *O. hatcheri* (Dyer, 2006; Somoza *et al.*, 2008; Amalfi, 2009). Both species hybridize in captivity (Strüssmann *et al.*, 1997*a*) and in natural habitat (Dyer, 2000*b*). Reciprocal hybrids between both species matured and F2 are viable, suggesting that complex degrees of hybridization might occur in natural habitats (Strüssmann *et al.*, 1997*a*). In addition, both species show a different degree of sexual determination by temperature (Strüssmann *et al.*, 1996, 1997*b*). Hybrids reveal strong interactions between genotype and temperature for sex determination and fertility (Inazawa *et al.*, 2011), and the mean summer air temper-ature (MSAT) in the distribution areas of both species shows that *O. bonariensis* is absent when MSAT is $<19°$ C (Liotta, 2006; Aigo *et al.*, 2008; Cussac *et al.*, 2009; Table I). Multidimensional morphology (Crespin & Berrebi, 1999) and geometric morphometrics (Valentin *et al.*, 2002) have proven to be useful tools to study the morphological transition across hybrid zones between species. The aim of this work is to analyse the cephalic morphological variations of *O. hatcheri* and *O. bonariensis* in Patagonia, in order to (1) explore traits with taxonomical meaning and presumptively related with resource polymorphism and (2) enhance the comprehension of the mor-phological variation of both species across a hybrid zone that is, thus far, undefined.

TABLE I. Sampling localities of individuals of *Odontesthes* spp. used in this study ordered from north to south, species identification based on key characteristics, percentage of individuals misclassified by the discriminant analysis of geometric morphometrics and body size

Sampling locality	Latitude and longitude	Species based on key characteristics (n) {% of misclassified}	L_S (cm) (mean and range)
Ullum Reservoir	31° 28' S; 68° 40' W	*O. bonariensis* (22) {18·2}	8·9 (6·1–13·7)
El Carrizal Reservoir	33° 20' S; 68° 43' W	*O. bonariensis* (33) {6·1}	17·1 (9·7–30·9)
Lake San Lorenzo	36° 05' S; 58° 01' W	*O. bonariensis* (40) {0·0}	20·1 (16·0–27·2)
Lake Urre Lauquen	38° 05' S; 65° 50' W	*O. bonariensis* (4) {25·0}	12·0 (7·0–16·4)
Lake Pellegrini	38° 41' S; 67° 59' W	*O. bonariensis* (7) {57·2}, *O. hatcheri* (32) {64·4}, IIC (6)	26·0 (22·3–29·6)
Lake Morenito	41° 03' S; 71° 31' W	*O. hatcheri* (42) {38·1}	26·7 (15·4–41·2)
Lake Carilafquen	41° 12' S; 69° 25' W	*O. hatcheri* (58) {1·8}	19·2 (29·6–11·4)
Lake Rivadavia	42° 30' S; 71° 45' W	*O. hatcheri* (26) {30·7}	7·1 (6·4–7·8)
Lake Rosario	43° 15' S; 71° 20' W	*O. hatcheri* (25) {8·0}	28·0 (15·9–33·0)
Florentino Ameghino Reservoir	43° 42' S; 66° 29' W	*O. hatcheri* (14) {28·5}	16·6 (13·1–24·4)
Chubut River (Los Altares)	43° 51' S; 68° 48' W	*O. hatcheri* (14) {14·3}	9·4 (6·1–11·2)
Lake Musters	45° 28' S; 69° 10' W	*O. hatcheri* (24) {12·5}	11·8 (6·0–22·9)
Lake Buenos Aires	46° 29' S; 71° 28' W	*O. hatcheri* (36) {2·8}	17·8 (10·7–27·1)
Lake Pueyrredon	47° 23' S; 71° 55' W	*O. hatcheri* (32) {6·2}	13·8 (9·2–39·0)

IIC, individuals with intermediate characteristics; n, sample size; L_S, standard length.

MATERIALS AND METHODS

Fishes were captured from eight lakes, three reservoirs and one river in the Pampean, Andean-Cuyan, and Patagonian provinces (López *et al.*, 2008), from 2006 to 2008. Individuals were captured by angling in Lake San Lorenzo, within the native range of *O. bonariensis*, with gillnets and seine in Carrilafquen, Morenito, Pellegrini, Rosario, Muster, Rivadavia and Buenos Aires lakes and with seine in Carrizal, Ullum and Florentino Ameguino reservoirs and in Chubut River, which are within the original area of distribution of *O. hatcheri* (Marrero, 1950; Table I).

All fishes ($n = 415$) were killed with an overdose of benzocaine (solution 1:10 000). Digital images of the left side of the head were captured (Nikon D70; www.nikon.com), taking care to minimize parallax error. Fishes were identified using the dichotomous key of Dyer (2006). In Lake Pellegrini, several individuals could not be assigned to one or another species (intermediate individuals) and were considered as a separate group. These fishes exhibit dorsal scale size (number of rows) typical of *O. hatcheri*, but dorsal fin position as expected for *O. bonariensis*.

Images were selected in order to obtain usable lateral views of the head. Geometric morphometrics analysis (GMA) of the head was performed using 11 homologous landmarks (Bookstein, 1991). The landmarks, digitized on digital images with the TpsDig 2.10 software (www.life.bio.sunysb.edu/morph/index.html), were: (1) dentary anterior tip, (2) premaxilla anterior ventral tip, (3) premaxilla anterior dorsal tip, (4) premaxilla posterior ventral tip, (5) centre of the anterior nostril, (6) maxilla posterior ventral tip (7, 8, 9 and 10), anterior, posterior, dorsal and ventral edge of the eye and (11) external tip of the angle between the vertical and horizontal shafts of the preopercle (Fig. 1). Morphometric characteristics were scored and analysed to quantify anatomical differences between species and to explore variation within species, testing for correlation with physical and chemical variables of their habitat.

Landmark configurations for each specimen were aligned, rotated, translated and scaled by a generalized Procrustes analysis (GPA; Rohlf & Slice, 1990) using a consensus configuration as a reference (Rohlf & Marcus, 1993). Partial (PW) and relative warps (RW) were calculated using TpsRelw v1.35 (Rohlf, 2003*a*). RWs allowed for the visualization of mean head shape of each population by means of deformation grids relative to the consensus shape. Cluster analysis (CA, centroid clustering and average linkage between groups) and discriminant analysis (DA) were performed with SPSS software (www-01.ibm.com/software/analytics/spss) employing the PW and uniform co-ordinates (weight matrix) in order to group fishes on the
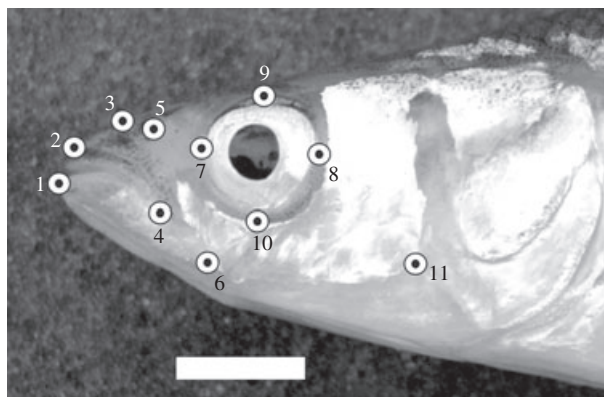

FIG. 1. Homologous landmarks on the left lateral view of the head of a just captured individual *Odontesthes* sp.: dentary anterior tip (1); premaxilla anterior ventral tip (2); premaxilla anterior dorsal tip (3); premaxilla posterior ventral tip (4); centre of the anterior nostril (5); maxilla posterior ventral tip (6); anterior (7), posterior (8), dorsal and ventral (9) edge of eye; external tip of the angle between the vertical and horizontal shafts of the preopercle (11). Bar = 10 mm.

basis of overall head shape and test differences between species and presumptive hybrids, and within species among capture sites, respectively.

TPSRegr 1.28 (Rohlf, 2003b) and polynomial regressions between discriminant functions (DF) among sampling sites and standard length (L_S) were assayed in order to assess size effects within each species. The grade of the polynomial regression was assessed on the basis of r^2 and the significance of the polynomial coefficients.

Assuming that morphology can give a good indication about genetic introgression (Crespin & Berrebi, 1999; Valentin *et al.*, 2002), quantifying the extent to which the original area of distribution of *O. hatcheri* was converted into a hybrid zone was attempted. Thus, individuals of *O. hatcheri* and *O. bonariensis* were identified by a taxonomic key, the predicted taxonomic group for each individual was obtained by applying DA on the PW and uniform co-ordinates, and the percentage of individuals misclassified by DA was calculated for each sampling site. Three hatcheries (Estación Hidrobiológica de Chascomús, Estación de Piscicultura de Embalse and Piscicultura Río Limay) have participated since 1930 in stocking programmes of *O. hatcheri* and *O. bonariensis* along the Andean Cuyan and Patagonian Provinces (González & Mastrarrigo, 1954; Amalfi, 2009). The hypothesis that morphological misclassification is the evidence of genetic introgression was tested using the correlation of the percentage of individuals of each species misclassified by DA *v.* the Euclidean distance from each of the three hatcheries, under the assumption that consequences of stocking programmes should be more conspicuous in locations closer to the hatchery.

Conversely, the existence of a morphometric pattern that could be contrasted with taxonomical classification was explored. Landmark information was separated for each sampling site and subjected to GMA. RW and consensus co-ordinates for each population were extracted, and CA (centroid clustering and squared Euclidean distance) was performed using the shape matrix.

Intraspecific morphological variation can result from natural genetic variation, different degrees of introgression and environmental effects. Differences between populations and intrapopulation variations within two lakes with enough individuals (Carrilafquen and Morenito Lakes) were analysed using the same landmarks. DAs were performed to test differences among populations using the shape matrix provided by GMA. In all cases, the description of shape variation in the first two RWs was performed visualizing the deformation grids in the extreme of each axis from left to right and from bottom to top. Environmental information for sampling sites was obtained from the available literature and from Servicio Meteorológico Nacional (www.smn.gov.ar). Ten environmental variables (Table II) were used in order to search for environmental dependence (in lakes and reservoirs) of the cephalic shape. The significance of these relationships was considered after Bonferroni correction.

RESULTS

INTERSPECIFIC DIFFERENCES

The first two RWs of the GMA ($n = 415$) explain 48% of the variance (RW1 = 31·68% and RW2 = 16·15%). The head shape variation explained by RW1 ranges between individuals with a longer maxilla and premaxilla, smaller eyes in an anterior position, tall and short heads and prognathism of the lower jaw in the negative semi-axis, and individuals with a shorter maxilla and premaxilla, larger eyes, longer heads and prognathism of the upper jaw in the positive semi-axis. The head shape variation explained by RW2, ranges between individuals with short heads and prognathism of the upper jaw in the negative semi-axis, and individuals with longer heads and prognathism of the lower jaw in the positive semi-axis.

When the taxonomic status (*O. hatcheri*, *O. bonariensis* and intermediate individuals) was considered, both species were mostly separated by RW2

Table II. Physicochemical variables for capture sites of *Odontesthes* spp. (ordered from north to south)

	Area (km^2)	Altitude (m a.s.l.)	Depth (m)	Perimeter (km)	Line coast development	Perimeter: area (km km^{-2})	Mean summer air temperature (°C)	Conductivity (μS cm^{-1})	Chlorophyll *a* (mg m^{-3})	Total N: total P
Ullum[a,b]	32	768	40	50	0.23	1.55	22	550	0.93	1020.0
Carrizal[a,b]	31	786		46	0.23	1.46	22	1670	4.79	925.0
San Lorenzo[a]	16		2	18	0.44	1.13	21			
Urre Lauquen[a,c]	95	219	7	29	0.05	0.31	23	122 100	7.60	2660.0
Pellegrini[a,b,c,d]	112	270	9	69	1.84	0.62	22	22 000		71.3
Morenito[a,e,f]	<1	758	5	2	1.38	9.58	13	78	7.09	
Carrilafquen[a,c,g]	6	825	2	11	1.25	1.85	17	894	23.70	0.30
Rivadavia[a,b]	22	527	104	31	1.83	1.37	13	55	1.69	253.0
Rosario[a,b]	14	650	25	21	1.56	1.45	13			76.6
Florentino Ameguino[a,b,c]	65	169	25	205	7.18	3.16	19	242	1.20	63.4
Chubut River		269	2				18	291	2.17	
Muster[a,b]	414	260	20	150	2.08	0.36	17	440	14.07	65.9
Buenos Aires[a,h]	1870	214	463	504	3.29	0.27	14	80		
Pueyrredón[a]	326	155		211	3.29	0.65	13	176	0.35	

aServicio Meteorológico Nacional (http://www.smn.gov.ar/), bQuirós (http://www.agro.uba.ar/users/quiros/), cIARH-INCYTH (1995), dAmalfi (2009), eMacchi *et al.* (1999), fModenutti *et al.* (2000), gDíaz & Pedrozo (1996), hDíaz *et al.* (2000).

(Fig. 2). DA differentiates *O. hatcheri*, *O. bonariensis* and intermediate individuals with 83·6% of cases correctly classified into the taxonomically assigned category, explaining 100% of the total variance. DF1 (Wilks' $\lambda = 0.353$, $n = 415$, $P < 0.001$) separates both species and DF2 (Wilks' $\lambda = 0.917$, $n = 415$, $P < 0.01$) separates the intermediate individuals from both *O. hatcheri* and *O. bonariensis* (Fig. 2).

Notwithstanding the excellent discrimination among taxonomic groups, the taxonomic categories of *O. hatcheri* and *O. bonariensis* include a non-negligible number of individuals considered in other categories by the DA, *i.e.* misclassified cases. Each sampling site shows a different proportion of misclassified cases (Table I). In particular, the number of individuals taxonomically predicted as *O. hatcheri* and misclassified by the DA for each sampling site [Fig. 3(a)] shows a negative and significant correlation with the Euclidean distance from Limay hatchery (Pearson $r = -0.674$, $n = 10$, $P < 0.05$) and Embalse hatchery (Pearson $r = -0.722$, $n = 10$, $P < 0.05$), and a marginal correlation regarding Chascomus hatchery (Pearson $r = -0.627$, $n = 10$, $P > 0.05$). The number of individuals taxonomically predicted as *O. bonariensis* and misclassified by the DA for each sampling site [Fig. 3(b)] shows only a marginal correlation with the distance from the Limay hatchery (Pearson $r = -0.867$, $n = 5$, $P > 0.05$).

The dendrogram based on CA applied on the consensus shape of each sampling site shows that the grouping of each species is mostly correct, with *O. hatcheri* and *O. bonariensis* being the two main groups (Fig. 4). The *O. bonariensis* group, however, includes the individuals of Lake Pellegrini (both species and intermediate individuals) while individuals of Lake Buenos Aires (*O. hatcheri*) are separate from the others.

INTRASPECIFIC MORPHOLOGICAL VARIATION OF *O. BONARIENSIS*

The first two RWs, considering 106 individuals in three lakes and two reservoirs, explain 54% of the variance (RW1 = 40% and RW2 = 14%). The head shape variation explained by RW1 ranges between individuals with a longer maxilla and premaxilla, smaller eyes, a shorter head and prognathism of the upper jaw in the negative semi-axis, and individuals with shorter maxilla and premaxilla, larger eyes, longer heads, a thicker anterior portion of the premaxilla and a slight prognathism of the lower jaw in the positive semi-axis. The head shape variation explained by RW2 ranges between individuals with a shorter maxilla and premaxilla, a shorter head and more anteriorly positioned eyes in the negative semi-axis, and individuals with a longer maxilla and premaxilla, longer head and more posteriorly positioned eyes in the positive semi-axis.

DA among the five capture sites produces three significant DFs that correctly classify 89·6% of the cases into the original lake or reservoir, and explain 97·2% of the variance. DF1 (Wilks' $\lambda = 0.022$, $n = 106$, $P < 0.001$) shows a significant relationship with L_S (Pearson correlation coefficient $= 0.874$, $n = 106$, $P < 0.01$) and does not differentiate any of the populations from the overall size-related trend (Fig. 5). DF2 and DF3 do allow differentiation of individuals from Ullum and Carrizal Reservoirs, Lake San Lorenzo and the group formed by Pellegrini and Urre Lauquen Lakes (Fig. 5).
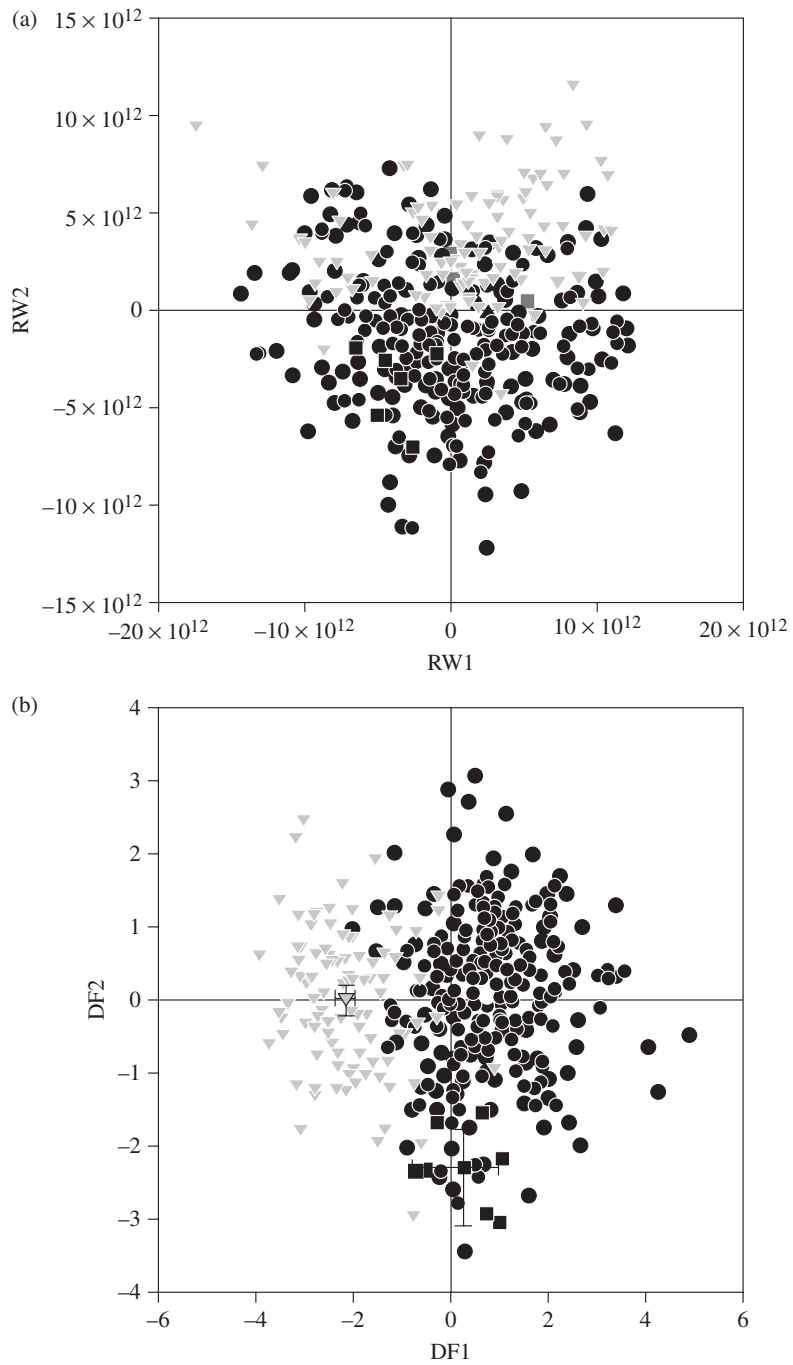
FIG. 2. Interspecific differences (geometric morphometrics): (a) RW1 *v*. RW2 [▽, *Odontesthes bonariensis*; ●, *Odontesthes hatchery*; ■, intermediate individuals (of Lake Pellegrini)] and (b) discriminant analysis among species and presumptive hybrids, discriminant function (DF) 2 *v*. DF1. Group centroids and 95% C.I. are indicated.
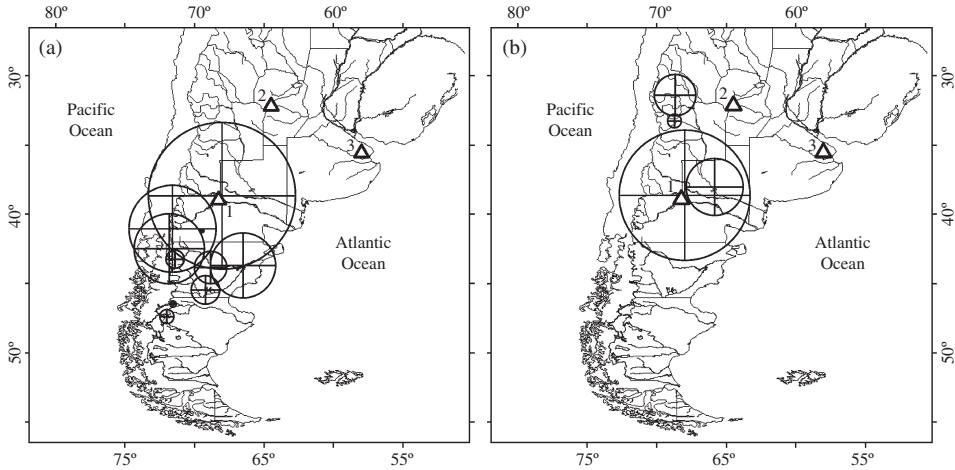
FIG. 3. Percentage of individuals taxonomically predicted as (a) *Odontesthes hatcheri* and (b) *Odontesthes bonariensis* and misclassified by the discriminant analysis (diameter of the circles), by capture site (crosses). △, the geographic position of stocking hatcheries (1, Limay, 38° 59′ S; 68° 14′ W; 2, Embalse, 32° 13′ S; 64° 29′ W; 3, Chascomús, 35° 36′ S; 58° 01′ W).

INTRASPECIFIC MORPHOLOGICAL VARIATION OF *O. HATCHERI*

The first two RWs explain 49% of the variation among eight lakes, one river and one reservoir, with 302 individuals, using the same 11 landmarks. The head shape variation explained by RW1 ranges between individuals with a longer maxilla and
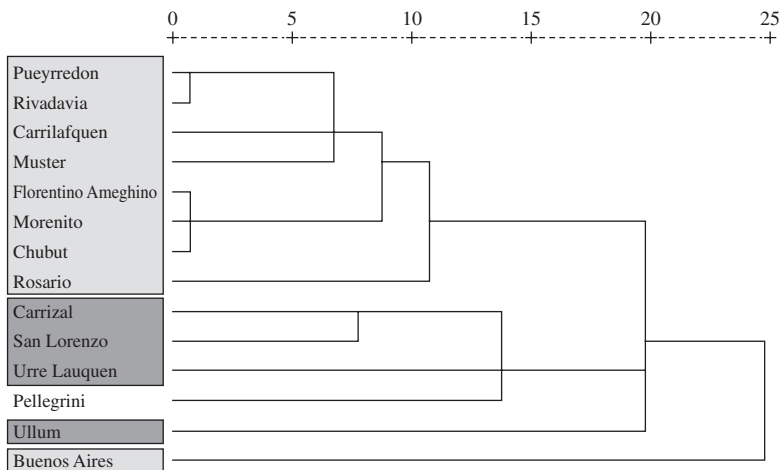
FIG. 4. Dendrogram based on cluster analysis applied on the consensus shape of each capture site. Distances are rescaled to fall in the range of 1–25. *Odontesthes hatcheri* (□) and *Odontesthes bonariensis* (■) are the two main groups. Note that all the individuals of Lake Pellegrini (both species and intermediate individuals) are clustered within the *O. bonariensis* group. Individuals of Lake Buenos Aires (*O. hatcheri*) are separated from all other groups.
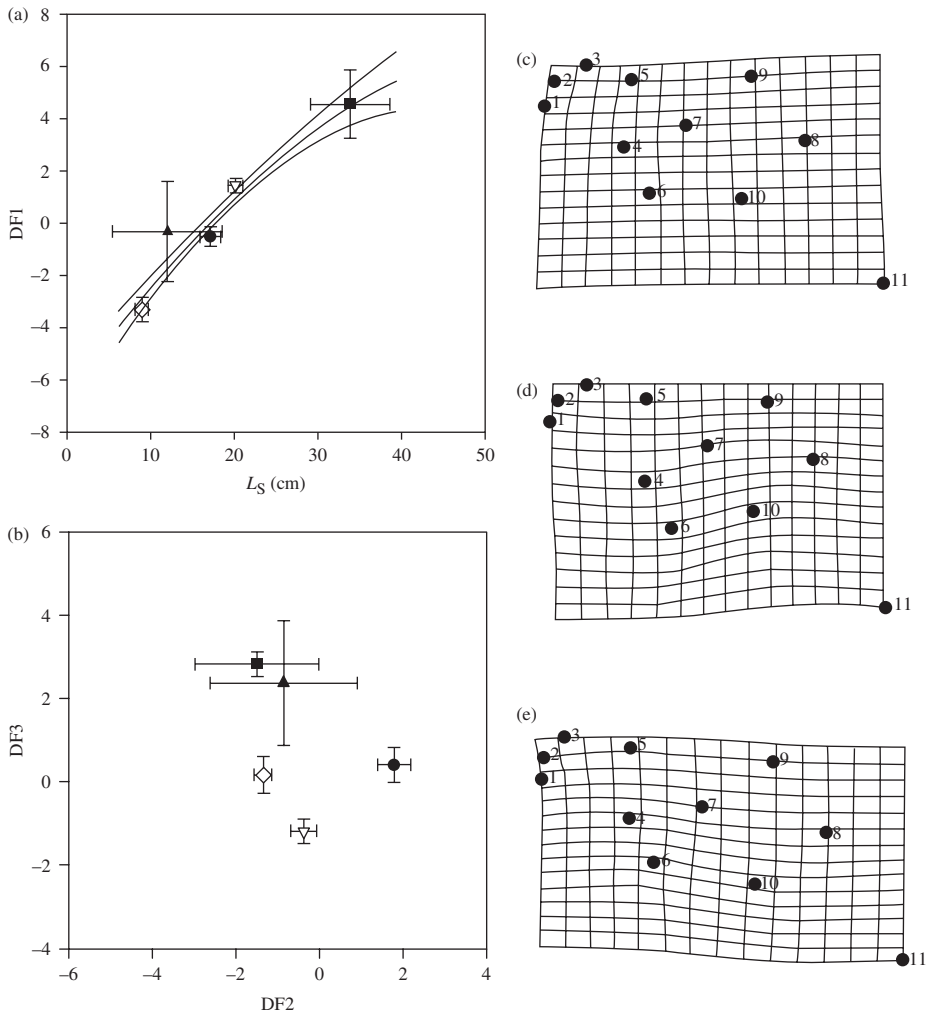
FIG. 5. *Odontesthes bonariensis*: (a) discriminant function (DF) 1 *v.* standard length (L_S) and (b) DF3 *v.* DF2. [Carrizal Reservoir (●), Lake San Lorenzo (△), Lake Pellegrini (■), Ullum Reservoir (◇) and Lake Urre Lauquen (▲)]. Fitted polynomial line, centroids and 95% C.I. are indicated. Deformation grids show the mean shape for (c) Carrizal Reservoir, (d) Lake San Lorenzo and (e) Ullum Reservoir (see Fig. 1).

premaxilla, smaller eyes and shorter head in the negative semi-axis, and individuals with shorter maxilla and premaxilla, larger eyes, longer head and prognathism of the upper jaw in the positive semi-axis. The RW2 shows individuals with shorter head, more anteriorly positioned eyes and prognathism of the upper jaw in the negative semi-axis and individuals with smaller eyes in positive semi-axis.

Regarding the size effect, a significant regression between RW1 and L_S ($t = -8·949$, $n = 302$, $P < 0·001$; Fig. 6) can be seen, indicating a clear allometric growth, with the small individuals (10 cm L_S) presenting a shorter maxilla and premaxilla, longer head and upper jaw prognathism, medium-sized individuals (25 cm) presenting a long maxilla and premaxilla, small head and slight upper jaw
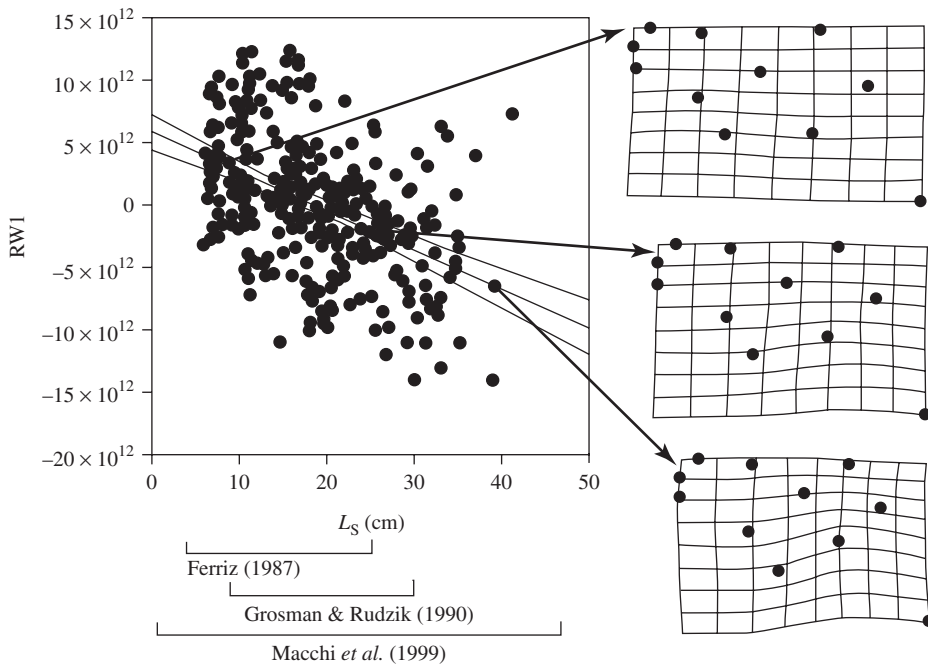
FIG. 6. Size effect in *Odontesthes hatchery*: RW1 *v*. standard length (L_S) showing fitted line, 95% C.I. and deformation grids for small (10 cm L_S), medium (25 cm L_S) and large (40 cm L_S) individuals. The L_S range references corresponded to the diet studies of Ferriz (1987), Grosman & Rudzik (1990) and Macchi *et al.* (1999) (see Fig. 1.).

prognathism, and large individuals (40 cm) with a longer maxilla and premaxilla, smaller eyes and shorter head.

The only significant correlation between RWs and environmental variables was RW1 *v*. line coast development $\{D_L = p \ [2 \ (\pi \ A)^{0.5}]^{-1}$, where p = perimeter and A = area; regression $t = -61.407$, $n = 9$, $P < 0.01\}$. DA among water bodies produces eight significant DFs that correctly classify 73·5% of the cases into the original lake, river or reservoir, and explain 99·1% of the variance. Morphological variation is related to L_S. DF1 (Wilks' $\lambda = 0.023$, $n = 302$, $P < 0.001$) and DF2 (Wilks' $\lambda = 0.077$, $n = 302$, $P < 0.001$) show a significant relationship to L_S (Pearson $r = 0.594$, $n = 302$, $P < 0.01$ and Pearson $r = -0.569$, $n = 302$, $P < 0.01$, respectively). Notwithstanding the size effect, DFs show different body shapes for Chubut River individuals and the populations of the lakes Buenos Aires, Rivadavia and Rosario (Fig. 7).

INTRALACUSTRINE MORPHOLOGICAL VARIATION OF *O. HATCHERI*

Within Lake Morenito ($n = 42$), RW1 explains 49·77% and RW2 13·82% of the variance. The head shape variation explained by RW1 ranges between individuals with a longer maxilla and premaxilla, smaller eyes, shorter head and prognathism of the lower jaw in the negative semi-axis, and individuals with shorter maxilla and
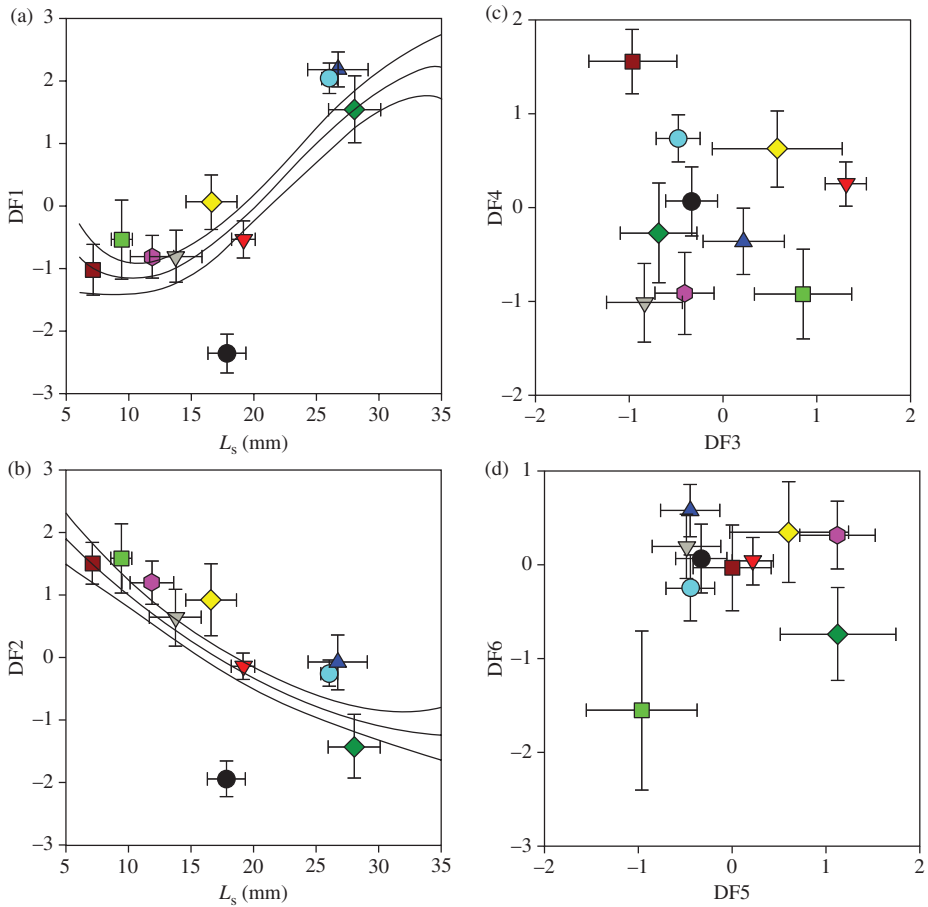
FIG. 7. Intraspecific morphological variation of *Odontesthes hatcheri*: (a, b) discriminant functions (DFs) 1 and 2 and standard length (L_S). Fitted polynomial line, centroids and 95% c.i. are indicated, (c) DF4 *v*. DF3 and (d) DF6 *v*. DF5 (●, Lake Buenos Aires; ▲, Lake Carrilafquen; ■, Chubut River; ◆, Florentino Ameghino reservoir; ▲, Lake Morenito; ⬢, Lake Muster; ●, Lake Pellegrini; ▽, Lake Pueyrredon; ■, Lake Rivadavia; ◆, Lake Rosario).

premaxilla, larger eyes and longer head in the positive semi-axis. The head shape variation explained by RW2 ranges between individuals with a longer maxilla and premaxilla, shorter head and more anteriorly placed eyes in the negative semi-axis, and individuals with shorter maxilla and premaxilla and prognathism of the upper jaw in the positive semi-axis.

Within Lake Carrilafquen ($n = 58$), RW1 explains 23·15% and RW2 19·67% of the variance. The head shape variation explained by RW1 ranges between individuals with a shorter maxilla and premaxilla, shallower head and posteriorly positioned eyes in the negative semi-axis, and individuals with a longer maxilla and premaxilla, more anteriorly placed eyes and deeper head in positive semi-axis. The head shape variation explained by RW2 ranges between individuals with larger eyes in the negative semi-axis and individuals with smaller eyes in the positive semi-axis.

Considering both lakes in the same TPS analysis, a negative regression ($t = -3.523$, $n = 41$, $P < 0.001$) between RW1 and L_S is obtained for individuals of Lake Morenito (regression, $F_{1,40} = 12.428$, $n = 41$, $P < 0.001$). In contrast, the regression of RW1 with size is positive ($t = -7.148$, $n = 57$, $P < 0.001$) in Lake Carrilafquen (regression, $F_{1,56} = 51.095$, $n = 57$, $P < 0.001$).

DISCUSSION

The present results represent the first evidence of introgression of the transplanted *O. bonariensis* with the native *O. hatcheri*. Turner (1999) gives a detailed discussion about hybridization and introgression in fishes. Deliberate stocking of hatchery fishes is likely to increase the number of introgressed and hybrid individuals, increasing the likelihood of misclassification. Head shape allows distinction between individuals of *O. hatcheri* and *O. bonariensis*. The closer the hatcheries participating in stocking programmes are to the sampling site, the higher the percentage of misclassified individuals, particularly for *O. hatcheri*. Thus, misclassification is a likely product of stocking, which probably occurs more frequently closer to hatcheries. Water bodies near Estación de Piscicultura Río Limay appear to have been stocked more times and with greater number of fishes than the more remote sites.

This complex landscape agrees with the genetic introgression predicted by Strüssmann *et al.* (1997*a*), and could be one of the causes for the observed morphological variation of *O. hatcheri* and *O. bonariensis* populations in the hybrid zone. Particularly, the presence of individuals with intermediate morphological and meristic characteristics only in Lake Pellegrini appears to rely on the existence of an artisan fishery in this lake, where artificial fertilization using captured fishes is a common practice among fishermen (Ringuelet, 1943), carried on up to the present. This practice might be constantly introducing hybrids in the system, probably preventing the isolating action of natural barriers. In the same way, considering the lateral view of the head as featured in this work, all the individuals of Lake Pellegrini (*O. hatcheri, O. bonariensis* and intermediate individuals) are found nested within the *O. bonariensis* group, and the individuals of the remote Lake Buenos Aires (*O. hatcheri*) are separated from all the others (Figs 3 and 8).

Cephalic shape is related to size in both species. In agreement with Dyer (2006), large-sized individuals of *O. hatcheri* have lower jaw prognathism and a shorter head in relation to small-sized individuals, within Lake Morenito. This might be related to ontogenetic feeding changes, specifically involving a shift to piscivory in individuals of L_T 300 mm or more (Macchi *et al.*, 1999). In contrast, in Lake Carrilafquen, where there are no data about piscivory (M. Alonso, pers. comm.), upper jaw prognathism for large individuals of *O. hatcheri* could be observed. All morphological analyses from this study (interspecific and intraspecific, between and within populations) show that variation affects the same anatomical structures: size of head, prognathism, size of upper and lower jaws and size and positions of eyes. This morphological variation, which is repeated within populations, between populations and between species has been named nested variation (Foster *et al.*, 1998). Nested variations might be generated by genetic or environmental causes. Many authors have documented body shape divergences involving fishes that inhabit shallow littoral *v.* deeper limnetic zones of temperate postglacial lakes (Schluter & McPhail, 1992;
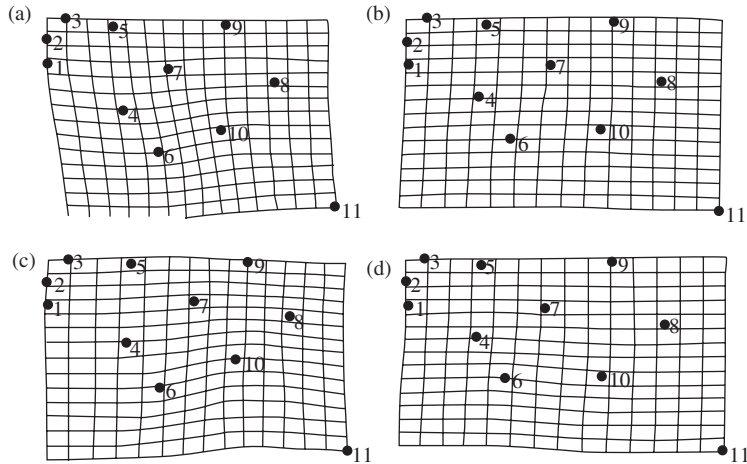
FIG. 8. Deformation grids corresponding to *Odontesthes hatcheri* populations in (a) Lake Buenos Aires, (b) Lake Rivadavia, (c) Lake Rosario and (d) Chubut River (see Fig. 1).

Robinson & Wilson, 1994; Ruzzante *et al.*, 1998; Riopel *et al.*, 2008). In addition to Aigo *et al.* (2008), who positively relate abundance of *O. hatcheri* with the area of the lake, the dependence of cephalic shape of *O. hatcheri* with D_L suggests an effect of the development of the littoral zone, nutrient input, macrophyte abundance and shelter availability on the head shape, probably mediated by food availability (Crichigno *et al.*, 2012).

Odontesthes hatcheri occupies almost all the Andean region (*i.e.* from 35° 04′ S in El Nihuil reservoir to 47° 18′ S in Lake Pueyrredon; Aigo *et al.*, 2008) and Shuter & Post (1990) propose that winter starvation constrains fish distribution at high latitudes. The ability of fishes to successfully face a given food availability could be understood in terms of the fit between morphology and function (prey capture and food processing). Two closely related types of prey capture mechanisms have been described for the atherinopsid genera *Cauque*, *Austromenidia*, *Basilichthys* and *Odontesthes*: premaxillary protrusion and premaxillary bite (Osse, 1989; De la Hoz, 1994; De la Hoz & Aldunate, 1994; De la Hoz *et al.*, 1994). In this way, the observed dependence between the cephalic morphology of *O. hatcheri* and body size agrees with the trophic niche shifts observed for the species (Cervellini *et al.*, 1993; Macchi *et al.*, 1999). The presumptive euriphagous characteristic of *O. hatcheri* (Ferriz, 1987; Grosman & Rudzik, 1990; Bello *et al.*, 1991; Macchi *et al.*, 1999) requires more consideration. The diet of the species changes greatly between lakes but not within them, with most of the intralacustrine variation ascribed to the ontogenetic shift of the diet (Ferriz, 1987; Grosman & Rudzik, 1990; Cervellini *et al.*, 1993; Battini *et al.*, 1995; Macchi *et al.*, 1999; Menni, 2004; Fig. 5). In consequence, the species shows a succession of more or less stenophagous ontogenetic periods: larva, juvenile and adult, that appear to be correlated with the trade-off between limnetic abundance of small-sized prey for larvae and shelter availability in the littoral area for larger fish (Werner & Hall, 1988; Cussac *et al.*, 1992; Barriga *et al.*, 2002, 2012).

Odontesthes hatcheri has diminished throughout Patagonia, probably due to diet overlap, competitive exclusion and active predation by salmonids, despite its previous

exposure to the native perch *Percichthys trucha* (Valenciennes 1833) (Macchi *et al.*, 2007; Lindegren *et al.*, 2012). Introgression thus adds a new threat to this species and deserves particular consideration in terms of local conservation management.

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