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Morphological and genetic variability in an alien invasive mussel across an environmental gradient in South America

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Abstract

Adaptation is an essential step in the establishment and spread of alien species in new environments, with phenotypic plasticity or genetic variability often contributing to this success. The golden mussel *Limnoperna fortunei* is a biofouling mollusc native to Southeast Asia that was introduced to South America near the Río de la Plata estuary, Argentina, though the species has subsequently spread more than 2000 km upstream. We analyzed morphological and genetic variation in 24 introduced populations of *L. fortunei* across its South American range. Relative gill area and shell morphology differed significantly, even among geographically proximate populations. Differences in relative gill area were especially marked across the species' range and were negatively correlated with total suspended solids. Whereas mean gill cilia length, filament width, and interfilamental ciliary junction distance did not differ significantly among populations, mean gill cilia density was significantly lower in populations from areas with high suspended solids. Conversely, morphological differences were not related to the number of haplotypes, haplotype diversity, or nucleotide diversity, based upon analyses of the mitochondrial cytochrome *c* oxidase subunit 1 gene. Our results indicate that introduced populations of golden mussels in South America exhibit pronounced morphological variation in shell and gill metrics that appear to result from developmental plasticity in relation to total suspended sediments, as has been observed in other mussel species. These adaptations may have facilitated spread of this species to a wide range of habitats.

The establishment of a viable population is an essential step in successful biological invasions (Blackburn et al. 2011), and adaptation to the new environment is a key factor contributing to establishment and spread of nonindigenous species (Daehler 2003). Adaptation itself may result from phenomena such as developmental or phenotypic plasticity, genetic variability, adaptive homeostasis, and maternal effects, among others (Ghalambor et al. 2007; Hulme 2008). In spatially or temporally variable environments, rapid adaptation to changing conditions could be decisive to the success of colonizing populations (Ghalambor et al. 2007). Rapid adaptation may be particularly important in river floodplains that experience pronounced spatial and temporal variation in flow rate and other environmental conditions (Wells and Pigliucci 2000).

Phenotypic plasticity is one of the most important mechanisms for rapid adaptation to changing environmental conditions (Schlichting and Pigliucci 1998). Significant phenotypic plasticity has been reported in a number of invasive bivalves, including the Asian clam *Corbicula fluminea,* zebra mussel *Dreissena polymorpha*, and quagga mussel *Dreissena rostriformis bugensis* (*Dreissena bugensis*, Marsden et al. 1996; Sousa et al. 2007; Peyer et al. 2010). In general, phenotypic plasticity in these species involves changes in shell morphology, mainly in width : length and width : height ratios. Variation in gill structure in response to changing environmental conditions also has been described in bivalves, especially in lamellibranchs; these molluscs possess high filtering abilities and respond to food type and quantity, suspended sediment, and other factors (Payne et al. 1995; Lei et al. 1996). However, in some cases morphological variation is related to genetic differences among populations rather than to phenotypic plasticity (Marsden et al. 1996).

The golden mussel *Limnoperna fortunei* was introduced to South America near the Rio de la Plata River, Argentina, around 1990 (Pastorino et al. 1993). The species has since colonized an array of natural and artificial environments (Darrigran 2002; Boltovskoy et al. 2006). *L. fortunei* has spread approximately 2000 km across almost the entire Rio de la Plata basin, as well as to inland lakes and reservoirs, at rates as high as 250 km per year (Boltovskoy et al. 2006). The extensive spread across the basin has been associated with downstream recruitment of settling, postmetamorphic larvae (Boltovskoy et al. 2006) and with upstream colonization by biofouling individuals on local vessels (Karatayev et al. 2007). However, factors underlying this species' remarkable adaptation to new

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environments have not been established. For example, a relatively high filtration activity was recorded for adults (Sylvester et al. 2005) and veligers (Gazulha 2010) in introduced areas, but the morphological, developmental, or physiological determinants associated with these observations have not yet been explored.

Evidence exists for genetic variation in *L. fortunei* populations across its South American range. For example, *L. fortunei* populations exhibited strong genetic structure based on nuclear microsatellite markers (Zhan et al. 2012), implying a possible genetic basis for its invasion success. On the other hand, morphological variation in these populations has not been addressed. Better understanding of the roles of genetic and phenotypic variation in these populations may help explain the spread of this highly successful invader.

In this study, we assess the morphological variation in 24 populations of *L. fortunei* throughout the entire invaded range in South America and relate findings to both genetic structure and environmental factors. We utilize conventional morphological measures, including shell morphology and gill macro- and ultrastructure, to compare populations living under different environmental conditions across the South American range. Additionally, we relate morphological variation to genetic diversity based upon mitochondrial cytochrome *c* oxidase subunit 1 gene (COI) sequences of individuals from different populations across the species' range.

Methods

Study area and sample collection and analysis—Sampling was designed to cover most of the invaded range of *L. fortunei* in South America (Fig. 1; Table 1). Samples were collected between 2007 and 2010 from the lower Paraná River (populations: Del Este Channel, EC; Carapachay River, CR; Tigre, TI; and San Fernando, SF) and the Río de la Plata estuary (populations: Buenos Aires, BA; Quilmes, QU; Punta Lara, PL; Santa Lucía River, SL; and Magdalena, MA), representing the species' southernmost range in South America. The lower Paraná River and the Río de la Plata estuary are strongly influenced by anthropogenic activities, including sewage discharge and industrial installations along the banks of the river and its tributaries. The invasion pathway was followed, sampling up to the northern limit of the species' distribution, over 2000 km upstream, in the upper Paraná River (populations: Rio Baía, RB; Itaipu Hydroelectric Power reservoir, IT; Yabebiry River, YR; Yaciretá Dam, YD; Paraná River, PR; Cayasta-1, CA-1; Cayasta-2, CA-2; and Setubal Lagoon, SA) and upper Paraguay River (population: Corumba, CO) in Brazil. One population from the western range limit was also collected in the Rio Tercero reservoir (RT), Argentina. Samples were also collected along the Uruguay River and Salto Grande Lake in Argentina, to the northern distributional limit (populations: Federación, FE; Salto Grande Dam, SG; and Puerto Luis, PU). Finally, a sample was collected from the Guaiba basin on the coastal plain of Rio Grande do Sul, Brazil (population Sao Gonçalo Channel, SO). In total, 24 populations distributed along the Río de la Plata and Guaiba basins were sampled in four countries: Argentina, Brazil, Paraguay, and Uruguay (Fig. 1; Table 1). All mussels were preserved in 95% ethanol and were later stored at 4°C prior to laboratory analyses.

Macrostructure analysis—A subsample of 15 mussels was randomly selected and analyzed from each population for morphometric analysis. Shell length, shell width, and shell height were measured to the nearest 0.1 mm using calipers (Electronic Digital) to estimate maximum anterior–posterior, dorsal–ventral, and lateral dimensions, respectively.

The right valve of each mussel was removed, and shell and lamella areas (Fig. 2) were delineated and calculated using a dissecting microscope (Leica S8APO) equipped with a digitizer and image processing software "QCapture" version 6.0 and AutoCAD 2000. Shell area and lamella area for the inner ascending demibranchs were measured to the nearest 0.1 mm^2 using a digital image at 16× magnification (Fig. 2). The average number of filaments per millimeter (filament density, FD) was calculated in each mussel (15 per population) from three randomly chosen gill sections from a 128× magnification image of the gill (Fig. 2). Shell-free dry weight (dry wt) for each mussel was measured after individuals were dried to constant weight at 60°C. Population FE (Salto Grande Lake, Argentina) was excluded from macrostructure and ultrastructure analysis (*see* below), although not from genetic analyses, owing to poor preservation of gill structures.

To avoid size-based differences among mussels (Reyment et al. 1984), similarly sized mussels were used and shell distances and gill : shell areas data were standardized using the following ratios: lamella area : shell area (relative gill area, R_{LS}), shell width : shell length (R_{WL}), shell width : shell height (R_{WH}), and shell height : shell length (R_{HL}). Ratio data was, in turn, log-transformed prior to statistical analysis. Analysis of variance (ANOVA) and Tukey's multiple comparison tests were applied to test differences in log-transformed ratios (dependent variables) among populations from different areas (independent variables). Additionally, these ratios and the remaining morphological variables, such as FD and shell-free dry wt, were used for multivariate analysis (*see* below).

Ultrastructure analysis—Individual mussels were examined using scanning electron microscopy (SEM) to evaluate whether differences in gill area (macrostructure patterns) were related to the gill ultrastructure. Previous work with *D. polymorpha* demonstrated that the contraction of interfilamental ciliary junctions modified the interfilamental distance and, in consequence, the total gill area (Medler and Silverman 1997; Medler et al. 1999). We measured the ultrastructure variables filament width, interfilamental ciliary junction distance, and cilia length and density in sections of tissue dissected from the tips of the gill from the same mussels (15 per population) used for morphological analysis (Fig. 3). Gill tissue was rehydrated from 70% ethanol to tap water and was mounted on small steel platforms with a drop of water. Both surfaces of the inner ascending lamellae were examined with a Hitachi S2500

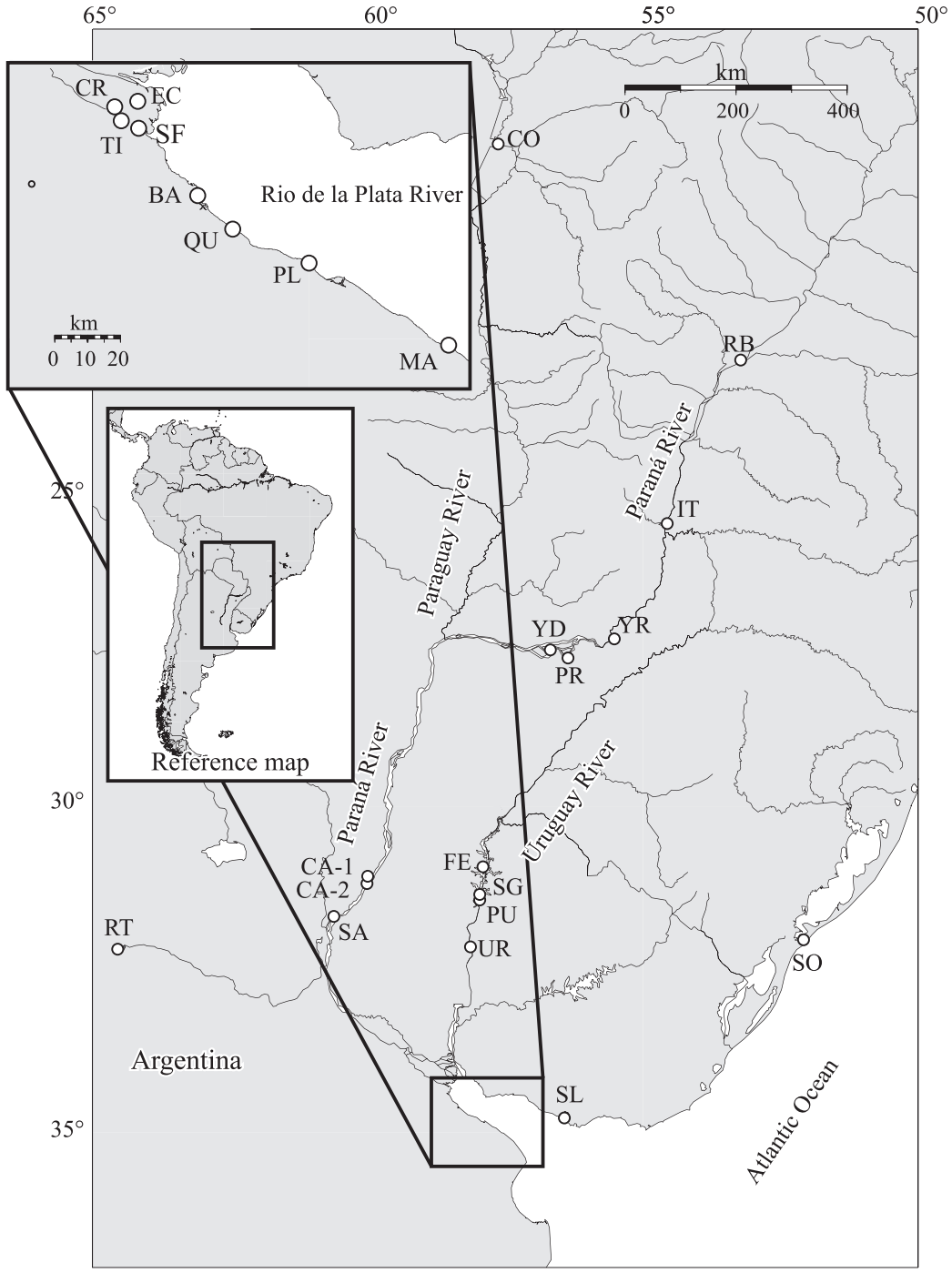

Fig. 1. Sampling sites for the invasive golden mussel *L. fortunei* in South America. Site names as per Table 1.

scanning electron microscope operated at 10 kV and under low vacuum conditions (130 Pa). We excluded mussels for which we were not able to measure at least one of the aforementioned variables, and repeated measures on single individuals were averaged to avoid pseudoreplication. Total gill area was estimated as lamella area × 8. Total shell length, gill area, and ultrastructure variables from the population group with the significantly highest R_{LS} based on ANOVA were compared to the other populations using a *t*-test.

Genetic analysis—Genomic deoxyribonucleic acid (DNA) was extracted from the posterior adductor muscle of another randomly selected subsample of more than 21 individuals from each population, following Elphinstone et al. (2003). Polymerase chain reaction (PCR) amplification, PCR product cleaning, and sequencing of the mitochondrial COI gene were performed according to Zhan et al. (2012). Sequences were checked and then aligned using CodonCode Aligner software version 2.0.6 (CodonCode Corp.). Subsequently, alignment was visually inspected and manually

Table 1. Environmental characteristics (annual mean) of sampling sites for *Limnoperna fortunei*. Full source references are given in Web Appendix, Table A2. WT, water temperature; Chl *a*, chlorophyll *a*; DO, dissolved oxygen; TSS, total suspended solids.

Sample site	Region or state, country	Latitude (°)	Longitude (°)	Collection year	WT (°C)	pH	Calcium (mg L^{-1})	Chl *a* (mg L^{-1})	DO (mg L^{-1})	TSS (mg L^{-1})	Source
CO	Corumba, Brazil	−18.997	−57.654	2007	28.1	6.4	3.5	0.78	4.1	39.8	Oliveira et al. 2011; Oliveira and Calheiros 2000
RB	Baía River, upper Paraná River, Brazil	−22.686	−53.252	2007	25.4	6.9	4.0	3.38	8.1	1.9	Velho et al. 2001; Rodrigues et al. 2009
IT	Itaipu Hydroelectric Power reservoir, Brazil	−25.407	−54.589	2009	24.3	7.6	—	3.80	8.2	6.9	Filho 2006
YR	Yabebiry River, Misiones, Argentina	−27.296	−55.543	2008	24.4	7.2	4.1	0.24	8.3	3.5	Neiff et al. 2000
YD	Yaciretá Dam, Brazil, Paraguay and Argentina	−27.471	−56.704	2008	24.1	7.4	4.3	0.70	8.2	1.2	
PR	Paraná River, Santa Tecla, Corrientes, Argentina	−27.605	−56.384	2008	24.1	7.4	4.0	0.70	8.2	1.2	
CA-1	Cayasta, Santa Fe, Argentina	−31.183	−60.080	2008	21.0	7.2	11.0	10.00	7.8	—	Maine et al. 2004
CA-2	Cayasta, Santa Fe, Argentina	−31.186	−60.033	2008	21.0	7.2	11.0	10.00	7.8	—	
FE	Federación, Salto Grande Lake, Argentina	−30.992	−57.915	2010	27.0	7.7	27.9	16.67	8.1	34.0	De León and Guillermo 2003
SG	Salto Grande Dam, Uruguay	−31.249	−57.952	2008	27.7	7.9	5.8	16.35	8.6	17.0	
PU	Puerto Luis, Salto Grande Lake, Argentina	−31.249	−57.951	2010	27.7	7.9	5.8	16.35	8.6	17.0	
SA	Setubal Lagoon, Santa Fe, Argentina	−31.635	−60.681	2007	20.2	7.2	27.9	5.40	7.7	—	Devercelli and Peruchet 2008
SO	Sao Gonçalo Channel, Brazil	−31.811	−52.388	2007	18.6	6.9	49.0	5.19	9.4	71.5	Yunes et al. 1996; Paiva et al. 2008; Da Rocha et al. 2009
UR	Uruguay River, Colon, Argentina	−32.152	−58.189	2010	19.9	7.2	5.8	2.50	7.9	17.0	L. J. Janiot unpubl.
RT	Río Tercero Dam, Cordoba, Argentina	−32.213	−64.473	2008	19.4	8.2	14.5	6.35	9.0	4.2	Mac Donagh et al. 2008; Boltovskoy et al. 2009
EC	Del Este Channel, Buenos Aires, Argentina	−34.346	−58.519	2010	20.9	7.1	10.8	2.78	—	41.9	Gómez et al. 1998; Cataldo et al. 2001
CR	Carapachay River, Buenos Aires, Argentina	−34.398	−58.594	2008	19.0	7.1	8.9	4.35	6.6	69.5	Pizarro and Alemanni 2005; Pizarro et al. 2007
TI	Lujan River, Tigre, Buenos Aires, Argentina	−34.416	−58.578	2010	19.0	7.1	8.9	4.35	6.6	69.5	Cataldo et al. 2001
SF	Lujan River, San Fernando, Argentina	−34.428	−58.552	2008	19.0	7.1	8.9	4.35	6.6	49.7	
BA	Buenos Aires city, Argentina	−34.606	−58.345	2008	23.1	7.3	23.5	4.09	5.7	70.4	Nagy et al. 2002; Calliari et al. 2005
QU	Quilmes, Buenos Aires, Argentina	−34.716	−58.214	2010	23.1	8.4	23.5	4.09	10.7	70.4	Calliari et al. 2005
PL	Punta Lara, Buenos Aires, Argentina	−34.782	−58.011	2010	23.1	8.4	23.5	4.09	9.4	70.4	
SL	Santa Lucía River, Canelones, Uruguay	−34.810	−56.431	2007	20.8	7.8	—	6.11	8.2	29.2	Nagy et al. 2002
MA	Magdalena, Buenos Aires, Argentina	−35.013	−57.536	2010	23.1	8.2	23.5	4.70	8.7	58.2	Calliari et al. 2005

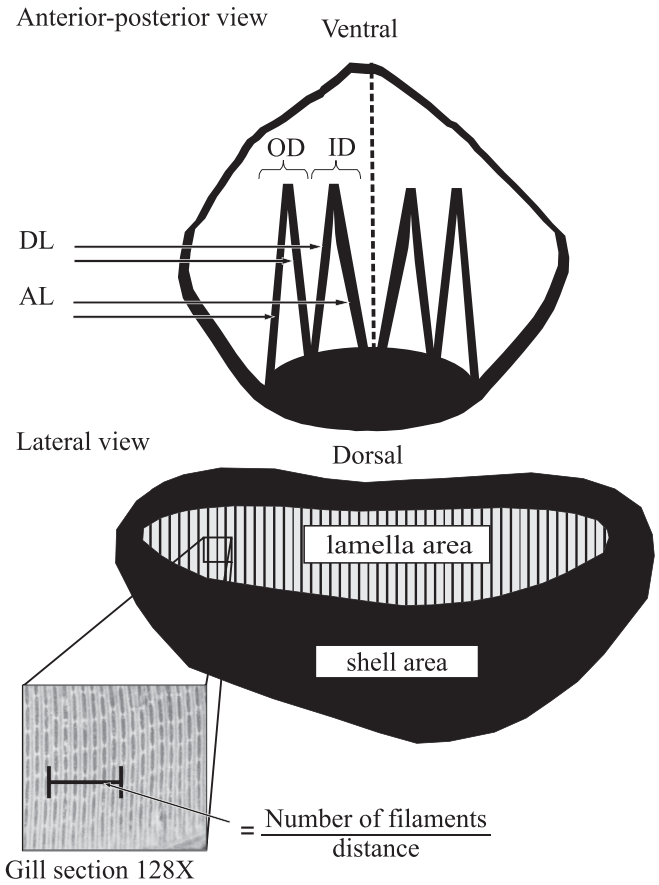

Fig. 2. Anteroposterior and lateral view of the *L. fortunei* gills. Inner demibranch (ID), outer demibranch (OD), descending lamella (DL), ascending lamella (AL). Detail of the gill section used to calculate the number of filaments per millimeter (lower left corner).

adjusted. Individual haplotypes were identified using DNA Sequence Polymorphism version 5 (Rozas et al. 2003).

Evolutionary relationships among haplotypes were examined using Bayesian inference (BI) and neighbor-joining (NJ) phylogeny reconstructions, with the brown mussel *Perna perna* used as an outgroup. All molecular evolution model parameters were estimated using MrModeltest version 3.7 (Posada and Crandall 1998) with the Akaike Information Criterion. Bayesian analysis was conducted with MrBayes version 3.2 (Ronquist and Huelsenbeck 2003). Trees were sampled every 100 generations for two million generations, and the first 25% of all the trees sampled before convergence were discarded as burn-in. NJ phylogenetic analyses were performed using Molecular Evolutionary Genetics Analysis version 4.0 (Tamura et al. 2007), based on nucleotide distances corrected using the Tamura–Nei model (Tamura and Nei 1993). Clade support was estimated using bootstrap analysis with 1000 replicates. Additionally, relationships among haplotypes were further examined using a statistical parsimony haplotype network generated at the 95% connection limit, using software developed by Templeton, Crandall, and Sing (TCS) version 1.21 (Clement et al. 2000).

Intrapopulation genetic diversity was measured by the number of haplotypes per population (*Nh*), haplotype diversity (h), and nucleotide diversity (π), using DNA Sequence Polymorphism version 5.00.07 (Rozas et al. 2003). As with the analysis applied to ultrastructure variables, *t*-tests were used to examine differences between groups with the most salient significant differences in macrostructure characteristics with respect to *Nh*, h, and π. A nonparametric Mann–Whitney *U*-test (Statistica 6.0 software) was used to determine differences in individual haplotypes frequencies (which represent a frequency of a given haplotype in a given population) among population groups with the most salient significant differences in macrostructure characteristics.

Morphological and environmental variables—Mean annual values of key environmental parameters, including water temperature, dissolved oxygen concentration (mg L^{-1}), total suspended solids (TSS, mg L^{-1}), pH, and chlorophyll *a* (Chl *a*) concentration (mg L^{-1}), were obtained from the published literature (Table 1). If environmental information was not available for a given sampling site, we looked for the closest available location to our site. Canonical correlation analysis (CCA) was used to evaluate the relationship between log-transformed morphometric data (dependent variables: dry wt, FD, R_{WL}, R_{WH}, and R_{LS}) and environmental parameters (independent variables). CCA allows the assessment of the relationship between two sets of variables and includes a chi-square test on successive canonical roots to assess how many roots should be considered. We used Statistica 6.0 to compute CCA.

Spearman rank correlation coefficients were calculated to explore relationships between the first significant canonical root (independent variable) and log-transformed morphological ratios (macrostructure) and environmental and genetic variables (dependent variables). Environmental and morphological variables with a significant Spearman rank correlation were compared using a *t*-test. Comparison groups were defined using populations separated by ANOVA.

Results

Macrostructure—We observed significant differences among populations with respect to a number of gill and shell morphology attributes, including R_{LS}, R_{WL}, and R_{WH}. Because these two last variables exhibited less pronounced variation among populations than R_{LS}, we used groups defined by the latter to carry out most of the remaining comparisons. ANOVA analysis of the most salient morphological difference, R_{LS}, found three population groups (Fig. 4). Fourteen populations formed the biggest group and had a significantly higher R_{LS} than populations in the other two groups ($F_{21,\ 323} = 31.3$, $p < 0.001$; ANOVA and Tukey's test; Fig. 4). Most of the abovementioned populations also had significantly lower R_{WL} ratios ($F_{21,\ 323} = 28.1$, $p < 0.001$; ANOVA and Tukey's test) and R_{WH} ratios ($F_{21,\ 323} = 14.5$, $p < 0.001$; ANOVA and Tukey's test) as compared to other populations (Fig. 4).

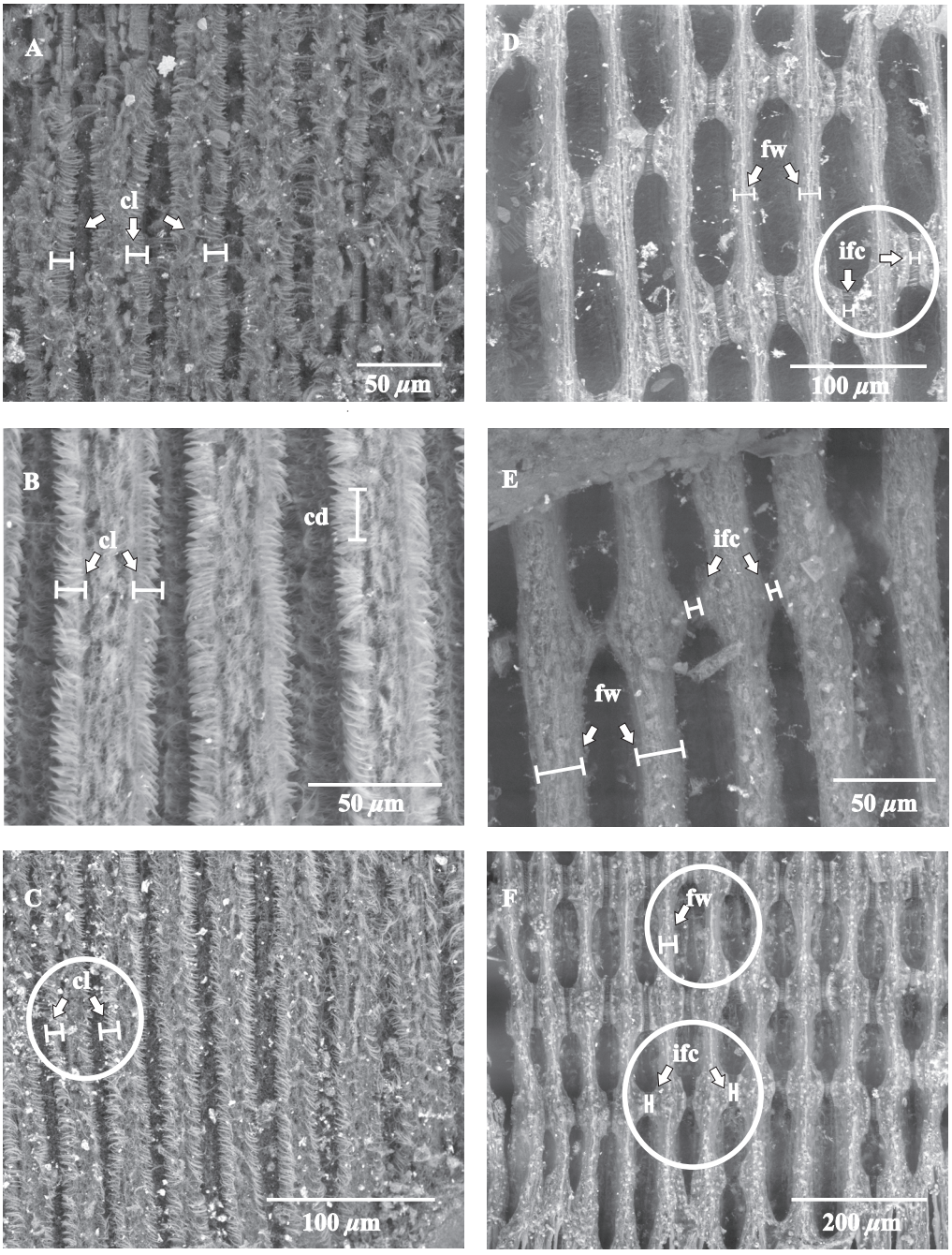

Fig. 3. Scanning electron micrographs of *L. fortunei* inner descending lamellae showing (A–C) outer surface with details of mean cilia length (cl) and mean cilia density (cd) measures: (A) 1000×, (B) 1600×, (C) 1000×; (D–F) inner surface with details of interfilamental ciliary junction distance (ifc) and filament width (fw): (D) 800×, (E) 1200×, (F) 400×.

For the other nine populations, which exhibited low R_{LS} ratios, two overlapped groups were observed. QU displayed the lowest value for R_{LS}, but it was not significantly different from any remaining populations. In addition, QU was not significantly different from other populations with respect to R_{WL} and R_{WH} ratios; therefore, we defined two comparison groups: populations with low R_{LS} (QU, MA, RB, PL, BA, SO, CR, CO, and TI) and high R_{LS} (UR, YR, SA, RT, EC, SL, CA1, CA2, SF, SG, YD, PR, IT, and PU).

Ultrastructure—Strong morphological differences observed among populations with respect to R_{LS} allowed us to group the populations into two main groups and test ultrastructure differences. Mean cilia length, filament width, and interfilamental ciliary junction distance did not differ significantly among populations (*t*-tests, $p > 0.05$; Table 2). However, populations with significantly lower R_{LS} (QU, MA, RB, PL, BA, SO, CR, CO, and TI) exhibited significantly lower mean cilia density and average

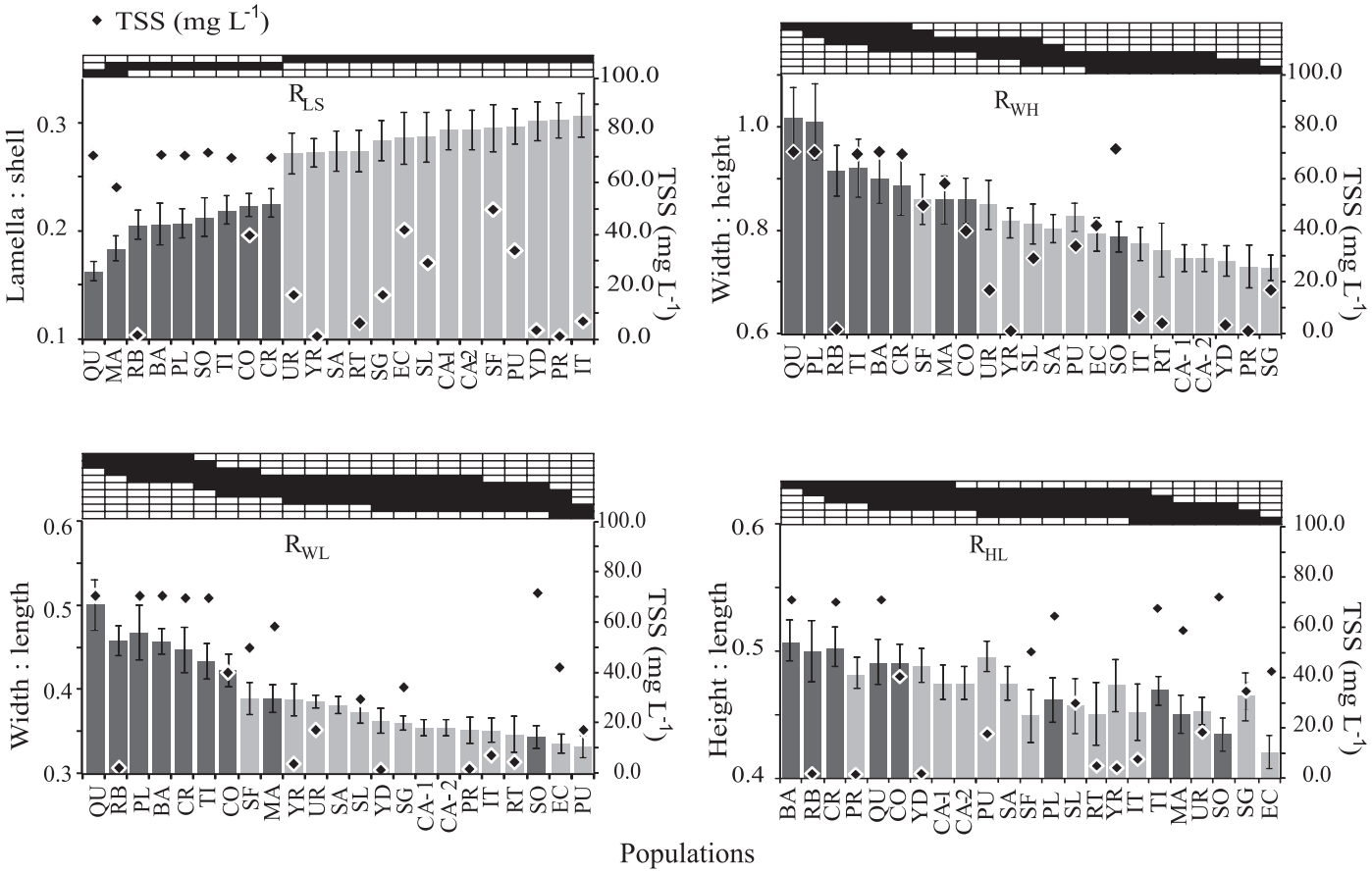

Fig. 4. Morphometric ratios of *L. fortunei* populations studied: R_{WL}, R_{WH}, R_{HL}, and R_{LS}. Populations were ordered to pool those with nonsignificant differences. The grid on top of the figures shows the statistical results (black cells indicate populations that were not significantly different based on Tukey's multiple comparisons test, $\alpha = 0.05$). Black diamonds indicate total suspended solids (TSS) for each population in mg L^{-1}. Bar colors represent groups of similar gill morphology (on all panels).

total gill area as compared to the remaining populations (*t*-tests, $p < 0.001$ and $p < 0.021$, respectively; Table 2).

Genetic analysis—The 510 base pair COI alignment contained 24 polymorphic sites, only 10 of which were parsimony informative. Across all samples analyzed, we identified 18 distinct haplotypes (GenBank accession numbers: HQ843794–HQ843808). The *Nh* varied from four to eight, and *h* ranged from 0.387 to 0.726 (Table 3). The most abundant haplotype (Lfm03) was detected at high frequency in all populations (60.5%). Haplotypes with frequencies higher than 2% were also shared among most populations. Frequencies of all population-specific haplotypes were extremely low (0.1–0.3%). The TCS network was highlighted by a star-shaped pattern with a dominant haplotype (Lfm03) at the center. The other haplotypes were connected to the dominant one (Lfm03) by several mutation steps (Fig. 5a). BI and NJ phylogenetic analyses resulted in very shallow phylogenies (Fig. 5b). Genetic variables, including *Nh*, *h*, and π, did not differ significantly between the two macrostructure morphological groups defined by R_{LS} (*t*-tests, $t = -0.261$, degrees of freedom [df] = 20, $p = 0.796$; $t = -0.827$, df = 20, $p = 0.418$; and $t = 0.366$, df = 20, $p = 0.718$, respectively). Additionally, these two groups did not differ significantly in haplotype frequencies (Mann–Whitney *U*-test, $p > 0.05$; Web Appendix, Table A1, www.aslo.org/lo/toc/vol_59/issue_2/0400a.html).

Morphological and environmental variables—pH and dissolved oxygen concentration exhibited little variation throughout the study area (*see* Table 1). In general, water was alkaline and well oxygenated. The lowest oxygen values were recorded in QU, which is close to a petrochemical complex. Low oxygen values (less than 6.6 mg L^{-1}) were also recorded on the upper Paraguay River (Corumba, Brazil), in the Lujan and Carapachay Rivers, and on the Rio de la Plata River near Buenos Aires. Water temperature followed a latitudinal gradient, with values between 19.0°C in the Río de la Plata River in the south and 28.1°C in Corumba, Brazil, in the north. TSS and Chl *a* concentration exhibited the greatest variation among sampling sites. Chl *a* values indicate oligotrophic conditions in the upper Paraguay and Paraná Rivers and eutrophic conditions in Salto Grande reservoir. Other sampling sites, such as the Middle Paraná River, Paraná delta, and Rio de la Plata estuary, had intermediate Chl *a* levels. TSS ranged from very high values (e.g., 70 mg L^{-1}) in the lower Paraná and Rio de la Plata

Table 2. Ultrastructure and total suspended solids values (mean ± standard deviation [SD]) for two groups of *Limnoperna fortunei* populations defined according to differences in gill macrostructure (*t*-test results). TSS, total suspended solids; *n*, total number of valid observations adding the two population groups (number of observations in each group); df, degrees of freedom; *p*, *p*-values; and *t*, *t* value for *t*-test analysis.

Population groups	Mean cilia length ± SD (μm)	Mean cilia density ± SD (number per μm)	Interfilamental ciliary junctions distance ± SD (μm)	Filament width ± SD (μm)	Average gill surface ± SD (mm^2)	Total shell length ± SD (mm)	TSS ± SD ($mg\ L^{-1}$)
QU, MA, RB, BA, PL, SO, TI, CO, CR	11.8±1.3	16.7±3.2	7.3±4.6	19.3±5	131.3±24.8	15.0±1.2	57.9±23.4
UR, YR, SA, RT, EC, SL, CA1, CA2, SF, SG, YD, PR, IT, PU	11.5±0.4	25.0±2.6	9.0±1.4	16.7±3.7	189.0±65.9	15.6±2.6	17.2±16.7
Statistical analysis							
n	68(29+39)	54(35+19)	33(8+25)	57(19+38)	23(9+14)	23(9+14)	20(9+11)
df	66	52	31	55	21	21	18
t	−0.86	−4.57	0.99	0.92	6.24	0.32	2.7
p	0.395	<0.001	0.328	0.364	0.021	0.576	0.014

Rivers, to intermediate values (40 mg L^{-1}) in the upper Paraguay River, to very low values (1–2 mg L^{-1}) in the upper Paraná River and Rio Tercero reservoir.

CCA performed using biological and environmental parameters revealed clear factor structure and significant correlations with the first canonical root (chi-square test < 0.01). The first canonical root was correlated with R_{LS} and TSS concentration, followed by shell metrics (i.e., R_{WL} and R_{WH} shell ratios). This component explained 24.7% of total variance (first canonical root; Table 4), with main loadings strongly positive with respect to TSS, R_{WH}, and R_{WL} and strongly negative with respect to R_{LS} (Table 4).

Spearman rank correlation analysis demonstrated that the scores of the first canonical root were positively correlated with TSS, R_{WH}, and R_{WL} (Spearman correlation: $r = 0.694$, $p < 0.01$; $r = 0.573$, $p < 0.01$; and $r = 0.635$, $p < 0.01$, respectively; Fig. 6), were strongly negatively correlated with R_{LS} (Spearman $r = -0.754$, $p < 0.01$; Fig. 6), and were not correlated with any measure of genetic diversity (i.e., *Nh*, *h*, or π). In addition to significantly lower R_{LS}, populations QU, MA, RB, PL, BA, SO, CR, CO, and TI had a significantly higher mean R_{WL}, R_{WH}, and TSS (*t*-tests, $t = 4.43$, df = 20, $p < 0.001$; $t = 4.68$, df = 20, $p < 0.001$; and $t = 2.70$, df = 18, $p = 0.015$, respectively).

Discussion

This is the first study to explore morphometric variation in *L. fortunei* throughout the species' introduced range in South America. Our analysis revealed clear differences with respect to gill structure and shell morphometry among populations, which corresponded to environmental factors rather than genetic composition. We observed significant variation among populations with respect to gill proportions and shell morphometry (including width : length and width : height ratios). Similar findings have been reported in other bivalve species (Drent et al. 2004), including the invasive bivalves *C. fluminea*, *Crassostrea gigas*, *D. polymorpha*, and *D. rostriformis bugensis* (Payne et al. 1995; Dutertre et al. 2009; Peyer et al. 2010). Morphometric variation thus appears to allow invasive molluscs to successfully colonize, become established in, and spread from novel environments, including those exhibiting substantial environmental gradients.

One of the most important differences that we observed among populations was with respect to R_{LS}, which was significantly lower in some populations, especially those exposed to a high concentration of TSS (i.e., QU, MA, RB, BA, PL, SO, TI, CO, and CR; Table 1; Fig. 4). Our results support a general tendency established in previous studies: bivalve suspension feeders, such as *D. polymorpha* and *C. gigas,* have smaller gill surfaces in turbid habitats as an adaptive mechanism for optimizing food intake and avoiding gill damage associated with elevated mineral turbidity (Payne et al. 1995; Schneider et al. 1998; Dutertre et al. 2009). Although other factors, such as low dissolved oxygen concentration and high contamination levels, may also play an important role (as can be observed on the significantly lower gill area of mussels found downstream from a petrochemical complex in QU) our analyses suggest

Table 3. Collection details: haplotype number per population (*Nh*); haplotype diversity (*h*); and nucleotide diversity (π) for *Limnoperna fortunei*.

Sample site	Region or state, country	Sample size	*Nh*	Haplotype code(s)	*h*	π
CO	Corumba, Brazil	29	5	Lfm01–05	0.416	0.0021
RB	Rio Baía, Alto Rio Paraná, Brazil	27	5	Lfm01–05	0.724	0.0059
IT	Itaipu Hydroelectric Power reservoir, Brazil	32	6	Lfm01–06	0.625	0.0033
YR	Yabebiry River, Misiones, Argentina	27	5	Lfm01–05	0.704	0.0037
YD	Yaciretá Dam, Brazil, Paraguay and Argentina	34	4	Lfm01, Lfm03–05	0.677	0.0029
PR	Paraná River, Santa Tecla, Corrientes, Argentina	26	6	Lfm01–06	0.609	0.0033
FE	Federación, Salto Grande Lake, Argentina	24	6	Lfm01, Lfm03–05, Lfm07–08	0.638	0.0031
CA-1	Cayasta, Santa Fe, Argentina	27	6	Lfm01–06	0.726	0.0051
CA-2	Cayasta, Santa Fe, Argentina	32	4	Lfm01, Lfm03–05	0.567	0.0022
SG	Salto Grande Dam, Uruguay	46	8	Lfm01, Lfm03–05, Lfm09–12	0.570	0.0031
PU	Puerto Luis, Salto Grande Lake, Argentina	24	6	Lfm01–05, Lfm11, Lfm18	0.630	0.0064
SA	Setubal Lagoon, Santa Fe, Argentina	30	5	Lfm01–05	0.618	0.0042
SO	Sao Gonçalo Channel, Brazil	34	5	Lfm03–06, Lfm10	0.631	0.0034
UR	Uruguay River, Colon, Argentina	23	4	Lfm02–03, Lfm05, Lfm17	0.387	0.0025
RT	Río Tercero Dam, Cordoba, Argentina	59	6	Lfm01, Lfm03–06, Lfm13	0.546	0.0022
EC	Del Este Channel, Buenos Aires, Argentina	24	6	Lfm01–03, Lfm05, Lfm07, Lfm14	0.594	0.0041
CR	Carapachay River, Buenos Aires, Argentina	29	7	Lfm01–07	0.700	0.0035
TI	Lujan River, Tigre, Buenos Aires, Argentina	24	5	Lfm01–05	0.540	0.0068
SF	Lujan River, San Fernando, Argentina	30	5	Lfm01–05	0.575	0.0064
BA	Buenos Aires city, Argentina	52	6	Lfm01–06	0.483	0.0056
QU	Quilmes, Buenos Aires, Argentina	22	4	Lfm03, Lfm05–07	0.541	0.0028
PL	Punta Lara, Buenos Aires, Argentina	21	4	Lfm03–05, Lfm15	0.414	0.0027
SL	Santa Lucía River, Canelones, Uruguay	26	5	Lfm01, Lfm03–05, Lfm10	0.634	0.0038
MA	Magdalena, Buenos Aires, Argentina	22	7	Lfm01–06, Lfm16	0.688	0.0036

that both total gill area and R_{LS} of *L. fortunei* were strongly influenced by the amount of TSS (Fig. 4).

Previous studies have shown physiologically based, temporal variation in gill structure in *D. polymorpha* as a result of increasing concentrations of Ca^{+2} and K^{+}. This resulted in muscle contraction, decreasing the interfilament distance and, consequently, the gill area (Medler and Silverman 1997; Medler et al. 1999). However, a lack of significant difference in interfilament spaces in gills among *Limnoperna* populations supports the idea that gill variation is not simply an ephemeral reaction to the environment at that moment, but rather appears to be a permanent feature developed during growth in different environments. Previous studies demonstrated that the relationship between pallial organs and turbidity is more complex and may involve other environmental and morphological factors, including availability and quality of food, or dimension of the labial palp (Payne et al. 1995; Drent et al. 2004; Dutertre et al. 2009). In general, a higher concentration of food does not cause a change in gill area; rather it effects a relative increase in the ratio of labial palp area to gill area, which results in mussels having a higher food-sorting capacity (Drent et al. 2004). It is also possible that the relative decrease in gill area was accompanied by a relative increase in palp area, as has been observed in preliminary data from the Uruguay River (E. M. Paolucci unpubl.).

Mussels in our study could be separated into two shell shape groups, which, as with gill structure, were correlated with TSS concentration. The first grouping had higher width : length and width : height shell ratios, whereas the second had a more elongate shell shape. Differences in shell morphology as an adaptation to new environments have been recorded in a number of alien bivalve species, such as *D. polymorpha*, *D. bugensis,* and *C. fluminea* (Marsden et al. 1996; Sousa et al. 2007; Peyer et al. 2010). Shell shapes, particularly the width : length and width : height ratios, may represent an adaptive response to different environmental conditions, such as population density, predation pressure, depth, substrate type, and food availability, among others. Despite the apparent complexity of the interaction between shell shape and environment, a recent experimental approach observed significant developmental plasticity in shell morphology (i.e., width : length and width : height ratios) of *D. rostriformis bugensis* (*D. bugensis*) exposed to different temperatures, regardless of food quantity or level of water motion (Peyer et al. 2010). The authors found that differences in temperature affect growth rate and result in production of two different morphotypes—shallow and deep quagga mussels—which differ with respect to both width : length and width : height ratios. Although we observed that TSS was better correlated than temperature with mussel morphology, the former factor may also affect mussel growth rate and change shell morphology in consequence.

Although mussels displayed mixtures of gill and shell characteristics in some populations (MA, CO, and SO), our results revealed an association between shell shape and R_{LS}, with more elongated organisms having higher R_{LS}s and shorter ones lower R_{LS}s (Fig. 2). This association appears to be related to developmental plasticity (Drent et al. 2004; Peyer et al. 2010). Some environmental factors

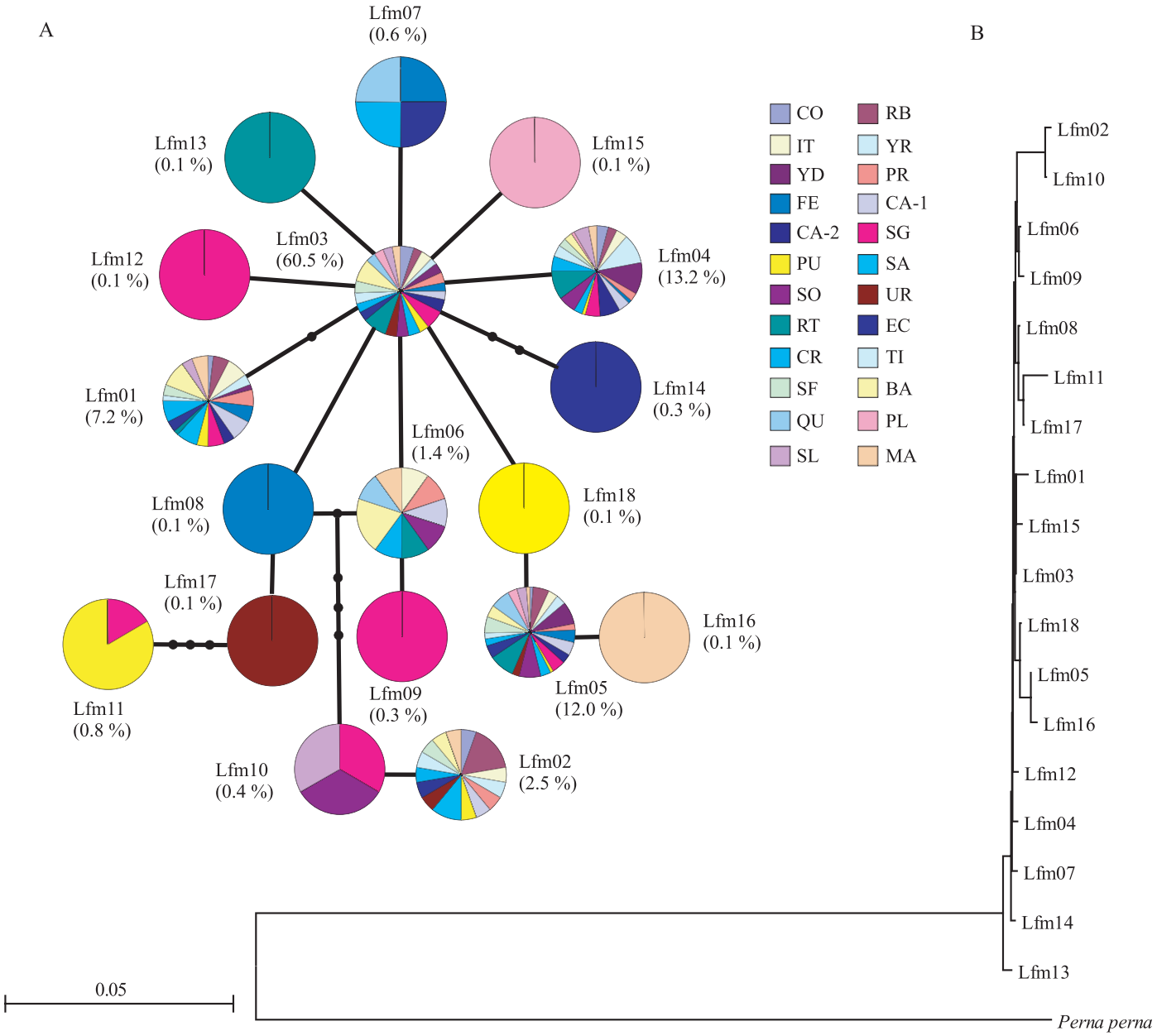

Fig. 5. (A) Parsimony haplotype network and (B) NJ phylogenetic tree based on mitochondrial COI haplotypes for *L. fortunei* in South America. Percentages indicate relative number of individuals carrying the haplotype. Dark circles indicate unsampled haplotypes. The green mussel *Perna perna* was used as an outgroup. Colors indicate sampled locations as per Table 1. Haplotype names as per Table 3.

can have a strong influence on growth rates and, consequently, on shell shape. The same factors that affect the development of gills in this study (e.g., TSS) may also affect growth and, consequently, modify shell proportions.

Our results indicate that, although *h* was relatively high, genetic structure was very low and could not account for morphological variability among populations. Similar findings have been reported for other bivalves (Sousa et al. 2007). A previous study observed substantial microsatellite variation among *Limnoperna* populations (Zhan et al. 2012), yet even these patterns do not coincide with our observed patterns of morphological variation. Rather, our combined results support the hypothesis that morphological differences result from phenotypic plasticity. However, this is the first study comparing morphological and genetic variation in *L. fortunei*, and further studies may be necessary in order to assess the importance of the genetic component on shell and gill morphology for this species.

Morphological variation in key characters, such as gill structure, may play an important role in the successful spread of *L. fortunei* and other invasive bivalves (Payne et al. 1995; Sousa et al. 2007; Dutertre et al. 2009). Although it is not clear whether developmental plasticity confers competitive advantage on these invasive molluscs relative

Table 4. The canonical loadings of the dependent and independent variables and eigenvalues on the first three canonical roots. TSS, total suspended solids; R_{WH}, width : height shell ratio; R_{WL}, width : length shell ratio; WT, water temperature; Chl *a*, chlorophyll *a*; FD, filament density; DO, dissolved oxygen; dry wt, dry weight; R_{LS}, lamella area : shell area ratio.

	Component		
Variable	Root 1	Root 2	Root 3
TSS	0.769	0.331	−0.094
R_{WH}	0.510	0.476	0.631
R_{WL}	0.347	0.358	0.859
pH	0.137	0.413	−0.215
WT	0.121	−0.887	−0.023
Chl *a*	0.116	0.270	−0.573
FD	−0.067	−0.469	−0.222
DO	−0.080	0.398	−0.659
Dry wt	−0.221	0.757	0.510
R_{LS}	−0.831	−0.249	0.041
Eigenvalues	0.247	0.174	0.079

to native or even other introduced species (Peyer et al. 2010), a comparison of morphologic characteristics and phenotypic plasticity of different invasive bivalves can help illuminate factors contributing to invasion success and the impacts of these species in invaded areas. *L. fortunei* in our study averaged 166.4 ± 60.2 mm² in R_{LS} for animals that averaged 15.4 ± 2.1 mm shell length (Table 2). These values are slightly higher than those reported for bivalves such as *D. polymorpha* (139.0 ± 76.0 mm² for individuals 14.6 ± 3.3 mm in length; Payne et al. 1995; Lei et al. 1996). This difference was even higher when only populations with high lamella : shell area ratios were considered (average 189.0 ± 65.9 mm², shell length 15.6 ± 2.6 mm). The higher filtering surface area of *L. fortunei* as compared to *D. polymorpha* was described earlier by Morton (1973) and may explain the higher filtration rate recorded by the former (Sylvester et al. 2005). Such plasticity would appear particularly advantageous in very dynamic environments, such as the Rio de la Plata basin, which, with its extensive wetland areas, can experience dramatic changes in environmental conditions. Given the dramatic changes in ecosystem characteristics caused by introduced *D. polymorpha* populations in invaded

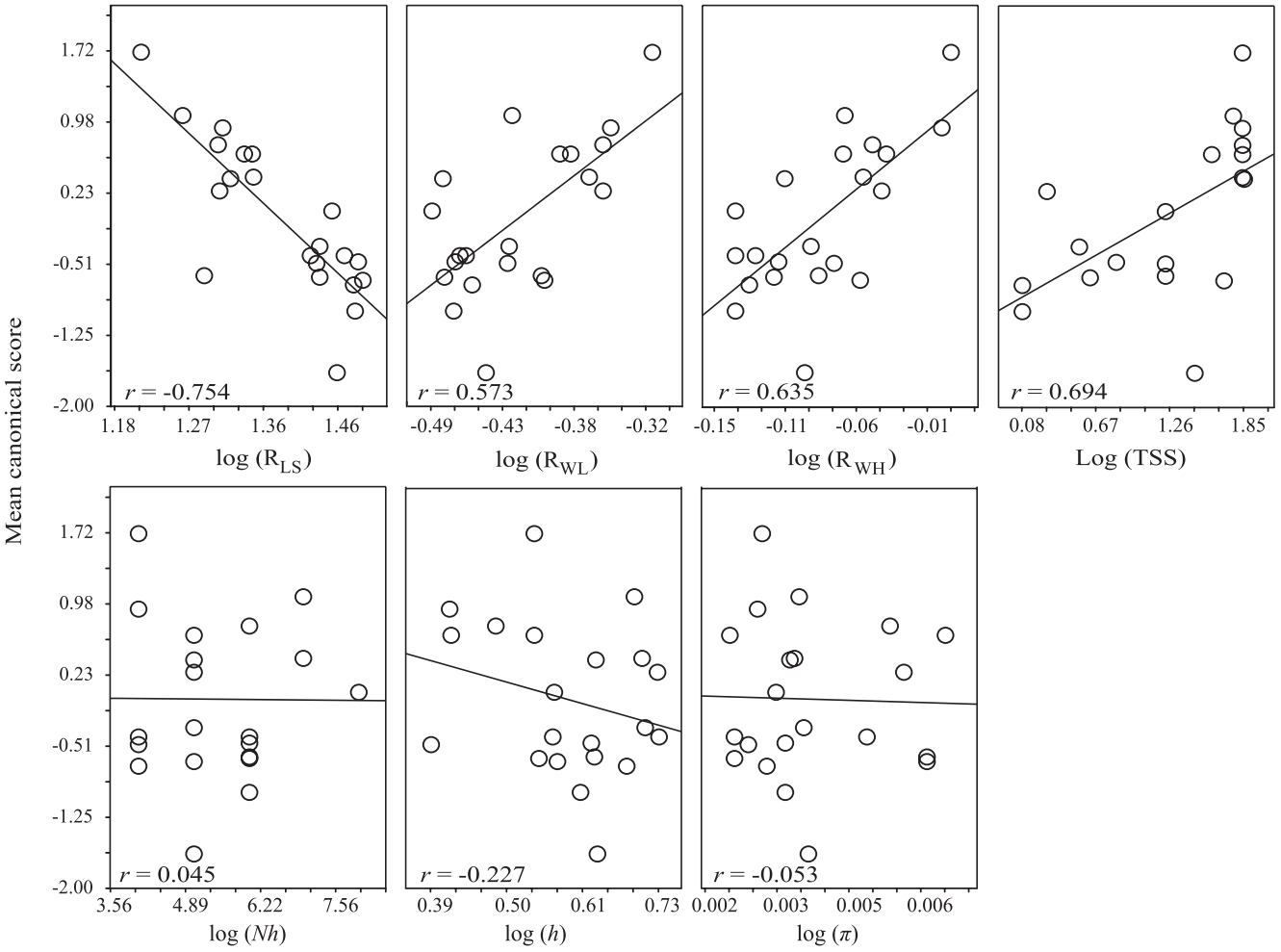

Fig. 6. Spearman correlation between the first canonical root and morphological, environmental, and genetic variables. Lamella area:shell area (R_{LS}), shell width:shell length (R_{WL}), shell width:shell height (R_{WH}), total suspended solids (TSS), haplotype number (*Nh*), haplotype diversity (*h*), and nucleotide diversity (π).

systems across North America and parts of Europe, colonization and spread of an even more potent ecological engineer—the golden mussel *L. fortunei*—must be prevented (Karatayev et al. 2007; Boltovskoy et al. 2009).

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