EVOLUTION & DEVELOPMENT 12:6, 628–634 (2010)
DOI: 10.1111/j.1525-142X.2010.00447.x

Heterochronic phenotypic plasticity with lack of genetic differentiation in the southeastern Pacific squat lobster *Pleuroncodes monodon*

Pilar A. Haye,[a,*] Pilar Salinas,[b] Enzo Acuña,[a] and Elie Poulin[b]

[a]Departamento de Biología Marina, Facultad de Ciencias del Mar, Universidad Católica del Norte and Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Larrondo 1281, Coquimbo, Chile
[b]Instituto de Ecología y Biodiversidad, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Santiago, Chile
*Author for correspondence (email: phaye@ucn.cl)

SUMMARY Two forms of the squat lobster *Pleuroncodes monodon* can be found along the Pacific coast of South America: a smaller pelagic and a larger benthic form that live respectively in the northern and southern areas of the geographic distribution of the species. The morphological and life history differences between the pelagic and benthic forms could be explained either by genetic differentiation or phenotypic plasticity. In the latter case it would correspond to a heterochronic phenotypic plasticity that is fixed in different environments (phenotype fixation). The aim of this study was to evaluate whether the two forms are genetically differentiated or not; and thus to infer the underlying basis—heritable or plastic—of the existence of the two forms. Based on barcoding data of mitochondrial DNA (the COI gene), we show that haplotypes from individuals of the pelagic and benthic forms comprise a single genetic unit without genetic differentiation. Moreover, the data suggest that all studied individuals share a common demographic history of recent and sudden population expansion. These results strongly suggest that the differences between the two forms are due to phenotypic plasticity.

INTRODUCTION

Evolutionary biologists and ecologists use the term phenotypic plasticity to describe the ability of an organism to alter its physiology, morphology, or development in response to changes in its environment (Bradshaw 1965; Callahan et al. 1997). Based on the adaptive plasticity hypothesis, phenotypic plasticity evolves to maximize fitness when the environment is variable and is a common response to environmental gradients (Via et al. 1995; Agrawal 2001). For marine invertebrates it has been shown that those with low dispersal potential have lower levels of phenotypic plasticity than those with high dispersal potential (i.e., long-lived planktonic larvae) (Hollander 2008), and that crustaceans often display phenotypic plasticity. For example, some brachyuran crab species that have a larva that lives in the plankton for several weeks, develop stronger and larger claws in response to diet (Smith and Palmer 1994; Smith 2004; Taylor et al. 2009), and neither the color forms nor the size differences could be attributed to genetic differentiation (Reuschel and Schubart 2007), demonstrating that the size differences correspond to phenotypic plasticity. Phenotypic plasticity has been reported in many marine species, even some with low dispersal potential, such as the isopod *Idotea granulose* that displays morphological variation correlated with rockpool algal diversity (Hull et al. 2001).

Phenotypic plasticity in the developmental pathway is an important mode of evolution for many marine organisms (e.g., Hunda and Hughes 2007; Nielsen 2008). Heterochronic phenotypic variation has been reported for several species. For crustaceans it has been associated with an abbreviated larval development (Makarov and Maslennikov 1981; Clark 2005; Delgado and Defeo 2006; Kavanagh et al. 2006; Ozawa and Ishii 2008). In some species, heterochronic plasticity translates into the existence of more than one form of adult, but without genetic differentiation between forms. Heterochronies leading to phenotypic plasticity have been generally associated with a response of the species to a varying environment (Scheiner 1993; Denoël and Joly 2000; Parsons et al. 2010).

The squat lobster *Pleuroncodes monodon* Milne Edwards 1837, has a high dispersal potential given by a long-lived larva and has a wide range of geographic distribution (at least from 6°S to 40°S) (Haig 1955; 1968; Acuña et al. 2003). There is a striking difference in morphology and life history/behavior between individuals of the north and south of the range of distribution of *P. monodon*. In the north there is a pelagic adult form (up to 25°25.48′S) (Barbieri et al. 2001) associated mainly to pelagic fisheries resources like anchovy (Rivera and Santander 2005; Gutiérrez et al. 2008). Southern adult individuals of *P. monodon* are benthodemersal and thus associated with a bottom trawl fishery (Acuña et al. 2003).

Fig. 1. *Pleuroncodes monodon*. Size difference between benthic (A) and pelagic (B) forms along the southeast Pacific.

Interestingly, the northern pelagic form is characterized by having a much smaller size when reaching reproductive maturity (Barbieri et al. 2001) than the benthic southern form (Fig. 1). Males and females captured in the northernmost region of Chile had between 12 and 22 mm cephalothoracic length (CL) (mean = 15.1 mm) and between 11 and 17 mm CL (mean = 14 mm), respectively (Barbieri et al. 2001). In contrast, individuals of the southern area are much larger at maturity (CL from 20.6 to 21.9 mm). Thus, pelagic northern forms are smaller than the southern benthic ones and do not go through a benthic stage. This pelagic form has been also described for the congener *Pleuroncodes planipes*, and Kashkina and Kashkin (1993) refer to the phenomenon as a recent pelagization based on progenesis, which is the acquisition of sexual maturity earlier in ontogeny.

The morphological and ecologic/behavioral differences between the pelagic and benthic forms of species of the genus *Pleuroncodes* are puzzling. Genetic differentiation and phenotypic plasticity are mutually exclusive ways of achieving adaptation to a heterogeneous environment (Thoday 1953; Jain 1979; Hazel et al. 1987; Borenstein et al. 2006; Crispo 2008). It may be that the two forms correspond to different species. In this scenario there would be strong genetic differentiation between the forms. A suitable marker for differentiating species is the COI gene of the mitochondrial DNA (mtDNA). The sequence of this gene has been shown to be a good barcoding marker to differentiate species (Hebert et al. 2003; Rubinoff 2005; Costa et al. 2007; Radulovici et al. 2009).

Alternatively, the two forms of *P. monodon* may be one species with strong developmental phenotypic plasticity. If the occurrence of two forms in *Pleuroncodes* spp. is a consequence of phenotypic plasticity, the mechanism of plasticity could be a heterochronic process such as paedomorphic plasticity, because in the northern area individuals either have slower growth rate (neoteny), or same growth rate but acquire maturity at smaller size (progenesis) (McNamara 1986; Klingenberg 1998). On the other hand, the phenotypic plasticity could correspond to phenotype fixation, a kind of plasticity wherein each environment

harbors one form (West-Eberhard 1989). In the case of *P. monodon*, only one form is present in each the pelagos and the benthos. West-Eberhard (1989) emphasizes that phenotype fixation "can occur with little or no genetic change, if the environment of the population uniformly induces or selects for a single alternative."

Analyses of the geographic distribution of the genetic diversity of species have proven insightful to discriminate between alternative scenarios that could explain morphological variation (e.g., Robinson and Wilson 1996; Snell-Rood et al. 2010). For example, Spivak and Schubart (2003) reported a lack of genetic differentiation between two forms of a brachyuran crab, and suggested that analyzed samples represent a single species that displays morphological differentiation. With the goal of assessing the degree of differentiation between pelagic and benthic forms of *P. monodon* and thus, determine the existence of two species or one species with heterochronic phenotypic plasticity, we undertook an analysis of partial sequences of the mtDNA COI gene, including samples of both forms.

MATERIALS AND METHODS

Individuals of *P. monodon* were collected between 2004 and 2006 from nine sites in the southeast Pacific from 06°30′S to 36°60′S, covering a wide portion of the species geographic range of distribution (Fig. 2A). The maximum linear distance between sampling sites was approximately 3500 km. Samples were obtained from pelagic purse seine tows in the northern area and from bottom trawl tows in the southern area. Twenty-two individuals of each form were used in this study. This sample size is large enough to obtain a representative barcode for a species since usually five to 10 individuals per species are used (Hajibabaei et al. 2007). Total genomic DNA was extracted using the QIAamp DNA mini kit following manufacturers standard protocol. DNA was eluted in 200 µl.

Polymerase chain reaction (PCR), using the universal primers HCO and LCO (Folmer et al. 1994), was used to amplify a portion of the COI gene of the mtDNA. PCR's were performed in 10 µl reactions with: 30 ng of DNA, 2.5 mM $MgCl_2$, 0.2 mM of a dNTP mix, 1 × buffer, and 0.8 µM of each primer. The cycling program consisted of a 10 min initial soak at 94°C, followed by 35 cycles of 1 min at 95°C, 1 min at 40°C, and 2 min at 72°C, ending with a final extension of 13 min at 72°C. Success of PCR was visualized after horizontal 1% agarose DNA electrophoresis with diluted ethidium bromide using a UV-transilluminator and a 100 bp ladder to determine amplicon size. Amplicons were purified using a PCR Purification Kit (Qiagen Inc., Valencia, CA, USA) following manufacturer's protocol. Sequencing reactions were performed in both directions with an ABI 3730XL capillary automated sequencer (Applied Biosystems, Carlsbad, CA, USA).

Unique sequences for each individual and alignment of sequences using default parameters were obtained using the software CODONCODE ALIGNER 2.0.4 (CodonCode). Alignment was visually verified and sequences were truncated at each extreme to produce equal size aligned sequences for each individual. Haplotype sequences were deposited in the NCBI GenBank database (GenBank accessions HQ284198-HQ284237).

Phylogenetic analyses were conducted using Maximum-Likelihood (ML) in PAUP* 4.0b10 (Swofford 2002) and Bayesian approach implemented in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), incorporating the model of molecular evolution HKY = I+G selected as the one that best fit the data by MrModeltest (Nylander 2004). For both analyses, the COI sequence of *Cervimunida johni* (GenBank HQ284238) was used as outgroup. For ML, branch support was obtained from 10,000 replicates of a bootstrap analysis. For Bayesian, the settings were two simultaneous runs of Markov chain Monte Carlo for seven million generations, with a burn-in of 25%.

To determine the genetic relationship between COI haplotypes and explore processes of population dynamics and represent genealogical relationships among haplotypes, a median-joining network was constructed using NETWORK 4.5 (Bandelt et al. 1999). The coalescent criteria of Crandall and Templeton (1993) were used to resolve loops. ARLEQUIN 3.11 (Excoffier et al. 2005) was used to calculate the Φ_{ST} value, and associated probability between pelagic and benthic forms, and determine if they are significantly genetically differentiated.

DNASP 4.10.2 (Rozas et al. 2003) was used to estimate genetic diversity indices (total and for each of the two forms), perform mismatch distribution analysis (MDA) and calculate neutrality statistics on all the data. The estimated genetic diversity indices were: number of segregating sites, number of haplotypes, haplotype diversity, nucleotide diversity, and mean number of pairwise differences. Tajima's *D* (Tajima 1989) and Fu's *F* (Fu 1997) statistics of neutrality were used to test for sudden changes in effective population size based on departures from neutrality given by excesses of rare alleles and young haplotypes. In the MDA, which is the distribution of the observed number of differences between pairs of haplotypes, statistically significant differences between observed and simulated sudden-expansion distribution were evaluated with Harpending Rugged Index (*r*). When mutations accumulate with low lineage loss, the MDA curve is smooth and unimodal (Rogers and Harpending 1992; Excoffier 2004).

RESULTS

The final aligned truncated data set was composed of 44 sequences (22 of each form) of 576 base pairs in length, consisting of 40 haplotypes. Phylogenetic analyses with ML and Bayesian approaches resulted in similar tree topologies. They show that there is no reciprocal monophyly of the COI sequences of each form. The position of some haplotypes varied between topologies resulting from ML and Bayesian approaches probably as a consequence of the very short genetic distances between haplotypes (Fig. 2B). As expected with such closely related haplotypes, only nine nodes had posterior probabilities over 0.5 and of those three had values ≥ 0.75. Within two of these three well-supported clades there are samples from the two forms included. Similarly, only six nodes showed bootstrap support values $>50\%$, and of those,

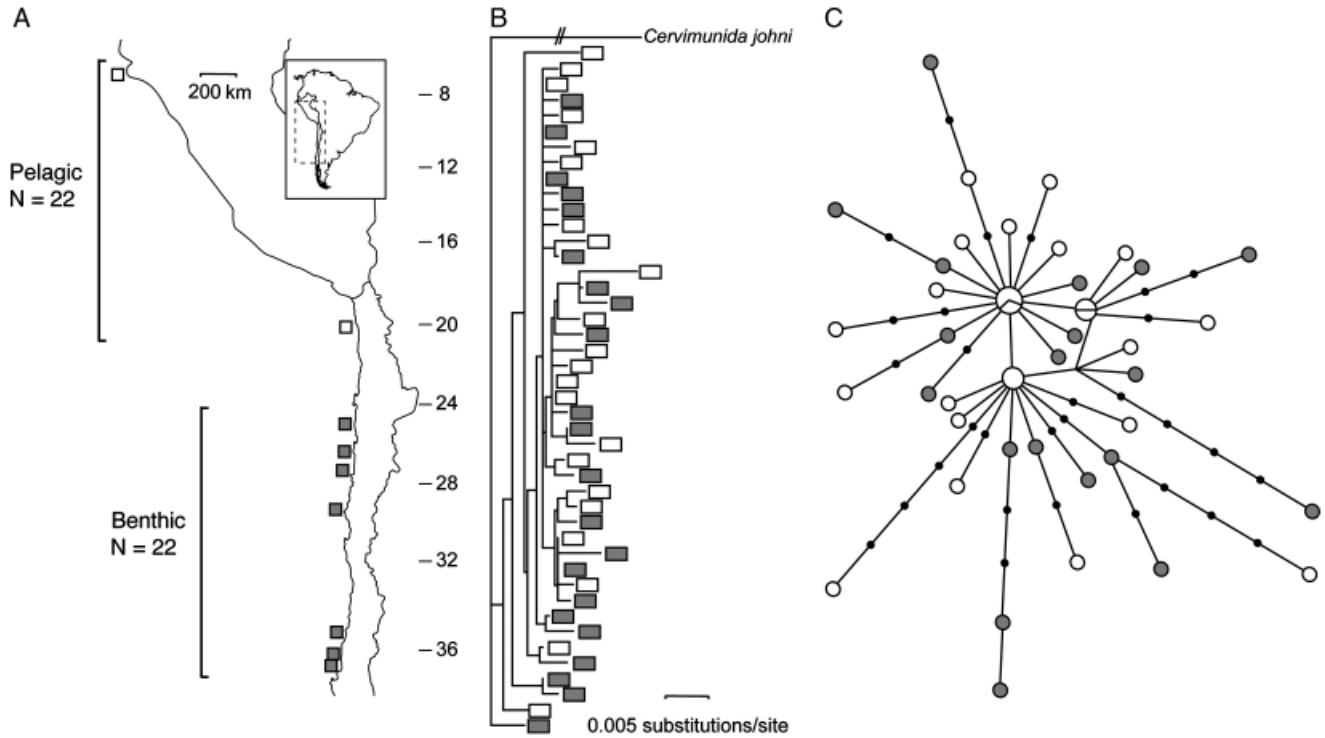

Fig. 2. *Pleuroncodes monodon*. (A) Map with sampling locations along the southeast Pacific. White squares represent sampling sites within the northern area that harbors the pelagic form and gray squares the southern area localities with benthic form. (B) Result of ML phylogenetic analysis. At the end of each branch there is a white or gray rectangle in concordance with the sampling areas shown in map (A). (C) Median-joining haplotype network. Circles represent haplotypes and their size is proportional to their frequency. Colors relate to northern and southern areas as shown in map (A). Small black circles represent unsampled or hypothetical haplotypes.

just one was >85%. The 40 haplotypes form a haplotype network shaped as a very ramified star where haplotypes from northern and southern areas are mixed (Fig. 2C). Both phylogenetic and haplotype network analyses show that individuals from the two forms belong to the same species and that they are highly genetically connected. Also, pairwise Φ_{ST} between forms ($\Phi_{ST} = -0.013$, $P = 0.876$) shows that there is no genetic differentiation between pelagic and benthic forms.

There is an overall very high genetic diversity that is similar among forms (haplotype diversity is 0.995 for the total samples and for each form separately) (Table 1). Differences between pairs of sequences, seen in a mismatch frequency distribution graph, show a similar distribution to the one expected for a population that has suffered fast growth approximately two mutational times ago ($r = 0.02$; $P = 0.68$). The significant and negative Tajima's D ($D = -1.875$) and Fu's F values ($F = -9.701$), give additional support for a population expansion. Demographic data is consistent with individuals of both forms sharing a demographic history of a population expansion.

DISCUSSION

The results show no evidence of genetic divergence or geographic genetic structure between the pelagic and benthic forms of *P. monodon*, suggesting that local populations comprise a single genetic unit along the coasts of Peru and Chile in the southeast Pacific. Thus, morphological and ecological/behavioural differences between forms of *P. monodon* are likely a consequence of heterochronic phenotypic plasticity with phenotype fixation.

The complete lack of genetic differentiation between forms (according to Φ_{ST} values) is compatible with a high gene flow scenario between environments that bear different squat lobster forms. Additionally, phylogenetic and haplotype network analyses show that the two squat lobster forms are not reciprocally monophyletic, and demographic genetic analyses suggest that both forms have a common population history. Since COI is usually highly variable within crustacean species (e.g., Sotka et al. 2004; Trontelj et al. 2005; Remerie et al. 2006; Beenaerts et al. 2010), and considering the lack of COI differentiation between forms, it is unlikely that another genetic marker (i.e., nuclear variable sequences) may yield significant genetic differences between the two forms of *P. monodon*.

Heterochrony has been recognized as a major kind of developmental dissociation that increases morphological evolvability and is thus an important mode of evolution (Cunningham and Buss 1993; Klingenberg 1998; Brigandt 2006; Moczek 2008). For an isopod species of the genus

Table 1. Genetic diversity indices of *Pleuroncodes monodon* along the SE Pacific for each of the forms and all individuals

Form	*N*	*S*	*H*	*h*	π	*k*
Pelagic	22	35	21	0.995	0.007	4.325
Benthic	22	30	21	0.995	0.007	4.893
Total	44	48	40	0.995	0.007	4.430

N, sample size; *S*, number of segregating sites; *H*, number of haplotypes; *h*, haplotype diversity; π, nucleotide diversity; *k*, mean number of pairwise differences.

Haplomesus, the absence of the seventh thoracic appendage is suggested to be due to heterochrony, that is, the ancestral juvenile character state of bearing six pereopods is retained resulting in a paedomorphic species (Kavanagh et al. 2006). There are varying examples of heterochrony within crustacean species. For example, there is paedomorphic plasticity in the carapace hingements and inner marginal carapace area of ostracods (Ozawa and Ishii 2008). Similar to the kind of heterochrony that may be occurring in *P. monodon*, some other species of Crustacea show variations in the timing of appearance and rates of character development, which occur at different stages in abbreviated larval development, such as euphausiids, brachyurans, and anomurans (Makarov and Maslennikov 1981; Clark 2005; Delgado and Defeo 2006). Particularly for the anomuran mole crab, *Emerita brasiliensis*, Delgado and Defeo (2006) state "The principal benefit to early maturation is demographic: sexually mature neotenic males are most likely to succeed since they have less chance of dying before maturation." In this species, both long and abbreviated ontogenetic pathways are found, and the long one is probably maintaining the basal population. The short pathway seems to be a response to population density and female biased sex ratio. In 1967, Efford had already defined neoteny as a characteristic of males of the genus *Emerita*. Species of the genus *Pleuroncodes* may have a similar strategy in which depending on environmental conditions, individuals undergo the long or abbreviated ontogenetic pathways.

What may be the environmental trigger of the paedomorphic phenotype fixation observed in *P. monodon*? Around 25°S is the southern limit of the geographic distribution of the pelagic form. Although apparently there are no sharp differences in environmental conditions (e.g., pronounced peninsula, particularly strong upwelling, current features) at approximately 25°S, some differences have been reported. For example, from 18°S to 24°S, the Chilean Counter Current, that is closer to the coast than the Humboldt Current and flows predominantly equatorward, has its seasonal maximum in the autumn while it becomes seasonally variable south of approximately 25°S (Blanco et al. 2001; Thiel et al. 2007). Also there are biotic differences north and south of 24–25°S. Both demersal and littoral fishes (Mann 1954; Sielfeld and Vargas 1996; Ojeda et al. 2000) as well as peracarids (Thiel 2002) show a break in the species distribution at 24–25°S (see Thiel et al. 2007). This latitude is not considered as a major biogeographic break of the coast of Chile, but it is remarkable that some taxa display a break at 24–25°S and that this coincides with a "break" in the distribution of the forms of *P. monodon*. Another relevant feature of the north southeast Pacific is that the upper boundary of the Oxygen Minimum Zone (OMZ) is very shallow, over 150 m, and when approaching the coast it extends into the shallow euphotic zone (Morales et al. 1999; Levin 2003; Helly and Levin 2004; Fuenzalida et al. 2009). Interestingly, the bathymetric distribution of *P. monodon* overlaps with the OMZ between 50 and 600 m (Roa et al. 1995). Even though oxygen concentration may have phenotypic and/or deleterious effects on organisms in general (Crispo and Chapman 2008; Ulloa and Pantoja 2009), it has been shown that *P. monodon* has physiological adaptations to live in low oxygen zones. The shallow OMZ may even represent a predation refuge for the species (Villarroel et al. 2001). It is unknown the extent of the OMZ and its effect on benthic communities (Thiel et al. 2007) so maybe, indirectly, it is still affecting *P. monodon* in spite of its own adaptability to low oxygen regimes. Whether one or several of these environmental features are associated with the observed phenotypic plasticity on *P. monodon* has yet to be explored.

Acknowledgments

This work was partially funded through grants FIP N° 2004-11 (EA and PAH) and by the Projects P05-002 ICM and PFB 023 (EP and PS). We thank Raúl Vera, Natalia Muñoz and Mary Gallardo for miscellaneous help given at some stage of this project. Mariano Gutiérrez of the Instituto del Mar del Perú (IMARPE) provided the northernmost samples.

REFERENCES

Acuña, E., González, M. T., and González, M. 2003. Pesquerías de langostinos y camarón nailon en el Norte de Chile. In E. Yañez (ed.). *Actividad Pesquera y de Acuicultura en Chile*. Pontificia Universidad Católica de Valparaíso, Escuela de Ciencias del Mar, Valparaíso, pp. 263–287.

Agrawal, A. A. 2001. Phenotypic plasticity in the interactions and evolution of species. *Science* 294: 321–326.

Bandelt, H. J., Forster, P., and Röhl, A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* 16: 37–48.

Barbieri, M. A., et al. 2001. Evaluación directa de langostino colorado de la I a IV Regiones, 1999. FIP Technical Reports, FIP/IT No. 99-30.

Beenaerts, N., et al. 2010. Phylogenetic diversity of Sri Lankan freshwater crabs and its implications for conservation. *Mol. Ecol.* 19: 183–196.

Blanco, J. L., Thomas, A. C., Carr, M. E., and Strub, P. T. 2001. Seasonal climatology and hydrographic conditions in the upwelling region off northern Chile. *J. Geophys. Res. Oceans* 106: 451–467.

Borenstein, E., Meilijson, I., and Ruppin, E. 2006. The effect of phenotypic plasticity on evolution in multipeaked fitness landscapes. *J. Evol. Biol.* 19: 1555–1570.

Bradshaw, A. D. 1965. Evolutionary significance of phenotypic plasticity in plants. *Adv. Genet*. 13: 115–155.
Brigandt, I. 2006. Homology and heterochrony: the evolutionary embryologist Gavin Rylands de Beer (1899–1972). *J. Exp. Zool*. 306B: 317–328.
Callahan, H. S., Pigliucci, M., and Schlichting, C. D. 1997. Developmental phenotypic plasticity: where ecology and evolution meet molecular biology. *BioEssays* 19: 519–525.
Clark, P. F. 2005. The evolutionary significance of heterochrony and the abbreviated zoeal development of pilumnine crabs (Crustacea: Brachyura: Xanthoidea). *Zool. J. Linn. Soc*. 143: 417–446.
Costa, F. O., et al. 2007. Biological identifications through DNA barcodes: the case of the Crustacea. *Can. J. Fish. Aquat. Sci*. 64: 272–295.
Crandall, K. A., and Templeton, A. R. 1993. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics* 134: 959–969.
Crispo, E. 2008. Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. *J. Evol. Biol*. 21: 1460–1469.
Crispo, E., and Chapman, L. J. 2008. Population genetic structure across dissolved oxygen regimes in an African cichlid fish. *Mol. Ecol*. 17: 2134–2148.
Cunningham, C. W., and Buss, L. W. 1993. Molecular evidence of multiple episodes of paedomorphosis in the family Hydractiniidae. *Biochem. Syst. Ecol*. 21: 57–69.
Delgado, E., and Defeo, O. 2006. A complex sexual cycle in sandy beaches: the reproductive strategy of *Emerita brasiliensis* (Decapoda: Anomura). *J. Mar. Biol. Assoc. U. K*. 86: 361–368.
Denoël, M., and Joly, P. 2000. Neoteny and progenesis as two heterochronic processes involved in paedomorphosis in *Trituris alpestris* (Amphibia: Caudata). *Proc. R. Soc. Lond. B* 267: 1481–1485.
Efford, I. E. 1967. Neoteny in sand crabs of the genus *Emerita* (Anomura, Hippidae). *Crustaceana* 13: 81–93.
Excoffier, L. 2004. Patterns of DNA sequence diversity and genetic structure after range expansion: lessons from the infinite-island model. *Mol. Ecol*. 13: 853–864.
Excoffier, L., Laval, G., and Schneider, S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol. Bioinf. Online* 1: 47–50.
Folmer, O., Black, M., Hoeh, R., Lutz, R. A., and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxydase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol*. 3: 294–299.
Fu, Y.-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147: 915–923.
Fuenzalida, R., Schneider, W., Garcés-Vargas, J., Bravo, L., and Lange, C. 2009. Vertical and horizontal extension of the oxygen minimum zone in the eastern South Pacific Ocean. *Deep-Sea Res. II* 56: 992–1003.
Gutiérrez, M., Ramirez, A., Bertrand, S., Móron, O., and Bertrand, A. 2008. Ecological niches and areas of overlap of the squat lobster 'munida' (*Pleuroncodes monodon*) and anchoveta (*Engraulis ringens*) off Peru. *Progr. Oceanogr*. 79: 256–263.
Haig, J. 1955. Reports of the Lund University Chile expedition 1948–49. 20. The Crustacea Anomura of Chile. *Acta Universitatia Lundensia* 51: 1–68.
Haig, J. 1968. A report on anomuran and brachyuran crabs collected in Peru during cruise 12 of R/V Anton Bruun. *Crustaceana* 15: 19–30.
Hajibabaei, M., Singer, G. A. C., Hebert, P. D. N., and Hickey, D. A. 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends Genet*. 23: 167–172.
Hazel, W., Brandt, R. B., and Grantham, T. 1987. Genetic variability and phenotypic plasticity in pupal color and its adaptive significance in the swallowtail butterfly *Papilio polyxenes*. *Heredity* 59: 449–455.
Hebert, P. D. N., Ratnasingham, S., and deWaard, J. R. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. Lond. B* 270: S96–S99.
Helly, J. J., and Levin, L. A. 2004. Global distribution of naturally occurring marine hypoxia on continental margins. *Deep-Sea Res*. 51: 1159–1168.
Hollander, J. 2008. Testing the grain-size model for the evolution of phenotypic plasticity. *Evolution* 62: 1381–1389.
Hull, S. L., Winter, L. J., and Scott, G. W. 2001. Habitat heterogeneity, body size and phenotypic diversity in *Idotea granulosa* (Isopoda) on the north-east coast of England. *J. Mar. Biol. Assoc. U.K*. 81: 949–954.
Hunda, B. R., and Hughes, N. C. 2007. Evaluating paedomorphic heterochrony in trilobites: the case of the diminutive trilobite *Flexicalymene retrorsa minuens* from Cuncunnatuan Series (Upper Ordovician), Cincinnati region. *Evol. Dev*. 9: 483–498.
Jain, S. K. 1979. Adaptive strategies: polymorphism, plasticity, and homeostasis. In O. T. Solbrig, S. K. Jain, G. B. Johnson, and P. H. Raven (eds.). *Topics in Plant Population Biology*. Columbia University Press, New York, pp. 160–187.
Kashkina, A. A., and Kashkin, N. I. 1993. Structure of the distribution range of the red crab *Pleuroncodes planipes* Stimpson 1860 (Crustacea: Galatheidae). *Okeanologiya* 33: 397–405.
Kavanagh, F. A., Wilson, G. D. F., and Power, A. M. 2006. Heterochrony in *Haplomesus* (Crustacea: Isopoda: Ischnomesidae): revision of two species and description of two new species. *Zootaxa* 1120: 1–33.
Klingenberg, C. P. 1998. Heterochrony and allometry: the analysis of evolutionary change in ontogeny. *Biol. Rev*. 73: 79–123.
Levin, L. A. 2003. Oxygen minimum zone benthos: adaptation and community response to hypoxia. *Oceanogr. Mar. Biol*. 41: 1–45.
Makarov, R. R., and Maslennikov, V. V. 1981. Ecology of larval development of the crustacean *Euphausia superba*. Change in dominant larval forms as a function of environmental conditions. *Mar. Ecol. Prog. Ser*. 4: 265–271.
Mann, G. 1954. *Vida de los peces en aguas chilenas*. Insituto de Investigaciones Veterinarias, Universidad de Chile, Chile.
McNamara, K. J. 1986. A guide to the nomenclature of heterochrony. *J. Paleontol*. 60: 4–13.
Moczek, A. P. 2008. On the origins of novelty in development and evolution. *BioEssays* 30: 432–447.
Morales, C. E., Hormazábal, S. E., and Blanco, J. L. 1999. Interannual variability in the mesoscale distribution of the depth of the upper boundary of the oxygen minimum layer off northern Chile (18-45S): implications for the pelagic system and biogeochemical cycling. *J. Mar. Res*. 57: 909–932.
Nielsen, C. 2008. Six major steps in animal evolution: are we derived sponge larvae? *Evol. Dev*. 10: 241–257.
Nylander, J. A. A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
Ojeda, F. P., Labra, F. A., and Muñoz, A. A. 2000. Biogeographic patterns of Chilean littoral fishes. *Rev. Chil. Hist. Nat*. 73: 625–641.
Ozawa, H., and Ishii, T. 2008. Taxonomy and sexual dimorphism of a new species of *Loxoconcha* (Podocopida: Ostracoda) from the Pleistocene of the Japan Sea. *Zool. J. Linn. Soc*. 153: 239–251.
Parsons, K. J., Skúlason, S., and Ferguson, M. 2010. Morphological variation over ontogeny and environments in resource polymorphic arctic charr (*Salvelinus alpinus*). *Evol. Dev*. 12: 246–257.
Radulovici, A. E., Sainte-Marie, B., and Dufresne, F. 2009. DNA barcoding of marine crustaceans from the estuary and Gulf of St Lawrence: a regional-scale approach. *Mol. Ecol. Res*. 9: 181–187.
Remerie, T., Bourgois, T., Peelaers, D., Vierstraete, A., Vanfleteren, J., and Vanreusel, A. 2006. Phylogeographic patterns of mysid *Mesopodopsis slabberi* (Crustacea, Mysida) in Western Europe: evidence for high molecular diversity and cryptic speciation. *Mar. Biol*. 149: 465–481.
Reuschel, S., and Schubart, C. D. 2007. Contrasting genetic diversity with phenotypic diversity in coloration and size in *Xantho poressa* (Brachyura: Xanthidae), with results on its ecology. *Mar. Ecol*. 28: 296–305.
Rivera, J., and Santander, E. 2005. Variabilidad estacional de la distribución y abundancia de larvas de langostino colorado en la zona norte de Chile (Decapoda, Anomura, Galatheidae). *Invest. Mar. Valparaíso* 33: 3–23.
Roa, R., Gallardo, V. A., Ernst, B., Baltazar, M., Cañete, J. I., and Enríquez-Brionnes, S. 1995. Nursery grounds, age structure and abundance of juvenile squat lobster *Pleuroncodes monodon* on the continental shelf off central Chile. *Mar. Ecol. Prog. Ser*. 116: 47–54.
Robinson, B. W., and Wilson, D. S. 1996. Genetic variation and phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (*Leomis gibbosus*). *Evol. Ecol*. 10: 631–652.

Rogers, A. R., and Harpending, H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Mol. Biol. Evol.* 9: 552–569.

Ronquist, F., and Huelsenbeck, J. P. 2003. MRBAYES 3: bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Rozas, J., Sánchez-deI Barrio, J. C., Messeguer, X., and Rozas, R. 2003. DNASP: DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 19: 2496–2497.

Rubinoff, D. 2005. Utility of mitochondrial DNA barcodes in species conservation. *Cons. Biol.* 20: 1026–1033.

Scheiner, S. M. 1993. Genetics and evolution of phenotypic plasticity. *Annu. Rev. Ecol. Syst.* 24: 35–68.

Sielfeld, W., and Vargas, M. 1996. Composición y estructura de la ictiofauna demersal de la zona norte de Chile. *Invest. Mar. Valparaíso* 24: 3–17.

Snell-Rood, E. C., Van Dyken, J. D., Cruickshank, T., Wade, M. J., and Moczek, A. O. 2010. Toward a population genetic framework of developmental evolution: the costs, limits, and consequences of phenotypic plasticity. *BioEssays* 32: 71–81.

Smith, L. D. 2004. Biogeographic differences in claw size and performance in an introduced crab predator *Carcinus maenas*. *Mar. Ecol. Prog. Ser.* 276: 209–222.

Smith, L. D., and Palmer, A. R. 1994. Effects of manipulated diet on size and performance of brachyuran crab claws. *Science* 264: 710–712.

Sotka, E. E., Wares, J. P., Barth, J. A., Grosberg, R. K., and Palumbi, S. R. 2004. Strong genetic clines and geographical variation in gene flow in the rocky intertidal banacle *Balanus glandula*. *Mol. Ecol.* 13: 2143–2156.

Spivak, E. D., and Schubart, C. D. 2003. Species status in question: a morphometric and molecular comparison of Cyrtograpsus affinis and C. altimanus (Decapoda, Brachyura, Varunidae). *J. Crustac. Biol.* 23: 212–222.

Swofford, D. L. 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0*. Sinauer Associates, Sunderland, Massachusetts.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123: 585–595.

Taylor, G. M., Keyghobadi, N., and Schmidt, P. S. 2009. The geography of crushing: variation in claw performance of the invasive crab *Carcinus maenas*. *J. Exp. Mar. Biol. Ecol.* 377: 48–53.

Thiel, M. 2002. The zoogeography of algae-associated peracarids along the Pacific coast of Chile. *J. Biogeogr.* 29: 999–1008.

Thiel, M., et al. 2007. The Humboldt current system of northern and central Chile: oceanographic processes, ecological interactions and socioeconomic feedback. *Oceanogr. Mar. Biol.* 45: 195–344.

Thoday, J. M. 1953. Components of fitness. *Symp. Soc. Expt. Biol.* 7: 96–113.

Trontelj, P., Machino, Y., and Sket, B. 2005. Phylogenetic and phylogeographic relationships in the crayfish genus *Austropotamobius* inferred from mitochondrial COI gene sequences. *Mol. Phylogenet. Evol.* 34: 212–226.

Ulloa, O., and Pantoja, S. 2009. The oxygen minimum zone of the eastern South pacific. *Deep-Sea Res. II* 56: 987–991.

Via, S., Gomulkiewicz, R., de Long, G., Scheiner, S. M., Shlichting, C. D., and Van Tienderen, P. H. 1995. Adaptive phenotypic plasticity: consensus and controversy. *Trends Ecol. Evol.* 10: 212–217.

Villarroel, J. C., Acuña, E. H., and Andrade, M. J. 2001. Feeding and distribution of the bigeye flounder *Hippoglossina macrops* off northern Chile. *Mar. Freshwater Res.* 52: 833–841.

West-Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* 20: 249–278.