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GENETIC VARIABILITY, SHELL AND SPERM MORPHOLOGY SUGGEST THAT THE SURF CLAMS *DONAX MARINCOVICHI* AND *D. OBESULUS* ARE ONE SPECIES

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ABSTRACT

The taxonomy of two sympatric surf clams *Donax marincovichi* Coan, 1983 and *Donax obesulus* Reeve, 1854, inhabiting the coastal Humboldt Current Upwelling System is revisited. Because both species are exploited by artisanal fisheries, it is essential to verify that they are indeed distinct species that have to be managed separately. In this study, both taxa were sampled across their shared distributional area and specimens were indentified according to their respective morphological characteristics. Although width/height and height/length ratios revealed significant differences within sampling areas, the two morphotypes were frequently incongruent for taxonomically important morphometric parameters. In addition, they showed no significant mitochondrial genetic differentiation within or among populations and exhibited indistinguishable sperm ultrastructure. We conclude that the two morphotypes do not represent distinct species and should be included together under *D. obesulus*.

INTRODUCTION

The bivalve genus *Donax* is distributed almost worldwide, except for polar regions, and can be locally very abundant on exposed sandy beaches (Ansell, 1983). Donacidae occupy a major role in nearshore trophic webs, as they feed on phytoplankton, while in turn they are prey for gastropods, demersal fish, birds and mammals (Ansell, 1983). These bivalves represent an important resource for artisanal fisheries and local markets (Paredes & Cardoso, 2001; Rey, 2006, 2007, 2008; Riascos, 2006) and are exported, e.g. to Europe. Two species, *Donax marincovichi* Coan, 1983 and *Donax obesulus* Reeve, 1854, are distributed along the Humboldt Current Upwelling System from northern Chile to northern Ecuador (Fig. 1, Table 1). Within their shared distributional range both species reveal a broad range of shell shapes (Coan, 1983; Guzmán, Saá & Ortlieb, 1998).

The taxonomic status of several species of Donacidae has been much debated (Loesch, 1957; Wade, 1967a, b; Chanley, 1969; Morrison, 1971; Narchi, 1983; Bonsdorff & Nelson, 1992; Nelson, Bonsdorff & Adamkewicz, 1993; Guzmán *et al*., 1998; Paredes & Cardoso, 2001; Laudien, Flint & van der Bank, 2003). The difficulty of species recognition is demonstrated by the reduction of 'valid' species from 64 (Ansell, 1983) to 45 in a recent taxonomic revision of the genus (Coan, Scott & Bernard, 2000). Clearly, confusion in taxonomical classification has led to numerous synonyms. The uncertain taxonomic status of the Chilean and Peruvian species *D. marincovichi* and *D. obesulus* is reflected in the current taxonomic literature (Olsson, 1961; Keen, 1971; Coan, 1983; Guzmán *et al*., 1998; Paredes & Cardoso, 2001). In Chile these clams are informally known as 'machilla' and in Peru as 'mariposa', 'palabrita', 'concha blanca' or 'marucha' (Huaraz & Ishiyama, 1980; Soto, 1985; Paredes & Cardoso, 2001; Rey, 2006). Both species are exploited by the artisanal fisheries and are managed as one evolutionarily significant unit referred to as *Donax peruvianus* (Paredes & Cardoso, 2001; Rey, 2006, 2007, 2008). However, to verify management strategies and to optimize sustainable exploitation it is essential to know if *D. marincovichi* and *D. obesulus* can be treated as one species or if they have to be managed separately.

To clarify the taxonomic status of *D. marincovichi* and *D. obesulus* three different taxonomic methods were applied: (1) shells were classified after Coan (1983), followed by statistical evaluation of two morphometric parameters; (2) morphometric comparison of sperm ultrastructure of both species was performed using transmission electron microscopy (TEM); (3) sequence data of partial cytochrome *c* oxidase subunit 1 gene (COI) were used to estimate inter- and intraspecific similarities among geographically distant populations along the northern Chilean and Peruvian distributional area.

Sperm ultrastructural studies have provided a useful tool for taxonomic and phylogenetic investigations of bivalves for >45 years (Galtsoff & Gallardo, 1960).

In particular, genetic tools have been shown to be very suitable for resolving taxonomic and systematic problems, and techniques such as DNA barcoding have revealed a great capacity to define species boundaries (Kimura & Weiss, 1964; Levinton & Suchanek, 1978; Koehn *et al*., 1984; Utter, 1991; Held, 2000; Hebert, Ratnasingham & DeWaard, 2003; Held & Wägele, 2005; Witt, Threloff & Hebert, 2006; Coghlan & Gosling, 2007). Genetic analyses are not only helpful in clarifying phylogenetic relationships, but also provide possibilities to analyse intraspecific relatedness between single populations and hence larval dispersal between regions and the dependence of recruitment on local stocks (Adamkewicz & Harasewych, 1994; Soares, 1999; Laudien *et al*., 2003; Coghlan & Gosling, 2007).

For the clarification of the taxonomic status a combination of both morphological and genetic analyses is particularly effective for species identification (Jaernegren *et al*., 2007).

MATERIAL AND METHODS

Material

Specimens ($n = 109$: *Donax marincovichi* $n = 56$ and *Donax obesulus* $n = 53$) were sampled at 10 locations along their

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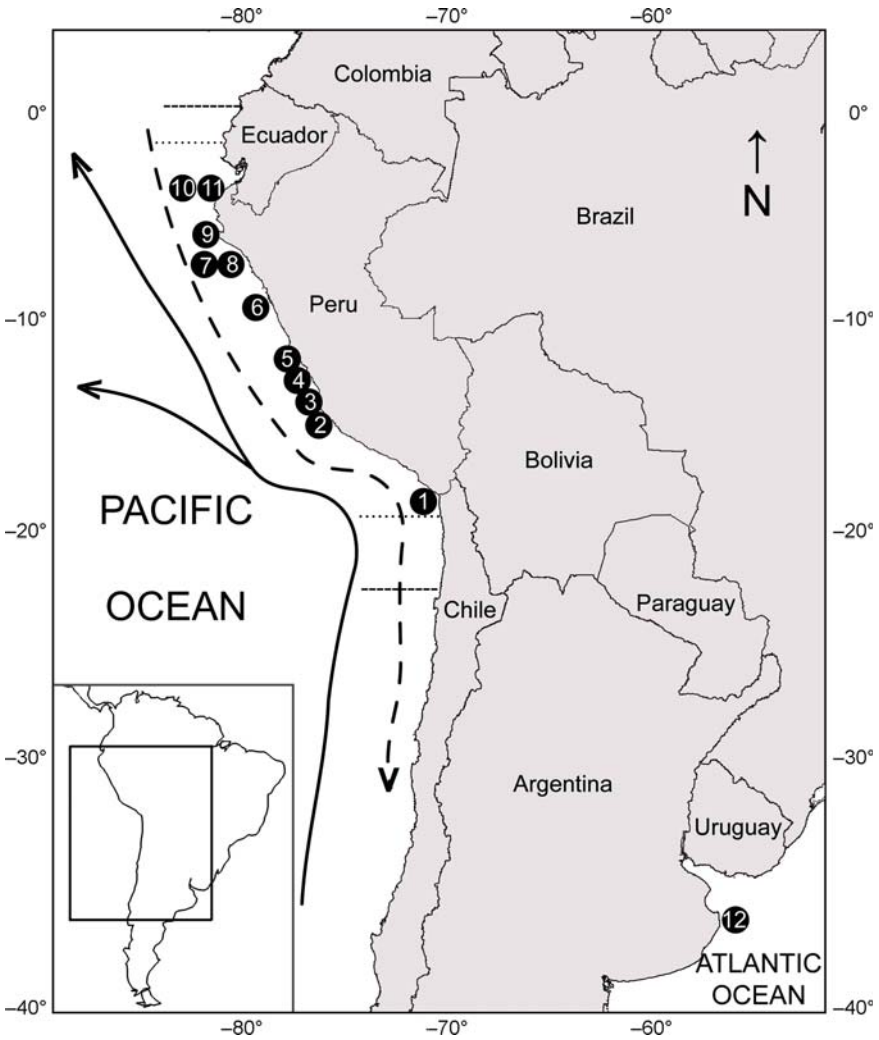

Figure 1. Distribution of *Donax marincovichi* (dotted line) (18°28′S; 70°20′W; 2°12′S; 80°58′W) and *Donax obesulus* (dashed line) (23°28′S; 70°31′W; 0°27′S; 80°7′W) and sampling stations (1–11) along the Chilean and Peruvian coast. Outgroups *Donax asper* and *Donax hanleyanus* were sampled at Stations 11 and 12. For further details see Table 1. Peru Coastal Current (solid arrows) and Peru Chile Undercurrent (dashed arrow), simplified after Tarazona & Arntz (2000).

Table 1. Sampling sites of *Donax marincovichi* ($n = 56$), *Donax obesulus* ($n = 53$) and *Donax* outgroups (*Donax asper* and *Donax hanleyanus*).

Station	Country	City nearby	Beach	Longitude	Latitude	*D. marincovichi*	*D. obesulus*
1	Chile	Arica	Chinchorro	S18°27′53.8″	W70°18′24.3″		11
2	Peru	Chincha	Violetas	S13°29′05.6″	W76°11′25.4″	11	
3	Peru	San Bartolo	Silencio	S13°24′33.0″	W76°11′49.1″	11	
4	Peru	Bujama	Sarapampa	S12°43′20.4″	W76°37′42.4″	10	1
5	Peru	Asia	Asia	S12°48′04.0″	W76°33′56.4″	10	1
6	Peru	Chimbote	El Dorado	S09°10′24.5″	W78°32′12.6″	8	2
7	Peru	Chiclayo	La Laguna	S07°04′08.7″	W79°44′00.2″		11
8	Peru	Chiclayo	La Laguna	S07°04′23.5″	W79°43′47.1″	6	5
9	Peru	Sechura	San Pedro	S05°29′49.8″	W80°53′53.7″		11
10	Peru	Tumbes	Hueso de Ballena	S03°30′20.5″	W80°29′04.0″		11
11	Peru	Tumbes	Hueso de Ballena	S03°30′20.5″	W80°29′04.0″	*Donax asper*	
12	Argentina	Villa Gesell	Mar Azul	S37°20′44.8″	W57°01′41.3″	*Donax hanleyanus*	

The numbers of the respective morphotypes indentified for each station are listed in the last two columns.

distributional range (Fig. 1, Table 1, Stations 1–10). Specimens of *Donax asper* from northern Peru (Tumbes; Fig. 1, Table 1, Station 11) and *Donax hanleyanus* from the Atlantic coast of Argentina (Villa Gesell; Fig. 1, Table 1, Station 12) were sampled as outgroups within the Donacidae. To avoid ontogenetic differences in shell morphology adult specimens (min, 15.02 mm; max, 29.97 mm) were chosen for analysis.

Table 2. Differentiation of *Donax marincovichi* and *D. obesulus* after Coan (1983).

	Donax marincovichi	*Donax obesulus*
1. Fig. 2A–D	Never has punctations	Punctations present on most specimens
2. Fig. 2E/F	Less inflated, W/H 0.57–0.69	More inflated, W/H 0.71–0.77
3. Fig. 2E/F	Beaks low	Beaks inflated
4. Fig. 2G/H	Periostracum dark	Periostracum light
5. Fig. 2G/H	Periostracum adherent in a wide marginal band	Periostracum light, marginal traces only
6. Fig. 2A–D	Surface silky	Surface shiny
7. Fig. 2I/J	Posterior lateral more distant from cardinals	Posterior lateral close to cardinals
8. Fig. 2K/L	Dorsal margin not produced above posterior lateral	Dorsal margin produced above posterior lateral
9. Fig. 2I–L	Anterior cardinal of right valve small, thin	Anterior cardinal of right valve thickened
10. Not considered	Maximum size 32 mm	Maximum size 38 mm

Shell morphology

Based on shell characteristics (following Coan, 1983) all individuals were identified as *D. marincovichi* or *D. obesulus* (Table 2). Specimens were measured with a vernier calliper (± 0.01 mm) to record length (anterior–posterior, L), height (ventro-dorsal, H) and width (left–right, W). Width/height (W/H) and height/length (H/L) ratios were calculated and the nonparametric Kruskal–Wallis test was used to evaluate statistical differences of each ratio among all stations. When the Kruskal–Wallis test revealed significant differences, *post hoc* comparisons were performed using the Dunn test at a level of 5% significance to evaluate paired (i.e. between every pair) differences (Zar, 1999). The character of 'maximum anterior–posterior shell length' specified by Coan (1983) for *D. marincovichi* (32 mm) and *D. obesulus* (38 mm) was not applied in this study as all specimens lay below these values (*D. marincovichi*, 29.75 mm; *D. obesulus*, 27.83 mm maximum lengths).

Sperm morphology

In order to compare sperm morphology, gonads of *D. marincovichi* and *D. obesulus* were dissected, *c.* 1 mm^3 of tissue (always from the same area) was fixed in 4% glutaraldehyde buffered with 0.1 M disodium phosphate buffer (pH 7.4) for 4 h at 4°C and then thoroughly rinsed in phosphate buffer. Tissue samples were dehydrated using an ascending series of ethanol (from 20% to absolute ethanol) and embedded in Araldite resin. Ultra-thin sections were produced using an LKB 2128 ultramicrotome after mounting the tissue on 200-μm mesh uncoated copper grids, stained with uranyl acetate (10–20 min) and lead citrate (10–20 min). For further details of preparation see Giménez *et al.* (2008). Ultrastructural sperm morphology was photographed with a TEM Philips EM 301 at 60 kV and a Jeol 1200 EX II at 80 kV. All measurements on sperm structures are based on TEM observations. As there are several important changes during maturation of sperm, such as changes in the acrosome and the positioning of the mitochondria (Pal, 1996), only mature sperm were analysed. Characterization of sperm morphology was realized by a precise identification of the general components of sperm (acrosome, nucleus, midpiece and flagellum).

Genetic analyses

For a subset of 79 *Donax* specimens (*D. asper*, $n = 9$; *D. hanleyanus*, $n = 9$; *D. marincovichi*, $n = 25$ and *D. obesulus*, $n = 36$) we sequenced a fragment of the mitochondrial COI gene. Individuals with opened and closed shells were preserved from each beach in >80% ethanol. Muscle tissue was extracted from the middle and apex region (*c.* 1 mm^2) of the foot and cleaned with ethanol (75%) to remove sand, detritus or external organic matter. The muscle tissue was cut into small pieces to decrease tissue lysis time. To avoid contamination of DNA, extraction was carried out under sterile conditions. DNA extraction was performed with the Qiagen DNA Mini kit using the standard tissue protocol. However, in a modification of this protocol, only 150 μl of elution buffer was used to increase the concentration of DNA.

Initially, DNA amplification of two mitochondrial genes (COI, 16S) was attempted using universal primers (Palumbi, Martin & Romano, 1991; Folmer *et al.*, 1994). The ribosomal large subunit gene (16S) did not amplify in many specimens and, therefore, further analyses focused on the COI gene, which amplified successfully using the primers LCO-1490 and HCO-2198 (Folmer *et al.*, 1994).

Amplification of the COI fragment was carried out in 25 μl reactions containing 1× HotMaster buffer, 0.2 mM dNTPs, 0.5 μM of each primer, 1–3 μl of DNA template, 0.02 U/μl HotMaster Taq polymerase (5 prime, Hamburg, Germany), filled up to 25 μl with sterile H_2O. The PCR temperature profile for the COI amplification was: 94°C for 2 min, 38 cycles of 20 s at 94°C, 15 s at 42°C, 80 s at 65°C, followed by a final extension step of 7 min at 65°C.

PCR products were checked on a 1.5% TAE agarose gel and purified using a Qiagen QIAquick PCR purification kit according to the manufacturers' recommendations. Cycle-sequencing of PCR products was carried out using terminal primers given above and the BigDye Terminator v3.1 kit of Applied Biosystems (ABI), purified using DyeEx-Kit (Qiagen) according to the modified protocol and sequenced on an ABI 3130xl automated sequencer.

Sequences were aligned using MUSCLE (Edgar, 2004). As outgroup for the alignment, a sequence of *Macoma balthica* was used (GenBank accession number EF044136). Phylogenetic analyses were performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). The optimal model of sequence evolution was identified using hierarchical likelihood ratio tests and the Akaike Information Criterion (AIC) as implemented in MrModeltest 2.2 (Nylander, 2004). The MCMC analyses was performed using two independent runs with 10 million generations each and sampling every 100th tree. Convergence was reached after 60.000 generations (discarded as burn-in). A statistical parsimony network was calculated from the *D. marincovichi* and *D. obesulus* data set using TCS 1.21 (Clement *et al.*, 2000) and a 95% connection limit.

RESULTS

Shell morphology

Based on Coan's (1983) morphological characters, specimens ($n = 109$) from 10 different locations from northern Chile to

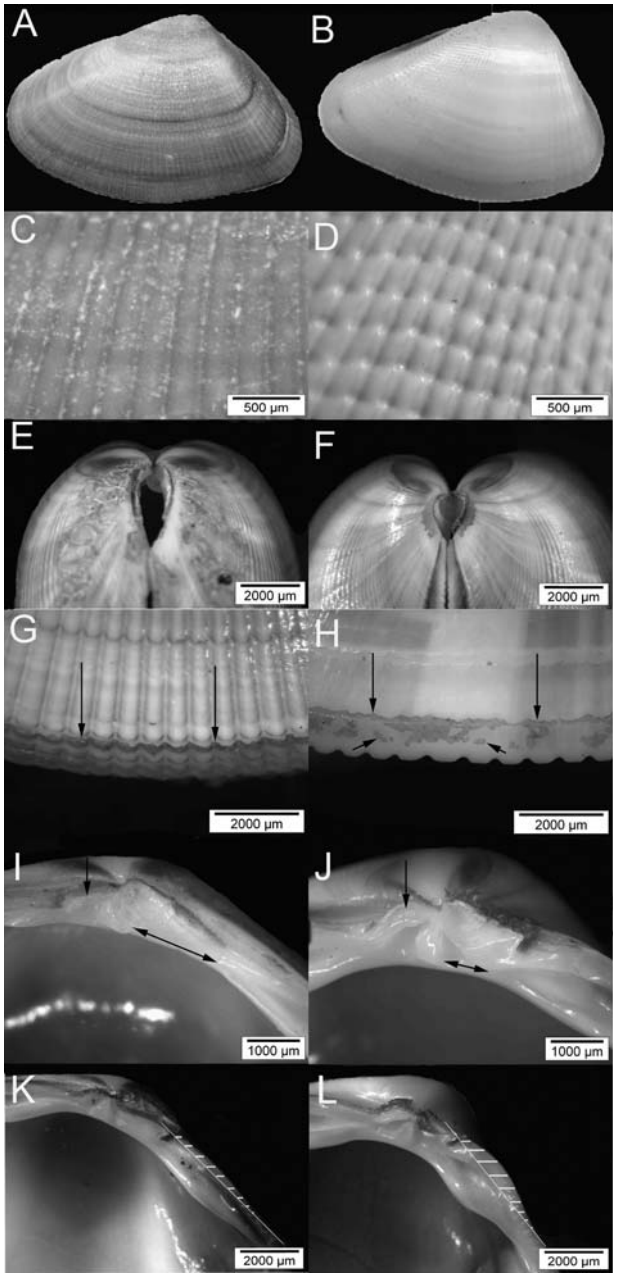

Figure 2. A. *Donax marincovichi* (left valve, 23 mm). Valve is less inflated, W/H between 0.57 and 0.69, maximum length 32 mm. **B.** *Donax obesulus* (left valve, 17 mm), valve is more inflated, W/H between 0.71 and 0.77. **C.** *Donax marincovichi* never has punctation, surface silky (32 ×). **D.** *Donax obesulus* shows punctation on most specimens, surface shiny (32 ×). **E.** *Donax marincovichi:* posterior dorsal half, beaks low (10 ×). **F.** *Donax obesulus:* posterior dorsal half, beaks inflated (10 ×). **G.** *Donax marincovichi:* left valve ventral margin: periostracum dark, adherent, in a wide marginal band (10 ×). **H.** *Donax obesulus:* left valve ventral margin: periostracum light, marginal traces only (10 ×). **I.** *Donax marincovichi:* right valve hinge region close to umbo: posterior lateral more distant (double-headed arrow) and anterior cardinal of right valve (single-headed arrow) small, thin (12.5 ×). **J.** *Donax obesulus:* right valve hinge region close to umbo: posterior lateral close to cardinals (double-headed arrow) and anterior cardinal (single-headed arrow) of right valve thickened (12.5 ×). **K.** *Donax marincovichi:* dorsal margin less (or not) produced above posterior lateral (hatched area) (7 ×). **L.** *Donax obesulus:* dorsal margin produced above posterior lateral (hatched area) (7 ×).

Table 3. Number of mismatches (inapplicable identification feature, as number and as percentage) for *Donax marincovichi* and *Donax obesulus* for each character (Fig. 2, Table 2).

	Donax marincovichi ($n = 56$)	%	*Donax obesulus* ($n = 53$)	%
1. Punctation	0	0	0	0
2. W/H	8	14	5	9
3. Beak	1	2	7	13
4. Periostracum colour	50	88	2	4
5. Periostracum marginal	20	35	13	25
6. Surface	9	16	1	2
7. Posterior lateral	37	65	5	9
8. Dorsal margin	0	0	13	12
9. Anterior cardinal	11	19	13	25
Total	133		58	

Maximal size (character 10, Table 2) was not considered as there were no specimens available reaching the maximum size given by Coan (1983).

northern Peru were identified to species level (Fig. 2, Table 1). However, only 20% of the specimens showed a perfect match with the morphotype expected from the species' description, while 80% of the specimens conflicted in at least one character by possessing a character state expected for the other species. Final assignment to species level was carried out by using the predominant number of morphological characteristics (Coan, 1983). The percentage of individuals exhibiting mismatches for each morphological character is presented in Table 3.

The northern Chilean population (Fig. 1, Table 1, Station 1) was composed only of clams identified as *Donax obesulus*. The central Peruvian beaches from Chincha to Chimbote (Fig. 1, Table 1, Stations 2–6) yielded predominantly *Donax marincovichi* morphotypes. In contrast, the population further north (Fig. 1, Table 1, Station 7) consisted exclusively of the *D. obesulus* morphotype, followed by Station 8 (Fig. 1, Table 1), which was inhabited by both morphotypes. The northernmost populations of Sechura and Tumbes (Fig. 1, Table 1, Stations 9 and 10) were composed of nominal *D. obesulus* morphotypes.

Among all the morphological characters used for identification in this study, only 'punctation' appeared to be reliable. As described by Coan (1983), it was predominant and/or prevalent on the *D. obesulus* morphotype, but never present on *D. marincovichi*. The most ambiguous identification features (Table 3) were 'colour' and 'distance from the posterior lateral to the cardinals' (respective mismatches of 46% and 38%), followed by 'location of the periostracum' (29%) and 'size of the anterior cardinal tooth' (18%). The characters 'dorsal margin produced or not produced above posterior lateral' and 'W/H ratio' conflicted in 12% of the cases. The characters 'surface' and 'shape of the beak' revealed 9% and 7% of mismatch, respectively (Table 3). Within the nine applied characters quantified by Coan (1983) the morphotype *D. marincovichi* ($n = 56$) possessed 133 mismatches, while morphotype *D. obesulus* ($n = 53$) revealed 58 mismatches (Table 3).

W/H, as well as the H/L ratio between all specimens, were tested and revealed significant differences ($P < 0.001$). Further comparisons between stations were made (Table 4), indicating that in terms of W/H and H/L ratios, stations from northern Chile and northern Peru (Fig. 1, Table 1, Stations 1, 7, 9 and 10), all dominated by *D. obesulus*, differed significantly from

Table 4. Pairwise comparison of different sampling sites (Table 1) by Dunn test applied to identify differences in W/H and H/L between single stations.

	W/H	H/L
1 *vs* 2	0.000*	0.003*
1 *vs* 3	0.000*	0.000*
1 *vs* 4	0.002*	0.018*
1 *vs* 6	0.003*	0.020*
2 *vs* 7	0.021*	0.037*
2 *vs* 8	0.151	0.025*
2 *vs* 9	0.000*	0.042*
2 *vs* 10	0.000*	0.000*
3 *vs* 7	1.000	0.008*
3 *vs* 8	1.000	0.005*
3 *vs* 9	0.000*	0.009*
3 *vs* 10	0.001*	0.000*
4 *vs* 9	0.001*	0.208
4 *vs* 10	0.013*	0.000*
5 *vs* 10	0.458	0.000*
6 *vs* 9	0.001*	0.211
6 *vs* 10	0.018*	0.000*
8 *vs* 9	0.044*	1.000

Asterisks indicate significant differences ($P < 0.05$).

central Peruvian stations (Fig. 1, Table 1, Stations 2–6 and 8), which are dominated by *D. marincovichi*. Specimens of the former stations are more wedge shaped (shorter and thicker) indicating a compacter shape than clams from the other sites (Fig. 3).

However, in addition to individuals displaying all morphological characters of one species and thus easily identified, individuals from all stations (except station 10) showed a number of transitional character states thus making unequivocal identification difficult or impossible (Fig. 3). Recent results increased the maximum value of the H/L ratio (Coan, 1983) (Table 2) for *D. marincovichi* to 0.62–0.73 and for *D. obesulus* to 0.66–0.85, respectively. Further sampling at Jahuay beach (Fig. 1, Table 1, Station 3) revealed that *D. marincovichi* reaches a maximum length up to 35 mm instead of 32 mm as reported by Coan (1983).

Sperm morphology

TEM observations of sperm structure of the two species *D. marincovichi* and *D. obesulus* (Coan, 1983) revealed that both demonstrate a typical primitive aquasperm type, containing an acrosome, nucleus, a short midpiece and a ring of mitochondria surrounding the flagellum (Fig. 4A). The pear-shaped acrosome settles in a nuclear fossa (Fig. 4A, B). A transverse section of the acrosome is provided in Figure 4C. It illustrates that the acrosome is differentiated into two regions with different electron densities. The surrounding outer crescent-shaped basal ring is electron dense and represents *c*. 50% of the total area of the acrosome. It is covered by an outer acrosomal membrane. The anterior, central and posterior region is electron lucent containing the axial rod in the centre. The electron-lucent part of the acrosome apex is arrow-shaped (Fig. 4B). The sperm nucleus capturing approximately one-third of the total size of the sperm is associated with the acrosome (Fig. 4D). The flagellum shows the structure typical of molluscs: the axoneme within the flagellum consists of a central pair of microtubules encircled by nine doublets (9 + 2 type). Its termination is surrounded by four mitochondria (Fig. 4E). Mean values of length and diameter of the acrosome, nucleus and midpiece are given in Table 5.

Genetic analyses

DNA of good quality was extracted from specimens whose shells were cracked open immediately before preservation, presumably because diffusion of ethanol into tissue depends mainly on the permeability of the surface texture of the organism (Held, 2000). In contrast, DNA of individuals preserved intact showed greater degradation and lower yields. DNA extracted from the middle piece of the foot was on average less in quantity and lower in quality, i.e. more fragmented. Consequently, all tissues used for DNA extraction were obtained from the anterior apex of the foot of previously sectioned individuals.

The alignment of the COI gene sequences yielded a 567-bp alignment. Analyses of uncorrected *P*-distances and haplotype sharing in the statistical parsimony network revealed no consistent differences between *D. marincovichi* and *D. obesulus* (Figs 5, 6). Strong differences were recorded for *D. marincovichi* and *D. obesulus* and the two Donax outgroup species (>15% *P*-distance, Fig 5). The pairwise genetic difference in *D. obesulus*–*D. obesulus* or *D. marincovichi*–*D. marincovichi* pairs was equal to within-species pairwise comparisons, i.e. *D. marincovichi*–*D. obesulus* pairs and *vice versa* (0–1.2%). In addition, no genetic differentiation was observed between geographically separated populations.

For details of analysed specimens ($n = 61$) and number of matches and mismatches of identification after Coan (1983) see Supplementary material.

DISCUSSION

The W/H and H/L ratios obtained during this study revealed significant differences between *Donax marincovichi* and *D. obesulus* among sampling areas (Table 4). However, following the key of Coan (1983) (Fig. 2 and Table 2) these as well as other distinguishing characteristics frequently revealed incongruence concerning identification of the two species. Of the identified specimens 80% displayed characters of both morphotypes (Table 3). In order to clarify the taxonomic status we applied genetic tools and observed the sperm morphology of both morphotypes.

The genetic analysis indicated that COI gene sequences are useful for species discrimination within the genus *Donax*. The observed divergence levels (0–1.2%) of both morphotypes pooled (Fig. 5) are typical of intraspecific levels (Held, 2000; Hebert *et al*., 2003; Cardenas, Castilla & Viard, 2009). No consistent spatial structure was found in the distribution of genetic variance among geographically separated populations, suggesting extensive and ongoing gene flow between geographically separated populations and also between nominal *D. obesulus* and *D. marincovichi*. The same molecular marker system was highly informative when distinguishing the two *Donax* species included in the outgroup (*D. asper* and *D. hanleyanus*).

In addition, the examination of sperm morphology showed that sperm of both morphotypes were of a similar aqua sperm type (Fig. 4), which is typical of most members of the family Veneroidea (Healy, 1995). This sperm type is characterized by a conical acrosome, a short nucleus, a midpiece consisting of a ring of round mitochondria surrounding the centrioles and a single simple flagellum with a 9+2 axoneme (Healy, 1995, 2000). Comparison of the main components of the respective sperm revealed no differences in morphology. The electron

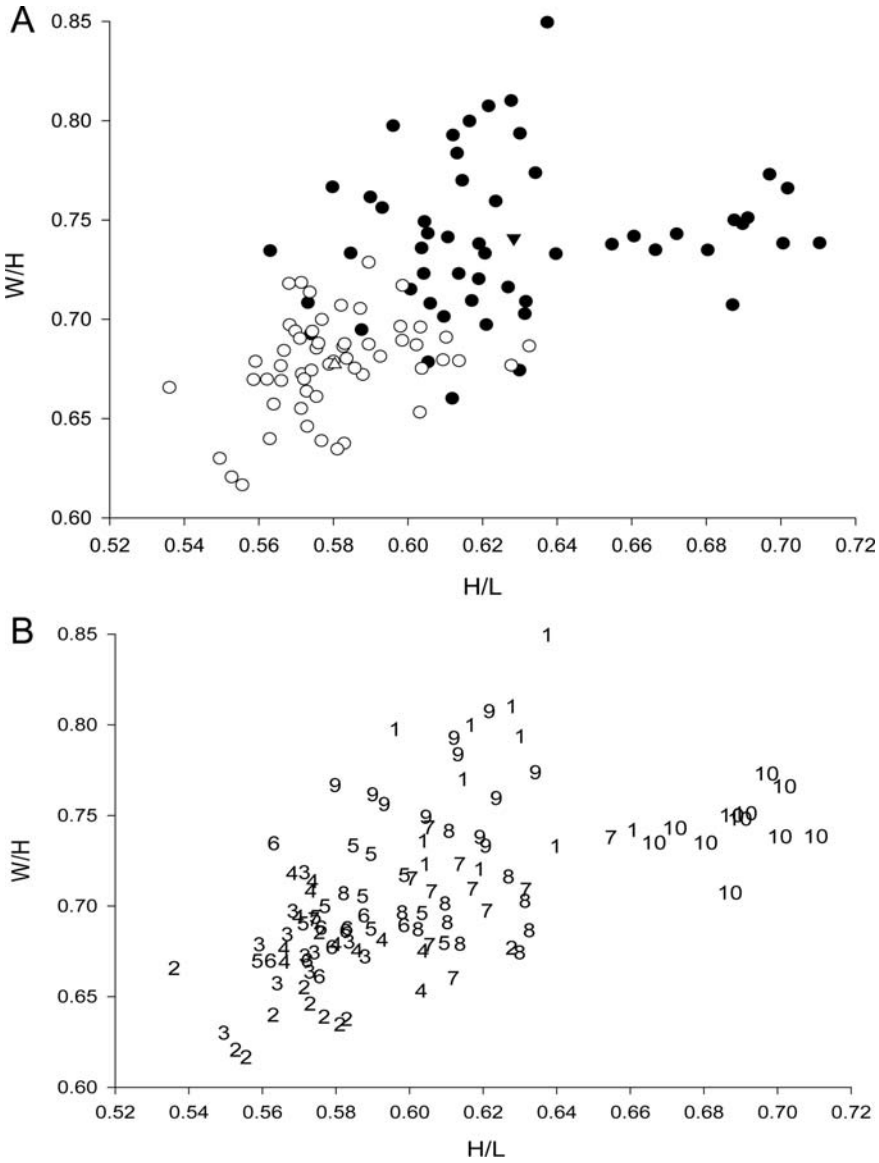

Figure 3. A. Shell shape variability of *Donax marincovichi* (open circles; mean: open triangle) and *Donax obesulus* (solid circles; mean: solid triangle) from stations along the Pacific coast of northern Chile and Peru. Width/height (W/H) ratios are plotted *vs* height/length (H/L). **B.** Shell shape variability of *Donax marincovichi* and *Donax obesulus* from Stations 1 to 10.

lucent part of the acrosome apex has an arrow-shaped point (Fig. 4B), which appears to be characteristic. A review of sperm of Donacidae (van der Horst *et al*., 1986; Hodgson, Bernard & van der Horst, 1990; Sousa & Oliveira, 1994; Healy, 1995; Matos *et al*., 1995; *D. hanleyanus*, M. Herrmann, unpublished) showed that the present sperm type differs in shape and size from those previously reported (Fig. 4, Table 5).

Neither significant mitochondrial genetic differentiation within or among populations, nor differences in sperm ultrastructure, separate the two morphotypes defined by Coan (1983) as distinct species. Therefore we propose that the differences between the morphotypes might be primarily the result of phenotypic plasticity caused by different local environments. We suggest that *D. marincovichi* and *D. obesulus* are better characterized as two different ecomorphs (sensu Nelson *et al*., 1993) of the species *D. obesulus*.

Within the study area geographically separated populations spanned approximately 1900 km (linear distance) from the southernmost station (Chinchorro Beach in Tarapacá, Chile) to the northernmost station (Sechura Bay in Tumbes, Peru). Meroplanktic larval development of *Donax* species takes about three to four weeks, depending on environmental conditions (food availability and temperature), and can be delayed under unfavourable conditions (Chanley, 1969; Helm, Bourne & Lovatelli, 2004). The Peruvian Coastal Current (Fig. 1) transports water masses at speeds of up to 15 cm s^{-1}, which is equivalent to approximately 400 km per month (Tarazona & Arntz, 2000), implying that the analysed sub-populations may be interconnected via stepping-stone larval exchange within a modest number of generations. Similar long-distance interpopulational genetic connectivity has been recorded for *D. serra* populations from the Benguela Current Upwelling System (Laudien *et al*., 2003) and for *D. deltoides* populations connected by the East Australian Current (Murray-Jones & Ayre, 1997).

Our results concur with numerous publications demonstrating that shell shape of Donacidae may vary greatly and that it is unreliable as a taxonomic feature (Olsson, 1961; Keen, 1971; Coan, 1983; Guzmán *et al*., 1998). This family is well known to

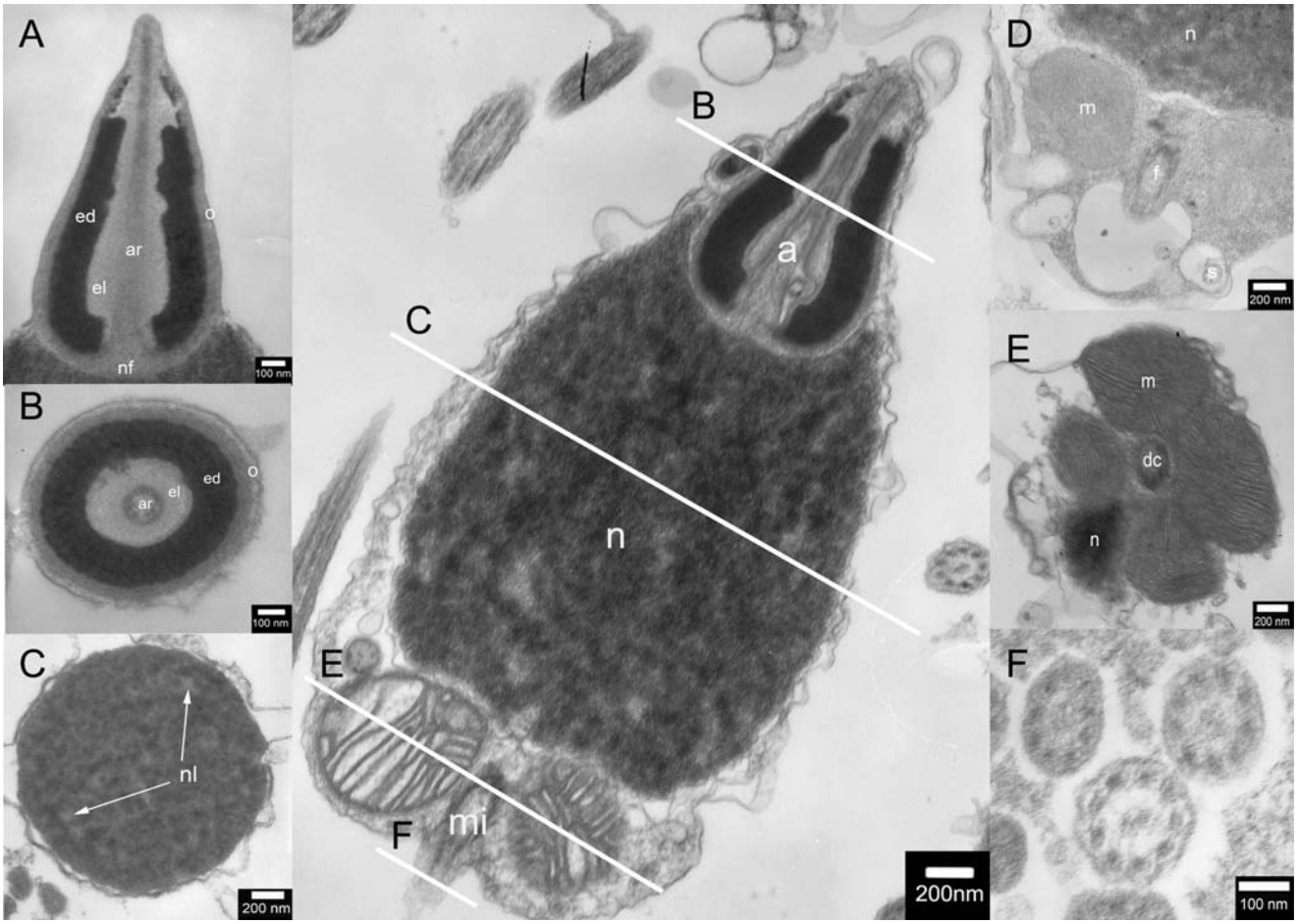

Figure 4. Overview in mid-longitudinal section through sperm type shared by *Donax obesulus* and *Donax marincovichi*, showing level of transverse sections (20,000 ×). **A.** Detail, mid-longitudinal section of acrosome (50,000 ×). **B.** Transverse section of acrosome (60,000 ×). **C.** Transverse section of nucleus with brighter spots of nuclear lacunae (20,000 ×). **D.** Detail, longitudinal section of satellite (30,000 ×). **E.** Transverse section of four mitochondria, part of the nucleus and distal centriole (30,000 ×). **F.** Transverse section of flagellum showing 9 + 2 axoneme (80,000 ×). Abbreviations: a, acrosome; ar, axial rod; dc, distal centriole; ed, electron-dense basal ring; el, electron-lucent region; f, flagellum; m, mitochondrion; mi, midpiece; n, nucleus; nf, nuclear fossa; nl, nuclear lacunae; o, outer plasma membrane; s, satellite.

Table 5. Comparison of sperm ultrastructure of *Donax* species: mean (± SD) measurements (length and diameter) of acrosome, nucleus and midpiece.

Species (*n*)	Acrosome (μm)	Nucleus (μm)	Midpiece (μm)	Author
Donax sp. (39)	1.38 × 0.83 (± 0.17/± 0.06)	2.11 × 1.83 (± 0.19/± 0.20)	0.68 × 1.54 (± 0.16/± 0.29)	This study
Donax deltoides (5)	1.18 × 0.88	0.67 × 0.75	0.6 × 1.75	Healy (1995)
Donax serra (20)	0.9 × 1.0	1.33 × 1.5	No data	Hodgson (1990)
Donax sordidus (20)	1.0 × 0.8	1.5 × 1.4	No data	Hodgson (1990)
Donax madagascariensis (20)	1.07 × 0.98	1.56 × 1.4	No data	Hodgson (1990)

span a wide spectrum of shell colour, pattern and shape. High heterogeneity has been documented for *D. variabilis* populations over short linear distances; at six stations covering 114 km of total distance, each population presented a unique combination in shell length and morph frequencies (Adamkewicz, 1989). Extreme diversity of shell characters and the absence of clear diagnostic features for Donacidae have also been reported in other studies (Wade, 1967a, b; Adamkewicz, 1989; Donn, 1990; Adamkewicz & Harasewych, 1994; McLachlan *et al*., 1995; Laudien *et al*., 2003), suggesting that variation in size, colour, shape and sculpture of the shells may be due to phenotypic plasticity rather than an expression of genetic differentiation between the populations. McLachlan *et al*. (1995) studied the influence of beach morphodynamics on shell shape. Reflective beaches favour smaller, uniform and more wedge-shaped shells, while dissipative (Dean's parameter 5–10; for details see Defeo & McLachlan, 2005) beaches instead favour larger valves with a broader spectrum of shapes. This was also documented for *D. serra* inhabiting reflective (Dean's parameter 0–2; Defeo & McLachlan, 2005) *vs* intermediate (Dean's parameter 2–5) to dissipative beaches (Laudien *et al*., 2003). Donn (1990) stressed the influence of locality and population density and documented that a high-intertidal population of *D. serra* at a higher density had thicker

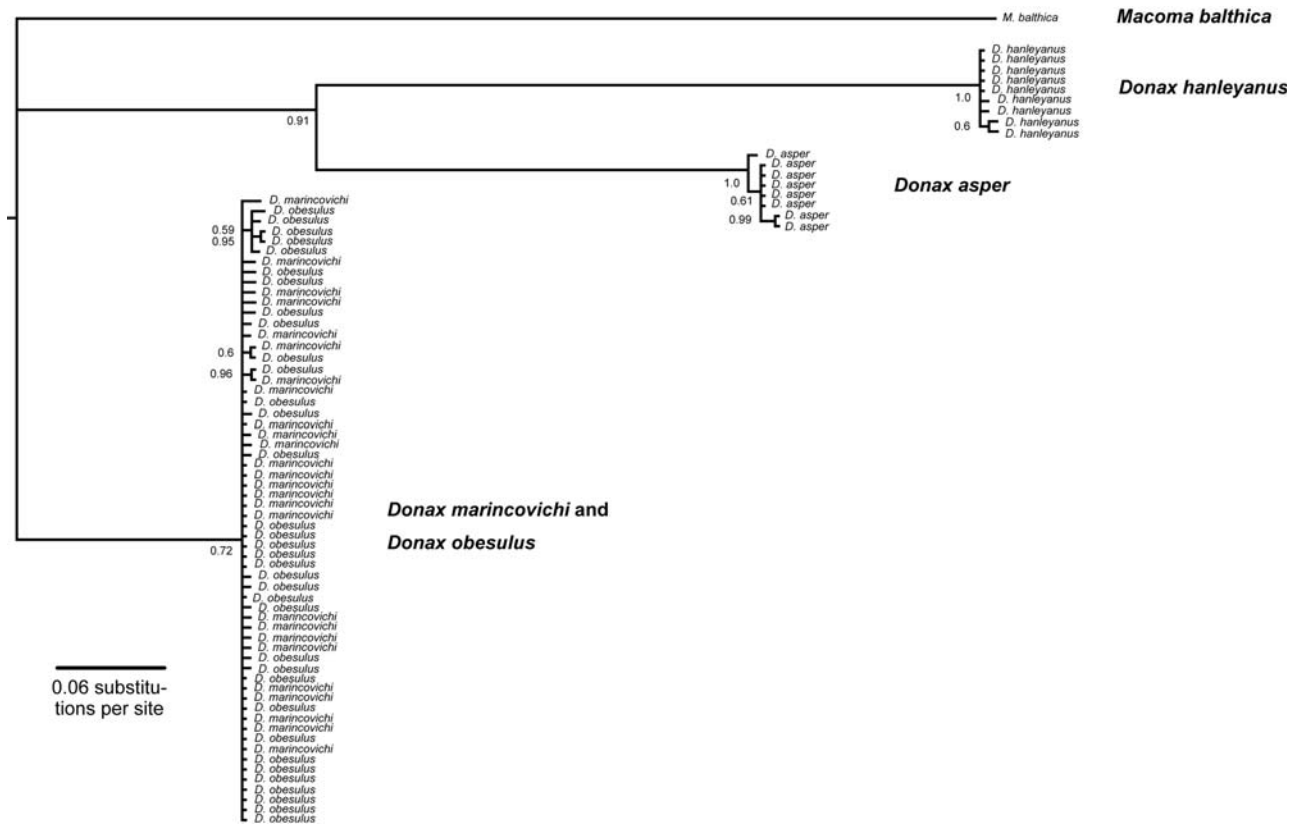

Figure 5. Majority rule (>50%) consensus tree obtained from Bayesian analysis of the mitochondrial COI gene sequence data for *Donax marincovichi*, *Donax obesulus* and the outgroups *Donax asper*, *Donax hanleyanus* and *Macoma balthica*. Numbers below branches are posterior probability values.

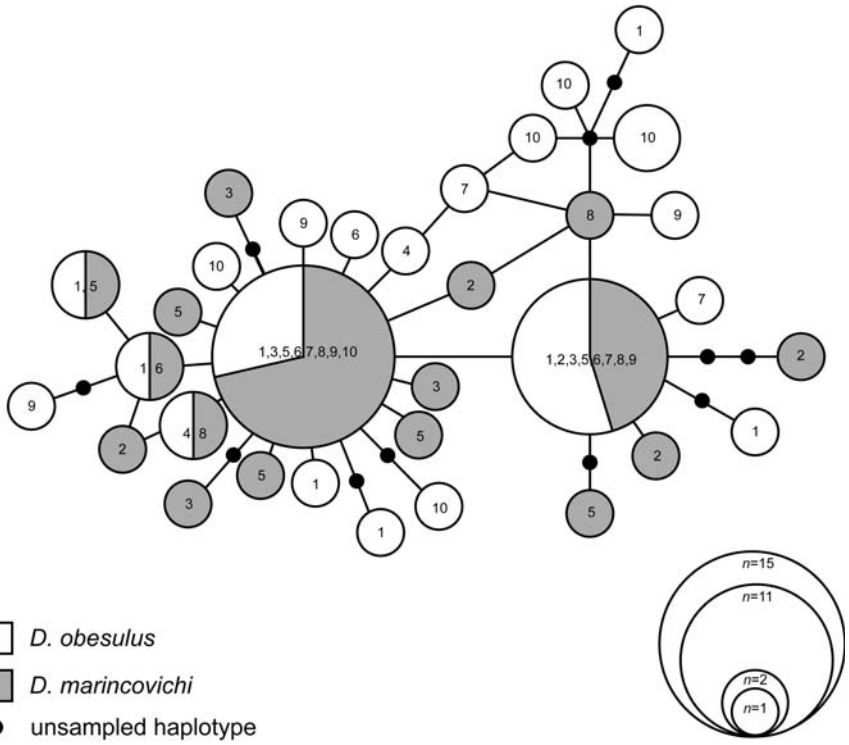

Figure 6. Statistical parsimony network (95% connection limit) for the putative *Donax marincovichi* and *Donax obesulus* specimens sampled at station 1–10. The diameter corresponds to the number of specimens found with a specific haplotype. The number(s) within each haplotype refer(s) to the station(s) at which the haplotype was found.

and heavier shells, whereas low-intertidal or subtidal populations possessed flatter, more rounded shells. Considering the observed specimens, it can be suggested that the *D. marincovichi* morphotype may be more adapted to intermediate beaches because it has a flatter and less wedge-shaped form (Fig. 3), whereas the *D. obesulus* morphotype from northern Chile and Peru may be more adapted to reflective beaches, because it is shorter and more wedge-shaped (Fig. 3) (cf. McLachlan *et al.*, 1995).

The diversity of synonyms of Donacidae reflects taxonomical confusion that is mainly due to the use of unreliable shell characteristics for species determination. Future research should include faster-evolving nuclear markers (e.g. AFLP, microsatellites) that could extend our findings based on mitochondrial sequences, and resolve more recent evolutionary events and at a finer geographic resolution than the present study (Held & Leese, 2007).

In conclusion, as a consequence of environmentally driven phenotypic plasticity, analysis of shell morphology may be unsuitable for the delimitation of *Donax* species. Phenotypic plasticity in shape, dimensions, sculpture and colour can be considered adaptive for species living in environments that are physically and biologically dynamic, where it can give rise to distinctive ecomorphs.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Molluscan Studies* online.

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