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SHORT COMMUNICATION

Recent introduction of the dominant tunicate, *Pyura praeputialis* (Urochordata, Pyuridae) to Antofagasta, Chile

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Abstract

The large sessile tunicate *Pyura stolonifera* (Pleurogona: Stolibranchiata: Pyuridae), has been regarded as a complex taxon with disjointed distributions, including Australia (*Pyura stolonifera praeputialis*), South Africa (*Pyura stolonifera stolonifera*) and South America (Chile, Antofagasta: *Pyura* sp., the 'piure de Antofagasta'), and has been cited under at least five taxonomic combinations. The 'piure de Antofagasta' is a competitively dominant species in rocky intertidal habitats and shows a limited geographical range (60–70 km) exclusively inside the Bay of Antofagasta. Using cytochrome oxidase I (COI) mitochondrial sequence data from *Pyura* specimens of the three taxa we tested whether the Chilean taxon represents: (i) a Gondwana relict; (ii) a more recently divergent species; or (iii) a recently introduced species. The results suggest that the Chilean taxon is a recent introduction to Chile from Australian populations and that *Pyura stolonifera praeputialis*, from Australia, and the 'piure de Antofagasta' are geographical populations of a single species: *Pyura praeputialis*; whereas the South African taxon represents a second species: *Pyura stolonifera*.

Keywords: Chile, introduction, mtDNA, *Pyura praeputialis*, tunicates

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Introduction

Southern hemisphere biogeographical patterns for benthic nearshore organisms, with limited larval dispersal, are understudied. Large sessile tunicates of the genus *Pyura* (Pleurogona: Stolibranchiata: Pyuridae) have very short larval dispersal (Clarke *et al*. 1999), form conspicuous rocky intertidal and shallow subtidal fringes and are characteristic biotic components of the temperate seas of South Africa, Australia and Chile, showing remarkable disjointed distributions (Castilla & Guiñez 2000). Based on morphological analysis there is no agreement upon the taxonomic relation between the southern hemisphere '*Pyura stolonifera* complex of species': the South African taxon ('red bait') cited as *P. stolonifera* (Heller 1878) by Day (1974) and Millar (1962), and as *P. stolonifera stolonifera* by Kott (1976, 1997); the Australian taxon ('cunjevoi'), cited as *P. praeputialis* (Heller) by Millar (1963), *P. stolonifera* (Heller, 1878) by Kott (1952, 1976, 1985) and *P. stolonifera praeputialis* Heller by Kott (1976, 1997); and the Chilean taxon *Pyura* sp. ('piure de Antofagasta'), which has been cited as *P. praeputialis* (Heller) by Millar (1963), *P. stolonifera* (Heller, 1878) by Kott (1985) and *P. stolonifera bradleyi* by Kott (1997). *P. bradleyi* (Van Name 1931) is a related South American species collected in 1866–1867 from Zorritos, Peru (3°40'?S) by the Peabody Museum, Yale University (Van Name 1931, 1945) but it has not been collected since then (see Clarke *et al*. 1999) and was synonimized with *P. stolonifera* from Australia and *Pyura* sp. from Chile by Kott (1985). The evolutionary and biogeography of these *Pyura* taxa and the taxonomic status of *Pyura* sp. from Antofagasta are currently unsettled (Castilla & Guiñez 2000). Three hypothesis can be put forward: (i) the 'piure de Antofagasta' represents a Gondwanaland relic of the taxonomic *Pyura* complex (Kott 1985, 1997); (ii) this *Pyura* was recently introduced to the Bay of Antofagasta (Monniot 1994; Monniot & Monniot 1994; Clarke *et al*. 1999;

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Table 1 Classification, collection area, date of sampling, species code and accession number for mtDNA sequences in this study. Classification based on Kott (1952, 1976, 1985, 1997). All individuals were collected by J. C. Castilla, except the individual from South Africa that was collected by Professor George Branch

Species	Location	sampling	Date of Species code	Sequence code†
Pyura sp.	El Way, Antofagasta, Chile (I)	November 1997	piure de Antofagasta C1	JCC1_Ant
Pyura sp.	Las Conchillas, Antofagasta (I)	July 1998	piure de Antofagasta C2	Py21_inN
Pyura sp.*	Las Conchillas, Antofagasta (I)	July 1998	piure de Antofagasta C3	Py22_inN
Pyura sp.	La Rinconada, Antofagasta (S)	July 1998	piure de Antofagasta C4	Py23_sub
Pyura sp.	El Edén, Antofagasta (I)	July 1999	piure de Antofagasta C5	Py24_int
Pyura sp.	El Edén, Antofagasta (I)	July 1999	piure de Antofagasta C6	Py25_int
P. stolonifera praeputialis	Botany Bay, Sydney, Australia (I)	May 1996	*P. s. praeputialis* A1	Py2p_Aus
P. s. praeputialis	Botany Bay, Sydney, Australia (I)	April 1999	*P. s. praeputialis* A2	Py3p_Aus
P. s. stolonifera	False Bay, C. Town, South Africa (I)	July 1998	*P. s. stolonifera* S1	Py_Safr
P. chilensis	Isla Santa María, Mejillones, Chile (S)	July 1998	*P. chilensis* 1	JCC9_PCh
P. chilensis	Isla Santa María, Mejillones, Chile (S)	July 1998	*P. chilensis* 2	Pch22-PUC
P. chilensis	Isla Santa María, Mejillones, Chile (S)	July 1998	*P. chilensis* 3	Pch23-PUC
Halocynthia roretzi	(GenBank: Accession no. S54796)		*H. roretzi* 1	Halo_ror

*Abnormal morph. I, intertidal; S, subtidal.

†Data sets are publicly available at the archived data web pages of the University of California Museum of Palaeontology, http://www.ucmp.berkeley.edu/archdata/Pyura.html, as well as upon request. Under the column headed Sequence are the corresponding archive names.

Castilla & Guiñez 2000); (iii) this *Pyura* represents a distinct species that diverged relatively recently. We use COI mitochondrial sequence data from *Pyura* specimens collected in Australia, South Africa and Chile (Antofagasta) to test among the competing hypotheses. The main goal of this study was to clarify the phylogenetic position of the 'piure de Antofagasta' (= *Pyura* sp.) relative to the Australian *P. stolonifera praeputialis* and the South African *P. stolonifera stolonifera*.

Materials and methods

Specimens from the lower intertidal fringe and the subtidal of the *Pyura stolonifera* complex from South Africa, Australia and Antofagasta, and *P. chilensis* (Antofagasta) were collected knife-detached for DNA analyses (Table 1). Data on the COI sequence, available from GenBank (Accession no. S54796, Yokobori *et al*. 1993) for *Halocynthia roretzi* was used as outgroup (Table 1). Tissue from the top portion of the inhalant siphon (1–1.5 cm) and of gill-sac tissue (1 × 1 cm) were removed and preserved in individual containers with 90% ethanol. DNA was extracted by maceration of preserved tissue in the reagent DNAzol (Chomczynski *et al*. 1997) followed by centrifugation and ethanol precipitation; and re-eluted in pure water. Double-stranded products for the COI mitochondrial gene were amplified via polymerase chain reaction (PCR) using the following primers from Folmer *et al*. (1994):

LCO-1490 (5′–3′ =) GGTCAACAAATCATAAAGATATTGG, and
HCO-2198 (5′–3′ =) TAAACTTCAGGGTGACCAAAAATCA

The PCR was carried out in 50-μL volumes. In addition to the DNA template from each specimen, PCR reactions included 5 μL 10× PCR buffer (Perkin–Elmer), 5 μL dNTPs (10 μM stock), 2 μL of each primer (10 mM stock), 3 μL $MgCl_2$ solution (25 μM stock, Perkin–Elmer), 0.2 μL AmpliTaq (5 U/mL stock, Perkin–Elmer) and 31.8 μL ddH_2O. Reactions were run for 37 cycles on Perkin–Elmer 9600 (U. California, Berkeley, USA) and PTC-100 MJ Research (P.U. Católica de Chile, Santiago) thermocyclers with the following parameters: an initial 1 min denaturation at 95 °C; then cycled at 95 °C for 40 s (denaturation), 42 °C for 40 s (annealing) and 72 °C for 60 s (extension). Wizard PCR Preps (Promega) cleaned products were visualized on 1% agarose gels, stained with ethidium bromide, visualized under UV light and concentrations estimated by comparison with a known standard. For automatic sequencing, double-stranded products were cycle sequenced at Berkeley using the Thermo Sequenase Dye Terminator Cycle Sequencing Pre-mix Kit (Amersham), with reaction volumes one half than those recommended in the manufacturer's protocols, and at Santiago using the ABI PRISM310 automatic sequencer (Perkin–Elmer), following the manufacturer's instructions. Each amplified mitochondrial DNA (mtDNA) product was sequenced in both directions and compared by overlap to assure accuracy using NAVIGATOR SEQUENCE software (Perkin–Elmer). COI sequences were translated to amino acids, based on the mitochondrial code for invertebrate animals, with ascidian exceptions (Yokobori *et al*. 1993), via MacVector7 (IBI) and aligned by eye. The resulting data matrix for analyses totalled 585 bases for 13 individuals. These data were analysed using PAUP Version 4.0b8 (Swofford 1998). Tree searches were

Table 2 Mean total and transversion only (bold) pairwise sequence differences within and between Pyuridae species. (1 SD is given in parentheses)

Species	*Pyura* sp.	*P. s. praeputialis*	*P. s. stolonifera*	*P. chilensis*	*H. roretzi*
Pyura sp.	3.00 (2.30)	2.67 (2.10)	109.83 (0.41)	149.67 (1.14)	142.67 (1.10)
	1.07 (0.70)	**1.00 (0.85)**	**39.67 (0.52)**	**62.67 (0.97)**	**66.00 (0.89)**
P. s. praeputialis		4.00 (0.00)	110.50 (0.71)	148.50 (0.55)	142.00 (0.00)
		2.00 (0.00)	**40.00 (0.00)**	**62.67 (1.21)**	**66.00 (1.41)**
P. s. stolonifera			0.00 (–)	141.67 (1.15)	143.00 (0.00)
			0.00 (–)	**52.67 (0.58)**	**40.00 (0.00)**
P. chilensis				2.67 (1.15)	159.00 (0.00)
				1.33 (0.58)	**66.67 (0.58)**
H. roretzi					0.00 (–)
					0.00 (–)

conducted using neighbour-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) methods. We employed the Kimura 2-parameter model for NJ searches and both unweighted and weighted (Ti/Tv = 3:1) parsimony for MP searches. The HKY85 + Γ model of nucleotide substitution was used for maximum likelihood optimality criteria with both the gamma shape and transversion ratio estimated (Hasegawa *et al*. 1985). Topological robustness was assessed using both bootstrap (1000 replicates) and Bremer support (ML, Bremer 1994).

Results

Of the 585 bases compared, 229 are variable, and 165 are parsimony informative (151 if the analysis is restricted to *Pyura* spp.). Of the 165 informative sites, 125 occur at third positions, 6 at second positions and 34 at first positions. Thirty-six of 195 amino acids are substituted (18.5%); 26 amino acid substitutions occur within *Pyura* spp. Of the 13 individuals compared, three sequences were identical: 'piure de Antofagasta' C5, C6 and *P.* s. *praeputialis* A2 from Australia. For all pairwise comparisons, the maximum number of sequence differences (= 159) was observed between the *Halocynthia roretzi* individual and the three individuals of *P. chilensis*. We estimated the total and transversion average differences within and between Pyuridae species (Table 2). The differences among *P. s. praeputialis* from Australia and 'piure de Antofagasta' are lowest: 2.67 and 3.0 for all, and 1.00 and 1.07 for transversions, representing 0.5% of total base difference. These differences are equal to or smaller than the intraspecific differences within both taxa (Table 2). However, the differences of *P.* s. *stolonifera* with both *P. s. praeputialis* and 'piure de Antofagasta' (110.5 and 109.8, 19%) are many-fold greater than the differences between the 'piure de Antofagasta' and *P.* s. *praeputialis*. The total base differences between each of the *P. stolonifera* complex taxa and *P. chilensis* (25%) are of the same magnitude as the differences with the outgroup species *H. roretzi* (24%) from a different Pyuridae genus.

Phylogenetic relationships among the *Pyura* taxa are consistently inferred for all three optimality search criteria: NJ, MP and ML. Figure 1(A) shows the maximum likelihood tree for the data using the HKY85 + Γ model for sequence evolution. Under ML, the transition/transversion ratio was estimated at 3.64 and the gamma shape estimated to be 0.313. Both equal weighting and 3:1 weighting for parsimony searches resulted in identical topologies to the ML tree. The eight most parsimonious topologies were found under MP. Although the network cluster, which includes the two *P. s. praeputialis* and the six 'piure de Antofagasta' is stable (Fig. 1B), rooting within it creates multiple topologies under MP. Within the network, haplotypes from Antofagasta fall between haplotypes from Australia. NJ searches also resulted in the same topology. Bootstrap support for the major lineages are all over 70% regardless of the search criteria used (Fig. 1A). Within the *Pyura* species sampled, three lineages are distinct: (i) the first contains individuals of both 'piure de Antofagasta' and *P.* s. *praeputialis* from Australia; (ii) the second is represented by *P. s. stolonifera* from South Africa; and (iii) the third is represented by *P. chilensis* (Fig. 1A).

Discussion

Our data suggest that the 'piure de Antofagasta' (= *Pyura* sp.), is not a distinct species or subspecies but a recently introduced population of *Pyura stolonifera praeputialis* from Australia. The divergence between Australian and Chilean individuals is extremely low (0.4% average). Moreover, one Australian individual is identical to two individuals from Chile. Haplotypes from Antofagasta fall within those from Australia within the haplotype network. Thus, even with our limited sampling, neither geographical population is monophyletic. Furthermore, the pattern of haplotype evolution indicates that these populations share

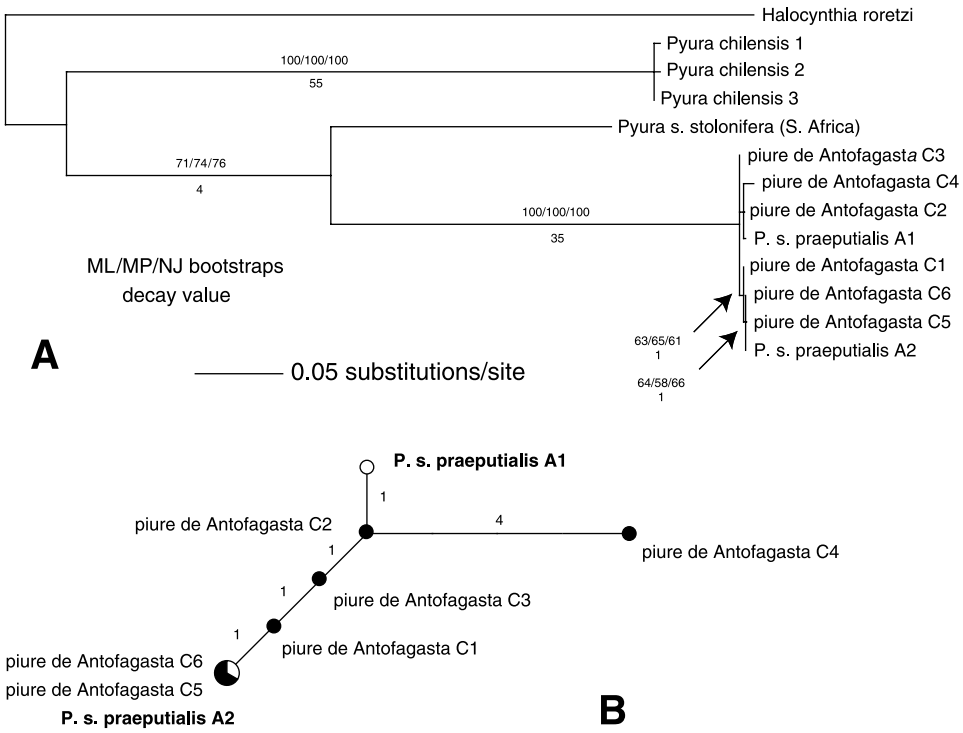

Fig. 1 Phylogenetic hypotheses of *Pyura* relationships. (a) Tree for which our data are most probable using neighbour-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) methods. For NJ we used a Kimura 2-parameter, for MP both unweighted and weighted (Ti/Tv = 3.1) parsimony and for ML the HKY85 + Γ model of nucleotide substitution. Bremer support indices shown below the nodes, and bootstrapping values (1000 bootstrap replicates) shown above the nodes. (b) Haplotypes network clustering including *P. s. praeputialis* and 'piure de Antofagasta' under maximum parsimony.

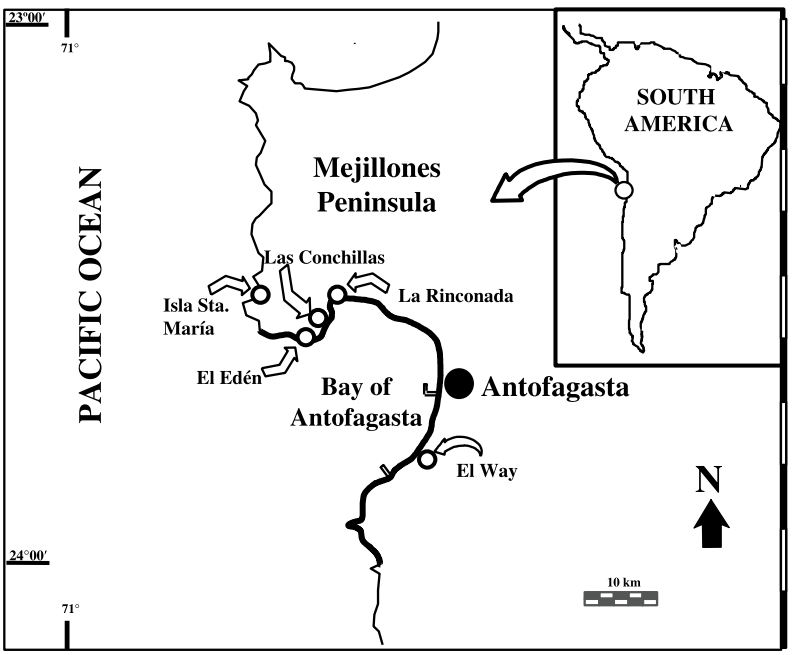

Fig. 2 Map of the Bay of Antofagasta and Mejillones Peninsula, showing in black the distribution of the intertidal 'piure de Antofagasta' (*Pyura* sp.) beds. Main sites of collection are shown.

an extremely recent common history. Molecular data also revealed that both, the Australian and Chilean taxa, are different lineages from the South African taxon (Castilla & Guiñez 2000). Although, we do not have a taxonomically appropriate calibration for COI mtDNA sequence divergence for ascidians, the divergence between *P. stolonifera praeputialis* and 'piure de Antofagasta' and *P. stolonifera stolonifera* is high at ≈ 19%. This amount of sequence divergence is consistent with other taxonomically distinct species for the COI gene. For instance, a difference of 5.26% separated the Portuguese oyster, *Crassostrea angulata* from the Pacific oyster, *Crassostrea gigas* (Ó Foighil *et al*. 1998), and genetic distances between nominal Asterinidae starfish species ranged from 2.5 to 24.5% (Hart *et al*. 1997).

With the caveat that using per cent differences is an arbitrary means of distinguishing species, we suggest that the status of the two subspecies proposed by Kott (1976, 1985, 1997), *P. s. praeputialis* from Australia and *P. s. stolonifera* from South Africa should be changed to species level: *P. praeputialis* and *P. stolonifera*, respectively. This agrees with Millar (1962, 1963) propositions. Furthermore, based upon phylogenetic analysis of COI and the near identity of COI sequences, we suggest that the 'piure de Antofagasta' taxon represents geographically isolated populations from the Australian species: *P. praeputialis*. This result is evident even though our sample sizes are relatively small, leading to an underestimate in the true amount of genetic diversity in the study taxa. The limited geographical distribution (60–70 km) of the 'piure de Antofagasta' population, exclusively inside the Bay of Antofagasta (Chile) (Fig. 2), along with its prominent level of ecological dominance where it occurs, suggests a recent introduction to Chile from the endogenous Australian *P. praeputialis*, presumably via ship fouling, ballast water or alternatively via rafting transport (Jackson 1986; Ó Foighil *et al*. 1999; Castilla & Guiñez 2000). There is a history of founder populations colonizing South America from Australia and New Zealand (Castilla & Guiñez 2000) and it is known that ascidian species may increase their geographical range partly owing to ship traffic (Monniot & Bitar 1983; Monniot 1994). The first reports of Australian ships arriving in the Bay of Antofagasta coincide approximately with the founding of the city in 1868 (Maino 1985; Arce 1997). The first scientific report of its presence inside the Bay of Antofagasta was made by Guiler (1959), who originally confounded the species with *P. chilensis*. Local elderly people in Antofagasta (personal communication to JCC) indicate that the species was present as an intertidal dominant organism as early as 1920–25. Successful introductions of exotic taxa to rocky shore communities are relatively rare compared with those of soft-bottom, fouling marine and estuarine habitats (Ruiz *et al*. 1997). One exception to this pattern is the mussel *Mytilus galloprovincialis* which has become well established along the rocky shores of the North Pacific and displaced the native *Mytilus trossulus* along the southern coast of California (Geller 1999). The introduction of *P. praeputialis* to Antofagasta, Chile has produced a similar pattern in its domination of space in the rocky shore community. In Antofagasta, the mid to low intertidal rocky shore fringe extends over several metres along flat platforms and are completely dominated by *P. praeputialis*, which out competes the mussel *Perumytilus purpuratus* for space (Paine & Suchanek 1983; Castilla 1998). The presence of *P. praeputialis* in the rocky shores of the Bay of Antofagasta is associated with a dramatic increase in local rocky shore biodiversity: whereas over 110 species (gamma diversity) of macro-invertebrates and algae coexist on and within the Antofagasta's *P. praeputialis* matrices, only 28 species (gamma diversity) live along adjacent coastlines (Cerda & Castilla 2002). It is somewhat surprising that these large mat-forming tunicates are so tightly restricted to this bay, showing very sharp boundaries (ecotones) both at its southern and northern distribution limits within the bay (Clarke *et al*. 1999; Castilla *et al*. 2000). The hypothesis that a water circulation retention mechanism occurs within the Bay of Antofagasta (Clarke *et al*. 1999), similar to a shadow upwelling (Graham & Largier 1997), may facilitate the retention of the tunicate larvae and explain the very restricted range of distribution of the species in Chile. We suggest that mitochondrial COI sequences may be used to clarify the systematic status and phylogenetic relationships of other tunicate species, such as the case of *P. bradleyi* (Peru & Ecuador, Van Name (1931), 1945), and the reported presence of *P. stolonifera* in Dakar and Morocco (Day 1974) and *P. praeputialis* in Tahiti (Kott 1985).

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