Phylogeography of *Pachygrapsus transversus* (Gibbes, 1850): The effect of the American continent and the Atlantic Ocean as gene flow barriers and recognition of *Pachygrapsus socius* Stimpson 1871 as a valid species

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Abstract

Genetic and morphometric comparisons among a few specimens of the littoral crab *Pachygrapsus transversus* have revealed marked intraspecific differences between three different coastlines (Cuesta and Schubart, 1998). Here we build on the previous study by presenting a more comprehensive analysis covering the entire range of this species from the Galápagos Islands to Israel, based on 195 specimens for morphometric analysis and 39 individuals for genetic comparisons of the 16S mtDNA. It is confirmed that marked genetic differences are present between three major coastlines (eastern Pacific, western and eastern Atlantic), whereas along single coastlines there is mostly high genetic homogeneity. Morphometric analyses also allow distinction of adult specimens from the three coastlines. In contrast, larval morphological and morphometric differences were less consistent and cannot be used to separate zoea I stages from the different megapopulations. In addition to the genetic separation of populations from different coastlines, this study provides new evidence for less marked, but consistent genetic differentiation between European and northern African populations of *P. transversus* on one hand, and central African ones on the other. However, by far the most prominent genetic differences within this complex are those between Pacific and all Atlantic populations, probably dating back to separation of these populations during the closure of the Panama Isthmus roughly three million years ago. Based on morphological and genetic differences outlined in this study, we revalidate the species name *Pachygrapsus socius* Stimpson, 1871 for all Pacific representatives of *P. transversus*.

Introduction

The tropical to subtropical littoral crab species *Pachygrapsus transversus* (Gibbes, 1850) (Decapoda: Brachyura: Grapsidae) is one of several marine coastal invertebrates occurring on both eastern and western sides of the Atlantic Ocean as well as the eastern Pacific Ocean (see Rathbun, 1918; Poupin *et al.*, 2005). This distribution pattern probably originates from a time when the two American continents were still separated and gene flow occurred between the tropical western Atlantic and eastern Pacific. Also, several million years ago, the Atlantic Ocean was a considerably narrower ocean basin than today, and current-mediated larval transport across it may have been feasible during the life span of a marine planktonic larva (Scheltema, 1986), especially if it consisted of several zoeal stages (Hines, 1986; Brossi-Garcia and

Rodrigues, 1997). With the widening of the Atlantic Ocean and the closure of the Panama Isthmus, most gene flow across the Atlantic and between the tropical Atlantic and Pacific ceased, and it must be assumed that present populations of tropical coastal invertebrates from the eastern Atlantic (EA), the western Atlantic (WA) and the eastern Pacific (EP) are for the most part genetically isolated from each other.

In the case of *P. transversus*, Cuesta and Schubart (1998) have previously pointed out morphological and molecular differences between the allopatric populations corresponding to the three coastlines. Most conspicuous is the coloration of the outer face of the chela in adult animals, which in Atlantic populations shows a dark blot in the area of articulation of the two fingers, whereas it is of a uniform light color in Pacific representatives (Stimpson, 1871; Cuesta and Schubart, 1998: Fig. 2). Morphometric comparisons between adult representatives from Cádiz (Spain, EA), Florida, Jamaica, Puerto Rico, Antigua, Grenada, Curaçao (WA) and Panama (WA, EP) suggested differences in ratios of carapace length and width, as well as length and width of the fourth pereiopod. First zoea larvae from Cádiz (EA) (see Cuesta and Rodríguez, 1994) were larger than their counterparts from Panama (WA, EP) and were the only ones with longer dorsal spines than rostral spines (Cuesta and Schubart, 1998: Table II). Also the dentition of the ventral margin of the larval carapace and the antennal spines showed consistent differences between populations (Cuesta and Schubart, 1998: Fig. 1).

For their molecular approach, Cuesta and Schubart (1998) used a 510-basepair fragment of the mitochondrial 16S rRNA gene to compare a total of four individuals from the three different coastlines. A single sequence from Cádiz (EA) differed in 1.0 % sequence divergence from two individuals with the same haplotype from the Caribbean (Antigua and Panama, WA) and in 3.5 % sequence divergence from a single sequence from Pacific Panama (EP). The transisthmian differences between the western Atlantic and the eastern Pacific amounted to 3.3 % divergence. For comparison, gene sequence divergence from the closely related species *Pachygrapsus crassipes* (Randall, 1840) ranged between 7.1 and 7.8 %. An example of genetic differences in a stretch of 16 contiguous basepairs was presented (Cuesta and Schubart, 1998: Table I).

All these differences indicated a lack of gene flow between three allopatric populations of *P. transversus* and a consequent morphological divergence. However, the results of Cuesta and Schubart (1998) were based on only a limited dataset in terms of the number of specimens and the geographic coverage. The described patterns may not be consistent throughout the respective coastlines. Before any taxonomic conclusions can be drawn, the degree of intrapopulational variation along the three different coastlines should be studied. Accordingly, Cuesta and Schubart (1998: 1505) pointed out: "Since differences were consistent within populations, a taxonomic separation might appear reasonable. On the other hand, we feel the need to study crabs from additional sites of this widely distributed species, before postulating new subspecies or species for entire coastlines." Similarly, Guerao *et al.* (2001) supported taxonomic separation of the Pacific populations of *Geograpsus lividus* (H. Milne Edwards, 1837), but only on the basis of a limited dataset which needs to be expanded in terms of sample size and geographic range.

The aim of the present study was to expand the dataset of Cuesta and Schubart (1998) by measuring and DNA-sequencing additional specimens of *Pachygrapsus transversus* from the three allopatric populations as defined above (EA, WA, EP). Samples were chosen to cover almost the entire distributional range of this species from Galápagos (Garth, 1946) to Israel (Holthuis and Gottlieb, 1958). Preliminary morphological and molecular differences as stated by Cuesta and Schubart (1998) could then be checked for consistency within entire coastlines and interpreted for taxonomic conclusions. The second wider aim of this paper was to assess the role of the American continent and the Atlantic Ocean as gene flow barriers for tropical coastal invertebrates, taking into account marked geological changes experienced in the distribution of land masses surrounding the Atlantic Ocean during the past 5 million years.

Material and Methods

Specimens of *Pachygrapsus transversus* were collected or obtained from museum collections and colleague donations for more than 30 localities, covering the entire range of distribution from Baja California to the Galápagos Islands and Peru in the eastern Pacific, from Florida to southern Brazil in the western Atlantic, and from Israel to the mouth of the Congo River in the eastern Atlantic. Our material also included 10 specimens from the type series of *Pachygrapus socius* Stimpson, 1871 from Cape St. Lucas (Baja California Sur, Mexico) and Panama, deposited at the Museum of Comparative Zoology at Harvard University (Cambridge, Massachusetts, USA). Rathbun (1918) and subsequent authors have treated this taxon as a synonym of *Pachygrapsus transversus* (see Poupin *et al.* 2005).

Specimens used for DNA analysis (museum and new collections, Table I) were preserved in 75-96% ethanol. This material consists of 15 individuals from the eastern Pacific, 14 from the eastern Atlantic and 10 from the western Atlantic. For extraction of DNA, PCR amplification and sequencing of the partial 16S mitochondrial DNA gene (encoding the large subunit rRNA) we generally followed the protocol of Schubart et al. (2000). The forward primer 16Sar (5'-CGCCTGTTTATCAAAAACAT-3') was thereby combined with the reverse primer 16Sbr (5'-CCGGTCTGAACTCAGATCACGT-3') (see Palumbi *et al.*, 1991) to amplify a 523 basepair fragment (e.g. Genbank AJ130800) or with the primer 1472 (5'-AGATAGAAACCAACCTGG-3') (see Crandall and Fitzpatrick, 1996) resulting in 551 basepairs of amplified DNA (e.g. Genbank AJ250641). In several cases, when older museum samples were used for DNA amplification (Cameroon, Mauritania, Republic of Congo, El Salvador, Galápagos, and a syntype of *P. socius* from Mexico), it was impossible to amplify the entire fragment at once, and internal primers designed for grapsoid crabs (5'-GACGATAAGACCCTATAAAGCTT-3' and 5'-TTATCRCCCCAATAAAATA-3', 16L15 and 16H16, respectively, see Schubart et al. 2001) were used to obtain the complete sequence. All sequences revealing new haplotypes were confirmed by sequencing both strands and using alternative primer combinations. Sequences were aligned manually with the multisequence editing program ESEE (Cabot and Beckenbach 1989). For phylogenetic analyses, the complete 16Sar-1472 region was used, whereas the haplotypes were defined and their differences established using the 16Sar-16Sbr region, because DNA of almost all specimens was available from this slightly shorter region. Previous to this study, only the 16S sequence of *Pachygrapsus transversus* from the western Atlantic (Panama, Louisiana, Mexico) had been deposited in molecular databases, without showing any difference in their sequence (accession numbers AJ225892, AJ130800, AJ250641). New sequences corresponding to populations from the other coastlines and additional haplotypes were submitted to the European Molecular Biology Laboratories database (EMBL accession numbers: AM180256- AM180262). The program MEGA2 (Kumar et al. 2001) was used to calculate Kimura 2-parameter distance estimates of sequence divergence and to infer phylogenetic relationships by neighbor-joining (NJ) (Saitou and Nei 1987). Statistical significance of groups within inferred trees was evaluated by the by the bootstrap method with 2000 pseudoreplicates.

For morphometric comparisons of *Pachygrapsus transversus* from the three coastlines, sixteen measurements were taken from 195 individuals (45 males and 32 females from EA, 40 males and 31 females from WA and 23 males and 24 females from EP). Among the measured animals were the following specimens from museum collections (* indicate new specimens added to collections for this study; MCZ: Museum of Comparative Zoology, Cambridge; MZUSP: Museu de Zoologia Universidade de São Paulo, Brazil; RMNH: Naturalis Museum, Leiden, Netherlands; SMF: Senckenberg Museum, Frankfurt a.M., Germany; ULLZ: University of Louisiana at

Lafayette Zoological Collection, Lafayette, USA; USNM: Smithsonian Institution and National Museum of Natural History, Washington DC, USA): **East Atlantic**: **Israel**. Tel Aviv, 1 male, 1 female (USNM 152561). – **Spain**. Cádiz, Cabo Trafalgar, coll. 25. Sept. 1996, 12 males, 10 females (*MCZ 61993); coll. 1. June 1996, 1 female (*SMF 23720); coll. 11. June 1998, 5 males, 3 females (*SMF 30127); Ibiza, Cala Vedella, 1 female (*SMF 30128); Ibiza, St. Vicent, 3 juveniles (*SMF 30129); Canary Islands, Gran Canaria, 3 males, 1 female (USNM 216989). – **Morocco**. Rabat, 8 males, 1 female (USNM 258363). – **Mauritania**. Nouadhibou, 1 male, 3 females (RMNH D 39732). – **Senegal**. Cap de Biche, 3 males, 2 females (RMNH D 39729). – **Ghana**. Takoradi, 1 female (*SMF 30126). – **São Tome and Principe**. Bom-Bom, 4 juveniles (*RMHN D 51197). – **Republic of Congo**. Congo River Mouth, 10 males, 8 females (USNM 54235). – **Cameroon**. North of Nimbe, 1 male (SMF 25979). – **West Atlantic**: **USA**. Florida, Fort Pierce, 1 male, 2 females (*ULLZ 3754); Boca Raton, 3 males (*MCZ 61995); Texas, Port Aransas, 2 males, 1 female (*RMNH D 48031), 2 females (*SMF 30130). – **Mexico**. Veracruz, Punta Delgada, 1 juvenile (*ULLZ 3723). – **Panama**. María Chiquita, 1 male, 1 female (*MCZ 61994), 1 male, 1 female (*SMF 23718). – **Cuba**. Sancti Spíritu, La Boca near Trinidad, 3 males (*SMF 30131). **Antigua**. English Harbour, 1 male (*SMF 23719). – **Brazil**. São Paulo, Ubatuba, 2 males, 2 females (*SMF 30132); Santa Catarina, Ilha Sta. Catarina, Praia Ingleses, 1 male (*MZUSP-16887). – **Pacific**: **Mexico**. Baja California Sur, Cape San Lucas 3 males, 5 females, syntypes of *P. socius* (MCZ 1312); Baja California Sur, Playa Santispac, 1 male (*MCZ 61997); Baja California Sur, Puerto San Carlos, 2 males, 1 female (*MCZ 61996), 3 males & 2 females (RMNH D 48032). – **El Salvador**. San Miguelito, 1 female (RMNH D 9594). – **Panama**. 1 male, 1 female, syntypes of *P. socius* (MCZ 1022); Naos, 1 male, 1 female (*SMF 23717), 1 male, 1 female (*ULLZ 3805). - **Peru**. Lima, Callao, 1 female (*SMF 30133). - Ecuador Galapagos (USNM 78224).

The following measurements were taken: maximum carapace width (CW), posterior carapace width (PCW), maximum carapace length (CL), body height (BH), intraorbital width (IW), chela merus length and width, chela propodus height (PrH) and length (PrL), chela dactylus length, third pereiopod merus length (3ML) and width (3MW), fourth pereiopod merus length (4ML) and width (4MW), fourth pereiopod total length (4Prp), and maximum pleon width. All morphometric data were statistically tested for normal distribution and subsequently compared with the parametric 1-Factor ANOVA and Discriminant Function Analyses.

First zoea larvae in addition to the six hatches (2 from Cape Trafalgar, Spain, 1 from Caribbean Panama, and 3 from Pacific Panama) from the study of Cuesta and Schubart (1998) were obtained from three western Atlantic populations (2 hatches from Louisiana, 1 from Florida and 1 from Brazil) and one eastern Pacific population (1 hatch from Baja California, Mexico), rendering a total of 11 hatches for morphometric comparisons. Up to 30 larvae per hatch were measured. Drawings and measurements were made using Wild MZ6 and Zeiss Axioskop compound microscopes, each equipped with a *camera lucida*. Rostrodorsal length (rdl) was measured from the tip of the rostral spine to the tip of the dorsal spine, carapace length (cl) from the base of the rostrum to the posterior margin of the carapace, dorsal spine length (ds) from spine base to tip, and rostral spine length (rs) from the deepest part of the ocular eave to spine tip.

Parental females and/or samples of zoea I of *Pachygrapsus tranversus* from Florida, Louisiana (both USA) and Baja California (Mexico) were deposited at the the University of Louisiana at Lafayette Zoological Collection, Lafayette (ULLZ 6370, 6369, 4135 respectively), and from Trafalgar (Spain), María Chiquita, Naos and Farfán (all Panama), at the Senckenberg Museum Frankfurt (SMF 23720, 23718, 23717, 30946 respectively). One parental female and a sample of zoeae I of *P. transversus* from Ubatuba, São Paulo (Brazil) are deposited at the Crustacean Collection of the Department of Biology, FFCLRP, University of São Paulo (catalog number CCDB/FFCLRP/USP # 1571).

Table I: Specimens of *Pachygrapsus transversus* (Gibbes, 1850) used for DNA sequencing, with locality and year of collection, genetic haplotype, length of sequenced fragment, and museum catalog number.

Locality of Collection	Year	Haplotype	length	Catalogue number
Israel: Tel Aviv	1966	EATL-1	551	USNM 152561
Spain: Ibiza: Cala Vedella	2001	EATL-1	551	SMF 30128
Spain: Ibiza: St. Vicent	2001	EATL-1	551	SMF 30129
Spain: Cádiz: Cabo de Trafalgar 1	1996	EATL-1	551	SMF 23720
Spain: Cádiz: Cabo de Trafalgar 2	1998	EATL-1	506	SMF 30127
Mauritania: Nouadhibou	1978	EATL-1	551	RMNH 39732
Spain: Canary Islands	1984	EATL-1	523	USNM 361562
Cape Verde Islands: Ilha de Santa Luzia	1982	EATL-2	492	RMNH 39744
Cameroon: near Nimbe	1984	EATL-2	551	SMF 25979
São Tomé & Principe: Bom Bom 1-3	2004	EATL-2	551	RMNH D51197
Ghana: Takoradi	2001	EATL-3	551	SMF 30126
Republic of Congo: Congo River Mouth	1915	EATL-4	551	USNM 54235
USA: Florida: Ft. Pierce	1998	WATL-1	500	ULLZ 3755
USA: Florida: Boca Raton 1-3	2004	WATL-1	506	MCZ 61995
USA: Louisiana: Grande Isle	1997	WATL-1	523	ULLZ 3782
Mexico: Veracruz: Punta Delgada	1998	WATL-1	551	ULLZ 3723
Panama: María Chiquita	1996	WATL-1	512	SMF 23718
Antigua: English Harbour	1994	WATL-1	492	SMF 23719
Brazil: São Paulo: Ubatuba	1996	WATL-1	523	SMF 30132
Brazil: Santa Catarina: Praia Ingleses	2004	WATL-1	551	MZUSP-16887
Mexico: Baja California Sur: C. San Lucas	1860	PAC-1	375	USNM 1312 syntype
Mexico: Baja California Sur: San Carlos 1	1999	PAC-1	551	RMNH 48032
Mexico: Baja California Sur: San Carlos 2	1999	PAC-1	503	MCZ 61996
Mexico: Baja California Sur: San Carlos 3	1999	PAC-1	551	MCZ 61996
Mexico: Baja California Sur: Mulegé	2001	PAC-1	504	ULLZ 5933
Mexico: Baja California Sur: Santispac 1	1999	PAC-2	551	ULLZ 4134
Mexico: Baja California Sur: Santispac 2	1999	PAC-1	551	ULLZ 4134
Mexico: Baja California Sur: Santispac 3	2001	PAC-1	504	ULLZ 5934
Mexico: Baja California Sur: Santispac 4	2001	PAC-3	551	ULLZ 5935
El Salvador: S. Miguelito: El Cuco	1953	PAC-1	500	RMNH 9594
Costa Rica: Puntarenas: Golfo Nicoya	1998	PAC-1	521	ULLZ 4303
Panama: Naos 1	1996	PAC-1	511	SMF 23717
Panama: Naos 2	1996	PAC-1	551	SMF 23717
Peru: Lima: Callao	1999	PAC-1	551	SMF 30133
Ecuador: Galápagos	1938	PAC-1	551	USNM 78224

Results

All mtDNA sequences of the large subunit ribosomal RNA of *Pachygrapsus transversus* clustered in one of three clearly divergent haplotype groups (Fig. 1). These haplotype groups are completely to relatively homogeneous in contrast to the pronounced differences found among the three groups. The distribution of the haplotype groups corresponds perfectly to the three major coastlines that comprise the distributional range of the studied species: eastern Pacific, western and eastern Atlantic. Overall, eight haplotypes were found, of which four are from the eastern Atlantic, three from the Pacific and only one from the western Atlantic. The only locality in the Pacific, where haplotypes other than the most common one were encountered was the Gulf of California. The eastern Atlantic population can be further subdivided into i) a European to north African haplotype group, sharing the same haplotype, ii) a more southern central African haplotype group, consisting of two different haplotypes and iii) a haplotype from the Republic of Congo, which is genetically closest to the western Atlantic populations. The first two haplotype groups are consistently separated by one transition. In contrast, the western and all eastern Atlantic haplotypes are consistently separated by at least three transitions (the European forms by five transitions, the Congo River form by three) and the Pacific haplotype group is consistently separated from all Atlantic haplotypes in as many as 15 diagnostic positions (12 transitions, 1 transversion, 1 indel). Thereby the differences between Pacific and western Atlantic populations sum up to exactly the same number (17 in total: 14 transitions, 1 transversion, 2 indels) as do the consistent differences between Pacific and eastern Atlantic populations.

To test for significant morphometric differences between adult crabs from three coastlines in single character ratios, as well as in overall discriminant analyses, we analysed (after confirming that all the morphometic data followed a normal distribution) whether females and males differed in some of the character ratios as suggested by Flores (1996) and Cuesta and Schubart (1998). Using the entire dataset of 195 individuals (108 males and 67 females), we found significant differences in all except one of the tested morphometric ratios between males and females: CL/CW, BH/CW, PCW/CW, IW/CW, 4Prp/CW, 3ML/3MW, PrH/CW, PrL/CW, PrH/CW. Therefore, all 1-Factor-ANOVA analyses were carried out only after separation of the sexes. Overall significant differences that were consistent in both sexes were found in the following ratios: carapace length to carapace width, posterior carapace width to maximum carapace width, fourth pereiopod length versus carapace width and fourth pereiopod merus length versus merus width (see Table II). There were no significant differences in chela morphometry or in frontal (intraorbital) width or body height. In order to test and visualize the overall differentiation between populations of *P. transversus,* a discriminant analysis was carried out using ten variables. The data set was subjected to canonical analyses (Fig. 2) in which discrimination between the groups was highly significant (Wilks' Lambda: 0.37439; F (30,352) = 7.4428; p<0,0001), resulting in a 76.17 % probability of correct classification. The western Atlantic population was correctly classified with a likelihood of 88.0 %, the eastern Atlantic population with a likelihood of 80.3 %, and the Pacific population with a likelihood of 51.1 %. The Mahalanobis distances (D^2) are highest between the East and West Atlantic (D^2 4.716) and lowest between East Atlantic and Pacific (D^2 3.286). Two discriminant functions (roots) accounted for 100% of the explained variance.

The inclusion of new larval morphometric data corresponding to the first zoeal stage from a total of five hatches from Brazil, Florida, Baja California, and Louisiana (2 hatches), to supplement previous results of Cuesta and Schubart (1998: Table II), resulted in the loss of consistent morphometric patterns that would allow distinction of the larvae from the three coastlines (Table III). First zoeal stages of the Atlantic populations seem overall larger (rdl >

0.74 mm) than Pacific ones (rdl < 0.65 mm), but with clear exceptions in the tropical waters of the Caribbean (0.62 mm) and the subtropical Gulf of Mexico (0.58 mm, the smallest of all examined). Also, the ratios of dorsal versus rostral carapace spines showed differences, being >1 in the Atlantic populations and < 1 in the Pacific ones, but again with exceptions represented by the Caribbean and Gulf of Mexico populations (<1). Dentition of the antennae and ventral carapace margins showed high variability among populations but no clear geographic pattern (Figs. 3-4).

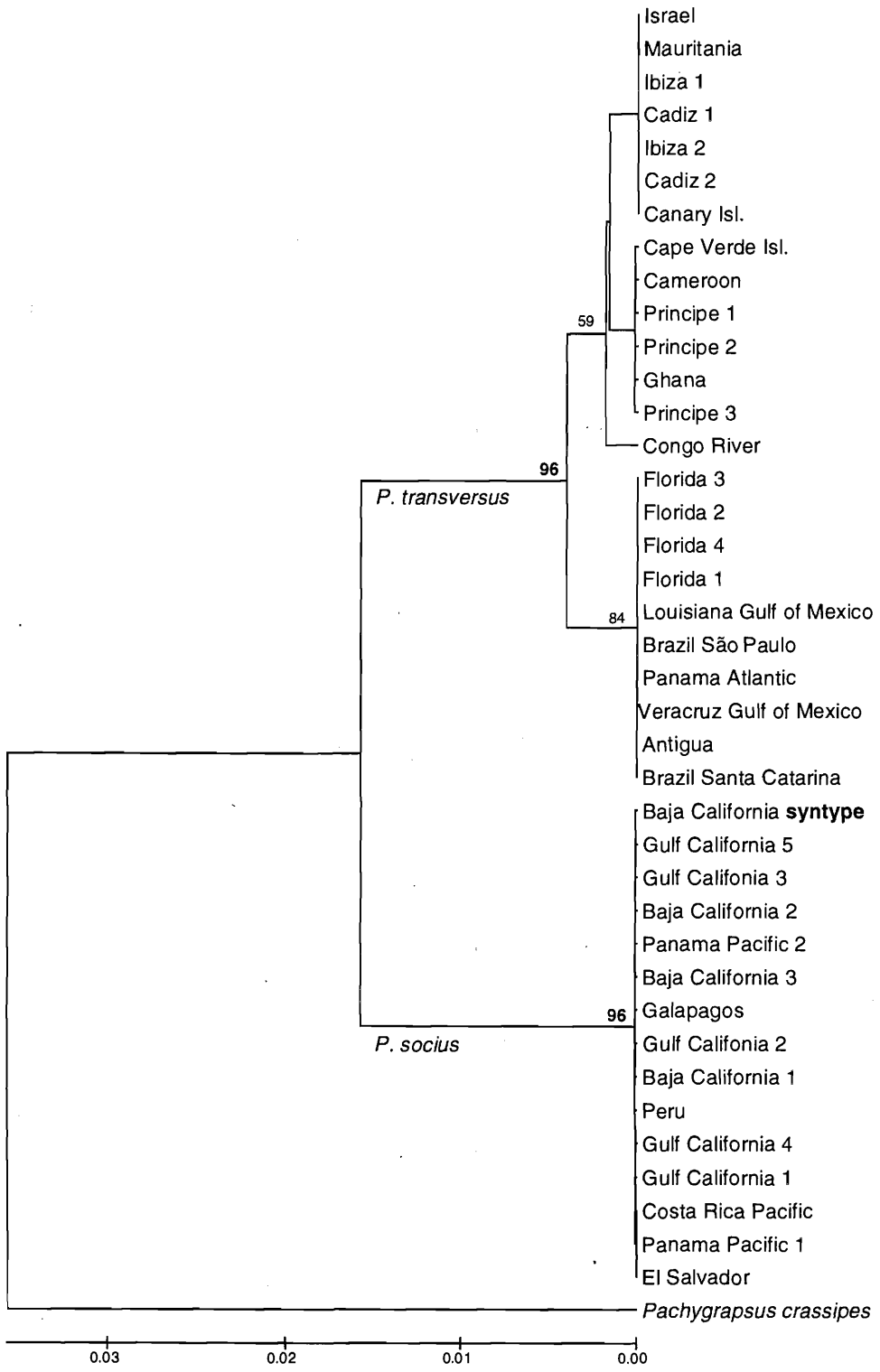

Figure 1: Linearized neighbor joining distance tree of 42 sequences (551 basepairs) of the mitochondrial 16S rRNA gene from the entire range of the species *Pachygrapsus transversus* (Gibbes, 1850) demonstrating its geographic subdivision.

Table II: Differences in adult morphometry among allopatric populations of *Pachygrapsus transversus* (Gibbes, 1850) from three coastlines. Numbers represent mean, standard deviation, number of individuals measured, F value from the 1-Factor ANOVA and Scheffe post-hoc tests.

		East Atlantic	West Atlantic	East Pacific	F	p	Scheffe
CL/CW	males	0.80 ± 0.02 (45)	0.77 ± 0.02 (40)	0.81 ± 0.02 (23)	40.79	< 0.0001	all
	females	0.78 ± 0.02 (32)	0.75 ± 0.05 (31)	0.78 ± 0.02 (24)	11.49	< 0.0001	WA-(EA+EP)
PCW/CW	males	0.77 ± 0.02 (45)	0.79 ± 0.02 (40)	0.81 ± 0.02 (23)	25.07	< 0.0001	all
	females	0.79 ± 0.02 (31)	0.80 ± 0.02 (31)	0.81 ± 0.02 (24)	8.77	0.0004	EA-EP
4Prp/CW	males	1.31 ± 0.06 (43)	1.34 ± 0.07 (38)	1.39 ± 0.05 (22)	15.07	< 0.0001	EP-(EA+WA)
	females	1.25 ± 0.05 (28)	1.27 ± 0.06 (28)	1.31 ± 0.06 (23)	7.32	0.0012	EP-(EA+WA)
4ML/MW	males	1.87 ± 0.08 (43)	1.95 ± 0.10 (38)	1.90 ± 0.09 (23)	6.89	0.0016	EA-WA
	females	1.84 ± 0.09 (29)	1.93 ± 0.09 (29)	1.90 ± 0.14 (24)	5.43	0.0062	EA-WA

Table III: Differences in zoea I morphometry between 7 populations (11 hatches) of *Pachygrapsus transversus* from the East and West Atlantic, and the East Pacific. Measurements include rostrodorsal length (rdl) and the ratio of dorsal *versus* rostral carapace spine lengths. Numbers represent means ± standard deviations, with the number of measured larvae in parentheses.

	East Atlantic		West Atlantic			East Pacific	
	Cádiz (Spain) 2 hatches	Florida (USA) 1 hatch	São Paulo (Brazil) 1 hatch	Maria Chiquita (Panama-Atl.) 1 hatch	Louisiana (USA) 2 hatches	Naos / Farfán (Panama-Pacific) 3 hatches	Baja California (Mexico) 1 hatch
Rostrodorsal length [mm]	0.749 ± 0.025 (20) 0.751 ± 0.020 (40)	0.77 ± 0.02 (20)	0.76 ± 0.01 (20)	0.62 ± 0.03 (20)	0.59 ± 0.01 (20) 0.57 ± 0.01 (20)	0.62 ± 0.02 (20) 0.66 ± 0.03 (20) 0.62 ± 0.02 (7)	0.63 ± 0.04 (20)
Dorsal/rostral spine ratio	1.070 ± 0.052 (20) 1.070 ± 0.047 (40)	1.03 ± 0.06 (20)	1.15 ± 0.06 (20)	0.94 ± 0.03 (20)	0.78 ± 0.13 (20) 0.74 ± 0.04 (20)	0.93 ± 0.05 (20) 0.74 ± 0.03 (20) 0.95 ± 0.03 (7)	0.88 ± 0.04 (20)

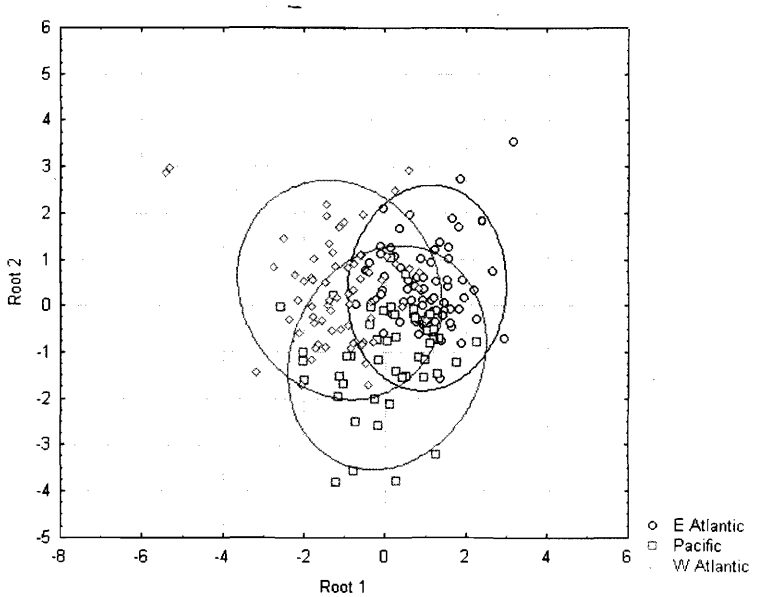

Figure 2: Canonical analysis depicting discrimination by morphometric measurements within *Pachygrapsus transversus* (Gibbes, 1850) from three different coastlines; plot of the first discriminant function (root 1) against the second (root 2).

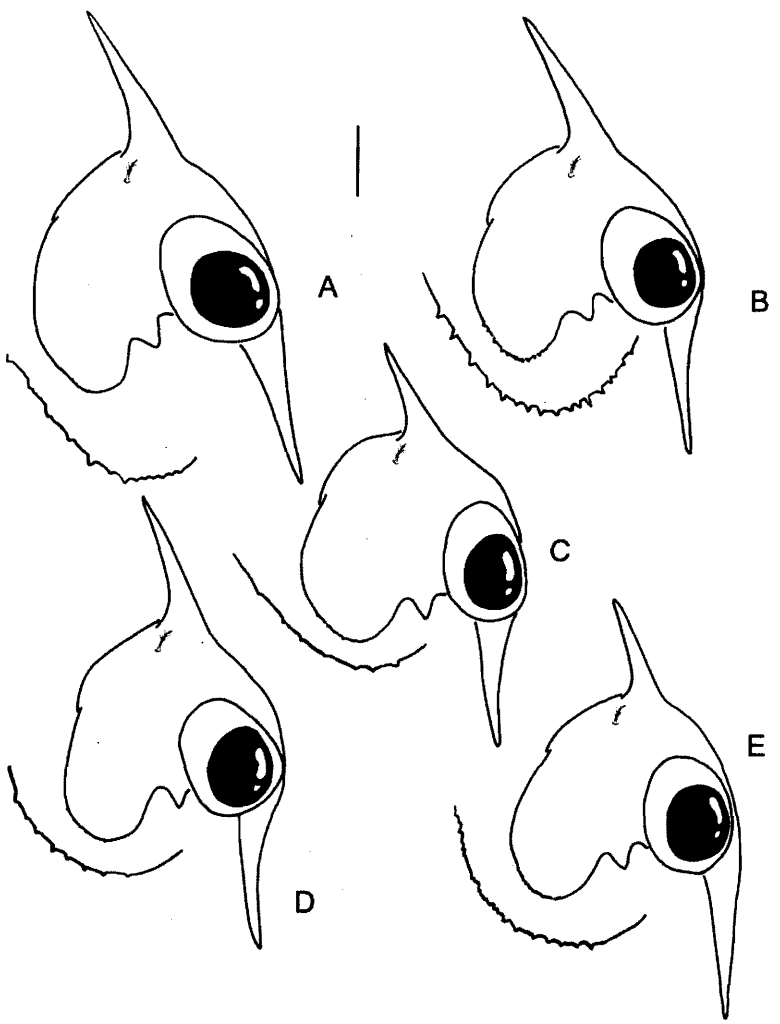

Figure 3: Lateral view of the carapace of the zoea I of *Pachygrapsus transversus* (Gibbes, 1850) with amplification of ventral carapace margin: (A) Cádiz, East Atlantic; (B) Maria Chiquita, Caribbean; (C) Isles Dernieres, Louisiana (USA), West Atlantic; (D) Santispac, Baja California (Mexico), East Pacific; (E) Naos (Panama), East Pacific. Scale bar 0.1 mm.

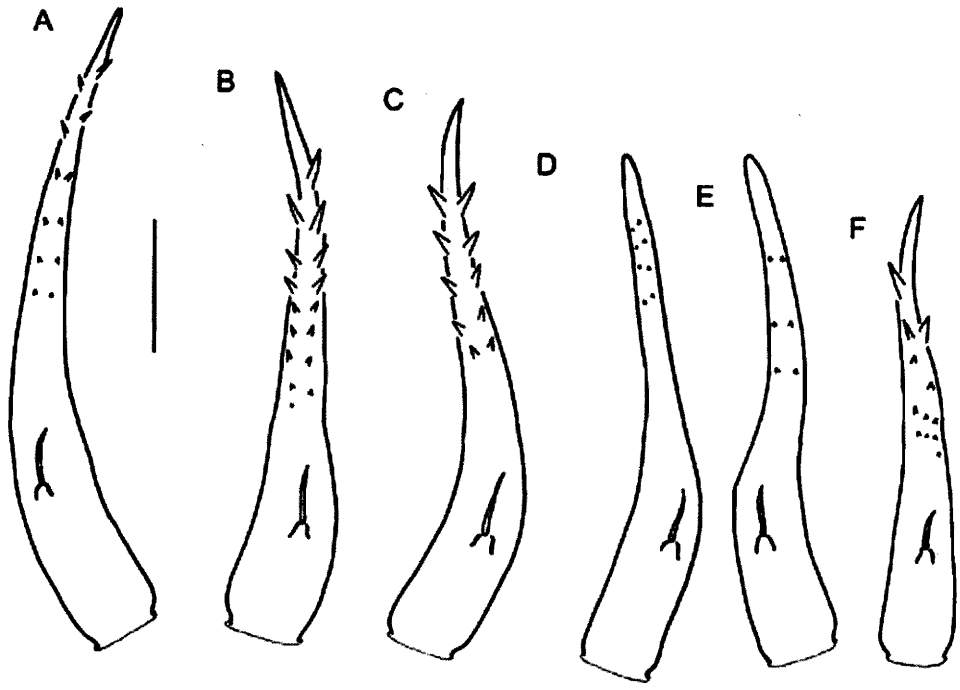

Figure 4: Different antennal spinulation patterns observed in seven allopatric populations of *Pachygrapsus transversus* (Gibbes, 1850): (A) Cádiz, Spain, East Atlantic; (B) Fort Pierce, Florida, USA, West Atlantic; (C) Ubatuba, São Paulo, Brazil, West Atlantic; (D) Maria Chiquita, Panama, West Atlantic; (E) Santispac, Baja California, Mexico, East Pacific; (F) Isles Dernieres, Louisiana, USA, West Atlantic. Scale bar 0.1 mm.

Discussion

Marked genetic differences in mtDNA, corresponding to the large subunit ribosomal rRNA, were found between populations of the tropical littoral crab *Pachygrapus transversus* from three different coastlines. These differences were previously noted by Cuesta and Schubart (1998) on the basis of preliminary results from four individuals. This pattern is now confirmed for entire coastlines stretching over several thousands of kilometres. The genetic homogeneity of the two American populations is remarkable. Ten western Atlantic individuals obtained from Florida to southern Brazil all share the exact same haplotype (WATL-1). Along the Pacific coast, three different haplotypes were found, but two of them (PAC-2 and PAC-3) were only found once in one locality in the Gulf of California (Sea of Cortez), where the most common haplotype PAC-1 was also present. Therefore, haplotype diversities of these coastlines are very low. In contrast the European-African Atlantic coast is genetically more heterogeneous and structured. So far, four different haplotypes have been found, of which two occurred more than once. Here the haplotype distribution follows a geographic pattern. Only one haplotype (EATL-1) is found in and restricted to European-north African waters, including the western and eastern Mediterranean, the Canary Islands and Mauritania. A second haplotype (EATL-2) differs in one position from EATL-1 and is predominant in the Gulf of Guinea. Derived from this are a third (EATL-3) and a fourth haplotype (EATL-4) found in Ghana and near the Congo River mouth, respectively, both of which differ from EATL-2 in one transition. Interestingly, the one consistent difference found between the three central African haplotypes and the European-north African haplotype, is shared between central Africa and the American Atlantic populations (WATL-1), and the one additional difference from the Congo River mouth is also shared with WATL-1. Therefore, genetic distance to the eastern American haplotype gradually decreases from southern Europe to tropical Africa.

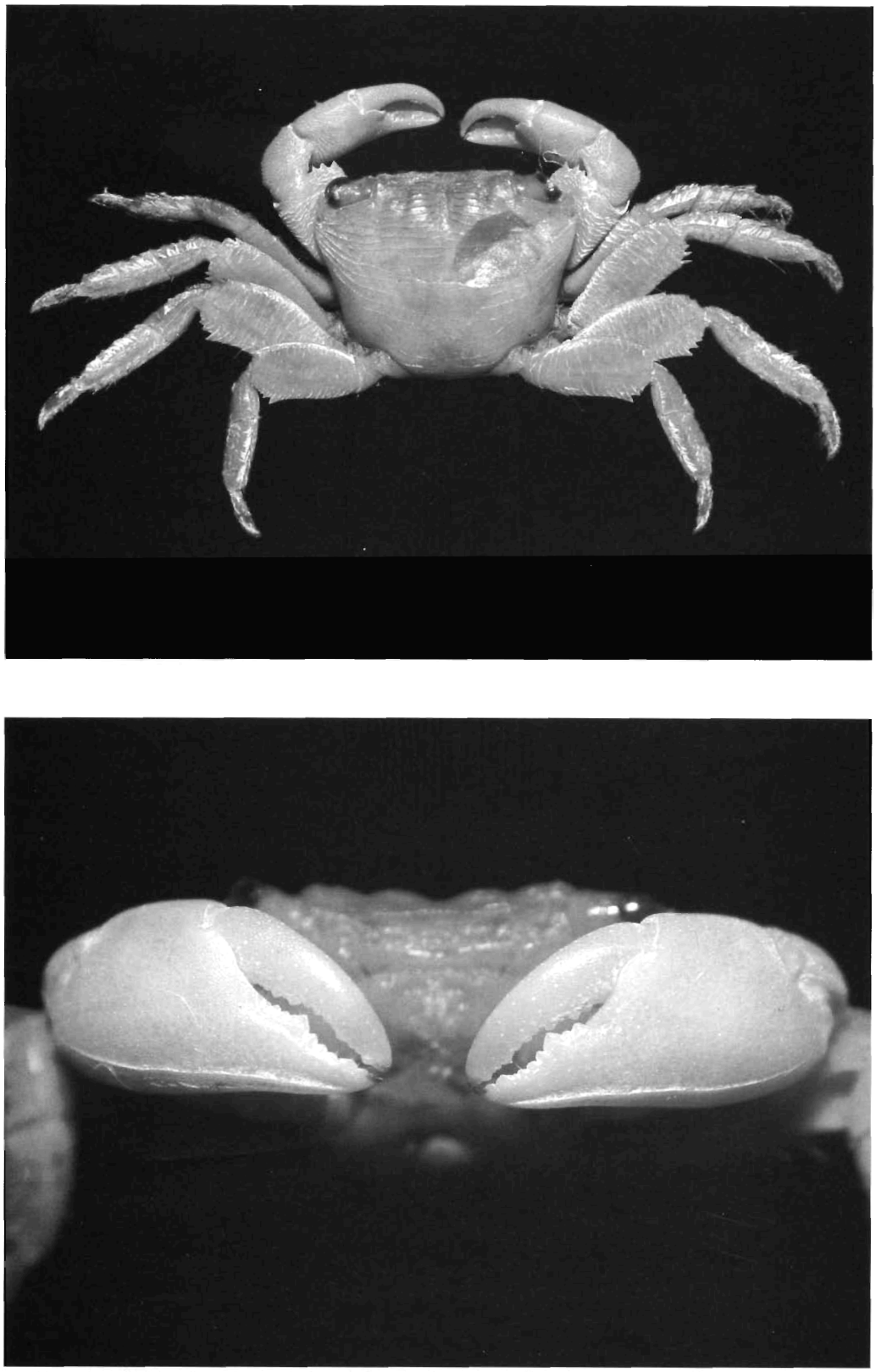

Figure 5: Dorsal and frontal view of designated male lectotpye of *Pachygrapsus socius* Stimpson, 1871 from Pacific Panama, 1860, MCZ 1022.

Genetic divergence across the American continent is far more pronounced than divergence across the Atlantic Ocean. It likely reflects the separation of at least 3 million years, which is the approximate time estimate for the closure of the Panama Isthmus and the separation of Atlantic and Pacific tropical waters (Keigwin, 1982; Knowlton and Weigt, 1998) and accounts for observed differences in adult morphology. Consequently, taxonomic separation seems

necessary at least for the Pacific representatives of this species complex. For this reason, we herewith validate the previously synonymized name of *Pachygrapsus socius* Stimpson, 1871 to be used for all representatives of *P. transversus* from the eastern Pacific. One syntype of Stimpson's type series from Pacific Panama (14.23 mm carapace width – 11.56 mm carapace length) collected by Agassiz on 15 March 1860 and in the holdings of the Museum of Comparative Zoology in Cambridge (MCZ 1022) is herewith designated as the lectotype (Fig. 5). A detached leg from the type series from Cape St. Lucas was used for DNA extraction. We were able to read 375 basepairs of this DNA and they matched perfectly the sequence of the common Pacific haplotype PAC-1 and differed from all Atlantic haplotypes. In the original description of *Pachygrapsus socius*, Stimpson (1871) wrote: "Closely allied to *P. transversus*, but differing in several minor particulars. The carapace is somewhat narrower, less convex, and more strongly striated; and the frontal region is more depressed and expanded. The propodal finger of the chelipeds never has the dark blot which is always more or less conspicuous in *P. transversus*." This consistent difference in color was already confirmed and shown as a photograph by Cuesta and Schubart (1998). In the present study, we include another photograph to emphasize the difference of the light color of the outer chela of *Pachygrapsus socius* (Fig. 6A) as compared to the darker chelae of three genetically distinct Atlantic populations of *P. transversus* (Fig. 6B-D). It can be observed that the dark blot is not only found on the propodal finger, as indicated by Stimpson (1871), but also extends onto the dactylus.

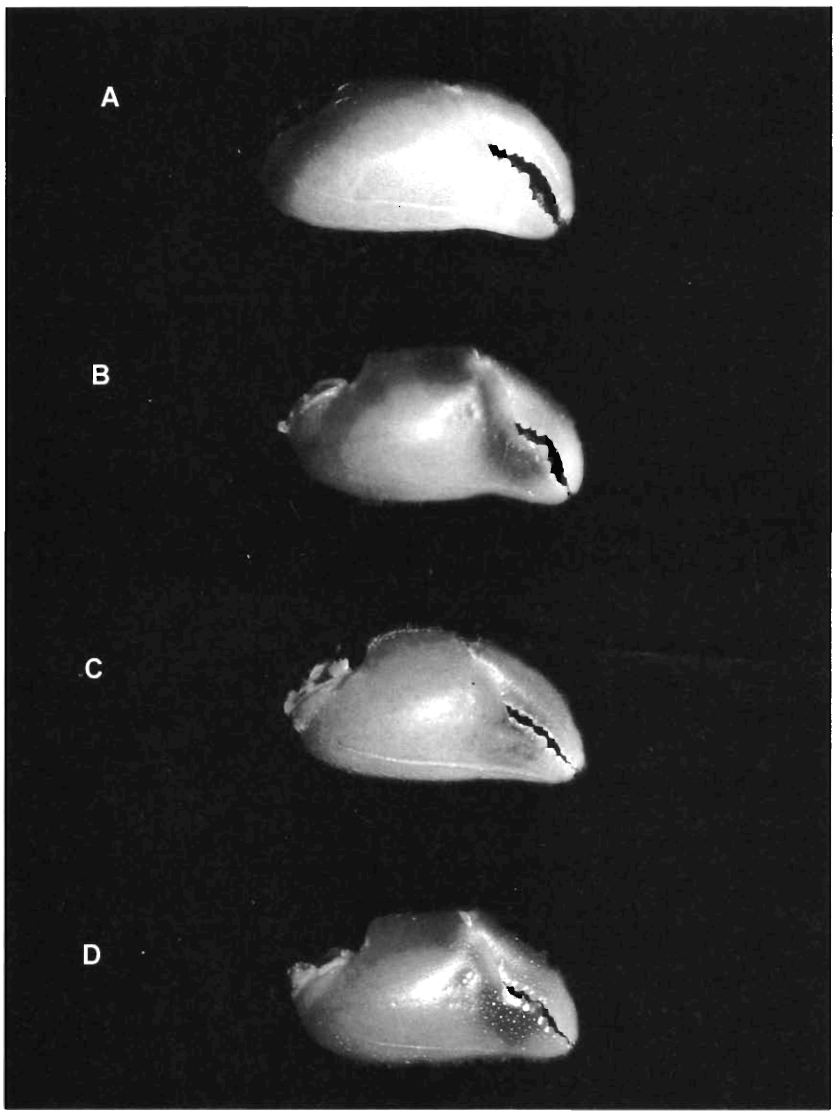

Figure 6: Coloration of outer face of chela palma and dactylus in *Pachygrapsus socius* Stimpson, 1871 (A) and *P. transversus* (Gibbes, 1850) (B-D) comparing four different populations: (A) Naos, Panama, East Pacific; (B) Antigua, Lesser Antilles, West Atlantic; (C) Cádiz, Spain, East Atlantic; (D) Takoradi, Ghana, East Atlantic.

Morphometric differences among adults of *Pachygrapsus transversus* from three coastlines were similar to the ones outlined in Cuesta and Schubart (1998). The same four character ratios that showed significant differences in the earlier study, were confirmed in the present study as the best separating values. Despite the much higher sample size (195 versus 77), F-values and standard deviations remained in a similar range. However, the post-hoc tests allowed a clearer separation, resulting in significant differences between all three populations in male CW/CL and PCW/CW. Overall it can be said that *P. socius* from the East Pacific has indeed a "somewhat narrower" carapace than the Atlantic *P. transversus* (larger CL/CW), as suggested by Stimpson (1871). This difference, however, is more obvious when comparing to the West Atlantic populations (i.e. the populations that Stimpson used for his comparisons) than to those of the East Atlantic. The ratio of posterior to anterior carapace width reveals that the Pacific *P. socius* is the less triangular in shape (largest PCW/CW) and that the East Atlantic *P. transversus* is the most triangular, perhaps reflecting what Stimpson (1871) referred to as "less convex". We could not confirm his observation that the frontal region is more expanded. Instead, we could show that *P. socius* has significantly longer fourth pereiopods (third walking legs) than *P. transversus* (4Prp/CW), even though the meri of these legs are only moderately slender and are not significantly different from any of the Atlantic populations (Table II). The discriminant function analysis showed that the combined analysis of ten variables better separated West and East Atlantic populations of *P. transversus* (highest Mahalanobis distances) than it separated *P. socius* from any of the two Atlantic populations (Fig. 2). This is different from the genetic analyses, demonstrating that morphometric differences cannot be used readily as a quantitative measure for genetic distinctness, but that they can be detected even at low levels of genetic isolation (see also Reuschel and Schubart, *in press*).

An increase in the sample size for morphological and morphometric analyses of larvae, showed that the initial conclusions of Cuesta and Schubart (1998) must be reconsidered. The differences pointed out in the previous study for rostrodorsal length and the ratio of dorsal to rostral carapace spines are still valid for separations between Atlantic (western and eastern) and the East Pacific populations (see Table III and Figure 3) when new hatches from Brazil, Florida (both WA) and Baja California (EP) are added to the analysis. However, they cannot be used to separate the first zoeae of *Pachygrapsus transversus* and *P. socius*, when zoeae from the Caribbean (Maria Chiquita) and the Gulf of Mexico (Louisiana) populations are included in the analysis. Based on a total of 11 hatches (see Table III) we can now say that there are no definitive larval morphometric features that would show consistent differences between coastlines. In the case of antennae and the ventral carapace margin, the high variability of dentition patterns is noteworthy (see Fig. 4), but apparently not related to geographic origin. The herewith documented variability of antennular denticulation has important implications, considering that this character has been used until now in larval descriptions to define, and in some cases even to separate, species (e.g. Cuesta and Schubart, 1999). This intraspecifically variable character, although perhaps constant at some population level (same pattern in different hatches from the same population), cannot be used for characterizing species due to its apparent phenotypic plasticity. Water tempertaure could be a possible factor to explain the similar size and ratio of the spines between tropical populations from both sides of Panama, Gulf of California and Gulf of Mexico, all of which clearly differ from populations in colder areas as such southern Spain and Brazil. Shirley *et al.* (1987) showed that water temperature during incubation had a considerable effect on zoeal morphology in *Cancer magister*. Additional hatches from more populations are needed to determine the degree of potential variability of zoeal morphology and possibly the underlying mechanisms.

In the future, the *Pachygrapsus transversus* species complex should be analysed with more variable molecular markers focused on genetic differentiation between potentially recent

separations, as for example along the African coast. These tools should also be applied to studies of potential endemism in the Mediterranean, Gulf of Mexico and Gulf of California. Furthermore, the phylogenetic position of the Atlantic species *Pachygrapsus loveridgei* Chace, 1966 from Ascension and St. Helena (Manning and Chace, 1990) should be investigated. These results could then be compared to those for other crab species or species complexes with similar distributions (e.g. *Grapsus*, *Geograpsus*, *Goniopsis*) to confirm whether the same allopatric separations have acted on these taxa in a similar way. Together with an ever improving knowledge of life histories for these species, such findings can help us to better understand differentiation processes in the marine environment.

Acknowledgements

For the help with material and other support we would like to thank the following students, colleagues and institutions: Kristina Beblo, John Christy, Edgardo Díaz, Augusto Flores, H. O. von Hagen, Fernando Mantelatto, Carsten Müller, Silke Reuschel, Antonio Rodríguez, Rita Vargas, Peter Wirtz, Ardis Johnston from the Museum of Comparative Zoology (Cambridge, USA), Karen Reed from the Smithsonian Institution (Washington DC, USA), Charles Fransen from Naturalis Museum (Leiden, Netherlands) and Gustavo Melo from the Museu de Zoologia Universidade de São Paulo (Brazil). Very cordially we would like to acknowledge Fernando Mantelatto for the excellent job during the organization of the 3rd Brazilian Crustacean Congress and 2004 TCS Midyear Meeting in Florianopolis (SC) Brazil and M.L.N.F. for editing this proceedings volume. Joseph Poupin and Gerhard Pohle provided valuable criticisms. Support was provided by the University of Regensburg (Prof. J. Heinze); U.S. Department of Energy grant no. DE-FG02-97ER12220, U.S. National Science Foundation grants no. DEB-0315995 and EF-0531603, and several small grants from the Smithsonian Marine Station at Ft. Pierce, Florida (to DLF); and by a research contract through "Ramon y Cajal" from the "Ministerio de Educación y Ciencia,"Spain (to JAC). This is contribution No. 110 of the UL Lafayette Laboratory for Crustacean Research and contribution No. 631 of the Smithsonian Marine Station at Ft. Pierce, Florida.

References

Brossi-Garcia, A. L. and Rodrigues, M. D. 1997. Zoeal morphology of *Pachygrapsus transversus* (Gibbes) (Decapoda, Grapsidae) reared in the laboratory. Revista Brasileira de Zoologia 14(4): 803-819.

Cabot, E. L. and Beckenbach, A. T. 1989. Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. Computer Applications in the Biosciences 5: 233-234.

Chace Jr., F. A. 1966. Decapod crustaceans from St. Helena Island, South Atlantic. Proceedings of the United States National Museum 118: 622-661.

Crandall, K. A. and Fitzpatrick Jr., J. E. 1996. Crayfish molecular systematics: using a combination of procedures to estimate phylogeny. Systematic Biology 45(1): 1-26.

Cuesta, J. A. and Rodríguez, A., 1994. Early zoeal stages of *Pachygrapsus marmoratus* (Fabricius), *P. transversus* (Gibbes) and *P. maurus* (Lucas) (Decapoda: Brachyura: Grapsidae) reared in the laboratory. Scientia Marina 58(4): 323-327.

Cuesta, J. A. and Schubart, C. D. 1998. Morphological and molecular differentiation between three allopatric populations of the littoral crab *Pachygrapsus transversus* (Gibbes, 1850) (Brachyura: Grapsidae). Journal of Natural History 32: 1499-1508.

Cuesta, J. A. and Schubart, C. D. 1999. First zoeal stages of *Geograpsus lividus* and *Goniopsis pulchra* from Panama confirm consistent larval characters for the subfamily Grapsinae (Crustacea: Brachyura: Grapsidae). Ophelia 51(3): 163-176.

Flores, A. A. V. 1996. Biologia de *Pachygrapsus transversus* (Gibbes, 1850) (Crustacea, Brachyura, Grapsidae) na região de Ubatuba, SP. M. Sc. dissertation, Universidade Estadual Paulista, Botucatu, Brasil, 143 pp.

Garth, J. S. 1946. Littoral brachyuran fauna of the Galapagos Archipelago. Allan Hancock Pacific Expeditions 5(10): 341-601.

Gibbes, L. R. 1850. On the carcinological collections of the cabinets of natural history in the United States; with an enumeration of the species contained therein, and descriptions of new species. Proceedings of the American Association for the Advancement of Science 3: 165-201.

Guerao, G., Schubart, C. D. and Cuesta, J. A. 2001. The first zoeal stages of *Grapsus grapsus* Linnaeus and *Geograpsus lividus* (H. Milne Edwards) (Decapoda, Brachyura, Grapsidae) from the western Atlantic. Nauplius 9(2): 111-121.

Hines, A. H. 1986. Larval patterns in the life histories of brachyuran crabs (Crustacea, Decapoda, Brachyura). Bulletin of Marine Science 39(2): 444-466.

Holthuis, L. B. and Gottlieb, E. 1958. An annotated list of the decapod Crustacea of the Mediterranean coast of Israel, with an appendix listing the Decapoda of the Eastern Mediterranean. Bulletin of the Research Council of Israel 7B: 1-126.

Keigwin, L. 1982. Isotopic paleoceanography of the Caribbean and East Pacific: role of Panama uplift in late neogene time. Science 217: 350-353.

Knowlton, N. and Weigt, L. A. 1998. New dates and new rates for divergence across the Isthmus of Panama. Proceedings of the Royal Society London B 265: 2257-2263.

Kumar, S., Tamura, K., Jakobsen, I.B., and Nei, M. 2001. MEGA2: Molecular Evolutionary Genetics Analysis software. Bioinformatics 17 (12):1244-1245.

Manning, R. B. and Chace Jr., F. A. 1990. Decapod and stomatopod Crustacea from Ascension Island, South Atlantic Ocean. Smithsonian Contributions to Zoology 503: 1-91.

Milne Edwards, H., 1837. Histoire naturelle des Crustacés, comprenant l'anatomie, la physiologie et la classification de ces animaux. Vol. 2. 532 pp. Atlas 32 pp. Librairie Encyclopédique de Roret, Paris.

Palumbi, S. R., Martin, A., Romano, S., Mcmillan, W. O., Stice, L. and Grabowski, G. 1991. The simple fool's guide to PCR. A collection of PCR protocols, version 2. University of Hawaii, Honolulu, 44 pp.

Poupin, J., Davie, P. J. F. and Cexus, J. C. 2005. A revision of the genus *Pachygrapsus* Randall, 1840 (Crustacea: Decapoda: Brachyura, Grapsidae), with special reference to the Southwest Pacific species. Zootaxa, 1015: 1-66.

Randall, J. W. 1840. Catalogue of the Crustacea brought by Thomas Nutall and J. K. Townsend, from the west coast of North America and the Sandwich Islands, with descriptions of such species as are apparently new, among which are included several species of different localities, previously existing in the collection of the Academy. Journal of the Academy of Natural Sciences of Philadelphia 8: 106-147.

Rathbun, M. J. 1918. The grapsoid crabs of America. Bulletin of the United States National Museum 97: 1-461.

Reuschel S. and Schubart C. D. *in press.* Geographic differentiation of two Atlanto-Mediterranean species of the genus *Xantho* (Crustacea: Brachyura: Xanthidae) based on genetic and morphometric analyses. Marine Biology 148(4).

Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406-425.

Scheltema, R. S. 1986. On dispersal and planktonic larvae of benthic invertebrates: an eclectic overview and summary of problems. Bulletin of Marine Science 39(2), 290-322.

Schubart, C. D., Neigel, J. E. and Felder, D. L. 2000. Use of the mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea. In: The biodiversity crisis and Crustacea. J. C. von Vaupel Klein & F. Schram, eds. Proceedings of the Fourth International Crustacean Congress, Amsterdam, Netherlands, 20-24 July 1998, vol. 2. Balkema, Rotterdam/Brookfield. Crustacean Issues 12: 817-830.

Schubart, C. D., Cuesta, J. A. and Rodríguez, A. 2001. Molecular phylogeny of the crab genus *Brachynotus* (Brachyura: Varunidae) based on the 16S rRNA gene. Hydrobiologia 449: 41-46.

Shirley, S. M., Shirley, T. C. and Rice, S. D. 1987. Latitudinal variation in the Dungeness crab, *Cancer magister*: Zoeal morphology explained by incubation temperature. Marine Biology 95: 371-376.

Stimpson, W. M. 1871. Notes on North American Crustacea in the Museum of the Smithsonian Institution, no. III. Annals of the Lyceum of Natural History of New York 10: 92-136.

Received: 14th Feb 2005
Accepted: 10th Nov 2005