# No evidence of host specialization in a parasitic pea-crab exploiting two echinoid hosts

**Q. Jossart[1,2,*], B. David[2], C. De Bruyn[1,2], C. De Ridder[1], T. Rigaud[2], R. A. Wattier[2]**

[1]Laboratoire de Biologie Marine (CP 160/15), Université Libre de Bruxelles (ULB), 50 avenue F. Roosevelt, 1050 Brussels, Belgium

[2]Biogéosciences, UMR CNRS 6282, Université de Bourgogne, 6 boulevard Gabriel, 21000 Dijon, France

ABSTRACT: The pinnotherid crab *Dissodactylus primitivus* lives parasitically on 2 burrowing echinoid species, *Meoma ventricosa* and *Plagiobrissus grandis*. The fecundity of female crabs varies between hosts, and is higher when parasitizing *P. grandis* than *M. ventricosa*. Moreover, the hosts present great variations in morphology (size and density of spines). These characteristics suggest the potential to differentiate crabs according to host species. We investigated the genetic (microsatellites) and morphometric (outline analysis) differentiation of this parasitic crab between 2 host species at 1 Jamaican site (Western Lagoon, Discovery Bay), and compared it with geographic differentiation among 4 sites along the north coast of Jamaica. Greater genetic differences between parasites of the 2 sympatric hosts than between parasites of a single host at different geographic locations would indicate host differentiation. Genetic analyses (microsatellites) did not detect spatial differentiation (probably due to local hydrography) or differentiation according to host species. This lack of host differentiation could be explained by mobility of adult crabs between hosts. However, there was weak but significant morphological differentiation between female crabs from the 2 hosts. This morphological difference may reflect constraints due to host morphology.

KEY WORDS:  Host specialization · Spatial scale · Ectoparasite · Population genetic structure · Microsatellite · Morphometry · Brachyuran decapods · Echinoid

## INTRODUCTION

The habitat of a parasite is, by definition, discontinuous and variable in time and space (Price 1980). Parasites can be associated with several hosts and can also pass across various surrounding environments during free stages of their life-cycle. Hence, differentiation among parasite populations can be variable, according to their degree of host specialization and the complexity of their life cycles. Parasite populations can be characterized along different spatial scales: between host individuals (infrapopulations), between host populations and even between host species when parasites are not strict specialists (Combes 2001, Poulin 2007). For instance, coloniza-

tion of a new host species can lead to host specialization (host-race formation) (McCoy 2003). Drès & Mallet (2002) defined host-races as 'genetically differentiated, sympatric populations of parasites that use different hosts, and between which there is appreciable gene flow'. Parasite differentiation according to host species (with variable degrees of sympatry) has been detected in different taxa, e.g. the frugivorous fly *Rhagoletis pomonella* (Bush 1969, Feder et al. 2003), the tick *Ixodes uriae* (Kempf et al. 2009), the barnacle *Wanella milleporae* associated with fire corals (Tsang et al. 2009), and the crab *Pinnotheres novaezelandiae* associated with bivalves (Stevens 1990). In contrast, other studies failed to demonstrate genetic differentiation according to host species; e.g.

in the parasitic fish *Rhodeus amarus* (Reichard et al. 2011), or in 3 lice species parasitizing shearwaters (Gómez-Díaz et al. 2007). Therefore, multi-host parasites provide a variety of case studies to explore alternative mechanisms influencing the degree of observed differentiation, such as host specialization and spatial differentiation, in a single model (Bouzid et al. 2008).

The pea crab *Dissodactylus primitivus* (Brachyura, Pinnotheridae) is an ectoparasite of 2 burrowing echinoids species, *Meoma ventricosa* and *Plagiobrissus grandis*, along the Caribbean and neighboring American coasts (Telford 1982, Hendler et al. 1995, De Bruyn et al. 2009). *Meoma ventricosa* is a relatively sluggish echinoid with uniformly short dense spines that burrows in the sediment during the day. It emerges at dusk to forage on the sediment's surface for the whole night (Kier & Grant 1965, Hammond 1982, Hendler et al. 1995). In contrast, *P. grandis* is a highly active, quickly moving echinoid covered in less dense spines, with 2 sets of long aboral spines. Its nyctohemeral rhythm is less pronounced than that of *M. ventricosa* (Kier & Grant 1965, Hammond 1982, Hendler et al. 1995).

*Dissodactylus primitivus* damages host tegument and negatively affects host fecundity (De Bruyn et al. 2009). This crab infects its 2 hosts asymmetrically: juveniles only occur on *Meoma ventricosa*, while adult crabs are found with similar mean burden and sex ratios on both echinoid hosts (De Bruyn et al. 2010). Parasite fecundity differs slightly according to host species; females brood more eggs (+17%) on *Plagiobrissus grandis* (De Bruyn et al. 2010). The adult crabs are also differentially attracted by the 2 host species. Crabs collected on *M. ventricosa* show a marked preference for chemical cues from this host in situation of choice. This is an example of imprinting, a factor that could promote specialization (De Bruyn et al. 2011). However, crabs collected on *P. grandis* do not show such a preference (De Bruyn et al. 2011). It remains unclear whether *D. primitivus* is showing incipient specialization between the 2 hosts. On one hand, factors promoting differentiation between hosts include the imprinting phenomenon, the higher fecundity for females on *P. grandis* and the different living conditions provided by the 2 host species with contrasting morphologies and behaviors. On the other hand, factors that may prevent differentiation include a shift of adult crabs from one host to the other and the loss of imprinting in crabs infecting *P. grandis*.

The study of genetic differentiation often aids our understanding of parasite life cycles (De Meeûs et al. 2007) and is necessary for detecting host specialization. Many studies analyze population genetics of crustaceans (e.g. Weber et al. 2000, Cassone & Boulding 2006, Herborg et al. 2007, Silva et al. 2009, Silva et al. 2010a,b, Fratini et al. 2011), but fewer deal with parasitic or symbiotic species (Stevens 1990, 1991, De Meeûs et al. 1992, Harrison 2004, Sotka 2005, Tsang et al. 2009).

We investigated the genetic differentiation of *Dissodactylus primitivus* in relation to its 2 hosts within a single location, and compared it with local scale geographic genetic differentiation in 1 host. Greater genetic differentiation between parasites of the 2 hosts living in sympatry than between single-host parasite populations from different geographic locations would indicate host-race formation.

In addition to population genetic analyses, morphology may also indicate differentiation among populations (Magniez-Jannin et al. 2000, Navarro et al. 2004, Pinceel et al. 2004, Garnier et al. 2005, Silva et al. 2010a,b). Recent developments in morphometrics allow researchers to quantify morphological disparity by building morphospaces in which distances are unbiased (notably for the size of the measured object), therefore facilitating comparison with population genetic data and distances (Laffont et al. 2011). For example, Silva et al. (2010b) found that in the crab *Perisesarma guttatum*, genetic and morphometric data both attested the subdivision of this species into 2 main clades on the East African coast. However, other studies found morphological differentiation without any genetic differentiation (e.g. Nice & Shapiro 1999, Magniez-Jannin et al. 2000). Therefore, we combined our population genetic survey with a morphometric analysis of morphological variation in *Dissodactylus primitivus*.

## MATERIAL AND METHODS

### Site description

We sampled 4 sites on the northern coast of Jamaica (Fig. 1). Two sites were located within the lagoon of Discovery Bay (Western Lagoon: WL, Eastern Lagoon: EL) and 2 were outside (Pear Tree Bottom: PTB, Chalet Caribe: CC) (Fig.1). Discovery Bay (180° 28′ N, 77° 24′ W) is partially closed by a fringing reef pierced by a 12 m deep channel to allow shipping traffic (Gayle & Woodley 1998). This channel extends into the lagoon so that WL and EL are located on opposite sides of the channel.
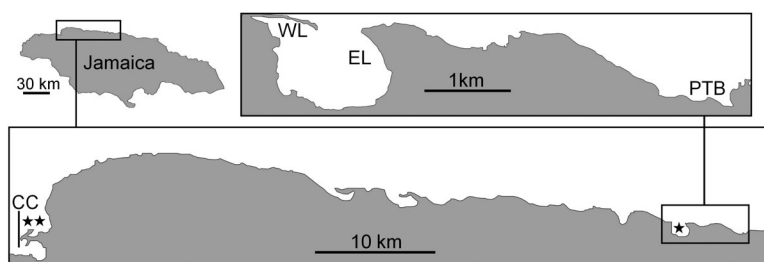
Fig. 1. Sampling sites on the north coast of Jamaica. WL: Western Lagoon, EL: Eastern Lagoon, PTB: Pear Tree Bottom, CC: Chalet Caribe. ★: Discovery Bay, ★★: Montego Bay

## Sample collection

In WL, crabs were sampled from sympatric hosts, *Meoma ventricosa* and *Plagiobrissus grandis* (the 2 echinoid species may be found within the same 10 m². In EL, PTB, and CC, *P. grandis* were very rare or absent and crabs were only collected from *M. ventricosa*.

For population genetic analyses, samples were collected by SCUBA diving or snorkeling at depths ranging from 2 to 4 m at WL, 5 to 6 m at EL, 12 to 18 m at PTB and 7 to 9 m at CC. In WL and EL, each individual host specimen (i.e. infrapopulation) was kept separately in a plastic bag that was immediately tied up after collection. In CC and PTB, crabs were directly collected under water without counting the number of sea urchins. In the laboratory, crabs were individually isolated and preserved in pure ethanol. We sampled 407 crabs, 169 from WL (82 from 33 *Plagiobrissus grandis* and 87 from 26 *Meoma ventricosa*), 132 from 32 hosts at EL, 83 at PTB and 23 at CC. Crabs were sampled from WL and EL in 2009, CC in 2005, and PTB in 2005 and 2009.

For morphometric analyses, 137 crabs were collected in 2007 (WL, EL) and 2005 (PTB, CC): 70 at WL (32 from *Plagiobrissus grandis* and 38 from *Meoma ventricosa*), 39 crabs at EL from 28 *M. ventricosa*, 13 at PTB and 15 at CC.

## Population genetic data collection

The cephalothorax of each crab was removed and the muscles at the basis of pereopods were collected, dried for 2 h at ambient temperature and frozen at −80°C. DNA was extracted using a Chelex chelating resin method (Walsh et al. 1991). First, each sample was crushed using 1 tungsten ball (3 mm diameter) and a mixer mill (1 min at 18 Hz). After adding 100 µl of Chelex solution (1 g of Chelex in 20 ml of sterile water), the sample was crushed again for 1 min. The samples were then placed at 85°C for 90 min and mixed every 30 min. Finally, after centrifuging (3 min at 13 200 g), the supernatant with DNA was collected.

Ten microsatellite loci (Anderson et al. 2010) were amplified by PCR using primers in 4 PCR multiplex (Table 1). Each reaction in multiplex (15 µl) included 7.5 µl of Master Mix Qiagen (Taq Polymerase, nucleotides), 1 µl of DNA, 0.3 µl (10 µM) of each forward or reverse primer and a variable volume of sterile water (5.3 µl for Multiplex a and d, 4 µl for Multiplex b, 4.7 µl for Multiplex c). The PCR conditions consisted of 40 cycles for each of the 3 temperature steps [30 s at 94°C (denaturation), 90 s at 51°C (annealing) and 30 s at 72°C (elongation)]. These cycles were preceded by a step of 15 min at 95°C (first denaturation) and were followed by a step of 10 min at 72°C (last elongation). Finally, 1 µl of amplified DNA was mixed with 0.4 µl of the size standard LIZ (http://appliedbiosystems.com) and 10 µl of formamide prior to electrophoresis with an AB 3730 DNA Analyzer.

Genotypes were deduced from electropherograms using the software Peak Scanner (https://products.appliedbiosystems.com). Allelic binning was done using the program Autobin, but each genotype was verified by eye (Guichoux et al. 2011).

## Statistical analyses of population genetic data

The software FSTAT (2.9.3.2) was used to estimate genetic variability i.e. allele frequencies, number of alleles, allelic richness (AR, a measure of the number of alleles adjusted for sample size), number of private alleles (alleles only found in a single group) and

Table 1. The 10 microsatellite loci used in our study. *A* = total number of alleles (see http://appliedbiosystems.com). The fluorochromes are part of the DS33 Applied Biosystems Standard Dye Set. bp = base pairs

| Locus | PCR Multiplex | Fluorochrome | Size range (bp) | *A* |
|---|---|---|---|---|
| DpC115 | a | NED | 162–188 | 7 |
| DpC118 | a | VIC | 265–291 | 12 |
| DpA5 | b | VIC | 210–238 | 11 |
| DpA113 | b | VIC | 108–134 | 12 |
| DpC4 | b | FAM | 152–184 | 14 |
| DpA101 | c | NED | 217–259 | 16 |
| DpD110 | c | PET | 236–276 | 17 |
| DpD111 | c | FAM | 251–309 | 11 |
| DpC9 | d | FAM | 178–220 | 8 |
| DpC110 | d | PET | 134–168 | 11 |

expected and observed heterozygosities (Goudet 1995). Differences between sites in mean AR, averaged over loci, were tested using non parametric statistics (Kruskal-Wallis test). Deviations from Hardy-Weinberg equilibrium ($F_{IS}$) and linkage disequilibrium were also assessed using FSTAT. In WL, $F_{IS}$ was tested both for separate host populations and for pooled data.

POWSIM (4.1) was implemented to measure the statistical power ($1 - \beta$) of detecting differentiation, taking into account the number of loci, the level of polymorphism and numbers of individuals in our study (Ryman & Palm 2006). The retained parameter values were selected according to the advices of POWSIM manual ($N_e$ of 2000; 10 generations of drift; 1000 runs). A power of 0.75 was considered sufficient to detect differentiation in our samples (Beninger et al. 2012, Olsson et al. 2012).

We measured differentiation using different complementary methods. Weir & Cockerham's (1984) estimator of multilocus Wright's fixation index $F_{ST}$ ($\theta_{wc}$) was calculated using FSTAT and was statistically tested against the null hypothesis of $F_{ST} = 0$, using 120 permutations of alleles between populations. The p-value of this test corresponds to the proportion of permutations leading to a $F_{ST}$ greater (or equal) than the observed one (Fratini & Vannini 2002). The nominal significance level (5%) was adjusted by a standard Bonferroni correction for multiple comparisons (Rice 1989). Following the recommendation of Waples & Gaggiotti (2006), we also applied a contingency test of allele frequency heterogeneity (hereafter Fisher's test of differentiation) using Arlequin 3.5 (Excoffier & Lischer 2010). This approach follows the method of Raymond & Rousset (1995). Finally, we also used Arlequin to run an analysis of molecular variance (AMOVA) to assess the relative variance contributions of genetic differences within and between groups (Excoffier & Lischer 2010).

In addition, 2 Bayesian clustering approaches were implemented to infer the more probable number ($K$) of genetic clusters. For a putative value of $K$ (1–5) and for 10 independent simulations, parameters were set in STRUCTURE 2.3.3 (Pritchard et al. 2000) as: running lengths of 100 000, admixture model (with prior sampling location), alpha inferred and allele frequencies correlated among populations. We also used DPART software which uses Dirichlet Process to infer $K$ values (Onogi et al. 2011). The parameter set for 5 independent simulations was: alpha equal to 0.51, lambda equal to 0, length of burn-in of 400 000 and length of iterations after burn-in of 100 000.

## Morphometric data collection

Body shape (cephalothorax outline) was assessed using discrete Fourier analysis (DFA) (Moellering & Raynor 1981). A Nikon measuring microscope (MM60) was used to take pictures of all individuals with the same capture method (same positioning, same point of reference). Then, outlines were extracted from cephalothorax pictures using the software Optimas 6.5 (www.mediacy.com). Each outline could be approximated by a sum of sine and cosine functions (i.e. harmonics). Discrete Fourier analysis was performed to determine the parameters (amplitude, phases, Fourier coefficients) of each harmonic ($H$) (software CDFT = Complex Discrete Fourier Transform) (Dommergues et al. 2007).

We measured 20 cephalothoraxes twice to assess measurement error (ME) (Bailey & Byrnes 1990). The amplitude parameter for the first 40 harmonics (20 conjugates) was retained. This allowed us to keep the maximum ME reasonably low. Indeed, ME was always >20%, except for 3 harmonics ($ME_{conjugate\ of\ H2} = 42\%$; $ME_{conjugate\ of\ H16} = 33\%$; $ME_{conjugate\ of\ H18} = 20\%$). The first measure was kept for analyses for these 20 crabs. For other crabs, each cephalothorax was measured only once to obtain the harmonic's parameters.

## Statistical analyses of morphometric data

Statistical analyses were performed using STATIS-TICA 7.0 (www.statsoft.com). Two principal component analyses (one for inter-hosts comparisons, another for inter-site comparisons) were performed to reduce the number of variables; the initial 40 amplitudes were transformed into 7 components. These 7 components explained 91% of the total variance in the 2 analyses. The 7 components followed a normal distribution in each group (non-significant Kolmogorov-Smirnov tests). Potential allometric effects were estimated using Spearman correlations between area of cephalothorax (taken as a proxy of size) and each of these components. Since all data met the homoscedasticity conditions (non-significant Levene's tests), multivariate analyses of variance (MANOVAs) were performed on the 7 components to appraise morphometric differentiation between hosts or sites. Two factors were simultaneously considered in the MANOVAs: Host and Sex of crabs for differentiation by host species, and Site and Sex of crabs for the differentiation among locations. Sex of crabs was always taken into account as a factor because the crabs are sexually dimorphic. Interactions between

these factors were also evaluated. Finally, multiple discriminant analyses (MDA) were performed on the 7 components to infer Mahalanobis distances and posterior probabilities between groups. Divergences expressed by MDA were visualized by reconstituting the outlines of individuals that were representative of the shape changes along the canonical axis.

## RESULTS

### Population genetic analysis

#### Variability

The 10 microsatellite loci were highly polymorphic; allele numbers per locus ranged from 7 to 17 (mean = 11.9). The minimum average number of alleles per site (7.6) was observed at CC (Table 2). Allelic richness per locus ranged from 5 to 10.6, with an average of 7.5 (Table 2), and was not significantly different between groups (hosts or sites) (Kruskal-Wallis test: $H$ = 0.358, p = 0.986). There were only 10 private alleles (2.1% of all alleles) and these were found in WL-P, EL and PTB (Table 2). The mean observed and expected heterozygosities per locus were 0.83 (range = 0.71–0.93) and 0.78 (range = 0.63–0.89), respectively. Multilocus $F_{IS}$ (including that of the pooled data for WL) ranged between −0.003 (CC) and −0.114 (EL). These values were not significantly different from zero (Table 2; 5% nominal threshold Bonferroni corrected to p = 0.001) and hence no deviations from HWE were observed. No linkage disequilibrium was detected between pairs of loci (45 pairwise comparisons, 5% nominal threshold Bonferroni corrected to p = 0.0011).

### Genetic differentiation of crabs among hosts

Population genetic differentiation of crabs among hosts was considered only at WL, where the 2 host species were abundant. The results of the software POWSIM gave a statistical power (1 − β) of 0.752 for Fisher's test of differentiation, associated with an $F_{ST}$ equal to 0.0025. $F_{ST}$ between crabs from the different host species was very low ($F_{ST}$ = −0.0015) and not significant (p = 0.80). In addition, Fisher's test did not find any differentiation (p = 1). AMOVA (results not shown) identified that all genetic variance was due to within-host variation. Bayesian clustering confirmed this absence of differentiation. STRUCTURE identified the highest posterior probability (p = 0.99) when

Table 3. *Dissodactylus primitivus.* STRUCTURE results for (a) crabs from different hosts within Western Lagoon (WL) and (b) all samples from all sites. $K$ is the number of genetic clusters, $X$ represents the genotypes of individuals and Pr is the posterior probability. For each $K$ value, ln Pr($X/K$), associated variance and Pr($K$) were calculated. Ten runs were done for each value of $K$ and only the average values are shown in this table

| Analysis of crabs | $K$ | Ln Pr($X/K$) | Variance ln Pr($X/K$) | Pr($K$) |
|---|---|---|---|---|
| (a) From different hosts | 1 | −5029.8 | 50.2 | 0.99 |
| | 2 | −5076.5 | 172 | $5 \times 10^{-21}$ |
| | 3 | −5080.8 | 182.2 | $7 \times 10^{-23}$ |
| | 4 | −5049.7 | 119.8 | $2 \times 10^{-9}$ |
| (b) From all sites | 1 | −12242.5 | 55.5 | 1 |
| | 2 | −12324 | 284.1 | $4 \times 10^{-36}$ |
| | 3 | −12344.4 | 337.9 | $5 \times 10^{-45}$ |
| | 4 | −12360.9 | 373.7 | $4 \times 10^{-52}$ |

Table 2. *Dissodactylus primitivus.* Number of alleles ($A$) including the number of private alleles in parenthesis, allelic richness ($AR$), and deviation from Hardy-Weinberg equilibrium ($F_{IS}$) for 10 microsatellite loci in crabs from 4 sites (WL: Western Lagoon, EL: Eastern Lagoon, PTB: Pear Tree Bottom, CC: Chalet Caribe). *D. primitivus* is present on: *Meoma ventricosa* (M) and *Plagiobrissus grandis* (P) in WL, while only *M. ventricosa* is present in EL, PTB, and CC

| Locus | $A$ | | | | | $AR$ | | | | | $F_{IS}$ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | WL-*P* | WL-*M* | EL | PTB | CC | WL-*P* | WL-*M* | EL | PTB | CC | WL-*P* | WL-*M* | EL | PTB | CC |
| DpC115 | 7 (1) | 6 | 6 | 6 | 6 | 5.4 | 5 | 5 | 4.8 | 5.6 | −0.113 | −0.184 | −0.267 | −0.173 | 0.093 |
| DpC118 | 12 | 10 | 11 | 10 | 8 | 8.9 | 8.4 | 8.2 | 8.7 | 7.7 | 0.024 | −0.035 | 0.005 | 0.010 | 0.061 |
| DpA113 | 10 | 9 | 9 | 10 (1) | 8 | 7.7 | 8.1 | 7.3 | 7.4 | 7.9 | −0.157 | −0.060 | −0.068 | −0.036 | −0.038 |
| DpC4 | 7 | 10 | 11 (1) | 10 (1) | 6 | 5.3 | 6.6 | 7.4 | 7.4 | 5.8 | −0.102 | −0.097 | −0.171 | −0.167 | 0.044 |
| DpA5 | 12 (2) | 12 | 12 | 10 | 9 | 8.6 | 8.6 | 8 | 7.5 | 9 | 0.003 | −0.085 | −0.107 | −0.165 | −0.171 |
| DpA101 | 15 | 13 | 15 | 14 (1) | 10 | 10.3 | 10.6 | 10.4 | 10.7 | 9.9 | 0.017 | −0.061 | −0.027 | 0.031 | 0.163 |
| DpD111 | 14 | 14 | 14 (1) | 15 (1) | 9 | 10.3 | 10.3 | 9.6 | 10.8 | 8.9 | 0.018 | 0.023 | 0.018 | −0.007 | 0.205 |
| DpD110 | 8 | 9 | 11 (1) | 9 | 7 | 6.5 | 7 | 7.1 | 7.1 | 6.9 | 0.049 | 0.001 | 0 | −0.060 | −0.071 |
| DpC110 | 7 | 8 | 7 | 8 | 6 | 5.6 | 6.2 | 5.7 | 5.9 | 5.9 | −0.195 | −0.209 | −0.343 | −0.244 | −0.214 |
| DpC9 | 9 | 11 | 10 | 8 | 7 | 5.5 | 6.1 | 6 | 6.1 | 6.7 | 0.002 | −0.036 | −0.271 | −0.085 | −0.159 |
| All loci | 10.1 | 10.2 | 10.6 | 10 | 7.6 | 7.4 | 7.7 | 7.5 | 7.6 | 7.4 | −0.040 | −0.069 | −0.114 | −0.083 | −0.003 |

$K = 1$ (Table 3 a). Other posterior probabilities (K = 2, 3, 4) were close to 0. Results from DPART (data not shown) and STRUCTURE were congruent.

### Population genetic differentiation among sites

At WL, the samples of crabs from the 2 host species were pooled because they showed no differentiation (see above). The statistical power $(1 - \beta)$ from POWSIM was 0.994 (associated with an average $F_{ST}$ of 0.0025) for Fisher's differentiation test. $F_{ST}$ between crabs collected in 2 different years in PTB (2005 and 2009) was equal to 0.0038 and was not significant (p = 0.35). Fisher's test of differentiation was congruent with this (p = 1). Consequently, temporal variation was not detected, and samples from PTB 2005 and 2009 were pooled in further analyses.

Overall, $F_{ST}$ among the 4 sites was equal to 0.003. Pairwise $F_{ST}$ values ranged from 0.0021 (EL-WL) to 0.0095 (EL-CC), but none were significantly different from zero, and Fisher's tests of differentiation were all non-significant (Table 4). The global Fisher's test of differentiation yielded a p-value of 0.28. The AMOVA revealed that almost all of the genetic variance (99.76%) occurred within sites (details not shown).

STRUCTURE detected no genetic structure. The highest posterior probability (p almost equal to 1) appeared when $K = 1$ (Table 3b). DPART also found only 1 genetic cluster (result not shown).

### **Morphometric analysis**

#### Morphological differentiation among hosts

Morphological differentiation between crabs from different host species was considered only at WL. Spearman correlations between components of the principle component analysis (PCA) and crab size (cephalothorax area) detected no strong allometric effect: 6 of the 7 correlations were non-significant and $r_S$ never exceeded 0.27. Therefore, the observed differences were not size dependent. Moreover, Component 4, for which the Spearman correlations were significant, did not contribute to the results of the MANOVA for host species effects.

The MANOVA revealed a highly significant effect of crab sex (Wilks'

lambda = 0.403; $F = 12.495$; p < 0.0001) and a marginally significant effect of the host factor (Wilks' lambda = 0.796; $F = 2.198$; p = 0.047). Interaction between these 2 factors was not significant (Wilks' lambda = 0.862; $F = 1.347$; p = 0.245) indicating that the variation between hosts was independent of sex. In order to discard sex-related differences, multiple discriminant analyses were performed separately for male and female crabs. For males, there was no significant effect of host (squared Mahalanobis distance = 1.299; $F = 1.371$; p = 0.256), but for female crabs, there was a marginally significant difference among host species (squared Mahalanobis distance = 2.662; $F = 2.481$; p = 0.043). Projecting specimens along the canonical axis of the multiple discriminant analysis showed that the small difference between female crabs of the 2 hosts concerned the anterolateral margin of the cephalothorax. Female crabs from *Plagiobrissus grandis* tended to have a more rounded margin than females from *Meoma ventricosa*, which had a more angular margin (Fig. 2).

Table 4. *Dissodactylus primitivus.* Pairwise $F_{ST}$ values (below the diagonal) and probabilities of Fisher's test of differentiation (above diagonal) among 4 sites (WL: Western Lagoon, EL: Eastern Lagoon, PTB: Pear Tree Bottom, CC: Chalet Caribe). ns: $F_{ST}$ value not significantly different from zero after Bonferroni correction (5% nominal threshold corrected to p = 0.0083). The probabilities associated with $F_{ST}$ were: 0.017 (WL-EL), 0.217 (WL-PTB), 0.083 (WL-CC), 0.100 (EL-PTB), 0.017 (EL-CC), 0.050 (PTB-CC)

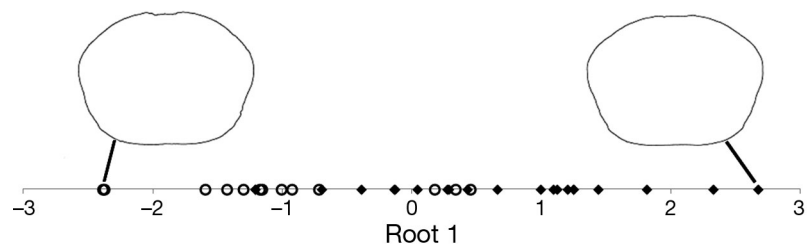| | WL | EL | PTB | CC |
|---|---|---|---|---|
| WL | | 1.00 | 1.00 | 0.77 |
| EL | $0.0021^{ns}$ | | 1.00 | 0.78 |
| PTB | $0.0022^{ns}$ | $0.0028^{ns}$ | | 0.67 |
| CC | $0.0037^{ns}$ | $0.0095^{ns}$ | $0.0028^{ns}$ | |



Fig. 2. *Dissodactylus primitivus.* Distribution of female cephalothorax shapes on the canonical axis (Root 1) of a multiple discriminant analysis. ○ = Crabs parasitizing *Plagiobrissus grandis*, ◆ = crabs from *Meoma ventricosa*. True outlines of extreme individuals show the differences in the shape of the anterolateral margin

### Morphological differentiation among sites

Morphometric differentiation among sites was studied only in crabs parasitizing *Meoma ventricosa*. As for inter-host comparisons, Spearman coefficients did not detect strong correlations between PCA components and size: $r_S$ never exceeded 0.37, and 5 of the 7 correlations were non-significant.

There were no morphological differences among crabs on *Meoma ventricosa* from different sites. The MANOVA revealed that site had no significant effect (Wilks' lambda = 0.716; $F$ = 1.538; p = 0.065) but sex did (Wilks' lambda = 0.612; $F$ = 8.257; p < 0.0001), though the interaction was not significant (Wilks' lambda = 0.788; $F$ = 1.077; p = 0.373).

## DISCUSSION

The question of host differentiation of the parasitic crab *Dissodactylus primitivus* arose from observations of its life cycle. These observations include the asymmetrical exploitation of 2 echinoid host species, and uncertainty of the rate and direction of adult crab movements between these 2 hosts (De Bruyn et al. 2009, 2010, 2011). Because there are no juveniles on *Plagiobrissus grandis*, the main question (about differentiation according to host species) was whether crabs issued from parents living on *P. grandis* would return to this host, or whether random movement occurs between hosts.

All our analyses revealed that population genetic differentiation of *Dissodactylus primitivus* among its 2 hosts species was not significant. This suggests random mating and high gene flow between crabs from the 2 hosts. At least 2 factors could explain this situation: an insufficient chemical preference for at least 1 of the 2 host species, and a high mobility of adult crabs between host species. De Bruyn et al. (2011) showed that crabs from *Meoma ventricosa* were more attracted by this host species than by *Plagiobrissus grandis*, but crabs from *P. grandis* were equally well attracted by both host species. These results, together with our new results, suggest that chemical attraction is insufficient to cause genetic divergence between crabs living on different host species. The lack of genetic differentiation according to host species could also be triggered by adult mobility. This situation contrasts with that of another pea-crab, *Pinnotheres novaezelandiae*, that lives in the mantle cavity of bivalves spends most of its adult life within a single individual host. Females are strictly host-bound and males show low mobility (Stevens 1990). This low mobility between hosts is associated with host-race differentiation.

Nevertheless, there was a small morphological differentiation between female *Dissodactylus primitivus* infecting the 2 different echinoid hosts. Two hypotheses (selection and phenotypic plasticity) could be associated with this situation of slight morphological variation associated with an absence of genetic differentiation in microsatellite markers (which are theoretically neutral). Indeed, if selection cannot be refuted with the current data, phenotypic plasticity (associated with host constraints during development) could represent a parsimonious explanation. *Meoma ventricosa* is densely covered by short stiff spines, while *Plagiobrissus grandis* has less dense, longer, more flexible spines (Hendler et al. 1995). It is therefore possible that, during the crab's molts, spine characteristics of *M. ventricosa* lead to more compressed carapaces. The fact that differences exist only for females can be related to differential mobility between sexes. Indeed, male crabs tend to move more frequently than females, the latter probably spending more time on a given host (De Bruyn 2010). This hypothesis of phenotypic plasticity should be verified in aquarium experiments (e.g. by maintaining individuals from juvenile to adult stages on one or the other host species).

Among the 4 sites investigated, there was no overall genetic differentiation; hence, the crabs on *Meoma ventricosa* constitute a single panmictic population. In addition, the absence of morphological differentiation may be indicative of similar environmental conditions between locations. Former studies show contrasting genetic structures (using various molecular markers) in crabs at similar spatial scales. For example, low but significant differentiation was detected in free-living crabs: e.g. in the spider-crab *Inachus dorsettensis* between 2 sites of the Isle of Man (Weber et al. 2000) and in *Pachygrapsus marmoratus* among sites of the Lusitanian and Italian coasts separated by <50 km (Silva et al. 2009, Fratini et al. 2011). In symbiotic species, genetic structuring was also detected in *Pinnotheres atrinicola* between locations separated by <100 km (Stevens 1991). However, population homogeneity was found in *Pachygraspus crassipes* in the North Pacific (Cassone & Boulding 2006) and in *Perisesarma guttatum* from African mangroves (Silva et al. 2010b). Genetic homogeneity over tens of kilometers, as observed in *D. primitivus*, is likely due to dispersal of pelagic larval stages.

*Dissodactylus primitivus* has a pelagic larval duration (PLD) of around 15 d (Pohle & Telford 1983),

which should favour dispersal, matching the classical hypothesis (still under debate) that a long PLD could decrease population genetic structure (Bohonak 1999, Kochzius et al. 2009, Selkoe & Toonen 2011, Faubry & Barber 2012). *D. primitivus* females have small brood sizes (<300 eggs per brood) compared to other crab species (Christensen & McDermott 1958, Telford 1978, Mantelatto & Fransozo 1997). Similar to a long PLD, the release of numerous pelagic small eggs, which is frequent in marine organisms (Bohonak 1999, Ni et al. 2011), is thought to favour dispersal by increasing the probability of settlement in many different locations. The present study shows that a small brood size does not necessarily represent a limiting factor to efficient gene flow. Finally, larvae of many decapod species are mobile, and despite their small size, are able to resist drifting due to currents (Bradbury & Snelgrove 2001, Yednock & Neigel 2011). This life history trait might reduce dispersal (Yednock & Neigel 2011). To our knowledge, no data exists on the swimming capability of *D. primitivus* larvae. However, we could speculate that active swimming is not effective enough to prevent genetic homogenization as observed in our study. In spite of these characteristics *a priori* limiting dispersal, and despite the discontinuous habitat associated with its symbiotic life, *D. primitivus* showed no population structuring, suggesting a major effect of current. We therefore propose that the east-west Caribbean Current, which sweeps the northern coast of Jamaica (Gayle & Woodley 1998), could promote dispersal of pelagic organisms among the different sites investigated here.

The overall absence of genetic and morphological differentiation according to host species or among crabs parasitizing *Meoma ventricosa* at 4 sites suggests a strong homogeneity within and among the North Jamaican locations investigated. Future work will consider crabs from other Caribbean islands with a hierarchical approach, ideally including sites with *Plagiobrissus grandis*. This will allow us to assess the scale needed to observe a significant differentiation and if population genetic structure correlates with that of the hosts, with the hydrography or with the history of Caribbean islands.

LITERATURE CITED

Anderson CM, Aparicio GJ, Atangana AR, Beaulieu J and others (2010) Permanent genetic resources added to Molecular Ecology Resources Database 1 December 2009–31 January 2010. Mol Ecol Resour 10:576–579

Bailey RC, Byrnes J (1990) A new, old method for assessing measurement error in both univariate and multivariate morphometric studies. Syst Zool 39:124–130

Beninger PG, Boldina I, Katsanevakis S (2012) Strengthening statistical usage in marine ecology. J Exp Mar Biol Ecol 426-427:97–108

Bohonak AJ (1999) Dispersal, gene flow, and population structure. Q Rev Biol 74:21–45

Bouzid W, Stefka J, Hypsa V, Lek S and others (2008) Geography and host specificity: Two forces behind the genetic structure of the freshwater fish parasite *Ligula intestinalis* (Cestoda: Diphyllobothriidae). Int J Parasitol 38:1465–1479

Bradbury IR, Snelgrove PVR (2001) Contrasting larval transport in demersal fish and benthic invertebrates: the roles of behaviour and advective processes in determining spatial pattern. Can J Fish Aquat Sci 58: 811–823

Bush GL (1969) Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). Evolution 23:237–251

Cassone BJ, Boulding EG (2006) Genetic structure and phylogeography of the lined shore crab, *Pachygrapsus crassipes*, along the northeastern and western Pacific coasts. Mar Biol 149:213–226

Christensen AM, McDermott JJ (1958) Life-history and biology of the oyster crab *Pinnotheres ostreum.* Biol Bull 114: 146–179

Combes C (2001) Parasitism: the ecology and evolution of intimate interactions. The University of Chicago Press, Chicago, IL

De Bruyn C (2010) Modalités fonctionnelles et évolutives des parasitoses développées par les crabes Pinnotheridae aux dépens des échinides fouisseurs. PhD dissertation, Université Libre de Bruxelles—Université de Bourgogne

De Bruyn C, Rigaud T, David B, De Ridder C (2009) Symbiosis between the pea crab *Dissodactylus primitivus* and its echinoid host *Meoma ventricosa*: potential consequences for the crab mating system. Mar Ecol Prog Ser 375: 173–183

De Bruyn C, David B, De Ridder C, Rigaud T (2010) Asymmetric exploitation of two echinoid host species by a parasitic pea crab and its consequences for the parasitic life cycle. Mar Ecol Prog Ser 398:183–191

De Bruyn C, De Ridder C, Rigaud T, David B (2011) Chemical host detection and differential attraction in a parasitic pea crab infecting two echinoids. J Exp Mar Biol Ecol 397:173–178

De Meeüs T, Marin R, Renaud F (1992) Genetic heterogeneity within populations of *Lepeophtheirus europaensis*

(Copepoda: Caligidae) parasitic on two host species. Int J Parasitol 22:1179−1181

De Meeûs T, McCoy KD, Prugnolle F, Chevillon C, Durand P, Hurtrez-Bousses S, Renaud F (2007) Population genetics and molecular epidemiology or how to 'débusquer la bête'. Infect Genet Evol 7:308−332

Dommergues CH, Dommergues JL, Verrecchia EP (2007) The discrete cosine transform, a Fourier-related method for morphometric analysis of open contour. Math Geol 39:749−763

Drès M, Mallet J (2002) Host races in plant-feeding insects and their importance in sympatric speciation. Philos Trans R Soc Lond B Biol Sci 357:471−492

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10: 564−567

Faubry S, Barber PH (2012) Theoretical limits to the correlation between pelagic larval duration and population genetic structure. Mol Ecol 21:3419−3432

Feder JL, Berlocher SJ, Roethele JB, Dambroski H and others (2003) Allopatric genetic origins for sympatric host-plant shifts and race formation in *Rhagoletis.* Proc Natl Acad Sci USA 100:10314−10319

Fratini S, Vannini M (2002) Genetic differentiation in the mud crab *Scylla serrata* (Decapoda: Portunidae) within the Indian Ocean. J Exp Mar Biol Ecol 272:103−116

Fratini S, Schubart CD, Ragionieiri L (2011) Population genetics in the rocky shore crab *Pachygrapsus marmoratus* from the western Mediterranean and eastern Atlantic: complementary results from mtDNA and microsatellites at different geographic scales. In Held C, Koenemann S, Schubart CD (eds) Phylogeography and population genetics in Crustacea. CRC Press, Boca Raton, FL

Garnier S, Magniez-Jannin F, Rasplus JY, Alibert P (2005) When morphometry meets genetics: Inferring the phylogeography of *Carabus solieri* using Fourier analyses of pronotum and male genitalia. J Evol Biol 18:269−280

Gayle PMH, Woodley JD (1998) Discovery Bay, Jamaica. In: Kjerfve B (ed) CARICOMP — Caribbean coral reef, seagrass and mangrove sites. Coastal region and small island papers, No. 3. UNESCO, Paris

Gómez-Díaz E, González-Solís J, Peinado MA, Page RDM (2007) Lack of host-dependent genetic structure in ectoparasites of *Calonectris* shearwaters. Mol Ecol 16: 5204−5215

Goudet J (1995) FSTAT (vers. 1.2): a computer program to calculate F-statistics. J Hered 86:485−486

Guichoux E, Lagache L, Wagner S, Chaumeil P and others (2011) Current trends in microsatellite genotyping. Mol Ecol Resour 11:591−611

Hammond LS (1982) Patterns of feeding and activity in deposit-feeding holothurians and echinoids (Echinodermata) from a shallow back-reef lagoon, Discovery Bay, Jamaica. Bull Mar Sci 32:549−571

Harrison JS (2004) Evolution, biogeography, and the utility of mitochondrial 16S and COI genes in phylogenetic analysis of the crab genus *Austinixa* (Decapoda: Pinnotheridae). Mol Phylogenet Evol 30:743−754

Hendler G, Miller JE, Pawson DL, Kier PM (1995) Sea stars, sea urchins, and allies. Echinoderms of Florida and the Caribbean. Smithsonian Institution Press, Washington, DC

Herborg LM, Weetman D, Van Oosterhout C, Hänfling B (2007) Genetic population structure and contemporary dispersal patterns of a recent European invader, the Chinese mitten crab, *Eriocheir sinensis.* Mol Ecol 16: 231−242

Kempf F, Boulinier T, de Meeûs T, Arnathau C, McCoy KD (2009) Recent evolution of host-associated divergence in the seabird tick *Ixodes uriae.* Mol Ecol 18: 4450−4462

Kier PM, Grant RE (1965) Echinoid distribution and habits, Key Largo coral reef preserve, Florida. Smithson Miscellaneous Collections 149(6). Smithsonian Institution, Washington, DC

Kochzius M, Seidel C, Hauschild J, Kirchhoff S and others (2009) Genetic population structures of the blue starfish *Linckia laevigata* and its gastropod ectoparasite *Thyca crystallina.* Mar Ecol Prog Ser 396:211−219

Laffont R, Firmat C, Alibert P, David B, Montuire S, Saucède T (2011) Biodiversity and evolution in the light of morphometrics: from patterns to processes. C R Palevol 10: 133−142

Magniez-Jannin F, David B, Dommergues JL, Su ZH, Okada TS, Osawa S (2000) Analysing disparity by applying combined morphological and molecular approaches to French and Japanese carabid beetles. Biol J Linn Soc 71: 343−358

Mantelatto FLM, Fransozo A (1997) Fecundity of the crab *Callinectes ornatus* Ordway, 1863 (Decapoda, Brachyura, Portunidae) from the Ubatuba region, São Paulo, Brazil. Crustaceana 70:214−226

McCoy KD (2003) Sympatric speciation in parasites — What is sympatry? Trends Parasitol 19:400−404

Moellering H, Raynor JN (1981) The harmonic analysis of spatial shapes using dual axis Fourier shape analysis (DAFSA). Geogr Anal 13:64−77

Navarro N, Zatarain X, Montuire S (2004) Effects of morphometric descriptor changes on statistical classification and morphospaces. Biol J Linn Soc 83:243−260

Ni L, Li Q, Kong L (2011) Microsatellites reveal fine-scale genetic structure of the Chinese surf clam *Mactra chinensis* (Mollusca, Bivalvia, Mactridae) in Northern China. PSZNI: Mar Ecol 32:488−497

Nice CC, Shapiro AM (1999) Molecular and morphological divergence in the butterfly genus *Lycaeides* (Lepidoptera: Lycaenidae) in North America: evidence of recent speciation. J Evol Biol 12:936−950

Olsson J, Florin AB, Mo K, Aho T, Ryman N (2012) Genetic structure of whitefish (*Coregonus maraena*) in the Baltic Sea. Estuar Coast Shelf Sci 97:104−113

Onogi A, Nurimoto M, Morita M (2011) Characterization of a Bayesian genetic clustering algorithm based on a Dirichlet process prior and comparison among Bayesian clustering methods. BMC Bioinformatics 12:263

Pinceel J, Jordaens K, Van Houtte N, De Winter AJ, Backeljau T (2004) Molecular and morphological data reveal cryptic taxonomic diversity in the terrestrial slug complex *Arion subfuscus/fuscus* (Mollusca, Pulmonata, Arionidae) in continental north-west Europe. Biol J Linn Soc 83:23−38

Pohle G, Telford M (1983) The larval development of *Dissodactylus primitivus* Bouvier, 1917 (Brachyura: Pinnotheridae) reared in the laboratory. Bull Mar Sci 33: 257−273

Poulin R (2007) Evolutionary ecology of parasites. Princeton University Press, Princeton, NJ

Price PW (1980) Evolutionary biology of parasites. Princeton University Press, Princeton, NJ

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49:1280–1283

Reichard M, Bryja J, Pola ik MIK, Smith C (2011) No evidence for host specialization or host-race formation in the European bitterling (*Rhodeus amarus*), a fish that parasitizes freshwater mussels. Mol Ecol 20:3631–3643

Rice WR (1989) Analysing tables of statistical tests. Evolution 43:223–225

Ryman N, Palm S (2006) POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. Mol Ecol 6:600–602

Selkoe KA, Toonen RJ (2011) Marine connectivity: a new look at pelagic larval duration and genetic metrics of dispersal. Mar Ecol Prog Ser 436:291–305

Silva IC, Mesquita N, Schubart CD, Alves MJ, Paula J (2009) Genetic patchiness of the shore crab *Pachygrapsus marmoratus* along the Portuguese coast. J Exp Mar Biol Ecol 378:50–57

Silva IC, Alves MJ, Paula J, Hawkins SJ (2010a) Population differentiation of the shore crab *Carcinus maenas* (Brachyura: Portunidae) on the southwest English coast based on genetic and morphometric analyses. Sci Mar 74:435–444

Silva IC, Mesquita N, Paula J (2010b) Genetic and morphological differentiation of the mangrove crab *Perisesarma guttatum* (Brachyura: Sesarmidae) along an East African latitudinal gradient. Biol J Linn Soc 99:28–46

Sotka EE (2005) Local adaptation in host use among marine invertebrates. Ecol Lett 8:448–459

Stevens PM (1990) A genetic analysis of the pea crabs (Decapoda:Pinnotheridae) of New Zealand. I. Patterns of spatial and host-associated genetic structuring in *Pin-notheres novaezelandiae* Filhol. J Exp Mar Biol Ecol 141: 195–212

Stevens PM (1991) A genetic analysis of the pea crabs (Decapoda:Pinnotheridae) of New Zealand. II. Patterns and intensity of spatial population structure in *Pin-notheres atrinicola.* Mar Biol 108:403–410

Telford M (1978) Post-larval growth in two species of *Dissodactylus* (Brachyura: Pinnotheridae). Bull Mar Sci 28: 645–650

Telford M (1982) Echinoderm spine structure, feeding and host relationships of four species of *Dissodactylus* (Brachyura: Pinnotheridae). Bull Mar Sci 32:584–594

Tsang LM, Chan BKK, Shih FL, Chu KH, Allen Chen C (2009) Host-associated speciation in the coral barnacle *Wanella milleporae* (Cirripedia: Pyrgomatidae) inhabiting the *Millepora* coral. Mol Ecol 18:1463–1475

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10: 506–513

Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Mol Ecol 15:1419–1439

Weber LI, Hartnoll RG, Thorpe JP (2000) Genetic divergence and larval dispersal in two spider crabs (Crustacea: Decapoda). Hydrobiologia 420:211–219

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38: 1358–1370

Yednock BK, Neigel JE (2011) Rethinking the mechanisms that shape marine decapod population structure. In Held C., Koenemann S. and Schubart C.D. (eds) Phylogeography and population genetics in Crustacea. CRC Press, Boca Raton, FL